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Article

Salacia campestris root bark extract: peroxidase inhibition, antioxidant and antiradical profile

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Reactive oxygen species (ROS) and free radical species have been implicated in initiating or accompanying many diseases in living organisms; there is thus, a continual need for antioxidants molecules to inactivate ROS/free radicals. Many studies of plants crude extracts have demonstrated free-radical scavenging and antioxidant action. *Salacia* species have long been used, in several countries, as traditional medicines against certain diseases and for their anti-inflammatory properties. In this study, *Salacia campestris* Walp (Hippocrateaceae) root bark ethanol extract (ScEtOH) was assessed for its ability to scavenge free radicals and reactive oxygen species; the results were expressed as percentage inhibition of the active species. ScEtOH was efficient against studied species: DPPH radical (obtained inhibition = 30%), $ABTS^{\bullet+}$ (IC_{50} = 1.8±0.8 µg/mL), HOCl (IC_{50} = 1.7 ± 0.1 µg/mL), $O_2^{\bullet-}$ (obtained inhibition = 32%), and $NO^{\bullet}$ (obtained inhibition = 18 %). Peroxidase activity inhibition was evaluated through the guaiacol oxidation reaction catalyzed by hemin, HRP and myeloperoxidase (MPO); data showed that ScEtOH at 10 µg/mL led to 54 and 51% of inhibition, respectively, for the hemin and HRP systems. In the MPO system, ScEtOH promoted a 50% inhibition at 8.9 µg/mL, whereas quercetin, a powerful MPO inhibitor, inhibited this system at 1.35 µg/mL.

Uniterms: Salacia campestris *Walp/antioxidant properties. Hippocrateaceae. Myeloperoxidase. Reactive oxygen species. Antioxidants. Free radicals.*

Espécies reativas do oxigênio (ERO) e radicais livres estão relacionados ao início ou à exacerbação de muitas doenças em organismos vivos; existindo portanto uma necessidade contínua por moléculas antioxidantes que inativem as ERO e radicais livres. Muitos estudos com extratos brutos de plantas têm demonstrado propriedades antioxidantes e seqüestradoras de radicais livres. Espécies de *Salacia* são utilizadas, em muitos países, como remédio tradicional contra certas doenças e por suas propriedades antiinflamatórias. Neste estudo, o extrato bruto etanólico da casca da raiz da *Salacia campestris* Walp (Hippocrateaceae) foi avaliado quanto à sua habilidade em seqüestrar radicais livres e espécies reativas do oxigênio; os resultados são expressos como porcentagem de inibição das espécies ativas. ScEtOH mostrou-se eficiente frente as espécies estudadas: radical DPPH (inibição obtida = 30%), $ABTS^{\bullet+}$ (IC_{50} = 1,8±0,8 µg/mL), HOCl (IC_{50} = 1,7 ± 0,1 µg/mL), $O_2^{\bullet-}$ (inibição obtida = 32%), and $NO^{\bullet}$ (inibição obtida = 18%). A inibição da atividade peroxidásica foi avaliada através da oxidação do guaiacol catalisada pela hemina, HRP e mieloperoxidase (MPO); os dados mostram que 10 µg/mL de ScEtOH promovem 54 e 51% de inibição, respectivamente para os sistemas da hemina e da HRP. No sistema da MPO, ScEtOH promoveu 50% de inibição na dose de 8,9 µg/mL, enquanto a quercetina, um potente inibidor da MPO promoveu tal inibição com 1,35 µg/mL.

Unitermos: *Salacia campestris* Walp/propriedades antioxidantes Hippocrateaceae. Mieloperoxidase. Oxigênio/espécies reativas. Antioxidantes. Radicais livres.

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INTRODUCTION

The oxidative burst from polymorphonuclear neutrophils (PMN) entail the production of reactive oxygen species (ROS) generated by two key enzymes, NADPH oxidase and myeloperoxidase (MPO). Free radicals may play an important role in the origin of life and in biological evolution, and have beneficial effects in living aerobes. For example, oxygen radicals act critically in signal transduction, gene transcription and regulation of soluble guanylyl cyclase activity in cells (Tepe *et al.*, 2005a). However, free radicals and other relative species can oxidize biomolecules (e.g., protein, amino acids, lipid, and DNA), leading to cell injury and death. Oxidative stress, a disturbance of the oxidant/antioxidant ratio in living aerobic organisms, may be involved in processes such as mutagenesis, carcinogenesis, lipid peroxidation, fragmentation of proteins, and carbohydrate damage (Ferreira, Matsubara, 1997; Babior, 2000). Experimental data have indicated that free radicals play a critical role in a variety of pathological processes, including aging, multiple sclerosis, inflammation, coronary heart and cardiovascular diseases, senile dementia, arthritis and atherosclerosis (Ferreira, Matsubara, 1997; Babior, 2000; Benedì *et al*., 2004; Vellosa *et al.*, 2007a).

A continual supply of antioxidant molecules is necessary for ROS inactivation. In an effort to prevent or diminish induced ROS damage, investigators have evaluated compounds that prevent their generation and reduce the damage. Recent studies shown that a number of plant products, including polyphenols, flavonoids and terpenes and various plant extracts, exert a free-radical scavenging and an antioxidant action (Corsino *et al.*, 2000; Pereira *et al.*, 2005; Carvalho *et al.*, 2005; Cui *et al.*, 2005; Benedì *et al.,* 2004;). Natural antioxidants obtained from plant extracts have attracted increasing interest, owing to consumer concern about the safety of the synthetic antioxidants in food. Extracts from fruits, vegetables, cereals and their by-products have shown effective antioxidant activity in model biological systems (Tepe *et al.,* 2005b; Sun, Ho, 2005).

Several representatives of the related *Hippocrateaceae* and *Celastraceae* families are claimed to be medically useful as antimicrobial, anticancer and antimalarial. Some *Salacia* species (Hippocrateaceae) have long been used in India, Sri Lanka, and China as traditional medicines for the treatment of rheumatism and skin diseases, and also for their anti-inflammatory properties (Carvalho *et al.*, 2005).

Quinonemethide triterpenes, secondary metabolites restricted to the higher plant families Celastraceae and Hippocrateaceae, have shown a variety of biological activity such as antitumoral, antimicrobial and antimalarial (Corsino *et al.*, 2000). Carvalho *et al.* (2005) described the occurrence of several quinonemethide triterpenes (salacin, friedelin, pristimerin, maytenin, 20α-hydroxymaytenin and netzahualcoyene) in *S. campestris* (Hippocrateaceae) root barks and compared their anti-radical properties by their DPPH scavenger potential.

Protection of the living organism against oxidative stress relies not only on endogenous antioxidants, but also on exogenous compounds taken in food and beverages. In view of the therapeutic actions of *Salacia* species against rheumatism and inflammation, it is important to evaluate the antioxidant and antiradical potential of *Salacia campestris Walp*. It would also be useful to know if crude extract or isolated compounds from *S. campestris* are able to inhibit ROS generation by peroxidases, mainly MPO.

MATERIALS AND METHODS

Experimental apparatus and analytical condition

This study was undertaken in order to assess the antioxidant activity of crude ethanol extract of *S. campestris* root bark (ScEtOH) by determining its capacity to scavenge free radicals and some reactive oxygen species, and inhibit peroxidase activity from hemin, HRP type VI (horseradish peroxidase; EC 1.11.1.7) and MPO (from polymorphonuclear neutrophils of *Rattus norvegicus* var. *albinus*). All assays were done with a HP 8453 Diode Array Spectrophotometer. Various doses of plant extract, uric acid (a natural antioxidant) and trolox (E vitamin analogue) were assayed and their scavenger capacities against oxidant species were calculated as mean values of triplicates assays and expressed as percentage of radical or ROS scavenged (% inhibition) calculated for HOCl assay by Eq.1, and by Eq.2 for DPPH, $ABTS^{\bullet+}$, $NO^{\bullet}$ assays.

$$\text{Inhibition (\%)} = \left(1 - \left(\frac{A_0 - A_T}{A_0 - A_1}\right)\right) \times 100 \qquad \text{Eq.1}$$

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.

$$\text{Inhibition (\%)} = \left(1 - \left(\frac{A_{\text{sample}}}{A}\right)\right) \times 100 \qquad \text{Eq.2}$$

Where: **A** is test absorbance without sample and $\mathbf{A_{sample}}$ is test absorbance with sample.

Plant material

Salacia campestris root barks were identified and supplied by Prof. Dr. Maria Helena de O. Antunes, from Fazenda Canchin at the São Carlos Federal University, São Carlos (SP, Brazil). The voucher specimens (No.2845) are deposited at the Herbarium of Botany Department of the Federal University of São Carlos (UFSCar), São Carlos (SP, Brazil). The bark root (51.20 g) from the specimen was dried over 40 °C and triturated. After that, the obtained powder was submitted to an ultrasound extraction with ethanol (300 mL) by 20 minutes in triplicate and concentrating the filtrate under reduced pressure for obtaining an ethanolic crude extract (8.6 g).

Chemicals

Quercetin, ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)], DTNB [5,5'-dithiobis(2-nitrobenzoic acid)], guaiacol, hemin, HRP (horseradish peroxidase), trolox, uric acid, phenazine methosulfate (PMS), NADH, NBT (nitrobluetetrazolium), and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma Chemicals Co. Griess Reagent was kindly provided by Professor Iracilda Zeppone Carlos (Clinical Immunology Lab. of the School of Pharmaceutical Sciences – UNESP at Araraquara, SP, Brazil). All other reagents were analytical grade and commercially available.

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. Ethanol solutions of *S. campestris* extracts and trolox and aqueous solutions of uric acid at various concentrations were evaluated against 60 μM DPPH. The reaction mixture (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 (Brand-Williams *et al*., 1995).

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

Pellegrini *et al.* (1999) used the $ABTS^{\bullet+}$ assay to evaluate antioxidant capacity of some fruits extracts using absolute ethanol as diluent. In this paper, ethanol was replaced by sodium phosphate buffer. $ABTS^{\bullet+}$ was prepared by reacting 5 mL of 7 mM ABTS aqueous solution with 88 μL of 140 mM potassium persulphate (molar ratio 1:0.35) and the mixture allowed to stand in the dark at room temperature for 12–16 h before use. Prior to assay this $ABTS^{\bullet+}$ stock solution was diluted with NaH_2PO_4/Na_2HPO_4 (100 mM, pH 7.0, diluted 1:10 before use) 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 s. Tubes were incubated for 30 min and then read at 734 nm.

HOCl scavenging activity

TNB (5-thio-2-nitrobenzoic acid) was produced from DTNB, as described by Ching and co-workers (1994). TNB (80 μM) is oxidized to DTNB by HOCl (22 μM) causing the absorbance at 412 nm to fall while DTNB absorbance (325 nm) appeared. Samples were incubated with HOCl for 5 min in NaH_2PO_4/Na_2HPO_4 (50 mM, pH 7.0) buffered solution. TNB was then added, following 15 min incubation on 25 °C (Ching *et al*., 1994).

Superoxide radical scavenging activity

Superoxide radicals, produced by NADH and PMS, reduce NBT and produce a formazan compound. The intensity of color is inversely proportional to the antioxidant concentration (Kakkar *et al.*, 1984). The assay was carried out in sodium pyrophosphate 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, plant extract (several volumes) and buffer to complete 1mL final volume. Reactions were started by adding NADH. After incubation at 25 °C for 90 s, 100 μL of glacial acetic acid and 900 μL of sodium pyrophosphate buffer were added. After vigorous homogenization, the color intensity of mixture was measured at 560 nm.

Nitric oxide radical scavenging activity

For this assay, several volumes of ScEtOH (10 μg/mL) 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 ^{0}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. Sodium nitroprusside is known to decompose in aqueous solution at physiological pH, producing $NO^{\bullet}$. Under aerobic conditions, $NO^{\bullet}$

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 chromophore (Yen *et al.*, 2001).

Hemin and HRP assays

We evaluated the effect of *S. campestris* extract (10, 1 and 0.1 µg/mL) on: i) Hemin, prosthetic group of peroxidases; the assay was performed in sodium phosphate buffered saline (PBS) pH 7.0, 25^{0}C, using 3 mM hemin, 2.5 mM H_2O_2, and 5 mM guaiacol; ii) HRP, an important and well studied peroxidase used as a model for peroxidase inhibition studies; we used Dulbecco's phosphate buffered saline (PBS-D), at 37 ^{0}C, 7 nM HRP, 2 mM guaiacol and 0.1 mM H_2O_2 which initiated the reaction, followed for 1 min at 470 nm. The kinetic constant v_0 was determined by graphical analysis. All reactions were monitored in the presence of the *S. campestris* extract (10, 1 and 0.1 µg/mL) and in its absence. Quercetin, a MPO inhibitor (Pincemail *et al.*, 1988) was used as a peroxidasic inhibitor pattern.

Myeloperoxidase (MPO) assay

Reactions with MPO were carried out by guaiacol oxidation, with several concentrations of *S. campestris* extract, in PBS buffer pH 7.0. The absorbance rate was followed at 470 nm. The final concentrations in the reaction mixture were 20 mM guaiacol and 0.2 mM H_2O_2 plus 75 mU MPO. In this system MPO inhibition from ScEtOH and quercetin was evaluated.

MPO extraction from PMN

These procedures were approved by the UNESP-School of Pharmaceutical Sciences Ethical Committee (process 09/2005). Neutrophils (PMN) were isolated according to Paino *et al.* (2005). PMN were obtained from 6 male adults (100-160 grams) *Rattus norvegicus* var. *albinus*. Each rat was intraperitoneally inoculated with 5 mL of 1% oyster glycogen in 0.85% NaCl. The animal was sacrificed 12 h later and its peritoneal cavity washed with 20 mL PBS-D buffer (calcium free) to obtain a suspension of neutrophils. This was centrifuged at 200 xg, for 3 min. The sediment was washed twice with PBS, then resuspended in 500 µL of this buffer, and kept at –20 ºC for 12 h. After cell rupture and centrifuging at 200g a crude extract containing MPO was obtained. MPO was determined by assaying guaiacol oxidation in PBS buffer at pH 7.0. The reaction was followed at 470 nm, with 100 mM guaiacol and 0.5 mM H_2O_2 at 25°C. The MPO activity (U/mL) was defined by eq. 3.

$$Activity\ (U/mL) = \frac{(A_{470\ nm}\ at\ 1\ minute\ -\ A_{470\ nm}\ at\ 0\ minute)\ x\ total\ volume\ (mL)}{(Path\ length)\ x\ crude\ extract\ volume\ (mL)}$$

RESULTS AND DISCUSSION

DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule; because of its odd electron, in the ethanol solution shows a strong absorption band at 531 nm (Brand-Williams *et al.*, 1995). As DPPH reacts with suitable reducing agents, this electron becomes paired off causing a color change from purple to yellow and the solution loses color stoichiometrically with the number of electrons taken up. This reactivity has been widely used to test either the ability of diverse compounds to act as free radical scavengers or the antioxidant activity of plant extracts (Soares *et al.*, 1997). DPPH reduction was estimated from the bleaching of the radical solution (decay of absorbance at 531 nm) in the presence of the extracts and substances assayed. Fig.1 shows dose-response curves obtained DPPH scavenging using trolox, uric acid or ScEtOH. Whereas the ethanol extract and uric acid did not promote 50% inhibition within the assayed doses, trolox was shown to be a good free radical scavenger with an IC_{50} of 3.3±0.4 µg/mL, raising 94% inhibition at about 13µg/mL. According to these data, ScEtOH was a slightly

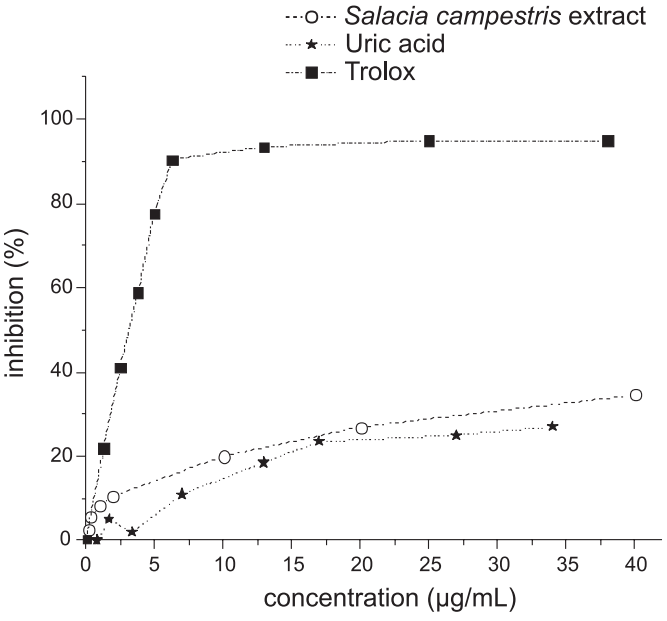

FIGURE 1 - DPPH radical (60 µM) scavenger action by *S. campestris* extract, uric acid and trolox. The reactions were made in ethanol, at 25 °C, and incubated by 15 min.

better DPPH scavenger than uric acid. The *S. campestris* ethanolic extract inhibition (30% in 40µg/mL) was similar to the one observed to the ethanolic extract from *Maytenus aquifolium* in a previous study (Vellosa *et al.*, 2007b).

The $ABTS^{\bullet+}$ assay has been used to screen the relative radical-scavenging capacities of flavonoids and phenolics, which act as electron- or H-donating agent or plant extracts that contains these substances (Pellegrini *et al*., 1999). The green cation radical $ABTS^{\bullet+}$ has absorbance peaks at 630, 734 and 812 nm. On interaction with antioxidants the radical is reduced, suppressing the absorbance of the green radical cation in a dose-dependent way. From the data in Fig. 2, the samples can be classified in order of descending scavenging power, by comparing IC_{50} values (in brackets): trolox (0.7 ± 0.04 µg/mL) > uric acid (1.2 ± 0.06 µg/mL) > ScEtOH (1.8 ± 0.08 µg/mL). Vellosa *et al*. (2006) and Oliveira *et al*. (2007) observed good DPPH scavenger action respectively to *Maytenus ilicifolia* (IC_{50} = 2 µg/mL) and *Agaricus Blazei* (IC_{50} = 23 µg/mL). The *S. campestris* ethanolic extract is so efficient scavenger of $ABTS^{\bullet+}$ as it was *M. ilicifolia* ethanolic extract and more efficient than *A. blazei* ethanolic extract. The *S. campestris* ethanolic extract has a bigger scavenger capacity than *Maytenus aquifolium* (IC_{50} = 4 µg/mL) (Vellosa *et al.*, 2007b).

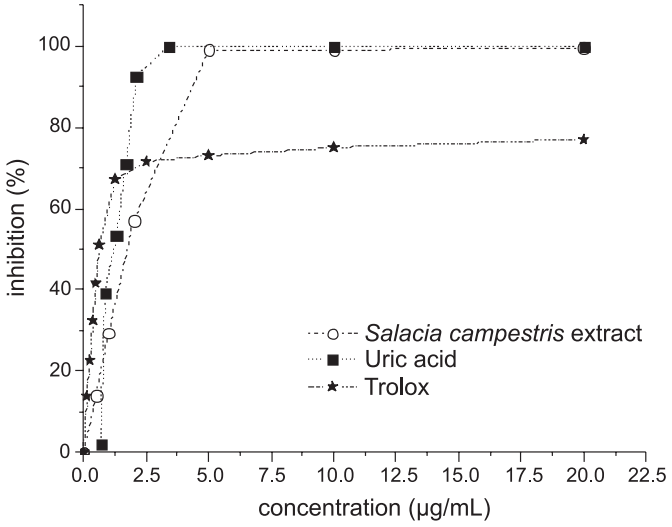

FIGURE 2 - $ABTS^{\bullet+}$ radical (55 µM) Scavenger action by *S. campestris* extract, uric acid and trolox. The reactions were made in 10mM sodium phosphate buffer, pH 7.0, 25 ^{0}C, and incubated by 30 min.

In biological systems, hypochlorous acid is the most toxic and abundant oxidant agent produced by PMN (Lapenna, Cuccurollo, 1996; Vellosa *et al*., 2007c). It can attack important biological molecules and generate other harmful ROS (Weiss, 1989; Eaton, 1993). This oxidant specie reacts with ammonia produced by *Helicobacter pylori* in human stomach to produce the microbicide monochloramine, which is also related to stomach injury observed in gastric ulcers (Lapenna, Cuccurollo, 1996). Hence, it is important to discover drugs and plant extracts that are able to fight HOCl. Here, we evaluated the potential of ScEtOH to scavenge HOCl, in a comparative study with trolox and uric acid. From the results in Fig. 3, the IC_{50} values can be obtained and used to rank the samples as HOCl scavengers: uric acid (0.3 ± 0.03 µg/mL) > ScEtOH (1.7 ± 0.1 µg/mL) > trolox (3.1 ± 0.3 µg/mL). These values were equal to those ones obtained to Maytenus species in previous studies (Vellosa *et al.*, 2006, 2007b). The results are very encouraging, because *S. campestris* leaves, like *Maytenus ilicifolia* and *Maytenus aquifolium*, are currently used in Brazilian folk medicine against gastric ulcers and any gastric protection obtained may be explained, at least partly, by scavenging HOCl.

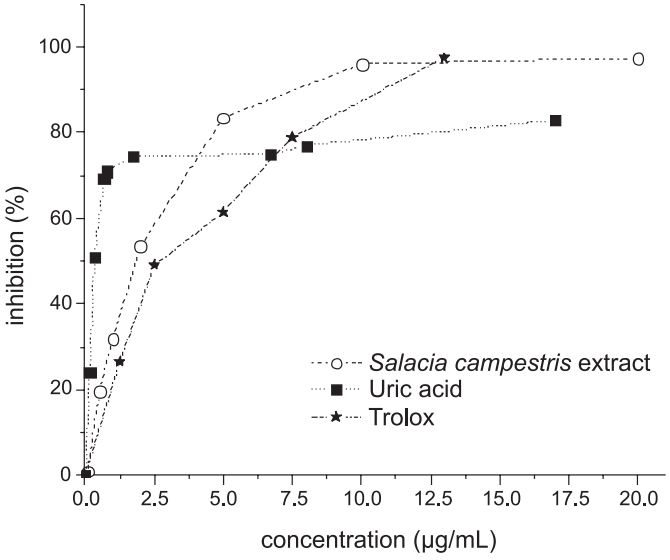

FIGURE 3 - HOCl (22 µM) Scavenger action by *S. campestris* extract, uric acid and trolox. The reactions were made in 50mM sodium phosphate buffer, pH 7.0, 25 ^{0}C, and incubated by 15 min.

ScEtOH action over superoxide anion was evaluated by a non-enzymatic method of generating superoxide by reacting phenazine methosulfate with NADH. The superoxide is stained by NBT. We used this system to evaluate if the samples tested are able to antagonize superoxide anion *in vitro*. It is important to note that in this method, NBT must be in excess to evaluate the real potential of samples to scavenge the superoxide anion. Any superoxide scavenger potential found for ScEtOH could be expected to contribute to a possible protective action in various tissues, including stomach. From Figure 4, it can be concluded that

S. campestris ethanol extract is much better than either uric acid or trolox at scavenging superoxide radical anion.

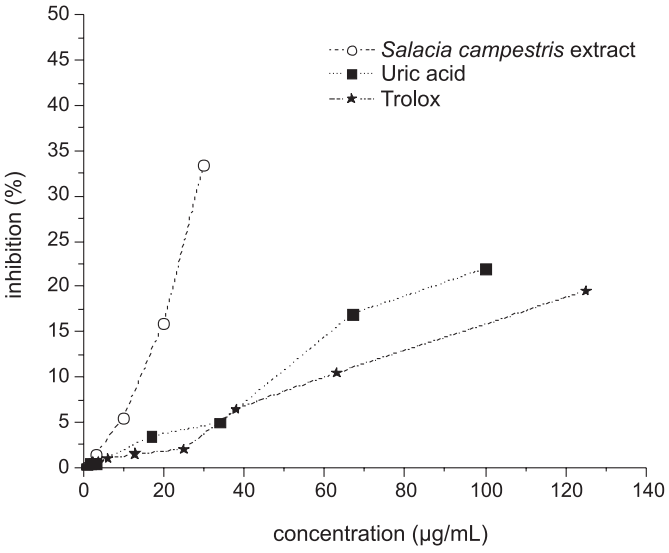

FIGURE 4 - Superoxide anion radical Scavenger action by *S. campestris* extract, uric acid and trolox. The reactions were made in 25 mM sodium pyrophosphate buffer, pH 8.3, 25^{0}C, and incubated by 90 s.

Recent studies have shown that reactive nitrogen intermediates, such as nitric oxide (NO$^{\bullet}$), peroxynitrite (ONOO$^{\bullet-}$) and nitrogen dioxide (NO_2), also play an important part in the inflammatory process and possibly in carcinogenesis (Yen *et al.*, 2001). We constructed an analytical curve with sodium nitrite to calculate the nitric oxide level in the sodium nitroprusside method described above. In this method, using sodium nitroprusside as a source of nitric oxide, control tubes (without scavengers) had absorbance values of 0.428±0.02 (n=40), representing about 21 µM NO$^{\bullet}$ (Figure 5). We evaluated the potential of trolox, uric acid and ScEtOH as nitric oxide scavengers (Figure 6). Note that trolox is unable to scavenge nitric oxide. The data show that ScEtOH and uric acid had similar scavenging action against the nitric oxide radical.

ScEtOH is effective over the evaluated systems - DPPH radical (obtained inhibition = 30%), ABTS$^{\bullet+}$ (IC_{50} = 1.8±0.8 µg/mL), HOCl (IC_{50} = 1.7 ± 0.1 µg/mL), $O_2^{\bullet-}$ (obtained inhibition = 32%), and NO$^{\bullet}$ (obtained inhibition = 18%). According to results previously published by Vellosa *et al.* (2007 B), ScEtOH have a similar potential to *Maytenus aquifolium* on scavenging these species (exception against ABTS$^{\bullet+}$) – DPPH radical (obtained inhibition = 36 %), ABTS$^{\bullet+}$ (IC_{50} = 3.6±0.3 µg/mL), HOCl (IC_{50} = 2.0±0.1 µg/mL), $O_2^{\bullet-}$ (obtained inhibition = 36 %), and NO$^{\bullet}$ (obtained inhibition = 18 %). In previous studies Carvalho *et al.* (2005) and Corsino *et al.* (2000) had isolated from *S. campestris* some quinonepethides triterpenoids that presented antioxidant properties that may be related to the results observed in this paper. However, due the hydrophobic nature of these compounds [(Carvalho *et al.* (2005) have extracted these compounds using CH_2Cl_2 as solvent, and Corsino *et al.* (2000) had used hexane], quinonemethides triterpenes must be at low levels in the evaluated ethanolic extract. The results observed must be explicated mainly by the hydrophilic flavonoids extracted by ethanol as solvent.

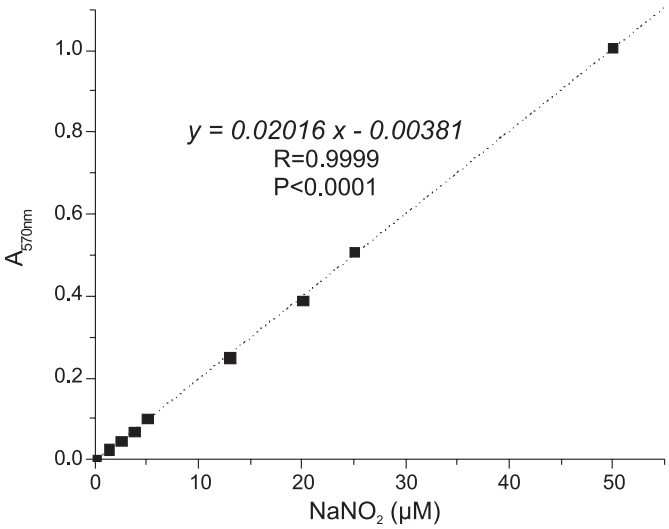

FIGURE 5 - Analytical curve of sodium nitrite developed by Griess Reagent. The reactions were made in 50 mM sodium phosphate buffer, pH 7.0, 25 ^{0}C, and incubated by 15 min.

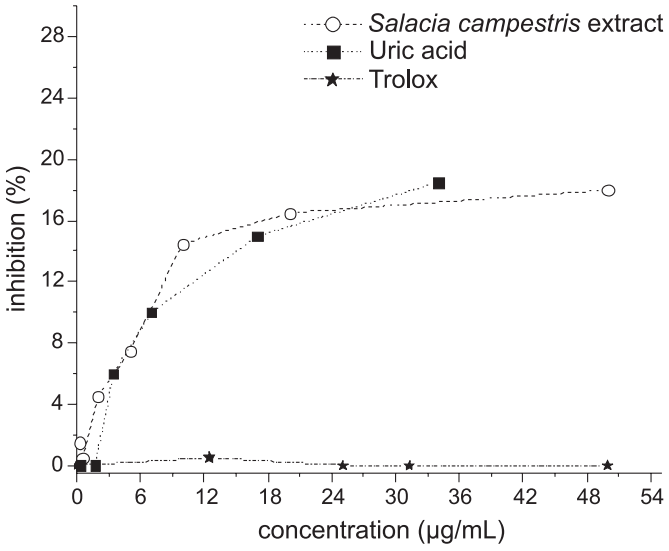

FIGURE 6 - Nitric oxide Scavenger action by *S. campestris* extract, uric acid and trolox. The reactions were made in 50 mM sodium phosphate buffer, pH 7.0, 25 ^{0}C, and incubated by 90 min before reaction with Griess Reagent.

Peroxidases are heme-containing enzymes that use

H_2O_2 to oxidize a variety of biomolecules and xenobiotics. The heme in the native enzyme is usually ferriprotoporphyrin IX, with four pyrrole nitrogens bound to the Fe(III). The fifth coordination position on the proximal side of the heme is usually occupied by the imidazole side chain of a histidine residue. The sixth coordination position is vacant in the native enzyme on the distal side of the heme. Plant peroxidases, e.g. horseradish peroxidase, consist of about 300 amino acids and a non-covalently bound heme, whereas mammalian peroxidases are much larger (576–738 amino acids) and the heme is covalently bound (O'Brien, 2000). In this paper, we evaluated the effect of quercetin, a powerful MPO inhibitor (Pincemail *et al.*, 1988), and ScEtOH on hemin (free heme from peroxidases) and HRP activity. The results are showed in Tables 1 and 2. At the highest concentration (10 µg/mL) ScEtOH inhibited guaiacol oxidation by hemin and by HRP. So, it is possible that the mechanism of peroxidase inhibition by ScEtOH is related to an action over the enzymes prosthetic group.

MPO is an important peroxidase from neutrophils that uses H_2O_2 and chloride ions to catalyze the production of the reactive and cytotoxic oxidant hypochlorous acid (Jerlich *et al.*, 2000). Effects of ScEtOH and quercetin on MPO inhibition were evaluated by guaiacol oxidation method. ScEtOH promoted a 50% inhibition at 8.9 µg/mL whereas quercetin (Pincemail *et al.*, 1988) inhibited by 50% at 1.35 µg/mL, both responses being clearly dose-dependent (Figure 7). Quercetin and ScEtOH were able to inhibit guaiacol oxidation by peroxidases (MPO and HRP) and their prostetic group, where quercetin was a more efficient inhibitor. The inhibition observed by the extract may be related to its flavonoids contends and further studies must clarify which flavonoids are able to act as MPO inhibitor and what would be their potential compared to quercetin, a well known flavonoid.

TABLE 1 - Guaiacol (5 mM)/H_2O_2 (2.5 mM) oxidation by hemin (3 µM) in presence and absence of *S. campestris* ethanolic extract or quercetin; v_0 values – mean±SD ($p<0.05$). The reactions were made in 50mM sodium phosphate buffer, pH 7.0, 25 °C, and incubated by 60 s

Sample	v_0 (1.10^{-4} s^{-1})		
Control (no sample)	54 ±7		
	10 µg/mL	1 µg/mL	0.1 µg/mL
S. campestris extract	23±2	56±7	51±1
quercetin	3±1	35±4	52±3

TABLE 2 – Guaiacol (2 mM)/H_2O_2 (0.1 mM) oxidation by HRP(7 nM) in presence and absence of *S. campestris* ethanolic extract or quercetin; v_0 values – mean±SD ($p<0.05$). The reactions were made in 50mM sodium phosphate buffer, pH 7.0, 37 °C, and incubated by 60 s

Sample	v_0 (1.10^{-4} s^{-1})		
Control (no sample)	144±10		
	10µg/mL	1 µg/mL	0.1 µg/mL
S. campestris extract	70±10	145±7	135±7
quercetin	15±2	129±2	138±9

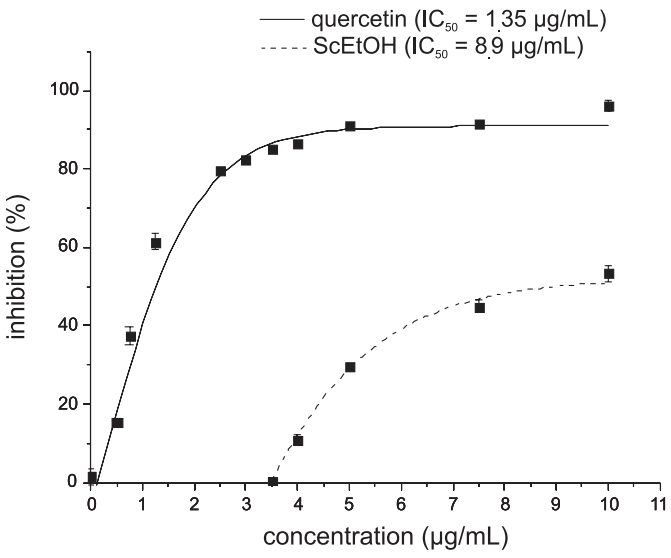

FIGURE 7 - Myeloperoxidase (75 mU) inhibition, by quercetin and ScEtOH, in a system with H_2O_2 (0.2 mM) and guaiacol (20 mM) The reactions were made in 50 mM sodium phosphate buffer, pH 7.0, 25 °C, and incubated by 60 s.

CONCLUSION

We demonstrated that *S. campestris* ethanol extract was able to scavenge various oxidant and radical species and inhibit myeloperoxidase, an enzyme involved in many physiological and pathological conditions. We conclude that ScEtOH is efficient against several species assayed. The results provide useful pharmacological information related to free radicals and oxidant species. It is possible that the use of this plant to treat certain pathologies could prevent or fight tissue damage when this involves an established oxidative stress. Of course, further studies are necessary, 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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