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Evaluation of antioxidant activity and cytotoxic potential of *Cryptocoryne ciliata*

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ABSTRACT

Cryptocoryne ciliata belonging to the Araceae family has been investigated for isolation of its secondary metabolites and evaluation of biological activities of the crude extractives with special emphasis to the antioxidant activity and brine shrimp lethality bioassay. The whole plant was extracted with methanol and concentrated extract was partitioned using petroleum ether, carbon tetrachloride and ethyl acetate. Aqueous soluble fraction of the methanolic extract showed the highest antioxidant activity. The carbon tetrachloride soluble fraction of the methanolic extract and the ethyl acetate soluble fraction of the methanolic extract showed moderate antioxidant activity as compared to free antioxidant activity of tert-butyl-1-hydroxytoluene. In the brine shrimp lethality bioassay, among all extracts of whole plant of *C. ciliata*, the carbon tetrachloride soluble fraction of the methanolic extract showed strong cytotoxic activity. Aqueous soluble fraction of the methanolic extract, methanolic crude and ethyl acetate soluble fraction of the methanolic extract showed mild cytotoxicity as compared to that of vincristine sulphate. The study confirms the mild to moderate antioxidant and moderate potent cytotoxic activities of *C. ciliata* plant extract as compared to reference standards and therefore demands the isolation of active principles and thorough bioassay.

Key Words: *Artemia salina*, bioactive compound, cytotoxicity, brine shrimp lethality bioassay, free radical scavenging activity, nauplii.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive compound, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982)

and natural products play an important role in drug development programs in the pharmaceutical industry (Baker *et al.*, 1995). In the present study whole plant of *Cryptocoryne ciliata* was used to evaluate antioxidant activity and cytotoxic potentiality of it. Antioxidant in compounds plays an important role as a health-protecting factor. The role of free radicals in our body generates reactive oxygen species and these are capable of damaging crucial bio-molecules. If they are not effectively scavenged by cellular constituents, they lead to disease conditions (Halliwell and Gutteridge, 1989).

Bioactive compounds are almost always toxic to living body at some higher doses. Brine shrimp lethality bioassay (McLaughlin *et al.*, 1998; Persoone, 1980) is a rapid and comprehensive bioassay for the bioactive compounds of natural and synthetic origin. By this method, natural product extracts, fractions as well as pure compounds can be tested for their bioactivity. In this method In vivo lethality in a simple zoological organism (Brine shrimp nauplii) is used as a favorable monitor for screening

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and fractionation in the discovery of new bioactive natural products (Meyer *et al.*, 1982). This bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, antiviral, pesticidal and anti-tumor etc. of the compounds (Meyer *et al.*, 1982; McLaughlin *et al.*, 1998).

MATERIALS AND METHODS

Plant sample of *Cryptocoryne ciliata* was collected from Khulna in September 2010. The whole plant part(s) was collected in fresh condition. It was sun-dried and then it was oven-dried at reduced temperature (not more than 50°C) to make it suitable for grinding purpose. The dried whole plant was then crushed into coarse powder by a high capacity grinding machine. The powdered material was extracted with methanol with the help of a soxlet apparatus in the Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka. The solvent was evaporated to obtain a solid residue. 5gm of methanol extract of whole plant was triturated with 90% methanol. The crude extract was dissolved completely. This was mother solution, which was fractioned successfully by three solvents of different polarity such as petroleum ether, carbon tetrachloride and ethyl acetate.

A simple method that has been developed to determine the antioxidant activity of plant extract utilizes the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Brand-Williams *et al.*, 1995). The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and purple in color and it is compared to that of tert-butyl-1-hydroxytolunene (BHT) by UV spectrophotometer. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured (Choi *et al.*, 2000; Desmarchelier *et al.*, 1997).

The methanolic extract of the whole plant (ME) and the different partitionate of the methanolic extract of the whole plant i.e. aqueous soluble partitionate (AQ), petroleum ether soluble partitionate (PE),

carbon tetrachloride soluble partitionate (CTC) and ethyl acetate soluble partitionate (EA) of *Cryptocoryne ciliata* were subjected to DPPH method. 2ml of a methanol solution of the extract at different concentration (0.500 to 0.977µg/ml) were mixed with 3ml of a DPPH methanol solution (20µg/ml). After 30 min reaction period at room temperature in dark place, the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. Inhibition free radical DPPH in percent (%) was calculated as follows:

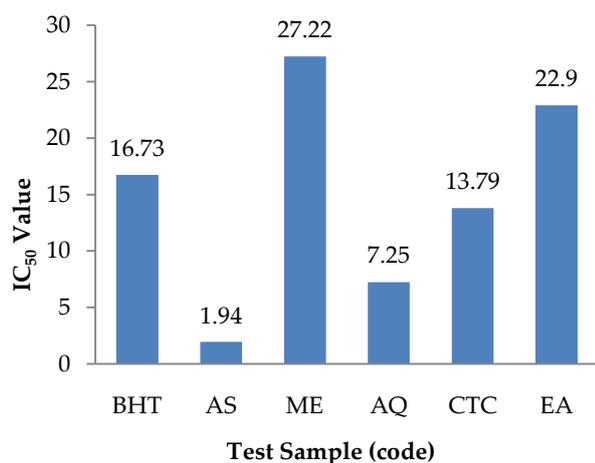
$$I\% = \frac{1 - A_{\text{sample}}}{A_{\text{blank}}} \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagent except the test material).

The methanolic extract of the whole plant (ME) and the different partitionate of the methanolic extract of the whole plant i.e. aqueous soluble partitionate (AQ), petroleum ether soluble partitionate (PE), carbon tetrachloride soluble partitionate (CTC) and ethyl acetate soluble partitionate (EA) of *Cryptocoryne ciliata* were subjected to brine shrimp lethality bioassay (Meyer *et al.*, 1982). All the test samples (ME, AQ, PE, CTC and EA) were taken in vials and dissolved in 100µl of pure dimethyl sulfoxide (DMSO) to get stock solution. Then 50µl of this solution was taken in the first test tube containing 5ml of sea water and 10 shrimp nauplii. Thus the final concentration of the prepared of the first test tube was 800µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In every case, 50µl of the test samples were added to the test tube and fresh 50µl DMSO was added to the vial. Thus different concentrations were found in the different test tubes. Control groups were used in the cytotoxicity study to validate the test method and ensure that the result obtained are only due to the activity of the test agent and the effects of the other possible factors are nullified. In the present study vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 20µg/ml from which serial dilutions were done using DMSO to get solutions of concentrations 10µg/ml, 5µg/ml, 2.5µg/ml, 1.25µg/ml, 0.625µg/ml, 0.3125µg/ml, 0.15625µg/ml, 0.078125µg/ml and 0.039µg/ml. The positive control

Table 1: IC₅₀ value of standard and different partitionates *Cryptocoryne ciliata*.

Code	Sample	IC ₅₀ (µg/ml)
BHT	Tert-butyl-1-hydroxytoluene (standard)	16.73
AS	Ascorbic acid (standard)	1.94
ME	Methanolic extract	27.22
AQ	Aqueous soluble partitionate of methanol extract	7.25
CTC	Carbontetrachloride soluble partitionate of methanol extract	13.79
EA	Ethyl acetate soluble partitionate of methanol extract	22.90

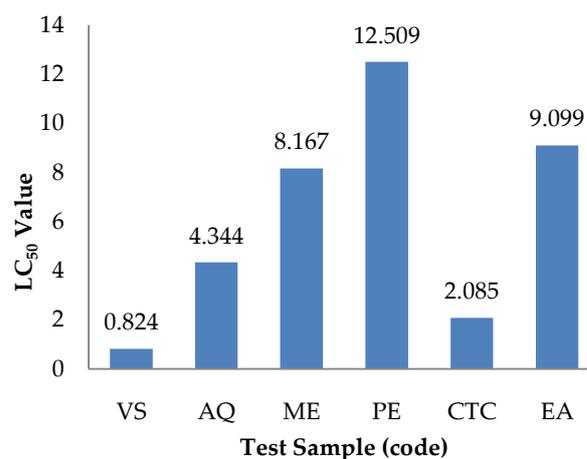
**Figure 1: IC₅₀ values of standards & different partitionates of *Cryptocoryne ciliata*.**

solution was added to the pre-marked vials containing 10 living shrimps nauplii in 5ml simulated sea water to get positive control group result. 50µl DMSO was added to each three pre-marked glass vials containing 10 living shrimps nauplii in 5ml simulated sea water to use as negative control group. If the brine shrimps in these vials showed a rapid mortality rate, the test was considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds.

RESULTS AND DISCUSSION

Extract concentration providing 50% inhibition (IC₅₀) was calculated from the graph plotted inhibition percentage against extract concentration and the results are given in Table 1 and Figure 1. Here BHT was used as positive control.

In this investigation, the methanolic extract (ME), aqueous (AQ), carbon tetrachloride (CTC) and ethyl acetate (EA) soluble partitionate of the whole plant showed the free radical scavenging activity with

**Figure 2: LC₅₀ values of the different extractives of *Cryptocoryne ciliata*.**

IC₅₀ value 27.22µg/ml, 7.25µg/ml, 13.79µg/ml and 22.90µg/ml, respectively.

The lethal concentration (LC₅₀) of the test samples after 24 hours was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis. The lethality of the extractives to brine shrimps was determined and the results are given in Table 2 and Figure 2.

Table 2: LC₅₀ and LC₉₀ values of the test samples of *Cryptocoryne ciliata*.

Test Samples	Regression equation	R ²	LC ₅₀ values	LC ₉₀ values
VS	y = 33.21x + 52.78	0.971	0.824	13.205
AQ	y = 22.95x + 35.36	0.974	4.344	240.341
ME	y = 29.8x + 22.82	0.976	8.167	179.623
PE	y = 33.22x + 13.55	1.000	12.509	200.136
CTC	y = 24.76x + 42.10	0.953	2.085	86.015
EA	y = 34.63x + 16.79	0.974	9.099	130.036

The LC₅₀ values of AQ, ME, PE, CTC and EA were found to be 4.344µg/ml, 8.167µg/ml, 12.509µg/ml, 2.085µg/ml and 9.099µg/ml, respectively. However, varying degree of lethality of *Artemia salina* was observed with exposure to different dose levels of the test samples ranging from 0-800µg/ml and further investigation regarding the cytotoxic property of *Cryptocoryne ciliata* needs to be studied.

CONCLUSION

The antioxidant and cytotoxic activities of various fractions of *Cryptocoryne ciliata* plant, found in this study, may explain some of the traditional medicinal uses of this plant. These could be of particular interest in relation to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates.

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