American Journal of Plant Sciences, 2013, 4, 1709-1712
http://dx.doi.org/10.4236/ajps.2013.49208 Published Online September 2013 (http://www.scirp.org/journal/ajps)

Evaluation of Antioxidant and Cytotoxic Capacity of *Croton bonplandianum*. Baill

Muhammad Naeem Qaisar*, Bashir Ahmad Chaudary, Muhammad Uzair, Sajid Nawaz Hussain

Faculty of Pharmacy, Bahauddun Zakariya University, Multan, Pakistan.
Email: *naeemqaisar78@uos.edu.pk

Received June 3rd, 2013; revised July 3rd, 2013; accepted July 31st, 2013

ABSTRACT

The antioxidant and cytotoxic activities of Methanolic and Dichloromethane extracts of *Croton bonplandianum* Baill were evaluated in a bid to provide better scientific basis for the isolation of bioactive compounds from the plant. The DPPH and hydroxyl radical scavenging activities of the Methanolic extract were 59.62%, and Dichloromethane extract was not toxic. Gallic acid used as reference compounds showed higher antioxidant activities to the plant extracts. Both methanolic and dichloromethane extracts were evaluated for their brine shrimp lethality. Methanolic extract showed cytotoxic activity *in vitro* with a LD_{50} value of 115.76 (0.0048 - 13.76) µg/ml. Etoposide was used as standard drug. Put together, these results confirm that *Croton bonplandianum* extracts possess appreciable natural antioxidant and cytotoxic potentials, thereby providing good justification for the isolation of pure bioactive compounds.

Keywords: Antioxidant Activity; Scavenging Activity; Cytotoxic Activity; Brine Shrimp; *Croton bonplandianum*

1. Introduction

Plants are serving human needs in the forms of foods and fruits. Plants also provide the important components of medicines, cosmetics and beverages. The most recent study by the World Health Organization (WHO) approximated that for the primary health care needs, four fifths of the total population still put confidence on the plant medicine [1], and most of these plant extracts or their bioactive components. Still today, a huge public is taking interest to use the herbal remedies.

Croton bonplandianum Baill (syn. *Croton sparsiflorus* Morong) is a monoecious woody shrub, which is 1 - 5 m in height, but more usually c. 30 - 40 cm, with whorled branches. The plant grows in sandy clay soil along roadside, irrigation canal banks, in plantations and on waste ground [2]. It has been reported that this plant grows in S. Balivia, Paraguay, S. W. Brazil, N. Argentina, Bangladesh, South America, South India and Pakistan [3,4]. In Pakistan, this plant is found near Khyber, Attock, Wah, Rawalpindi, Sargodha, Gujarat, Sialkot, Lahore and Karachi.

Among the medicinal benefits of plants, antioxidant properties have received increasing attention due to their role in preventing or down-regulating myriads of oxidative damages caused by free radicals in the body [5]. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases [6,7].

The antioxidant capacities of *Croton bonplandianum* Baill consumed locally in Pakistan have not been clearly presented. Such information, if provided, will not only possibly introduce the plants as cost effective and accessible sources of natural antioxidants but also justify the need for renewed domestication efforts on them. Against this back-drop, this study is aimed at evaluating the antioxidant activities of *Croton bonplandianum* Baill in selected *in vitro* assay systems.

Apart from already documented traditional uses, traditional healers claim to be using the leave extracts for treating cancer, although it was difficult to establish the type of cancer being treated. This indicated that the plant might contain potentially useful anticancer compounds. However, up to now there is little phytochemical or pharmacological work done on this. Therefore in this study

*Corresponding author.

we present results of the brine shrimp lethality test (BST) of Methanolic and Dichloromethane extracts of *Croton bonplandianum.*

2. Materials and Methods

2.1. Collection and Extraction of Plant Material

The plant material was collected from surroundings of Sargodha district. The plant was identified as *Croton bonplandianum by* Professor Dr. Altaf Hussain Dasti, Institute of pure and applied Biology, Bahauddin Zakariya University, Multan. For the purpose of effective extraction, whole plant material was shade dried for 15 days. Then dried plant material was ground in blender and weighed. The extraction of this finely ground material was affected by simple maceration. The weighed amount of plant material was taken in extraction bottle and measured volume of dichloromethane was added to it. Ultrasonication of this mixture was carried out occasionally in order to achieve maximum possible extraction. Filtration was carried out after 24 hours of addition of solvent. The process was repeated three times with dichloromethane. The extraction of the marc was done by methanol in the same manner. The Dichloromethane and methanol extracts were concentrated separately under reduced pressure by using rotary evaporator. The dichloromethane (20.2 g) and methanol (48.9 g) extracts were collected in separate sample bottles and designated with codes as CBD and CBM respectively.

2.2. Chemicals

DPPH (1,1-diphenyl-2-picryl hydrazyl) radical and Rutin were purchased from Sigma Aldrich Chemical Company, USA; Folin and Ciocalteau's Phenol reagent and Trichloroacetic acid (TCA) from Qualikems Fine Chemical Pvt. Ltd., New Delhi, India; Gallic acid monohydrate from Kem Light Laboratories Pvt. Ltd., Mumbai, India. Solvents and other chemicals used for this study were of analytical grade, while water was glass distilled.

2.3. DPPH (1,1-Diphenyl-2-Picryl-Hydrazyl) Radical Scavenging Assay

Antioxidant activity of the extracts of *Croton bonplandianum* were measured in this assay as ability to scavenge stable DPPH radicals according to [8]. A concentrations (0.5 µg/ml) of the test extracts were prepared in methanol. To 2.5 ml solution of each extract concentration was added 1 ml of 0.3 mM of freshly prepared DPPH solution in methanol and allowed to react in the dark at room temperature for 30 min. Absorbance of the resulting solution was measured at 518 nm. Methanol (1 ml) added to 2.5 ml of each extract concentration was used as blank, while 1 ml of 0.3 mM DPPH solution added to 2.5 ml of methanol served as a negative control. Gallic acid, prepared in the same concentrations as the test extracts, were used as reference standards (positive controls) for comparison.

Percentage DPPH scavenging activities of the extracts and reference standards were determined using the formula.

$$\% \text{ scavenging activity } 100 - \left[\left(A_s - A_b \right) / A_c \times 100 \right]$$

where A_s = Absorbance of sample (extract or reference standard), A_b = Absorbance of blank and A_c = Absorbance of negative control [8].

2.4. Brine Shrimp Lethality Test (BST)

The brine shrimp lethality test (BST) was used to predict the presence, in the extracts, of cytotoxic activity [9]. Both the crude extracts were tested for brine shrimp lethality. Solutions of the extract were made in DMSO and incubated in duplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the duplicate vials. Control brine shrimp larvae were placed in a third vial which contained sea water and DMSO only. After 24 h the nauplii were examined against a lighted background, and the average number of survived larvae in each triplicate was determined. The mean percentage mortality was plotted against the logarithm of concentrations and the concentration killing fifty percent of the larvae (LC_{50}) was determined from the graph by taking the antilogarithm of the concentration corresponding to 50% mortality rate of the test organisms. Etoposide was used as a standard test drug.

3. Results

3.1. DPPH Radical Scavenging Assay

In this assay, the ability of the investigated extracts to act as donors of hydrogen atoms or electrons in transformation of DPPH radical into its reduced form was investigated. The results indicated that methanolic extract (CBM) had IC_{50} 396.205 and % radical scavenging activity (%RSA) of 59.62% and while the dicholoromethane extract (CBD) is inactive (**Table 1**). The scavenging activities of the extracts were, however, lower than those tested of gallic acid (93.13%).

Table 1. Antioxident activity of extracts.

Extract code	Conc. mg/ml	IC_{50} ± SEM. µg/ml	% RSA
CBM	0.5	396.205 ± 4.6	59.629
CBD	0.5	inactive	39.37
STD Gallic acid	0.094	4.3 ± 0.43	93.13

3.2. Brine Shrimp Lethality Test

In the brine shrimp lethality test methanolic extracts was toxic with an with a LD_{50} value of 115.76 (0.0048 - 13.76) µg/ml. Dichloromethane extract was not toxic (**Table 2**).

4. Discussion

Plants have been mentioned as one of the most important targets to search for natural antioxidants from the point of view of safety [10,11]. The activities of antioxidants have been attributed to various mechanisms including prevention of chain initiation, decomposition of peroxides, radical scavenging and reducing capacity [12]. Consequently, these activities vary with assay methods and a single assay may be inadequate. It is for this reason that antioxidant activities of the extracts in this study were evaluated Phenols and flavonoids represent two phytochemicals whose relative abundance in plant extracts has been profusely linked to antioxidant activities. Phenols and flavonoids in extracts may explain their high antioxidant activities [12]. DPPH radical scavenging assay provides an easy, rapid, and convenient method to evaluate antioxidants and radical scavengers [13]. It is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515 nm and also for the visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, and this effect can be quantitatively measured from the changes in absorbance [14]. The extracts tested in this study showed DPPH radical scavenging activities.

Bioactive compounds are often toxic to shrimp larvae. Hence, *in vivo* lethality to shrimp larvae can be used as a rapid and simple preliminary monitor for bioactive compounds during the isolation of natural products. The eggs of the brine shrimp *Artemia salina* (Leach) are readily available as fish food in pet shops. When placed in artificial sea water, the eggs hatch within 48 hours, providing large numbers of larvae. These tiny shrimp larvae have been extensively used as a tool to monitor the cytotoxicity of samples under study. This is a rapid, inexpensive, in-house, general bioassay which has been developed for screening, fractionation and monitoring of physiologically active natural products[9].The methanolic extract was toxic with LD_{50} value of 115.76 (0.0048 - 13.76) µg/ml against *Artemia salina* when tested *in vitro*, indicated a possibility that the extract may contain a toxic compounds.

Table 2. Brine shrimp lethality test of extracts.

Extract code	Dose µg/ml	No. of shrimp	No. of survivors	LD 50 µg/ml	STD. Drug	LD 50 µg/ml
	1000	30	04			
CBM	100	30	20	115.76	*Etoposide*	7.4625
	10	30	23			
	1000	30	16			
CBD	100	30	19	1327.85	*Etoposide*	7.4625
	10	30	24			

5. Conclusion

The results of the present study indicate that the extracts showed significantly different but appreciably potent antioxidant and cytotoxic activity that cannot be neglected. These results support the traditional healers claim, but it could also mean that methanolic extract is potentially toxic, since brine shrimp lethality activity can also be used as an indicator for toxicity. There is need to test this compound on cancer cell lines and other tests in order to establish its safety and the possibility of developing an anticancer agent. The study therefore not only reveals these as accessible reservoirs of natural antioxidants and cytotoxic compounds, but very importantly, provides good scientific justification for the isolation of pure bioactive compounds.

6. Acknowledgements

The authors are grateful to the Pharmacy Department, Bahauddin Zakariya University Multan, Pakistan. International Center of Chemical And Biological Sciences, University of Karachi is also acknowledged for antioxidant activity and brine shrimp lethality tests.

REFERENCES

[1] N. R. Farnsworth, O. Akerele, A. S. Bingel, D. D. Soejarto and Z. Guo, *Bulletin of the World Health Organization*, Vol. 63, 1985, pp. 965-981

[2] E. Nasir and S. I. Ali, "Flora of Pakistan No. 172," Shamim Printing Press, Karachi, 1986, p. 43

[3] C. S. Pande and J. D. Tewari, *Journal of the Indian Chemical Society*, Vol. 39, 1962, pp. 545-552.

[4] S. Satish and D. S. Bhakuni, "Constituents of Indian and Other Plants," *Phytochemistry*, Vol. 11, No. 9, 1972, pp. 2888-2890. doi:10.1016/S0031-9422(00)86539-5

[5] Y. Abiy, "Antimicrobial Flavonoids from the Stem Bark of *Erythrina burtii*," *Fitoterapia*, Vol. 96, 2005, pp. 496-499.

[6] A. Braca, C. Sortino, M. Politi, J. Morelli and J. Mendez, "Antioxidant Activity of Flavonoids from *Licania licaniaeflor*," *Journal of Ethnopharmacology*, Vol. 79, No. 3, 2002, pp. 379-381. doi:10.1016/S0378-8741(01)00413-5

[7] U. Ozgen, A. Mavi, Z. Terzi, A. Yildrim, M. Coskun and P. J. Houghton, "Antioxidant Properties of Some Medi-

cinal Laminaceae Species," *Pharmaceutical Biology*, Vol. 44, No. 2, 2006, pp. 107-112. doi:10.1080/13880200600592061

[8] L. L. Mensor, F. S. Menezes, G. G. Leitao, A. S. Reis, T. C. dos Santos, C. S. Coube and S. G. Leitao, "Screening of Brazilian Plant Extracts for Antioxidant Activity by the Use of DPPH Free Radical Method," *Phytotherapy Research*, Vol. 15, No. 2, 2001, pp. 127-130. doi:10.1002/ptr.687

[9] B. N. Meyer, N. R. Ferrigni, L. B. Jacobsen, D. E. Nicholas and L. McLaughling, "Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents," *Planta Medica*, Vol. 45, No. 5, 1982, pp. 31-34. doi:10.1055/s-2007-971236

[10] D. E. Okwu, "Phytochemicals and Vitamin Content of Indigenous Spices of South Eastern Nigeria," *Journal of Sustainable Agriculture and Environment*, Vol. 6, No. 2, 2004, pp. 30-34.

[11] M. A. Hala, "Comparative Antioxidant Activity Study of Some Edible Plants Used as Spices in Egypt," *Journal of American Science*, Vol. 7, No. 1, 2011, pp. 1118-1122

[12] N. C. Cook and S. Samman, "Flavonoids-Chemistry, Metabolism, Cardioprotective Effects and Dietary Sources," *Journal of Nutritional Biochemistry*, Vol. 7, No. 2, 1996, pp. 66-76. doi:10.1016/0955-2863(95)00168-9

[13] V. Roginsky and E. A. Lissi, "Review of Methods to Determine Chain-Breaking Antioxidant Activity in Food," *Food Chemistry*, Vol. 92, No. 2, 2005, pp. 235-254. doi:10.1016/j.foodchem.2004.08.004

[14] M. R. Saha, S. M. R. Hasan, R. Akter, M. M. Hossain, M. S. Alam, M. A. Alam and M. E. H. Mazunder, "*In Vitro* Free Radical Scavenging Activity of Methanol Extract of the Leaves of *Mimusops elengi* Linn," *Bangladesh Journal of Veterinary Medicine*, Vol. 6, No. 2, 2008, pp. 197-202.

