Stamford Journal of Pharmaceutical Sciences

BRIEF COMMUNICATION

Phytochemical and Cytotoxic Investigation of *Codiaeum variegatum* Linn. Leaf

*Nadia Saffoon[1], Md. Ashraful Alam[2] and Golam Mezbah Uddin[2]

[1]Department of Pharmacy, The University of Asia Pacific, Dhanmondi, Dhaka-1209, Bangladesh.
[2]Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh.

***Corresponding Author**
Nadia Saffoon
Department of Pharmacy
The University of Asia Pacific
Dhanmondi, Dhaka-1209
Bangladesh.
Contact no.: +880 1745 924 800
E-mail: nadia_saffoon@yahoo.com

Received - 01 June 2010 *Accepted for Publication - 23 September 2010*

Phytotherapy, the treatment of diseases by the use of plants, was the beginning of pharmacotherapy or treatment of diseases by means of drugs (Ghani, 1998). The number of plants with medicinal properties included in material medica of traditional medicine in this subcontinent at present stand at about 2000 plants (Chopra et al., 1958). More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh (Yusuf et al., 1994). According to some generous estimates, almost 80% of the present day medicines are directly or indirectly obtained from plants (Ghani, 1998). The large family of euphorbiaceae composed of 321 genera, 7950 species is cosmopolitan except for arctic areas, with centers of distribution in tropical America and Africa (Raffauf, 1996). Its native habitats include India, Philippines, Sri Lanka, Thailand, Indonesia, Malaysia and some other Pacific Islands. They are also popular in East Asia and Java, Australia and now world over in the tropics (Stamps and Osborne, 2003; Taylor, 1938). The garden Crotons is an example of the most common plants in this group that have not been fully explored. The garden Croton, *Codiaeum variegatum* Linn. is a group of beautifully variegated leafy perennial, tropical ornamental herbs, shrubs or trees with glabrous branches and prominent leaf scars (Dutta, 2004). There are only six known basic species of *C. variegatum* (Taylor, 1938). Aside its ornamental value, it is also used for medicinal purposes. Root decoction is taken to treat gastric ulcers. Its leaves contain antibacterial and antiamoebic properties (Moundipa et al., 2005). Freeze-dried leaf decoction of *C. variegatum* is taken as tea by Filipinos (Gertrudes, 2006). Drinking of crushed leaves cures diarrhoea. Young leaves of *C. variegatum*, *Pandanus macroieacceretia* (white part), coconut milk and sap of the root of *Areca catechu* is drunk for gonorrhea treatment. Also, sap of the leaves is pressed out and mixed with coconut milk and spread over affected area of syphilis (Robert et al., 1988). In the present study two different types of leaves, called Spiral and Curly Boy have been used to evaluate their chemical constituents and their cytotoxic potentiality. Methods used in phytochemical screening should be simple, rapid and can be done with the help of minimum equipment and reasonably selective classes of compounds under study (Sofawora, 1993). The phytochemical screening of plant materials to determine the presence of bioactive constituents is vital in the knowledge of therapeutic properties of plants (Ogunwenmo et al., 2007). Bioactive compounds are almost always toxic in high doses. The *in vivo* lethality in a simple zoological organism can be used as a convenient monitor for screening and fractionation in the discovery and monitoring of bioactive natural products. (Meyer et al., 1982). A general bioassay that appears capable of detecting a board spectrum of bioactivity present in crude extracts is the brine shrimp lethality bioassay (BSLT). The technique is easily mastered, costs little, and utilizes small amount of test material. The aim of this method is to provide a front-line screening that can be backed up by more specific and more expensive bioassays once the active compounds have been isolated. It appears that BSLT is predictive of cytotoxicity and pesticidal activity (Ghisalberti, 1993).

Two types of leaves of *C. variegatum* were collected from Siddeswari campus of Stamford University Bangladesh at day time and was identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka where the voucher specimen no: 31,988 has been deposited for reference. Then the leaves were garbled and dried by air in tray dryer for 15 days. After drying the leaves were grinded by hammer mill. Fine powder was obtained after grinding which was almost green colored and was extracted with 80% of methanol with the help of a Soxhlet apparatus. The

solvent was completely removed and the dried crude extracts (of both Curly boy and Spiral) were obtained.

Photochemical screening of the methanolic extracts of leaves of Curly Boy and Spiral was done according to the methods described by Ghani (1998).

Brine shrimp lethality bioassay (McLaughlin et al., 1998; Meyer et al., 1982; Persoone, 1980) technique was applied for the determination of cytotoxic property of the plant extracts. Vincristine sulphate was used as the positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 µg/ml from which serial dilutions were made using DMSO to get 10 µg/ml, 5 µg/ml, 2 µg/ml, 1 µg/ml, 0.8 µg/ml, 0.6 µg/ml, 0.4 µg/ml, 0.2 µg/ml, 0.1 µg/ml. Then the solutions were added to the pre-marked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water. 100 µl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii. If the brine shrimps in these vials show a rapid mortality, then the test is considered as invalid as the nauplii died due to some reasons other than the cytotoxicity of the compounds. 2 mg of each of the extracts was dissolved in DMSO and solutions of varying concentrations such as 200, 150, 100, 50, 20, 15, 10, 8, 4, and 2 µg/ml were obtained by serial dilution technique. Then the solutions were added to the pre-marked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

From different group tests of methanolic leaf extracts of *Cordiaeum variegatum* Linn. (Curly Boy), presence of saponins, reducing sugars, tannins and gums were revealed. No alkaloids, steroids or flavonoids were found from the tests. Methanolic extracts of leaves of *Cordiaeum variegatum* Linn. (Spiral) also provided the presence of saponins, reducing sugars, tannins and gums. In addition group tests for steroids, and flavonoids found to be positive incase of spiral leaves.

Table 1: Results of the test samples of *C. variegatum*

Sample	LC_{50} (µg/ml)	LC_{90} (µg/ml)	Regression Equation	R^2
Vincristine Sulfate (Positive control)	0.072	3.20	y = 24.27x + 77.745	0.9522
C. variegatum **(Curly Boy extract)**	4.24	38.71	y = 41.657x + 23.858	0.9147
C. variegatum **(Spiral extract)**	0.968	16.49	y = 32.479x + 50.465	0.926

The phytochemical investigation of *C. variegatum* Linn. for two types of leaves named Curly Boy and Spiral supported the previous claim of tannin content of these plants. Two types of leaves only differed in their steroids and flavonoids content. The brine shrimp lethality bioassay indicates that this plant has got cytotoxic properties. LC_{50} for Spiral was bellow 1 µg/ml. In comparison to the positive control it can be said that both Curly Boy and Spiral crude extracts exhibited promising cytotoxic activities. It was reported by Nonaka et al. that tannin gallo and ellagic acid were inhibitors of HIV replication. The results from the phytochemical investigation and the cytotoxicity tests thus can be correlated with each other. A further investigation regarding the principle compound(s) involved in the cytotoxic property of *C. variegatum* needs to be studied.

REFERENCES

Chopra, RN. (1958), Indigenous drugs of India. UN Dhar & Sons Pvt. Ltd., Calcutta.

Dutta AC. (2004), *Botany for Degree Students*, 6th Ed, Oxford University Press, pp. 415-417, 592.

Gertrudes QB. (2006), Phytochemical analysis and toxicity test of functional foods. 24th Annual PAASE Meeting: Philippine-American Bonds in Science and Technology.

Ghani A. (1998), Medicinal plants of Bangladesh: Chemical constituents and uses. Asiatic Society of Bangladesh.

Ghisalberti EL. (1993), Detection and isolation of bioactive natural products. In S. M. Colegate, & R. J. Molyneux (Eds.), Bioactive natural products: detection, isolation and structure elucidation, pp. 15-18. Boca Raton: CRC Press.

McLughilin JL, Rogers LL. (1998), The use of Biological assays to evaluate botanicals. *Drug Information J.* 32: 513-524.

Meyer BN, Ferringni NR,. Puam JE, Lacobsen LB, Nicols DE, McLaughilin JL. (1982), Brine Shrimp: A convenient general bioassay for active constituents. *Planta Med*. 45: 31-32.

Moundipa P, Kamini G, Charles F, Iris B (2005), Medicinal Plants from Cameroon with amoebicidal activity: *Codieaum variegatum*, a potential source of new products against Amoebiasis. *Afr. J. Tradit. Complement. Altern. Med*. 2: 113-121.

Nonaka G, Nishioka I , Yamagishi T, Kashiwada Y , Dustschman GE, Bodner AJ et al. (1990). Anti-AIDS agents, 2: Inhibitory effects of tannins on HIV reverse transcriptase and HIV replication in H9 lymphocytes cells. *J. Nat. Prod.* 53(3): 587-595.

Olusola KO, Idowu OA, Innocent C, Esan EB, Oyelana OA. (2007), Cultivars of *Codiaeum variegatum* (L.) Blume (Euphorbiaceae) show variability in phytochemical and cytological characteristics. *African Journal of Biotechnology.* 6(20): 2400-2405

Persoone G. (1980), Proceeding of the International Symposium on brine shrimp*, Artemia salina*, Vol. 1-3, Universa Press, Witteren, Belgium.

Raffauf RF (1996), Plant Alkaloids; A guide to their discovery and distribution. Food products Press, pp. 82-84

Robert A, Shorley L, Leslie A. (1988), The Palauan and Yap Medicinal plant studies of Masayoshi Okabe. *ATOLL Res. Bull*. No. 317.

Sofowara A. (1993), *Medicinal plants and traditional medicine in Africa*. John Wiley and Son Ltd., pp. 150-153.

Stamps RH, Osborne LS. (2003), Croton production and use. Circular ENH 878, Series of the Environmental Hort. Dept., Florida COOP. Ext. services. IFAS, Univ. Florida EIDS, http://edis.ifas.ufl.edu

Taylor N. (1938), *The garden dictionary and the practical encyclopedia of garden horticulture and landscape design*. De Lux ed., Garden City Publishing Company Inc., Garden City, New York, USA.

Yusuf M, Chowdhury JU, Wahab MA Begum J. (1994), *Medicinal Plants of Bangldesh*. 1st ed. Dhaka, Bangladesh Council of Scientific and Industrial Research (BCSIR).