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ORIGINAL PAPER

Antioxidant Effects of Different Extracts from *Melissa officinalis, Matricaria recutita* and *Cymbopogon citratus*

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Abstract Considering the important role of oxidative stress in the pathogenesis of several neurological diseases, and the growing evidence of the presence of compounds with antioxidant properties in the plant extracts, the aim of the present study was to investigate the antioxidant capacity of three plants used in Brazil to treat neurological disorders: *Melissa officinalis, Matricaria recutita* and *Cymbopogon citratus.* The antioxidant effect of phenolic compounds commonly found in plant extracts, namely, quercetin, gallic acid, quercitrin and rutin was also examined for comparative purposes. Cerebral lipid peroxidation (assessed by TBARS) was induced by iron sulfate (10 μM), sodium nitroprusside (5 μM) or 3-nitropropionic acid (2 mM). Free radical scavenger properties and the chemical composition of plant extracts were assessed by 1′-1′ Diphenyl-2′ picrylhydrazyl (DPPH) method and by Thin Layer Chromatography (TLC), respectively. *M. officinalis* aqueous extract caused the highest decrease in TBARS production induced by all tested pro-oxidants. In the DPPH assay, *M. officinalis* presented also the best antioxidant effect, but, in this case, the antioxidant potencies were similar for the aqueous, methanolic and ethanolic extracts. Among the purified compounds, quercetin had the highest antioxidant activity followed by gallic acid, quercitrin and rutin. In this work, we have demonstrated that the plant extracts could protect against oxidative damage induced by various pro-oxidant agents that induce lipid peroxidation by different process. Thus, plant extracts could inhibit the generation of early chemical reactive species that subsequently initiate lipid peroxidation or, alternatively, they could block a common final pathway in the process of polyunsaturated fatty acids peroxidation. Our study indicates that *M. officinalis* could be considered an effective agent in the prevention of various neurological diseases associated with oxidative stress.

Keywords Oxidative stress · Iron sulfate · 3-Nitropropionic acid · Sodium nitroprusside · Medicinal plants

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Introduction

Reactive oxygen species (ROS) are generated by normal metabolic processes in all organisms utilizing oxygen [1–3]. However, excessive ROS production can overcome cellular antioxidant defenses and can lead to a condition termed oxidative stress. Of particular importance, oxidative stress has been implicated in the installation and progression of several degenerative diseases, via either DNA mutation, protein oxidation and/or lipid peroxidation [3–6].

Literature data have given a special attention to the role of ROS and oxidative stress in chronic neurodegenerative

disorders such as Parkinson's and Alzheimer's diseases [7, 8]. In this context, several studies have focused in the potential use of natural and synthetic antioxidant compounds in a variety of in vitro and in vivo models of human pathologies, including neurotoxicity models [9–13].

Medicinal plants have been traditionally used in the treatment of several human diseases and their pharmacological and therapeutic properties have been attributed to different chemical constituents isolated from their crude extracts. Of particular importance, chemical constituents with antioxidant activity can be found at high concentrations in plants and can be responsible for their preventive effects in various degenerative diseases, including cancer, neurological and cardiovascular diseases [14–27]. Thus, the antioxidant properties of plants have a full range of perspective applications in human healthcare [2]. Interestingly, literature data have indicated that the pharmacological properties of crude extracts of plants can be lost after isolation of specific compounds, indicating that part of their pharmacological properties can be related to a combination of different classes of compounds [28, 29].

Lemon balm, *Melissa officinalis* L. (Lamiaceae) *(M. officinalis)* is widely used as herbal tea to treat or to relieve nervous disturbance of sleep and functional gastrointestinal disorders. Of particular importance, some studies have demonstrated antitumoral and neuroprotective effects of *M. officinalis* [30–33]. *Cymbopogon citratus* (DC) Stapf (Gramineae) *(C. citratus)* is an herb worldwide known as lemongrass. The tea made from its leaves is popularly used as antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative [34]. However, the mechanisms involved in its pharmacological properties are not well understood. *Matricaria recutita* L. (Asteraceae) *(M. recutita)*, particularly the dried flower heads of the plant, is widely used in traditional and herbal medicine because of its anti-inflammatory, spasmolytic, antipeptic, sedative, antibacterial and antifungal properties [35–37]. Nonetheless, the mechanisms involved in the therapeutic properties of these plants are still not elucidated.

In this context, considering the importance of the oxidative stress in the pathogenesis of various diseases, including those related to the central nervous system and the presence of a number of compounds with antioxidant properties in the plant extracts, the aim of the present study was to investigate, in a comparative way, the antioxidant capacity of the three popularly worldwide used plants on the oxidative stress induced by different agents in brain of rats. We have also investigated the effect of purified compounds, namely, quercetin, quercitrin, gallic acid and rutin. They are commonly found in plant extracts and could be involved in the antioxidant activity of plant extracts against in vitro iron sulfate-, sodium nitroprusside- and nitropropionic acid- induced cerebral lipid peroxidation.

Experimental Procedure

Chemicals

Tris–HCl, thiobarbituric acid (TBA), 3- nitropropionic acid (3-NPA), 1′-1′ diphenyl-2′ picrylhydrazyl (DPPH), rutin, quercetin, gallic acid and malonaldehyde bis- (dimethyl acetal) (MDA) were obtained from Sigma (St. Louis, MO, USA). Sodium nitroprusside (SNP) was obtained from Merck (Darmstadt, Germany). Iron sulfate (Fe_2SO_4), ascorbic acid, chloridric and acetic acid were obtained from Merck (Rio de Janeiro, RJ, Brazil). Quercitrin was isolated from *Solidago microglossa* D.C. and the purity of the isolated compound was 99.3% [38].

Extract Preparation

The plants were obtained from commercial sources. Ethanolic and methanolic extracts were obtained from 5 g of dried plant material (leaves of *C. citratus*, aerial parts of *M. officinalis* and flowers of *M. recutita*). These parts of the plants were macerated in the dark for 7 days with 50 ml of methanol or ethanol. After this, the extracts were evaporated to dryness under reduced pressure. The dry extracts were suspended in the same solvent. The aqueous extracts were obtained by infusion in hot water and they were prepared just before use.

Animals

Male Wistar rats (3.0–3.5 months of age and weighing 270–320 g) were maintained groups of 3–4 rats 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 a.m. The animals were maintained and used in accordance to the guidelines of the Brazilian Association for Laboratory Animal Science (COBEA).

Tissue Preparation

Rats were killed and the encephalic tissue was rapidly dissected and placed on ice. Tissues were immediately homogenized in cold 10 mM Tris–HCl, pH 7.5 (1/10, w/v). The homogenate was centrifuged for 10 min at 4,000 × g to yield a pellet that was discarded and a low-speed supernatant (S1) that was used for the TBARS assay [39].

TBARS

An aliquot of 100 μl of S1 was incubated for 1 h at 37°C with freshly prepared Fe_2SO_4 (10 μM), SNP (5 μM) or 3-NPA (2 mM) in the presence or absence of plant extracts

Table 1 Tested concentrations in the TBARS assay for each plant extract and isolated compounds

Plant extracts or isolated compounds	Tested concentrations (μg/ml)
M. officinalis aqueous extract	83.3–1666.7
M. officinalis methanolic extract	97.143–914.2
M. officinalis ethanolic extract	194.3–1828.6
M. recutita aqueous extract	83.3–1666.7
M. recutita methanolic extract	133.3–1255
M. recutita ethanolic extract	183.8–1729.7
C. citratus aqueous extract	83.3–1666.7
C. citratus methanolic extract	133.3–1255
C. citratus ethanolic extract	183.8–1729.7
Quercetin	0.015–2
Quercitrin	0.5–25
Gallic Acid	0.5–25
Rutin	5–25

or purified quercetin, gallic acid, quercitrin and rutin. Then, TBARS production was determined as described by Ohkawa et al. [40] and Puntel et al. [39]. The extracts and purified compounds were tested in the range indicated in Table 1. Ethanol and methanol had no effect in TBARS production. Indeed the levels of TBARS production in the presence of water, ethanol and methanol were in the range indicated in Table 2.

Radical-Scavenging Activity-DPPH Assay

The antioxidant activity of the extracts was evaluated by monitoring their ability in quenching the stable free radical DPPH, according Choi et al. with minor modifications [41]. Free radical scavenging capacity (FRSC) of plant extracts was calculated as their IC_{50} values (the concentration necessary to inhibit 50% radical formation), using the method of Dixon and Web [19]. Six different ethanol dilutions of each extract (7.8, 15.6, 31.2, 62.5, 125 and 250 μg/ml) were mixed with 1.0 ml of a 0.3 mM DPPH ethanol solution. Ethanol (1.0 ml) plus plant extract solution was used as a blank. The absorbance was measured at 518 nm after 30 min of reaction at room temperature. DPPH was prepared daily and protected from light. Relative activities were calculated from the calibration curve of L-ascorbic acid standard solutions working in the same experimental conditions. Scavenging or inhibitory capacity in percent (IC%) was calculated using the equation:

$$IC\% = 100 - \left[\left(Abs_{sample} - Abs_{blank}\right) \times 100/Abs_{control}\right]$$

where Abs_{sample} is the absorbance obtained in the presence of different extract concentrations and $Abs_{control}$ is that obtained in the absence of extracts. Tests were carried out in triplicate.

Total Phenolic Compounds Determination

The total phenol content was determined by mixing the extracts with 1.25 ml 10% Folin-Ciocalteu's reagent (v/v) which was followed by the addition of 1.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated at 45°C for 15 min, and the absorbance was measured at 765 nm. Gallic acid (GA) was used as standard for phenolic compounds [42].

TLC Analysis

Concentrated extracts were chromatographed on silica gel TLC plates. Mixtures of hexane: acetone (9:1), dichloromethane: ethanol (9:1), hexane: ethyl ether (7:3), ethyl acetate: ethanol: water (77:15:8), ethyl acetate: formic acid: water (65:15:20) and *n*-butanol: acetic acid: water (40:10:50) were used as eluents. Sitosterol, sinapic acid, quercetin and rutin were used as standard compounds. After elution, the TLCs were observed under UV light at

Table 2 TBARS levels in the presence of different pro-oxidants and distinct solvents (water, methanol or ethanol)

Plants	Extractor solvent	Pro-oxidants		
		Iron	SNP	3-NPA
M. officinalis	Water	673.4 ± 26.5	449.3 ± 31.9	298.4 ± 29.6
	Ethanol	740.6 ± 13.1	410.1 ± 52.5	269.2 ± 20.1
	Methanol	741.6 ± 38.8	447.9 ± 40.4	268 ± 17.1
M. recutita	Water	750 ± 24.7	569.7 ± 47.7	316.7 ± 21.1
	Ethanol	826 ± 116.9	569.9 ± 62.2	317.3 ± 66.9
	Methanol	819 ± 31.7	516.6 ± 127.9	289 ± 30.1
C. citratus	Water	711.7 ± 29.9	416.1 ± 34.7	375.8 ± 33.5
	Ethanol	776.5 ± 40.9	418.9 ± 47.1	341.5 ± 30.6
	Methanol	778.5 ± 21.6	461.7 ± 92.5	375.6 ± 98.6

254 and 366 nm. Afterwards, the compounds were detected by anisaldehyde sulphuric acid, oxaloboric solution and phosphomolibdic acid. It was also carried out a bidimensional TLC to confirm the presence of rutin in small amounts in the aqueous extract from *C. citratus*. In this case, the eluting solvent used were ethyl acetate: formic acid: water (80:8:12) two runs, in both directions [43, 44].

Statistical Analysis

Data from TBARS and DPPH were statistically analyzed by one-way ANOVA, followed by Duncan's multiple range tests when appropriated. Data from IC_{50} and Phenolic compounds were analyzed by *t*-test. When these data did not present variance homogeneity, they were log transformed. The results were considered statistically significant for $P < 0.05$.

Results

Effects of *Melissa officinalis, Matricaria recutita* and *Cymbopogon citratus* on TBARS Production Induced by 10 μM of Iron Sulfate

Aqueous, methanolic and ethanolic extracts obtained from *M. officinalis* (Fig. 1a), *M. recutita* (Fig. 1b) and *C. citratus* (Fig. 1c) significantly inhibited iron-induced TBARS production in brain preparations (for all plants and extracts *P* values were between 0.001 and 0.01). However, the inhibitory potency of the different types of extracts varied from plant to plant. For *M. officinalis* the potency order was aqueous > methanolic > ethanolic extracts (Fig. 1a; Table 3, $P < 0.01$). For *M. recutita,* the order was methanolic > aqueous and ethanolic (Fig. 1b; Table 3, $P < 0.01$), whereas for *C. citratus* the potency order was methanolic > ethanolic > aqueous (Fig. 1c; Table 3, $P < 0.01$).

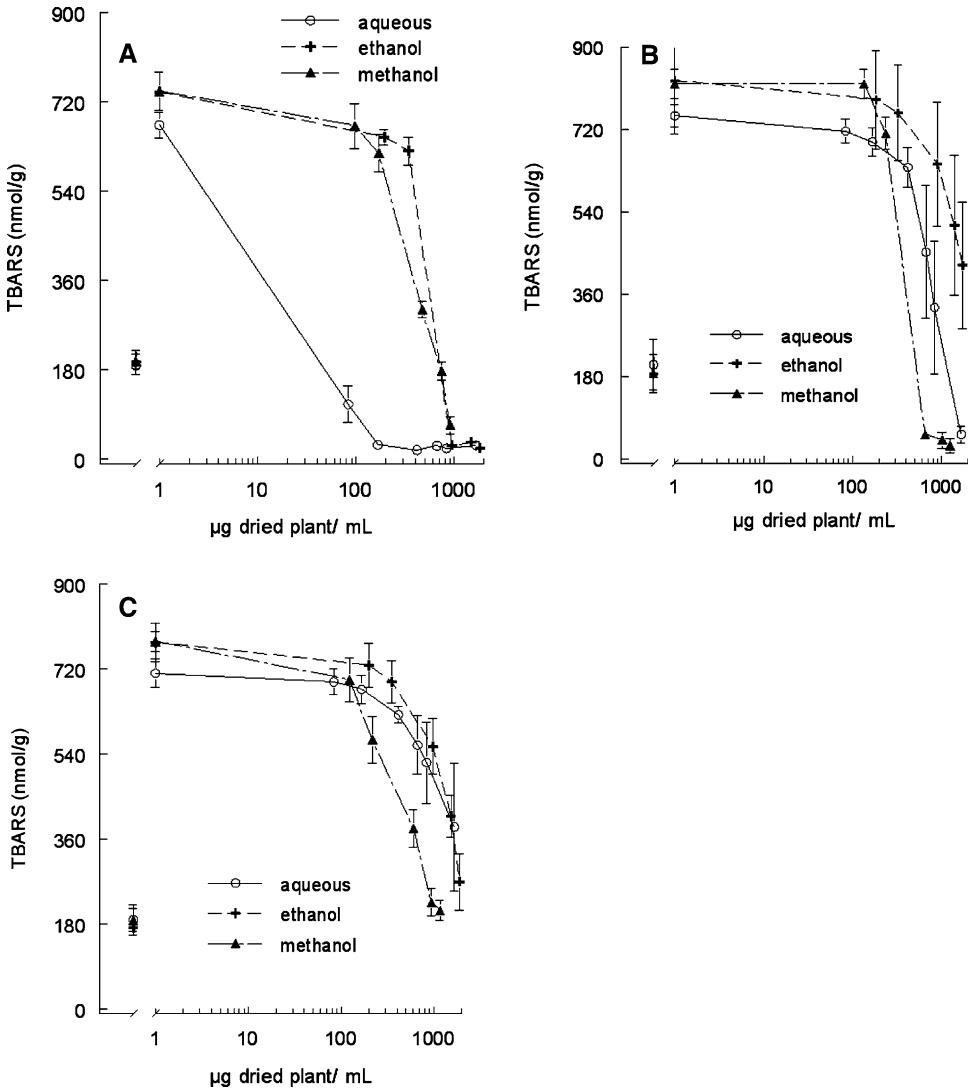

Fig. 1 Effects of different concentrations of aqueous, ethanolic and methanolic extracts from **a** *M. officinalis*, **b** *M. recutita* and **c** *C. citratus* on Iron (10 μM)-induced TBARS production in brain homogenates. The homogenates were incubated for 1 h with Iron and the plant extracts or without (basal). Data show means ± SEM values average from 3 to 4 independent experiments performed in duplicate

Table 3 IC_{50} (μg/ml) values for inhibition by plant extracts of TBARS production induced by different pro-oxidants in brain preparations

Plants	Extractor solvent	Pro-oxidants		
		Iron	SNP	3-NPA
M. officinalis	Water	15.67 ± 2.03^{a}	11 ± 0.6^{a}	77.4 ± 13.1^{a}
	Ethanol	568.5 ± 10.4^{b}	186.5 ± 51.3^{c}	512.4 ± 103.9^{c}
	Methanol	483 ± 25.5^{c}	22.3 ± 1.9^{b}	210.9 ± 24^{b}
M. recutita	Water	848.9 ± 169.8^{a}	58.4 ± 4.7^{a}	202 ± 31.5^{a}
	Ethanol	1874.3 ± 691^{a}	826.3 ± 70.3^{c}	1107.4 ± 49.4^{c}
	Methanol	415 ± 14.2^{b}	299.2 ± 8.1^{b}	590.9 ± 25.5^{b}
C. citratus	Water	2518.5 ± 913.8^{c}	476.5 ± 200.2^{a}	813.4 ± 236.9^{b}
	Ethanol	1549.9 ± 124.9^{b}	208.8 ± 28.2^{a}	1270.3 ± 101.9^{b}
	Methanol	535.8 ± 49.2^{a}	313.5 ± 8.8^{a}	355 ± 39.2^{a}

Different alphabets indicate statistical significance among different extracts of the same plant against the same pro-oxidant

Effects of *Melissa officinalis, Matricaria recutita* and *Cymbopogon citratus* on TBARS Production Induced by 5 μM of Sodium Nitroprusside (SNP)

Aqueous, methanolic and ethanolic extracts obtained from *M. officinalis* (Fig. 2a), *M. recutita* (Fig. 2b) and *C. citratus* (Fig. 2c) inhibited significantly SNP-induced TBARS production in brain preparations (for all plants and extracts P values were between 0.001 and 0.006). However, for *M. officinalis* and *M. recutita* the inhibitory potency of the different types of extracts varied in the following order: aqueous > methanolic > ethanolic extracts (Fig. 2a and b; Table 3, $P < 0.01$).

Effects of *Melissa officinalis, Matricaria recutita* and *Cymbopogon citratus* on TBARS Production Induced by 2 mM of 3-Nitropropionic Acid (3-NPA)

Aqueous, methanolic and ethanolic extracts obtained from *M. officinalis* (Fig. 3a), *M. recutita* (Fig. 3b) and *C. citratus* (Fig. 3c) inhibited 3-NPA-induced TBARS production in brain (for all plants and extracts P values were between 0.001 and 0.003). However, for *M. officinalis* and *M. recutita,* the inhibitory potency of the different types of extracts varied in the following order: aqueous > methanolic > ethanolic extracts (Fig. 3a and b; Table 3), whereas for *C. citratus* the potency order was methanolic > aqueous and ethanolic extracts (Fig. 3c; Table 3, $P < 0.01$).

DPPH Radical-Scavenging Activity of *Melissa officinalis, Matricaria recutita* and *Cymbopogon citratus*

M. officinalis aqueous, methanolic and ethanolic extracts promoted an inhibition of DPPH radical with similar potency (Fig. 4a; Table 4, $P < 0.01$). The inhibitory potency of DPPH radical by different extracts of *M.recutita* was in the following order: methanol > ethanol > water (Fig. 4b; Table 4, $P < 0.01$). *C. citratus* methanolic and ethanolic extracts promoted an inhibition of DPPH radical with similar potency, which was higher than that obtained with aqueous extract (Fig. 4c; Table 4, $P < 0.01$).

Total Phenolic Compounds Determination

The amount of phenolic compounds for *M. officinalis* and *M. recutita* was in the following order: aqueous > methanolic > ethanolic extracts (P values were between 0.001 and 0.01). However, for *C. citratus*, the order was ethanolic > aqueous > methanolic extracts (Table 5, P values were between 0.001 and 0.05).

Effects of Quercetin, Gallic Acid, Quercitrin and Rutin on TBARS Production Induced by 10 μM of Iron Sulfate, 5 μM of Sodium Nitroprusside (SNP) or 2 mM of 3-Nitropropionic Acid (3-NPA)

Iron, SNP and 3-NPA-induced TBARS production in brain preparations was significantly decreased by Quercetin ($P < 0.001$), Gallic Acid ($P < 0.001$), Quercitrin ($P < 0.001$) and Rutin ($P < 0.01$) (Fig. 5). Quercetin exhibited the highest antioxidant activity as indicated by the IC_{50} values (Table 6).

TLC Analysis

The TLC analysis indicated the presence of terpenoids in the ethanolic extract of *M. officinalis*. Furthermore, greater amounts of flavonoids were found in the aqueous extract of *M. officinalis*. In line with this, the aqueous extracts from

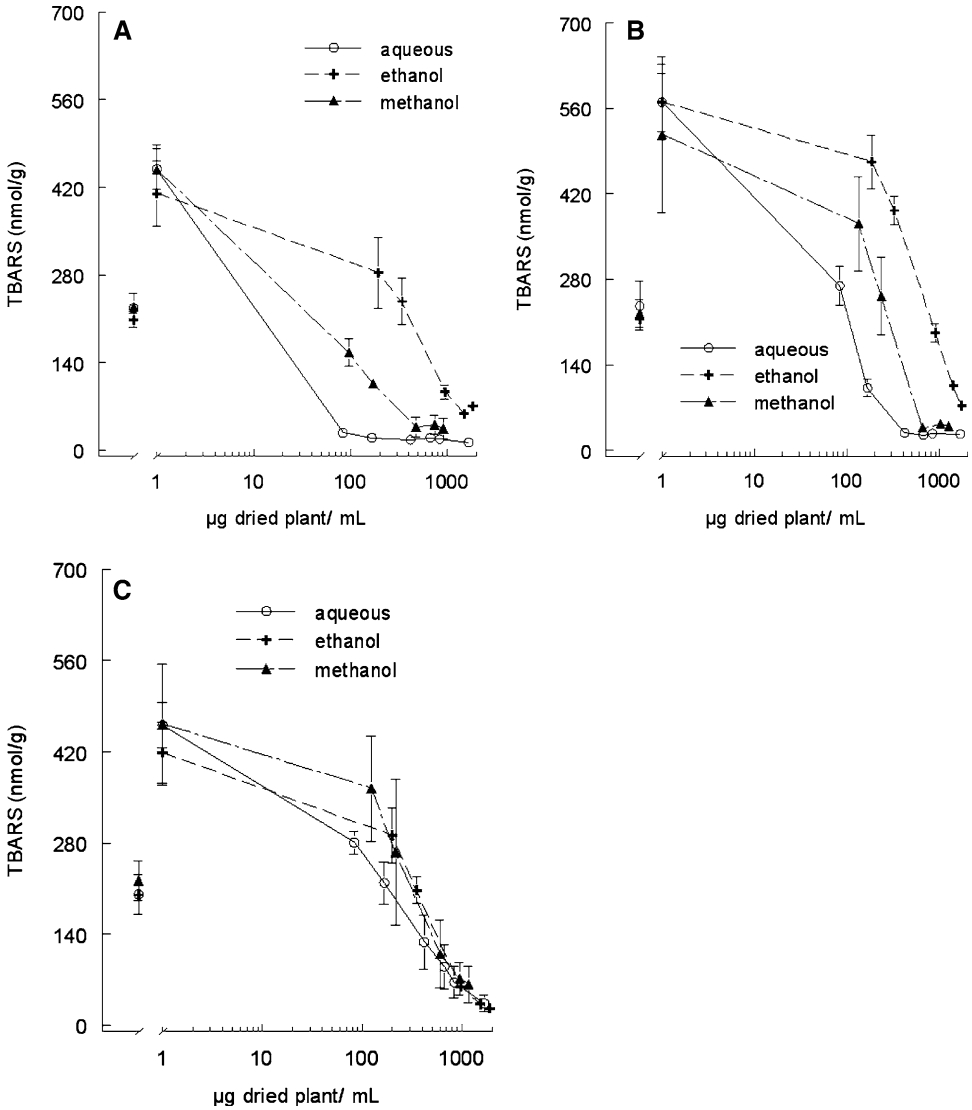

Fig. 2 Effects of different concentrations of aqueous, ethanolic and methanolic extracts from **a** *M. officinalis*, **b** *M. recutita* and **c** *C. citratus* on SNP (5 µM)-induced TBARS production in brain homogenates. The homogenates were incubated for 1 h with SNP and the plant extracts or without (basal). Data show means ± SEM values average from 3 to 4 independent experiments performed in duplicate

all these three plants presented more flavonoids than their respective ethanolic and methanolic extracts. For the ethanolic and methanolic extracts of *M. recutita*, simple phenolic compounds and flavonoids were detected in great amounts (data not shown).

Reducing agents were detected in all extracts. However they were more abundant in the aqueous extract of *M. officinalis*. This fact can explain the higher antioxidant activity of this extract. It was also possible to identify the presence of rutin in *C. citratus* aqueous extract by the bidimensional TLC (data not shown).

Discussion

In this study, we have tested the effect of three different plant species, *M. officinalis, M. recutita* and *C. citratus*, against well-known pro-oxidants, to investigate new potential antioxidants from the natural sources for the possible use in the diseases prevention.

The brain is particularly susceptible to free radical damage because of its high consumption of oxygen and its relatively low concentration of antioxidants enzymes and free radicals scavengers. Then, in this study, we used encephalic tissue for the TBARS assay and determine the quantity of phenolic compounds in the plant extracts to verify a possible relation with the antioxidant activity. These compounds are one of the largest and most ubiquitous groups of plant metabolites and there are current interest in their antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic activity [45–49].

In this work, the aqueous extract of *M. officinalis* had the highest activity against TBARS production induced by all tested agents, when compared with ethanolic and methanolic extracts. Interestingly, the inhibition of lipid peroxidation by *M. officinalis* extracts showed a relation

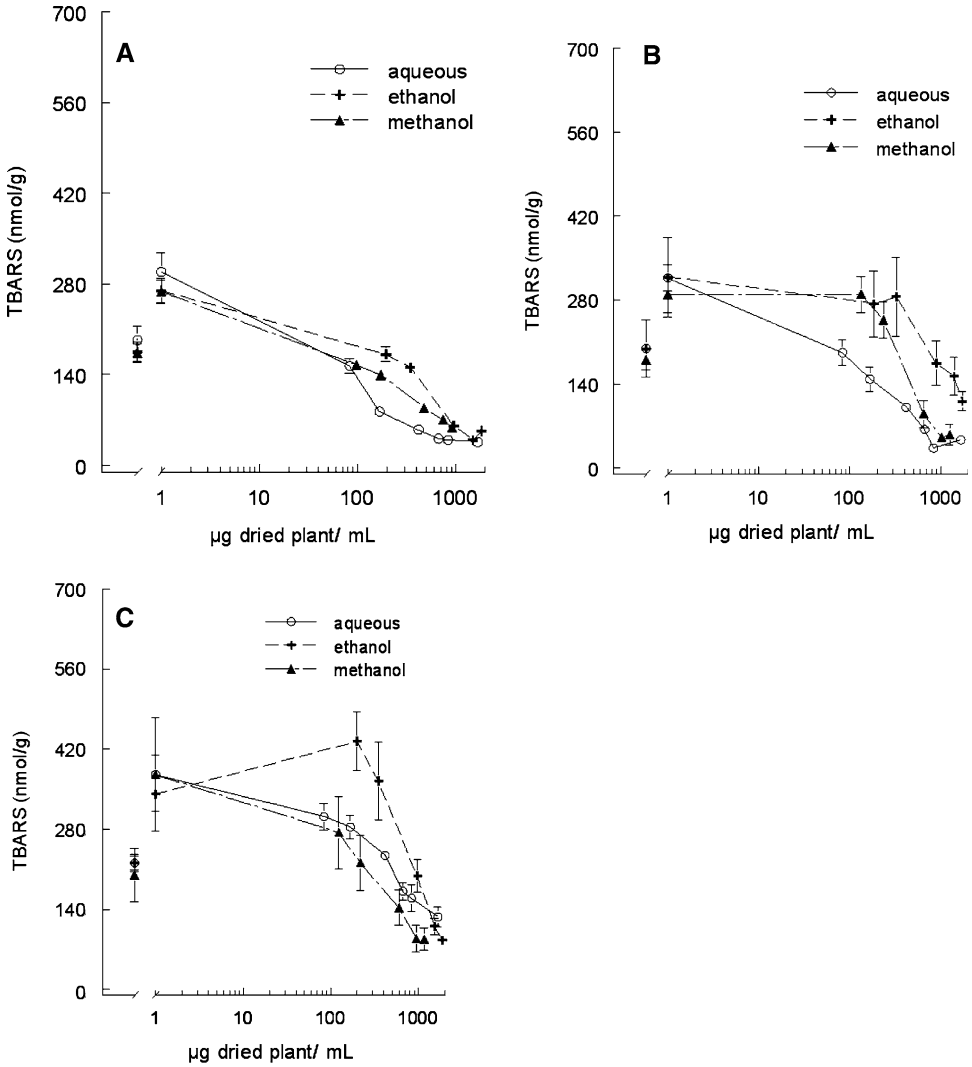

Fig. 3 Effects of different concentrations of aqueous, ethanolic and methanolic extracts from **a** *M. officinalis*, **b** *M. recutita* and **c** *C. citratus* on 3-NPA (2 mM)-induced TBARS production in brain homogenates. The homogenates were incubated for 1 h with 3-NPA and the plant extracts or without (basal). Data show means ± SEM values average from 3 to 4 independent experiments performed in duplicate

with its phenol content. However, in the DPPH assay, the three different extracts obtained from this plant (aqueous, ethanolic and methanolic) presented similar effect.

For *M. recutita* and *C. citratus*, TBARS inhibitory potency varied depending on the pro-oxidant used in a rather complex way. In contrast to *M. officinalis*, there was no clear relation between the antioxidant activity and phenolic contents. In the DPPH test, *M. recutita* methanolic extract presented lower IC_{50} than the ethanolic and aqueous extracts. Furthermore the free radical scavenger potency was not related to phenol concentrations. For *C. citratus*, the IC_{50} values for methanolic and ethanolic extracts were lower than aqueous extract. As in *M. recutita,* the free radical scavenger potency was not related with phenol concentrations.

Here we have used pro-oxidant agents that induce lipid peroxidation by different mechanisms. Free iron can induce neurotoxicity [50] via stimulation of Fenton reaction [51] and its levels are increased in some degenerative diseases [52–54]. SNP can cause oxidative stress and citotoxicity either by releasing cyanide and/or nitric oxide (NO) which can generate peroxynitrite [55–58]. Nitropropionic acid is thought to induce oxidative stress via inhibition of succinate dehydrogenase [59]. Although at first glance, the distinct antioxidant properties of plant extracts could indicate that they were acting via distinct mechanism. Although this can be the case, plant extracts could be inhibiting a common final (or downstream) pathway in polyunsaturated fatty acids peroxidation. Thus, we cannot exclude that a single mechanism is involved in the antioxidant of the tested extract.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical has been widely used to test the free radical scavenging ability of various natural products [60] and has been accepted as a model compound for free radicals originating in lipids [61, 62]. In the present study, the extracts obtained from *M. officinalis* exhibit lowest IC_{50} values, indicating the highest potential as free radical scavengers.

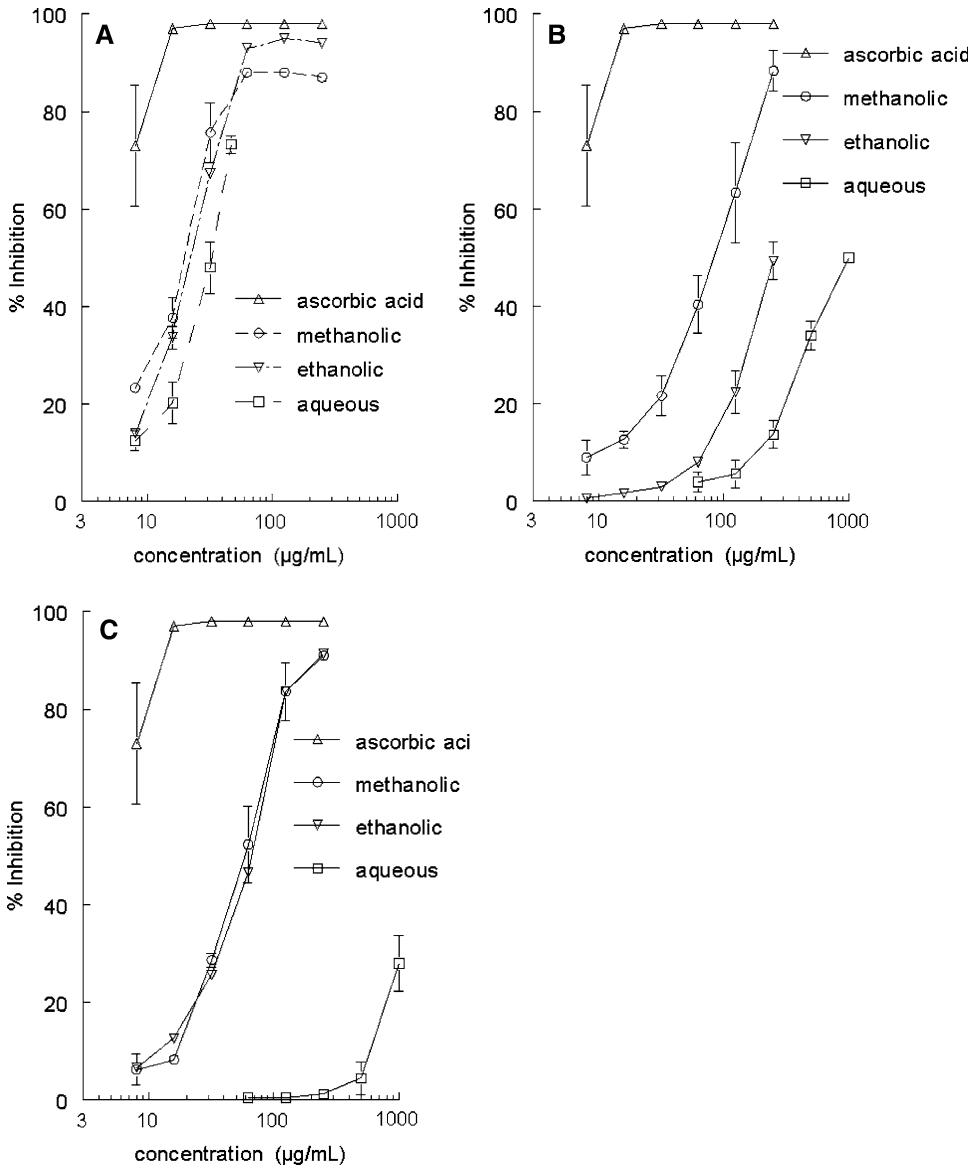

Fig. 4 Effects of different concentrations of aqueous, ethanolic and methanolic extracts from **a** *M. officinalis*, **b** *M. recutita* and **c** *C. citratus* on DPPH test. The results are expressed as percentage of inhibition and Ascorbic Acid was used as a positive control. Data show means ± SEM values average from 3 to 4 independent experiments performed in triplicate

Table 4 IC_{50} (µg/ml) values of tested plant extracts obtained by the reaction with DPPH free radical

Plants	Extractor solvent	IC_{50} (µg/ml)
M. officinalis	Water	32.9 ± 1.2^{b}
	Ethanol	28.2 ± 0.4^{a}
	Methanol	24.3 ± 2.1^{a}
M. recutita	Water	947.2 ± 22.5^{c}
	Ethanol	258.9 ± 13.3^{b}
	Methanol	115.9 ± 16.3^{a}
C. citratus	Water	1615.7 ± 302.2^{b}
	Ethanol	97.7 ± 0.2^{a}
	Methanol	85.7 ± 12.2^{a}

Different alphabets indicate statistical significance among different extracts of the same plant

Flavonoids are plant secondary metabolites widely distributed in the plant kingdom, and can be subdivided into six classes: flavones, flavanones, isoflavones, flavonols, flavanols, and anthocyanins based on their structure and conformation of the heterocyclic oxygen ring (C ring) of the basic molecule [63]. It has been demonstrated that flavonoid compounds in several aqueous extracts have very strong antioxidant and free radical scavenging activities, and are much more effective than vitamins C and E in protecting cells from free radical damage [24, 64]. Our study demonstrate the presence of flavonoid compounds in the extracts by TLC analysis, mainly in the aqueous extracts, which also presented important antioxidant activity, suggesting that these extracts could offer various health benefits, since flavonoids have been linked to

Table 5 Phenolic compounds determination in aqueous, ethanolic and methanolic extracts from *Melissa officinalis*, *Matricaria recutita* and *Cymbopogon citratus*

Plants	Extractor solvent	Phenol (nmol GA/g plant) mean ± SEM
Melissa officinalis	Water	389.65 ± 99.15[a]
	Ethanol	26.41 ± 0.09[c]
	Methanol	166.32 ± 18.92[b]
Matricaria recutita	Water	74.65 ± 12.23[a]
	Ethanol	18.71 ± 0.07[c]
	Methanol	30.01 ± 1.15[b]
Cymbopogon citratus	Water	64.24 ± 8.56[b]
	Ethanol	103.72 ± 6.43[a]
	Methanol	28.28 ± 1.60[c]

The results are expressed as nmol Gallic Acid (GA)/g dried plant. Data show means ± SEM values average from 3 to 4 independent experiments performed in triplicate. Different alphabets indicate statistical significance among different extracts of the same plant

Table 6 IC_{50} (μg/ml) values of compounds against different pro-oxidant agents- induced TBARS production in brain preparations

Compounds	Pro-oxidants		
	Iron	SNP	3-NPA
Quercetin	1.4 ± 0.03[a]	0.17 ± 0.03[a]	0.10 ± 0[a]
Gallic Acid	16.3 ± 4.5[b]	1.6 ± 0.2[b]	1.2 ± 0.2[b]
Quercitrin	12.2 ± 2.8[b]	1.4 ± 0.46[b]	1.3 ± 0.2[b]
Rutin	25.8 ± 5.8[b]	10.57 ± 2.1[c]	14.4 ± 1.3[c]

Different alphabets indicate statistical significance among different compounds against the same pro-oxidant

benefits in reducing the risk of certain cancers [22–25] and cardiovascular diseases [26–28]. Our data demonstrated also that the tested isolated compounds (flavonoids and phenolic compounds), that are present at a high quantity in plant extracts, showed an excellent activity against TBARS production induced by different agents, which promote

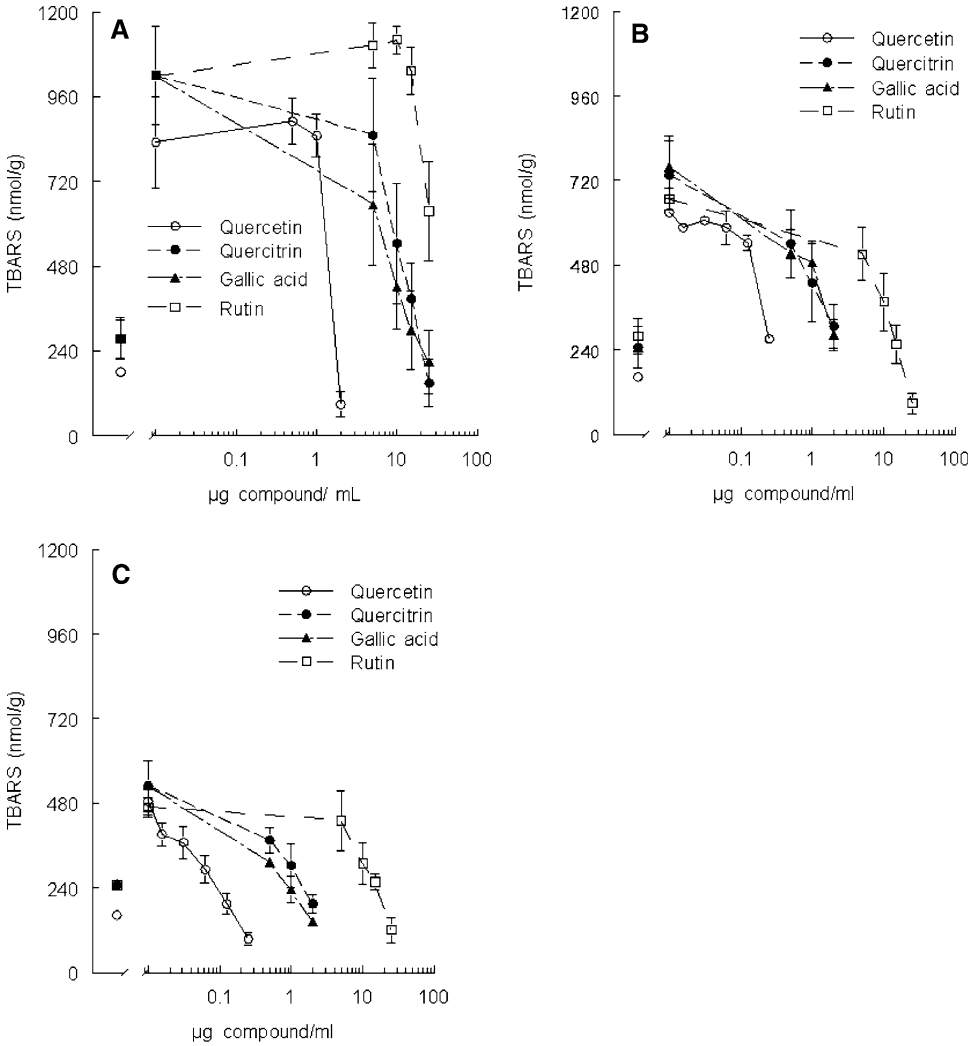

Fig. 5 Effects of different concentrations of Quercetin, Gallic Acid, Quercitrin and Rutin on **a** Iron (10 μM), **b** SNP (5 μM) or **c** 3-NPA (2 mM)-induced TBARS production in brain homogenates. The homogenates were incubated for 1 h with Iron, SNP or 3-NPA and compounds or without (basal). Data show means ± SEM values average from 3 to 4 independent experiments performed in duplicate

lipid peroxidation by different process. Quercetin was the most effective among the purified tested compounds, followed by gallic acid, quercitrin and rutin. This could be explained by the highest lipophilic characteristics of quercetin, which could increase its potency as a blocker of lipid peroxidation. In contrast, the lower antioxidant activity of rutin can be related to the presence of the glycoside hydrophilic group in its structure [65]. Interestingly, plant extracts are sources of a variety of potentially beneficial compounds, including the purified phenolic compounds tested here. The superior activity of the purified compounds in comparison with plant extracts can be explained in the basis of the lower concentration of the antioxidant compounds in the extracts. In spite of these, the use of crude plant extracts can be considered of pharmacological importance both in view of its easy availability and to the presence of different compounds that can have synergic effects in vivo.

In conclusion, all extracts tested here are effective inhibitors of TBARS production and also presented DPPH scavenger activity. In part, these effects can be related to their phenolic content, including the presence of flavonoids. Interestingly, *M. officinalis* aqueous extract presented the best antioxidant activities and the highest content of reducing agents, when compared to *M. recutita* and *C. citratus*. Consequently, this plant could be used as a potential agent for the prevention of various neurological diseases associated with oxidative damage. In line with this, recent data from literature have supported a protective role for *M. officinalis* intake against Alzheimer disease [29]. It is important emphasize that the aqueous extracts from plants tended to present highest antioxidant activities, which is the preparation used by the general population.

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