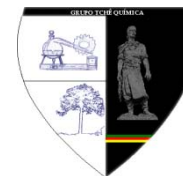




TEOR TOTAL DE FENÓLICOS E FLAVONOIDES, ATIVIDADES ANTIOXIDANTE E ALELOPÁTICA DOS EXTRATOS DOS FRUTOS VERDES DE *Solanum lycocarpum*



TOTAL PHENOL AND TOTAL FLAVONOID CONTENT, ANTIOXIDANT AND ALLELOPATHIC ACTIVITIES OF EXTRACTS OF THE UNRIPE FRUITS OF *Solanum lycocarpum*

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RESUMO

Solanum lycocarpum é conhecida popularmente como lobeira, sendo bastante utilizada na medicina popular. No presente estudo, os extratos hexânico e metanólico obtidos dos frutos verdes de *S. lycocarpum*, por extração em Soxhlet, foram avaliados quanto às atividades antioxidante e alelopática e tiveram determinados os teores de compostos fenólicos e flavonoides. Os extratos apresentaram atividade antioxidante nas sete concentrações testadas. Os valores de CE₅₀ obtidos para os extratos puderam ser correlacionados diretamente com a quantidade de compostos fenólicos e flavonoides presentes nas amostras. Para a atividade alelopática, as amostras apresentaram efeitos heterogêneos sobre o crescimento do hipocótilo e da radícula das sementes de cebola e efeitos inibitórios sobre as sementes de alface. Esses resultados incentivam novos estudos com a espécie, no sentido de se isolar e identificar os compostos responsáveis por essas atividades para que, no futuro, possam ser usados como antioxidantes ou aleloquímicos de origem natural.

Palavras-chave: *Solanum lycocarpum*, antioxidante, alelopatia, flavonoides, fenólicos.

ABSTRACT

The species *Solanum lycocarpum* is popularly known as “fruit of the wolf”, and is widely used in traditional medicine. In the present study, the hexane and methanol extracts obtained from the unripe fruits of *S. lycocarpum*, using a Soxhlet extractor, were evaluated for allelopathic and antioxidant activities. In addition, the content of phenolic compounds and flavonoids was determined. The extracts showed antioxidant activity at the seven concentrations tested. The EC₅₀ values obtained for the extracts could be correlated directly with the content of the phenolic compounds and flavonoids present in the samples. For the allelopathic activity, the samples showed heterogeneous effects on the growth of the hypocotyl and radicle of onion seeds, as well as inhibitory effects against lettuce seeds. These results encourage further studies of this species in order to isolate and identify the compounds responsible for these activities that, in the future, could be used as antioxidants or allelochemicals of a natural origin.

Keywords: *Solanum lycocarpum*, antioxidant, allelopathic, flavonoids, phenolic.

INTRODUCTION

Foods of plant origin, such as teas, herbs, oilseeds, beans, fruits, and vegetables, have been suggested as natural sources for antioxidants (Ara and Nur, 2009). Antioxidants are substances that can delay or inhibit lipid oxidation, acting as free radical scavengers, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors and synergists. *In vivo*, lipid oxidation may play a role in coronary heart disease, atherosclerosis, cancer, and the aging process (Rufino *et al.*, 2011). Vitamins A, C and E, carotenoids, flavonoids and other simple phenolic compounds found in cereals, fruits and vegetables are known to possess antioxidant potential (Arnao *et al.*, 2001). This explains why the search for natural antioxidants, especially of plant origin, has greatly increased in recent years (Lima *et al.*, 2010).

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, such as templates, has been widely attempted in recent years. The potential of phytochemicals and allelochemicals (natural plant toxins) in agriculture has been the subject of research that has the main purpose of finding phytotoxic substances to use as new herbicidal templates (Macías *et al.*, 2008).

The Solanaceae family is comprised of 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 and 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 *et al.*, 2003). It is widely used in traditional medicine as a sedative, in the treatment of epilepsy, asthma, diabetes, obesity, high cholesterol levels, and abdominal and renal pains (Munari *et al.*, 2012). Phytochemical studies of this species showed that solasonine and solamargine are two

predominant steroidal glycoalkaloids that have a common aglycone, solasodine (Miranda *et al.*, 2013).

Our group studied this species, reported the presence of caffeic and chlorogenic acids in ripe fruits (Morais *et al.*, 2015) and apigenin and kaempferol in the leaves (Costa *et al.*, 2015), and also anti-inflammatory, antioxidant, anti-tumour, cytotoxic, genotoxic, antibacterial, allelopathic and larvicidal (Chiavegatto *et al.*, 2017; Costa *et al.*, 2015; Morais *et al.*, 2013, 2015, 2017; Pereira *et al.*, 2014; Silva *et al.*, 2015). The aim of this present study was to evaluate the total phenolic compounds, flavonoid contents and antioxidant and allelopathic activities of hexane and methanol extracts of the unripe fruits of *Solanum lycocarpum*.

MATERIALS AND METHODS

Chemicals

2,6-di-tert-butyl-4-methylphenol (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin, 2-(*N*-morpholino) ethanesulfonic acid (MES), gallic acid, Folin-Ciocalteu reagent and aluminum trichloride were obtained from Sigma (St. Louis, MO, USA). Methanol, hexane, ethanol and dimethylsulfoxide were obtained from Vetec (Duque de Caxias, RJ, Brazil).

Plant material and extraction

The unripe fruits of *S. lycocarpum* A. St. Hil. were collected in São Sebastião do Oeste (20°14'38.96"S, 45°2'14.38"W), Minas Gerais, Brazil, in August 2013. Dr. Alexandre Salino identified the plant material and a voucher specimen (BHCB 159397) was deposited at the Instituto de Ciências Biológicas Herbarium at the Universidade Federal de Minas Gerais in Belo Horizonte, MG, Brazil. As *S. lycocarpum* a Brazilian native genetic material, the present study had been approved by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) through Access and Shipment Component of Genetic Heritage for scientific research purpose (no. 010655/2011-5).

Hexane and methanol were used as solvents to obtain the extracts from 74.62 g of dried and powdered unripe fruits, using a Soxhlet extractor. The extracts were then concentrated in a rotary evaporator at 40 °C under reduced pressure to yield the hexane (HEX, 2.26 g) and methanol (MET, 10.23 g) extracts. The extracts were screened for the presence of different

phytoconstituents, such as saponins, tannins, alkaloids, sterols, terpenes, coumarins, and flavonoids (Wagner *et al.*, 2001).

Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) was estimated using the Folin-Ciocalteu test in hexane and methanol extract (Zielinski *et al.*, 2014), with modifications. The Folin-Ciocalteu aqueous solution (2,250 μL ; 1:4 v:v) was added to the standard solution or samples (250 μL) and, subsequently, 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 the reference compound and the Total Phenolic Contents were expressed as mg of gallic acid equivalents (GAE)/g of extract. All assays were performed in triplicate.

Total Flavonoid Content (TFC)

The Total Flavonoid Content was estimated according to the Dowd method (Morais *et al.*, 2014). Exactly 2 mL of 2% aluminum trichloride (AlCl_3) in ethanol was mixed with the same volume of the extract solution (1 mg/mL). 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 solution with 2 mL methanol without AlCl_3 . Quercetin was used as the reference compound to produce the standard curve, and Total Flavonoid Contents were expressed as mg of quercetin equivalents (QE)/g of extract. All assays were performed in triplicate.

DPPH radical scavenging assay

The radical-scavenging abilities of *S. lycocarpum* extracts were based on reactions with a 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and were compared to the standard, 2,6-di-tert-butyl-4-methylphenol (BHT). 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, 500, 1000 and 2000 $\mu\text{g/mL}$) 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 $^{\circ}\text{C}$). After 30 min, the absorbance at 517 nm was measured with a spectrophotometer (Biotek Power Wave XS2, USA), and 80% methanol was used for the baseline correction.

Scavenging ability was expressed as the inhibition percentage and was calculated by equation (Burda and Oleszek, 2001):

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

where $\text{Abs}_{\text{control}}$ is the absorbance of the DPPH radical in 80% methanol and $\text{Abs}_{\text{sample}}$ is the absorbance of samples and standards in 80% methanol + DPPH. The antioxidant activity of all of the samples was expressed as IC_{50} , which was defined as a concentration (in $\mu\text{g/mL}$) of samples required to inhibit the formation of the DPPH radicals by 50%. IC_{50} values were calculated using the Probit analysis (Finney, 1980). All assays were performed in triplicate.

Allelopathic assay

The allelopathic activity of extracts obtained from unripe fruits of *S. lycocarpum* was evaluated through the effects on the growth of lettuce (*Lactuca sativa* cv cabbage, Feltrin, Brazil) and onion (*Allium cepa* cv Red Creole, Topsed Garden, Brazil) seeds. The dried extracts were dissolved in distilled water and their pH values were buffered with 10 mM 2-(*N*-morpholino) ethanesulfonic acid, adjusted to 6.0-6.2 with a NaOH solution. Concentrations lower than 200 $\mu\text{g/mL}$ 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 in each dish with 7 mL of the test (50, 100 and 200 $\mu\text{g/mL}$) 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 $^{\circ}\text{C}$ in a controlled-environment growth chamber (Fanem 346-MD, Brazil) for seven days. After this time, the lengths of the radicle and hypocotyl were measured. During the measurement process, the dishes were kept at 4 $^{\circ}\text{C}$ to avoid subsequent growth (Freitas *et al.*, 2015).

The effects on the growth can be calculated using the following formula (Pinto *et*

al., 2013):

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

Ma is the mean value of the seeds with samples tested and Mc is the mean value of the control (seeds grown without the 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. The differences between the experiment and control were significant at a *p*-value < 0.05.

RESULTS AND DISCUSSION

In this work, phytochemical tests revealed the presence of alkaloids, terpenes, sterols and flavonoids in the hexane extract. Alkaloids and flavonoids were present in the methanol extract. In a previous study on the green fruits of *S. lycocarpum*, alkaloids and coumarins were found in the hexane fraction. In addition, flavonoids, tannins and coumarins were found to be present in the methanol extract (Pereira *et al.*, 2014).

The 1,1-diphenyl-2-picrylhydrazyl (DPPH)-scavenging activity of hexane and methanol extracts obtained from *S. lycocarpum* are presented in Table 1. The extracts and positive control 2,6-di-tert-butyl-4-methylphenol (BHT) were capable of scavenging DPPH radicals in a concentration-dependent manner. The samples exhibited more activities compared to the positive control BHT at concentrations of 1 and 10 µg/mL. As can be observed from the IC₅₀ values (Table 2), methanol extract was found to be the most potent antioxidant, followed by hexane extract. The extracts were less active than was the BHT. The IC₅₀ values determined for the extracts of *S. lycocarpum* on the DPPH radical were statistically significant when compared with BHT (*p*-value < 0.05).

The antioxidant activity of *S. lycocarpum* was previously reported in the literature. Martins *et al.* (2013) recent study investigated the optimization of the extraction condition and the evaluation of the antioxidant activity of the extracts from the *S. lycocarpum* fruits. Their study also demonstrated that the polar solvents (50% ethanol) possessed high levels of antioxidant activity. In a previous study on the ripe fruits of *S. lycocarpum*, the ethanol extracts and fractions exhibited antioxidant activity using the DPPH

method, with IC₅₀ values estimated to be between 2.96 and 172.00 µg/mL (Morais *et al.*, 2015).

The methanol extract had the highest level of total phenolic compounds and flavonoids: 8.797 mg GAE/g of extract and 3.028 mg QE/g of extract, respectively, confirming the screening for the presence of flavonoids. The total phenolic compounds and flavonoids of hexane extract were estimated to be 2.332 mg GAE/g of extract and 0.616 mg QE/g of extract, respectively (Table 2). The IC₅₀ values determined for the extracts could be correlated with the total phenolic compounds and flavonoids. A previous study on the ripe fruits of *S. lycocarpum* revealed the presence of phenolic acids, as caffeic and chlorogenic acids (Morais *et al.*, 2015).

The allelopathic effects of the hexane and methanol extract from unripe *S. lycocarpum* fruits on the radicle and hypocotyl of *L. sativa* and *A. cepa* were evaluated in three different concentrations, and the results are shown in Figures 1 and 2. The methanol extract stimulated the growth of the *Allium cepa* (onion) radicle at concentrations of 50 and 200 µg/mL, with 48.5% at a concentration of 50 µg/mL. The hexane extract showed inhibitory activity on the growth of the radicle in 41.3% at a concentration of 200 µg/mL. Regarding the growth of the hypocotyl, the methanol extract stimulated growth at the three concentrations tested, with 43.4% at a concentration of 50 µg/mL. The hexane extract inhibited hypocotyl growth in 52.7% at a concentration of 200 µg/mL (Figure 1).

Figure 2 shows the growth of the *Lactuca sativa* (lettuce) radicle and hypocotyl. The hexane and methanol extracts showed inhibitory activities against the radicle and hypocotyl at the three concentrations tested. The best results were the hexane extract inhibitory activity on the growth of the radicle at a concentration of 100 µg/mL and of the hypocotyls at a concentration of 200 µg/mL.

The extract and fractions from ripe *S. lycocarpum* fruits showed allelopathic potential, with inhibition germination of *L. sativa* and *A. cepa* (Morais *et al.*, 2013). Phytochemical studies showed the presence of alkaloids in a large number of *Solanum*, including *S. lycocarpum* (Miranda *et al.*, 2013). The observed allelopathic effects from the ripe *S. lycocarpum* fruits are probably caused by the alkaloids detected in the hexane and methanol extracts.

CONCLUSION

The results demonstrated that ripe *Solanum lycocarpum* fruits possessed antioxidant potential. The EC₅₀ values obtained for the extracts could be directly correlated with the content of phenolic compounds and flavonoids present in the samples. For allelopathic activity, the samples showed heterogeneous effects on the growth of onion seed hypocotyls and radicles, and inhibitory effects against lettuce seeds. These results encourage further studies of this species in order to isolate and identify the compounds responsible for these activities that in the future, could be used as antioxidants or allelochemicals of a natural origin.

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CONFLICT OF INTEREST

The authors do not have any conflicts of interest.

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Table 1. DPPH-scavenging activity of *Solanum lycocarpum* fruit extracts.

Samples ($\mu\text{g/mL}$)	HEX (%)	MET (%)	BHT (%)
1	38.99 \pm 0.33*	39.11 \pm 0.83*	18.50 \pm 0.24
10	40.81 \pm 0.76*	39.36 \pm 0.15*	25.90 \pm 0.64
100	42.99 \pm 0.71*	41.85 \pm 0.95*	86.00 \pm 0.56
250	44.25 \pm 0.47*	42.86 \pm 0.75*	91.40 \pm 0.28
500	45.11 \pm 0.47*	44.43 \pm 0.69*	94.02 \pm 0.64
1000	51.84 \pm 0.35*	53.01 \pm 0.78*	99.12 \pm 0.82
2000	61.66 \pm 0.89*	64.17 \pm 0.85*	99.99 \pm 0.01

HEX: hexane extract; MET: methanol extract; and BHT: 2,6-di-tert-butyl-4-methylphenol. Each value in the table is the mean \pm standard deviation ($n = 3$) of % DPPH-scavenging activity of *Solanum lycocarpum* fruit extracts. * $p < 0.05$ compared with BHT.

Table 2. IC_{50} values, total phenolic compounds and flavonoids content of *Solanum lycocarpum* fruit extracts.

Samples	IC_{50} ($\mu\text{g/mL}$)	TPC ^A	TFC ^B
HEX	559.86 \pm 7.85*	2.332 \pm 0.439	0.616 \pm 0.011
MET	526.19 \pm 6.76*	8.797 \pm 0.876	3.028 \pm 0.177
BHT	16.36 \pm 1.63	-	-

HEX: hexane extract; MET: methanol extract; and BHT: 2,6-di-tert-butyl-4-methylphenol. IC_{50} : concentration (in $\mu\text{g/mL}$) of samples required to inhibit the formation of DPPH radicals by 50%.

^ATotal phenolic content: results expressed as mg of gallic acid equivalents/g of extract.

^BTotal flavonoids content: results expressed as mg of quercetin equivalents/g of extract.

Each value in the table is the mean \pm standard deviation ($n = 3$).

* $p < 0.05$ compared with BHT

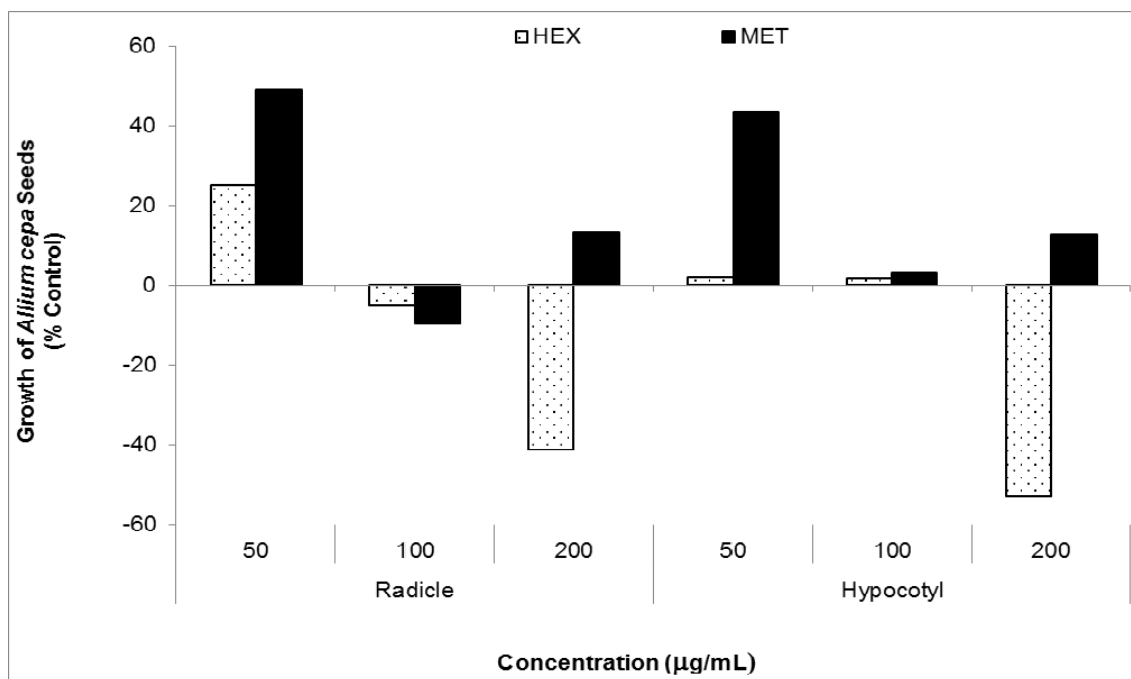


Figure 1. Growth of *Allium cepa* (onion) seeds exposed to *Solanum lycocarpum* fruit extracts at concentrations of 50, 100 and 200 µg/mL.

HEX: hexane extract; MET: methanol extract. All values are statistically significant ($p < 0.05$) comparing to the control.

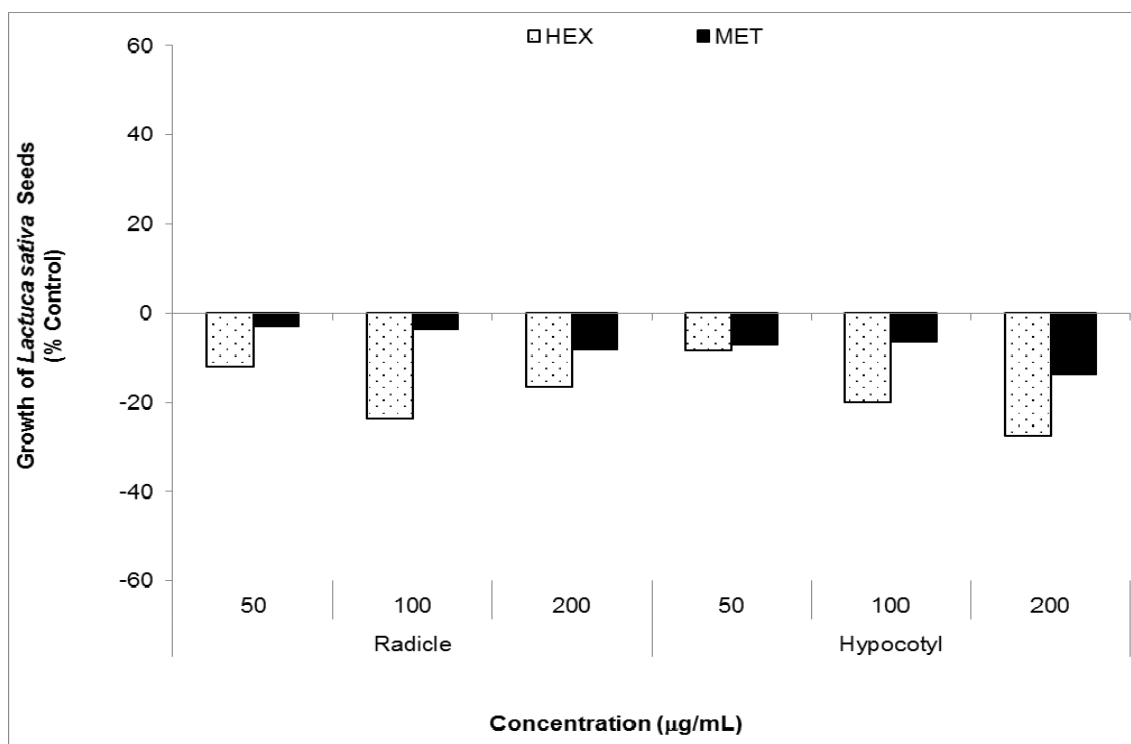


Figure 2. Growth of *Lactuca sativa* (lettuce) seeds exposed to the *Solanum lycocarpum* fruit extracts at concentrations of 50, 100 and 200 µg/mL.

HEX: hexane extract; MET: methanol extract. All values are statistically significant ($p < 0.05$) comparing to the control.