Antioxidant properties of *Taraxacum officinale* fruit extract are involved in the protective effect against cellular death induced by sodium nitroprusside in brain of rats

Dirleise Colle, Letícia Priscilla Arantes, Ricardo Rauber, Sérgio Edgar Campos de Mattos, João Batista Teixeira da Rocha, Cristina Wayne Nogueira & Félix Alexandre Antunes Soares

Published online: 06 Apr 2012.

Submit your article to this journal

View related articles

informa
healthcare

RESEARCH ARTICLE

Antioxidant properties of *Taraxacum officinale* fruit extract are involved in the protective effect against cellular death induced by sodium nitroprusside in brain of rats

Dirleise Colle[1], Letícia Priscilla Arantes[1], Ricardo Rauber[2], Sérgio Edgar Campos de Mattos[2], João Batista Teixeira da Rocha[1], Cristina Wayne Nogueira[1], and Félix Alexandre Antunes Soares[1]

[1]*Universidade Federal de Santa Maria, Centro de Ciências Naturais e Exatas, Departamento de Química, Programa de Pós-graduação em Ciências Biológicas: Bioquímica Toxicológica, Santa Maria, RS, Brazil and* [2]*Universidade de Cruz Alta, Cruz Alta, RS, Brazil*

Abstract

Context: *Taraxacum officinale* Weber (Asteraceae), known as dandelion, is used for medicinal purposes due to its choleretic, diuretic, antitumor, antioxidant, antiinflammatory, and hepatoprotective properties.

Objective: We sought to investigate the protective activity of *T. officinale* fruit extract against sodium nitroprusside (SNP)-induced decreased cellular viability and increased lipid peroxidation in the cortex, hippocampus, and striatum of rats *in vitro*. To explain the mechanism of the extract's antioxidant activity, its putative scavenger activities against NO•, DPPH•, OH•, and H_2O_2 were determined.

Methods: Slices of cortex, hippocampus, and striatum were treated with 50 μM SNP and *T. officinale* fruit ethanolic extract (1–20 μg/mL) to determine cellular viability by MTT reduction assay. Lipid peroxidation was measure in cortical, hippocampal and striatal slices incubates with SNP (5 μM) and *T. officinale* fruit extract (1–20 μg/mL). We also determined the scavenger activities of *T. officinale* fruit extract against NO•, DPPH•, OH•, and H_2O_2, as well as its iron chelating capacity.

Results: The extract (1, 5, 10, and 20 μg/mL) protected against SNP-induced decreases in cellular viability and increases in lipid peroxidation in the cortex, hippocampus, and striatum of rats. The extract had scavenger activity against DPPH• and NO• at low concentrations and was able to protect against H_2O_2 and Fe^{2+}-induced deoxyribose oxidation.

Conclusion: T. officinale fruit extract has antioxidant activity and protects brain slices against SNP-induced cellular death. Possible mechanisms of action include its scavenger activities against reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are attributed to the presence of phenolic compounds in the extract.

Keywords: *Taraxacum officinale*, antioxidant activity, reactive oxygen species, phenolic compounds, neuroprotection

Introduction

The generation of reactive oxygen species (ROS), which is a consequence of aerobic metabolism, and reactive intermediates occurs in various physiological and pathophysiological conditions (Jeon et al., 2008). Under normal conditions, ROS production is controlled by endogenous antioxidant systems present in the body that establish equilibrium between ROS and antioxidants. As such, overproduction of ROS or inadequate antioxidant content can cause oxidative stress (Kaur et al., 2006). ROS induce lipid peroxidation, damage biomolecules, such as DNA and proteins, and affect cellular viability (Jeon et al., 2008).

Oxidative stress resulting from an increase in free radical generation is an important factor in various neurodegenerative diseases (Beal, 1996). The brain is highly susceptible to free radical damage because of its high utilization of oxygen and the presence of relatively low

concentrations of antioxidant enzymes and free radical scavengers (Muralidhara, 2008).

In fact, sodium nitroprusside (SNP) is a pro-oxidant agent that impairs cellular viability and induces lipid peroxidation in brain tissue (Rauhala et al., 1998). SNP has been suggested to cause cytotoxicity through the release of nitric oxide radical ($NO^•$), the generation of ferricyanide anions $[(CN)_5\text{-}Fe]^{-3}$ that can react with hydrogen peroxide (H_2O_2) via the Fenton reaction, and generating hydroxyl radicals ($OH^•$) in tissue preparations (Beal, 1996). $NO^•$ induces oxidative stress, neuronal toxicity, and apoptotic cell death, which is accompanied by mitochondrial dysfunction (Riobo et al., 2001; Fukushima et al., 2006; Romero et al., 2010). $NO^•$ is a known mediator of inflammatory processes that play an important role in the pathogenesis of several neurodegenerative diseases (Brown, 2007; Calabrese et al., 2007); therefore, models of $NO^•$-mediated cytotoxicity may serve as tools to evaluate active compounds for further treatment (Romero et al., 2010).

In an attempt to obtain protection against ROS and RNS, and to avoid the development of neurodegenerative diseases, different research groups have been interested in screening new therapies using antioxidant substances with scavenger activity (Wang et al., 2008).

In recent years, considerable attention has been directed towards the identification of plants with antioxidant activity that may be used for human consumption (Kaur et al., 2006). The therapeutic properties of some plant extracts used in traditional medicine have been linked to their capacity to interact with reactive species and produce antioxidant activities. Such antioxidant properties found in plant extracts have been attributed to polyphenols (Peschel et al., 2006). Polyphenols act as antioxidant through several mechanisms, including free radical scavenging, metal ion chelation, hydrogen donation, and action as a substrate for radicals, such as superoxide anion and hydroxyl (Barreira et al., 2008).

Taraxacum officinale Weber (Asteraceae), commonly known as dandelion, has long been used for medicinal purposes due to its choleretic, diuretic, antitumor, antioxidant, antiinflammatory, antinociceptive, and hepatoprotective properties (Baba et al., 1981; Kisiel & Barszcz, 2000; Hu & Kitts, 2003; Jeon et al., 2008). Flavonoids and phenolic compounds, such as luteolin, caffeic acid, and cholorgenic acid, are found in extracts of *T. officinale* (Hu & Kitts, 2003; Domitrovic et al., 2010; Koh et al., 2010). These compounds, which are generally found in plants, protect cells from oxidative stress by preventing the formation of free radicals or by detoxifying free radicals, resulting in the prevention of a variety of pathophysiological processes (Mates & Sanchez-Jimenez, 2000).

Considering the previously reported antioxidant and pharmacological properties of the extract as well as the involvement of ROS and RNS in several brain disorders, we investigated whether a fruit extract of *T. officinale* possesses the capacity to protect brain slices against SNP-induced decreases in cellular viability. The antioxidant activity of the extract in brain structures was determined by measuring lipid peroxidation induced by SNP, a pro-oxidant agent. To explain the mechanisms by which *T. officinale* fruit extract displays antioxidant activity, we conducted *in vitro* experiments to demonstrate its putative scavenger activity against different free radicals, such as $NO^•$, $DPPH^•$, $OH^•$, and H_2O_2, and its iron chelating capacity.

Methods

Chemicals

Thiobarbituric acid (TBA), malonaldehyde-bis-dimethyl acetal (MDA), 1,1-diphenyl-1-picrylhydrazyl (DPPH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), deoxyribose, *ortho*-phenanthroline and iron chloride ($FeCl_3$) were purchased from Sigma (St. Louis, MO, USA). Sodium nitroprusside (SNP) was obtained from Merck (Darmstadt, Germany) and iron sulphate ($FeSO_4$) from Reagen (Rio de Janeiro, RJ, Brazil). All other reagents were obtained from local suppliers.

Animals

Male Wistar rats weighing 250 ($\pm$ 35.36) g, approximately 3 months of age, from our own breeding colony were kept in cages with continuous access to water and food, in a room with controlled temperature (22 ± 3°C) and in 12 h light/dark cycle with lights on at 7:00 am. The animals were maintained and used in accordance with the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of Federal University of Santa Maria, Brazil.

Plant material

The fresh fruit of *T. officinale* Weber (Asteraceae), known as dandelion, were used as the plant material and obtained from Cruz Alta University (Rio Grande do Sul, Brazil). The collection of *T. officinale* fruit was carried out during the winter and early spring. The taxonomic identification was confirmed by Department of Botany of Cruz Alta University, and registered under the number 157 in the Herbarium of Cruz Alta University.

Preparation of the extract

For the preparation of the ethanolic extract, fruits (1 g) were extracted with ethanol (70%) for 2 weeks at room temperature. The extract was evaporated and concentrated to dryness under reduced pressure using a rotary evaporator and redissolved in ethanol (EtOH; 70%) to a concentration of 2 mg/mL.

Determination of total phenolic content

The total phenolic content was determined by adding 0.5 mL of the extract to 2.0 mL of 7.5% sodium carbonate and 2.5 mL of 10% Folin-Ciocalteu reagent. The reaction mixture was incubated at 45°C for 15 min. Afterwards, the absorbance was measured at 765 nm using a spectrophotometer. Gallic

acid was used as a phenol standard (Singleton et al., 1999). The total phenolic content was expressed as micrograms of gallic acid equivalent/mg extract.

Cellular viability

Cellular viability in cortex, hippocampus and striatum slices was quantified by measuring the reduction of MTT to a dark violet formazan product by mitochondrial dehydrogenases (Mosmann, 1983). Slices (0.4 mm) of the brain structures were obtained by transversally cuts using a McIlwain chopper. SNP was used in the concentration of 50 µM to induce a decrease in cellular viability (Ávila et al., 2008). Slices were preincubated with the *T. officinale* fruit extract (1, 5, 10 and 20 µg/mL) or vehicle (EtOH) and SNP (50 µM) for 2 h at 37°C in oxygenated PBS buffer (124 mM NaCl, 5 mM KH_2PO_4, 10 mM Na_2HPO_4, 5 mM NaH_2PO4, 10 mM Glucose). After incubation, the slices were washed twice with 0.5 mL of buffer. MTT reduction assay was performed in plates containing 500 µL of buffer, and the reaction was started by adding 0.5 mg/mL MTT. After 1 h of incubation at 37°C, the medium was removed and the slices dissolved in dimethylsulfoxide (DMSO). The rate of MTT reduction was measured spectrophotometrically at 570 nm. The slices were homogenized in SDS 1% and NaOH 0.1 N solution and separated to carry out protein measurement according to the method of Lowry (Lowry et al., 1951).

Lipid peroxidation (LPO)

Animals were killed by decapitation and the brain was removed. The whole brain was used or dissected into three specific structures (cortex, hippocampus and striatum) and then homogenized (1:10) in Tris-HCl 10 mM buffer, pH 7.4 and centrifuged at 4,000× g at 4°C for 10 min. The low speed supernatant fraction obtained (S1) was used for TBARS measurements. The antioxidant effect of *T. officinale* fruit extract was evaluated against SNP (5 µM) through measurements of thiobarbituric acid reactive substances (TBARS) production, according to Ohkawa et al. (1979). The S1 fractions were preincubated at 37°C for 1 h in a buffered medium containing the *T. officinale* fruit extract (1, 5, 10 and 20 µg/mL) or vehicle (ethanol) and SNP (5 µM). The basal TBARS levels were determined in the absence of SNP (autooxidation of the homogenates). Then, SDS 8.1%, acetic acid/ HCl buffer and thiobarbituric acid 0.6% were added to the tubes and incubated at 95°C for 1 h. TBARS formation was determined spectrophotometrically at 532 nm, using malondialdehide (MDA) as standard.

Free radical scavenging assays
Scavenging activity of NO•

NO• scavenging activity was determined using the Griess reagent (Green et al., 1981). SNP (5 mM, in PBS) was incubated at 25°C with different concentrations of *T. officinale* fruit extract (1, 5, 10, 20, 50, 75 and 100 µg/mL) or vehicle (EtOH). After 120 min, 0.5 mL of incubation solution was sampled and mixed with 0.5 mL of Griess reagent [0.1% N-(naphthyl) ethylenediamine dihydrochloride,

1% sulphonilic acid in 5% of phosphoric acid]. The absorbance was measured at 550 nm. The values were compared with the control to determine the percentage of inhibition of nitrite reaction with Griess reagent, depicted by the *T. officinale* extract as an index of its NO• scavenger activity (Marcocci et al., 1994). NO• scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following equation:

$$\% \text{ inhibition} = \frac{(A_0 - A_{sample})}{A_0} \times 100$$

where A_0 was the absorbance of the control (blank, without extract) and A_{sample} was the absorbance in the presence of different extract concentrations. Tests were carried out in duplicate.

DPPH• scavenging activity

The measurement of the *T. officinale* fruit extract scavenger activity against the DPPH• radical was performed in accordance with Cho et al. (2002). DPPH• solution (85 µM) was added to a medium containing different concentrations of the extract (10, 20, 30, 40, 50, 75 and 100 µg/mL) or vehicle (EtOH) and incubated at room temperature for 30 min. The decrease in the absorbance measured at 518 nm depicted the scavenger activity of the extract against DPPH• radical. Ascorbic acid was used as positive control to determine the maximal decrease in DPPH• absorbance. DPPH• scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following equation:

$$\% \text{ inhibition} = \frac{(A_0 - A_{sample})}{A_0} \times 100$$

where A_0 was the absorbance of the control (blank, without extract) and A_{sample} was the absorbance in the presence of different extract concentrations. Tests were carried out in duplicate. The IC_{50} values (the concentration necessary to inhibit 50% radical formation) of ascorbic acid and plant extract were calculated using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA) and are expressed as mean (95% confidence intervals).

Determination of oxidative damage to deoxyribose

The deoxyribose degradation assay was performed according to Puntel et al. (2005). Deoxyribose oxidation was induced with both H_2O_2 and $FeSO_4$ alone and in combination. Briefly, the reaction medium was prepared containing the plant extract (1, 5, 10 and 20 µg/mL) or vehicle (EtOH), 3 mM deoxyribose, potassium phosphate buffer (0.05 mM, pH 7.4), 50 µM $FeSO_4$, and 500 µM H_2O_2. Solutions of $FeSO_4$ and H_2O_2 were made prior to use. Reaction mixtures were incubated at 37°C for 30 min and stopped by the addition of 0.8 mL of trichloroacetic acid (TCA; 2.8%), followed by the addition of 0.4 mL of thiobarbituric acid (TBA; 0.6%). Next, the medium was incubated at 100°C for 20 min and the absorbance was recorded at

532 nm (Halliwell & Gutteridge, 1981). Standard curves of MDA were constructed for each experiment. The values are expressed as percentage of control values.

Iron chelating property and reducing power

The iron chelating property of the extract (Minotti & Aust, 1987; Bucher et al., 1993) was determined according to Puntel et al. (2005). The method is based on the reaction of free Fe^{2+} with ortho-phenantroline, forming a colored complex. First, the mixture containing Fe^{2+} (150 μM) and different concentrations of the extract (1, 5, 10, 20, 50, 75 and 100 μg/mL) or vehicle (EtOH) was used to form a complex. Afterwards, a solution of *ortho*-phenantroline was added to the mixture (62.5 μg/mL, equivalent to 0.25%). The formation of complexes between Fe^{2+} and the extract was estimated by a decrease in the color reaction at 510 nm when compared to a control tube without extract. The values are expressed as a percentage of chelating activity.

To examine the reducing power of the *T. officinale* fruit extract, the same method (*ortho*-phenantroline) was used. The method is based on the power of the extract in reducing free Fe^{3+} to form Fe^{2+}. Fe^{2+} reacts with *ortho*-phenantroline forming a colored complex. $FeCl_3$ (150 μM) solution and different concentrations of the extract (1, 5, 10, 20, 50, 75 and 100 μg/mL) or vehicle (EtOH) were used. Afterwards, a solution of *ortho*-phenantroline was added to the mixture (62.5 μg/mL equivalent to 0.25%). The extract reducing power was estimated by an increase in the color reaction at 510 nm when compared to a control tube without extract. The values were expressed in absorbance. The solutions were prepared freshly for each experiment.

Statistical analysis

Statistical significance was assessed by one-way ANOVA, followed by Duncan *post hoc* test. Alternatively, the reducing power test was analyzed by two-way ANOVA followed by the Bonferroni *post hoc* test. Results are expressed as mean ± SEM. The differences were considered significant when $p < 0.05$.

Results

Total phenolic content

Total phenolic content, as assayed by the method of Folin-Ciocalteau, was expressed in microgram equivalents of gallic acid per milligram of each fraction of extract. We detected 180 ± 0.0157 μg gallic acid equivalents of phenols in 1 mg of ethanolic fruit extract.

Cellular viability

SNP induced a decrease in cellular viability in the cortex (about 83%), striatum (about 72%), and hippocampus (about 68.2%) when compared to the control ($p < 0.05$, Figures 1A–1C). The extract (5–20 μg/mL) protected
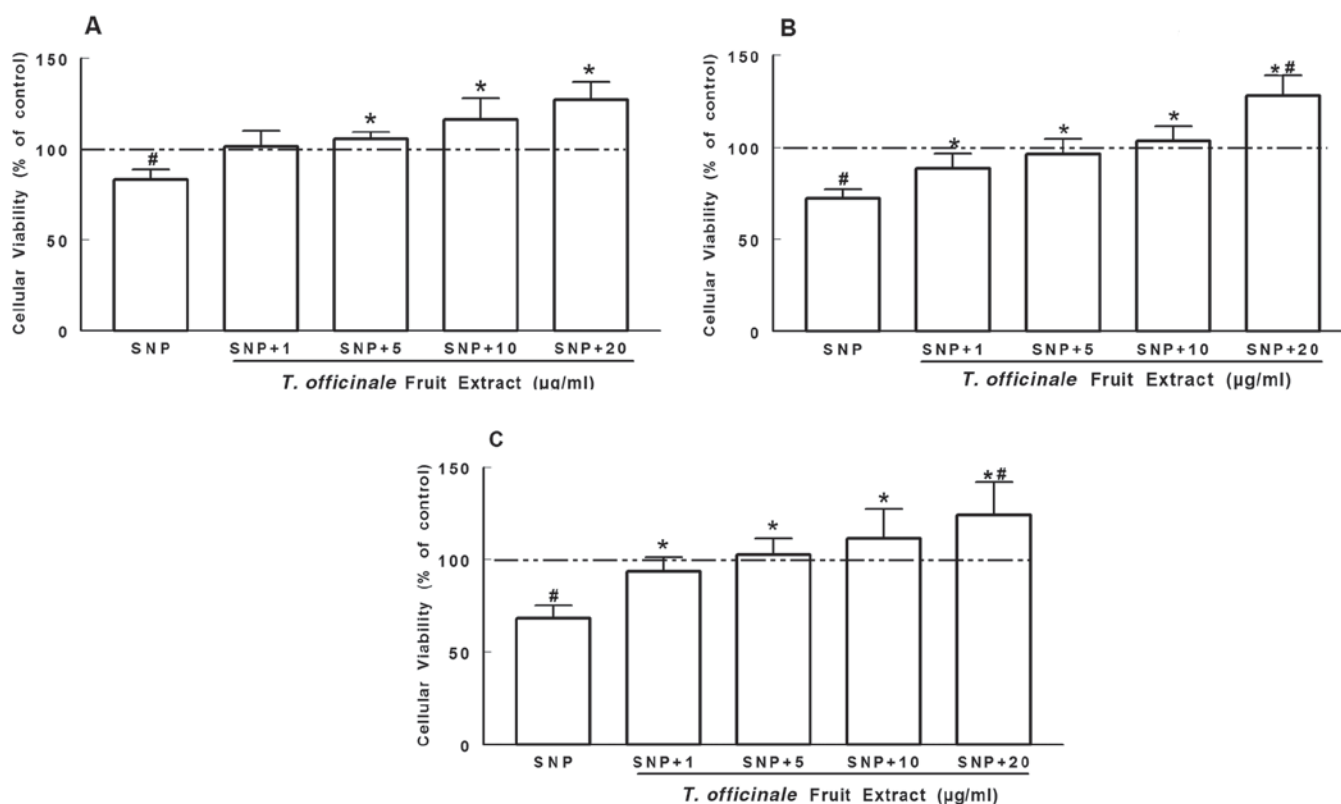

Figure 1. Protective effect of *T. officinale* fruit extract on the decrease of cellular viability induced by SNP. Slices of cortex (A), striatum (B) and hippocampus (C) were preincubated with *T. officinale* fruit extract (1, 5, 10 and 20 μg/mL) or vehicle (EtOH) and SNP (50 μM) for 2 h at 37°C in oxygenated PBS buffer. Cellular viability was evaluated by the MTT reduction method. Results are expressed as the percentage of control (dotted line). # Indicates statistical difference from control group. * Indicates statistical difference from SNP group by one-way ANOVA, followed by Duncan's *post hoc* test ($p < 0.05$). All experiments were performed in duplicate ($n = 6$).

cortical slices against SNP-induced decreased cellular viability ($p < 0.05$, Figure 1A). However, in the striatum and hippocampus, the protective effect of the extract was seen at concentrations of 1–20 μg/mL ($p < 0.05$, Figures 1B–1C).
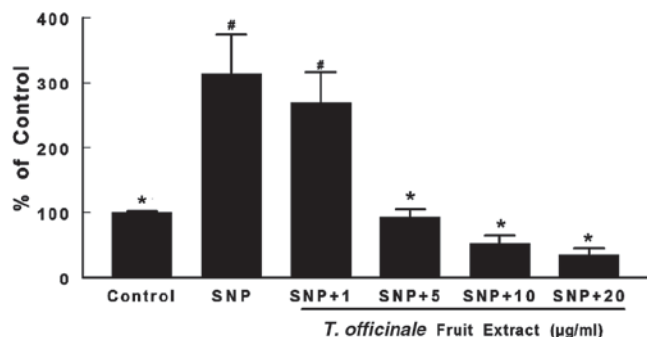

Figure 2. Effects of *T. officinale* fruit extract on SNP-induced lipid peroxidation in whole brain of rats. Brain homogenates were pre-incubated at 37°C for 1h in a buffered medium containing *T. officinale* fruit extract (1, 5, 10 and 20 μg/mL) or vehicle (EtOH) and SNP (5 μM). Lipid peroxidation was evaluated through measurements of thiobarbituric acid reactive substances (TBARS) production. Results are expressed as percent of control (without SNP and extract). 100% of control corresponds to 3.92 ± 0.37 nmol MDA/g of tissue. # Indicates statistical difference from control group. * Indicates statistical difference from SNP group by one-way ANOVA, followed by Duncan's *post hoc* test ($p < 0.05$). All experiments were performed in duplicate ($n = 5$).

Lipid peroxidation

SNP induced a 300% increase in TBARS levels in S1 of whole brain ($p < 0.05$, Figure 2). At concentrations of 5, 10, and 20 μg/mL, the extract significantly reduced TBARS levels when compared to the SNP-treated group ($p < 0.05$, Figure 2).

Figure 3 shows the antioxidant activity of the extract against lipid peroxidation in the cortex, striatum, and hippocampus of rats. SNP induced a significant increase in TBARS formation in all brain structures to approximately 200% above the control level ($p < 0.05$, Figures 3A–3C). The extract significantly reduced SNP-induced TBARS formation to control levels from 5 μg/mL in all brain structures analyzed ($p < 0.05$, Figures 3A–3C). In the hippocampus, the highest concentration used (20 μg/mL) showed a significant difference when compared with the control ($p < 0.05$, Figure 3C). Basal TBARS levels were also decreased in all brain structures analyzed by the extract at 5 μg/mL (data not shown).

Free-radical scavenging activity

The capacity of *T. officinale* fruit extract to scavenge NO$^•$ and DPPH$^•$ is shown in Figure 4. The extract showed significant NO$^•$ scavenging activity at concentrations of 5–100 μg/mL (Figure 4A). At a concentration of
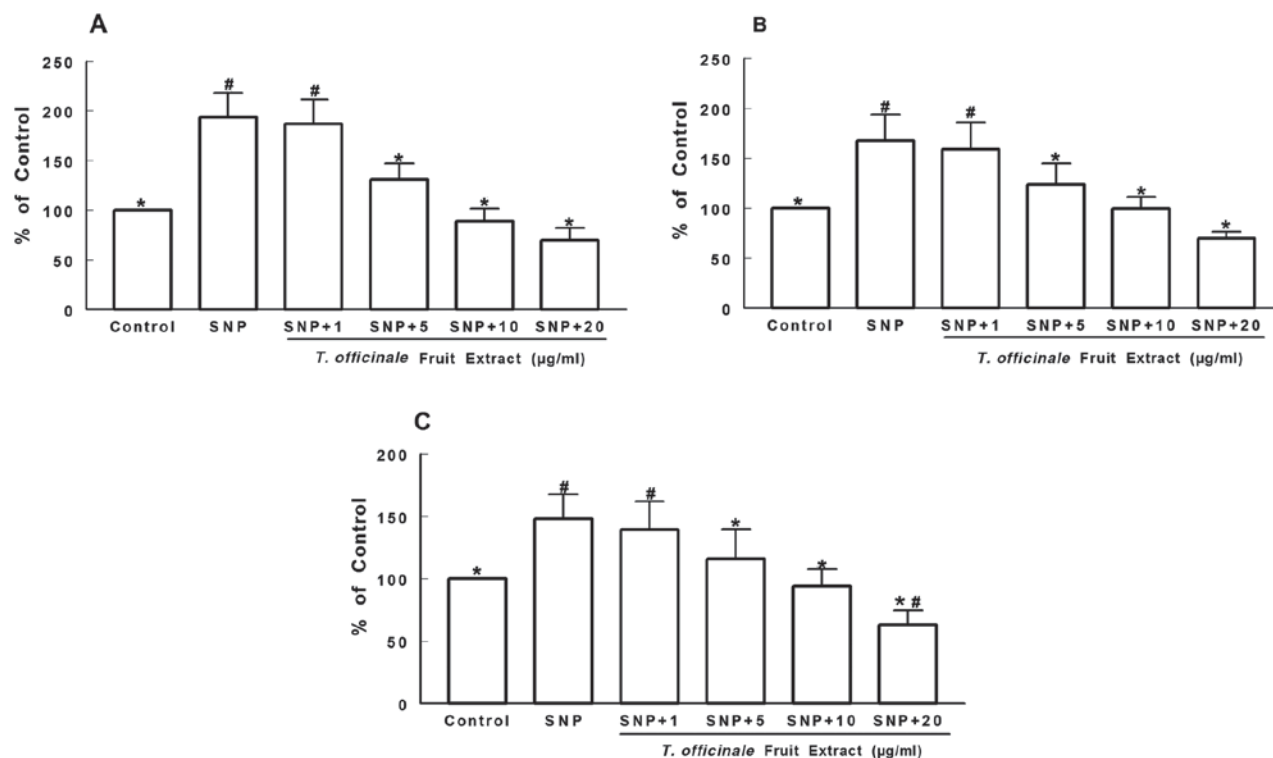

Figure 3. Effects of *T. officinale* fruit extract on SNP-induced TBARS production in cortex (A), striatum (B) and hippocampus (C) of rats. The homogenates of brain structures were preincubated at 37°C for 1h in a buffered medium containing *T. officinale* fruit extract (1, 5, 10 and 20 μg/mL) or vehicle (EtOH) and SNP (5 μM). Lipid peroxidation was evaluated through measurements of thiobarbituric acid reactive substances production (TBARS). Results are expressed as percent of control (without SNP and extract). 100% of control corresponds to 1.63 ± 0.21 nmol MDA/g of tissue (A), 1.63 ± 0.34 nmol MDA/g of tissue (B) and 1.58 ± 0.06 nmol MDA/g of tissue (C). # Indicates statistical difference from control group. * Indicates statistical difference from SNP group by one-way ANOVA, followed by Duncan's *post hoc* test ($p < 0.05$). All experiments were performed in duplicate ($n = 5$).
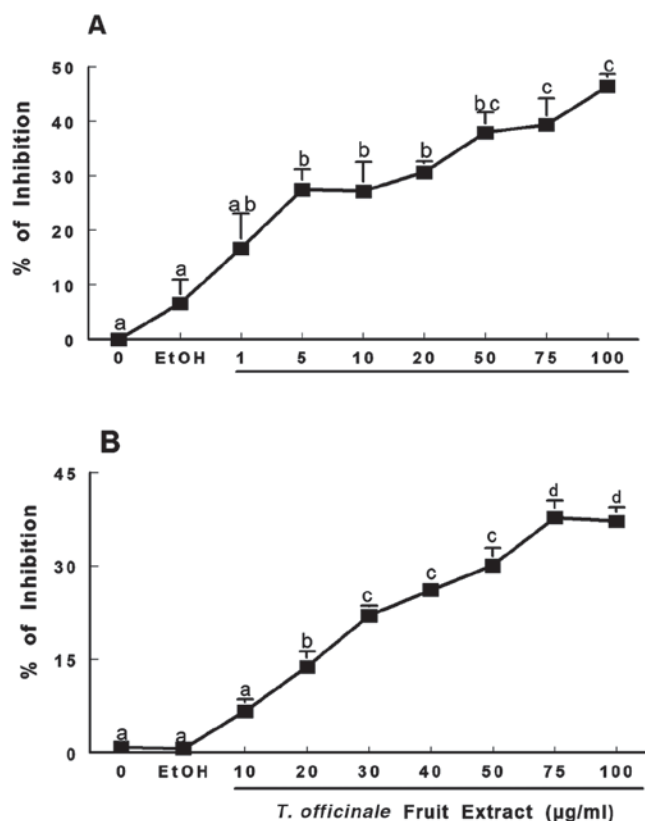
Figure 4. Scavenging activity of *T. officinale* fruit extract on NO•
(A) and DPPH• (B). For NO• analysis, SNP (5 mM) was incubated
at 25°C for 120 min with *T. officinale* fruit extract (1, 5, 10, 20, 50,
75 and 100 μg/mL) or vehicle (EtOH) and NO• scavenging activity
was determined using the Griess reagent. For DPPH• scavenger
activity, *T. officinale* (10, 20, 30, 40, 50, 75 and 100 μg/mL) or
vehicle (EtOH) were incubated at room temperature for 30 min
and the decrease in the absorbance measured at 518 nm depicted
the scavenger activity of the extract against DPPH• radical. Results
are expressed as percent of inhibition in relation to control without
extract. The mean control value is 21.85 ± 1.33 μM of nitrite (A) and
0.904 ± 0.0138 ABS (B). [a], [b], [c] and [d] indicate statistical significance
among different concentration of the extracts by one-way ANOVA,
followed by Duncan's *post hoc* test ($p < 0.05$). All experiments were
performed in duplicate ($n = 4$).

75 μg/mL, *T. officinale* extract inhibited NO• formation by
39.35%.

Moreover, the extract possesses significant DPPH•
radical scavenging activity at 20–100 μg/mL (Figure 4B).
At a concentration of 75 μg/mL, the extract showed 37.7%
inhibition. The IC_{50} (the concentration necessary to
inhibit 50% radical formation), values were 123.94 (98.22–
147.66) and 8.77 (6.23–10.23) μg/mL for *T. officinale* fruit
extract and ascorbic acid, respectively.

Deoxyribose oxidation

The capacity of the extract to inhibit deoxyribose oxidation
is shown in Figure 5. The extract was able to protect against
deoxyribose oxidation induced by H_2O_2 or Fe^{2+} at concen-
trations of 1, 5, 10, and 20 μg/mL ($p < 0.05$; Figures 5A–5B).
A similar protective effect was observed only at concentra-
tions of 10 and 20 μg/mL of the extract when both H_2O_2
and Fe^{2+} were added to the medium ($p < 0.05$, Figure 5C).

Iron chelating property and reducing power

Figure 6 shows the reducing power of *T. officinale* fruit
extract. The extract demonstrated reducing power in a
concentration- and time-dependent manner as indi-
cated by a significant effect in two-way ANOVA analysis
($F_{32, 72} = 510$, $p \leq 0.0001$; Figure 6). Moreover, one-way
ANOVA showed a significant effect of the extract at 20 μg/
mL ($p < 0.01$) and 1 h of incubation. However, after 24 h, a
significant effect of the extract was observed at 1 μg/mL
($p < 0.001$). The extract did not have iron (Fe^{2+})-chelating
activity (data not shown).

Discussion

Phytochemicals isolated from fruit, vegetables, and
herbs have been extensively studied both *in vivo* and *in
vitro* to characterize potential antioxidant and bioactive
properties (Hu & Kitts, 2003). Phenolic compounds have
significant importance because they are responsible
for scavenging free radicals and sequestering transition
metal ions (Hu & Kitts, 2003). The content of phenolic
compounds in ethanolic extract was 180 ± 0.015 μg/
mg plant extract as expressed in gallic acid equivalents.
Considering that similar levels were found in the flower
extract, which also possesses antioxidant activity (Hu &
Kitts, 2005), we believe that the protective effects of the
fruit extract against SNP-induced decreases in cellular
viability are probably due to the phenolic compounds.

Cellular viability is affected by oxidative stress, since
the production of reactive species causes damage to the
inner and outer mitochondrial membranes and opens
the mitochondrial permeability transition pores, thereby
inducing apoptosis (Sas et al., 2007). SNP is described a
pro-oxidant agent that impairs mitochondrial function
and decreases MTT reduction (Nie et al., 2006).

In this study, the exposure of cortical, hippocampal,
and striatal slices to SNP, a NO• donor, decreased cel-
lular viability. This result is consistent with previous
observations in brain slices (Ávila et al, 2008) and cor-
tical neurons, since SNP induces cell death and apop-
tosis-related mitochondrial dysfunction (Fukushima
et al., 2006; Romero et al., 2010). The neurotoxic effect of
SNP has been attributed to the production of ROS and
release of NO•.

Recent studies have demonstrated a role of NO•, which
is an important neuronal messenger in the central ner-
vous system, in the pathophysiology of disorders, such
as Alzheimer's and Parkinson's diseases (Castillo et al.,
2000). High NO• levels generated in response to cytok-
ines or the excitatory neurotransmitter glutamate can
result in neuronal cell death (Coyle et al., 1993; Bolanos
et al., 1997). Several mechanisms have been proposed for
NO•-induced cell death, including oxidative stress and
mitochondrial alterations (Riobo et al., 2001; Fukushima
et al., 2006; Romero et al., 2010). NO•, is a free radical that
combines with superoxide radical (O^{2-}) to form peroxyni-
trite ($ONOO^-$), a potent oxidant capable of inducing lipid
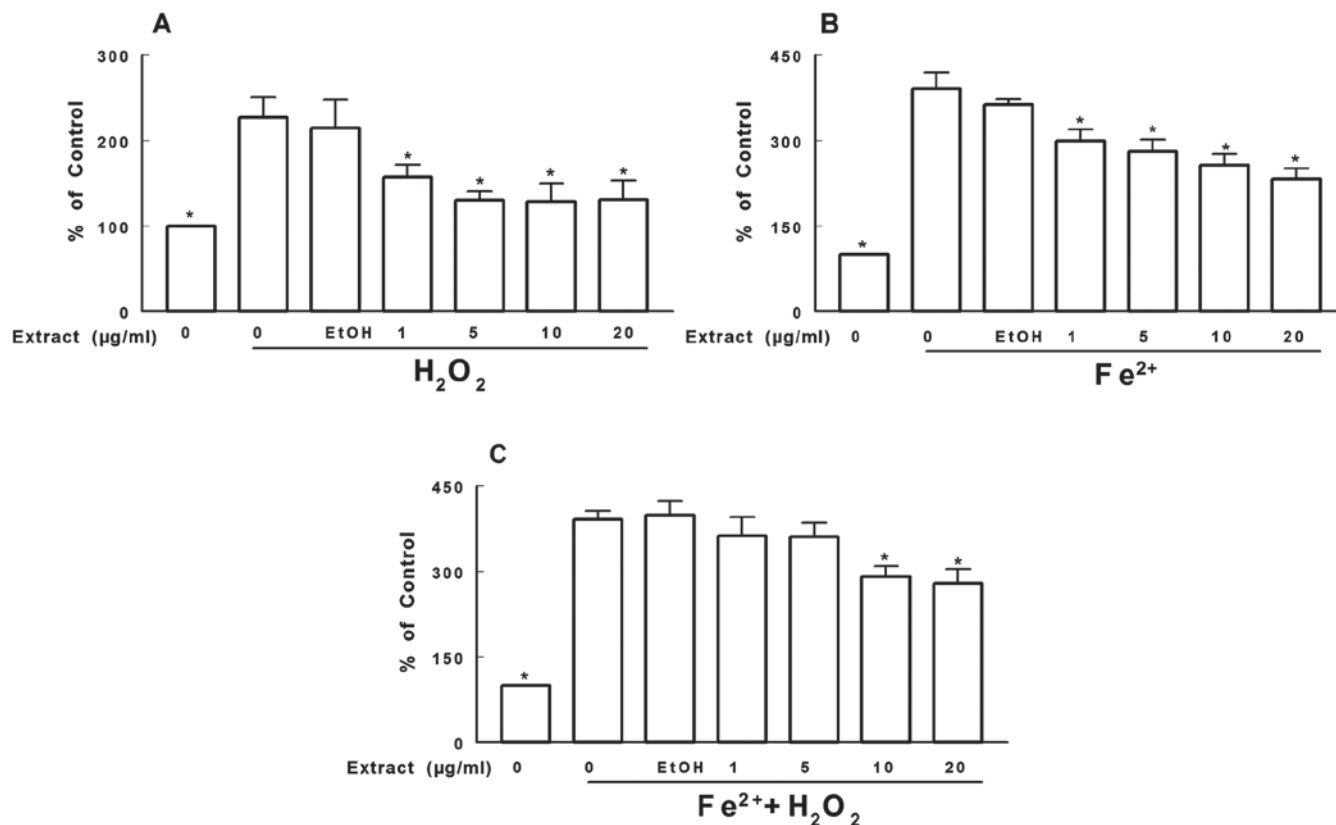peroxidation (Sah et al., 1995). In this study, SNP reduced

Figure 5. Effect of *T. officinale* fruit extract on the deoxyribose oxidation induced by H_2O_2 (A), Fe^{2+} (B) and Fe^{2+} + H_2O_2 (C). *T. officinale* fruit extract (1, 5, 10 and 20 µg/mL) or vehicle (EtOH) were incubated with 3 mM deoxyribose, 50 µM FeSO$_4$ and/or 500 µM H_2O_2 at 37°C for 30 min. Results are expressed as percent of control. The mean control value is 1.065 ± 0.193 nM of MDA/g of deoxyribose. * Indicates a statistically significant difference from H_2O_2 (A), Fe^{2+} (B) or Fe^{2+} + H_2O_2 (C) by one-way ANOVA, followed by Duncan's *post hoc* test ($p < 0.05$). All experiments were performed in duplicate ($n = 4$).
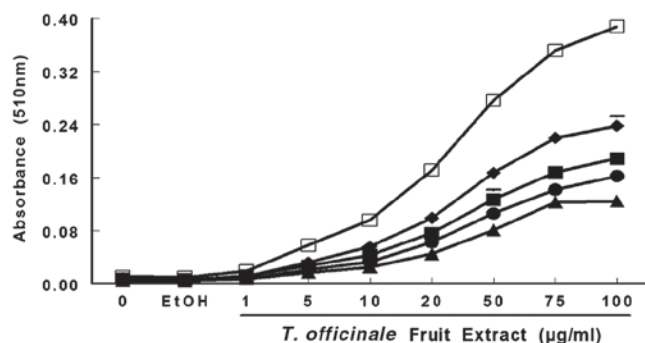

Figure 6. Reducing power of *T. officinale* extract was evaluated by spectrophotometer detection of Fe^{3+} to Fe^{2+} transformation. *T. officinale* fruit extract (1, 5, 10, 20, 50, 75 and 100 µg/mL) or vehicle (EtOH) were incubated with 150 µM FeCl$_3$, 62.5 µg/mL *ortho*-phenantroline. The extract reducing power was estimated by an increase in the color reaction at 510 nm when compared to a control in different times, 1 hour (▲); 2 hours (●); 3 hours (■); 6 hours (◆) and 24 hours (□). The mean control value is 0.01 ± 0.00051 ABS. All experiments were performed in duplicates ($n = 3$).

cellular viability, and this effect was prevented by the extract in all brain structures. This may be attributed to the antioxidant activity of the extract, acting as a scavenger of the reactive species generated by SNP.

The antioxidant effect of the extract was demonstrated by its efficient ability to reduce lipid peroxidation

generated by SNP in whole brain and brain structures. This antioxidant activity may be attributed to the phenolic compounds present in the extract, as they have the ability to accept electrons, which can combine with free radicals generated by SNP, thereby protecting lipids against oxidation. Similar antioxidant ability has been shown by *T. officinale* flower extracts, which suppressed lipid oxidation *in vitro* in linoleic acid emulsions (Hu & Kitts, 2005).

In the present study, the fruit extract effectively reduced nitrite formation, indicating decreased levels of NO• and suggesting a possible scavenger activity against reactive nitrogen species (RNS). Hu and Kitts reported an activity against RNS in ethanolic flower extracts, which was attributed to the phenolic content (Hu & Kitts, 2005). In previous studies, the ethanolic extract of *T. officinale* flowers demonstrated suppressive effects on NO• production in macrophage cells (Jeon et al., 2008). Two flavonoid compounds, luteolin and luteolin-7-*O*-glucoside, in *T. officinale* suppress the production of NO• in LPS-activated RAW264.7 macrophage cells, which was attributed to the suppression of inducible nitric oxide synthase (iNOS; Hu & Kitts, 2003).

In addition, the fruit extract shows significant scavenger activity against DPPH• radicals. Similarly, the *T. officinale* flower ethanolic extract showed scavenging

activity against DPPH• radicals in a concentration-dependent manner (Jeon et al., 2008). The DPPH• scavenging ability of the extract may be attributed to its ability to donate hydrogens. The donation of a hydrogen to the free radical and then reduction to an unreactive species is one mechanism by which an antioxidant can remove a free radical (Wang et al., 2008). DPPH• radical scavenging activity in the *T. officinale* flower extract was attributed to a reducing activity derived from the phenolic content of the extract (Hu & Kitts, 2005). Thus, the *T. officinale* fruit extract also acted as a scavenger of DPPH• radicals.

Moreover, in the present study, low concentrations of the dandelion fruit extract protected deoxyribose from H_2O_2- and Fe^{2+} -induced oxidation. Several plant extracts/constituents exert their antioxidant activities by chelating catalytic metals (Niu et al., 2000). For example, flavonoids possess high Fe^{2+}-chelating activity. Their scavenging potential and metal chelating ability are dependent on their unique phenolic structure as well as the number and position of their hydroxyl groups (Pazos et al., 1999). Nevertheless, we observed that the dandelion fruit extract did not show significant Fe^{2+}-chelating activity. However, *T. officinale* flower extract is a potential transitional metal ion chelator (Hu & Kitts, 2005). Flavonoids present in the fruit extract are unlikely to have the required structure as in the flower extract, which could explain this result.

The activity of the extract in preventing deoxyribose oxidative degradation induced by Fenton reagents was also established. The Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^•$) generates $OH^•$ radicals, which induce severe oxidative damage to cellular components (Halliweel, 1995). The mechanism of antioxidant action of polyphenols is usually attributed to $OH^•$-scavenging activity (Sah et al., 1995). However, the antioxidant efficiency of polyphenols against deoxyribose degradation is not caused by the neutralization of $OH^•$ radicals, but by chelating Fe^{2+} (Lopes et al., 1999).

The extract reduced Fe^{3+} to Fe^{2+} ions, demonstrating concentration- and time-dependent reducing power. Studies have reported that several polyphenols can reduce Fe^{3+} to Fe^{2+} (Moran et al., 1997). The reducing properties are generally associated with the presence of reducing molecules, which exert antioxidant activity by donating a hydrogen atom and breaking the free radical chain (Wang et al., 2008). Electron-donating agents contribute to antioxidant activity by donating an electron to the free radical, which results in the neutralization of the radical. The reduced species then acquires a proton from the solution (Wang et al., 2008).

Taken together, our data demonstrate that *T. officinale* fruit extract is a potent antioxidant at low concentrations, as evident by the decrease in lipid peroxidation and protection against SNP-induced cellular dysfunction. One possible mechanism by which *T. officinale* fruit extract protects against oxidative stress is through ROS- and RNS-scavenger activity, which is attributed to phenolic compounds. The phenolic compounds in *T. officinale* fruit extract act as neuroprotective antioxidants or reducing agents.

As the extract was protective against the decrease in cellular viability and the increase in lipid peroxidation induced by SNP in brain structures, further studies on the neuroprotective activity of *T. officinale* fruit extract *in vivo* are needed. Further investigations are also necessary to determine the specific phenolic compounds present in the extract and their quantity.

Conclusion

In conclusion, *T. officinale* fruit extract has antioxidant activity at low concentrations and protects against cell death in the brain. One possible mechanism of action of *T. officinale* fruit extract is as a scavenger of ROS and RNS. These effects might be attributed to the phenolic content present in the extract.

Declaration of interest

References

Avila DS, Gubert P, Palma A, Colle D, Alves D, Nogueira CW, Rocha JB, Soares FA. (2008). An organotellurium compound with antioxidant activity against excitotoxic agents without neurotoxic effects in brain of rats. *Brain Res Bull*, 76, 114–123.

Baba K, Abe S, Mizuno D. (1981). [Antitumor activity of hot water extract of dandelion, *Taraxacum officinale*-correlation between antitumor activity and timing of administration (author's transl)]. *Yakugaku Zasshi*, 101, 538–543.

Barreira JC, Ferreira IC, Oliveira MB, Pereira JA. (2008). Antioxidant activity and bioactive compounds of ten Portuguese regional and commercial almond cultivars. *Food Chem Toxicol*, 46, 2230–2235.

Beal MF. (1996). Mitochondria, free radicals, and neurodegeneration. *Curr Opin Neurobiol*, 6, 661–666.

Bolaños JP, Almeida A, Stewart V, Peuchen S, Land JM, Clark JB, Heales SJ. (1997). Nitric oxide-mediated mitochondrial damage in the brain: mechanisms and implications for neurodegenerative diseases. *J Neurochem*, 68, 2227–2240.

Brown GC. (2007). Mechanisms of inflammatory neurodegeneration: iNOS and NADPH oxidase. *Biochem Soc Trans*, 35, 1119–1121.

Bucher JR, Tien M, Morehouse LA, Aust SD. (1983). Redox cycling and lipid peroxidation: The central role of iron chelates. *Fundam Appl Toxicol*, 3, 222–226.

Calabrese V, Mancuso C, Calvani M, Rizzarelli E, Butterfield DA, Stella AM. (2007). Nitric oxide in the central nervous system: Neuroprotection versus neurotoxicity. *Nat Rev Neurosci*, 8, 766–775.

Castillo J, Rama R, Dávalos A. (2000). Nitric oxide-related brain damage in acute ischemic stroke. *Stroke*, 31, 852–857.

Cho SY, Park JY, Park EM, Choi MS, Lee MK, Jeon SM, Jang MK, Kim MJ, Park YB. (2002). Alternation of hepatic antioxidant enzyme

activities and lipid profile in streptozotocin-induced diabetic rats by supplementation of dandelion water extract. *Clin Chim Acta*, 317, 109–117.

Coyle JT, Puttfarcken P. (1993). Oxidative stress, glutamate, and neurodegenerative disorders. *Science*, 262, 689–695.

Domitrovic R, Jakovac H, Romic Z, Rahelic D, Tadic Z. (2010). Antifibrotic activity of Taraxacum officinale root in carbon tetrachloride-induced liver damage in mice. *J Ethnopharmacol*, 130, 569–577.

Fukushima T, Koide M, Ago Y, Baba A, Matsuda T. (2006). T-817MA, a novel neurotrophic agent, improves sodium nitroprusside-induced mitochondrial dysfunction in cortical neurons. *Neurochem Int*, 48, 124–130.

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. (1982). Analysis of nitrate, nitrite, and [15^N] nitrate in biological fluids. *Anal Biochem*, 126, 131–138.

Halliweel B. (1995). How to characterize an antioxidant: An update. *Free radical and oxidative stress: Environments, Drugs and Food Additives*. Portland Press, 72–102.

Halliwell B, Gutteridge JM. (1981). Formation of thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. *FEBS Lett*, 128, 347–352.

Hu C, Kitts DD. (2003). Antioxidant, prooxidant, and cytotoxic activities of solvent-fractionated dandelion (*Taraxacum officinale*) flower extracts in vitro. *J Agric Food Chem*, 51, 301–310.

Hu C, Kitts DD. (2005). Dandelion (*Taraxacum officinale*) flower extract suppresses both reactive oxygen species and nitric oxide and prevents lipid oxidation in vitro. *Phytomedicine*, 12, 588–597.

Jeon HJ, Kang HJ, Jung HJ, Kang YS, Lim CJ, Kim YM, Park EH. (2008). Anti-inflammatory activity of *Taraxacum officinale*. *J Ethnopharmacol*, 115, 82–88.

Kaur G, Alam MS, Jabbar Z, Javed K, Athar M. (2006). Evaluation of antioxidant activity of *Cassia siamea* flowers. *J Ethnopharmacol*, 108, 340–348.

Kisiel W, Barszcz B. (2000). Further sesquiterpenoids and phenolics from *Taraxacum officinale*. *Fitoterapia*, 71, 269–273.

Koh YJ, Cha DS, Ko JS, Park HJ, Choi HD. (2010). Anti-inflammatory effect of *Taraxacum officinale* leaves on lipopolysaccharide-induced inflammatory responses in RAW 264.7 cells. *J Med Food*, 13, 870–878.

Lopes GK, Schulman HM, Hermes-Lima M. (1999). Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. *Biochim Biophys Acta*, 1472, 142–152.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the Folin phenol reagent. *J Biol Chem*, 193, 265–275.

Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. (1994). The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. *Biochem Biophys Res Commun*, 201, 748–755.

Mates JM, Sanchez-Jimenez FM. (2000). Role of reactive oxygen species in apoptosis: implications for cancer therapy. *Int J Biochem Cell Biol*, 32, 157–170.

Minotti G, Aust SD. (1987). An investigation into the mechanism of citrate-Fe2+-dependent lipid peroxidation. *Free Radic Biol Med*, 3, 379–387.

Moran JF, Klucas RV, Grayer RJ, Abian J, Becana M. (1997). Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: Prooxidant and antioxidant properties. *Free Radic Biol Med*, 22, 861–870.

Mosmann T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*, 65, 55–63.

Muralidhara GKS. (2008). Effect of *Centella asiatica* leaf powder on oxidative markers in brain regions of prepubertal mice *in vivo* and *in vitro* efficacy to ameliorate 3-NPA-induced oxidative stress in mitochondria. *Phytomedicine*, 15, 971–984.

Nie BM, Yang LM, Fu SL, Jiang XY, Lu PH, Lu Y. (2006). Protective effect of panaxydol and panaxynol on sodium nitroprusside-induced apoptosis in cortical neurons. *Chem Biol Interact*, 160, 225–231.

Niu XL, Ichimori K, Yang X, Hirota Y, Hoshiai K, Li M, Nakazawa H. (2000). Tanshinone II-A inhibits low density lipoprotein oxidation in vitro. *Free Radic Res*, 33, 305–312.

Ohkawa H, Ohishi N, Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, 95, 351–358.

Pazos M, Gallardo JM, Torres JL, Medina I. (1999). Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. *Food Chem*, 92, 547–557.

Peschel W, Sánchez-Rabaneda F, Diekmann W, Plescher A, Gartzía I, Jiménez D. (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chem*, 97, 137–150.

Puntel RL, Nogueira CW, Rocha JB. (2005). Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain *in vitro*. *Neurochem Res*, 30, 225–235.

Rauhala P, Khaldi A, Mohanakumar KP, Chiueh CC. (1998). Apparent role of hydroxyl radicals in oxidative brain injury induced by sodium nitroprusside. *Free Radic Biol Med*, 24, 1065–1073.

Riobó NA, Clementi E, Melani M, Boveris A, Cadenas E, Moncada S, Poderoso JJ. (2001). Nitric oxide inhibits mitochondrial NADH:ubiquinone reductase activity through peroxynitrite formation. *Biochem J*, 359, 139–145.

Romero C, Benedí J, Villar A, Martín-Aragón S. (2010). Involvement of Hsp70, a stress protein, in the resistance of long-term culture of PC12 cells against sodium nitroprusside (SNP)-induced cell death. *Arch Toxicol*, 84, 699–708.

Sah NK, Kumar S, Subramanian M, Devasagayam TP. (1995). Variation in the modulation of superoxide-induced single-strand breaks in plasmid pBR322 DNA by biological antioxidants. *Biochem Mol Biol Int*, 35, 291–296.

Sas K, Robotka H, Toldi J, Vécsei L. (2007). Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *J Neurol Sci*, 257, 221–239.

Singleton VL, Orthofer R, Lamuela-Raventos RM. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. *Methods Enzymol*, 299, 152–178.

Wang H, Gao, XD, Zhou GC, Cai L, Yao WB. (2008). *In vitro* and *in vivo* antioxidant activity of aqueous extract from *Choerospondias auxillaris* fruit. *Food Chem*, 106, 888–895.