ISSN 1996-3351

Asian Journal of Biological Sciences

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Asian Journal of Biological Sciences 5 (5): 268-272, 2012
ISSN 1996-3351 / DOI: 10.3923/ajbs.2012.268.272
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Antioxidant Potential, Total Phenolic and Flavonoid Contents from the Stem Bark of *Guazuma ulmifolia* Lam.

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ABSTRACT

The species *Guazuma ulmifolia* Lam., popularly known in Brazil as chico-magro, belongs to Sterculiaceae, is used in the form of a tea as hypolipidemic and to reduce weight. In this work, antioxidant potential, total phenolic and flavonoids contents from the stem bark of the plant were investigated. Antioxidant potential was determined using DPPH assay. Total phenolic was measured using Folin-Ciocalteu and total flavonoid, using aluminium chloride. The stem bark of *G. ulmifolia* exhibited high antioxidant capacity, as well as a large content of phenolic and flavonoid compounds. IC_{50} obtained with DPPH assay varied from 8.09±0.14 to 67.69±0.31 μg mL^{-1}. Total phenolic varied from 160.00±0.71 to 373.44±0.36 mg g^{-1} DF (Dry Fraction) and flavonoid contents, from 23.50±0.48 to 33.20±0.27 mg g^{-1} of DF. Results obtained indicated that the stem bark extracts and fractions from *G. ulmifolia* have a good potential to prevent disease caused by free radicals and also might be used as a potential source of natural antioxidant agents.

Key words: *Guazuma ulmifolia* Lam., antioxidant, DPPH, hypolipidemic

INTRODUCTION

Plants can show excellent natural antioxidants properties. The antioxidant substances may be defined as substances, which suffer oxidation over others and this is the most different mechanisms (Janovik *et al*., 2011). Living organisms possess endogenous antioxidant systems to keep the formation of free radicals in tolerable levels (Gordon, 1996; Sabir *et al*., 2012). Such substances are involved in preventing the development of various diseases related to oxidative stress, including cancer, cardiovascular diseases, in addition to its role in delaying the aging of cells (Mensor *et al*., 2001). Several compounds of plant origin have this property, but the most effective belong to the group of polyphenols, which have carbon skeleton conducive to the stabilization of free radicals (Larrauri *et al*., 1996; Sati *et al*., 2010; Boligon *et al*., 2012).

The family Sterculiaceae is widespread in the tropics of the world, especially in America and Africa, with about 68 classes and 430 species. *Guazuma ulmifolia* Lam. known as "chico-magro" or "mutamba" occurs in all of Latin America and measure 16 m in height and 50 cm in diameter of the trunk. It is popularly used for the treatment of dandruff, hypercholesterolemic and to reduce weight (Nunes *et al*., 2003). Several anthocyanidins were isolated from the ethyl acetate fraction of the stem bark of *G. ulmifolia*, some of them with antiulcer activity (Hor *et al*., 1996). Camporese *et al*. (2003) observed the antimicrobial activity of hexanic fraction obtained from the leaves and stem bark against *Escherichia coli*. Considering the importance of quantifying

compounds with capacity antioxidant, this study were determined the levels of total phenolic, total flavonoids and antioxidant potential in crude extract and dichloromethane, ethyl acetate and butanolic fractions from the *Guazuma ulmifolia* Lam. stem bark.

MATERIALS AND METHODS

Plant collection and extractions: Stem bark of *Guazuma ulmifolia* Lam. was collected in Tangará da Serra (Mato Grosso State in Brazil) in August of 2007 (coordinates 14°37"25'S and 57°29"15'W). Exsiccate was archived as voucher specimen in the herbarium of Department of Biology at Federal University of Santa Maria by register number SMBD 7508. The parts of the plant were dried at room temperature and powdered in a knife mill. The powder of stem bark was macerated at room temperature with ethanol 70% for a week with daily shake-up. After filtration, the extract was evaporated under reduced pressure to remove the ethanol. Each extract was suspended in water and fractionated successively with dichloromethane, ethyl acetate and n-butanol (3×200 mL for each solvent).

Determination of total phenolics: The determination of total phenolic contents was performed by the Folin-Ciocalteu method (Boligon *et al.*, 2012). Briefly, 0.5 mL of 2 N Folin-Ciocalteu reagent was added to 1 mL of each sample (0.15 mg mL^{-1}) and this mixture was allowed to stand for 5 min before the addition of 2 mL of 20% Na_2CO_3. The total phenolic contents were expressed in milligrams equivalents of gallic acid (GAE) per gram of each fraction. The equation obtained for the calibration curve of gallic acid was:

$$Y = 34.443X-0.0942 \ (r = 0.9998)$$

The determination of total flavonoids: The determination of total flavonoids from stem bark was done by the method developed by Boligon *et al.* (2012). In this model, added 0.5 mL of aluminum chloride 2% to 0.5 mL concentrations of sample in 1000 μg mL^{-1} diluted in methanol. 2.5 mL of methanol was added to mixture. After 1 h, the reading is done in spectrophotometer at 420 nm. The positive control was used was rutin. The equation obtained for the calibration curve of rutin was:

$$Y = 20.394X-0.2033 \ (r = 0.9998)$$

Radical-scavenging activity-DPPH assay: The antioxidant activity of the fractions and the crude extracts was evaluated by monitoring its ability in quenching the stable free radical DPPH, according to a slightly modified method previously described by Boligon *et al.* (2009). Spectrophotometric analysis was used to measure the free radical scavenging capacity and to determine the scavenging concentration or inhibitory concentration (IC_{50}-concentration required to inhibit 50% of the DPPH in the assay). The DPPH quenching ability was expressed as IC_{50}. Six different ethanol dilutions of each fractions and crude extracts at 250, 125, 62.5, 31.25, 15.62 and 7.81 μg mL^{-1} were mixed with 1.0 mL of DPPH 0.3 mM in ethanol solution. After 30 min, the readings were made in spectrophotometer at 518 nm. A solution of DPPH (1 mL; 0.3 mM) in ethanol (2.5 mL) was used as a negative control and ascorbic acid in the same concentrations used for the fractions and the crude extracts provided the positive control. The test was performed in triplicate and the calculation of the antioxidant activity followed the equation:

$$\text{Inhibition } \% = 100 - \frac{\left[\left(\text{Abs}_{\text{sampel}} - \text{Abs}_{\text{blank}}\right) \times 100\right]}{\text{Abs}_{\text{control}}}$$

where, $\text{abs}_{\text{sampel}}$ is $\text{abs}_{\text{orbance}}$ of each fraction, $\text{abs}_{\text{blank}}$ is $\text{abs}_{\text{orbance}}$ of fractions without adding the DPPH and $\text{abs}_{\text{control}}$ is $\text{abs}_{\text{orbance}}$ of solution of ethanol in DPPH.

RESULTS AND DISCUSSION

Antioxidant profiles of stem bark fractions from the method DPPH are shown in Fig. 1. In this study, ethyl acetate was the most active fraction, IC_{50} (the amount of extract of the plant tested necessary to decrease the concentration of initial DPPH absorbance by 50%) value obtained was 8.09±0.14 μg mL^{-1}. Butanolic fraction showed more IC_{50} value (12.19±0.25 μg mL^{-}) than ethyl acetate fraction, being also very active and the crude extract IC_{50} was 20.61±0.19 μg mL^{-1}. The lowest antioxidant activity (IC_{50} = 67.69±0.31 μg mL^{-1}) was observed for dichloromethane fraction (Table 1). Ethyl acetate antioxidant activity was found to be higher than the well known antioxidant ascorbic acid, commonly used as a standard (IC_{50} = 9.01±0.08 μg mL^{-1}). On the other hand, butanolic, dichloromethane and crude extract from stem bark were inferior to ascorbic acid performance. This could be explained on the basis of the similarity between compounds with high antioxidant activity extracted by these organic solvents (Boligon *et al*., 2012).

Ethyl acetate and butanolic fractions from the stem bark exhibited 96.01 and 95.04%, respectively of inhibition of DPPH in the concentration of 250 μg mL^{-1} (Fig. 1). This behavior can be understood by the different composition of each fraction, since there are compounds that react quickly with the DPPH and others that have a slower reaction mechanism (Tsimogiannis and

Table 1: Biochemical analysis of different fractions of *Guazuma ulmifolia* Lam. extracts

	Stem bark			
G. ulmifolia fraction	Crude extracts yield (%)	Phenols[1]	Flavonoids[2]	IC_{50}[3]
Butanolic	5.61	333.33±0.97[a]	32.6±0.13[a]	12.19±0.25[a]
Ethyl acetate	7.13	373.44±0.36[b]	33.2±0.27[a]	8.09±0.14[b]
Dichloromethane	4.89	146.66±0.15[c]	23.5±0.48[b]	67.69±0.31[c]
Crude extract	11.07	160.00±0.71[d]	29.4±0.06[a]	20.61±0.19[d]

[1]Phenols: Expressed as mg g^{-1} gallic acid equivalents (GAE), [2]Flavonoids: Expressed as rutin mg g^{-1} fraction, [3]IC_{50}: Concentration (μg mL^{-1}) required to inhibit 50% of the DPPH in the assay, Values (Mean±SD) followed by different letters in each column differ by Tukey test at $p<0.001$

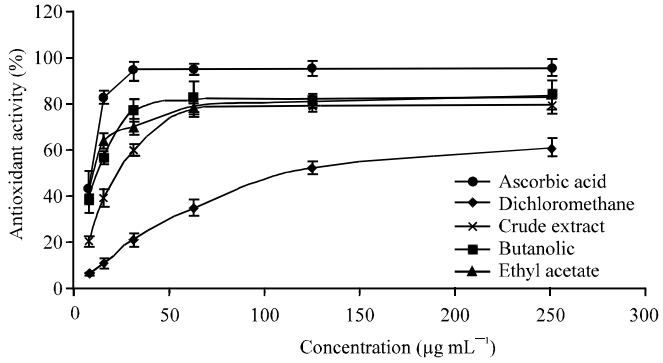

Fig. 1: Antioxidant activities of different fractions the of *G. ulmifolia* stem bark

Oreopoulou, 2004; Aliyu *et al.*, 2011). The DPPH assay is used as a tool *in vitro* evaluation of extracts and fractions and its results can be associated to the presence of phenolic and flavonoid compounds (Rice-Evans *et al.*, 1996; Janovik *et al.*, 2012).

Total phenolic contents assayed by the method of Folin-Ciocalteu expressed in milligrams equivalent of gallic acid per gram of each fraction, total flavonoids contents expressed in milligrams equivalent of rutin per gram of each fraction. For the determination of phenolic compounds, the ethyl acetate also exhibited the highest value (373.44±0.36 mg g^{-1}), dichloromethane fraction showed the lowest phenolic content (146.66±0.15 mg g^{-1}). When comparing the results obtained with phenolics and flavonoids contents assays, a correlation between both dosages for ethyl acetate and butanolic fractions was observed (Table 1).

Comparing the phenolic compounds, flavonoids contents and IC_{50} obtained for stem bark, it was observed that the fraction of ethyl acetate had higher content of flavonoids, polyphenols and lower IC_{50}. Several researchers described a positive correlation between phenolic and flavonoids contents and antioxidant activity using the same assaying systems (Shyamala *et al.*, 2005; Boligon *et al.*, 2012; Janovik *et al.*, 2011). The beneficial properties of flavonoids may be attributed to its ability to sequester the free radicals (Decker, 1997). These compounds can inhibit the process of lipid peroxidation (Halliwell *et al.*, 1995). Therefore, it is possible that much of the antioxidant activity of *G. ulmifolia* is due to the high presence of phenolic and flavonoid compounds.

CONCLUSION

Present results suggest that the species has strong free radical scavengers and can be considered as good sources of natural antioxidants. Values for antioxidant potential and concentrations of total phenolic and flavonoid obtained for crude extract and fractions from *Guazuma ulmifolia* exhibited positive relation. Ethyl acetate and butanolic fractions presented the highest phenolic and flavonoid contents indicate that these compounds contributed to the antioxidant potential. All results obtained are reported for the first time for *G. ulmifolia*.

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