Allelopathic potential of the ripe fruits of *Solanum lycocarpum* A. St. Hil. (Solanaceae)

Potencial alelopático dos frutos maduros de *Solanum lycocarpum* A. St. Hil. (Solanaceae)

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ABSTRACT
The species *Solanum lycocarpum* A. St. Hil., popularly known as the “fruit of the wolf”, is widely distributed in the Brazilian Cerrado. The fruits are usually consumed ‘in natura’ or used in jellies, jams or pasta preparations. The objective of this study was to evaluate the allelopathic potential of ethanol extract and fractions obtained from ripe fruits of *S. lycocarpum* against seeds of onion and lettuce. The ethanol extract and fractions showed allelopathic potential, especially on the growth of radicle of *Allium cepa* (onion). The dichloromethane and hydroethanol fractions showed inhibitory activity on the growth of hypocotyl of lettuce in concentrations at 125 and 250 μg/mL. The hydroethanol fraction inhibited growth of radicle of lettuce, at the three concentrations tested. At the concentration of 500 μg/mL, the dichloromethane fraction inhibited the radicle of lettuce in 86%. The ethanol extract and fractions showed inhibitory activity on hypocotyl of onion, in concentration at 500 μg/mL, and radicle at the three concentrations tested. The dichloromethane fraction showed better allelopathic activity on the growth of lettuce and onion seeds. These results should encourage additional studies of extract and fractions from the ripe fruits of *S. lycocarpum* for the isolation of bioactive compounds with allelopathic potential.

Keywords: *Solanum lycocarpum*; ripe fruits; allelopathic.

RESUMO
A espécie *Solanum lycocarpum* A. St. Hil., popularmente conhecida como "fruto-do-lobo", é amplamente distribuída no Cerrado brasileiro. Os frutos geralmente são consumidos ‘in natura’ ou utilizados em geleias, compotas ou na preparação de massas. O objetivo deste estudo foi avaliar o potencial alelopático do extrato etanólico e frações obtidas dos frutos maduros de *S. lycocarpum* sobre as sementes de alface e cebola. O extrato etanólico e frações apresentaram potencial alelopático, especialmente sobre o crescimento da radícula de *Allium cepa* (cebola). As frações diclorometânica e hidroetanólica inibiram o hipocótilo de alface nas concentrações de 125 e 250 μg/mL. A fração hidroetanólica inibiu o crescimento da radícula de alface nas três concentrações testadas. Na concentração de 500 μg/mL, a fração diclorometânica inibiu a radícula em 86%. O extrato etanólico e frações apresentaram atividade inibitória sobre o hipocótilo da cebola na concentração de 500 μg/mL e da radícula nas três concentrações testadas. A fração diclorometânica apresentou maior atividade alelopática sobre o crescimento das sementes de alface e cebola. Estes resultados incentivam estudos adicionais com o extrato e as frações dos frutos maduros de *S. lycocarpum* para o isolamento de compostos bioativos com potencial alelopático.

Palavras-chave: *Solanum lycocarpum*; frutos maduros; alelopático.

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Allelopathy refers to any process involving secondary metabolites produced by plants, algae, bacteria and fungi, which have positive or negative effects on the growth and development of the natural and agricultural system (IAS, 1996). The development of new bioactive molecules with potential application in pharmacology and agriculture by using natural products as templates has been a widely used in the recent years. The potential of phytochemicals and allelochemicals (natural plant toxins) in agriculture has been the subject of research with the main purpose of finding phytotoxic substances to use as new herbicidal templates (MACÍAS et al., 2008).

The Solanaceae family comprises about 3000 species and 150 genera. It is prevalent in tropical and subtropical regions of South America, and has economic importance because several species of the *Solanum* genus are cultivated for food, such as *Solanum tuberosum* (potato), *Solanum lycopersicum* (tomato), *Solanum melongena* (eggplant), and *Solanum gilo* (gilo) (SOUZA; LORENZI, 2008). The species *Solanum lycocarpum* A. St. Hil., popularly known as the "fruit of the wolf", is widely distributed in the Brazilian Cerrado. The fruits are usually consumed 'in natura' or used in jellies, jams, or pasta preparations (VIEIRA JR. et al., 2003). It is widely used in traditional medicine as a sedative, in the treatment of epilepsy, asthma, diabetes, obesity, reduction of cholesterol levels, and abdominal and renal pains (MUNARI et al., 2012). However, in our knowledge, there are few reports in the literature of biological activities of ripe fruits of *S. lycocarpum*. In the present work, the ethanol extract and fractions obtained from ripe fruits of *S. lycocarpum* were evaluated by allelopathic potential on the growth of seeds of *Lactuca sativa* (lettuce) and *Allium cepa* (onion).

The ripe fruits of *S. lycocarpum* A. St. Hil. were collected in São Sebastião do Oeste, Minas Gerais, Brazil, in August 2011. The plant material was identified by Dr. Alexandre Salino and a voucher specimen (BHCB 159397) was deposited at the Instituto de Ciências Biológicas Herbarium, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Extraction of the dried and powdered ripe fruits (250.58 g) by percolation (EtOH, 7 L, 72 h) gave the ethanol extract (EE, 48.02 g), after concentrated in a rotary evaporator at 40°C under reduced pressure. Part of this extract (11.90 g) was dissolved in EtOH/H_2O (1:1) and then successively extracted with C_6H_{14}, CH_2Cl_2, and EtOAc (Vetec, Brazil), resulting in 1.00, 0.58, 1.84, and 7.87 g of hexane (Hex), dichloromethane (DCM), ethyl acetate (Ac), and hydroethanol (HE) fractions, respectively.

Allelopathic activity of the ethanol extract and fractions obtained from ripe fruits of *S. lycocarpum* were evaluated by the effects on the growth of seeds of lettuce (*Lactuca sativa* cv cabbage, Feltrin, Brazil) and onion (*Allium cepa* cv Red Creole, Topsed Garden, Brazil). The ethanol extract and fractions dried were dissolved with deionized water and their pH values buffered with 10 mM 2-(*N*-morpholino) ethanesulfonic acid (MES, Sigma, St. Louis, USA) were adjusted to 6.0-6.5 with NaOH solution. Concentrations lower than 500 μg/mL were obtained by dilution series. Growth was conducted in 100 mm Petri dishes containing a 9.0 cm sheet of Whatman no. 1 filter paper as support. Then, 25 lettuce or onion seeds were placed per dish with 5 mL of a test (500, 250 and 125 μg/mL) or a control solution (deionized water with MES). All tests were performed in triplicate and repeated at least once. The dishes were covered with Parafilm to reduce evaporation and incubated in the dark at 25 ^{o}C, in a controlled-environment growth chamber, for 7 days. After this time, the lengths of radical and hypocotyl were measured. During the measurement process, the dishes were kept at 4 ^{o}C to avoid subsequent growth (VIEIRA et al., 2005).

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

% on the growth = [(Ma - Mc) / Mc] x 100

Ma is mean values of seeds with samples tested and **Mc** is mean values for 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. The data were evaluated using Student's *t* tests and the differences between the experiment and control were significant at a value of $P < 0.05$.

The allelopathic effects of the ethanol extract and fractions from ripe fruits of *S. lycocarpum* on the radicle and hypocotyl of *L. sativa* and *A. cepa*

were evaluated in three different concentrations, and the results are shown in Fig. 1 and 2. The EE and Hex fraction stimulated growth of the hypocotyl of *Lactuca sativa* (lettuce), at the three concentrations tested (Figure 1A). The Ac fraction, at a concentration of 125 μg/mL, stimulated the growth of hypocotyl, while at the concentrations of 250 and 500 μg/mL inhibited growth. The DCM and HE fractions showed inhibitory activity on the growth of hypocotyl at the concentrations of 125 and 250 μg/mL. At the concentration of 500 μg/mL, the DCM fraction showed the better inhibition, with 76%. The Figure 1B shows the results for radicle when seeds of lettuce were treated to ethanol extract and fractions, at the three different concentrations. Regarding on the growth of radicle, the samples presented heterogeneous allelopathic effects. The EE, Hex and Ac fractions stimulated growth of radicle, at a concentration of 125 μg/mL, and EE and DCM fraction at a concentration of 250 μg/mL. The HE fraction inhibited growth of radicle, at the three concentrations tested. At the concentration of 500 μg/mL, the DCM fraction inhibited the radicle of lettuce in 86%.

Figure 2A shows the growth of hypocotyl of the onion. The EE, Hex and HE fractions also presented heterogeneous allelopathic effects on the growth of hypocotyl, while the DCM and Ac fractions showed inhibitory activity. At the concentration of 500 μg/mL, the EE and HE fraction inhibited the growth by 84% and 44%, respectively. The Hex fraction inhibited growth at the concentrations of 125 and 500 μg/mL. The DCM and Ac fractions showed inhibitory activity at all concentrations evaluated, especially in the concentration of 500 μg/mL, with inhibition of 70% and 74%, respectively.

The EE and fractions showed high percentage of inhibition on the growth of radicle of *Allium cepa* (onion), at all concentrations evaluated (Figure 2B). The best inhibition effects were for concentrations 125 and 500 μg/mL. At the concentration of 500 μg/mL, the percentage inhibition was higher than 75% for all samples, where the HE fraction showed higher activity, with inhibition 96% of radicle.

The results of allelopathic effects show that inhibition or stimulation of growth of lettuce and onion seeds when treated with the samples are not dose-dependent. However, the seeds of *Allium cepa* (onion) present greater inhibition in presence of the EE and fractions of *S. lycocarpum*. There is no explanation for these response profiles, but our results corroborate with those obtained by PINTO et al. (2013) that show heterogeneous profiles and greater inhibition of onion seeds when treated with the samples.

Analysis of the differences between the experiment and control were significant at a value of $P < 0.05$, except in hypocotyl of lettuce for HE fraction at 500 μg/mL, and radicle of lettuce for Hex fraction at 250 and 500 μg/mL. Significant differences ($P > 0.05$) from the allelopathic effects not were observed: a) in hypocotyl of lettuce for HE at 125 and 250 μg/mL, EE at 125 and 250 μg/mL, and Hex at 125 μg/mL and EE at 500 μg/mL, b) in radicle of lettuce for Hex at 250 and 500 μg/mL, and Hex and Ac at 125 μg/mL, c) in radicle of onion for HE at 125 and 250 μg/mL, Hex and DCM at 125 μg/mL, DCM and HE at 250 μg/mL, and DCM and Ac at 500 μg/mL.

Study with *S. lycocarpum* showed that the leaves extracts were able to inhibit the growth of seeds of various plant species, such as tomato, watercress, onion and lettuce (OLIVEIRA et al., 2012). Another study determined the allelopathic effects of extracts of leaves and fruits of *S. lycocarpum* on *Sesamun indicum* seeds (AIRES et al., 2005). The ripe fruits of *S. lycocarpum* showed allelopathic potential with inhibition germination of *Cosmos sulphureus* Cav., being this activity attributed of glycoalkaloids (BORGHETTI; PESSOA, 1997). Phytochemical studies showed the presence of alkaloids in a large number of *Solanum*, including *S. lycocarpum* (MIRANDA et al., 2013). The observed allelopathic effects of *S. lycocarpum* can be probably caused by the alkaloids detected in EE and fractions. These results confirmed the allelopathic potential by *S. lycocarpum* and also showed for the first time, as far as we know, the allelopathic effects of the extract and fractions from the ripe fruits of *S. lycocarpum* against lettuce and onion seeds. The EE and fractions showed allelopathic potential, especially of inhibition on the growth of radicle of *Allium cepa* (onion). The DCM fraction showed better inhibition activity on the growth of lettuce and

onion seeds. These results should encourage additional studies of extract and fractions from the ripe fruits of *S. lycocarpum* for the isolation of bioactive compounds with inhibition potential for use as new herbicides.

Figure 1 - Growth of seeds of *Lactuca sativa* (lettuce) exposed to the samples at concentrations of 125, 250 and 500 μg/mL. **A)** Hypocotyl; **B)** Radicle. EE: ethanol extract, Hex: hexane fraction, DCM: dichloromethane fraction, Ac: ethyl acetate fraction, HE: hydroethanol fraction. Each value is the mean ± standard deviation. **P* < 0.05 compared to control group.

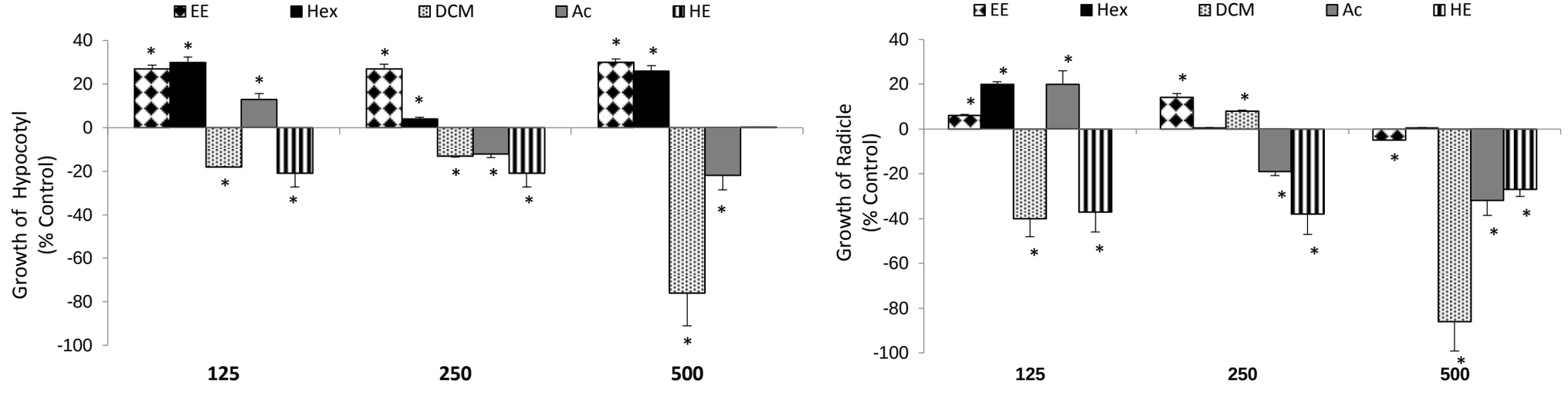
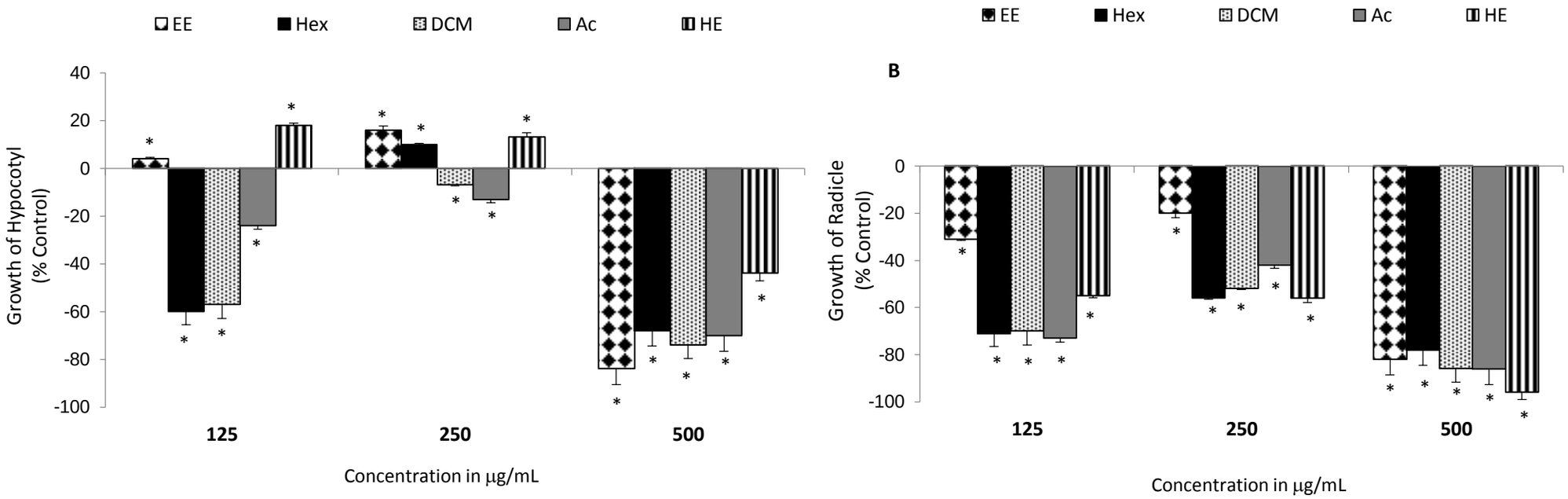


Figure 2 - Growth of seeds of *Allium cepa* (onion) exposed to the samples at concentrations of 125, 250 and 500 μg/mL. **A)** Hypocotyl; **B)** Radicle. EE: ethanol extract, Hex: hexane fraction, DCM: dichloromethane fraction, Ac: ethyl acetate fraction, HE: hydroethanol fraction. Each value is the mean ± standard deviation.**P* < 0.05 compared to control group.



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REFERENCES

AIRES, S. S.; FERREIRA, A. G.; BORGHETTI, F. Efeito alelopático de folhas e frutos de *Solanum lycocarpum* A. St. -Hil. (Solanaceae) na germinação e crescimento de *Sesamun indicum* L. (Pedaliaceae) em solo sob três temperaturas. **Acta Botanica Brasilica**, v. 19, n. 2, p. 339-344, 2005.

BORGHETTI, F.; PESSOA, D. M. A. Autotoxicidade e alelopatia em sementes de *Solanum lycocarpum* St. Hil. (Solanaceae). In: LEITE, L. L.; SAITO, C. H. (Orgs.). **Contribuição ao conhecimento ecológico de cerrado**. Brasília: Editora da UnB, 1997, p. 54-58.

IAS. INTERNATIONAL ALLELOPATHY SOCIETY CONSTITUTION. **First World Congress on Allelopathy. A Science for the Future**. Cadiz, Universidad de Cadiz, 1996.

MACÍAS, F. A.; OLIVEROS-BASTIDAS, A.; MARÍN, D.; CARRERA, C.; CHINCHILLA, N.; MOLINILLO, J. M. G. Plant biocommunicators: their phytotoxicity, degradation studies and potential use as herbicide models. **Phytochemistry Reviews**, v. 7, n. 1, p. 179-194, 2008.

MIRANDA, M. A.; TIOSSI, R. F. J.; SILVA, M. R.; RODRIGUES, K. C.; KUEHN, C. C.; OLIVEIRA, L. G. R.; ALBUQUERQUE, S.; MCCHESNEY, J. D.; LEZAMA-DAVILA, C. M.; ISAAC-MARQUEZ, A. P.; BASTOS, J. K. *In vitro* leishmanicidal and cytotoxic activities of the glycoalkaloids from *Solanum lycocarpum* (Solanaceae) fruits. **Chemistry & Biodiversity**, v. 10, n. 4, p. 642-648, 2013.

MUNARI, C. C.; OLIVEIRA, P. F.; LIMA, I. M. S.; MARTINS, S. P. L.; COSTA, J. C.; BASTOS, J. K.; TAVARES, D. C. Evaluation of cytotoxic, genotoxic and antigenotoxic potential of *Solanum lycocarpum* fruits glicoalkaloid extract in V79 cells. **Food and Chemical Toxicology**, v. 50, n. 10, p. 3696-3701, 2012.

OLIVEIRA, S. C. C.; GUALTIERI, S. C. J.; DOMÍNGUES, F. A. M.; MOLINILLO, J. M. G.; MONTOYA, R. V. Estudo fitoquímico de folhas de *Solanum lycocarpum* A. St. -Hil. (Solanaceae) e sua aplicação na alelopatia. **Acta Botanica Brasilica**, v. 26, n. 3, p. 607-618, 2012.

PINTO, M. E. A.; SILVA, N. L.; SIQUEIRA, E. P.; SANTOS, F. J. L.; LIMA, L. A. R. S. Allelopathic effect of fatty acid methyl esters from corn and sunflower oils. **BBR - Biochemistry and Biotechnology Reports**, v. 2, n. 1, p. 44-48, 2013.

SOUZA, V. C.; LORENZI, H. **Botânica Sistemática – Guia ilustrado para identificação das famílias de angiospermas da flora brasileira, baseado em APG II.** Nova Odessa: Instituto Plantarum, 704 p., 2008.

VIEIRA JR., G.; FERREIRA, P. M.; MATOS, L. G.; FERREIRA, E. C.; RODOVALHO, W.; FERRI, P. H.; FERREIRA, H. D.; COSTA, E. A. Anti-inflammatory effect of *Solanum lycocarpum* fruits. **Phytotherapy Research**, v. 17, n. 8, p. 892-896, 2003.

VIEIRA, H. S.; TAKAHASHI, J. A.; PIMENTA, L. P. S.; BOAVENTURA, M. A. D. Effects of kaurane diterpene derivatives on germination and growth of *Lactuca sativa* seedlings. **Zeitschrift für Naturforschung C: A Journal of Biosciences**, v. 60, n. 1-2, p. 72-78, 2005.

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