Acta Brasiliensis 4(1): 45-48, 2020
http://revistas.ufcg.edu.br/ActaBra

Artigo Original
http://dx.doi.org/10.22571/2526-4338232

Phytochemical screening and evaluation of phytotoxic activity of *Solanum lycocarpum* (Solanaceae) ripe fruit

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Received: August 26, 2019 / Accepted: October 22, 2019 / Published online: January 20, 2020

Abstract

The species *Solanum lycocarpum* A. St. Hil. (wolf fruit), belonging to the Solanaceae family, is used in folk medicine for the treatment of obesity, diabetes, and cholesterol reduction. The aim of this study was to perform phytochemical screening of the ethanol extract from the ripe fruit of *S. lycocarpum* to verify the presence of the main groups of secondary metabolites and to evaluate the phytotoxic effect on *Lactuca sativa* (lettuce) and *Allium cepa* (onion) seeds exposed to different concentrations of extract (125, 250 and 500 μg per plate). The fruits of *S. lycocarpum* were collected, crushed and percolated with anhydrous ethanol and the solvent was dried in a rotatory evaporator to obtain the ethanol extract. The ethanol extract was able to completely inhibit the growth of hypocotyl and radicle of *L. sativa* (lettuce) seeds in all concentrations tested. In *A. cepa* (onion) seeds, a concentration of 500 μg per plate was able to inhibit the growth of the hypocotyl and radicle by 100%. The metabolites detected in the extract were alkaloids, coumarins, flavonoids, and condensed tannins. The phytotoxic activity can be correlated to the presence of these compounds.

Keywords: Allium cepa, *Lactuca sativa*, secondary metabolites, wolf fruit, ethanol extract.

Triagem fitoquímica e avaliação da atividade fitotóxica do fruto maduro de *Solanum lycocarpum* (Solanaceae)

Resumo

A espécie *Solanum lycocarpum* A. St. Hil. (lobeira ou fruto do lobo), pertencente à família Solanaceae, é utilizada na medicina popular para o tratamento da obesidade, do diabetes e na redução do colesterol. O objetivo deste estudo foi realizar a triagem fitoquímica do extrato etanólico do fruto maduro de *S. lycocarpum* para verificar a presença dos principais grupos de metabólitos secundários e avaliar o efeito fitotóxico sobre sementes de *Lactuca sativa* (alface) e de *Allium cepa* (cebola) expostas a diferentes concentrações do extrato (125, 250 and 500 μg por placa). Os frutos de *S. lycocarpum* foram coletados, triturados e extraídos por percolação com etanol anidro e, o solvente foi secado em evaporador rotatório, obtendo-se o extrato etanólico. O extrato etanólico foi capaz de inibir completamente o crescimento do hipocótilo e da radícula de sementes de *L. sativa* (alface), em todas as concentrações testadas. Nas sementes de *A. cepa* (cebola), a concentração de 500 μg por placa foi capaz de inibir em 100% o crescimento do hipocótilo e da radícula. Os metabólitos detectados no extrato foram alcaloides, cumarinas, flavonoides e taninos condensados. A atividade fitotóxica pode ser correlacionada à presença desses compostos.

Palavras-chave: Allium cepa, *Lactuca sativa*, metabólito secundário, lobeira, extrato etanólico.

Introduction

The growth of the world's population, combined with a greater demand for food production, has increased the use of herbicides for weed control. Synthetic herbicides cause damage to human health and the environment, such as triggering changes in resistance to invasive species, contamination of aquatic systems, and toxicity to humans. Researchers have been increasingly interested in finding natural compounds that can be used as bioherbicides to replace synthetic herbicides, and that are less environmentally harmful, water-soluble and less toxic (Matsumoto, Ribeiro, Takao, & Lima, 2010).

Secondary metabolites synthesized by plants and belonging to various classes (terpenes, alkaloids, phenolic compounds, steroids, long-chain fatty acids, unsaturated lactones) may act as allelochemicals and are regulated or altered by various environmental factors such as climate, and soil. Allelochemicals interfere in different ways in plant

communities to generate phytotoxic effects, such as decreasing plant growth due to inhibition of mitosis (Sanchez-Moreiras, De la Pena, & Reigosa, 2008), affecting the conservation, dormancy, germination, vegetative vigor, growth of seedlings and adult plants, and influencing species competition (Yamagushi, Gusman, & Vestena, 2011). Thus, the search for herbicides with different mechanisms of action from those already existing is of great importance, and plants with allelopathic effects could be a natural herbicide source (Alvarenga *et al*., 2009).

The species *S. lycocarpum* is found in the Brazilian Cerrado and is popularly known as cerrado eggplant or wolf fruit (Munari *et al*., 2012). This species is widely used in folk medicine as a sedative, in the treatment of epilepsy, diabetes, obesity, reduction of cholesterol levels, and in the preparation of influenza syrups (Dall'Agno & Von Poser, 2000). Previous studies have shown that fruits of *S. lycocarpum* exhibit allelopathic, anti-inflammatory, antimicrobial, antioxidant, antitumoral, cytotoxic, genotoxic and larvicidal activities (Morais, Silva, Oliveira, Ferreira, & Lima, 2013; Pereira, Silva, Ribeiro Neto, Alves, & Lima, 2014; Morais *et al*., 2015; Silva, Ribeiro Neto, Alves, & Lima, 2015; Chiavegatto, Chaves, Silva, Lima, & Techio, 2017; Morais *et al*., 2017; Bahia *et al*., 2018; Morais *et al*., 2018; Silva *et al*., 2018; Silva, Fonseca, Coimbra, Duarte-Almeida, & Lima, 2019).

Due the biological effects and low cytotoxicity of ripe fruits from *S. lycocarpum*, the contribution of this work is important to verify the phytotoxic effect of ethanol extract on lettuce and onion seeds.

Material and Methods

Plant material and extraction

S. lycocarpum ripe fruits were collected in the Cerrado region São Sebastião do Oeste, Midwest Minas Gerais State, Brazil (20°14'38.96" S and 45°2'14.38" W) (SISBIO n. 30006). Fertile samples were collected, and vouchers were identified by Dr. Alexandre Salino, and deposited in the Institute of Biological Sciences Herbarium (BHCB 159397) at the Federal University of Minas Gerais (UFMG). This research has access permission to the components of plant genetic heritage (n. 010655/2011-5/CNPq/CGEN/MMA) and it is registered in the SisGen Platform (Register AEF6C95), according to Brazilian Biodiversity Law (13.123/2015).

Extraction of the dried and powdered fruits (2086.13 g) by percolation (ethanol, 7 L) produced 48.02 g of ethanol extract (EE).

Phytochemical analysis

Phytochemical screening of the extract was performed to evaluate the presence of the main classes of secondary metabolites: flavonoids, coumarins, alkaloids (Silva, Miranda, & Conceição, 2010), saponins, tannins, steroids and triterpenoids (Matos, 2009).

Phytotoxic activity test by bioautography

Phytotoxicity was evaluated using seeds of *Lactuca sativa* L. var. Winter Curly (lettuce) (Feltrin®, Lot 46631196, Brazil) and *Allium cepa* cv. Baia Periforme (onion) (Feltrin®, Lot 450865, Brazil) according to the methodology described by Tonelli *et al*. (2014). The ethanol extract applied in chromatography plates of silica gel matrix (5.5 x 5.5 cm) with fluorescent indicator staining (Sigma-Aldrich) was eluted into the chromatography chamber using ethyl acetate/acetic acid/water (100:22:26) and visualized under UV light at 254 nm. The chromatographic profile of the ethanol extract by TLC plate exhibited four spots with different retention factors (Rfs). Rf 0.0 corresponded to the beginning of the chromatographic run, and Rf 0.9 (last spot in the plate). The intermediate profile was found between Rfs 0.0 and 0.9, and was a portion apparently free of substances. At the end of the chromatographic run was Rf 1.0.

$$\mathrm{Rf} = \frac{\text{distance spot moved}}{\text{distance solvent moved}} \qquad \text{(Eq. 1)}$$

The chromatography plates with ethanol extract then were placed into Petri dishes (10.0 Ø). One filter paper (9 cm in diameter) was placed over the extract-containing chromatography plates, 25 seeds were placed on top, and 10 mL of MES buffer (pH 6.0-6.2) was then added. In the control group was added the same amount of buffer to a Petri dish, containing a blank chromatography plate and the same amount of seeds. The test was performed in triplicate for different concentrations of extract (125, 250 and 500 µg per plate) and control group.

After a week of incubation in the dark, the Petri dishes were frozen at -10 °C for 24 h to stop plant growth. The size of hypocotyls and radicles of germinated *A. cepa* and *L. sativa* seeds were measured (Tonelli *et al*., 2014). The effects on growth of hypocotyls and radicles were calculated according by Pinto, Silva, Siqueira, Santos, & Lima (2013), which zero represents the control, positive values represent stimulation of growth, and negative values represent inhibition of growth.

Chemicals

For this study, 2-(*N*-morpholino) ethanesulfonic acid (MES) purchased from Sigma-Aldrich® (USA) was used. Sulfuric acid, ethanol, ferric chloride, sodium hydroxide, ethyl acetate, toluene and acetic anhydride purchased from Cromato Produtos Químicos® (Brazil). The reagents basic bismuth nitrate, potassium iodide and acetic acid were obtained from Vetec® (Brazil).

Statistical analysis

A Student's t-test was used to evaluate the statistical difference between the control group and the group exposed to ethanol extract of *S. lycocarpum.* The Tukey test was used to evaluate the statistical difference between growth of hypocotyl and radicle. The analyses were performed using GraphPad Prism 5.0 software (San Diego, CA, USA). Values were considered statistically significant at $p < 0.05$.

Results and Discussion

Phytochemical tests revealed the presence of alkaloids, coumarins, flavonoids, and condensed tannins in the ethanol extract, and absence of anthraquinones, steroids, triterpenes,

and saponins. A previous study with ripe fruits of this species (Morais *et al*., 2013) detected the presence of similar compounds in the ethanol extract as in our research; the exception was the absence of tannins. Phytochemical tests also showed the presence of coumarins, flavonoids, and tannins in the methanol extract from unripe fruits (Pereira *et al*., 2014). Many biological activities are correlated to the bioactive secondary metabolites present in plants (Oliveira, Gualtieri, Domíngues, Molinillo, & Montoya, 2012).

The ethanol extract exhibited strong phytotoxic effects on the hypocotyl and radicle of *L. sativa*, inhibiting 100% of growth in all Rfs, at all three concentrations tested. The hypocotyl and radicle growth of *A. cepa* seeds was also strongly inhibited (Table 1). The best inhibitory effects were observed at 500 µg per plate in all Rfs, and 250 µg per plate in the application (Rf 0.0), with 100% inhibition of hypocotyl and radicle growth.

Table 1. Phytotoxic effects of the ethanol extract from *S. lycocarpum* fruits on the hypocotyl and radicle growth of *A. cepa*.

Samples	Growth (% Control)					
	Hypocotyl			Radicle		
	125	250	500	125	250	500
RF 0.9	91.38 ± 0.21^{a}	90.62 ± 0.23^{a}	100.00 ± 0.00^{b}	79.43 ± 0.19^{c}	80.09 ± 0.18^{c}	100.00 ± 0.00^{b}
Intermediate	81.35 ± 0.46^{c}	85.40 ± 0.36^{d}	100.00 ± 0.00^{b}	64.44 ± 0.34^{e}	80.89 ± 0.18^{c}	100.00 ± 0.00^{b}
Application	79.14 ± 0.51^{c}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}	43.13 ± 0.54^{f}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}

Different concentrations of extract (125, 250 and 500 µg per plate). The results are means ± SD (n = 3). Means followed by the same letter do not differ according to the Tukey test ($p < 0.05$).

These results suggest that the ethanol extract presented great phytotoxic potential. In the phytotoxic assay, the ethanol extract (in all Rfs) was statistically significant when compared to the control ($p < 0.05$).

The growth inhibition of hypocotyl and radicle in *L. sativa* and *A. cepa* seeds when exposed to ethanol extract can be related to the secondary metabolites detected in phytochemical screening. Flavonoids, alkaloids, and coumarins can interfere with development by affecting the plant growth process (Soares, Scalon, Pereira, & Vieira, 2002). Coumarins are indicated as potent inhibitors of plant growth and seed germination by possessing the ability to block mitosis (Rice, 1984; Abenavoli *et al*., 2006; Willis, 2007).

There are some reports about the allelopathic activity of *S. lycocarpum*. Morais *et al*. (2013) showed the allelopathic potential of the ethanol extract and fractions (125, 250 and 500 µg/mL) from ripe fruits on *L. sativa* and *A. cepa* seeds, with inhibition in the range of 5-86% and 7-96%, respectively. Silva *et al*. (2019) evaluated the allelopathic effect of unripe fruit (50, 100 and 200 µg/mL) and the best results were for the hexane extract on hypocotyl and radicle of *A. cepa* seeds, with inhibitions of 52.7% and 41.3%, respectively, at a concentration of 200 µg/mL.

Conclusion

The ethanol extract from ripe fruits of *S. lycocarpum* showed potential phytotoxicity to the seeds of *A. cepa* and *L. sativa*. To the best of our knowledge, reports about the phytotoxic potential of the *S. lycocarpum* have not found.

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