Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 08, Issue, 10, pp.6106-6109, October, 2017

RESEARCH ARTICLE

ASSAY ON OSMOTIC FRAGILITY AND ANTIOXIDANT POTENTIAL OF THE METHANOLIC EXTRACT OF *CROTON HELIOTROPIIFOLIUS* KUNTH (EUPHORBIACEAE)

[1,*]Jéssica de Andrade Gomes Silva, [1]Gibbelly Cavalcante da Silva, [1]CibelyTenorio de Oliveira, [1]Tainá Maria Santos da Silva, [1]Marcos Aurélio Santos da Costa, [1]Daniel Francisco Pereira Júnior, [1]Izabela Rangel Lima, [2]José Adalberto da Silva Filho and [1]Sônia Pereira Leite

[1]Departamento de Histologia e Embriologia, Centro de Biociências, Universidade Federal de Pernambuco, Brasil
[2]Deprtamento de Engenharia Ambiental, Universidade Federal Rural de Pernambuco, Brasil

ARTICLE INFO

Article History:
Received 23rd July, 2017
Received in revised form
19th August, 2017
Accepted 02nd September, 2017
Published online 17th October, 2017

Key words:

Croton heliotropiifolius,
Osmotic fragility,
Antioxidant,
2,2-diphenyl-1-picrylhydrazyl (DPPH).

ABSTRACT

The expressive use of medicinal plants promotes a growing need to understand the properties of vegetal compounds and their possible biologically active behaviors. Studies focusing on *Croton heliotropiifolius* have reported a predominant presence of alkaloids, polyphenols and reducing compounds. This species is reported as useful in relieving stomach pain and dysentery and as an antipyretic. This study aimed to evaluate the hemolytic capacity of the methanolic extract of *C. heiotropiifolius*by*in vitro*osmotic fragility assay in erythrocytes, and determine the antioxidant potential of this extract using the 2,2-diphenyl-1-picrylhydrazyl (DPPH)method. The osmotic fragility assay was performed at the concentrations 50, 100, 250, 500, 750 and 1,000 µg/mL of extract. For the *in vitro* photocolorimetry of free radical sequestration using DPPH, the concentrations used were 50, 100 and 200 µg/mL. The methanolic extract of *C. heliotropiifolius* showed a low *in vitro* hemolytic activity under the test conditions. The highest concentration (1,000 µg/mL) showed a statistically significant difference ($p<0.001$) in relation to the other concentrations, but reached a percentage of 2.95%,a low percentage to confirm hemolysis. Regarding antioxidant capacity, all the concentrations tested presented statistical differences at a level of significance of $p<0.001$. The improvement in the antioxidant activity index followed an increase in extract concentration. Thus, it is probable that there is no damage to the erythrocyte membrane and that the extract has compounds able to stabilize free radicals. Our study provides important data, contributing to a possible development of herbal medicines in addition to improving the scientific knowledge on *Croton heliotropiifolius*.

INTRODUCTION

Many species of herbs are used as phytotherapics by the population (Bagatini *et al*., 2007). Due to an expressive use of medicinal plants, there is a growing need to understand the properties of vegetal compounds and their possible biologically active behaviors (Del Ré *et al*., 2012). Studies aiming to analyze the performance of compounds in cellular homeostasis evaluate the ability of substances to affect membranes, which may lead to cellular damage (Mohandas *et al*., 2008). Cytotoxic analyses using hematological components, cells required for hemodynamic maintenance, have been demonstrating that several herbs are capable of causing osmotic disturbances and morphological changes in erythrocytes (Maiworm *et al*., 2008). In contrast, the potential of some plant extracts to retard or inhibit the oxidation of molecules by suppressing chain oxidation reactions provides a protective effect which enables the use of such plants in complementary medicine (MahboubI *et al*, 2013). Chronic oxidative stress is responsible for many degenerative diseases, such as asthma, gastrointestinal diseases, heart disease, autoimmune diseases and Alzheimer's (Lushchak, 2014; Sies, 2015). Such antioxidant capacity is present in several natural constituents, such as α-tocopherol (vitamin E), β-carotene, ascorbate (vitamin C) and phenolic compounds (phenolic acids and flavonoids) (Sousa *et al.*, 2007). The species *Croton heliotropiifolius* Kunth, popularly known as "velamen" due to its tiny hairs, is endemic to the Brazilian Northeast region and can be found frequently in the Caatinga, swamps, Restingas and the Cerrado (Randau, 2001). Studies focusing on *Croton heliotropiifolius* reported a predominant presence of alkaloids, polyphenols and reducing compounds. This species is reported as useful in relieving stomach pain and dysentery and as an

***Corresponding author:* Jéssica de Andrade Gomes Silva,**
Departamento de Histologia e Embriologia, Centro de Biociências, Universidade Federal de Pernambuco, Brasil

antipyretic (Randau, 2001). Since few studies have been conducted to improve the understanding of the biological activities and the toxic behavior of *Croton heliotropiifolius* Kunth, this study aims to evaluate the cytotoxic capacity of the methanolic extract of this species through an *in vitro* osmotic fragility assay using erythrocytes. This test is capable of conducting a preliminary evaluation of plant toxicity besides determining the antioxidant potential of the extract using the free radical sequestration method (DPPH).

MATERIALS AND METHODS

Plant material

Leaves of *C. heliotropiifolius* were obtained in the urban area of the municipality of Garanhuns, Pernambuco (PE) state, Brazil. An exsiccate was prepared and deposited at the Andrade Lima Dárdano Herbarium of the Agronomic Research Institute (IPA) under the catalog number 90440, and identified by a botanist of that institution.

Obtaining the methanolic extract

The extract was obtainedusing the maceration method described by Filho, Yunes (1998).Leaves (100 g) were macerated for 10 days in methanol (1,000 mL) at room temperature and subjected to sporadic stirring. After this time, the mixture was filtered and the resulting filtrate was processed using a BUCHI Switzerland rotary evaporator at a temperature of 60°C until the total evaporation of the solvent.

Osmotic Fragility Assay

The osmotic fragility assay performed was based on the methodology described by Darcie and Lewis (1975). Commercial lamb blood samples (Laborclin®) were exposed to the extract for 60 minutes at room temperature and at different concentrations: 50 µg/mL, 100 µg/mL, 250 µg/mL, 500 µg/mL, 750 µg/mL and 1,000 µg/mL, diluted in isotonic sodium chloride solution (0.9%). Then, the solutions containing blood and extract were centrifuged (2,500 rpm/3 min) and the supernatant was analyzed using a Shimadzu UV-vis 1800 spectrophotometer, resulting in a dose-response curve containing the percentage of estimated hemolysis using the absorbance values obtained. A negative control was established using an isotonic solution of sodium chloride (0.9%), and a positive control was established using distilled water. Both underwent the same procedures as test samples. The assay was performed in duplicate, and the hemolytic percentage was determined using the absorbance of the positive control, designated as 100%.

Antioxidant Activity

The antioxidant activity of the leaf extract of *C. heliotropiifolius* was determined using *in vitro* photocolorimetry, which was performed by sequestrating free radicals using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Mensor *et al*., 2001). This analysis was based on the ability of the compounds to donate a proton to DPPH, thus stabilizing the free radical. To perform the assay, the samples were prepared by adding 1 mL of DPPH solution (60 µM, Sigma, Germany) into 2.5 mL of extract solutions, which were diluted in ethanol at the concentrations 50, 100 and 200 µg/mL. After a reaction time of 30 min, the absorbances of the samples were read using the UV-Vis UV Spectrophotometer (Shimadzu UV-vis 1800) with a wavelength of 520 nm. As a negative control, the mixture of 1 mL of the DPPH solution and 2.5 mL of ethanol was used (Mensor *et al*., 2001). All readings were performed in triplicate and, using the mean of the dataobtained, the difference in absorbance between the samples and the negative control was calculated. The percentages of antioxidant activities were determined by the equation:

Inhibition of DPPH activity (%) = [(A-B) / A] x 100

where A = Absorbance of the DHPP solution of the control sample, B = Absorbance of the DHPP solution in the presence of the extract.

Statistical analyses

Hemolytic percentages and antioxidant activity data were analyzed by analysis of variance (ANOVA) followed by Tukey test. Results with a $p<0.001$ were considered significant. The software used was ASSISTAT version 7.7.

RESULTS AND DISCUSSION

Osmotic Fragility Assay

The osmotic fragility assay of *C. heliotropiifolius* extract, under the tested conditions, obtained low percentages of hemolysis (Figure 1), considering that the hemolytic action should be considered high when the percentages reach values higher than 40% and low when such values are lower than 10% (Nofiani *et al*., 2011).

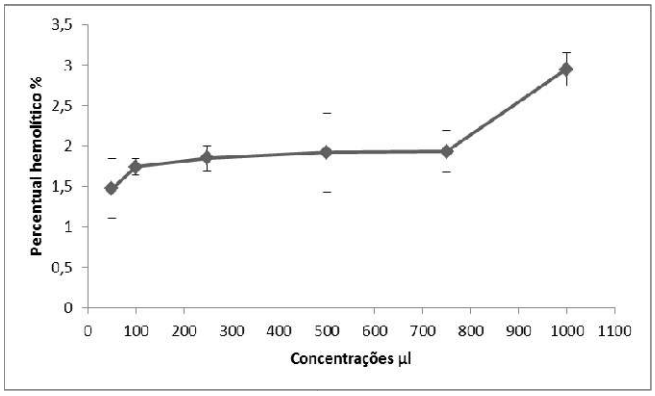

Figure 1. Dose-response curve showing hemolytic percentages of erythrocytes subjected to *C. heliotropiifolius* extract

The highest concentration tested, 1,000µg / mL, showed a significant statistical difference ($p<0.001$) in relation to the other concentrations, but reached a percentage of 2.95%, which is a value low to confirm hemolysis. Thus, there is likely no damages to the erythrocyte membrane. The hemolytic action of the different plant compounds is attributed to a number of non-specific mechanisms, for example, surfactant compounds that produce a hemolytic effect by solubilizing the plasma membrane or osmotic lysis, which promotes changes in the permeability of red blood cells (Aparicio *et al*., 2005). Saponins exert a hemolytic effect resulting from their ability to interact with the elements of the cell membrane of red blood cells, especially with cholesterol

molecules, causing a deformation in the membrane and, as a consequence, extravasation of the intracellular content (Dewick, 2002; Karabaliev *et al*., 2003). This compound was absent in the phytochemical analyses of the species *C. heliotropiifolius* (Silva *et al*., 2016).

Antioxidant Activity

Physiological processes such as respiration and metabolism result in the formation of free radicals (Tegeli *et al*., 2014). Although there are many antioxidant mechanisms in the body, occasionally such mechanisms are not sufficient to eliminate reactive species when there is an overproduction of reactive species, leading to an imbalance called oxidative stress (Cervellati *et al*., 2014, Kumar, 2011; Lushchak, 2014; Sies, 2015). Research has shown that the intakeof antioxidantsis very important to reduce the damage caused by reactive species (Rezaire *et al*., 2014). Natural products contain several compounds with an antioxidant activity (Almeida *et al*., 2011). This study evidenced that the methanolic extract of *C. heliotropiifolius* showed a DPPH clearance activity. All concentrations tested presented statistical differences at a level of significance of $p<0.001$. The improvement in the antioxidant activity index followed an increase in the extract concentration (Table 1), suggesting that the extract has compounds with the ability to stabilize free radicals.

Table 1. Results for the antioxidant activity of methanolic extracts of *C. heliotropiifolius* leaves using the DPPH radical

Methanolic extract of *Croton heliotropiifolius* (µg/mL)	Antioxidant Activity (%)
50	10.6 ± 0.33
100	13.8 ± 0.21
200	20.2 ±0.7

Extracts with an antioxidant potential are generally rich in phenolic and polyphenolic compounds (Conforti *et al*., 2005). Such compounds derived from medicinal herbs have been isolated in several plant families (Boudet, 2007; Razavi *et al*., 2008). Flavonoids are metabolites mostly associated with antioxidant activity (Van Den Berg *et al*., 2000) since they have a carbon skeleton favorable for the stabilization of free radicals. Studies have been demonstrating the presence of this class of compounds in *C. heliotropiifolius* (Randau, 2001; Silva *et al*., 2016). Other species of the genus *Croton*alsohave an antioxidant ability: *C. celtidifolius* (Nardi *et al*., 2003), *C. nepetaefolius* (Morais *et al*., 2006) and *C.argyrophylloides* (Catunda Jr *et al*., 2002). The antioxidant potential of *C. heliotropiifolius* was previously reported by Evangelica (2011), who reported higher values of antioxidant activity in the ethanolic extract when compared to our results. This can be attributed to the presence and the concentration of phenolic compounds found in the extract tested. Variations in the content of bioactive compounds are associated to factors such as solvent type, methodology used for the extraction process, plant physiology, climate, soil, luminosity, temperature, rainfall, nutrition, time and collection time (Gobbo-Neto; Lopes, 2007; Morais, 2009). Thus, it is possible to distinguish the intensity of the antioxidant action shown by plants. It is mainly determined by the number and the positions of hydroxyls of phytochemical compound molecules present in their composition (Melo *et al*., 2008). Our study provides important data, contributing to a possible development of herbal medicines in addition to improving the scientific knowledge on *Croton heliotropiifolius*.

Conflict of interests

The authors declare no conflicts of interest.

Acknowledgements

The authors would like to thank the Postgraduate Program in Morphotechnology (UFPE), the Centro Universitário Tabosa de Almeida (ASCES - UNITA), the Laboratory of Chemical Biophysics of UFPE and the Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support.

REFERENCES

Almeida M M B, Sousa P H M, Arriaga A M C, Prado G M, Carvalho M C E, Maia G A, Lemos T L G. 2011. Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. *Food Research International*, *44* (7), 2155-2159. http://doi.org/10.1016/j.foodres.2011.03.051

Angélico E C. 2011. Evaluation of Antibacterial and Antioxidant Activities of Crotonheliotropiifolius Kunteand Crotonblanchetianus Baill. Dissertation Master in Animal Science, Federal Universityof Campina Grande, Patos.

Aparicio R M, García-Celma M J, Vinardell M P, Mitjans M 2005. In vitro studies of the hemolytic activity of microemulsions in human erythrocytes. *Journal of Pharmaceutical and Biomedical Analysis*, 39(5), 1063-1067. http://doi.org/10.1016/j.jpba.2005.06.013PMID:16054795

Bagatini M D, Silva A C F D, Tedesco S B. 2007. The use ofAllium cepa test as a bioindicatorofgenotoxicityof medicinal plantsinfusions. *RevBrasFarmacogn*, *17*(3), 444-7.http://doi: 10.1590/S0102-695X2007000300019

Boudet A M 2007. Evolutionandcurrent status ofresearch in phenoliccompounds. *Phytochemistry*, 68(22), 2722-2735.http://doi.org/10.1016/j.phytochem.2007.06.012PMID:17643453

Catunda Junior F E A, Luciano J H S, Moraes S M 2002.. Antioxidant activity of essential oils from plants in Northeast Brazil. *Science and Technology*, 4, 23-29.

Cervellati C, Romani A, Seripa D, Cremonini E, Bosi C, Magon S, Zuliani G (2014). Oxidativebalance, homocysteine, and uricacid levels in older patients with Late Onset *al*zheimer's Diseaseor Vascular Dementia. *Journal of the Neurological Sciences*, 337(1), 156-161. http://doi.org/10.1016/j.jns.2013.11.041PMID: 24321755

Conforti F, Sosa S, Marrelli M, Menichini F, Statti G A, Uzunov D, Della Loggia R. 2008. In vivo anti-in flammatory and in vitro antioxidant activities of Mediterraneandietary plants. *Journal of ethno pharmacology*, 116(1), 144-151. http://doi.org/10.1016/j.jep.2007.11.015PMID:18164564

Dacie J V, Lewis S M 1975. PracticalHaematology (5th edit.) Churchill Livingstone. Edinburgh, London and New York.

Del Ré P V, Jorge N 2012. Spices as natural antioxidants: applications in food and implication in health. *Brazilian Journal of Medicinal Plants*, 389-399. http://dx.doi.org/10.1590/S1516-05722012000200021

Dewick, P. M. 2002. Medicinal natural products: a biosynthetic approach. John Wiley& Sons.

Filho V C. and Yunes R A. 1998. Strategies for obtaining pharmacologically active compounds from edicinal plants. Química nova, v.21, n.1. http://dx.doi.org/10.1590/S0100-40421998000100015

Gobbo-Neto L, Lopes N P 2007. Medicinal plants: factors influencing the content of secondary metabolites. Química nova, 30(2), 374.

Karabaliev M, Kochev V. 2003. Interaction of solid supported thin lipid films with saponin. Sensors and Actuators B: Chemical, *88*(1), 101-105. http://doi.org/10.1016/S0925-4005(02)00311-8

Kumar S 2011. Free Radicals and Antioxidants: Human and Food System. *Adv. Appl. Sci. Res*., 2(1):129.

Lushchak VI 2014. Free radicals, reactive oxygenspecies, oxidative stress and its classification. Chem. Biol. Interact. 224:164-175. http://doi.org/10.1016/j.cbi.2014.10.016 PMID: 25452175

Mahboubi M, Haghi G, Kazempour N, Hatemi A R. 2013. Total phenolic content, antioxidant and antimicrobial activities of Blepharisedulis extracts. *Songklanakarin J. Sci. Technol*., 35(1), 11-16.

Maiworm A I, Presta G A, Santos-Filho S D, Paoli S D, Giani T S, Fonseca A S, Bernardo-Filho M. 2008. Osmotic and morphological effects on red blood cell membrane: action of an aqueous extract of Lantanacamara. *Brazilian Journal of Pharmacognosy*; 18(1), 42-46.http://dx.doi.org/10.1590/S0102-695X2008000100010

Melo E A, Maciel M I S, Lima V A G L, Nascimento R J 2008. Antioxidant capacity of fruits. *Brazilian Journal of Pharmaceutical Sciences*, São Paulo, v. 44, n.2, p. 193-201.http://dx.doi.org/10.1590/S1516-93322008000200005

Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, Leitão SG. 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother Res*., 15: 127- 130. http://doi.org/10.1002/ptr.687

Mohandas N, Gallagher P G. 2008. Red cell membrane: past, present, and future. Blood, 112(10), 3939-3948. https://doi.org/10.1182/blood-2008-07-161166PMID:18988878

Morais L A S 2009. Influence of abiotic factors on the chemical composition of essentialoils. *Brazilian Horticulture*, v. 27, n. 2, p. 4050-4063.

Morais S M D, Catunda Júnior F E A, Silva A R A D, Neto M, Stone J, Rondina D, Cardoso J H L. 2006. Antioxidant activity of essential oils from Northeastern Brazilian Crotonspecies. Química Nova, 29(5), 907-910. http://dx.doi.org/10.1590/S0100-40422006000500004

Nardi G M, Felippi R, DalBó S, Siqueira-Junior J M, Arruda D C, Delle Monache F, Ribeiro-do-Valle R M. 2003. Anti-inflammatory and antioxidant effects of Crotoncel tidifoliusbark. *Phytomedicine*, 10(2-3), 176-184. https://doi.org/10.1078/094471103321659906PMID:12725573

Nofiani R, Kurniadi R, Ardiningsih P. 2017. Antimicrobial, Antioxidant, Hemolytic Activities and Toxicity of Ethyl Acetate Extract Froman Unidentified Coral-Associated Fungus, Aspergillus brevipes RK06. *Indonesian Journal of Cancer Chemoprevention*, 2(2).

Randau K P. 2001. Pharmacognostic study (pharmaco botanical and pharmacochemical) and biological activity of Crotonrhamnifolius H.B.K. And Crotonrhamnifolioides Paxand Haffm. (Euphorbiaceae). 143p. Dissertation Master in Pharmaceutical Sciences. Federal University of Pernambuco. Recife- PE.

Razavi SM, Nazemiyeh H, Hajiboland R, Kumarasamy Y, Delazar A, Nahar L, Sarker SD 2008. Coumarins from the aerialparts of Prangosuloptera (Apiaceae). *Rev Bras Farmacogn.,* 18: 1-5.

Rezaire A, Robinson J C, Bereau D, Verbaere A, Sommerer N, Khan M K, Fils-Lycaon B (2014). Amazonianpalm Oenocarpusbataua ("patawa"): Chemical and biological antioxidant activity–Phytochemical composition. *Food chemistry*, 149, 62-70. http://doi:10.1016/j.foodchem.2013.10.077PMID:24295677

Sies H. 2015. Oxidative stress: a concept in redox biologyand medicine. Redox Biol. 4:180-183. http://doi:10.1016/j.redox.2015.01.002PMID:25588755

Silva J A G, Silva G C, Silva TM S, Rangel I 2016. Estudo Fitoquímico das Folhas de Crotonheliotropiifolius Kunth (Euphorbiaceae). Anais I Conbracis. v. 1.

Sousa C D M, Silva H R, Vieira-Jr G M, Ayres M C C, Costa C D, Araújo D S, Chaves M H. 2007. Total phenols and antioxidant activity of five medicinal plants. *Química nova*, 30(2), 351-355.

Tegeli, V, Karpe P, Katve V. 2014. Importance of free radical and antioxidant on human health. IJPCBS, 4(4), 1038-1050.

Van Den Berg, R., Haenen, G. R., van den Berg, H., van der Vijgh, W. & Bast, A. 2000. The predictive value of the antioxidant capacity of structurally related flavonoids using the Trolox equivalent antioxidant capacity (TEAC) assay. *Food Chemistry,* 70(3), 391-395. http://doi.org/10.1016/S0308-8146(00)00092-3

****