Tropical Journal of Pharmaceutical Research June 2012; 11 (3): 437-444

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v11i3.13

Research Article

Effect of Boiling on the Cytotoxic and Antioxidant Properties of Aqueous Fruit Extract of Desert Date, *Balanites aegyptiaca* (L) Delile

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Abstract

***Purpose**: To evaluate the effect of boiling on in vitro bioactivities potency of Balanites aegyptiaca L. Delile (desert date) aqueous extract, a juice used traditionally for cooking ready-to-eat millet flour paste.*
***Methods**: Desert date fruits (1.5 kg) were soaked in water (1:2, fruit: water) for 24 h and sieved. The extract was divided into two parts - fresh extract (Fext) and boiled extract (Bext) which was obtained by boiling a portion of Fext for 10 min. The extracts were tested against the stomach cancer cell line SGC7901 and for antioxidant activity by 1,1-diphenyl-2-icrylhydrazyl DPPH, hydroxyl radical and ferric reducing power methods.*
***Results**: Both fresh extract (Fext) and boiled extract (Bext) exhibited pronounced antioxidant activity with DPPH values of 88.2 and 97.0 %, respectively, at hydroxyl radical concentration of 5 mg/ml. The extract contained a significant amount of vitamin C (42.3 and 38.9 mg/100 g for Fext and Bext, respectively). Boiling had significant effect ($p < 0.01$) on its antioxidant activity and also on its cytotoxic effect (56 % and 44 % dead cells respectively for Bext and Fext at respectively, at a concentration of 200 µg/ml).*
***Conclusion**: It is concluded that B. aegyptiaca aqueous extracts have remarkable cytotoxic activity against stomach cancer cell SGC7901.*

***Keywords:** Balanites aegyptiaca, Desert date, Cytotoxicity, Antioxidant activity, Stomach cancer cell line, DPPH radical*

Received: 23 October 2011 *Revised accepted: 24 March 2012*

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INTRODUCTION

Balanites aegyptiaca (L.) Delile (*Balanitaceae*), popularly known as Desert date, is a spiny, evergreen tree commonly grown in the arid regions of Africa, the Middle East, and southern Asia [1]. It is a multi-branched, spiny shrub or tree which grows up to 10 m in height [1,2]. Almost all the parts of *B. aegyptiaca* plant are traditionally used in several folk medicines. In the Sahara region of Africa, the fruits are used as oral hypoglycemic drug [3] while the stem, root and leaf extracts of *B. aegyptiaca* have commonly been used as various traditional folk medicines especially in Africa and southern Asia. The fruits are also commonly used as purgative, antiparasitic and schistosomicide. The fruit mesocarp contains a large variety of phytochemicals such as pregnane glycosides, coumarins, flavonoids, alkaloids, 6-methyl-diosgenin and furostanol saponins [3,4].

There is a wide range of oxygen-free radicals and other reactive oxygen species (ROS). They include free radicals such as superoxide anion radicals (O_2^{-}•), hydroxyl radicals (HO•), and non-free-radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), which may form in the human body and in foods. These radicals induce not only lipid peroxidation that causes deterioration of foods, but also cause oxidative damage by oxidizing biomolecules leading to cell death and tissue damage, such as atherosclerosis, cancer, emphysema, cirrhosis and arthritis [5,6]. Currently, the natural antioxidant *α*-tocopherol and some synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole and propyl gallate are commonly used to mop up free radicals in food and biological systems. However, the use of synthetic antioxidants in food products is regulated owing to their potential health hazards [5].

Cancer is a disorder that develops due to some molecular changes within the cell. It is the third leading cause of death worldwide, after cardiovascular, and infectious and parasitic diseases [6,7]. Stomach cancer is one of the most common causes of malignancy-related death worldwide [1,8]. Gnoula *et al* reported diosgenyl saponins isolated from *Balanites aegyptiaca* Del., consisting of a mixture of balanitin-6 (28 %) and balanitin-7 (72 %) and which has appreciable anticancer affects on human cancer cell [8]. In China, the annual average mortality rate of gastric carcinoma is as high as 16 per 100 thousand [9]. Chemoprevention and chemotherapy, including the use of natural products, synthetic compounds or dietary supplements, are promising ways to stop or reverse the process of carcinogenesis [8]. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for lead drugs because of the unmatched availability of chemical diversity [7,10].

The present study aimed to test the effect of boiling *B. aegyptiaca* aqueous extract on the stomach cancer cell line SGC7901 and on its antioxidant properties. This traditional food is used for cooking millet flour ready-to-eat meal in the Sahel region of Africa [1,2]. Furthermore, the chemical composition of *B aegyptiaca* aqueous extracts, such as polyphenol, flavonoids, soluble protein and vitamin C, were also determined.

EXPERIMENTAL

Materials

The fruits of *Balanites aegyptiaca* L. Delile were collected from Gao, Mali in September 2010 and supplied by Mss. Foutouma Tounkara, Department of Food Sciences, University of Bamako, Mali. The fruits were identified by Dr Chiacka Diakite, Section Médecine Traditionnelle, Institut National de Recherche en Santé Publique, Mali, and Dr Tan Ya Li of School of Medicine and Pharmaceutics, Jiangnan University,Wuxi, China. A voucher specimen (no. INRSP

7402) has been kept in the herbarium at the Institut National de Recherche en Santé Publique, Bamako, Mali. Stomach cancer cell line SGC7901 was provided by the Cell Bank of Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China) while 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich, Inc (Shanghai, China). Vitamin C was purchased from Zhejiang Weishi Biotechnology Co., Ltd (Zhejiang, China). All other reagents used were of analytical grade.

Extract preparation

The fruits (1.5 kg) were pealed, soaked in 3 L of distilled water (1:2) for 24 h in conical flask, sieved and then filtered through Whatman filter paper no 1. The extract was divided into two parts - fresh extract (Fext) and boiled extract (Bext) which boiled for 10 min and cooled. The extracts were concentrated to dryness with a rotary evaporator at reduced pressure, lyophilised, lyophilised (Floor-model freeze dryer, serial No. 050639219 A, Labconco Co., Kansas, USA) and stored at -20 ^{o}C until used

Determination of total phenolic content

Folin–Ciocalteu method was used for total phenolic content determination as described by Vázquez *et al* [11]. Folin–Ciocalteu reagent (2.5 ml) was diluted with water (1:10, v/v), and mixed with 2 ml of 75 g/l aqueous solution of sodium carbonate. The resultant solution was added to 0.5 ml of the aqueous desert date extract. The mixture was kept for 5 min at 50 ^{o}C before measuring the absorbance at 760 nm. The total phenolic content was determined from the calibration curve ($y = 0.1536x – 0.1433$; $R^2 = 0.9845$) of gallic acid standard solutions (1–20 mg/l) and expressed as mg gallic acid equivalent (GAE)/100g of *B. aegyptiaca* extract.

Flavonoid content determination

The flavonoid content of the extracts was determined according to the method of Meda *et al*.[12] with slight modification. The desert date extract (0.5 ml) was mixed with 0.5 ml methanol, 50 µl of 10 % $AlCl_3$, 50 µl of 1M potassium acetate and 1.4 ml distilled water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was then measured at 415 nm and total flavonoid was calculated using quercetin as standard ($y = 0.289x – 0.0036$; $R^2 = 0.998$) as mg of quercetin equivalent QE.100/mg of extract.

Ascorbic acid and soluble protein determination

Ascorbic acid was determined according to the 2,6-dichlorophenol-indophenol titration method [13]. The results (titre readings) were expressed as mg/100g of the extract. Soluble protein was determined according to the method of Bradford [14] which employed bovine serum albumin as standard for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.

Anticancer activity assay

Tryphan blue dye assay method [15] was used to evaluate the *in vitro* anticancer activity of the extracts. Both extracts (Fext and Bext) were aseptically passed through 0.02 µm microbe-free filter (Sigma-Aldrich, Shanghai, China) prior to use. Two samples per concentration (200, 150, 100, 50 and 25 µg/ml) of Fext and Bext extracts were prepared and 100 µl of each was transferred to the required number of graduated tubes. Phosphate buffered saline was added up to make up the volume to 800 µl. Finally 100 µl ($2x10^5$ cells/ml) of stomach cancer Cell (SGC7901) was added to each of the test tubes which were then were incubated at 37 ^{o}C under 5 % CO_2 atmosphere for 3 h. A 100 µl aliquot of tryphan blue dye was added to each of the test tubes and the number of dead cells was counted using a haemocytometer under a compound microscope. Cytotoxicity was calculated in percentage using Eq 1.

Cytotoxicity (%) = 100DC/(DC + LC) ..... (1)

where DC is the number of dead cells and LC is the number of living cells.

Evaluation of hydroxyl radical-scavenging activity

Hydroxyl radical-scavenging assay was carried out using the method described by de Avellar *et al* [16] with some modifications. Both 1,10-phenanthroline (0.75 mM) and $FeSO_4$ (0.75 mM) were dissolved in phosphate buffer (pH 7.4) and mixed thoroughly. H_2O_2 (0.01%) and *B. aegyptiaca* extract were added and mixed well. The mixture was incubated at 37 ^oC for 60 min and the absorbance measured using a spectrophotometer at 536 nm and hydroxyl radical scavenging activity ((HRSA) determined using Eq 2.

HRSA (%) = $[(A_s - A_1)/(A_0 - A_1)] \times 100$ - (2)

where A_S is the absorbance of the extract A_1 the absorbance of control solution containing 1,10-phenanthroline, $FeSO_4$ and H_2O_2, and A_0 the absorbance of blank solution containing 1,10-phenanthroline and $FeSO_4$.

DPPH radical scavenging activity assay

The scavenging effect of *B. aegyptiaca* extracts on DPPH free radical was measured according to the method of Shimada *et al* [7] with little modification. Two milliliters of extract (1.5, 3. 2 or 5 mg/ml) were added to 2 ml of 0.1 mM DPPH dissolved in 95 % ethanol. The mixture was shaken, left for 30 min at room temperature and the absorbance read at 517 nm. A lower absorbance represents a higher DPPH scavenging activity. Scavenging activity was calculated as in Eq 3.

DPPH scavenging activity (%) = $100(A_b - A_e)/A_b$ ..... (3)

where A_b is the absorbance of blank solution and A_e the absorbance of the extract solution.

Reducing power assay

The reducing power of the extracts was measured according to Wu *et al* [17]. The extract (0, 0.5, 1, 1.4, 1.8 or 2 mg/ml) was added to 2 ml of 0.2 M phosphate buffer (pH 6.6) and 2 ml of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 ^oC for 20 min, 2 ml of 10 %w/v trichloroacetic acid (TCA) added and the mixture centrifuged for 10 min at 3000 *g*. The supernatant (2 ml) was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) $FeCl_3$. After allowing reaction to take place for 10 min, the absorbance of the solution was determined using a spectrophotometer at 700 nm. High absorbance values of the reaction mixture indicate high reducing power.

Statistical analysis

Statistical analysis was carried out by paired t-test and significant difference was set at $p < 0.01$ between boiled and fresh extracts. SPSS software, version 18.0 (SPSS, Chicago, IL, USA) was used for the analysis.

RESULTS

Phytochemical composition of extracts

The phytochemical composition of the extracts is shown in Table 1 The protein content of the fresh extract (Fext, 4.1 %) was higher than that of grapes (3.4 %). The polyphenol and flavonoid contents of *B. aegytiaca* fresh extract (3.51 and 3.21 %, respectively) were lower than those of the boiled extracts (4.67 and 3.80 %, respectively). Vitamin C content was 42.3 and 38.9 mg/100g for Fext and Bext, respectively, and these values are higher than those for orange fruits.

Hydroxyl radical-scavenging activity

The results presented in Table 3 show significant difference ($p < 0.01$) between the Bext and Fext at all extract concentrations. However, Fext exhibited superior hydroxyl

Table 1: Proximate phytochemical composition of aqueous extracts of *B. aegyptiaca* fruit

Extract	Soluble protein (%)*	Polyphenols (%)*	Flavonoids (%)*	Vitamin C (%)**
Boiled (Bext)	5.77±1.71	4.67±0.36	3.80±0.49	1.86±0.17
Fresh (Fext)	4.09±1.12	3.51±0.62	3.21±0.59	0.98±0.44

Values are means ± standard deviation of triplicate determinations. Mean values in column are significantly different at p < 0.01 and p < 0.01*, between extract types*

radical scavenging activity to Bext (79.0 – 97.0 % compared with 61.6 – 82.5 % for the latter) over the concentration range tested (1.5 – 5.0 mg/ml).

Anticancer activity

The results of *in vitro* anticancer test are presented in Table 2. The aqueous extracts showed remarkable cytotoxic activity against stomach cancer cell line SGC7901. Both Fext and Bext showed significant effects ($p < 0.01$) on cell proliferation. Cytotoxic activity at extract concentration of 200 µg/ml were pronounced (56 and 44 % for Bext and Fext, respectively).

Tables 2: Effect of boiling *B. aegyptiaca* fruit aqueous extracts on stomach cancer Cell SGC7901 (2 x 10^5 cells/ml)

Extract (*µg.ml^{-1}*)	Sample no.	Number of cells		Dead cells (%)*
		Live	Dead	
Bext				
200	a	45	55	56
	b	43	57	
150	a	55	45	44
	b	58	42	
100	a	66	34	35
	b	64	36	
50	a	74	26	25
	b	77	23	
25	a	78	22	21
	b	80	20	
Control**	a	91	9	9
	b	92	8	
Fext				
200	a	54	46	44
	b	68	42	
150	a	62	38	39
	b	60	40	
100	a	72	28	30
	b	69	31	
50	a	75	25	26
	b	73	27	
25	a	81	19	18
	b	83	17	
Control**	a	90	10	10
	b	91	9	

*Bext = boiled extract; Fext = fresh extract; *Mean values in column are significantly different at (p < 0.01) between extracts; **Cell suspension without extract was used as control.*

Tables 3: Scavenging activity of *B. aegyptiaca* fruit aqueous extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals

Extract (mg/mL)	DPPH scavenging (%)*		Hydroxyl radical scavenging (%)*	
	Bext	***Fext***	***Bext***	***Fext***
1.5	31.3±2.3	49.1±1.1	61.6±2.8	79.0±1.9
2.0	35.7±4.1	64.1±2.9	65.4±2.3	86.4±0.5
3.0	60.3±2.6	80.0±3.1	77.2±3.0	89.4±1.2
5.0	68.2±2.1	88.2±2.7	82.5±2.4	97.0±1.2

DPPH radical-scavenging activity

Table 3 shows that Fext exhibited significantly higher ($p < 0.01$) DPPH radical scavenging activity (49.1 – 88.2 %) than Bext (31.3 – 68.2 %. Fext and Bext radical scavenging activities increased ($p < 0.01$) with increase in extract concentrations. Similar observations were reported by Amadou *et al* [6] and Vázquez *et al* [11] for aqueous extracts of fermented soybeans, and chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts, respectively.

Reducing power

Reducing power data indicate that the aqueous extracts are capable of donating electrons that can react with free radicals to convert them into stable products that strongly inhibit radical chain reaction [8,19]. Boiling the extract had a significant effect on its reducing power, as shown in Figure 1. Fext and Bext extracts displayed higher reducing power than vitamin C at low concentrations.

DISCUSSION

The antioxidants contained in foods, especially vegetables, are phenolic compounds, flavonoids, ascorbic acid, carotenoids and tocopherol. They are important protective agents for humans; they are also the most plentiful classes of constituents in the plant kingdom, and have been reported to have multiple biological effects [3,11]. Vitamin C consumption has been associated with antioxidant and neuroprotective effects [20]. Although boiling significantly reduced vitamin C content, the pulp of *B. aegytiaca* fruit is still a significant source of a variety of beneficial phytochemicals, including vitamin C.

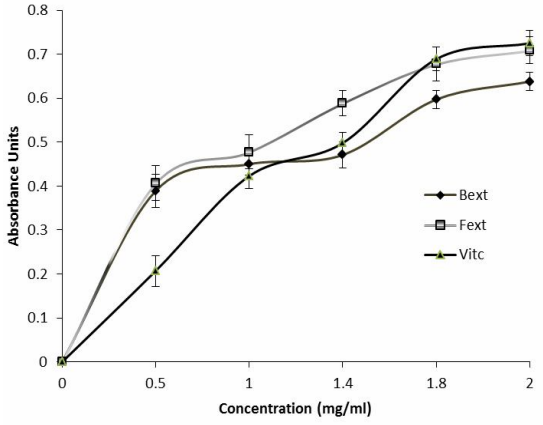

Figure 1: Reducing power of *B. aegyptiaca* fruit aqueous extracts; error bars = standard deviation (n = 3)

Interestingly, the boiled extract exhibited higher lethal activity on the stomach cancer cell SGC790 at all concentrations than the fresh extract. Gastric cancer is one of the most common causes of malignancy-related death worldwide, and dietary substances are promising ways to stop or reverse the process of carcinogenesis [10]. Despite the solvent used for the extraction, and the extract being boiled, *B. aegytiaca* fruit could be useful in reducing stomach cancer cell proliferation. The effect of boiling on the extract activity against stomach cancer cell SGC790 cell proliferation correlate with increase in polyphenol and flavonoid contents and decrease in vitamin C level. Various studies have shown that purified compounds

derived from *B. aegytiaca* fruit extract have significant biological activities [4,7,9].

Free radicals such as hydroxyl radical are generated from sequential reduction of oxygen during the normal course of aerobic metabolism. Over-abundant radicals cause oxidative stress which can lead to cell injury and tissue damage [5]. *Balanites aegyptiaca* extract can be a potential source of natural antioxidant, and incorporation of these extracts into foods could enhance their nutritional and antioxidant potentials. Our data corroborate those reported by Amadou *et al* [6] and Shimada *et al* [7].

DPPH radicals are widely used to investigate the scavenging activity of natural compounds. These free radicals are stable in ethanol and show maximum absorbance at 517 nm. When DPPH radicals encounter a proton-donating substance such as an antioxidant, the radicals are scavenged and their absorbance reduced [6]. *B. aegyptiaca* extracts showed significant scavenging activities against DPPH radicals. This is not surprising since the fruit mesocarp contains a large variety of phytochemicals amongst which are the pregnane glycosides, coumarins, flavonoids, 6-methyl-diosgenin and furostanol saponins [3,4], which could be electron donors, and hence can react with free radicals to convert them to more stable products and terminate the radical chain reaction.

Previous studies indicate that antioxidant activity and reducing power are directly related [6,19]. The reducing power of fermented foxtail millet extracts increased with increasing concentrations and it was observed that boiling the extract lowered reducing power over a concentration range of 0.5 to 2 mg/ml.

CONCLUSION

The aqueous extract of *B. aegyptiaca* fruit showed remarkable cytotoxic activity against the stomach cancer cell SGC7901. The activities were most likely due to bertain phytochemical constituents of the extracts. Evaluation of both biological properties and boiling conditions that can influence the stability and activity of millet flour mixed with the aqueous extract of *B. aegyptiaca* fruit is underway, with a view to formulating food products with optimum health benefits.

ACKNOWLEDGEMENT

This research was supported by the National Natural Science Foundation of China (no. 30671525), and the National High Technology Research and Development Program ("863" Program) of China (no. 2007. AA10Z325), 111 project-B07029. Thanks to Bian Yuan Yuan for making cultured stomach cancer cells SGC7901 available for the experiment. The helpful suggestions and comments of Mohamed Beva Kelfala Foh, Amza Tidjani and Maureen J Cheserek, are gratefully acknowledged. The authors also wish to thank Dr Chiacka Diakite of Section Médecine Traditionnelle, Institut National de Recherche en Santé Publique, (Bamako, Mali), for the collection and botanical identification of the fruits used in this study.

REFERENCES

1. *Yadav JP, Panghal M. Balanites aegyptiaca (L.) Del. (Hingot): A review of its traditional uses, phytochemistry and pharmacological properties. Int J Green Pharm. 2010; 4: 140–146.*
2. *Chothani DL, Vaghasiya HU. A review on Balanites aegyptiaca Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity. Pharmacog Rev 2011; 5: 55–62.*
3. *Kamel MS. A furostanol saponin from fruits of Balanites aegyptiaca. Phytochemistry 1998; 48: 755–757.*
4. *Sarker SD, Bartholomew B, Nash RJ. Alkaloids from Balanites aegyptiaca. Fitoterapia 2000; 71: 328–330.*
5. *Halliwell B. Gutteridge JMC. Free radical in Biology and Medicine, 3rd ed., UK: Oxford University Press, 1999.*
6. *Amadou I, Guo-Wei L, Yong-Hui S, Sun J. Reducing, radical scavenging, and chelation properties of fermented soy protein meal hydrolysate by Lactobacillus plantarum Lp6. Int J Food Properties 2011: 14(3): 654–665.*

7. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem. 1992; 40: 945–948.
8. Gnoula C, Megalizzi V, Neve ND, Sauvage S, Ribaucour F, Guissou P, Duez P, Dubois J, Ingrassia L, Lefranc F, Kiss R, Mijatovic T. Balanitin-6 and 7: Diosgenyl saponins isolated from Balanites aegyptiaca Del. display significant anti-tumor activity in vitro and in vivo. Int J Oncol. 2008; 32: 5–15.
9. Xue FB, Xu YY, Wan Y, Pan BR, Ren J, Fan DM. Association of H. pylori Infection with gastric carcinoma: a Meta-analysis. World J Gastroenterol. 2001; 7: 801–804.
10. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. J Ethnopharmacol. 2006; 106: 290–302.
11. Vázquez G, Fontenl E, Santo J, Freir MS, González-Álvarez J, Antorrena G. Antioxidant activity and phenolic content of chestnut (Castanea sativa) shell and eucalyptus (Eucalyptus globulus) bark extracts. Ind Crops Products 2008; 28: 279–285.
12. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 2005; 91: 571–577.
13. James CS. Analytical chemistry of foods. Chapman and Hall, New York, 1995; pp 137–139.
14. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72: 248–254.
15. Rajkapoor B. Jayakar B. Murugesh N. Antitumour activity of Bauhinia variegata on Dalton's ascitic Lymphoma. J Ethnopharmacol. 2003: 83:107–109.
16. de Avellar IG, Magalhaes MM, Silva AB, Souza LL, Leitao AC, Hermes-Lima M. Reevaluating the role of 1,10-phenanthroline in oxidative reactions involving ferrous ions and DNA damage. Biochimica et Biophysica Acta 2004; 1675: 46–53.
17. Wu HC, Chen HM, Shiau CY. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus). Food Res Int. 2003; 36: 949–957.
18. USDA. National Nutrient Database for Standard Reference, Release 23 (2010). Accessed May 24. http://www.ars.usda.gov/ba/bhnrc/ndl
19. Owolabi OJ, Amaechina FC, Okoro M. Effect of ethanol leaf extract of Newboulda laevis on blood glucose levels of diabetic rats. Trop J Pharm Res. 2011; 10(3): 249–254.
20. Santos LFL. Freitas RLM. Xavier SML. Saldanha GB. Freitas RM. Neuroprotective actions of vitamin C related to decreased lipid peroxidation and increased catalase activity in adult rats after pilocarpine-induced seizures. Pharmacol Biochem Behv. 2008; 89: 1–5.