Programa de Pós-graduação em Ciências Farmacêuticas[1], Núcleo de Tecnologia Farmacêutica, Laboratório de Pesquisa em Neuroquímica Experimental, Universidade Federal do Piauí (NTF/LAPNEX/UFPI), Teresina-Piauí; Departmento de Fisiologia e Farmacologia da Universidade Federal do Ceará[2], Fortaleza, Ceará, Brazil

Effects of an aqueous extract of *Orbignya phalerata* Mart on locomotor activity and motor coordination in mice and as antioxidant *in vitro*

A. P. dos S. Silva[1], G. S. Cerqueira[2], L. C. C. Nunes[1], R. M. de Freitas[1]

Received July 19, 2011, accepted August 23, 2011

Dr. Rivelilson M. De Freitas, Laboratório de Pesquisa em Neuroquímica Experimental, Universidade Federal do Piauí - UFPI, Campus Universitário Ministro Petrônio Portella, Curso de Farmácia, Bairro Ininga, Teresina, Piauí, Cep: 64.049-550
rivelilson@pq.cnpq.br

Pharmazie 67: 260–263 (2012) *doi: 10.1691/ph.2012.1105*

The antioxidant activities of aqueous extract (AE) of *Orbignya phalerata* were assessed *in vitro* as well as its effect on locomotor activity and motor coordination in mice. AE does not possesses a strong antioxidant potential according to the scavenging assays; it also did not present scavenger activity *in vitro*. Following oral administration, AE (1, 2 and 3 g/kg) did not significantly change the motor activity of animals when compared with the control group, up to 24 h after administration and did not alter the remaining time of the animals on the Rota-rod apparatus. Further studies currently in progress will enable us to understand the mechanisms of action of the aqueous extract of *Orbignya phalerata* widely used in Brazilian flok medicine.

1. Introduction

The palm tree *Orbignya phalerata* Mart (family Arecaceae) is populary known in Piauí and Maranhão, northeasten Brazil, as babassu. This palm tree is the most abundant species of the genus (Cantanhede 2005; Teixeira 2008).

The fruit of the babassu palm is fully exploited, with its various parts being used as energy, food and remedy (Prance 1987). The use of products such as mesocarp and the residue derived from babassu for the tratment of gastritis and for woundhealing is part of popular knowledge acumulated over centuries (Andrade 1986).

Souza et al. (2011) showed that, in ethnopharmacological studies, 68% of the nut breakers used products derived from babassu for the tratment of disease, collaborating with previous results that show various pharmacological activities such as antiinflammatory, treatment of gastritis, immunomodulatory action and anti-tumor activity (Silva 2001; Rennó et al. 2008).

Free radicals and related reactive species are strongly involved in several pathological and physiological processes, including seizures, cancer, cell death, inflammation and pain (Freitas et al. 2004; Xavier 2007; Santos et al. 2010). Many natural products exert significant antioxidant activities, which are related to their therapeutical properties or even a possible toxic effect (Guimarães et al. 2010). The evaluation of the redox properties of such compounds is crucial for both understanding the potential mechanisms of their biological actions and to determine possibly toxic or harmful side-effects. Considering the lack of experimental evidence and scientific investigations about possible therapeutic and/or redox properties of *Orbignya phalerata*, the purpose of the present study was to evaluate the redox properties and possible effects in locomotor activity and motor coordination of aqueous extract of the mesocarp of *Orbignya phalerata* Mart (Fig. 1) in mice.

2. Investigations and results

We investigated the antioxidant potential of aqueous extract of *Orbignya phalerata* against two different reactive species *in vitro*. Aqueous extract of *Orbignya phalerata* did not demonstrate a significant scavenging effect against NO (Fig. 3).

Aqueous extract of *Orbignya phalerata* also did not have a strong scavenging effect against hydroxyl radicals generated *in vitro* (Fig. 4). These results did not suggest a protection against lipoperoxidation chain reactions observed in TBARS assay. *Orbignya phalerata* components extract may interact with hydroxyl radicals, which are reactive oxygen species (ROS), instead with NO, which is a reactive nitrogen species (RNS).

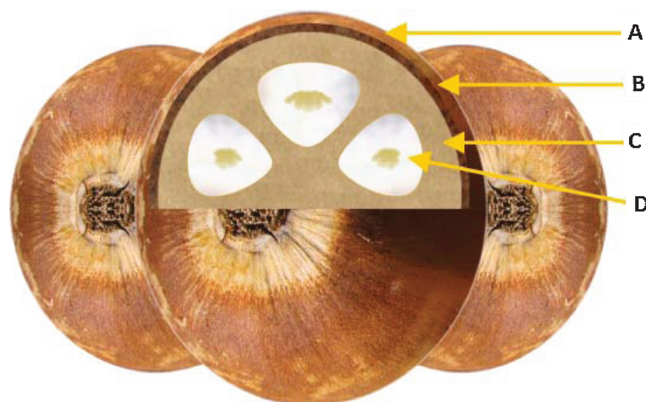

Fig. 1: Babassu: A) epicarp, B) mesocarp, C) endocarp and D) nut

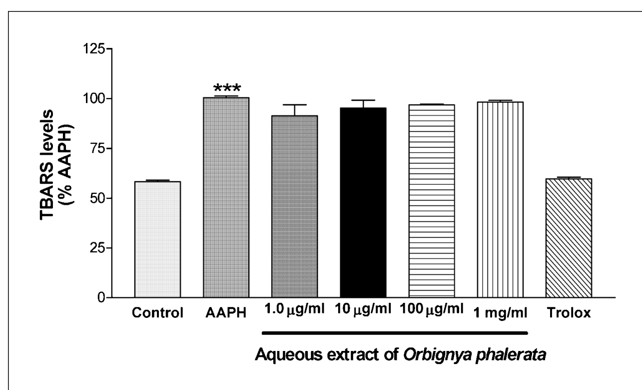

Fig. 2: Effects of aqueous extract (AE) of *Orbignya phalerata* on thiobarbituric acid-reactive substances (TBARS) *in vitro*. Control is incubation medium without AAPH; other groups contained AAPH alone or in the presence of different concentrations of aqueous extract (AE) of *Orbignya phalerata*. Trolox was used as standard antioxidant. Bars represent mean ± SEM. $*p < 0.05$, $***p < 0.0001$ (1-way ANOVA followed by Tukey's multiple comparison post hoc test)

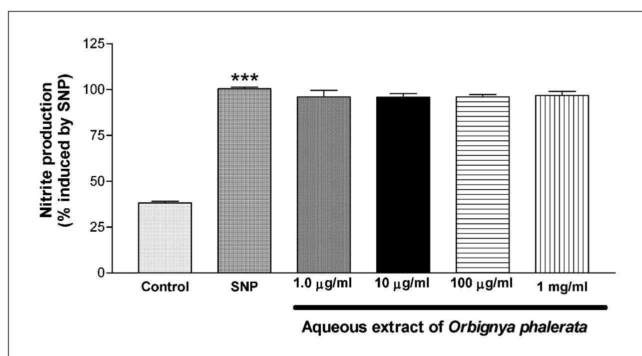

Fig. 3: Effects of aqueous extract (AE) of *Orbignya phalerata* on nitric oxide (NO) scavenging assay. Nitric oxide is generated from spontaneous decomposition of sodium nitroprusside (SNP) and interacts with oxygen to produce nitrite ions, which are measured by the Griess reaction. SNP group is sodium nitroprusside alone; other groups denote nitrite production by SNP in the presence of different concentrations of aqueous extract (AE) of *Orbignya phalerata*. Bars represent mean ± SEM. $***p < 0.0001$; $*p < 0.05$. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to all data

In doses of 1, 2 or 3 g/kg the aqueous extract of *Orbignya phalerata* did not cause significant changes in ambulation (number of crossings) at 24 h after administration (Fig. 5).

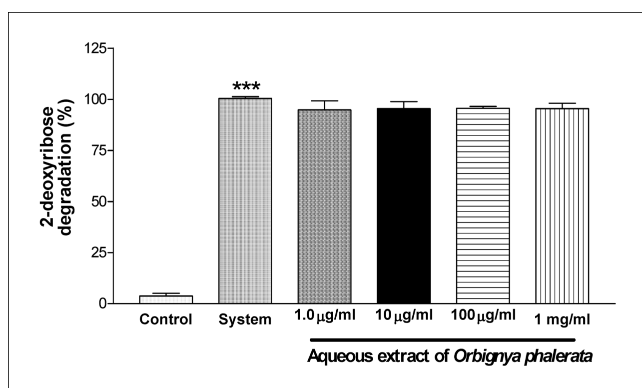

Fig. 4: Effects of aqueous extract (AE) of *Orbignya phalerata* on hydroxyl radical-scavenging activity quantified using 2-deoxyribose oxidative degradation *in vitro*, which produces malondialdehyde (MDA) by condensation with 2-thiobarbituric acid (TBA). System is MDA production from 2-deoxyribose degradation with $FeSO_4$ and H_2O_2 alone. Other groups denote MDA production by $FeSO_4$ and H_2O_2 in the presence of different concentrations of aqueous extract (AE) of *Orbignya phalerata*. Bars represent mean ± SEM. $***p < 0.0001$; $*p < 0.05$. One-way ANOVA followed by Tukey's multiple comparison post hoc test was applied to all data

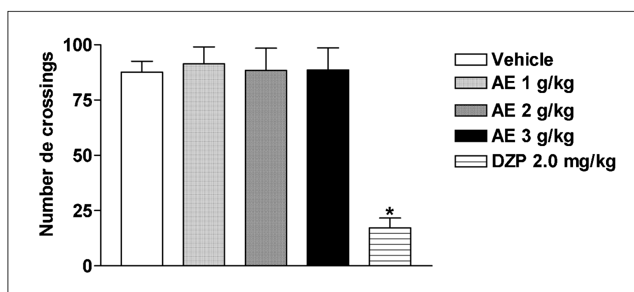

Fig. 5: Effects of aqueous extract (AE) of *Orbignya phalerata* (1, 2 and 3 mg/kg, o.r.) or diazepam (DZP, 2.0 mg/kg, i.p.) on locomotor activity of mice. The parameters evaluated were the total number of pulses of crossings in activity cage. Values are the mean ± S.E.M. for 7 mice (per group). $*p < 0.001$ as compared to control (Vehicle), one way ANOVA followed by t-Student-Neuman-Keuls test

Twenty-four hours after administration, the aqueous extract of *Orbignya phalerata* did not change the remaining time of animals on the Rota-rod apparatus (Fig. 6).

3. Discussion

The powder of babassu mesocarp is containing 68.3% starch, 1.54% protein, 0.27% lipid, 1.25% soluble carbohydrates, 2.51% fiber and amino. Hemicellulose and pentosans, are said to be also present among other organic substances (Rosenthal 1975; Garros-rosa 1986). The phytochemical screaning did not show any chemical groups that may be related to pharmacological activity, however a positive reaction was observed for polyphenols.

Membrane lipids are the most susceptible target of free radicals attack and propagation in biological systems (Freitas et al. 2005; Halliwell et al. 2008). Additionally, free radicals and related reactive species are strongly involved in several pathological and physiological processes, including cancer, cell death, inflammation and pain (Reanmongkol et al. 1997; Halliwell et al. 2007). Thus, we assessed the antioxidant potential of an aqueous extract of *Orbignya phalerata* by testing its ability to prevent oxidative damage to lipids induced by a free radical source *in vitro* (AAPH). Quantification of TBARS demonstrated that aqueous extract of *Orbignya phalerata* neither exerted a significant antioxidant effect against peroxyl radicals generated by AAPH, nor protected lipids from oxidation in a dose-dependent manner (Fig. 2). Trolox (300 µM), a synthetic analogue of vitamin E, which protects membranes from oxidative damage *in vivo*, was used as a general antioxidant standard for comparison. Therefore, it is possible that the aqueous extract of *Orbignya phalerata* did not interact more strongly with specific types of lipids, and in a lipid-rich system such as in the TBARS assay lipids with lesser affinity to aqueous extract of *Orbignya phalerata* and/or

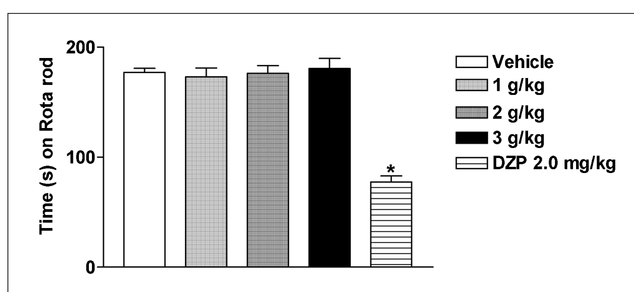

Fig. 6: Time (s) on the Rota-rod observed in mice after oral route treatment with vehicle (control), aqueous extract of *Orbignya phalerata* (1, 2 and 3 mg/kg, o.r.) or DZP (2.0 mg/kg, i.p.). The motor response was recorded for the following 180 s after drug treatment. Values are the mean ± S.E.M. for 7 mice (per group). $*p < 0.001$ as compared to control (vehicle), one way ANOVA followed by t-Student-Neuman-Keuls test

hydrophilic portions of amphipatic lipids are more susceptible to radical attack, allowing the initiation of lipoperoxidation chain reaction (Guimarães et al. 2010).
Further studies currently in progress will enable us to understand the precise action mechanisms of aqueous extract of *Orbignya phalerata* widely used in Brazilian ethnomedicine.

4. Experimental

4.1. Preparations of extracts Orbignya phalerata

The plant material was collected in February 2010, at the city of Parnarama, state of Maranhao, Brazil, and a sample was deposited at the Graziella Barroso Herbarium of the Federal University of Piaui under the voucher number 26.453. The aqueous extract of the mesocarp of *Orbignya phalerata* was prepared in the Laboratory of Pharmaceutical Thecnology of this University.
The collected fruits of *Orbignya phalerata* were dried under shade and extracted mechanically. The yield of powder mesocarp (Fig. 1) was approximately forty percent. The powder obtained was suspended in demineralized water at a concentration of 20%. The suspension of the mesocarp was kept at 4 °C in a refrigerator in air tight bottles until use. A weighed amount of the aqueous extract was taken for experiments.

4.2. In vitro tests

Phytochemical screening, described below for search saponins, steroids and terpenoids, alkaloids, anthraquinones, flavonoids and tannins was based on the method developed by Wall et al. the chemical classification of more than 4.000 different plant species. Data were obtained through the results of qualitative color reactions and precipitation, based on the chemical and physical-chemical properties of substances and tracked the major groups of chemical constituents that make up the plant material (Matos 1997; Agra 1990).
Sodium nitroprusside, $FeSO_4$, Griess' modified reagent, 2-deoxyribose, 2-thiobarbituric acid, AAPH (2,2'-azobis[2-methylpropi onamidine]dihydrochloride), trichloroacetic acid, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid), acetic acid, norhydroguaiaretic acid and polyoxyethylene-sorbitan monolate (Tween 80) were purchased from Sigma Co. (USA). All drugs and the aqueous extract of *Orbignya phalerata* were administered orally (o.r.) in volumes of 0.1 ml/10 g (mice).
TBARS (thiobarbituric acid reactive species) assay was employed to quantify lipid peroxidation (Freitas et al. 2004; Esterbauer et al. 1990) and an adapted TBARS method was used to measure the antioxidant capacity of aqueous extract of *Orbignya phalerata* using egg yolk homogenate as lipid rich substrate (Guimarães et al. 2010). Briefly, egg yolk was homogenized (1% w/v) in 20 mM phosphate buffer (pH 7.4); 1 ml of homogenate was sonicated and then homogenized with 0.1 ml of the aqueous extract of *Orbignya phalerata* at different concentrations. Lipid peroxidation was induced by addition of 0.1 ml of AAPH solution (0.12 M). Control was only aqueous extract vehicle (ethanol 0.1%). Reactions were carried out for 30 min at 37 °C. After cooling, samples (0.5 ml) were centrifuged with 0.5 ml of trichloroacetic acid (15%) at 1200 × g for 10 min. An aliquot of 0.5 ml from supernatant was mixed with 0.5 ml TBA (0.67%) and heated at 95 °C for 30 min. After cooling, samples absorbance was measured using a spectrophotometer at 532 nm. The results were expressed as percentage of TBARS formed by AAPH alone (induced control).
The formation of •OH (hydroxyl radical) from Fenton reaction was quantified using 2-deoxyribose oxidative degradation (Lopes et al. 1999). The principle of the assay is the quantification of the 2-deoxyribose degradation product, malondialdehyde, by its condensation with 2-thiobarbituric acid (TBA). Briefly, typical reactions were started by the addition of Fe^{2+} ($FeSO_4$ 6 mM final concentration) to solutions containing 5 mM 2-deoxyribose 100 mM H_2O_2 and 20 mM phosphate buffer (pH 7.2). To measure the antioxidant activity of the aqueous extract of *Orbignya phalerata* against hydroxyl radical, different concentrations of aqueous extract of *Orbignya phalerata* were added to the system before Fe^{2+} addition. Reactions were carried out for 15 min at room temperature and were stopped by the addition of 4% phosphoric acid (v/v) followed by 1% TBA (w/v, in 50 mM NaOH). Solutions were boiled for 15 min at 95 °C, and then cooled at room temperature. The absorbance was measured at 532 nm and results were expressed as MDA equivalents formed by Fe^{2+} and H_2O_2.
Nitric oxide was generated from spontaneous decomposition of sodium nitroprusside in 20 mM phosphate buffer (pH 7.4). Once generated NO interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction (Basu, 2006). The reaction mixture (1 ml) containing 10 mM sodium nitroprusside (SNP) in phosphate buffer and aqueous extract of *Orbignya phalerata* at different concentrations were incubated at 37 °C for 1 h. A 0.5 ml aliquot was taken and homogenized with 0.5 ml Griess reagent. The absorbance of chromophore was measured at 540 nm. Percent inhibition of nitric oxide generated was measured by comparing the absorbance values of negative controls (only 10 mM sodium nitroprusside and vehicle) and assay preparations. Results were expressed as percentage of nitrite formed by SNP alone.

4.3. Animal studies

Male Swiss mice (25–30 g), with 2 months of age, were used throughout this study. The animals were randomly housed in appropriate cages at 22 ± 1 °C on a 12 h light/dark cycle (lights on 06:00 a.m. -18:00 p.m.) with free access to food (Purina) and water. They were used in groups of 7 animals each. Experimental protocols and procedures were approved by Ethics Committe on Animal Experiments of the Federal University of Piaui (CEEA/UFPI No 044/09).
Mice were divided into four groups of 7 animals each. Vehicle (saline/Tween 80 0.5%; control group) and aqueous extract of *Orbignya phalerata* (1, 2 and 3 g/kg, o.r.) were injected. The spontaneous locomotor activity of the animals was assessed in a cage (50 cm × 50 cm × 50 cm) after 30 days of treatment (Asakura et al. 1993).
A *Rota-rod* tread mill device (AVS®, Brazil) was used for the evaluation of motor coordination. Initially, the mice able to remain on the *Rota-rod* apparatus longer than 180 s (9 rpm) were selected 24 h before the test. Thirty minutes after 30 days of administration of either aqueous extract of *Orbignya phalerata* (1, 2 and 3 g/kg, o.r.), vehicle (saline/Tween 80 0.5%; control group) or diazepam (DZP, 2.0 mg/kg, i.p.), each animal was tested on the Rota-rod apparatus and the time (s) remained on the bar for up to 180 s was recorded after 30 days of treatment.

4.4. Statistical analyses

The obtained data were evaluated by one-way analysis of variance (ANOVA) followed by Student-Neuman-Keuls *t*-test Dunnett's or Fisher's tests. In all cases differences were considered significant if $p < 0.05$.
The percent of inhibition by an antinociceptive agent was determined for the acetic acid-induced writhing and formalin tests using the following formula: Inhibition % = 100 . (control-experiment)/control (Reanmongkol et al. 1994).

Acknowledgements: This research was supported by Ativa Vida® Company, which provided the samples of Babassu coconut mesocarp.

References

Andrade MCA (1986) Terra e o homem no Nordeste: contribuição ao estudo da questão agrária no Nordeste, 5th ed. Atlas, São Paulo.
Agra MF, Barbosa Filho JM (1990) Levantamento da flora medicinal da Paraíba e triagem fitoquímica. Rev Bras Farmacog 71: 72–76.
Asakura W, Matsumoto K, Ohta H, Watanabe H (1993) Effect of alpha-adrenergic drugs on REM sleep deprivation-induced ineases in swimming activity. Pharmacol Biochem Behav 46: 111–115.
Basu S, Hazra B (2006) Evaluation of nitric oxide scavenging activity, *in vitro* and ex vivo, of selected medicinal plants traditionally used in inflammatory diseases. Phytother Res 20: 896–900.
Cantanhede LB (2005) Avaliação da capacidade de adsorção do epicarpo de babaçu (*Orbygnia phalerata*) in natura frente a diferentes adsortivos. Masters thesis, Universidade Federal do Maranhão, São Luis, 115f.
Esterbauer H, Cheeseman KH (1990) Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Meth Enzymol 186: 407–421.
Freitas RM, Sousa FCF, Vasconcelos SMM, Viana GSB, Fonteles MMF (2004) Pilocarpine-induced seizures in adult rats: lipid peroxidation level, nitrite formation, GABAergic and glutamatergic receptor alterations in the hippocampus, striatum and frontal cortex. Pharmacol Biochem Behav 78: 327–332.
Freitas RM, Souza FCF, Vasconcelos SMM, Viana GSB, Fonteles MMF (2005) Oxidative stress in the hippocampus after status epilepticus in rats. FEBS J 272: 1307–1312.
Garros-Rosa I (1986) Estudo químico qualitativo e quantitativo do resíduo amiláceo do coco babaçu. Rev Quim Ind 647: 15–18.
Guimarães AG, Oliveira GF, Melo MS, Cavalcanti SCH, Antoniolli AR, Bonjardim LR, Silva FA, Santos JPA, Rocha RF, Moreira JCF, Araújo AAS, Gelai DP, Quintans-Júnior LJ (2010) Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. B Clin Pharmacol Toxicol 107: 949–957.
Halliwell B (2008) Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and *in vivo* studies? Arch Biochem Biophys: 476: 107–112.

Halliwell B, Gutteridge JMC (2007) Free radicals in biology and medicine, 4th ed. Oxford University Press, Oxford.

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

Matos FJA (1997) Introdução a fitoquímica experimental, 2 ed. Fortaleza: UFC.

Prance GT, Balée W, Boom BM, Carneiro RC (1987) Quantitative ethnobotany and case for conservation in Amazonia. Conserv boil 1: 296–310.

Reanmongkol W, Matsumoto K, Watanabe H, Subhadhirasakul S, Sakai SI (1994) Antinociceptive and antipyretic effects of alkaloids extracted from the stem bark of *Hunteria zeylanica*. Biol Pharmac Bull 17: 1345–1350.

Rennó MN, Barbosa GM, Zancan P, Veiga VF, Alviano CS, Penna MS, Menezes FS, Holandino C (2008) Crude ethanol extract from babassu (*Orbignya speciosa*): cytotoxicity on tumoral and non-tumoral cell lines. Ann Braz Acad Sci 80: 467–476.

Rosenthal FRT (1975) O amido do coco de babaçu, algumas propriedades dos grânulos e das pastas. Rev Bras Tecnol 6: 29–33.

Santos IMS, Freitas RM, Silva EP, Feitosa CM, Saldanha GB, Souza GF, Tomé AR, Feng D, Freitas RM (2010) Effects of ubiquinone on hydroperoxide concentration and antioxidant enzymatic activities in the rat hippocampus during pilocarpine-induced seizures. Brain Res 1315: 33–40.

Silva BP, Parente P (2001) An antiinflammatory and immunomodulatory polysaccharide from *Orbignya phalerata.* Fitoterapia 72: 887–893.

Souza MH, Monteiro CA, Figueredo PM., Nascimento FR, Guerra RN (2011) Ethnopharmacological use of babassu (*Orbignya phalerata* Mart) in communities of babassu nut breakers in Maranhão, Brazil. J Ethnopharmacol 133: 1–5.

Xavier SML, Barbosa CO, Barros DO, Silva RF, Oliveira AA, Freitas RM (2007) Vitamin C antioxidant in hippocampus of adult Wistar rats after seizures and status epilepticus induced by pilocarpine. Neurosci Lett 420: 76–79.

Teixeira MA (2008) Babassu: A new approach for an ancient Brazilian biomass. Biomass Bioenerg 32: 857–886.