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Cytotoxic and thrombolytic activity of the aerial part of *Cestrum diurnum* L. (Solanaceae)

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Abstract

The methanol extract of *Cestrum diurnum* L. belonging to the family Solanaceae has been investigated for the presence of its secondary metabolites and evaluation of biological activities of the crude extractive for the brine shrimp lethality bioassay and thrombolytic activity. The methanol extract of the plant indicated the presence of alkaloids, steroids and tannin. The plant exhibited significant cytotoxic effect on brine shrimp lethality bioassay, where the plant extracts showed LC_{50} of 0.074 µg/ml and LC_{90} of 4.85µg/ml after 2 hrs and later the all shrimp died after 4hrs at the same concentration. For the standard chloramphenicol LC_{50} and LC_{90} were found 2.56µg/ml and 4.82µg/ml respectively after 2hrs. After 4hrs LC_{50} and LC_{90} were found 1.772µg/ml and 4.364µg/ml respectively. The percentages of clot lyses were found 8.783% for test sample, 2.44% for blank and 80.43% for standard streptokinase in thrombolytic assay.

Key words: *Cestrum diurnum,* Cytotoxic, Brine shrimp, Thrombolytic, Streptokinase

Introduction

Cestrum diurnum L. (Family: Solanaceae), Day-blooming Jasmine in English, is an erect evergreen woody shrub numerous leafy branches. The branches, which are green and with well-marked white lenticels when young, fawn with age. The younger parts are covered with a very sparse glandular scruf, leaves are simple, flower are short clusters of sweet white-smelling and fruits are black, nearly globular berry[1]. The genus *Cestrum* (Solanaceae) has many species that are used in the Chinese traditional medicine for the treatment of burns and swellings[2]. The plant is reported for alkaloids[3], a nor-lignan glycoside- cestrumoside[2], ursoloic acid[4]and some steroidal saponins named diurnoside[4], cesdiurins I-III[5]. The plant is also evaluated for antioxidant, hepatoprotective[6] and antimicrobial activity[7]. The plant is also reported to enhance calcium and phosphate uptake[8-9].

Materials and Methods

Collection and identification of plant material

Cestrum diurnum L. was collected from Dumuria, Khulna Bangladesh in February 2013. The species was confirmed a scientific officer, Bangladesh National Herbarium, Mirpur, Dhaka and a voucher specimen of the plant has been deposited in the library of the same institution for further reference.

Preparation of methanol extract

The collected aerial parts plant were separated from undesirable materials and shade-dried for ten days. The dried plant materials were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powdered sample was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 135 g of powered plant was taken to a clean, dry, flat-bottomed glass container and soaked in 400 ml of 99% methanol. Then the container was sealed and kept for a period of 10 days with occasional shaking or stirring[10]. The whole mixture then underwent a coarse filtration by cotton. It was then filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate was concentrated under air and dried. It rendered a greenish black color and was designated as crude methanol extract.

Chemicals and reagents

Standard chromogenic reagents used for chemical group test were of reagent grade and purchased from Sigma-Aldrich Co. LLC, Missouri, United States. Cholarphenicol, used as a standard drug in the cytotoxic assay was collected from the Square Pharmaceuticals Limited, Bangladesh. Methanol supplied by Laboratory Patterson Scientific, U.K. was used as solvent. Dimethyl sulfoxide DMSO, ≥99.9% purchased Sigma-Aldrich, India was used as solvent to dissolve the extracts. Laboratory reagent grade phosphate buffered saline (PBS) used as buffering ingredients was purchased from Fisher Scientific, U.K. Commercially available lyophilized streptokinase vial (Shanghai SIPI Pharmaceutical Co. Ltd., China) was used in thrombolytic activity assay. Sodium Chloride Crystal GR from Merck Ltd., Mumbai, India was used to prepare sea water in brine shrimp lethality bioassay.

Brine Shrimp

Brine shrimp eggs were purchased from Carolina Biological Supply Company, Burlington, NC, USA.

Instruments and equipment

pH Meter (pHep-HI 98107, Hanna Instruments , Romania), electronic balance (serial no.- 1508, OHAUS, Germany), vortex mixer (VM-2000, 220 V, Digisystem Laboratory Instruments Inc. Taiwan), centrifuge machine (Model 800, 50 W, 4000 rpm, China) were used for this study.

Test for different chemical groups

The crude methanol extract of *Cestrum diurnum* L. was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins using standard protocol[11]. 10% (w/v) solution of the extract in methanol was used for each of the above test.

Test for cytotoxic activity

The cytotoxicity assay was performed on brine shrimp nauplii using the modified method of Mayer *et al*[12] determining the 50% lethal dose (LC_{50}) and 90% lethal dose (LC_{90}) of the extract. Brine shrimp nauplii were obtained by hatching brine shrimp eggs in artificial sea-water (3.8% NaCl solution) for 22 hrs. Sample was prepared by dissolving of 50 mg of plant extract in 5ml of artificial sea water containing Dimethyl sulfoxide (DMSO) to have concentration of 10 µg/µl. From this solution 10, 20, 40, 80, 160 and 320 µl were transferred to each

10 ml vial and using artificial sea water volume was adjusted to 10 ml water to give concentrations of compound of 10, 20, 40, 80, 160 and 320 µg/ml respectively. Brine shrimp nauplii were grown in these solutions and observed their mortality for 24 h. But all nauplii died in the test sample. Then the starting time of observation was reduced gradually. Finally the % mortality was counted after 2 hrs and 4 hrs. The resulting data were transformed to probit analysis software (LdP Line software, USA)[13, 14] for the determination of LC_{50} and LC_{90} values of the extract. Artificial sea-water medium containing DMSO used for the analysis was employed as negative control. Chloramphenicol was used as standard in this assay.

***In vitro* thrombolytic activity**

5 ml of phosphate buffered saline (PBS) was added to the lyophilized streptokinase vial (15, 00,000 I.U.) and mixed properly. This suspension was used as a stock from which appropriate dilutions were made to observe the thrombolytic activity[15]. 2 ml venous blood drawn from healthy volunteers was distributed in three different pre weighed sterile microcentrifuge tube (0.5 ml/tube) and incubated at 37°C for 45 minutes. After formation of clot, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each microcentrifuge tube containing pre-weighed clot, 100 µl of methanol extract of *Cestrum diurnum* L. (10 mg/ml) was added. As a positive control, 100 µl of streptokinase was added to a test tube labeling positive control. 100 µl of distilled water were separately added to the test tube numbered as a negative non thrombolytic control. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lyses. After incubation, released fluid was removed by cotton carefully and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lyses was expressed as percentage of clot lyses and calculated by the following equation-

% of clot lysis= (weight of released clot/ clot weight) × 100

Results

Chemical group test

Different chemical tests on the methanol extract of *Cestrum diurnum* L. showed the presence of steroids, alkaloids and tannins (Table 1).

Cytotoxic activity

Table 2 shows the cytotoxic effect of the methanol extract *Cestrum diurnum* L. using brine shrimp lethality bioassay. In the test, the extract showed LC_{50} and LC_{90} of 0.074 µg/ml and 4.85 µg/ml after 2 hours respectively. Where, standard chloramphenicol showed LC_{50} and LC_{90} of 2.56 µg/ml and 4.82µg/ml after 2 hour respectively. After 4 hour, all nauplii died in the test sample. The LC_{50} and LC_{90} were found 1.772µg/ml and 4.364µg/ml respectively for chloramphenicol after 4 hour. Control group did not show any mortality. An approximate linear correlation was observed when concentrations versus percentages of mortality were plotted on graph paper.

Thrombolytic activity

Table 3 shows the effect of the methanol extract on clot lyses activity. The percentage (%) clot lysis was statistically significant ($p<0.001$) when compared with vehicle control. The plant extract showed mild colt lyses activity of 8.78% whereas standard streptokinase showed 80.43% clot lyses activity.

Discussion

The methanol extract of *Cestrum diurnum* L. demonstrated the presence of alkaloids, steroids and tannins as secondary metabolites. Brine shrimp lethality bioassay is an easy and straight forward bench top screening method for predicting important pharmacological activities like enzyme inhibition, ion channel interference, antimicrobial and cytotoxic activity[12, 16]. In the present study the extract showed LC_{50} at a very low concentration with very quick response indicating that the extract is significantly potent. Ideally, any agent useful in the treatment of cancer should not be toxic to normal cell. However, in reality, anticancer agents are often toxic to normal cells, particularly towards rapidly growing cells[17]. It is necessary to test this extract in low concentration to evaluate its potency and also against various cancer cell lines as well as normal cell lines to justify the potential to further investigate this plant for anticancer activity.

Further investigation is required to find the responsible compound(s) for the cytotoxic activity observed for *C. diurnum*. In the thrombolytic bioassay result suggested that the extract showed very mild activity. The plant may be evaluated again for both thrombolytic and thrombotic activity.

Present study is based on the report of preliminary phytochemical and biological screening of *C. diurnum* extract. Advanced studies including LC-MS can be carried out to get a bigger picture of the chemical constituents present in the plant. On the basis of the results from above studies, bioassay guided approach can be undertaken to isolate and identify the active component(s).

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References

1. Bor NL, Raizada MB. Some beautiful Indian climbers and shrubs. First edn. Bombay Natural History Society, Mumbai, India 1954;130-131
2. Khaled MM, Mostafa AF, Matsunami K, Kamel MS, Otsuka H. A new norlignan glycoside from *Cestrum diurnum* L. ARKIVOC 2007;8:63-70
3. Halim AF, Collins RP, Berigari MS. Alkaloids produced by *Cestrum nocturnum* and *Cestrum diurnum*. Planta Med 1971;20(1):44-53
4. Chakravarti RN, Datta S, Mitra MN. Tigogenin and ursolic acid from *Cestrum diurnum* Linn. Experientia 1964;20(4):200
5. Mostafa AF, Khaled MM, Kamel MS, Matsunami K, Otsuka H. Cesdiurins I-III, steroidal saponins from *Cestrum diurnum* L. J Nat Med 2008;62(2):168-73
6. Begum AS, Goyal M. Study of antioxidant and hepatoprotective activities of standardized *Cestrum diurnum* leaf extract. Inventi Rapid: Ethnopharmacology 2010;1(1).
7. Panghal M, Kaushal V, Yadav JP. *In vitro* antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. Ann Clin Microbiol Antimicrob 2011;10:21
8. Mello JR, Habermehl GG. Effects of calcinogenic plants–qualitative and quantitative evaluation. Dtsch Tierarztl Wochenschr 1998;105(1):25-29
9. Peterlik M, Wasserman RH. Stimulatory effect of 1,25-dihydroxycholecalciferol-like substances from *Solanum malacoxylon* and *Cestrum diurnum* on phosphate transport in chick jejunum. J Nutr 1978;108(10):1673-1679
10. Nayeem AA, Khatun A, Rahman MS, Rahman M. Evaluation of phytochemical and pharmacological properties of *Mikania cordata* (Asteraceae) leaves. J of Pharmacognosy and Phytotherapy 2011;3(8):111-123
11. Khatun A, Rahman M, Kabir S, Akter MN, Chowdhury SA. Phytochemical and pharmacological properties of the methanolic extract of *Ardisia humilis* Vahl. (Myrsinaceae). Int J of Res in Ayur and Pharm 2013;4(1):38-41
12. Meyer BN, Ferrigni NR, Putnam JB, Jacobsen LB, Nichols DE, JL M. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 1982;45:31-34
13. Uddin SJ, Grice ID, Tiralongo E. Cytotoxic Effects of Bangladeshi Medicinal Plant Extracts. Evid Based Complement Alternat Med 2011;1-6.
14. Finney DJ. Probit analysis. 3rd ed. Cambridge: University Press; 1971, 18, 37-77.
15. Khatun A, Rahman M, Jahan S. Preliminary phytochemical and pharmacological screening of *Murraya exotica* Linn. leaves extract. Orient Pharm Exp Med 2014; DOI: 10.1007/s13596-014-0150-x
16. Anderson JE, Goetz CM, McLaughlin JL, Suffness M. A blind comparison of simple bench-top bioassay and human tumour cell cytotoxicities as antitumor prescreens. Phytochem Analysis 1991;2:107-111
17. Priestman, T. Cancer Chemotherapy in Clinical Practice. London: Springer-Verlag 2008; 130-136..

Table 1: Results of phytochemical screening of *Cestrum diurnum* L. extracts.

Phytoconstituents	**Methanol extract of** *Cestrum diurnum* L.
Steroids	+
Carbohydrates	-
Flavonoids	-
Gums	-
Saponins	-
Alkaloids	+
Tannins	+

+: Positive result; - : Negative result;

Table 2: Brine shrimp lethality bioassay of the methanol extract of *Cestrum diurnum* L.

Sample	**After 2 hrs**		**After 4 hrs**		**Regression equation**	
	LC_{50} (µg/ml)	LC_{90} (µg/ml)	LC_{50} (µg/ml)	LC_{90} (µg/ml)	After 2 hrs	After 4 hrs
Methanol extract of *C. diurnum* L.	0.074	4.85	-	-	y = 9x + 49.333	-
Choramphenicol	2.56	4.82	1.772	4.364	y = 17.714x + 4.6667	y = 15.429x + 22.667

Table 3: *In vitro* thrombolytic activity of the methanol extract of *Cestrum diurnum* L.

Sample	**% clot lyses**	**Average**
Blank	2.44	
Methanol extract of *Cestrum diurnum* L. for volunteer 1	10	
Methanol extract of *Cestrum diurnum* L. for volunteer 2	8.51	8.78
Methanol extract of *Cestrum diurnum* L. for volunteer 3	7.84	
Streptokinase	80.43	