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Full Length Research Paper

Relative antioxidant activity of Brazilian medicinal plants for gastrointestinal diseases

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The free radical scavenging capacity of Brazilian medicinal plants and some of their constituents was examined i*n vitro* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) quantitative assay. Twelve medicinal plants, used to treat gastrointestinal disorders (*Alchornea glandulosa, Alchornea triplinervia, Anacardium humile, Byrsonima* crassa, *Byrsonima cinera, Byrsonima intermedia, Davilla elliptica, Davilla nitida, Mouriri pusa*, *Qualea grandiflora*, *Qualea parviflora* and *Qualea multiflora*), were selected because they showed antiulcerogenic activity in previous studies. The radical scavenging methanolic extracts activity demonstrated to be dose-dependent. The efficient concentration, which represents the amount of the antioxidant able of decrease the initial DPPH radical by 50%, vary from < 5 to 17.2 µg/ml. The lowest efficient concentration values among the analyzed plant were shown by *A. humile, B. crassa* and *Q. parviflora*. Purified phenolic compounds (amentoflavone, (+)-catechin, methyl gallate, quercetin 3-O-alpha-L-arabinopyranoside, and quercetin 3-O-beta-D-galactopyranoside) were also tested and the greatest antioxidant activities were obtained with (+)-catechin and methyl gallate, similar to quercetin, a phenolic compound used as standard.

Key words: Brazilian medicinal plants, antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gastric ulcers.

INTRODUCTION

Gastric and duodenal ulcers affect a large proportion of the world population and are induced by several factors, including stress, smoking, alcoholic beverages, nutritional deficiencies, ingestion of nonsteroidal anti-inflammatory drugs and *Helicobacter pylori* infection (O`Malley, 2003). Studies reveal an extensive variety of chemical compounds isolated from medicinal plants with antiulcer activity (Borrelli and Izzo, 2000). An ethnopharmacological survey carried out in the Cerrado of Central Brazil showed a high number of medicinal plants used to treat gastric pain and gastritis (Silva et al., 2000). Among the plants used in Brazilian popular medicine for the treatment of gastrointestinal disorders, several have shown antiulcerogenic and/or anti-*Helicobacter pylori* activities (Bonacorsi el al., 2009; Lima et al., 2008; Luiz-Ferreira et al., 2008; Moraes et al., 2008). There are evidences in support the participation of reactive oxygen species and other free radicals in the etiology and pathophysiology of several human diseases, including gastrointestinal inflammation and gastric ulcer (Repetto and Llesuy, 2002). Compounds with multiple mechanism of protective action may be highly effective in minimizing tissue injury in human diseases (Umamaheswari et al., 2007). A number of methods and variations in methods to measure antioxidants capacity in botanicals have been

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proposed. The radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is still highly utilized in antioxidant research due to its simple reaction system, which involves only the direct reaction between the radical and the antioxidant, without other interference, such as enzyme inhibition or the presence of multiple radicals (Cheng et al., 2006). The aim of this work was to evaluate the antioxidant capacity of methanolic (MeOH) extracts and isolated phenolic compounds from Brazilian medicinal plants for the treatment of gastroduodenal diseases using the DPPH free radical quantitative assay.

MATERIALS AND METHODS

Extract preparation

The MeOH extracts used in this study were the same employed in others studies and the preparation have already been described by Calvo et al. (2007) for *Alchornea glandulosa,* Lima et al. (2008) for *Alchornea triplinervia,* Luiz-Ferreira et al. (2008) for *Anacardium humile,* Cardoso et al. (2006) for *Byrsonima* crassa, Moleiro (2007) for *Byrsonima basiloba*, Sannomiya et al. (2007) for *Byrsonima intermedia,* Biso et al. (2010) for *Davilla* species, Santos et al. (2008) for *Mouriri pusa,* and Nasser (2008) for *Qualea* species. Briefly, the air-dried and powdered leaves were extracted with methanol at room temperature (48 h). Solvent was evaporated at temperature of 60°C under reduced pressure to yield the methanol solid extract. The reference material, extract and phytochemical screening of all plants are synthesized on Table 1. The plants employed in this study are part of the BIOTA/FAPESP Program.

Purified phenolic compounds

The air-dried and powdered leaves (2.0 kg) of *B. crassa* were extracted with methanol at room temperature (48 h). The solvent was evaporated at 60°C under reduced pressure to produce the MEOH extract. The yield (w/w) of the extract from the dried powdered *B. crassa* leaves was 7.91% (158.3 g). An aliquot of the extract (4.0 g) was permeated on a Sephadex LH-20 column (100 x 5 cm) and then eluted with MeOH. Fractions (8 ml) were collected and analyzed by thin-layer chromatography on silica gel eluted with $CHCl_3$/MeOH (80:20) and revealed by spraying with either NP/PEG (diphenylaminoborate/polyethyleneglycol) or an anisaldehyde/sulfuric acid solution. Fractions 129 to 141 (95.0 mg) were purified by repeated column chromatography (CC) on microcrystalline cellulose using with $CHCl_3$/MeOH (80:20) as the eluent, yielding the biflavonoid amentoflavone (6.0 mg). Fractions 88 to 95 (69.0 mg) were further purified by high-performance liquid cromatograph (HPLC), with MeOH/H_2O (1:1) as the eluent, to yield quercetin-3-O-beta-D-galactopyranoside (15.0 mg). Fractions 82 to 87 (122.0 mg) were purified by silica CC using EtOAc/n-PrOH/H_2O 140:8:80 (upper phase) as the eluent, yielding quercetin-3-O-alpha-L-arabinopyranoside (14.0 mg) and a mixture of (-)-epicatechin and (+)-catechin (30.0 mg). Epicatechin and (+)-catechin were separated by HPLC with MeOH/H_2O (20:80) as the eluent to yield 10 mg of each purified compound. Fractions 55 to 60 (112.0 mg) were purified by silica gel CC with $CHCl_3$/MeOH (75:25) as the eluent, yielding methyl gallate (8.0 mg), which was confirmed by NMR and TLC (Sannomiya et al., 2004). The extract and the purified major constituents were solubilized in dimethyl sulfoxide (DMSO).

DPPH photometric assay

The free radical scavenging activity of MeOH extracts and the phenolic compounds were measured *in vitro* by DPPH assay. Briefly, ethanol solutions of the methanol solid extracts and phenolic compounds (50 µl), at different concentrations (5 to 100 µg/ml), were mixed with 100 µl of DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich, USA) solution (0.3 mM, in methanol). The absorbance was measured at 517 nm (ABS sample) after 30 min of reaction at 25°C. Quercetin (Sigma-Aldrich, USA) was used as a standard antioxidant and DPPH solution plus ethanol was used as negative control (ABS control). Measurements were performed at least in triplicate. The capability to scavenge the DPPH radical was calculated as DPPH scavenging effect (%) = $[(ABS_{control} - ABS_{sample}/ABS_{control}) \times 100]$. The efficient concentration (EC_{50}), concentration of the antioxidant required to decrease the initial DPPH radical concentration by 50%, was also determined. A lower value of EC_{50} indicates a higher antioxidant activity (Siddhuraju and Manian, 2007).

RESULTS

The percentages of antioxidant activity for the MeOH extracts are shown on Table 2. The DPPH radical scavenging activity was dose-dependent. In terms of efficient concentration, the radical scavenging activity varied from < 5 to 17.2 µg/ml. The lowest efficient concentrations among the analyzed plants were shown by *A. humile, B. crassa* and *Q. parviflora*. The most effective purified phenolic compounds studied for antioxidant activities were (+)-catechin and methyl gallate, similar to quercetin, the flavonol used as standard (Table 3).

DISCUSSION

Gastritis and peptic ulcers are important gastrointestinal disorders that affect a considerable number of people. Products on the market for the treatment these diseases, including antacids, proton pump inhibitors, anticholinergic and histamine H_2-antagonists, can produce several adverse reactions (Yacyshyn and Thomson, 2002). Medicinal plants are among the most attractive sources of new drugs and have been shown to give promising results in treatment of gastric ulcers (Borrelli and Izzo, 2000). There is considerable interest in the antioxidant activity of plants, due to the fact that free radicals have been related to some diseases, as well as to the aging process (Nijveldt et al., 2001; Willcox et al., 2004). In this study, differences on the free radical scavenging activity of the MeOH extract from plants used in Brazilian

Table 1. Plants employed in this study, reference material, obtained extract, phytochemical screening, and phenolic compounds.

Plant	Herbarium voucher number	Plant part	Methanolic extract residue (%)	Phytochemical screening	Phenolic compound
Alchornea glandulosa	Campinas University Nº 132828	Leaf	11.8 (Calvo et at., 2007)	Phenolic acids, alkaloids, steroids, flavonoids, isocumarins, saponins, tannins, terpenes (Calvo, 2007; Calvo et al., 2007; Lopes et al., 2005)	Amentoflavone, methyl gallate, quercetin-3-O-alpha-L-arabinopyranoside, quercetin-3-O-beta-D-galactopyranoside (Calvo et al.., 2007)
Alchornea triplinervia	São Paulo State University Nº 14873	Leaf	15 (Lima et al., 2008)	Phenolic acids, ellagic acids, alkaloids, flavonoids, isocumarins, saponins, tannins (Calvo 2007; Lima et al., 2008)	Amentoflavone, methyl gallat, quercetin-3-O-alpha-L-arabinopyranosid, quercetin-3-O-beta-D-galactopyranoside (Calvo, 2007)
Anacardium humile	Tocantins University Nº 1922	Leaf	29.7 (Luiz-Ferreira et al., 2008)	Phenolic acids, catechins, flavonoids, saponins, tannins (Luiz-Ferreira et al., 2008)	Amentoflavone, (+)-catechin, methyl gallate, quercetin-3-O-beta-D-galactopyranoside (Luiz-Ferreira et al., 2008)
Byrsonima basiloba	São Paulo State University Nº 24163	Leaf	9.4 Moleiro, 2007	Phenolic acids, catechins, flavonoids, steroids, tannins (Lira et al., 2008; Moleiro, 2007)	Amentoflavone, (+)-catechin, methyl gallate, quercetin-3-O-alpha-L-arabinopyranoside, quercetin-3-O-beta-D-galactopyranoside (Lira et al., 2008)
Byrsonima crassa	Tocantins University Nº 3377	Leaf	7.91 (Cardoso et al., 2006)	Phenolic acids, catechins, steroids, flavonoids, tannins, terpenes (Cardoso, 2006; Cardoso et al., 2006; Sannomiya et al., 2005)	Amentoflavone, (+)-catechin, methyl gallate, quercetin-3-O-alpha-L-arabinopyranoside, quercetin-3-O-beta-D-galactopyranoside (Cardoso et al., 2006; Sannomiya et al., 2005)
Byrsonima intermedia	São Paulo State University Nº 24164	Leaf	4.3 (Cardoso, 2006)	Phenolic acids, catechins, steroids, flavonoids, tannins (Cardoso, 2006; Sannomiya et al., 2007)	Amentoflavone, (+)-catechin, methyl gallate, quercetin-3-O-alpha-L-arabinopyranoside, quercetin-3-O-beta-D-galactopyranoside (Sannomiya et al., 2007)

Table 1. Contd.

Davilla elliptica	Tocantins University Nº 4593	Leaf	7.1 (Rodrigues, 2007)	Phenolic acids, catechins, steroids, flavonoids, tannins, terpenes (Biso et al., 2010; Carlos et al., 2005; Michelin et al., 2005)	Quercetin-3-O-alpha-L-arabinopyranoside, quercetin-3-O-beta-D-galactopyranoside (Rodrigues, 2007)
Davilla nitida	Tocantins University Nº 3849	Leaf	11.7 (Rodrigues, 2007)	Phenolic acids, catechins, flavonoids (Biso et al., 2010; Rodrigues, 2007)	
Mouriri pusa	Tocantins University Nº 3341	Leaf	10 Santos et al. (2008)	Catechins, flavonoids, tannins (Santos et al., 2008; Vasconcelos et al., 2008)	(+)-catechin, quercetin-3-O-alpha-L-arabinopyranoside, quercetin-3-O-beta-D-galactopyranoside (Andreo et al., 2007; Santos et al., 2008)
Qualea grandiflora	Tocantins University Nº 4158	Bark	10.9 Nasser (2007)	Ellagic acids, catechins, steroids, flavonoids, saponins, tannins, terpenes (Nasser, 2007; Nasser et al., 2008)	
Qualea multiflora	Tocantins University Nº 9226	Bark	10.2 Nasser (2007)	Phenolic acids, ellagic acids, flavonoids, saponins, tannins, terpenes (Nasser, 2007; Nasser et al., 2008)	
Qualea parviflora	Tocantins University Nº 3379	Bark	9.2 Nasser (2007)	Ellagic acids, steroids, flavonoids, saponins, tannins, terpenes (Nasser et al., 2006; Nasser, 2007; Nasser et al., 2008)	

popular medicine to treat gastritis and ulcers were demonstrated. It is apparent from this data that levels of potential antioxidant activity have a wide variation in lower concentrations. Previously published investigations have demonstrated the effects of MeOH extract of *A. glandulosa* (Calvo et al., 2007) and *B. crassa* (Sannomiya et al., 2005) on different models of gastric lesion induced in mice. The pathogenesis of ethanol-induced gastric mucosal damage is still unknown but the formation of oxygen-derived free radicals and neutrophil infiltration into gastric mucosa is considering the main sources of mucosal damage (Chow et al., 1998). Plants containing antioxidant properties are effective in preventing this kind of lesion and a presence the phenolic compounds may probably explain the antiulcerogenic effect of these plants (Calvo et al., 2007; Luiz-Ferreira et al., 2008; Sannomiya et al., 2005). Although, the DPPH assay is usually classified as single electron transfer reaction, this radical may be

Table 2. Percentage of DPPH radical scavenging activity and antioxidant efficient concentration for methanolic extract of Brazilian plants used to treat gastritis.

Botanical name (popular name)	Scavenging (%)[a]							
	Methanolic extract concentration (µg/ml)							EC_{50} [b]
	5	10	20	40	50	80	100	
Alchornea glandulosa (tapiá)	28.5 ± 0.8	50.3 ± 0.4	72.4 ± 0.9	78.3 ± 1.1	80.1 ± 0.9	80.6 ± 0.9	83.8 ± 1.2	10
Alchornea triplinervia (tanheiro)	46.0 ± 0.5	67.2 ± 0.5	77.4 ± 0.9	79.8 ± 1.0	76.9 ± 0.9	79.6 ± 0.7	78.5 ± 1.0	5.9
Anacardium humile (cajuzinho-do-cerrado)	59.4 ± 0.7	73.2 ± 1.2	74.3 ± 0.5	74.1 ± 0.9	73.7 ± 0.8	73.4 ± 1.0	73.7 ± 1.3	< 5
Byrsonima basiloba (murici-de-ema)	22.6 ± 0.6	42.1 ± 1.1	64.8 ± 0.6	73.4 ± 0.8	73.2 ± 0.7	74.2 ± 1.0	74.6 ± 0.9	14.2
Byrsonima crassa (murici)	61.9 ± 1.2	81.9 ± 1.5	85.2 ± 1.1	86.4 ± 0.9	85.9 ± 0.7	86.3 ± 1.2	86.2 ± 0.6	< 5
Byrsonima intermedia (murici-do-campo)	27.0 ± 1.3	47.1 ± 1.1	73.1 ± 1.0	76.3 ± 0.9	77.7 ± 1.2	77.8 ± 1.3	78.0 ± 0.6	12.0
Davilla elliptica (cipó-de-carijó)	36.0 ± 0.7	59.7 ± 0.8	76.3 ± 1.1	76.9 ± 1.1	77.0 ± 1.1	77.1 ± 0.9	77.8 ± 0.7	8.8
Davilla nitida (cipó-de-carijó)	16.8 ± 0.7	31.2 ± 0.8	57.6 ± 0.7	77.0 ± 0.7	78.4 ± 0.8	78.7 ± 0.9	78.4 ± 0.9	17.2
Qualea grandiflora (pau-da-terra)	34.5 ± 0.8	62.5 ± 0.8	77.5 ± 0.5	80.1 ± 1.2	80.0 ± 0.9	80.5 ± 0.4	81.5 ± 0.9	8.6
Qualea parviflora (ipê-cascudo)	53.0 ± 0.7	71.7 ± 1.2	74.1 ± 1.0	77.0 ± 1.0	78.8 ± 1.0	81.3 ± 0.9	81.2 ± 1.0	< 5
Qualea multiflora (Cerra-do-campo)	50.9 ± 1.1	72.7 ± 1.1	74.6 ± 0.7	77.4 ± 1.3	78.6 ± 0.9	81.3 ± 0.7	80.7 ± 0.7	5
Mouriri pusa (puçá)	39.9 ± 0.7	57.4 ± 0.8	59.6 ± 0.8	63.0 ± 0.6	64.2 ± 0.5	67.5 ± 0.5	67.1 ± 0.8	7.9

[a]means of triplicate readings; [b]antioxidant efficient concentration.

neutralized either by direct reduction via electron transfers or by radical quenching via H atom transfer (Jiménez et al., 2004). Based on the mechanism of reduction of the DPPH molecule and on previous knowledge of the chemistry of some plants, it is possible to infer that the strong antioxidant activity of some polar extracts is due, at least in part, to the presence of substances with an available hydroxyl group. Compounds able to donate hydrogen are derived from the shikimate pathway, as for example, flavonoids (Huang et al., 2002). Flavonoids and gallic acid derivatives (methyl gallate, ethyl gallate, propyl gallate and others gallate esters) are major classes of phenolic compounds that are widely spread throughout the plant kingdom, whose structure-antioxidant activity relationships have been reported (Nenadis et al., 2004). In general, the free-radical scavenging and antioxidant activities of phenolics depend primarily on the number and positions of the hydrogen-donating hydroxyl groups on the aromatic ring of these molecules, but is also affected by other factors, such as glycosylation of aglycones and other H-donating groups (Cai et al., 2004). The results of this study showed that the flavonoid quercetin (a standard antioxidant) had a better antioxidant activity than its 3-O-glycoside derivatives (quercetin-3-O-alpha-L-arabinopyranoside and quercetin-3-O-beta-D-galactopyranoside). This corroborates reports that flavonoid aglycones are more potent antioxidants than their corresponding glycosides (Heim et al., 2002).

Catechin and quercetin belong to the flavanol and flavonol classes, respectively and they have been studied intensely because of their high ROS scavenging activities. These polyphenol molecules have the same number of hydroxyl groups on the A and B rings. In the chemical structure of quercetin there is a double bond at position 2,3 and an oxo group at position 4 in the C ring which seem to provide better antioxidant activity then that of catechin (Mamede and Pastore, 2004). Our data demonstrated that (+)-catechin and methyl gallate had a similar

Table 3. Percentage of DPPH radical scavenging activity and antioxidant efficient concentration for the phenolic compounds.

Phenolic compound	Scavenging (%)[a] Concentration (µg/ml)							EC_{50}[b]
	5	10	20	40	50	80	100	
Amentoflavone	36.4 ± 0.6	50.3 ± 0.7	67.2 ± 1.0	90.6 ± 1.1	95.7 ± 1.0	95.8 ± 1.3	95.6 ± 1.5	10
(+)-Catechin	93.6 ± 1.1	95.3 ± 1.0	96.3 ± 1.0	94.9 ± 0.9	94.7 ± 0.9	94.3 ± 1.1	94.0 ± 1.2	< 5
Methyl gallate	95.1 ± 0.5	97.2 ± 0.5	96.6 ± 0.5	96.8 ± 0.5	97.1 ± 0.5	97.3 ± 0.5	96.9 ± 0.6	< 5
Quercetin-3-O-alpha-L-arabinopyranoside	40.8 ± 0.6	56.6 ± 0.7	83.7 ± 0.9	94.1 ± 0.8	96.1 ± 1.1	95.6 ± 1.0	96.0 ± 1.1	8
Quercetin-3-O-beta-D-galactopyranoside	38.3 ± 0.3	53.9 ± 0.7	80.3 ± 0.7	92.2 ± 0.9	94.0 ± 1.0	93.4 ± 1.1	93.4 ± 0.9	9.9
Quercetin (standard)	94.6 ± 1.1	97.2 ± 0.9	96.9 ± 1.8	97.2 ± 1.8	96.9 ± 1.5	97.4 ± 1.5	97.0 ± 1.7	< 5

[a]means of triplicate readings; [b]antioxidant efficient concentration.

radical-scavenging activity even at a lower concentration. Methyl gallate has been shown to be an effective antioxidant in a variety of acellular experiments (Hsieha et al., 2004). The lower antioxidant activity of amentoflavone was consistent with other studies (Baggett et al., 2005; Hyun et al., 2006). This work has no intending to prove the total antioxidant capacity in the studied plants because multiple reaction characteristics and mechanisms are involved and no single assay will accurately reflects all of the radical sources or all antioxidants in a mixed or complex system. The antioxidant capacity demonstrated by a single method (DPPH) using different concentrations, may be useful to demonstrate the variable levels of compounds with free radical (DPPH) scavenging activity present in plants tissue but not necessarily correspondent to total of phenolic constituents and derivatives.

However, it is apparent from this data that levels of potential antioxidant activity have a wide variation at low concentration. These results suggested that the optimal effective dose for evaluating the potential of medicinal plants for its antiulcer activity on experimental models may be related, at least in part, to the concentration of antioxidants required to decrease the DPPH radical, since the antiulcerogenic potential of *A. glandulosa, A. humile* and *B. crassa* has also been attributed to the presence of oxygen radical scavengers (Calvo et al., 2007; Luiz-Ferreira et al., 2008; Sannomiya et al., 2005). The high free radical scavenging activity of some tested extracts in the DPPH test suggest that the antioxidant potential may be one of the mechanisms that explains the popular use of plants for the treatment of gastrointestinal diseases, as ulcer, because both ulcerous and inflammatory process are related to an increase of free radicals. The most effective species confirmed for their lowest antioxidant efficient concentration were *A. humile, B. crassa* and *Q. parviflora*. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in discovering the significance of folkloric remedies. Our results give a contribution to the pharmacological validation for the use of these species as Brazilian medicinal plants with antioxidant and antiulcerogenic potential.

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REFERENCES

Baggett S, Protiva P, Mazzola EP, Yang H, Ressler ET, Basile MJ (2005). Bioactive benzophenones from *Garcinia xanthochymus* fruits. J. Nat. Prod., 68: 354-360.

Biso FI, Rodrigues CM, Rinaldo D, Reis MB, Bernardi CC, De

MJC, Caldeira-de-Araújo A, Vilegas W, Cólus IM, Varanda EA (2010). Assessment of DNA damage induced by extracts, fractions and isolated compounds of *Davilla nitida* and *Davilla elliptica* (Dilleniaceae). Mutat. Res., 702: 92-99.

Bonacorsi C, Raddi MS, Carlos IZ, Sannomiya M, Vilegas W (2009). Anti-*Helicobacter pylori* activity and immunostimulatory effect of extracts from *Byrsonima crassa* Nied. (Malpighiaceae). BMC Complement. Altern. Med., 9: 2-8.

Borrelli F, Izzo AA (2000). The plant kingdom as a source of anti-ulcer remedies. Phytother. Res., 14: 581-591.

Cai Y, Luo Q, Sun M, Corke H (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., 74: 2157-2184.

Calvo TR (2007). Sustainable use of biodiversity in Brazil -chemical-pharmaceutical prospecting in higher plants: Alchorneaglandulosa, triplinervia Alchornea (Euphorbiaceae), andtruxillensis Indigofera suffruticosa Indigofera (Fabaceae). Doctoral Thesis, Institute of Chemistry of Araraquara, Universidade Estadual Paulista, p. 217.

Calvo TR, Lima ZP, Silva JS, Ballesteros KVR, Pellizzon CH, Hiruma-Lima CA, Tamashiro J, Souza-Brito AM, Takahira RK, Vilegas W (2007). Constituents and antiulcer effect of *Alchornea glandulosa*: activation of cell proliferation in gastric mucosa during the healing process. Biol. Pharm. Bull., 30: 451-459.

Cardoso CR (2006). Mutagenic activity and activating the cellular immune response induced by Byr Byrsonima crassa Niedenzu and Byrsonimaintermedia A. Juss. (Malpighiaceae). Thesis, Faculty ofPharmaceutical Sciences of Araraquara, Universidade Estadual Paulista, p. 129.

Cardoso CRP, Cólus IMS, Bernardi CC, Sannomiya M, Vilegas W, Varanda EA (2006). Mutagenic activity promoted by amentoflavone and methanolic extract of *Byrsonima crassa* Niedenzu. Toxicology, 225: 55-63.

Cheng Z, Moore J, Yu L (2006). High-throughput relative DPPH radical scavenging capacity assay. J. Agric. Food Chem., 54: 7429-7436.

Chow JY, Ma L, Cho CH (1998). Effect of cigarette smoke on ethanol-induced gastric mucosal lesions: the role of nitric oxide and neutrophils. Eur. J. Pharmacol., 342: 253-260.

Heim KE, Tagliaferro AR, Bobilya DJ (2002). Flavonoids antioxidants: chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem., 13: 572-584.

Hsieha T, Liub T, Chiac Y, Chernd C, Lue F, Chuangd M (2004). Protective effect of methyl gallate from *Toona sinensis* (Meliaceae) against hydrogen peroxide-induced oxidative stress and DNA damage in MDCK cells. Food Chem. Toxicol., 42: 843-850.

Huang D, Ou B, Hampsch-Woodill M, Flanagan JA, Deemer EK (2002). Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated beta-cyclodextrin as the solubility enhancer. J. Agric. Food Chem., 50: 1815-1821.

Hyun SK, Jung HA, Chung HY, Choi JS (2006). *In vitro* peroxynitrite scavenging activity of 6-Hydroxykynurenic acid and other flavonoids from *Gingko biloba* yellow leaves. Arch. Pharm. Res., 29: 1074-1079.

Jiménez A, Selga A, Torres JL, Julià L (2004). Reducing activity of polyphenols with stable radicals of the TTM series. Electron transfer versus H-abstraction reactions in flavan-3-ols. Org. Lett., 6: 4583-4583.

Lima ZP, Calvo TR, Silva EF, Pellizzon CH, Vilegas W, Brito AR, Bauab TM, Hiruma-Lima CA (2008). Brazilian medicinal plant acts on prostaglandin level and *Helicobacter pylori*. J. Med. Food, 11: 701-708.

Lira WM, Dos SFV, Sannomiya M, Rodrigues CM, Vilegas W, Varanda EA (2008). Modulatory effect of *Byrsonima basiloba* extracts on the mutagenicity of certain direct and indirect-acting mutagens in Salmonella typhimurium assays. J. Med. Food, 11: 111-119.

Lopes FCM, Calvo TR, Vilegas W, Carlos IZ (2005). Inhibition of hydrogen peroxide, nitric oxide and TNF-α production in peritoneal macrophages by ethyl acetate fraction from *Alchornea glandulosa*. Biol. Pharm. Bull., 28: 1726-1730.

Luiz-Ferreira A, Cola-Miranda M, Barbastefano V, Hiruma-Lima CA, Vilegas W, Souza-Brito AR (2008). Should *Anacardium humile* St. Hil be used as an antiulcer agent? A scientific approach to the traditional knowledge. Fitoterapia, 79: 207-209.

Mamede MEO, Pastore GM (2004). Wine phenolic compounds: structure and antioxidant action. Bull Pesqui Center. Process Aliment., 22: 233-252.

Michelin DC, Iha SM, Rinaldo D, Sannomiya M, Santos LC, Vilegas W, Salgado HRN (2005). Antimicrobial activity of *Davilla elliptica* St. Hill (Dilleniaceae). Rev. Bras. Farmacogn., 15: 209-211.

Moleiro FC (2007). Evaluation of the antiulcer activity of extracts and fractions from the leaves of Mouriri elliptica Mart. (Melastomataceae) andByrsonima basiloba A. Juss. (Malpighiaceae). Thesis, Institute of Biosciences of Botucatu, Universidade Estadual Paulista, p. 117.

Moraes TM, Rodrigues CM, Kushima H, Bauab TM, Villegas W, Pellizzon CH, Brito AR, Hiruma-Lima CA (2008). Hancornia speciosa: indications of gastroprotective, healing and anti-Helicobacter pylori actions. J. Ethnopharmacol., 120: 161-168.

Nasser ALM, Mazzolin LP, Hiruma-Lima CA, Santos LS, Eberlin MN, Souza-Brito ARM, Vilegas W (2006). Preparative droplet counter-current chromatography for the separation of the new nor-seco-triterpene and pentacyclic triterpenoids from *Qualea parviflora*. Chromatographia, 64: 695-699.

Nasser ALM (2007). Sustainable use of biodiversity in Brazil: drug-chemicalprospecting in higher plants: which SSP (Vochysiaceae). 197p.Doctoral Thesis, Graduate Program in Chemistry, Universidade Estadual Paulista.

Nasser AL, Carli CB, Rodrigues CM, Maia DC, Carlos IZ, Eberlin MN, Hiruma-Lima CA, Vilegas W (2008). Identification of ellagic acid derivatives in methanolic extracts from *Qualea* species. Z. Naturforsch. C., 63: 794-800.

Nenadis N, Wang LF, Tsimidou M, Zhang HY (2004). Estimation of scavenging activity of phenolic compounds using the ABTS assay. J. Agric. Food Chem., 52: 4669-4674.

Nijveldt RJ, Van NE, Van HDE, Boelens PG, Van NK, Van LPA (2001). Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr., 74: 418-245.

O`Malley P (2003). Gastric ulcers and GERD: the new “plagues” of the 21st century update for the clinical nurse specialist. Clin. Nurse Spec., 17: 286-289.

Repetto MG, Llesuy SF (2002). Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz. J. Med. Biol. Res., 35: 523-534.

Rodrigues CM (2007). Qualitative and quantitative characterization of secondary metabolites in plant extracts. Doctoral Thesis, Institute of Chemistry of Araraquara, Universidade Estadual Paulista, p. 199.

Sannomiya M, Rodrigues CM, Coelho RG, Dos SLC, Hiruma-Lima CA, Souza-Brito AR, Vilegas W (2004). Application of preparative high-speed counter-current chromatography for the separation of flavonoids from the leaves of *Byrsonima crassa* Niedenzu (IK). J. Chromatogr., 1035: 47-51.

Sannomiya M, Fonseca VB, Da SMA, Rocha LR, Dos SLC, Hiruma-Lima CA, Souza-Brito ARM, Vilegas W (2005). Flavonoids and antiulcerogenic activity from *Byrsonima crassa* leaves extracts. J. Ethnopharmacol., 97: 1-6.

Sannomiya M, Cardoso CR, Figueiredo ME, Rodrigues CM, Dos SLC, Dos SFV, Serpeloni JM, Cólus IM, Vilegas W, Varanda EA (2007). Mutagenic evaluation and chemical investigation of *Byrsonima intermedia* A. Juss. leaf extracts. J. Ethnopharmacol., 112: 319-326.

Santos FV, Tubaldini FR, Cólus IM, Andréo MA, Bauab TM, Leite CQ, Vilegas W, Varanda EA (2008). Mutagenicity of *Mouriri pusa* Gardner and *Mouriri elliptica* Martius. Food Chem. Toxicol., 46: 2721-2727.

Siddhuraju P, Manian S (2007). The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc. Food Chem., 105: 950-958.

Silva EM, Hiruma-Lima CA, Lólis SF (2000). Etnobotânica in the municipality of Porto Nacional. In: Symposiumof Brazilian Medicinal Plants. Cuiaba, Brazil.

Umamaheswari M, Asokkumar K, Rathidevi R, Sivashanmugam AT, Subhadradevi V, Ravi TK (2007). Antiulcer and in vitro antioxidant activities of Jasminum grandiflorum L. J. Ethnopharmacol., 110: 464-470.

Vasconcelos PC, Kushima H, Andreo M, Hiruma-Lima CA, Vilegas W, Takahira RK, Pellizzon CH (2008). Studies of gastric mucosa regeneration and safety promoted by *Mouriri pusa* treatment in acetic acid ulcer model. J. Ethnopharmacol., 115: 293-301.

Willcox JK, Ash SL, Catignani GL (2004). Antioxidants and prevention of chronic disease. Crit. Rev. Food Sci. Nutr., 44: 275-295.

Yacyshyn BR, Thomson ABR (2002). The clinical importance of proton pump inhibitor pharmacokinetics. Digestion, 66: 67-78.