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Full Length Research Paper

Maytenus ilicifolia as source of antioxidants and anti-radicals agents and its action on neutrophils peroxidase (myeloperoxidase)

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Maytenus ilicifolia **is an important plant with a great potential for cancer treatment and has a popular and diffused use, as tea, in Brazil and in other countries against gastric ulcers, dyspepsia and others gastric problems. In this paper, the antioxidant and anti-radical activities of this plant were compared with trolox and uric acid by their abilities as scavengers of DPPH$^{\bullet}$, ABTS$^{\bullet+}$, $O_2^{\bullet-}$, HOCl and NO. In all systems, a significant correlation existed between concentration of extracts and percentage inhibition of free radicals. Moreover, these extracts could inhibit oxidation of guaiacol catalyzed by peroxidases (horseradish peroxidase and myeloperoxidase) or by their prosthetic group, the hemin. The results provide useful pharmacological information against free radicals and reactive oxygen species, which are involved in a variety of pathological events like diabetes, cancer and aging. The crude extract and the hydroalcoholic fraction were able to act efficiently over all species assayed. This ability to scavenge radicals and endogenous reactive oxygen species, as well as to act as inhibitors of the enzymes that participate in their generation, would be useful for the treatment of various diseases mediated by radical species.**

Key words: Myeloperoxidase, celastraceae, oxidative damage, free radical, *Maytenus ilicifolia.*

INTRODUCTION

Free radicals are species that contain unpaired electrons. The oxygen radicals, such as superoxide radical ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$) and non-free radical species that participate on oxidative processes, such as H_2O_2 and singlet oxygen ($^1O_2$), are various forms of Reactive Oxygen Species (ROS). They are generated in many physiological or pathological redox processes (Vellosa et al., 2007c).

The $OH^{\bullet}$ inactivates many proteins, by oxidizing sulfhydryl groups, DNA, promoting mutations by changing purines and pirimidines basis for it (Ferreira and Matsubara, 1997). It can initiate too a lipoperoxidation process on cellular membranes which causes cellular death (Halliwell and Gutteridge, 1986). By using H_2O_2 and chloride ions as substrates, the enzyme Myeloperoxidase (MPO; donor hydrogen peroxide oxidorreductase, EC 1.11.1.7) produces Hypochlorous acid (HOCl), an extremely strong oxidant that attack important biomolecules such as amines, amides, thiols, amino acids and nucleotides (Arnhold, 2004; Auchére and Capelleire-Blandin, 1999; Eaton, 1993, Weiss, 1989). Then, besides participating on bacterial killing, HOCl can produce some other oxidant species (Mutza et al., 2003; Lapenna and Cuccurollo, 1996).

Usually there are different systems that trap and destroy these species, such as the enzymes superoxide dismutase, catalase and glutathione peroxidase. Over-produc-

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tion of free radicals associated with low concentrations of A, C and E Vitamins and a reduced level of the above mentioned enzymes, is considered to be the main contributor to oxidative stress (Banerjee et al., 2005, Halliwell, 1995).

An antioxidant is defined as any substance that can delay or inhibit an oxidative process (e.g. lipoperoxidation) when in low concentrations (Atoui et al., 2005; Chun et al., 2005) Phenolic antioxidants from plants secondary metabolism are good sources of natural antioxidants agents. The use of plants with pharmaceutical properties has received increased interest nowadays. Besides, these medicinal plants play an important role in public health, especially in developing countries. *Celastraceae* family comprises 55 different genera with 850 species spread throughout the tropics and sub-tropics. The *Maytenus* genus is one of the largest in this family, where 77 species have been found in Brazilian flora (Mossi et al., 2004).

Numerous compounds isolated from *Maytenus ilicifolia* have been tested for their antitumoral activity, however, the infusion of the leaves from *M. ilicifolia* have been used in Brazil by the folk medicine for their antiacid and antiulcerogenic effects (Quieroga et al., 2000). The presence of phenolic metabolites, such as condensed tannins and triterpenes could justify the usage of some species of *Maytenus* as anti-inflamatory and antiulcerogenic agents Jorge et al., 2004). Our previous published study has shown potential antioxidant and anti-radical activities of *M. ilicifolia* (Vellosa et al., 2006), so we decide to make a more detailed study on this potential action.

MATERIALS AND METHODS

This study was undertaken in order to assess *M. ilicifolia* as potential source of antioxidants and free radicals` scavengers. All spectrophotometric assays were read with a HP 8453 Diode Array Spectrophotometer. Chemiluminescent assay were made using the Bio Orbit Luminometer model 1251. Various doses of plant extract, uric acid (a natural antioxidant) and trolox (E vitamin analogue) were assayed and their response were expressed by percentage inhibition calculated comparing the responses on presence and absence of the tested samples according to Vellosa et al. (2007a).

Plant material and plant extracts preparation

The specimen (*M. ilicifolia*) was collected on UNAERP Experimental Farm (Campus Ribeirão Preto) and identified by Professor Rita Maria de Carvalho from Campinas University (UNICAMP) Bioscience Institute, where the specimen is now registered as HPM-BR 0059. The bark root from the specimen was dried over 40^{o}C and triturated. After that, the obtained powder (200 g) was submitted to an ultrasound extraction with ethanol (1000 mL) by 20 min. This procedure was repeated three times to the same powder. Then the extracts were pooled together and concentrated by filtration under reduced pressure for obtaining an ethanolic crude extract (35 g). Then, the crude ethanolic extract was submitted to a liquid-liquid separation on hexane (100 mL) and hexane:acetate 2:8 (100 mL) to produce the hexanoic (1.83 g) and hydroalcoholic (29,6 g) fractions [an hexane:acetate fraction (1,99 g) was not assayed].

Chemicals

DPPH (1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-Azinobis(3-ethylenebenzothiazoline-6-sulfonic acid), DTNB (5,5'-dithio-2-nitrobenzoic acid), sodium borohydride, EDTA (ethylenediaminetetraacetic acid), NADH (reduced nicotinamide adenine dinucleotide), PMS (phenazine methasulfate), NBT (nitroblue tetrazolium), sodium nitroprusside, guaiacol, luminol, HRP (horseradish peroxidase), hemin and hydrogen peroxide were purchased from Sigma Chemicals Co. All other reagents were analytical grade and commercially available. Myeloperoxidase (MPO) was used as crude extract from neutrophils`rats (*Ratus norvegicus albinus*).

Crude extract from neutrophil's rats

Neutrophils were obtained as described by Paino et al. (2005). The neutrophils suspension was cooled in refrigerator in about four hours and, after being thawed, it was centrifuged to separate the pellet. The supernatant were called the neutrophil crude extract containing myeloperoxidase. MPO content was evaluated through the guaiacol oxidation described by SIGMA Chemical Co.

Guaiacol oxidation

The plant extract interference over guaiacol oxidation catalyzed by peroxidase plus hydrogen peroxide was evaluated through two systems:

A) Myeloperoxidase inhibitions were evaluated through 100 mM guaiacol, 0.1U MPO and 0.5 mM H_2O_2.

B) HRP inhibitions were evaluated through 2 mM guaiacol, 7nM HRP and 0.1 mM H_2O_2. HRP concentration was determined by its ε at 403 nm (1.02×10^5 M^{-1} cm^{-1}) (Ohlson and Paul, 1976).

Guaiacol oxidations were followed at 470 nm. Reactions had started when hydrogen peroxide was added. The rates $\Delta A/\Delta t$ were calculated from the initial linear rate (v_0) of reactions (Desser et al., 1972). Samples were assayed in three doses: 10, 1 and 0.1µg/mL. The initial rates in the presence and absence of the samples were compared (mean $\pm$ standard deviation).

Hemin inhibition by chemiluminescence

Hemin, the prosthetic group of peroxidases, is able to oxidize different compounds in presence of hydrogen peroxide (Desser et al., 1972). Samples were assayed in three doses: 10, 1 and 0.1 µg/mL. Hemin inhibitions were evaluated through:

A) Spectrophotometric assay – guaiacol oxidation (470 nm): 5 mM guaiacol, 3 µM hemin and H_2O_2 (2.5 mM).
B) Chemiluminescent assay – l uminol oxidation: 0.1 mM, l uminol, 80 nM hemin and 0.05 mM hydrogen peroxide.

DPPH radical scavenging activity

DPPH is a free radical that, when dissolved in ethanol, has purple color. Loss of this color indicates radical scavenging activity. *M. ilicifolia* extracts and trolox ethanolic solutions and uric acid aqueous solution were evaluated against 60 μM DPPH. The aqueous solution were evaluated against 60 μM DPPH. The reac-

tion mixture (sample plus DPPH; total volume 1.0 mL) was shaken vigorously and allowed to react at room temperature. After 15 min, remaining DPPH was determined colorimetrically at 531 nm, using absolute ethanol as a blank (Assis et al., 2008; Vellosa et al., 2007a; Soares et al., 1997). The scavenger capacities against oxidant species were calculated as mean values of triplicates assays and expressed as percentage of radical or ROS scavenged (% inhibition) calculated through Eq.1 DPPH, $ABTS^{\bullet+}$, $O2^{\bullet-}$ and NO assays.

$$\text{Inhibition (\%)} = 1 - \left(\left(\frac{A_{\text{sample}}}{A} \right) \right) \text{ x } 100 \qquad \text{Eqn 1}$$

Where A is test absorbance without sample and A_{sample} is test absorbance with sample.

$ABTS^{\bullet+}$ radical scavenging activity

$ABTS^{\bullet+}$ was prepared according to Pellegrini et al. (1999) modify-cated (ethanol was replaced by 10 mM potassium phosphate buffer, pH 7.0). Prior to assay the $ABTS^{\bullet+}$ stock solution was diluted with the buffer solution (ratio 1:88) to give an absorbance at 734 nm of 0.414±0.013 (n = 40). One milliliter $ABTS^{\bullet+}$ was then added to glass test tubes containing various concentrations of each extract and mixed for 15 sec. Tubes were incubated for 30 min and then read at 734 nm (Vellosa et al., 2006; Vellosa et al., 2007a Oliveira et al., 2007) and inhibition percentage was calculated through Equation 1.

HOCl scavenging activity

The scavenger capacity against HOCl was studied by the TNB oxidation method described by Ching et al. (1994). Samples were incubated with HOCl for 5 min in KH_2PO_4/K_2HPO_4 (50 mM, pH 7.0) buffered solution. TNB (80 µM) was then added, following 15 min incubation on 25^{0}C (Assis et al., 2008; Vellosa et al., 2007a,b). TNB is oxidized to DTNB by HOCl (22 µM) causing the absorbance decreasing at 412 nm (DTNB absorbance at 325 nm raises at same time). The scavenger capacity was expressed as mean values of triplicates assays and expressed as percentage of the ROS scavenged (% inhibition) calculated through Equation 2.

$$\text{Inhibition (\%)} = 1 - \left(\left(\frac{A_0 - A_T}{A_0 - A_1} \right) \right) \text{ x } 100 \qquad \textbf{Eqn 2}$$

Where $\mathbf{A_0}$ is test absorbance at 412 nm without HOCl or sample, $\mathbf{A_1}$ is test absorbance at 412 nm with HOCl, but no sample and $\mathbf{A_T}$ is test absorbance at 412 nm with HOCl and sample.

Superoxide radical scavenging activity

Superoxide radicals, produced by NADH and PMS (phenazine methasulphate), reduce NBT (nitro blue tetrazolium salt) and pro-duce a formazan compound. The intensity of color is inversely proportional to the antioxidant concentration (Vellosa et al., 2007a; Kakkar et al., 1984). The assay was carried out in sodium pyro-phosphate buffer (0.025 M, pH 8.3) and the mixture contained 25 µL of 372 µM PMS, 75 µL of 600 µM NBT, 50 µL of 1560 µM NADH, in the presence of different concentrations of plant extracts (final volume = 1 mL). Reactions were started by adding NADH. After incubation at 25^{0}C for 90 sec, 100 µL of glacial acetic acid and 900 µL of sodium pyrophosphate buffer were added. After homogenization, the color intensity of mixture was measured at 560 nm and inhibition percentage was calculated through Eq.1.

Nitric oxide radical scavenging activity

For this assay, several samples solutions concentrations were added to test tubes with sodium nitroprusside solution (25 mM) to give a final volume of 1 mL, and the tubes were incubated at 25^{o}C for 1.5 h. An aliquot (0.25 mL) of the solution was then withdrawn and diluted with 0.15 mL Griess Reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore, produced during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride, was immediately read at 570 nm and inhibition percentage was calculated through Equation 1. Sodium nitroprusside is known to decompose in aqueous solution at physiological pH, producing NO. Under aerobic conditions, NO reacts with oxygen to produce the stable products nitrate and nitrite, and the nitrite can be determined with Griess reagent. The final absorbance at 570 nm is diminished by an NO scavenger, because less nitrite is produced to form the chro-mophore (Vellosa et al., 2007a; Yen et al., 2001).

RESULTS

The crude ethanolic extract of *M. ilicifolia* had already been previously studied by $ABTS^{\bullet+}$ and HOCl systems (Vellosa et al., 2006). The previous study indicated that it was a possible source of antioxidants and anti-radicals. In this new paper, after producing two fractions from the crude extract and studying new scavenger capacities assays, we complement the previous study tracing an action profile of this plant action. Results are shown in Tables 1 - 7.

The extract and hydroalcoholic fraction inhibited the peroxidasic activity of MPO, HRP (Table 1) and Hemin (Tables 1 and 2). The hydroalcoholic fraction was much better inhibitor than the hexanoic one, as soon as the last one did not inhibit HRP or MPO and its action over hemin chemiluminescent assay was worst than hydroalcoholic extract (Table 2).

The crude extract and its hydroalcoholic and hexanoic fractions were able to act over luminol oxidation by inhi-biting the peroxidases prostetic group activity in the presence of hydrogen peroxide (Table 2) . This result may be due a possible action directly over hemin or by a radical scavenger action leading to a decreasing of the radical oxidized luminol product. Its is possible to observe that by the guaiacol oxidation method the samples were not able to act over hemin through the studied conditions, exception made to the hydroalcoholic fraction of *M.*

Table 1. The effect of *M. ilicifolia* extracts over guaiacol oxidation by HRP, MPO or hemin and hydrogen peroxide.

		Samples		
	Final concentration (mg/mL)	**Crude extract**	**Hydroalcoholic fraction**	**Hexanoic fraction**
HRP	0 (control)		0.0144 ± 0.0010	
	0,0001	0.0140 ± 0	0,0130 ± 0.0010	0.0130 ± 0.0010
	0,001	0.0135 ± 0.0007	0.0140 ± 0.0010	0.0150 ± 0.0010
	0,01	0.0105 ± 0.0007*	0.0115 ± 0.0007*	0.0140 ± 0
MPO	0 (control)		0.0040 ± 0.0004	
	0,0001	0.0042 ± 0.0003	0.0041 ± 0.0002	0.0040 ± 0.0001
	0,001	0.0029 ± 0.0005*	0.0033 ± 0.0004*	0.0042 ± 0.0001
	0,01	0.0005 ± 0.00007*	0.0008 ± 0.00002*	0.0042 ± 0.0004
HEMIN	0 (control)		0.0054 ± 0.0007	
	0,0001	0.0055 ± 0	0.0052 ± 0.0004	0.0053 ± 0.0004
	0,001	0.0053 ± 0.0008	0.0055 ± 0.0006	0.0049 ± 0.0002
	0,01	0.0053 ± 0.0004	0.0039 ± 0.0004*	0.0057 ± 0.0004

*Statistically different compared to control ($p<0.05$)
Values (initial rate, s^{-1}) are expressed as mean ± standard deviation.

Table 2. The effect of *M. ilicifolia* extracts over luminol oxidation by hemin and peroxide

Samples			
Final concentration (mg/mL)	**Crude extract**	**Hydroalcoholic fraction**	**Hexanoic fraction**
0 (control)	51711 ± 347	51711 ± 347	51711 ± 347
0,0001	14904 ± 1253*	18755 ± 4347*	33658 ± 2662*
0,001	2090 ± 316*	1106 ± 40*	3983 ± 117*
0,01	38 ± 1*	40 ± 4*	209 ± 28*

*Statistically different compared to control ($p<0.05$)
Integral values (mean ± SD) from the plot (t = 1 min) of chemiluminescence luminol dependent were compared to the control tubes

ilicifolia (Table 1).

It is possible to observe that there is different potentials of the studied extract to act over different reactive species. The two model free radicals interacted with the samples in different intensities. $ABTS^{\bullet+}$ was scavengered more efficiently than $DPPH^{\bullet}$. About the reactive species naturally occurring in living organisms is possible to see that the samples had greater action over hypochlorous acid than superoxide radical and nitric oxide.

The best $DPPH^{\bullet}$ scavenger was trolox by scavenging almost 100% of the radical in the solution (Table 3) with low concentrations (0.013 mg/mL). The crude extract and its hydroalcoholic fraction had equal potential as $DPPH^{\bullet}$ scavengers, and were better than uric acid a well known human serum antioxidant (Table 3). The less effective agent against $DPPH^{\bullet}$ was the hexanoic fraction.

Uric acid was the best scavenger agent over $ABTS^{\bullet+}$ followed by trolox (Table 4). The hexanoic fraction was again the worst scavenger agent when evaluated by $ABTS^{\bullet+}$ method. The hydroalcoholic fraction was a little less effective than the crude extract (Table 4).

Although the hexanoic fraction was good HOCl scavenger, it did not raise the same effectiveness than the hydroalcoholic one or the crude extract that were so good as trolox (Table 5). Uric acid promoted good inhibition on low levels but its capacity had raised a constant inhibition on bigger concentrations (Table 5).

Table 6 shows low potential of the patterns uric acid and trolox as superoxide anion scavengers. This table shows too that the hydroalcholic fraction was more active than the crude extract. This result is probably due an improvement on superoxide scavengers level by the extraction process. The *M. ilicifolia* hexanoic fraction were not able to act over superoxide anion (Table 6) and nitric oxide (Table 7) meanwhile the crude extract and hydroalcoholic fraction scavengered all studied reactive species. Although trolox is a well known antioxidants agent, it was not able to act over NO (Table7).

Table 3. The effect of *M. ilicifolia* extracts, uric acid and trolox over the DPPH radical scavenging activity.

Samples				Standards			
Final concentration (mg/mL)	Crude extract	Hydroalcoholic fraction	Hexanoic fraction	Final concentration (mg/mL)	Uric acid	Final concentration (mg/mL)	Trolox
0.040	38 ± 2.8*	40 ± 2.8*	19 ± 1.4*	0.034	27 ± 1.4*	0.013	93.5 ± 0.7*
0.020	32.5 ± 2.1*	36 ± 0*	12 ± 0*	0.027	25 ± 1.4*	0.006	90.5 ± 0.7*
0.010	27.5 ± 0.7*	28.5 ± 0.7*	7.5 ± 0.7*	0.017	23.5 ± 0.7*	0.005	77.5 ± 3.5*
0.002	9 ± 1.4*	8.5 ± 2,1*	3 ± 0.8	0.013	18,5 ± 0.7*	0.004	59 ± 4.2*
0.001	6 ± 1.4*	5.5 ± 0.7*	2 ± 1.4	0.007	11 ± 1.4*	0.003	41 ± 5.7*
0.0004	4 ± 2.8	4 ± 0	0 ± 0	0.003	2 ± 1.4	0.001	22 ± 2.8*

*Statistically different compared to control ($p<0.05$)
The values (scavengered radical percentage compared to the control; A_{532nm} = 0,413 ± 0,035) are expressed as mean ± standard deviation.

Table 4. The effect of *M. ilicifolia* extracts, uric acid and trolox over the $ABTS^{\bullet+}$ radical scavenging activity.

Samples				Standards			
Final concentration (mg/mL)	Crude extract	Hydroalcoholic fraction	Hexanoic fraction	Final concentration (mg/mL)	Uric acid	Final concentration (mg/mL)	Trolox
0.020	100 ± 0*	100 ± 0*	19.5 ± 2.1*	0.0034	100 ± 0*	0.0025	71.5 ± 2.1*
0.010	99.5 ± 0.7*	100 ± 0*	7.5 ± 0.7*	0.0021	92.5 ± 0.7*	0.0006	51 ± 4.2*
0.005	99 ± 0*	73.5 ± 2.1*	3.5 ± 0.7	0.0017	71 ± 2.8*	0.0005	41.5 ± 2.1*
0.002	61.5 ± 2.1*	37.5 ± 0.7*	1.5 ± 0.7	0.0013	53.5 ± 4.9*	0.00038	32.5 ± 0.7*
0.001	33 ± 0*	19.5 ± 0.7*	0 ± 0	0.0008	39 ± 5.7*	0.00025	22.5 ± 0.7*
0.0005	21 ± 1.4*	11 ± 2.8*	0 ± 0	0.0007	2 ± 0,8	0.00013	14 ± 0*

*Statistically different compared to control ($p<0.05$)
The values (scavengered radical percentage compared to the control; A_{734nm} = 0,430 ± 0,026) are expressed as mean ± standard deviation

Table 5. The effect of *M. ilicifolia* extracts, uric acid and trolox over the HOCl scavenging activity.

Samples				Standards			
Final concentration (mg/mL)	Crude extract	Hydroalcoholic fraction	Hexanoic fraction	Final concentration (mg/mL)	Uric acid	Final concentration (mg/mL)	Trolox
0.020	100 ± 0*	96 ± 5.7*	54 ± 5.7*	0.008	77 ± 0*	0.013	98 ± 2.1*
0.010	100 ± 0.7*	95 ± 5.7*	36 ± 3.5*	0.007	75 ± 1.4*	0.008	79 ± 1.4*
0.005	89 ± 2.8*	88 ± 3.5*	15 ± 1.4*	0.002	75 ± 0.7*	0.005	60 ± 2.1*

Table 5 continued

0.002	58 ± 7.3*	68 ± 2.8*	4 ± 0	0.0008	71 ± 1.4*	0.003	49 ± 1.4*
0.001	30 ± 0.7*	36 ± 2.8*	1 ± 0	0.0007	70 ± 0.7*	0.001	26 ± 0.7*
0.0005	5 ± 1.4	16 ± 0*	0 ± 0	0.0003	51 ± 2.8*	0.0005	7 ± 2*

* Statistically different compared to control ($p<0.05$)
The values (scavengered oxidant percentage compared to the control; ΔA_{412nm} = 0.715 ± 0.033) are expressed as mean ± standard deviation.

Table 6. The effect of *M. ilicifolia* extracts, uric acid and trolox over the $O_2^{\bullet-}$ radical scavenging activity.

Samples				Standards			
Final concentration (mg/mL)	**Crude extract**	**Hydroalcoholic fraction**	**Hexanoic fraction**	**Final concentration (mg/mL)**	**Uric acid**	**Final concentration (mg/mL)**	**Trolox**
0.0030	49 ± 6.6*	61 ± 2.8*	2 ± 1.7	0.125	24 ± 2*	0.125	19.5 ± 4.9*
0.0020	40 ± 6.8*	48 ± 1.4*	1 ± 2.1	0.100	22 ± 1.4*	0.0625	10.5 ± 0.7*
0.0010	6 ± 1.4*	26 ± 2*	0 ± 2.8	0.67	17 ± 2.8*	0.0375	6.5 ± 2.1*
0.0003	0 ± 0	15 ± 2*	0 ± 0	0.34	5 ± 1.4*	0.025	2 ± 1.4
0.0002	0 ± 0	10 ± 4.9*	0 ± 2.1	0.17	3.5 ± 2.1	0.0125	1.5 ± 2.1

*Statistically different compared to control ($p<0.05$)
The values (scavengered radical percentage compared to the control; A560nm = 0,342 ± 0,023) are expressed as mean ± standard deviation.

Table 7. The effect of *M. ilicifolia* extracts, uric acid and trolox over the NO radical scavenging activity.

Samples				Standards			
Final concentration (mg/mL)	**Crude extract**	**Hydroalcoholic fraction**	**Hexanoic fraction**	**Final concentration (mg/mL)**	**Uric acid**	**Final concentration (mg/mL)**	**Trolox**
0.050	22 ± 0.7*	17.5 ± 0.7*	3.5 ± 0.7	0.034	18.5 ± 3.5*	0.313	0 ± 2.1
0.020	19.5 ± 3.5*	17 ± 1.4*	2 ± 0	0.017	15 ± 2.8*	0.125	-0.5 ± 0.7
0.010	15 ± 0*	14.5 ± 0.7*	0.7 ± 1	0.007	3 ± 9.9	0.031	0 ± 2.8
0.005	10.5 ± 0.7*	7 ± 0*	0.5 ± 2.1	0.003	6 ± 1.4	0.025	0 ± 0
0.002	5 ± 1.4	2.5 ± 0.7	0 ± 0	0.002	0 ± 1.4	0.013	0.5 ± 0.7
0.0005	0.5 ± 0.7	0 ± 0.7	0 ± 0	0.001	0 ± 0	0.005	0 ± 0

* Statistically different compared to control ($p<0.05$)
The values (scavengered radical percentage compared to the control; A_{570nm} = 0,462 ± 0,022) are expressed as mean ± standard deviation

Nitric oxide was hard to being scavengered. Trolox and the plant hexanoic fraction were not able to act over this specie. Uric acid, the crude extract and its hydroalcoholic fraction promoted similar inhibition of this system (Table 7).

DISCUSSION

Plant extracts studies are important for the searching of antioxidant and anti-radical molecules. In this paper, the potential evaluated can be understood as occurring a more efficient extraction of antioxidant agents by the hydroalcoholic solution than the one made with hexane. The hexanoic fraction has a weak potential as antioxidant source and didn't show action as inhibitor of myeloperoxidase, HRP or hemin. The best potential concerns to the hydroalcoholic fraction of *M. ilicifolia*. So a later isolation must follow with hydroalcoholic fraction. The crude extract, as well its hydroalcoholic fraction, contains compounds that can act as antioxidant and/or anti-radical. Moreover, they contain molecules that may be able to inhibit radical generation and oxidative process catalyzed by peroxidases, mainly myeloperoxidase, which generates hypochlorous acid in living organisms.

The results provide useful pharmacological information related to free radicals and oxidant species. Free radicals and reactive oxygen species are involved in a variety of pathological events like diabetes, cancer and aging. The ability of plant extracts and isolated compounds to scavenge radicals and endogenous reactive oxygen species like HOCl, as well as to act as inhibitors of the enzymes that participate in their generation (like peroxidases and oxidases), would be useful for the treatment of various diseases mediated by radical species. The crude extract and the hydroalcoholic fraction were able to act efficiently over all species assayed.

Moreover, the crude extract and its hydroalcoholic fraction were better than the patterns uric acid and trolox in some systems, mainly superoxide radical (Table 3) and nitric oxide (Table 5) . It is important to note that besides their antioxidant and anti-radical action, the crude extract and its hydroalcoholic fraction were similarly able to inhibit the enzymatic oxidation of guaiacol in the presence of H_2O_2 (Table 7).

Despite of the fact of some Celastraceae hydrophobic molecules (salacin, friedelin, pristimerin, maytenin, 20 -hydroxymaytenin and netzahualcoyene) had already been proved to be antioxidant agents (Carvalho et al., 2005) . In this work, they were not able to produce a signi-ficant action for the hexanoic extract. So, the hydroalco-holic fraction from the crude extract containing hydrophilic components (probably flavonoids and related molecules) must be essential to the observed effects.

Conclusion

We demonstrated that *M. ilicifolia* hydrophilic extracts were able to scavenge various oxidant and radical species and inhibit myeloperoxidase, an enzyme involved in many physiological and pathological conditions. It may be possible that the use of this plant to treat certain pathologies could prevent or fight tissue damage when this involves an established oxidative stress. Anyway, new studies are necessary to understand the mechanism(s) of this inhibition, including: i) isolation and characterization of active compounds from this plant; ii) *in vivo* studies of biological properties that confirm the possibility suggested here; and iii) toxicological studies to evaluate the safety of this plant as a medicinal agent.

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