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Chemical

Phytochemical profiling, antioxidant, and phytotoxic potentials of *Erythrina speciosa* Andrews leaves

Perfil fitoquímico, potencial antioxidante e fitotóxico das folhas de *Erythrina speciosa* Andrews

Alda Ernestina dos Santos[I], **Naomi Kato Simas**[II], **Ricardo Machado Kuster**[III]

[I] Instituto Federal de Minas Gerais, Departamento de Ciências e Linguagens, Bambuí, MG, Brasil
[II] Universidade Federal do Rio de Janeiro, Faculdade de Farmácia, Rio de Janeiro, RJ, Brasil
[III] Universidade Federal do Espírito Santo, Departamento de Química, Vitória, ES, Brasil

ABSTRACT

In order to enhance the chemical and biological understanding of the genus *Erythrina*, this study evaluated the chemical composition, phytotoxicity, and antioxidant potential of the hexane (Hex), dichloromethane (DCM), and ethyl acetate (EtOAc) phases from the methanolic extract of *E. speciosa* leaves. The DCM and EtOAc phases exhibited significant antioxidant activity, with DPPH radical reduction percentages exceeding 90%. Phytotoxicity tests revealed the phytotoxic potential of the DCM and EtOAc phases, inhibiting the growth of *L. sativa* seedlings by more than 40% and 30%, respectively, at concentrations of 1000 ppm and 500 ppm. Phytochemical analysis revealed a high total phenolic content in the DCM and EtOAc phases, where flavonoids such as apigenin, abyssinone II, wighteone, sigmoidin I, orientanol E, vitexin, and quercitrin were detected through techniques such as high-performance liquid chromatography (HPLC), electrospray ionization mass spectrometry (ESI-MS/MS), and thin layer chromatography (TLC). These compounds may be associated with the observed antioxidant potential and the inhibitory effects observed on *L. sativa*. However, further research on the isolated effects of these metabolites is warranted.

Keywords: *Erythrina*; Phytochemistry; Flavonoids; Phytotoxicity; Antioxidant activity

RESUMO

Visando aprimorar o conhecimento químico e biológico do gênero Erythrina, neste estudo avaliou-se a composição química, a fitotoxicidade e o potencial antioxidante das frações hexânica (Hex), diclorometano (DCM) e acetato de etila (EtOAc) do extrato metanólico das folhas de E. speciosa. As frações DCM e EtOAc exibiram atividade antioxidante significativa, com percentuais de redução do radical DPPH superiores a 90%. Os testes de fitotoxicidade revelaram o potencial fitotóxico das frações

DCM e EtOAc, que nas concentrações de 1000 ppm e 500 ppm inibiram o crescimento de mudas de L. sativa em mais de 40% e 30%, respectivamente. A análise fitoquímica revelou um elevado teor de fenóis totais para as frações DCM e EtOAc, nas quais foram detectados os flavonoides apigenina, abyssinona II, wighteona, sigmoidina I, orientanol E, vitexina e quercitrina. Esses compostos podem estar relacionados ao potencial antioxidante e aos efeitos fitotóxicos observados sobre L. sativa. No entanto, são necessários estudos futuros avaliando o efeito isolado desses metabólitos.

Palavras-chave: *Erythrina*; Fitoquímica; Flavonoides; Fitotoxicidade; Atividade antioxidante

1 INTRODUCTION

Medicinal plants have been used for centuries to treat various ailments based on their diverse biological activities, including antioxidant, cytotoxic, antibacterial (Viana et al., 2022) and antitumoral (Viana et al., 2023). Their therapeutic properties are derived from a diverse array of specialized metabolites evolved as part of plant defense or adaptation strategies. These bioactive compounds include phenolics, terpenes and alkaloids and that exhibit potent pharmacological activities. However, the complex phytochemistry poses challenges in fully characterizing plants' pharmacological potential.

With growing interest in natural products research and plant-based drug discovery, medicinal plants present an important source of leads for drug development and the optimization of extraction methods, particularly solvent selection, is crucial to recover bioactives and enable discovery of lead compounds (Viana et al., 2022a). Proper selection of extracting solvent based on a plant's known phytochemistry and desired applications is imperative for medicinal plant research. This enhances recovery of bioactive compounds and leads to more reproducible results.

The *Erythrina* genus (Fabaceae) holds significant medicinal value with a history of popular use since ancient times (Gilbert and Favoretto, 2012). Therefore, species of this genus play a crucial role in folk medicine across different countries, where they are used to treat a wide range of diseases, including infections (Wintola et al., 2021), malaria (Dkhil et al., 2020), inflammations (Thomgmee and Itharat, 2016), asthma (Amorim et al., 2018), bronchitis (Almeida, 1993), and depression

(Martins and Brijesh, 2020). The medicinal properties of *Erythrina* species are commonly associated with the presence of alkaloids and flavonoids, metabolites widely distributed in the genus (El-Masry et al., 2010).

Although sedative and calming activities are the most commonly reported for *Erythrina* (Garín-Aguiar et al., 2000; Rosa et al., 2012; Fahmy et al., 2018; Martins and Brijesh, 2020), the literature highlights a broad spectrum of biological effects for species of this genus. These encompass anti-inflammatory properties (Bhagyasri et al., 2017), antimicrobial effects (Mohanta et al., 2017; Sadgrove et al., 2020; Ahmed et al., 2020), cytotoxic (Mohanta et al., 2017; Ahmed et al., 2020) and antioxidant potentials (Mohanta et al., 2017; Bedane et al., 2016), antitumor activity (Passreiter et al., 2015) and enzyme inhibition (Santos et al., 2012; Hikita et al., 2015).

The genus *Erythrina* encompasses more than 100 species, which due to the striking beauty of their inflorescences are cultivated as ornamentals in various regions worldwide (Martins, 2014). These plants are predominantly found in tropical and subtropical regions (Vasconcelos et al., 2013). In Brazil, there are reports of around 11 species of *Erythrina*, distributed across biomes such as the Atlantic Forest, Cerrado, Caatinga, and Amazon (Lima and Martins, 2015).

Erythrina speciosa Andrews is a native and endemic Brazilian specie, found in the Cerrado and the Atlantic Forest, with geographic distribution in states on Northeast, Midwest, Southeast, and South regions of the country (Lollato et al., 2010). Despite its recognized medicinal potential, this specie is particularly renowned for its ornamental use. This is especially evident in the state of São Paulo, where *E. speciosa* is the specie of *Erythrina* most used in urban ornamentation (Martins, 2014).

From a phytochemical perspective, *E. speciosa* remains relatively unexplored, as there is a lack of studies evaluating the foliar flavonoids of this species. To contribute to the phytochemical and biological knowledge of the genus *Erythrina*, this study aimed to assess the phytochemical profile of the *E. speciosa* Andrews leaves. We also determined the total phenolic content and antioxidant potential of the different fractions from the

methanolic extract from the leaves. Finally, was evaluated the phytotoxicity of extracts as its impact on germination and early development of *Lactuca sativa*.

2 MATERIALS AND METHODS

2.1 Chemicals

Menadione (PubChem CID: 4055), quercetin (PubChem CID: 5280343), gallic acid (PubChem CID: 370), polyethylene glycol (PEG; PubChem CID: 481110092) and DPPH (2,2-diphenyl-1-picrylhydrazyl; PubChem CID: 15911) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Silica gel 60 F_{254} (20x20 cm) chromatoplates and dimethyl sulfoxide (DMSO: 99%; PubChem CID: 679) from purchased from Merck (Germany). NP reagent (2-aminoethyl diphenylborinate; PubChem CID: 1598) and Folin-Ciocalteau reagent (sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonate; PubChem CID: 10636) were purchased from Spectrum Chemical (New Brunswick, NJ, USA). The solvents acetic acid, dichloromethane, ethyl acetate, hexane, methanol, and phosphoric acid were obtained from Tedia (Fairfield, OH, USA).

2.2 Phytochemical study

2.2.1 Plant material

E. speciosa leaves were collected in a rural area of Silva Jardim, Rio de Janeiro, Brazil. The botanical identification of the collected sample was performed by profª Dra. Cássia Sakuragui and a voucher specimen was deposited in the Herbarium of the Botany Department of the Biology Institute, Federal University of Rio de Janeiro, under the number 39498.

2.2.2 Plant extraction

The fresh leaves (1300 g) of *E. speciosa* were dried in a hot air oven at 40 ± 1°C for

48 h. The dried leaves (1130 g) were then powdered and extracted exhaustively with methanol (MeOH) for 7 days. Thereafter, the crude methanolic extract was filtered through a Whatman paper (WHA1440150; Sigma-Aldrich) and evaporated to dryness under reduced pressure at a temperature below 45 °C in a Pemer rotatory evaporator coupled to a Cole Parmer vacuum pump, model 7049-50, resulting in 83 g of crude extract. Finally, the crude extract (45 g) was dissolved in a hydroalcoholic solution (MeOH-H_2O, 9:1) and subjected to partitioning with solvents in an increasing polarity gradient, using hexane, dichloromethane, and ethyl acetate (Santos et al., 2014), resulting in the respective phases: Hex (6.6 g), DCM (10.5 g) and EtOAc (4.3 g).

2.2.3 TLC and HPLC-DAD analysis

The Hex, DCM, and EtOAc phases were analyzed by Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography coupled with Diode Array Detector (HPLC-DAD). The TLC analyses were performed using silica gel 60 F_{254} chromatoplates eluted with an eluent system composed of EtOAc:MeOH:H_2O:AcOH (80:10:5:5, v/v) and revealed with the NP reagent and PEG.

In HPLC-DAD analysis, phenolic compounds were determined using a Shimadzu CBM-10A system chromatograph attached to an SPD-M10A ultraviolet photodiode beam detector. A 250 mm x 5 mm, 5 µm, Lichrosorb RP-18 (Phenomenex) column was chosen for chromatographic analysis. The eluents H_2O:H_3PO_4 (99:1, v/v) (solvent A) and MeOH:H_3PO_4 (99:1, v/v) (solvent B) were used in gradient mode at a flow rate of 1 mL/min. The composition of B was increased from 50% to 100% in 40 minutes. UV spectra were taken at 254 and 365 nm.

2.2.4 ESI-MS/MS analysis

The DCM and EtOAc phases were examined by Electrospray Ionization Mass Spectrometry (ESI-MS) using a methodology applied to flavonoid analysis. ESI-MS analyses were performed on a high-resolution Bruker micrOTOF II spectrometer

operating in the negative ionization mode for a mass range of *m/z* 100-1500. Samples were analyzed by direct insertion into the ionization source of the spectrometer, operating with nebulizer gas pressure at 0.6 bar, capillary voltage at 4.0 kV, and capillary temperature transfer at 180 °C.

The spectra obtained were processed using Bruker Compass Data Analysis 4.0 software. The elemental composition of the substances detected was determined based on the *m/z* values of the pseudomolecular ions. The *m/z* value and the suggested molecular formulas were used in the search for the probable structures in the literature and flavonoids database. The fragmentation of the main pseudomolecular ions of each metabolite was analyzed to confirm the compatibility between the detected substance and the substance suggested by the literature.

2.2.5 Total phenolic content determination

The Folin-Ciocalteau method adapted for microplates was employed to assess the Total Phenolic Content (TPC) of the Hex, DCM, and EtOAc phases from *E. speciosa* leaves. For this purpose, 100 µL of sample and 100 µL of MeOH were mixed in a tube. Then 100 µL of Folin-Ciocalteau reagent and 700 µL of 20% sodium carbonate solution were added. The reaction occurred for 20 minutes in the dark at room temperature. Following this, the samples underwent centrifugation for 5 minutes, and an aliquot of 250 µL was transferred to each well of the microplate. The absorbance was read at 760 nm using an automatic ELISA microplate reader (Molecular Devices). The TPC of the extracts was expressed as mg of gallic acid equivalent/g extract (Kenny et al., 2013), and calculated based on the calibration curve equation obtained with gallic acid solutions at varying concentrations (10 mg/L-200 mg/L).

2.3 Antioxidant potential evaluation

The *in vitro* antioxidant capacity of the extracts was determined using the DPPH (1,1-diphenyl2-picryl hydrazyl) radical scavenging method (Chatatikun and

Chiabchalard, 2013). Therefore, 50 µL of DPPH solution (0.3 mM) was mixed with 175 µL of extract prepared at different concentrations (5 mg/L-500 mg/L). Following incubation in complete darkness for 30 minutes, the absorbance was measured at 517 nm. The experiment was replicated three times, and the antioxidant potential was expressed by EC_{50}, the antioxidant concentration necessary to reduce the original amount of DPPH radicals by 50%. Quercetin served as the positive control.

2.4 Phytotoxicity evaluation

The phytotoxicity of the Hex, DCM, and EtOAc phases on *L. sativa* (lettuce) was assessed according to Baratelli *et al*. (2012). The evaluation considered two primary parameters: seed germination and seedling development, based on hypocotyl and root growth. The experiments were conducted in triplicate.

L. sativa seeds were chosen as model seedlings due to their rapid germination, uniformity, and sensitivity (Tigre et al., 2012), making them an ideal target specie for preliminary tests of substances or extracts with potential for use in weed control.

To assess the impact of *L. sativa* seed germination, the extracts were tested at different concentrations (125-1000 ppm). Each sample was dissolved in MeOH, and 500 µL of the solution was applied in a Petri dish (diameter = 6.0 cm; height = 1.5 cm) containing a filter paper disc. A methanolic solution of menadione at 143 ppm served as a positive control. After allowing the organic solvent to evaporate at room temperature for 24 hours, 2.5 mL of a 0.1% DMSO aqueous solution and 10 seeds of *L. sativa* were added to each plate. The Petri dishes were then placed in a germination chamber (model 708 NT; Novatecnica) at 25 ± 2 °C with a photoperiod of 12 hours for 5 days. Subsequently, the length of the seedlings' roots and hypocotyls was measured using a digital caliper (King Tools). The growth parameters were calculated as the mean percentage difference compared to the negative control (0.1% DMSO aqueous solution) treatments using the following Formula:

$$\text{Percentage difference (\%)} = \frac{\text{negative control} - \text{organic extract}}{\text{negative control}} \text{ x } 100 \quad (1)$$

2.5 Statistical analysis

The obtained data were submitted to statistical analysis using the GraphPad Prism version 5.0 (GraphPad Inc., La Jolla, CA, USA), with mean comparisons performed using the Tukey test. Differences were considered significant when $p<0.05$. Results were expressed as mean ± standard deviation (SD) of three independent and parallel measurements.

3 RESULTS AND DISCUSSION

3.1 Phytochemical profile

Preliminary TLC analysis revealed the presence of flavonoids only in the DCM and EtOAc phases, which were submitted to HPLC-DAD and ESI-MS/MS analyses for chemical characterization.

HPLC-DAD and ESI-MS/MS analyses indicated a significant similarity in the chemical profile of the DCM and EtOAc phases, where flavonoids from different classes were detected as aglycones and/or glycosides. Table 1 illustrates that the detected chemical classes of flavonoids include flavones, flavanones, flavonols, isoflavones, and isoflavanones. Detected compounds were tentatively identified based on mass fragmentation data compared to those already reported in the literature. Seven flavonoids were successfully characterized.

The ion with $[M - H]^-$ at *m/z* 269 is consistent with the aglycone of an isoflavone or a flavone. The mass spectrum of this ion revealed characteristic signals of the isomers apigenin and genistein, both flavonoids previously described for the genus *Erythrina*.

The *m/z* 161 fragment results from the ^{0,4}B-type fragmentation of the flavonoid C ring. This fragmentation is typical of flavones and rarely observed for isoflavones, whose characteristic fragmentations in negative mode are ^{1,3}B and ^{0,3}B types. Thus, this flavonoid was tentatively identified as apigenin, a flavone present in *E. vogelii* (Waffo et al., 2006), *E. caffra* (El-Masry et al., 2010) and *E. falcata* (Oliveira et al., 2014).

Table 1 – ESI-MS data of the detected flavonoids in the DCM and EtOAc phases

Compatible flavonoid	Molecular formula	$[M - H]^-$	MS/MS *m/z*	Reference
apigenin [b]	$C_{15}H_{10}O_5$	269.0487	251, 176, 161	Oliveira et al., (2014)
abyssinone II [a]	$C_{20}H_{20}O_4$	323.1264	305, 253, 189, 173	Chacha et al., (2005)
prenyl flavonoid [b]	$C_{20}H_{16}O_5$	335.0936	198, 136	Juma and Majinda (2006)
wighteone [b]	$C_{20}H_{18}O_5$	337.1075	319, 267, 219, 177	Djiogue et al., (2009)
sigmoidin I [a,b]	$C_{21}H_{22}O_5$	353.1366	335, 283, 201	Nkengfack et al., (1994)
orientanol E [a]	$C_{25}H_{28}O_6$	423.1785	405, 353, 271, 200	Tanaka et al., (1998)
vitexin/isovitexin [a,b]	$C_{21}H_{20}O_{10}$	431.0997	413, 341, 311	Oliveira et al., (2014)
quercitrin [a,b]	$C_{21}H_{20}O_{11}$	447.0958	429, 301, 151	Santos et al., (2014)
di-glycosyl flavonoid [a,b]	$C_{26}H_{28}O_{14}$	563.1433	353, 191	Ganbaatar et al., (2015)
di-glycosyl flavonoid [a,b]	$C_{26}H_{28}O_{15}$	579.1391	561, 447, 285	Krenn et al., (2003)

Source: Authors (2024)

[a] DCM phase; [b] EtOAc phase

The ion with $[M - H]^-$ at *m/z* 323 is compatible with a prenylated flavonoid. This is supported by the presence of the fragment at *m/z* 253 resulting from the loss of the isoprenyl unit. Additionally, the fragment at *m/z* 189 results from ^{0,4}B-type fragmentation followed by the loss of a C_3H_6 unit from the isoprenyl group. The signals observed in the spectrum are consistent with the fragmentation pattern of abyssinone II, a flavanone found in several species of *Erythrina*, including *E. abyssinica* (Nakanishi, 1982), *E. latissima*, (Chacha et al., 2005) e *E. addisoniae* (Watjen et al., 2008).

Three other prenylated flavonoids were characterized: wighteone, sigmoidin I, and orientanol E, with their $[M - H]^-$ ions observed at *m/z* 337, 353 and 423, respectively. For the $[M - H]^-$ ion at *m/z* 337, characteristic signals of an isoflavone were observed. The signal at *m/z* 219 resulted from ^{1,3}A-type fragmentation, confirming the presence of the

isoprenyl group in ring A. In turn, the fragment at *m/z* 177 is due to the simultaneous elimination of the isoprenyl group and ring B, consistent with the fragmentation pattern of the prenylated isoflavone wighteone.

Wighteone, also known as Erythrinin B, is an isoflavone commonly found in *Erythrina* species. Previous studies have reported the presence of this flavonoid in *E. orientalis* (Tanaka et al., 1998), *E. arborescens* (Yu et al., 2000), *E. indica* (Nkengfack et al., 2001), *E. lysistemon* (Pillay et al., 2001), *E. suberosa* (Tanaka et al., 2001), *E. poeppigiana* (Djiogue et al., 2009), *E. addisoniae* (Nguyen et al., 2010) e *E. subumbrans* (Rukachaisirikul et al., 2014).

The $[M - H]^-$ ion at *m/z* 353 showed MS/MS fragmentation at *m/z* 283 and *m/z* 201 related to the loss of the prenyl group and ^{0,3}B-type fragmentation with simultaneous elimination of the methyl group, respectively, suggesting the identification of the substance as sigmoidin I, an isoflavanone found in the roots of *E. sigmoidea* (Nkengfack et al., 1994).

A diprenyl flavonoid with $[M - H]^-$ ion at *m/z* 423 was detected in the DCM phase. The signal at *m/z* 353 observed in the MS/MS spectrum of this ion is related to the elimination of one of the prenyl groups. In turn, the signals at *m/z* 287 and *m/z* 271 are the result of fragmentations of ^{1,3}A and ^{0,3}A types, respectively, and confirm the presence of two isoprenyl units in ring A. These signals are compatible with the fragmentation of orientanol E, a diprenyl isoflavanone found in the roots of *E. orientalis* (Tanaka et al., 1998) and *E. suberosa* (Tanaka et al., 2001).

Finally, two monoglycosylated flavonoids were observed in the DCM and EtOAc phases. The $[M - H]^-$ ions at *m/z* 431 and *m/z* 447 correspond to a flavone and a flavonol, respectively. The MS/MS spectrum of the ion at *m/z* 431 revealed signals consistent with the fragmentation of vitexin or isovitexin, isomeric flavones found in *E. caffra* (El-Masry et al., 2010) and *E. falcata* (Oliveira et al., 2014). The signal at *m/z* 341 arises from the ^{0,2}X-type fragmentation of a hexose. The absence of a signal at *m/z* 268 confirms that the flavonoid in question is a *C*-glycosyl flavone since complete elimination of the hexose does not occur.

The ion at *m/z* 447 was identified as quercetin-3-*O*-ramnoside (quercitrin), with signals observed at *m/z* 301 and *m/z* 151 resulting from rhamnose elimination and ^{1,3}B-type fragmentation, respectively. Quercitrin is a monoglycosylated flavonol present in the roots of *E. mulungu* (Oliveira, 2009).

In terms of flavonoids, *Erythrina* exhibits great diversity, with more than 370 flavonoids reported for the genus (Chacon et al., 2022). However, *Erythrina* is primarily known for producing prenylated flavonoids, which are metabolites of limited occurrence (Cui et al., 2010), and of significant medicinal interest (Nguyen et al., 2012; Passreiter et al., 2015; Nguyen et al., 2020). As a result, prenyl flavonoids constitute the focus of most phytochemical studies evaluating the flavonoids of this genus (Nyandoro et al., 2017; Tuenter et al., 2019; Koch et al., 2019). Therefore, the characterization of flavonoids in the *Erythrina* genus holds significance from both phytochemistry and taxonomical perspectives.

In the present study, it is observed that the DCM and EtOAc phases contain similar flavonoids. The most abundant group consisted of prenylated flavonoids represented by four compounds compatible with the metabolites abyssinone II, wighteone, sigmoidin I and orientanol E, flavonoids described for different species of *Erythrina*, such as *E. latissima* (Chacha et al., 2005), *E. poeppigiana* (Djiogue et al., 2009), *E. sigmoidea* (Nkengfack et al., 1997) and *E. orientalis* (Tanaka et al., 1998), respectively.

As a result of our study, valuable flavonoids were detected in *E. speciosa* leaves consistent with the literature. However, some unidentified substances have also been detected, such as diglycosides flavonoids with molecular formulas $C_{26}H_{28}O_{14}$ and $C_{26}H_{28}O_{15}$. Further studies are required to characterize these metabolites.

3.2 Total phenolic content

The evaluation of phenolic content revealed a higher TPC for the DCM and EtOAc phases, which showed no significant difference ($p < 0.05$) between them, with values of 127.4 ± 1.2 and 119.2 ± 2.2 mg EAG/g of extract, respectively (table 2). Such

values are considerably higher than those observed for the DCM and EtOAc extracts of *E. neillii* leaves (Gabr et al., 2019), whose TPC were 9.1 ± 1.2 and 9.6 ± 0.3 mg EAG/g, respectively.

The TPC of DCM and EtOAc phases were also superior to that found by Sakat and Juvekar (2010) when evaluating the total phenolic content of the aqueous and the methanolic extracts from the leaves of *E. indica*, for which values of 24.9 ± 0.0 and 25.6 ± 0.0 mg EAG/g were observed, respectively.

Table 2 – Total phenolic content of Hex, DCM, and EtOAc phases

Phase	TPC (mg EAG/g)
Hex	46.3±1.1[b]
DCM	127.4 ± 1.2[a]
EtOAc	119.2 ± 2.2[a]

Source: Authors (2024)

Means followed by the same letter do not differ statistically by Tukey's test, for independent samples ($p<0.05$)

The Hex phase exhibited lower TPC compared to the DCM and EtOAc phases. However, the TPC of the Hex phase from *E. speciosa* leaves is higher than that observed for the DCM and EtOAc extracts of *E. indica* leaves (Sakat and Juvekar, 2010), as well as the aqueous and methanolic extracts of *E. neillii* leaves (Gabr *et al*., 2019).

3.2 Antioxidant potential

According to the results presented in Table 3, all samples were active and their CE_{50} could be calculated (Table 3). The DCM and EtOAc phases showed the strongest antioxidant potential, with DPPH reduction percentages above 90% for the highest concentration evaluated. There was no significant difference between the DCM and EtOAc phases, with EC_{50} values of 173.1 ± 0.2 and 163.9 ± 0.4 mg/L, respectively.

The DCM and EtOAc phases of the methanolic extract from *E. speciosa* leaves exhibited significantly higher antioxidant potential compared to the aqueous extract and methanolic extracts from *E. indica* leaves (Sakat and Juvekar, 2010), with EC_{50} values of 342.6 ± 19.6 and 283.2 ± 12.3 mg/L, respectively. Furthermore, the EtOAc

phase from *E. speciosa* leaves demonstrated greater activity than the EtOAc extract from *E. vogelii* leaves (Tauseef et al., 2013), which exhibited an EC_{50} > 200 mg/L by the DPPH method.

Table 3 – Antioxidant potential of the Hex, DCM, and EtOAc phases

Phase	CE_{50} (mg/L)
Hex	484.8 ± 0.3[a]
DCM	173.1 ± 0.2[b]
EtOAc	163.9 ± 0.4[b]

Source: Authors (2024)

Means followed by the same letter do not differ statistically by Tukey's test, for independent samples ($p<0.05$)

Despite the significant antioxidant potential observed for the DCM and EtOAc phases, other extracts of *Erythrina* species are more active in reducing the DPPH radical. For example, the acetone extract from the root bark of *E. burttii* exhibited an EC_{50} = 12.0 ± 2.6 mg/L (Yenesew et al., 2012), while the methanolic extract from *E. variegata* leaves showed an EC_{50} = 89.3 ± 1.5 mg/L (Alam et al., 2020).

Antioxidants are substances capable of minimizing or preventing the damage caused by the oxidation of macromolecules or cellular structures, thus protecting biological systems from harmful actions caused by free radicals (Mishra et al., 2012). Secondary metabolites are one of the main sources of antioxidant substances, especially flavonoids (Fahmy et al., 2018; Chatatikun and Chiabchalard, 2013). Therefore, many of the pharmacological effects observed for this class of substances are related to their antioxidant potential resulting from their ability to scavenge free radicals, chelate metal ions and/or act synergistically with other antioxidants (Silva et al., 2002).

The antioxidant potential of flavonoids is well-known (Banjarnahor and Artanti, 2014; Wang et al., 2020), and studies evaluating the structure-activity relationship reveal that factors such as the arrangement of functional groups around the nuclear structure and the total number of hydroxyl groups substantially influence the *in vitro* antioxidant activity presented by flavonoids (Heim et al., 2020; Moalin et al., 2011).

In this sense, the greater antioxidant potential presented by the DCM and EtOAc

phases is likely due to the flavonoids present in these samples, which consist mainly of aglycones and monoglycosides. These compounds have one or more free hydroxyl groups, which are structural factors that contribute to the antioxidant potential of phenolic compounds (Wang et al., 2020; Rice-Evans et al., 1996).

3.3 Phytotoxicity

The phytotoxicity results indicate that only the Hex and DCM phases exhibited significant inhibitory effects on the germination of *L. sativa* seeds, with inhibition percentages of 13.3% and 21.1% observed at 1000 ppm, respectively (Chart 1).

Chart 1 – Effects on the germination and initial development of *L. sativa*

Phase	Concentration (ppm)	Germination inhibition (%)	Hypocotyl inhibition (%)	Root inhibition (%)
Hex	1000	13.3 ± 0.8^{a}	10.3 ± 0.4^{b}	23.9 ± 1.8^{b}
	500	4.44 ± 0.4^{b}	4.94 ± 0.2^{c}	21.6 ± 0.5^{b}
	250	5.56 ± 1.3^{b}	0.82 ± 0.3^{c}	17.2 ± 0.9^{b}
	125	3.33 ± 0.9^{b}	0.64 ± 0.4^{c}	8.33 ± 0.8^{c}
DCM	1000	21.1 ± 1.2^{a}	25.1 ± 1.2^{a}	47.0 ± 2.2^{a}
	500	6.67 ± 0.9^{b}	8.23 ± 1.0^{b}	36.6 ± 1.5^{a}
	250	4.44 ± 1.0^{b}	2.47± 0.9^{c}	16.7 ± 0.9^{b}
	125	3.33 ± 0.4^{b}	1.64 ± 0.5^{c}	8.87 ± 0.6^{c}
EtOAc	1000	4.44 ± 0.9^{b}	17.7 ± 0.2^{a}	41.4 ± 2.1^{a}
	500	5.56 ± 1.2^{b}	9.05 ± 0.2^{b}	35.8 ± 1.9^{a}
	250	4.44 ± 1.0^{b}	3.29 ± 0.2^{c}	18.0 ± 0.8^{b}
	125	3.33 ± 0.9^{b}	2.46 ± 0.3^{c}	7.63 ± 1.0^{c}

Source: Authors (2024)

Means followed by the same letter do not differ statistically by Tukey's test, for independent samples ($p<0.05$)

The data reveals a higher activity for the DCM and EtOAc phases in the initial growth of *L. sativa* seedlings. The effects on root development were more pronounced than on hypocotyl growth. At 1000 ppm, inhibition rates of 25.1% and 17.7% for hypocotyl growth were observed for the DCM and EtOAc phases, respectively.

The root growth of the seedlings was significantly affected by all phases evaluated, as the treatment at 1000 ppm resulted in inhibition percentages exceeding 40%, thus

demonstrating the phytotoxic potential on the root growth of *L. sativa*. Such activity may be related to the presence of allelochemical substances in the leaves of *E. speciosa*.

Root growth is generally considered a better indicator of the phytotoxic effects of plant extracts. The higher sensitivity of roots is widely reported in the literature (Nakamura et al., 2021; Ogunsanya et al., 2022; Pinto et al., 2023). According to Alves et al. (2022), this is due to the direct contact of the roots with the extracts, exposing them to high concentrations of phytochemical compounds.

In a study conducted by Soares et al. (2002), evaluating the phytotoxicity of aqueous extracts from the leaves of six legumes, including *E. speciosa*, on the germination and development of *L. sativa*, no inhibitory effects were observed. However, treatment with the aqueous extract of this species showed inhibitory activity on the root growth of *L. sativa*. The authors attributed this activity to the presence of phenolic compounds, as the treatment with the aqueous extract after filtering with polyvinylpyrrolidone (PVP) to remove phenolic compounds showed no activity on seedling growth.

Garcia-Mateos et al. (2002) evaluated the impact of three alkaloids isolated from *E. americana* seeds on the germination of maize (*Zea mays*) and common bean (*Phaseolus vulgaris*) seeds, and no inhibitory effects were observed on either of the two target species.

In another study, Gris et al. (2019), examined the phytotoxic potential of hydroalcoholic extracts from leaves, bark, roots, and seeds of *E. fusca* on *L. sativa*. They found that leaves exhibited greater activity, which was attributed to the presence of *C*-glycosylated flavonoids detected in the extract. Treatment with *E. fusca* leaf extract significantly inhibited seed germination and root growth of *L. sativa* seedlings, demonstrating phytotoxicity similar to that induced by 2,4-dichlorophenoxyacetic acid (2,4-D), used as a positive control.

The allelochemicals inhibit and alter the growth or developmental patterns of plants, through selective action (Merino et al., 2018). There is a wide variety of allelochemicals, including flavonoids. These metabolites exert an inhibitory effect on

the germination and growth of various plant species (Weston and Mathesius, 2013). In the literature, several studies demonstrate the phytotoxic potential of different classes of flavonoids, including flavones, flavanones (Napal and Palacios, 2013), flavonols (Imatomi et al., 2013), flavan-3-ols (Nebo et al., 2014), and isoflavonoids (Shajib et al., 2012).

The phytotoxic potential of flavonoids is widely recognized, impacting seed germination and root growth of different seedlings (Weston and Mathesius, 2013; Bertoldi et al., 2009; Hooper et al., 2010; Mierziak et al., 2014; Gris et al., 2019). Considering the predominance of flavonoids in the DCM and EtOAc phases, these compounds are likely responsible for the observed phytotoxic effects on *L. sativa*.

4 CONCLUSIONS

This study characterized the phytochemical profile and investigated the antioxidant and phytotoxic potential of *E. speciosa* leaves. A total of seven flavonoids were characterized, including apigenin, abyssinone II, wighteone, sigmoidin I, orientanol E, vitexin and quercitrin.

The phytotoxic effect was primarily characterized by the inhibition of root development. However, Hex and DCM phases also affected the germination of *L. sativa* seeds. Currently, inhibiting seed germination and seedling growth is a significant way to assess the effectiveness of compounds with the potential to be used in weed control. Given the phytotoxicity exhibited by the leaves of *E. speciosa*, further must be conducted to purify and elucidate the metabolites responsible for the phytotoxic effect, which could contribute to a better understanding of the biological potential of the genus *Erythrina* and provide environmentally friendly weed management.

Although the genus *Erythrina* has been extensively studied, many species of this genus still lack scientific research, including *E. speciosa*, which remains relatively unexplored, particularly regarding its phytochemical and biological potential. In the present study, a total of seven flavonoids from the leaves of *E. speciosa* were characterized, and the antioxidant and phytotoxic potential of this specie was confirmed.

Given the ornamental and medicinal significance of *E. speciosa*, this study contributes to a better understanding of the phytochemistry and biological properties of an underexplored *Erythrina* species. Finally, the chemical diversity displayed by *E. speciosa* holds significance for understanding its traditional medicinal uses and discovering new applications.

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Authorship contributions

1 – Alda Ernestina dos Santos

Instituto Federal de Minas Gerais, PhD in Natural Product Chemistry
https://orcid.org/0000-0001-8086-7170 • alda.santos@ifmg.edu.br
Contribution: Writing – original draft

2 – Naomi Kato Simas

Universidade Federal do Rio de Janeiro, PhD in Natural Product Chemistry
https://orcid.org/0000-0002-9929-2714 • naomisimas@yahoo.com
Contribution: Writing – review and editing

3 – Ricardo Machado Kuster

Universidade Federal do Espírito Santo, PhD in Natural Product Chemistry
https://orcid.org/0000-0002-8961-5348 • kusterrm@gmail.com
Contribution: Supervision, writing – review and editing

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