RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2015.282.292

Antioxidant Activity of some Medicinal Plant Extracts: Implications for Neuroprotection

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ABSTRACT

Background and Objective: This study was conducted in order to evaluate the effect of aqueous and ethanolic extracts of *Phyllanthus niruri*, *Uncaria tomentosa* and *Mentha pulegium* against Lipid Peroxidation (LP). Additionally, it was determined the ability of these plants to scavenge specific free radicals and the interaction with iron ions. **Methodology:** The LP, scavenging activity, total phenolics and flavonoids were determined by colorimetric methods, whereas HPLC analysis was used to characterize the phytoconstituents. **Results:** Extracts significantly prevented both basal or induced (by Fe^{2+}, malonate, sodium nitroprusside or ferrocyanide) LP. Accordingly, the extracts were better antioxidants at higher used concentrations, the effect of aqueous seems to be higher than ethanolic; the effect of plants were found to be *P. niruri*>*M. pulegium*>*U. tomentosa*; extracts were better antioxidants when used in rat brain homogenates, as compared to yolk phospholipids. Extracts also presented significant scavenging activity, prevented deoxyribose degradation and chelate Fe^{2+}. The HPLC analysis revealed that the extracts have different types and quantities of phytoconstituents. **Conclusion:** Altogether, presented results support the idea that antioxidant activities of extracts are related to the ability to scavenge specific free radicals and/or interaction with redox chemistry of iron ions. Thus, the broad range of antioxidant activity of these plants indicates the use with potential application to reduce LP associated to neurodegenerative disorders.

Key words: *Phyllanthus niruri*, *Uncaria tomentosa*, *Mentha pulegium*, scavenger, phenolic compounds, flavonoids

Pharmacologia 6 (7): 282-292, 2015

INTRODUCTION

Excessive Reactive Oxygen Species (ROS) production can overmatch cellular antioxidant defenses leading to a deleterious condition called Oxidative Stress (OS) which has been implicated in the initiation and progression of several human degenerative diseases via deleterious modification of DNA, protein and/or lipids[1,2]. Of particular importance, during last years it has been given a special attention to the role of OS in neurodegenerative disorders[3]. Accordingly, as compared to other organs, brain is characterized by its high oxygen consumption rate, high levels of fatty acids, reduced ability of cell regeneration and low levels of antioxidant enzymes[4]. Thus, pharmacological approach to resist OS seems to be a promising therapeutic strategy to counteract neurodegenerative disorders. In this context, studies have focused on the potential use of natural compounds in a variety of conditions associated with OS, with special emphasis on medicinal herbs (and/or the metabolites) that are traditionally used by population[5-8].

Indeed, it has been reported that the consumption of medicinal plant extracts may exert positive effects in a variety of *in vitro* and *in vivo* models of human disorders[5,9]. Accordingly, the diet supplies antioxidants that act by augmenting cellular defenses protecting the cell against damage caused by ROS[10,11]. Besides, the mechanism(s) involved in antioxidant activity of natural compounds remains to be further characterized. Thus, the purpose of this study was to evaluate the antioxidant activity (and subsequent implications for neuroprotection) of three different plant species, namely *Phyllanthus niruri* (Linn.), *Uncaria tomentosa* (Willd.) DC. and *Mentha pulegium*, frequently used worldwide in folk medicine, as an herbal tea, to treat many disorders.

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P. niruri (Linn.), *U. tomentosa* (Willd.) DC. and *M. pulegium* aqueous and ethanolic extracts, have been shown to have several pharmacological properties[12-22]. Nevertheless, few studies have evaluated the effects of aqueous extracts, prepared as a tea, from these plants. Accordingly, aqueous herbal extracts have attracted attention since they can be consumed in a daily basis as infusions. Additionally, the mechanism(s) involved in those therapeutic properties[12-22], especially on antioxidant effect from these plants, are not fully understood. Of particular importance, it could be emphasized that supporting scientific data, concerning the neuroprotective potential of these plants, especially from aqueous extracts, are still scarce.

So, considering the: (1) Importance of the OS (and the consequent lipid peroxidation) in various neurological disorders and (2) Presence of a number of compounds with antioxidant properties in the plant extracts; the aim of this study was to investigate the antioxidant capacity of the aqueous and ethanolic extracts of these plants, on lipid peroxidation induced by different pro-oxidants in homogenate of rat brain or yolk phospholipids. Additionally, in order to better understand the mechanism(s) by which extract could act, it was tested the hypothesis that the antioxidant capacity of the extracts, under *in vitro* conditions, could be due to the ability to scavenge Nitric Oxide (NO) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and/or due to the interaction with the redox chemistry of iron ions.

MATERIALS AND METHODS

Chemicals: Tris–HCl, thiobarbituric acid (TBA), 1'-1'diphenyl-2'picrylhydrazyl (DPPH), quercetin, gallic acid and deoxyribose were obtained from Sigma (St. Louis, MO, USA). All the other chemicals were commercial products of the highest purity grade available.

Extracts preparation: Plants marketed and distributed by Oly e Orty Ind. e Com. Ltda-Novo Hamburgo/RS/Brazil were obtained from local commercial sources. Accordingly, three independent batches were randomly purchased and used in this study. Ethanolic extracts were obtained from 0.9 g of aerial parts of dried plant material (*P. niruri*, *U. tomentosa* and *M. pulegium*). Plants were macerated and maintained in the dark for 7 days with 10 mL of solvent. After this, the extracts were evaporated to dryness under reduced pressure. Dry extracts were suspended in the same volume (10 mL) of distilled water. Aqueous extracts were prepared mimicking the traditional use by population-by infusion (10 min) in hot water (0.9/10, w/v; 96°C). Aqueous extracts were prepared immediately just before use.

Animals and tissue preparation: Male adult Swiss rats (weighing 250-300 g) were maintained in groups of 3-4 animals per cage. They had continuous access to food and water in a room with controlled temperature (22±3°C) and on a 12 h light/dark cycle with lights on at 7:00 am. The animals were maintained and used in accordance to the guidelines of the Committee on Care and Use of Experimental Animal Resources. Rats were euthanized by using sodium phenobarbital (100 mg kg^{-1} i.p.) and the encephalic tissue was rapidly dissected and placed on ice. Tissues were immediately homogenized in cold NaCl 0.9% (1/10, w/v) and used for the lipid peroxidation assay.

Lipid peroxidation assay in brain homogenates: Lipid peroxidation was determined by measuring thiobarbituric acid-reactive species (TBARS) as previously described[23], with slight modifications. In short, aliquots of the homogenate (25 mL) from brain were incubated for 60 min in a medium containing 10 mM Tris-HCl, pH 7.4 in the absence (control) or presence of plant extracts (10-1000 μg mL^{-1}); or extracts/Fe^{2+} (10 μmol L^{-1}); or extracts/malonate (4 mmol L^{-1}); or extracts/sodium nitroprusside (SNP) (4 mmol L^{-1}); or extracts/ferrocyanide (4 mmol L^{-1}). The mixtures were incubated at 37°C for 60 min. The reaction was stopped by addition of 0.5 mL of acetic acid buffer and lipid peroxidation products were measured by the addition of 0.5 mL of TBA 0.6% and 0.2 mL of SDS 8.1%. The color reaction was developed by incubating tubes in boiling water for 60 min. The data are expressed as percent of inhibition in relation to respective control (without plant extract). Where indicated, solutions of $FeSO_4$ were made just before use in distilled water.

Phospholipids preparation and TBARS assay: Production of TBARS from phospholipids were determined using the method previously described[23], adapted to phospholipids. Briefly, the egg yolk was weighed to 1 g and diluted to 100 mL with 100 mM Tris-HCl, pH 7.4 and used as a homogenate. The remaining procedure was the same as that mentioned for the tissue samples except that the color reaction was carried out without SDS by adding 0.6 mL of TBA and 0.6 mL of acetic acid (pH 3.4). After incubation (1 h in boiling water) 0.2 mL of SDS 8.1% was added. An

aliquot of the supernatant was taken and the absorbance was read at 532 nm in a spectrophotometer. The data are expressed as percent of inhibition in relation to respective control (without plant extract).

DPPH-scavenging activity assay: Antioxidant activity of the ethanolic and aqueous extracts was evaluated by monitoring the ability to quench the stable free radical DPPH, according to previously described method[24], with minor modifications. Each extract (final concentration at 10-1000 μg mL^{-1}) was mixed with 200 mL of 0.3 mM DPPH ethanol solution. Water (800 μL) plus 200 μL of DPPH was used as blank (control). The absorbance was measured at 518 nm after 30 min of reaction at room temperature. Relative activities were calculated from the calibration curve of L-ascorbic acid standard solutions working in the same experimental conditions.

Degradation of deoxyribose: The ability of the plant extracts to prevent Fe^{2+}/H_2O_2-induced decomposition of deoxyribose was carried out using a method previously described[25]. The data are expressed as percent of inhibition in relation to control (without plant extract).

Nitric Oxide (NO) scavenging assay: The scavenging of NO was assessed by incubating sodium nitroprusside (1 mM, in potassium buffer) with different extracts (aqueous or ethanolic) concentrations (10-1000 μg mL^{-1}) accordingly to previous described method[26]. The values were compared to a control (without extracts) to determine the percentage of inhibition of nitrite reaction with Griess reagent, depicted by the different extracts, as an index of the NO scavenging activity.

Fe^{2+} chelation assay: The ability of the aqueous or ethanolic extracts to chelate Fe^{2+} was determined using a method previously described, with a slight modification[27]. Briefly 150 μL of freshly prepared 1 mM $FeSO_4$ was added to the aqueous extract of studied plants (10-1000 μg mL^{-1}). The reaction mixture was incubated for 10 min before the addition of 13 μL of 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the spectrophotometer. The values were compared to a control (without extracts) to determine the percentage of chelation depicted by the different extracts.

Total phenolic and flavonoid compounds determination by colorimetric methods: The total phenol content was determined accordingly to previously described method[28]. The total phenolic content was expressed as milligrams of GA equivalents/g of dried plant extract.

Total flavonoid content was measured by the aluminum chloride colorimetric assay, accordingly previously described method[29]. The total flavonoid content was expressed as milligrams of quercetin equivalents/g of dried plant.

Quantification of compounds by HPLC-DAD: High Performance Liquid Chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (YL9100) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu YL9110 reciprocating pumps connected to an YL9101 degasser with an YL9150 integrator and YL9160 diode array detector.

The phenolic compound profiles were determined according to the following procedure: The extracts were analyzed using a reversed phase carried out under gradient conditions using Synergi Fusion-RP 80A column (4.6 mm×250 mm). The mobile phase was composed of water (pH = 3): Acetonitrile (5:95, v/v) in a gradient mode, until 35 min, in which the mobile phase was 100% acetonitrile. At 38 min water (pH-3): acetonitrile (5:95, v/v) was used again in isocratic mode, as a mobile phase, until 50 min. A flow rate of 0.8 mL min^{-1} was used, 20 μL of sample were injected and the wavelengths were 220 nm for gallic acid, benzoic acid, syringic and vanillic acid and rutin, 320 nm for caffeic acid, cumaric acid and ferulic acid and 368 nm for quercetin. Phenolic compounds were identified and quantified by comparing the retention time and UV-visible spectral data to known previously injected standards. The chromatography peaks were confirmed by comparing the retention time with those of reference standards and by DAD spectra. Calibration curve for gallic acid: Y = 330.61x - 77.697 (r = 0.9981); benzoic acid: Y = 292.22x-75.86 (r = 0.9926); rutin: Y = 75.008x-7.5152 (r = 0.9993); caffeic acid: Y = 236.39x-66.902 (r = 0.9943); syringic and vanillic acid: Y = 815.48x-14.686 (r = 0.9998); cumaric acid: Y = 352.51x-20.303 (r = 0.9983); ferulic acid: Y = 279.43x-3.2422 (r = 0.9998); quercetin: Y = 148.32x-42.629 (r = 0.9948). All chromatography operations were carried out at ambient temperature and in triplicate.

Statistical analysis: Data (Figures and Table 1-3) were statistically analyzed by one-way ANOVA, followed by Tukey's multiple range tests when appropriate and are

Table 1: Effect of aqueous extracts from studied plants on basal or induced TBARS production in rat brain homogenates

Plants and that extracts conc.	Pro-oxidants None	Iron	SNP	Ferrocyanide	Malonate
P. niruri					
Basal (no extract)	100.31±9.81	160.66±8.71	157.40±15.41	156.46±10.73	155.56±10.37
10 mg mL^{-1}	46.22±5.83*	73.50±8.99*	N.D	N.D	74.92±9.780*
100 mg mL^{-1}	47.00±6.82*	124.44±4.28	N.D	N.D	54.03±9.780*
1000 mg mL^{-1}	41.40±6.56*	41.12±8.95*	41.06±7.38*	61.83±10.42*	59.06±11.29*
U. tomentosa					
10 mg mL^{-1}	79.99±13.45	160.01±15.87	N.D	N.D	93.10±8.18*
100 mg mL^{-1}	87.73±9.87	134.93±20.43	N.D	N.D	75.62±18.29*
1000 mg mL^{-1}	66.93±7.95*	77.60±10.89*	35.52±6.94*	46.98±2.50*	57.23±4.57*
M. pulegium					
10 mg mL^{-1}	60.62±8.68	102.41±13.37*	N.D	N.D	102.10±6.27*
100 mg mL^{-1}	41.90±9.68*	91.82±8.71*	N.D	N.D	39.89±7.14*
1000 mg mL^{-1}	39.5±12.10*	63.80±11.23*	45.70±11.23*	51.17±5.91*	40.12±3.65*

Values are expressed % of control (control without extract or pro-oxidant agent was considered 100%), (n = 3-5), *From respective control by one-way ANOVA following by Tukey's test, N.D: Not determined

Table 2: Effect of ethanolic extracts from studied plants on basal or induced TBARS production in rat brain homogenates

Plants and that extracts conc.	Pro-oxidants None	Iron	SNP	Ferrocyanide	Malonate
P. niruri					
Basal (no extract)	100.16±4.160	149.40±8.120	171.11±18.85	154.91±20.88	150.29±16.01
10 mg mL^{-1}	72.30±8.770	121.02±11.46	N.D	N.D	151.75±8.070
100 mg mL^{-1}	62.05±8.390*	94.43±9.72*	N.D	N.D	138.78±0.790
1000 mg mL^{-1}	53.33±9.540*	50.93±9.93*	112.14±22.56	75.65±10.18*	42.73±7.750*
U. tomentosa					
10 mg mL^{-1}	87.86±8.240	118.97±9.880	N.D	N.D	147.97±4.210
100 mg mL^{-1}	64.95±11.22	98.63±12.45*	N.D	N.D	58.14±0.86*
1000 mg mL^{-1}	57.77±9.680	73.98±13.39*	64.60±12.21*	68.53±14.61*	46.56±7.65*
M. pulegium					
10 mg mL^{-1}	77.48±9.860	117.26±11.50	N.D	N.D	167.87±6.47
100 mg mL^{-1}	70.51±10.39	110.59±11.68	N.D	N.D	152.54±4.91
1000 mg mL^{-1}	60.07±7.260	102.39±10.48*	96.04±3.75*	108.43±33.15	81.42±1.80*

Values are expressed % of control (control without extract or pro-oxidant agent was considered 100%), (n = 3-5), *From respective control by one-way ANOVA following by Tukey's test, N.D: Not determined

Table 3: Effect of aqueous or ethanolic extracts from studied plants on basal or induced TBARS production in isolated phospholipids

Plants and that extracts conc.	Aqueous None	Iron	Ethanolic None	Iron
P. niruri				
Basal (no extract)	100.91±19.98	234.01±62.73	100.65±17.95	214.23±58.94
10 mg mL^{-1}	90.40±6.81	204.14±49.04	85.58±10.96	196.63±51.77
100 mg mL^{-1}	85.06±7.20	169.80±26.10	84.88±20.28	183.44±46.63
1000 mg mL^{-1}	73.18±11.80	90.54±9.91*	118.63±58.92	135.48±22.28
U. tomentosa				
10 mg mL^{-1}	84.15±7.91	200.44±47.28	108.42±27.27	187.69±37.99
100 mg mL^{-1}	72.70±12.28	146.12±12.50	85.46±10.67	160.68±33.16
1000 mg mL^{-1}	72.42±19.31	85.76±7.39*	94.48±29.08	99.58±25.74*
M. pulegium				
10 mg mL^{-1}	88.44±8.38	178.98±43.44	94.48±17.90	199.87±47.80
100 mg mL^{-1}	80.43±11.38	164.20±61.83	72.06±11.46	182.01±59.26
1000 mg mL^{-1}	92.31±20.41	90.29±11.06*	67.63±8.970	157.21±45.99

Values are expressed % of control (control without extract or pro-oxidant agent was considered 100%), (n = 3-5), *From respective control by one-way ANOVA following by Tukey's test

presented as Means±SEM values average from 3-4 independent experiments performed in duplicate. (*) indicates a significant difference at $p<0.05$ from respective control (without extract), by Tukey's multiple range test. Phenolic compounds and flavonoids (Table 4 and 5) were analyzed by Kruskal-Wallis test,

Table 4: Total phenolic compounds in aqueous and ethanolic extracts from studied plants

Total polyphenol content (nmol GA g plant)	Aqueous extract	Ethanolic extract
Phyllanthus niruri	46.64±1.32[a]	90.53±2.50[c]
Uncaria tomentosa	44.61±3.42[a]	53.98±2.47[a]
Mentha pulegium	24.10±0.39[b]	53.18±2.95[a]

Table 5: Total flavonoids contents in aqueous and ethanolic extracts from studied plants

Total flavonoids content (nmol quercetin/g plant)	Aqueous extract	Ethanolic extract
Phyllanthus niruri	31.76±1.00[a]	11.12±0.60[b]
Uncaria tomentosa	12.54±0.52[b]	9.67±0.20[b]
Mentha pulegium	10.46±0.20[b]	9.80±0.20[b]

followed by Dunn's multiple comparisons test where appropriate. These data are presented as Means±SEM values average from 3-4 independent experiments performed in triplicate. Different alphabets indicate statistical significance ($p<0.05$) among extracts.

RESULTS

Effects of *P. niruri, U. tomentosa* and *M. pulegium* extracts on basal or Induced TBARS Production in rat brain tissues: Aqueous (Table 1) and ethanolic (Table 2) extracts from *P. niruri*, *U. tomentosa* and *M. pulegium* significantly decreased both basal, Fe^{2+}, malonate, sodium nitroprusside (SNP) or ferrocyanide induced TBARS production, in rat brain tissue preparations. Summarizing these data, it was found that: (1) The efficacy of different extracts varies depending on the concentration (higher antioxidant activity seen at higher concentrations used), (2) The effect of aqueous>ethanolic; indeed, all aqueous extracts (at higher concentration) were able to significantly inhibit both basal or induced-TBARS production, whereas ethanolic extracts work better under induced-conditions and (3) The effect of plants were found to be *P. niruri* > *M. pulegium* > *U. tomentosa*.

Effects of *P. niruri*, *U. tomentosa* and *M. pulegium* extracts on basal or Induced TBARS Production in phospholipids: Both extracts from *U. tomentosa* decreased, in a similar way, Fe^{2+}-induced TBARS production in phospholipids, whereas only aqueous extracts from *P. niruri* and *M. pulegium* decreased Fe^{2+}-induced TBARS production at higher concentration (Table 3). In contrast, none of studied extracts exert significant effect on basal TBARS levels (Table 3).

Effects of *P. niruri*, *U. tomentosa* and *M. pulegium* extracts on DPPH-scavenging assay: Aqueous and ethanolic extracts obtained from *P. niruri* (Fig. 1a), *U. tomentosa* (Fig. 1b) and *M. pulegium* (Fig. 1c) significantly inhibited DPPH radical oxidation at higher

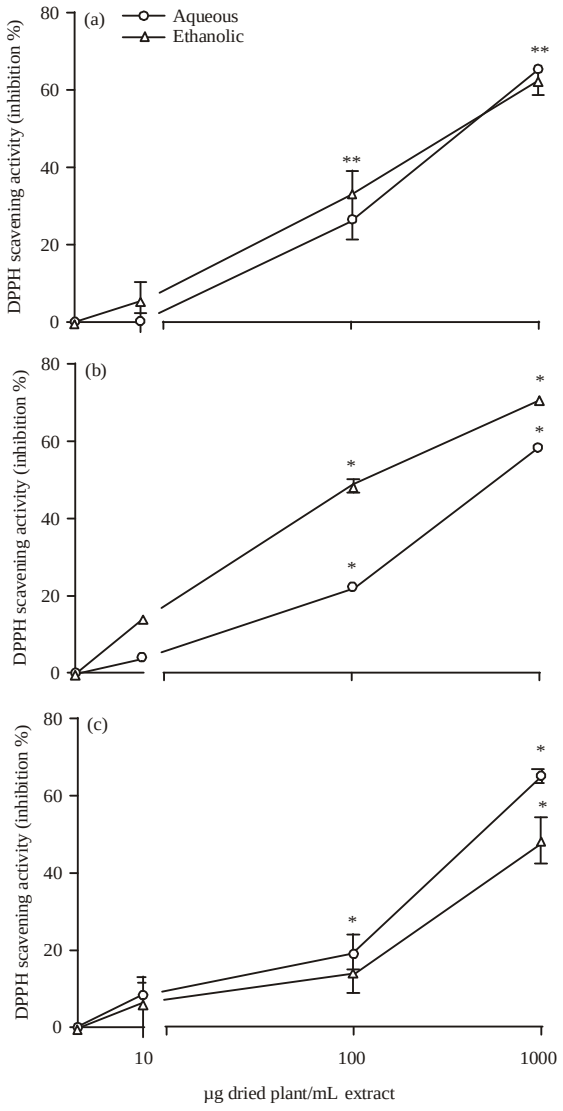

Fig. 1(a-c): Effects of aqueous and ethanolic extracts from (a) *P. niruri*, (b) *U. tomentosa* and (c) *M. pulegium* on DPPH-scavenging assay. The results are expressed as percentage of inhibition and ascorbic acid was used as a positive control

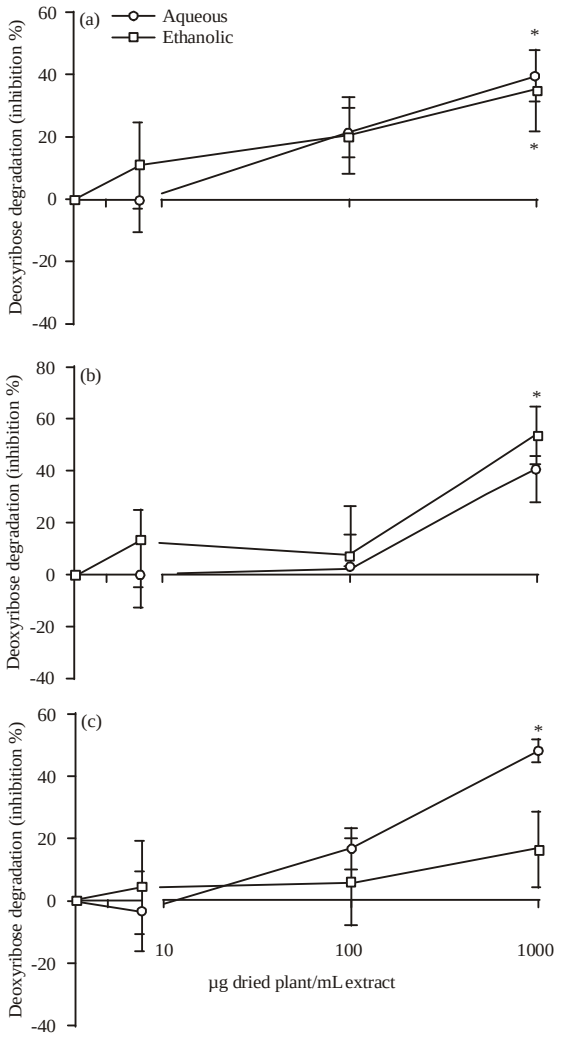

Fig. 2(a-c): Effects of aqueous and ethanolic extracts from (a) *P. niruri*, (b) *U. tomentosa* and (c) *M. pulegium* on deoxyribose degradation test

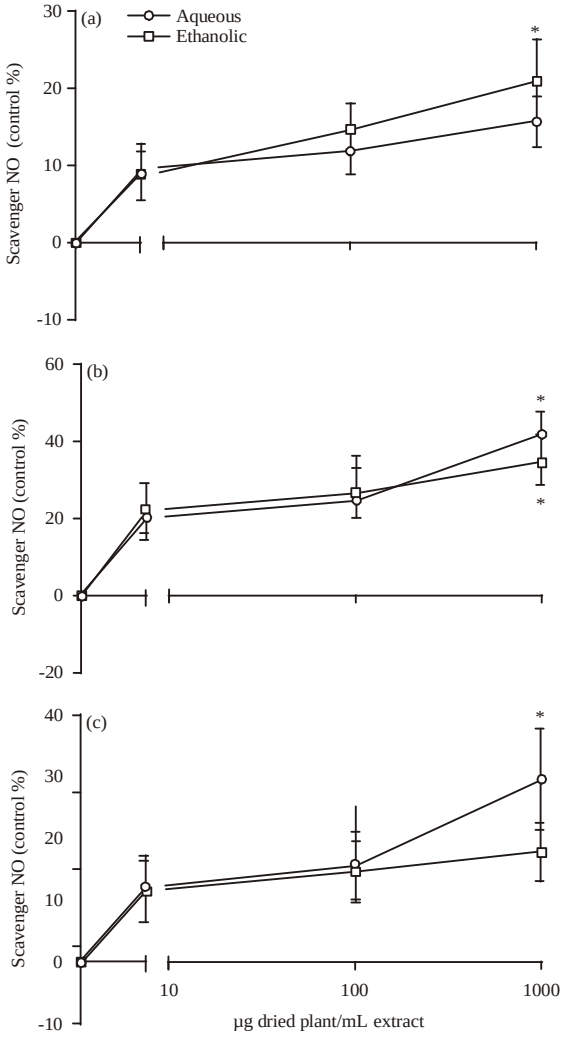

Fig. 3(a-c): Effects of aqueous and ethanolic extracts from (a) *P. niruri*, (b) *U. tomentosa* and (c) *M. pulegium* on NO-scavenging assay

concentrations (100-1000 µg mL^{-1}) with similar potency (reaching up to 60% of inhibition as compared to ascorbic acid).

Effects of *P. niruri, U. tomentosa* and *M. pulegium* extracts on deoxyribose degradation assay: *P. niruri* and *U. tomentosa* aqueous and ethanolic extracts promoted a significant inhibition of deoxyribose degradation with similar potency (30-50%, Fig. 2a and b, respectively) at 1000 µg mL^{-1}. Likewise, *M. pulegium* aqueous extracts also significantly inhibited deoxyribose degradation at 1000 µg mL^{-1} (40-50% of inhibition; Fig. 2c). Neither aqueous nor ethanolic extracts studied here were able to decompose H_2O_2 per se (i.e., catalase-like activity).

Effects of *P. niruri*, *U. tomentosa* and *M. pulegium* extracts on NO Scavenging Assay: *P. niruri* ethanolic (Fig. 3a) and *M. pulegium* aqueous (Fig. 3b) extracts promoted a slight (20-30%) but significant, scavenging of NO radical at 1000 µg mL^{-1} (Fig. 3c). Additionally, both extracts from *U. tomentosa* significantly scavenged NO radical at 1000 µg mL^{-1} (30-40%, Fig. 3c).

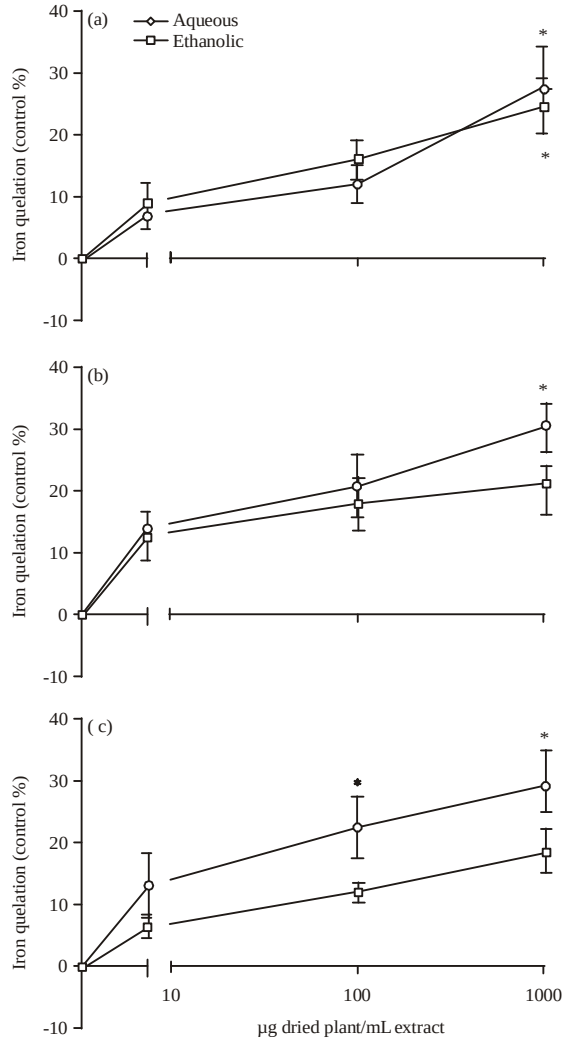

Fig. 4(a-c): Effects of aqueous and ethanolic extracts from (a) *P. niruri*, (b) *U. tomentosa* and (c) *M. pulegium* on iron chelation assay

Effects of *P. niruri*, *U. tomentosa* and *M. pulegium* extracts on Fe^{2+} Chelation: *P. niruri* aqueous and ethanolic extracts presented a slight but significant Fe^{2+} chelation ability at 1000 µg mL^{-1} (Fig. 4a, 25%). Similarly, aqueous extracts from both *U. tomentosa* and *M. pulegium* presented significant Fe^{2+} chelation ability (Fig. 4b and c, respectively).

Total Phenolic and Flavonoids Compounds in Studied Extracts: The amount of total phenolic compounds for *P. niruri* and *M. pulegium* was in the following order: Ethanolic >aqueous extracts. However, in *U. tomentosa* extracts (aqueous and ethanolic) the total phenolic content was found to be similar (Table 4).

In contrast, the amount of flavonoids compounds for *P. niruri* was found to be aqueous>ethanolic. Nevertheless, *U. tomentosa* and *M. pulegium* flavonoid content was found to be similar in both extracts (Table 5).

The HPLC fingerprinting of different aqueous and/or ethanolic extracts revealed the presence of the gallic acid, benzoic acid, rutin and syringic and vanillic acid at different concentrations (Table 6). However, caffeic acid, ferulic acid, cumaric acid and quercetin were not found on these extracts.

DISCUSSION

Present data clearly supports the notion that the extracts of three plants included in this study have potential to exert antioxidant activity under presented experimental conditions. Thus, it could be suggested that both three plants could be considered as effective agents in the prevention of various neurological disorders, where lipid peroxidation and/or OS are involved. Of particular importance, the findings concerning the effect of *U. tomentosa* aqueous extract are in accordance to previous data, where it was found to present neuroprotective effect against 6-OHDA induced cell damage[22]. However, this study seems to be the first report regarding the putative use of *P. niruri* and *M. pulegium* aqueous extracts, to prevent either basal or induced, by various agents, lipid peroxidation in rat brain homogenates. Additionally, presented data confirms the initial hypothesis that the antioxidant capacity of these plants could be related to the ability to scavenge specific free radicals. Moreover, evidence was found that the extracts could chelate iron ions (Fe^{2+}), potentially interfering with iron-driven free radical generation and exerting antioxidant activity. This conclusion is further supported by the protective effect found in the deoxyribose degradation assay.

Phenolic compounds which include flavonoids, are one of the largest and most ubiquitous groups of plant metabolites and there is current interest in the antioxidant activity of this class of phytochemicals[30, 31]. Indeed, flavonoids present free radical scavenging properties by acting as hydrogen-donating molecules via., the phenolic hydrogen, in addition to other chemical characteristics such as the lower reduction potentials than some free radicals[32]. Taking into account presented data, it could be speculated that phenolic compounds which include the flavonoids, present in the plant extracts (Table 4-6), are the putative candidates by

Table 6: Composition of studied plant extracts

	Phyllanthus niruri (mg L^{-1})		*Uncaria tomentosa* (mg L^{-1})		*Mentha pulegium* (mg L^{-1})	
Compounds	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
Gallic acid	297.00	1.760	73.02	35.8	N.D	N.D
Benzoic acid	2.120	0.709	N.D	N.D	0.931	2.929
Rutin	9.398	4.359	N.D	N.D	60.742	11.758
Syringic and vanillic acid	9.364	0.114	N.D	N.D	N.D	11.191

Results are expressed as mean of three different determinations, N.D: Not detected

exerting the antioxidant effect reported here. However, it is noteworthy that the composition of extracts, in terms of the type of phenolic and/or flavonoids presents and not just the amount, is responsible for the reported effects. Indeed, data present on Table 6, shows that the type and quantity of phytochemicals found here, varied among different plant extracts making impossible to point, based on these data which is/are the major contributor(s) by the presented results. Thus, it could be speculated that the individual and synergistic bioactivities of compounds with strong antioxidant properties which are present in the plant extracts (Table 6 and others that were not identified and/or found under used assay conditions), would have contributed to the observed antioxidant effects.

Accordingly, it was found that both extracts (aqueous and ethanolic) obtained from *P. niruri, U. tomentosa* and *M. pulegium* exhibited significant DPPH scavenging activity (Fig. 1), indicating the potential use of these plant extracts as scavengers[9]. Moreover, it was found that the plant extracts significantly inhibited deoxyribose degradation (Fig. 2). Thus, considering that these plant extracts do not present "Catalase-like" activity (i.e., do not interact with H_2O_2), it could be suggested two putative mechanisms to explain presented data: (1) That these extracts are able to interact, at least in part, with hydroxyl radical and (2) That these extracts interfere with the iron-driving free radical generation. Regarding the first putative mechanism which is supported by data presented on Fig. 2, the degradation of deoxyribose is supposed to be resultant of attack of the Fenton-reaction generated hydroxyl radicals[33,34]. Therefore, the antioxidant effect of plant extracts could be understood as a competitive reaction of deoxyribose and the plant constituents, with the reactive hydroxyl radicals[33,34].

Concerning the second plausible mechanism and in a non exclusive way, it could be suggested that the mixture of plant metabolites present in the extracts interfere with the redox chemistry of iron ions (Fig. 4) which, in turn, could contribute to the antioxidant activity in biological assays presented here (Table 1-3). Indeed, many plant metabolites present functional groups (unsaturated bonds, nitrogen and oxygen) that are important for the complexation of iron[35]. Thus, phenolic compounds have been reported to chelate Fe^{2+} given the hydroxyl and carboxyl moieties found in the chemical structures and also due to nucleophilicity exerted by the aromatic ring system[32]. As a result of iron chelation, interference on iron-driving free radical generation is believed to occur, thus interfering in the occurrence of the Fenton reaction[32,36].

Additionally, the antioxidant activity, against basal or induced-TBARS production, confirms the biological significance of data presented here. Indeed, a neuroprotective effect of other plant extracts and/or from the major constituents, under different experimental conditions was previously reported[37-41]. Taking into account this, it could be speculated that the extracts studied here could potentially prevent damage to lipids in brain tissue under different conditions (i.e., iron overload and/or during energetic deficit conditions, such as during stroke and ischemia/reperfusion). However, the most evident effect of extracts, under presented experimental conditions, was in the assays performed with rat brain tissue, as compared to assay with phospholipids. Considering this result, it could be speculated that an enzymatic alteration in the plant compounds might occur before the antioxidant activity in the TBARS assay. In fact, phospholipids are a system devoid of enzymes, as compared to brain homogenates. Thus, these results suggest that the metabolite(s) of plants are, potentially, better antioxidants than the non-metabolized phytochemicals. Anyway, accordingly to Albarracin *et al*.[42] more studies are necessary to better understand the real significance of this to biological systems, given that there is little evidence that these compounds and/or the metabolites, can cross blood brain barrier to exert neuroprotective effect[42]. Despite of this, several reports showing the neuroprotective effect of natural compounds[37-41].

Finally, the plant extracts also presented NO-scavenging activity (Fig. 3). Accordingly, presented data, concerning *P. niruri* effect against NO radical, reinforces the previous study[43]. On the other hand, this data clearly shows, for the first time, that *U. tomentosa*

and *M. pulegium* also presented NO-scavenging activity. Of particular importance, inhibition of NO radical is also considered a measurement of antioxidant activity[44], once antioxidants inhibit nitrite formation by directly competing with oxygen[44,45].

Thus, the broad range of antioxidant activity, evaluated here by the effect against different pro-oxidant agents, of studied plants indicates the potential use (especially from aqueous extract, prepared as an herbal tea) with potential application to reduce lipid peroxidation and consequent health benefits, with special emphasis on neurodegenerative disorders. Of particular importance, it could be supposed that positive effects offered by these plant extracts suggest that they may be useful in the treatment of various neurological disorders resulting from iron overload (mimicked here by Fe^{2+}, SNP or sodium nitroprusside addition) and/or metabolic deficit (mimicked here by malonate-a classical inhibitor of mitochondrial complex II-addition). Finally, these data supports the notion that the antioxidant activity of these plants could be due to the ability to scavenge specific free radicals and/or due to the interaction with redox chemistry of iron ions. Meanwhile, because of the complexity of pathways involved in iron-driving free radicals generation, this explanation is plausible but certainly not exclusive.

ACKNOWLEDGMENT

The authors are grateful to FAPERGS, CAPES, CNPq, FINEP, INCT-EN and UNIPAMPA. Additional support was given by CNPq/FAPERGS/DECIT/SCTIE-MS/PRONEM No. 11/2029-1. TT and CAOG are grateful to CAPES and CNPq for the scholarship.

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