



Palmatine antioxidant and anti-acetylcholinesterase activities: A pre-clinical assessment

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Abstract: There is evidence that palmatine (PA), an alkaloid isolated from the *Guatteria friesiana* plant, has some important biological activities, including anti-inflammatory and antidepressant effects. In this study, the antioxidant and anti-acetylcholinesterase (AChE) effects of PA were assessed. The antioxidant capacity was evaluated *in vitro* and *in vivo* through 7 distinct assays, and the anti-AChE activity was determined *in vitro*. The standards, trolox and ascorbic acid were used for the *in vitro* antioxidant test, while hydrogen peroxide was selected as a stressor for the *Saccharomyces cerevisiae* test. Additionally, PA was also combined with trolox and ascorbic acid to determine the likelihood of synergistic effects occurrence to what concerns to antioxidant potential. PA exhibited a potent and concentration-dependent antioxidant potential, although a stronger antioxidant activity was stated using the PA + trolox combination. PA was also found to inhibit AChE activity when compared to the negative control. Thus, PA may be viewed as a promissory phytotherapeutic agent to manage oxidative stress-mediated neurological diseases, especially the Alzheimer's and Parkinson's diseases.

Key words: Palmatine; Antioxidant; Acetylcholinesterase; Oxidative stress; Neurodegenerative diseases.

Introduction

The use of medicinal plants in the treatment of various diseases is ancient and, by these days, plant-derived compounds and their usages have received an increasing attention. Scientific reports suggest that oxidative stress-mediated diseases are numerous, and among them, the neurodegenerative disorders (NDs), such as Alzheimer's (AD) and Parkinson's (PD) diseases are well-known (1).

The acetylcholinesterase (AChE) enzyme catalyzes the hydrolysis of the neurotransmitter acetylcholine to terminate signals across cholinergic synapses, including those at neuromuscular junctions. Thus, AChE inhibition

is conceived as a strategy for the treatment of NDs, especially AD. Being plant-derived molecules widely recognized for their excellent antioxidant effects, dietary supplements-rich in these bioactive substances have increasingly been viewed as promising tools in the treatment of NDs (2).

Guatteria genus belongs to the Annonaceae family and is widely-distributed in the Amazonian region of Brazil, as well as in Colombia. Costa et al. suggested that plants from this genus are rich in alkaloids with promising biological activities (3). Among them a quaternary alkaloid called palmatine (PA) isolated from *G. friesiana* leaves is reported to have anti-inflammatory and anticancer effects (4). In addition, it should be un-

derlined that, phytochemicals having antioxidant capacity may impart an anti-inflammatory activity, such as essential oils (5). Thus, this study aims to assess the antioxidant and anti-AChE capacities of PA by adopting a number of pre-clinical (*in vitro* and *in vivo*) testing methods.

Materials and Methods

Source of palmatine

G. friesiana leaves were collected in January (2016) in Amazon, Brazil. A voucher specimen (#7341) was also deposited in the Department of Biology, Institute of Biological Sciences UFAM, Brazil. PA extraction and isolation processes were carried out as previously reported by Costa *et al.* (3).

Antioxidant assays

Sample preparation

For antioxidant assays, PA and the standards, ascorbic acid (AA) and trolox (TRO) were diluted with 0.05% Tween 80 dissolved in 0.9% NaCl (vehicle) solution to attain a concentration range of 1-50 $\mu\text{g/ml}$.

In vitro antioxidant tests

1,1-diphenyl-2-picrylhydrazyl (DPPH \bullet) radical scavenging

DPPH radical scavenging activity was assessed using the method described by Manzocco *et al.* (6). Briefly, 0.3 ml of sample (PA / TRO / AA / PA + TRO / PA + AA) was added to 2.7 ml of ethanolic solution of DPPH (0.5 mM). After 30 min, the absorbance was measured by using a spectrophotometer at 517 nm. The co-treatment groups were performed with AA or TRO at 10 $\mu\text{g/ml}$. For negative control (NC), 0.3 ml of vehicle (0.05% Tween 80 dissolved in 0.9% NaCl) was added to the DPPH solution. The DPPH radical scavenging potential was calculated using the following equation:

% scavenging of DPPH \bullet = $[(A_{br} - A_{ar})/A_{br}] \times 100$, where A_{br} and A_{ar} are the absorbance of DPPH \bullet before and after the reaction, respectively.

2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS \bullet^+) radical scavenging

The ABTS radical scavenging activity was performed using the method described by Seeram *et al.* (7), with a slight modification. Briefly, the ABTS \bullet^+ was produced by the addition of solid manganese dioxide (80 mg) to an aqueous solution of 5 mM ABTS in buffer Na^+/K^+ (pH 7.0). Then, 2.8 ml of the sample was added to 0.2 ml of ABTS solution. After 5 min, the absorbance was measured at 750 nm. The ABTS \bullet^+ scavenging capacity was determined using the following equation:

% scavenging of ABTS \bullet^+ = $[(A_{br} - A_{ar})/A_{br}] \times 100$, where A_{br} and A_{ar} are the absorbance of ABTS \bullet^+ before and after the reaction, respectively.

Hydroxyl ($\bullet\text{OH}$) radical scavenging

The ability of studied samples to scavenge $\bullet\text{OH}$ was determined according to the method described by Ruch *et al.* (8). Briefly, a solution of 40 mM H_2O_2 was prepared in phosphate buffer saline (PBS: 50 mM, pH 7.4). The initial absorbance was read at 230 nm by using a

spectrophotometer, following to the addition of specific concentrations of samples and co-treatments. The final absorbance was measured at 230 nm after 10 min of sample addition. The percentage scavenging of $\bullet\text{OH}$ was calculated by using the following equation:

% scavenging of $\bullet\text{OH}$ = $[(A_0 - A)/A_0] \times 100$, where A_0 and A are the absorbance of control and test sample, respectively.

Nitric oxide (NO) radical scavenging

In this assay, 0.375 ml of the test sample was added to 1.5 ml of sodium nitroprusside (10 mM) and 0.375 ml of PBS (pH 7.4) and the absorbance (A_{br}) was read at 546 nm in a spectrophotometer. After incubating the reaction mixture at 37 $^\circ\text{C}$ for 1 h, 1 ml of the aliquot was mixed with 1 ml of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 2 ml of naphthylethylenediamine dichloride (0.1% w/v)]. The reaction mixture was then incubated at room temperature for 30 min and the final absorbance (A_{ar}) was measured at the same wavelength. For NC, 0.375 ml of the vehicle was treated accordingly (9). The NO scavenging capacity was calculated using the following equation:

% scavenging of NO \bullet = $[(A_{br} - A_{ar})/A_{br}] \times 100$, where A_{br} and A_{ar} are the absorbance of NO before and after of the addition of Griess reagent, respectively.

Lipid peroxidation inhibition

The thiobarbituric acid reactive substances (TBARS) formation assay was performed by using egg lipoprotein re-constituted in phosphate buffered saline (PBS; 20 mM, pH 7.4). Briefly, 0.1 ml of sample was added to 1 ml egg yolk (1% v/v), following to an addition of 0.1 ml of 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH; 0.12 M). The reaction mixture was incubated at 37 $^\circ\text{C}$ for 15 min. After cooling, 0.5 ml of trichloroacetic acid (TCA; 15%) was added to 0.5 ml of the aliquot of the sample and the mixture was centrifuged at 1,200 rpm for 10 min. Then, an aliquot of 0.5 ml of the supernatant was mixed with 0.5 ml of thiobarbituric acid (TBA; 0.67%) and heated at 95 $^\circ\text{C}$ for 30 min. Finally, the absorbance was measured at 532 nm by using a spectrophotometer. The lipid peroxides levels were expressed as nmol TBARS/mg of egg yolk using a coefficient 1.56×10^5 ml/cm, and the results were expressed as lipid peroxidation inhibition percentage (10).

Reducing potential (RP)

The RP test was performed according to the method described by Oyaizu (11), with slight modifications. Briefly, 0.2 ml of the sample was added to 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of $\text{K}_3\text{Fe}(\text{CN})_6$ (1% w/v) and the reaction mixture was heated at 50 $^\circ\text{C}$ for 20 min. Then, 0.5 ml of TCA (10% w/v) was added to a constant stirring, following to the addition of 1.175 ml of distilled water and 0.125 ml of FeCl_3 (0.1% w/v). After 5 min, the absorbance was measured at 700 nm. Blank contained no sample, while 0.2 ml of the vehicle was added to the NC marked tube. The RP percentage was calculated as follows:

%RP = $[(A_{ts} - A_{bs})/A_{ts}] \times 100$, where A_{ts} and A_{bs} are the absorbance of test sample and blank, respectively.

In vivo oxidant/antioxidant assay

S. cerevisiae strains

The *Saccharomyces cerevisiae* EG118 strain is deficient in the enzymatic system involving the cytoplasmic superoxide dismutase (SOD; CuZn-SOD - *SOD1* gene product), and EG110 for mitochondrial SOD (Mn-SOD - *SOD2* gene product). EG133 is the double mutant and deficient on both *SOD1* and *SOD2*. EG103 corresponds to the wild type (SOD-WT), while EG to the *SOD1* (cytosolic SOD) and catalase (*CAT-1* (cytosolic CAT) defective strains. The strains used were kindly provided by Genetic Toxicology research group from the Federal University of Rio Grande do Sul, Brazil.

Central disk test in S. cerevisiae strains

The test was performed alone-, pre-, co- and in a post-treatment manner, using hydrogen peroxide (H₂O₂) as a stressor (STR) and saline solution as NC group. Strains sources and identification were as previously demonstrated (12). Briefly, the strains were maintained in yeast extract-peptone-dextrose (YEPD) medium, and PA (10 µl) at each specified dosage (1-50 µg/ml) was added to the sterile paper disc (13). In the pre-treatment assay, the PA-treated discs were incubated in bacteriological oven for 3 h at 35 °C. Then, 10 µl of STR was added in the discs. Co-treatment was compiled with a subsequent treatment of PA + STR, while post-treatment with the STR prior to a 3 h of PA treatment. STR was used at 10 mM. All dishes were then incubated at 35 °C for 48 h. The inhibition zones (mm) were measured at a range of 0 mm (full growth) to 40 mm (no growth). All tests were triplicated.

Test for anti-acetylcholinesterase (AChE) activity

Sample preparation

PA was diluted in the same above referred vehicle to attain a concentration range from 0.0625 to 1 mg/ml, while TRO in di-methylsulphoxide (DMSO) to 50 mM. Ascorbic acid (AA) and caffeine (CAF) were also used as standards for qualitative anti-AChE assay.

Qualitative anti-AChE assay

Approximately 2.0 µl of sample was applied to a silica gel plate with the aid of a capillary tube and eluted with chloroform-methanol (9:1) solvent system. Then, plates were sprayed with DTNB (5,5'-dithiobis [2-nitrobenzoic acid])/Atci (acetylthiocholine iodide) (1 mM DTNB, and 1 mM Atci in tris buffer at pH 8) and incubated for 15 min at 37 °C, following to dry for 3-5 min and re-spraying with 10 U/ml cholinesterase enzyme. White spots appearance in yellow plates confirmed the AChE inhibitory capacity (14).

Ellman's method for anti-AChE activity (quantitative)

Ellman's method was used and adapted from Pohancka *et al.* (15). Birefly, a disposable cuvette was filled with 0.4 ml of 0.4 mg/ml DTNB, 0.025 ml of AChE solution (0.5 µkat in 1 mM acetylthiocholine), 0.425 ml of PBS, and 0.050 ml of sample (PA/TRO/PA+TRO). The reaction was started by adding 0.1 ml of acetylthiocholine chloride at a given concentration for the assessment of K_m and V_{max} or 1 mM for toxicological and pharmacological investigations. Absorbance at 412 nm was

measured immediately and after 1 min. Enzyme activity was calculated through estimation of the extinction coefficient, $\epsilon = 14,150 \text{ M/cm}$.

Statistical analysis

The results are expressed as mean \pm standard deviation (SD). The analysis of variance (ANOVA) followed by Newman-Keul's *post-hoc* test *t*-student test using GraphPad Prism software (version 6.0, San Diego, California, U.S.A., Copyright ©), considering $p < 0.05$.

Results and Discussion

Oxidative stress is triggered by an imbalance between reactive species production [e.g., reactive oxygen/nitrogen species (ROS/RNS), free radicals, oxidizable ions and metals] and of antioxidants. Although, reactive species play a number of important physiological roles, an augmented production triggers a wide number of oxidative stress-mediated disorders (12). In our study, PA markedly ($p < 0.05$) scavenged the DPPH•, ABTS•+, •OH and NO radicals in a concentration-dependent manner. PA also reduced the TBARS formation, attributed to a strong reducing capacity. In the *in vitro* antioxidant tests, the half-maximal effective concentrations (EC₅₀) of PA were found between 2.17 and 14.4 µg/ml, and a potent antioxidant capacity was observed combining PA and TRO.

Among the reactive species, the peroxynitrite radical (ONOO•), formed by the reaction between NO and O₂ may oxidize cell macromolecules, including lipids, proteins, carbohydrates and genetic materials (e.g., DNA/RNA), at same time that increases β -amyloid peptide (A β) aggregation (1). The A β aggregation is evident to accumulate in AD patients. In this sense, substances with NO scavenging capacity may be helpful in AD treatment (16). In this study, PA alone and/or with TRO/AA revealed promising NO scavenging abilities.

The simple eukaryotic system, *S. cerevisiae* strains are commonly used to determine the oxidant/antioxidant capacities of numerous substances. The membrane constitution of *S. cerevisiae* facilitates the up-take of test substances (12). In our study, in comparison to the NC group, both STR and PA concentration-dependently inhibited all *S. cerevisiae* strains growth. This may be due to their antioxidant-mediated pro-oxidative effect on yeast cells; this is because, when PA was co-treated (pre-, co- and post-treatment) with STR significantly ($p < 0.05$) increased the survival capacity of the test strains (Table 2-5). Thus, we suppose that PA-mediated •OH scavenging capacity may be linked to this effect.

Galanthamine, a potent AChE inhibitor, widely used in AD treatment has shown significant antioxidant properties in some *in vitro* models through reduction of ROS, especially the NO generation in human neuroblastoma cells treated with H₂O₂ (17). This drug also has protective capacity against H₂O₂-induced oxidative damage in human lymphocytes (18). The oxidative stress, more specifically, the lipid peroxidation phenomenon may augment the A β levels. Moreover, the tau protein hyperphosphorylation is also known as another important cause of AD. In a study, TRO has been reported to prevent tau protein hyperphosphorylation (19). In this study, PA exhibited AChE inhibitory capacity. The

Table 1. Antioxidant capacity of palmatine and of positive controls.

Treatments	EC ₅₀ (µg/ml) [CI (µg/ml), R ²]					
	DPPH test	ABTS test	OH test	NO test	TBARS test	RP test
PA	3.48 ± 0.48 [0.57-24.87, 0.97]	10.64 ± 0.74 [5.72-19.81, 0.94]	10.91 ± 0.70 [5.2222.81, 0.92]	14.47 ± 0.82 [8.11-25.83, 0.95]	3.97 ± 0.30 [2.12-7.43, 0.96]	2.17 ± 0.40 [1.04-4.51, 0.95]
TRO	0.87 ± 0.38 [0.11-6.66, 0.81]	2.32 ± 0.56 [0.56-9.64, 0.83]	4.74 ± 0.48 [1.08- 20.93, 0.79]	5.02 ± 0.50 [2.34-10.76, 0.93]	1.02 ± 0.30 [0.29-3.55, 0.90]	0.14 ± 0.22 [0.02-0.83, 0.93]
AA	1.74 ± 0.40 [1.23-7.33, 0.89]	3.85 ± 0.54 [1.76-8.39, 0.94]	4.56 ± 0.36 [2.25-9.23, 0.95]	6.23 ± 0.46 [3.27-11.85, 0.95]	3.56 ± 0.28 [2.01-6.32, 0.97]	1.48 ± 0.39 [0.29-7.55, 0.83]
PA + TRO	3.12 ± 0.54 [0.15-64.90, 0.53]	3.70 ± 0.24 [2.38-5.73, 0.98]	6.25 ± 0.44 [2.98-13.11, 0.94]	1.73 ± 0.36 [0.35-5.46, 0.87]	0.49 ± 0.22 [0.15-1.59, 0.94]	0.44 ± 0.22 [0.14-1.35, 0.94]
PA + AA	0.06 ± 0.30 [0.002-1.92, 0.85]	3.74 ± 0.50 [1.71-8.18, 0.94]	7.13 ± 0.56 [3.45-14.70, 0.93]	3.41 ± 0.40 [1.15-10.15, 0.89]	0.97 ± 0.24 [0.25-2.49, 0.93]	1.54 ± 0.22 [0.96-2.47, 0.98]

PA: palmitine; TRO: trolox; AA: ascorbic acid; EC₅₀: half-maximal effective concentration; CI: confidence interval; R²: coefficient of determination at 95% confidence level.

Table 2. Antioxidant capacity of palmatine and of controls in *S. cerevisiae*

Strains	NC	H ₂ O ₂ (50 mM)	PA (µg/ml)				
			50 µg/ml	25 µg/ml	10 µg/ml	5 µg/ml	1 µg/ml
SOD-WT	0.75 ± 0.47	30.75 ± 5.058 ^a	29.25 ± 2.98 ^a	28.25 ± 5.85	27.50 ± 9.74	17.00 ± 3.55	9.00 ± 3.46
Sod1Δ	1.75 ± 1.70	25.50 ± 4.20 ^b	20.25 ± 8.53	11.50 ± 3.87	14.00 ± 2.44	12.00 ± 2.30	10.25 ± 2.06
Sod2Δ	0.50 ± 0.28	25.25 ± 6.18 ^b	24.75 ± 11.38 ^a	14.50 ± 2.10	17.50 ± 4.12	15.75 ± 4.34	9.75 ± 0.50
Sod1ΔSod2Δ	2.75 ± 3.59	27.00 ± 5.09 ^c	18.50 ± 5.44 ^a	15.00 ± 1.55	11.50 ± 2.5	12.00 ± 3.36	12.25 ± 0.50
Cat1Δ	0.50 ± 0.57	24.25 ± 4.03 ^c	16.75 ± 2.36 ^a	11.50 ± 4.04	10.50 ± 1.91	11.75 ± 2.06	9.75 ± 0.50
Sod1ΔCat1Δ	1.25 ± 1.50	22.25 ± 4.57 ^b	16.25 ± 2.75 ^a	12.00 ± 1.82	11.25 ± 0.95	11.50 ± 1.73	12.25 ± 3.94

CAT, catalase; H₂O₂, hydrogen peroxide; NC, negative control (vehicle); PA, palmatine; SOD, superoxide dismutase. Values are mean ± SD (n = 4); ^ap <0.05, ^bp <0.001 and ^cp <0.0001 compared to the NC.

Table 3. Evaluation of antioxidant capacity of palmatine co-treated to the H₂O₂ controls in *S. cerevisiae*.

Strains	NC	H ₂ O ₂ (50 mM)	PA + 50 mM H ₂ O ₂				
			50 µg/ml	25 µg/ml	10 µg/ml	5 µg/ml	1 µg/ml
SOD-WT	0.75 ± 0.95	38.50 ± 1.91 ^a	31.25 ± 6.13 ^a	29.23 ± 2.87	30.75 ± 4.64	25.25 ± 2.06	21.25 ± 2.68 ^{a,c}
Sod1Δ	1.75 ± 0.85	35.00 ± 4.76 ^b	35.00 ± 4.24 ^a	32.00 ± 3.65 ^a	30.75 ± 4.78	24.50 ± 5.26	21.00 ± 5.7
Sod2Δ	0.50 ± 0.28	37.25 ± 3.77 ^b	31.75 ± 2.06 ^a	34.00 ± 4.69 ^a	28.00 ± 2.44	31.75 ± 2.06	23.00 ± 2.44
Sod1ΔSod2Δ	2.75 ± 3.59	30.75 ± 1.50 ^a	34.75 ± 3.73 ^a	34.75 ± 6.80 ^a	34.50 ± 3.69	27.25 ± 6.80	24.00 ± 3.55
Cat1Δ	0.50 ± 0.28	36.75 ± 4.55 ^a	32.00 ± 4.23 ^a	33.00 ± 4.96 ^a	29.75 ± 9.32	27.50 ± 7.18	26.25 ± 6.50
Sod1ΔCat1Δ	1.25 ± 1.50	36.10 ± 4.97 ^a	34.75 ± 4.99 ^a	33.00 ± 4.96 ^a	35.50 ± 5.196	31.25 ± 6.29	29.75 ± 6.94

CAT, catalase; H₂O₂, hydrogen peroxide; NC, negative control (vehicle); PA, palmatine; SOD, superoxide dismutase. Values are mean ± SD (n = 4); ^ap <0.05 and ^bp <0.001 compared to NC; ^cp <0.05 compared to the H₂O₂.

Table 4. Evaluation of antioxidant capacity of palmitate pre-treated to the H₂O₂ controls in *S. cerevisiae*.

Strains	NC	H ₂ O ₂ (50 mM)	PA (µg/ml) + H ₂ O ₂ (50 mM)				
			50 µg/ml	25 µg/ml	10 µg/ml	5 µg/ml	1 µg/ml
SOD-WT	0.75 ± 1.75	39.00 ± 5.05 ^b	30.75 ± 6.50	27.00 ± 6.05	29.75 ± 4.50	22.75 ± 6.00	23.00 ± 6.05
Sod1Δ	1.75 ± 1.70	37.00 ± 4.76 ^b	31.50 ± 5.06	30.25 ± 11.32	29.00 ± 4.54	27.50 ± 4.04	24.75 ± 4.57
Sod2Δ	0.50 ± 0.57	37.25 ± 3.77 ^b	32.50 ± 3.00	31.25 ± 10.11	30.75 ± 6.50	27.00 ± 6.05	28.00 ± 6.075
Sod1ΔSod2Δ	2.75 ± 3.59	37.75 ± 3.86 ^a	35.75 ± 5.31	31.25 ± 2.98	33.75 ± 8.008	32.00 ± 9.27	27.00 ± 8.75
Cat1Δ	0.50 ± 0.28	36.75 ± 4.50 ^a	30.50 ± 4.00	31.00 ± 10.52	29.00 ± 8.36	28.50 ± 8.58	26.75 ± 9.03
Sod1ΔCat1Δ	1.25 ± 1.50	36.00 ± 4.69 ^a	35.25 ± 3.20 ^a	32.25 ± 6.99	27.75 ± 2.63	30.75 ± 6.39	26.25 ± 7.13

CAT, catalase; H₂O₂, hydrogen peroxide; NC, negative control (vehicle); PA, palmitate; SOD, superoxide dismutase. Values are mean ± SD (n = 4); ^ap <0.05 and ^bp <0.001 compared to NC.

Table 5. Evaluation of antioxidant capacity of palmitate post-treated to the H₂O₂ controls in *S. cerevisiae*.

Strains	NC	H ₂ O ₂ (50 mM)	PA (µg/ml) + H ₂ O ₂ (50 mM)				
			50 µg/ml	25 µg/ml	10 µg/ml	5 µg/ml	1 µg/ml
SOD-WT	0.75 ± 0.95	39.00 ± 1.41 ^c	30.50 ± 7.72	28.00 ± 5.71	32.00 ± 2.94	30.35 ± 4.64	23.50 ± 3.10
Sod1Δ	1.75 ± 1.70	37.00 ± 4.76 ^b	32.00 ± 6.97 ^{*a}	25.00 ± 2.16	27.75 ± 7.63	22.25 ± 5.60	17.00 ± 4.96
Sod2Δ	0.50 ± 3.77 ^b	37.25 ± 3.77 ^a	34.50 ± 4.20	33.50 ± 4.65	28.75 ± 3.86	23.50 ± 5.74	24.75 ± 6.94
Sod1ΔSod2Δ	2.75 ± 3.5 ^b	37.75 ± 3.86 ^b	34.00 ± 5.83	30.25 ± 5.9	23.25 ± 2.75	28.50 ± 9.4	22.00 ± 8.12
Cat1Δ	0.50 ± 0.57 ^a	36.75 ± 4.57 ^a	30.00 ± 6.16	27.25 ± 6.6	24.25 ± 4.50	22.00 ± 8.08	20.00 ± 2.16
Sod1ΔCat1Δ	1.25 ± 1.50	36.00 ± 4.69 ^b	33.00 ± 6.92 ^a	29.25 ± 4.99	24.75 ± 3.50	23.75 ± 2.87	18.25 ± 9.60

CAT, catalase; H₂O₂, hydrogen peroxide; NC, negative control (vehicle); PA, palmitate; SOD, superoxide dismutase. Values are mean ± SD (n = 4); NC: negative control (vehicle); ^ap <0.05, ^bp <0.001 and ^cp <0.0001 compared to the NC.

IC₅₀ values of PA and TRO in the anti-AChE assay were measured at 0.294 and 2.256 µg/ml, respectively. PA co-treated with TRO/AA showed better ($p < 0.05$) anti-AChE and antioxidant activity, including TBARS, than using PA and TRO/AA alone.

In short, PA exhibited potent antioxidant and anti-AChE abilities. Strong •OH scavenging capacity appears to be linked to the antioxidative defense in *S. cerevisiae* cells. The PA' anti-AChE effect seems to be attributed to its •OH and NO scavenging effects, as well as to its prominent TBARS formation inhibition capacity. Thus, PA may be conceived as a good phytotherapeutic tool to manage oxidative stress-mediated NDs, especially AD and PD. Our data also show that the co-administration of PA with other antioxidants, such as TRO and AA, seems to be a key strategy and with promising potential.

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Conflict of interest

The authors declare no conflict of interests.

References

- Islam MT, Silva CB, Alencar MVOB, Paz MF, Almeida FR, Melo-Cavalcante AA. Diterpenes: Advances in Neurobiological Drug Research. *Phytother Res* 2016; 30:915-28.
- Ota K, Oishi N, Ito K. Effects of imaging modalities, brain atlases and feature selection on prediction of Alzheimer's disease. *J Neurosci Meth* 2015; 256:168-83.
- Costa EV, Cruz PEO, Pinheiro MLB, Marques FA, Ruiz ALTG, Marchetti GM, Carvalho JE, Barison A, Maia BHLNS. Aporphine and tetrahydroprotoberberine alkaloids from the leaves of *Gutteria friesiana* (Annonaceae) and their cytotoxic activities. *J Braz Chem Soc* 2013; 24:788-96.
- Vrba J, Papouskova B, Pyszkova M, Zatloukalova M, Lemr K, Ulrichova J, Vacek J. Metabolism of palmatine by human hepatocytes and recombinant cytochromes P450. *J Pharm Biomed Analysis* 2015; 102:193-8.
- Islam MT, Mata AMOF, Aguiar RPS, Paz MFCJ, Alencar MVOB, Melo-Cavalcante AAC. Therapeutic Potential of Essential Oils Focusing on Diterpens. *Phytother Res* 2016; 30:1420-44.
- Manzocco L, Anese M, Nicoli MC. Antioxidant properties of tea

extracts as affected by processing. *Lebensmittel-Wissenschaft und -Technologie* 1998; 31:694-8.

- Seeram NP, Henning SM, Lee R, Niu Y, Scheuller HS, Heber D. Catechin and caffeine contents of green tea dietary supplements and correlation with antioxidant activity. *J Agric Food Chem* 2006; 54:1599-603.
- Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogen* 1989;10:1003-8.
- Marcocci I, Marguire JJ, Droy-lefaiz MT, Packer L. The nitric oxide scavenging properties of Ginkgo biloba extract. *Biochem Biophys Res Commun* 1994; 201:748-55.
- Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. *Meth Enzymol* 1990; 186:407-21.
- Oyaizu, M. (1986). Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucosamine. *J Nutri* 1986; 44:307-15.
- Islam MT, Streck L, Paz MFCJ, Sousa JMC, Alencar MVOB, Mata AMOF, Carvalho RM, Santos JVO, Silva-Junior AA, Ferreira PMP, Melo-Cavalcante AAC. Preparation of phytol-loaded nano-emulsion and screening for antioxidant capacity. *Int Arch Med* 2016; 9:1-15
- Fragoso V, Nascimento NC, Moura DJ, Silva ACR, Richter MF, Saffi J. Antioxidant and antimutagenic properties of the monoterpene indole alkaloid psychollatine and the crude foliar extract of *Psychotria umbellata* Vell. *Toxicol Vitro* 2008; 22:559-66.
- Rhee IK, Meent MV, Ingkaninan K, Verpoorte R. Screening for acetylcholinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. *J Chromatogr A* 2001; 915:217-23.
- Pohanka M, Hrabanova M, Kuca K, Simonato J-P. Assessment of Acetylcholinesterase Activity Using Indoxylacetate and Comparison with the Standard Ellman's Method. *Int J Mol Sci* 2011; 12:2631-40 .
- Mestres G, Santos CF, Engman L, Persson C, Ott MK. Scavenging effect of trolox released from brushite cements. *Acta Biomaterialia* 2015; 11:459-66.
- Barrera G. Oxidative Stress and Lipid Peroxidation Products in Cancer Progression and Therapy. *ISRN Oncol* 2012; 2012:137289.
- Triana-Vidal LE, Carvajal-Varona SM. Protective effect of galantamine against oxidative damage using human lymphocytes: A novel *in vitro* model. *Arch Med Res* 2013; 44:85-92.
- Warner TA, Kang JQ, Kennard JA, Harrison FE. Low brain ascorbic acid increases susceptibility to seizures in mouse models of decreased brain ascorbic acid transport and Alzheimer's disease. *Epilep Res* 2015; 110:20-5.