ANALGESIC, ANTIBACTERIAL AND CYTOTOXIC ACTIVITY OF *CORDIA DICHOTOMA*

[1] Shazid Md. Sharker*, [2] Khadiza Pervin, [2] IZ Shahid

[1] Department of Pharmacy, BGC Trust University Bangladesh, Chittagong. Bangladesh.
[2] Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh

Summary

The crude ethanol extract of leaves of *Cordia dichotoma* (Family-Borginaceae) was screened for its analgesic, antibacterial and cytotoxic activities. The extract produced significant writhing inhibition in acetic acid induced writhing in mice at the oral dose of 500 mg/kg body weight respectively (P<0.001), which was comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. When tested for its antibacterial effects disc diffusion method, it significant zone of inhibition of against both Gram negative and Gram positive bacteria against *Streptococcus aureus* , *Streptococcus pyogenes, Vibrio cholerae, Streptococcus epidermis, Hafnia* and *Escherichia coli* which is compareable with Kanamycin (30µg/ml).. Moreover, when tested for toxicity using brine shrimp lethality bioassay, the extract showed potent activity against the brine shrimp *Artemia salina* (LC_{50}: 20 µg/ml and LC_{90}:180µg/m). The overall results tend to suggest the analgesic, antibacterial and cytotoxic activities of the extract.

Key words: analgesic, antibacterial and cytotoxic; *Cordiad dichotoma;*

***Corresponding Author:**
Shazid Md. Sharker
Lecturer,
Department of Pharmacy,
BGC Trust University Bangladesh,
"BGC Biddynagar" Chandanaish,
O.R. Nizam Road, 1327/1418, BGC Tower (Fazal Tower),
Chittagong. Bangladesh.
E-mail: pharm_shazid@yahoo.com

Introduction

Cordia dichotoma (Local name- Kalahuza. Bohari, Bella in Bangladesh; Lasora in India) is a medium evergreen, grey, upto 15 m height tree. Its leaves is simple, egg shaped epileptic-lanceolate, 2.5 to 7.5 X 2.5 to 5 cm. its stem are long and branched and dropping. Flowers are seen at November to March and color is korimbuje-cyme, 3-4 flowers gather together. Fruit are white korimbuje-cyme and structure is capsule containing gummy pulp, small. Its wood is grayish in color. It occurs wildly all over Bangladesh (Moist sites, along watercourses), India (Several states), China, Taiwan, Australia, and North America [1]. Its fruit is used in cough as expectorant, purgative. Ripe fruit is used as tonic. Seed is used in ringworm. Bark is used as tonic. Leaves used in infection and headache [1].

Material and Method

For this present investigation the plant part (leaf) of *Cordia Dichotoma* was collected from the village of Shitly of Horinakundu Upazilla of Jhenidah District, Bangladesh in June 2006 and was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession number- 31363) and a voucher specimen was also deposited there. About 500 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 1000 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (ethanol extract) obtained was evaporated by a cooling fan which is used as a computer parts. It rendered a reddish crystal type of 5 (yield 1 %) gm. The gummy concentrate was designated as crude extract or extract of ethanol.

Drugs

Diclofenac Sodium (Opsonin Chemical Industries Ltd, Bangladesh), Kanamycin (Square Pharmaceuticals Ltd., Bangladesh)

Preliminary phytochemical analysis

The crude extracts were subjected to preliminary phytochemical screening for the detection of major chemical groups. In each test 10% (w/v) solution of the extract in ethanol was used unless otherwise mentioned in individual test (Table-1) 2-3.

Animals

Young Swiss-albino mice of either sex, weighing 20-25gm, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the tests. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55- 65%, room temperature 25.0±2.0^{o}C and 12 h light/dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water.

Pharmacological studies

Analgesic activity

Analgesic activity of the crude ethanol extract of *Cordia dichotoma* was tested using the model of acetic acid induced writhing in mice [4-5]. The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25 mg/kg of body weight; and group III were test groups and were treated with the extracts at the doses of 500 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7%) injection. Then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min. (Table-2)

Test of antibacterial activity

Antimicrobial activity of the crude extract as well as different solvent fractions was determined by disk diffusion method [6-7].

Preparation of disks

Three types of disks were used for antibacterial screening.

Sample disks

Sterile filter paper disks (5 mm in diameter) were taken in a blank petridish. 5 µl of sample solution (prepared by dissolving 1 g of the extract in 10 ml of ethanol) of the desired concentration (100 µg/µl) was applied on the disks with the help of a micropipette in an aseptic 1 condition. These discs were left for few minutes in aseptic condition for complete removal of solvent.

Standard disks

These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that produced by test samples. In this investigation standard kanamycin (30 µg disk) 1 disks(Oxoid, U.K.) were used as the reference.

Blank disks

These were used as negative control. They ensure that the residual solvents (left over the disks even after air drying) and the filter paper were not active themselves. 5 µl of methanol was applied on the sterile filter paper disk with the help of a micropipette and left for few minutes for complete removal of solvent.

Preparation of media

14 g dried Nutrient Agar Media (Oxoid, UK) was dissolved in 500 ml of distilled water and a clear medium was obtained by thorough shaking and heating in a water bath. The media was then sterilized in an autoclave at a temperature of 121°c and pressure of 15 lbs. (sq-inch) for 20 min.

Preparation of the seeded test plates

16 ml of the sterilized medium was poured to each (sterilized) test tube aseptically, under laminar air hood. Each of the test organisms was transferred from the subculture to the test tube with the help of the sterilized inoculating loop at 45°C under laminar air hood. The test tubes were shaken by rotation to get a uniform suspension of organisms. The bacterial suspensions were immediately transferred to the sterile petri-dishes and the petri-dishes were rotated several times, first clockwise and then anticlockwise, to assure homogeneous distribution of the test organisms to give a uniform layer of depth of approximately 4 mm. After the medium became cooled to room temperature, it was stored in a refrigerator (4°C) for 2 h.

All of the three disks (sample, standard and blank) were then placed in the seeded test Plates using sterile transfer loop for antibacterial screening. The plates were then kept at 4-8°C facilitating maximum diffusion. The plates are then kept in an incubator at 37°C for 12-18 h to allow the growth of bacteria. The experiments were carried out more than twice and the mean of the reading were recorded.

Cytotoxic activity

The brine shrimps used were obtained by hatching 5mg of eggs of *Artemia salina* in natural seawater after incubatedion at about 29^0C for 48h. The larvae (nauplii) were allowed another 48h in seawater to ensure survival and maturity before use. Five dose of plant extract (20, 40, 60, 80, 100, 120 and 140µg/ml) in 5% DMSO (Di-methyl sulfo-oxide) and/ or seawater was tested. Each extract preparation was dispensed into clean test tubes in 10µl/ml. for control; same procedure was followed except test samples. After marking the test tubes properly, 10 living shrimps were added to each of the 18 vials with the help of a Pasteur pipette [8]. The test tube containing the sample and control were then incubated at 29^0C for 24h in a water bath, after which each tube was examined and the surviving brine shrimps counted and recorded. From this, the percentage of mortality was calculated at each concentration.

Statistical Analysis

Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

Results

Preliminary phytochemical analysis

Results of different chemical tests on the ethanol extract of *Cordia dichotoma* showed the presence of steroids, tannins and glycosides. (Table1).

Table 1**:** Results of different chemical group tests of *Cordia dichotoma*

Plant Extract	Steroids	Alkaloids	Reducing Sugars	Tannins	Gums	Flavonoids	Saponins	Glycosides
Ethanol extract of *Aegiceras corniculatum*	+	-	-	+	-	-	-	+

+: Positive result; -: Negative result

Analgesic activity

Table 2 showed the effect of the ethanol extract of *Cordia dichotoma* on acetic acid induced writhing in mice. At the dose of 500 mg/kg of body weight, the extract produced about 71.75% writhing inhibition in test animals, respectively. The results were statistically significant (P<0.001) and were comparable to the standard drug diclofenac sodium, which showed 84.37% writhing inhibition at the dose of 25 mg/kg of body weight (P<0.001) (Table 2).

Table 2: Effect of ethanol extract of *Cordia dichotoma* on acetic acid induced writhing in mice

Animal Group/Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 in water 10 ml/kg, p.o.	19.20±0.735 (100)	---
Positive control Diclofenac sodium 25 mg/kg, p.o.	3±1.8 [a] (15.63)	84.37
Test group-I Ethanol extract 500 mg/kg, p.o.	5.5±1.73[a] (28.65)	71.75

Values are expressed as mean ± S.E.M (n=5); *, P- value were determined using student t-test, a<0.001 vs. control; %, percentage. p.o., per oral.

Antibacterial activity

The result of the antibacterial activity measured in term of zone of inhibition in mm. the ethanol extract for antibacterial activity was used single concentration 500 µg/disc. This extract show activity against both Gram negative and Gram positive bacteria against *Streptococcus aureus* , *Streptococcus pyogenes, Vibrio cholerae, Streptococcus epidermis, Hafnia, Escherichia coli* which is compareable with Kanamycin (30µg/ml) (Table 3).

Cytotoxic activity

In this bioassay, the extract showed lethality against the brine shrimp nauplii. The extract showed different mortality rate at different concentrations. The plot of percent mortality versus log concentration on the graph paper produced an approximate linear correlation between them. From the graph, the concentrations at which 50 and 90% mortality occurred were obtained by extrapolation (LC_{50}: 10 µg/ml; LC_{90}: 39.81 µg/ml) (Table 4).

Table 3: In-vitro anti-bacterial activity of ethanol extracts of leaves of *Cordia dichotoma*

Bacterial strains	Diameter of zone of inhibition in mm		
	Kanamycin (30µg/ml)	Ethanol extract (500µg/ml)	Blank (30µg/ml)
Escherichia coli	35	15	0
Plesiomonas			
Shigella dysenteriae	25	0	0
Hafnia	23.4	10.13	0
Vibrio cholerae	26.3	12.66	0
Shigella sonnei	31	0	0
Pseudomonas spp	26.25	0	0
Enterococci			
Streptococcus saprophyticus	30	0	0
Streptococcus aureus	26.3	12.66	0
Streptococcus pyogenes	25.4	12	0
Streptococcus epidermis	25	7.14	0

Table 4: Result of Brine shrimp lethality bioassay of ethanolic extract of leaves of *Cordia dichotoma*.

Test sample	Conc. (µg/ml)	Log (Conc.)	No. of alive shrimp	% mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Ethanol extract of *Cordia dichotoma*	20	1.30	5	50	20	180
	40	1.60	4	60		
	80	1.90	3	70		
	160	2.2	2	80		
	320	2.50	0	100		

Discussion

Since *Cordia dichotoma* belongs to the coastal forests, part of the plant constituents may be polar in nature. Ethanol was used which has a wide range of solubility in both polar and non-polar region. To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness.

Analgesic activity of the ethanol extract of *Cordia dichotoma* was tested by acetic acid induced writhing model in mice. Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings [9]. Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid [10]. The ethanol extract of *Cordia dichotoma* produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 2). On the basis of this result it can be concluded that the ethanol extract of *Cordia dichotoma* might possess analgesic activity.

The 95% ethanol extract of the leaves of *Cordia dichotoma* was tested for antibacterial activity against a number of both Gram positive and Gram negative bacteria. Standard antibiotic discs of Kanamycin were used for comparison purpose. The ethanol extract of this plant show antibacterial activity against most test organisms under experiment (Table 3).

The cytotoxic activity of the ethanol extract of *Cordia dichotoma* was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc. [11]. The extract was found to show potent activity against the brine shrimp nauplii (Table 4). Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds.

In conclusion, it could be suggested that the crude ethanol extract of *Cordia dichotoma* may possess analgesic, antibacterial and cytotoxic activities. However, further studies are necessary to find out the active principles responsible for these activities.

References

1. Dey TK., 2006. useful Plants of Bangladesh (Bangla version), 2nd edition, pp. 199, 281, 368, 875, 946, 961, 979.

2. Evans WC. (1989) *Trease and Evan's Textbook of Pharmacognosy*. 13 th edition. Cambridge University Press, London.

3. Ghani A. (1998) *Medicinal Plants of Bangladesh*. The Asiatic Society of Bangladesh, Dhaka, Bangladesh, 1st edition.

4. Ahmed F, Selim MST, Das AK, Choudhuri MSK. (2004) Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. *Pharmazie* **59,** 329-330.

5. Whittle BA. (1964), The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. *Br. J. Pharmacol. Chemother*. **22,** 246-253.

6. Ahmed, F., G.M.S. Rahman and A.K. Das, 2001. Antimicrobial activity of *Commelina benghalensis*. K. U. Studies, 3: 531-532.

7. Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by standardized single disk method. Am. J. Clin. Pathol., 45: 492-493.

8. Meyer BN, Ferrigni NR, Putnam JB, Jacobsen LB, Nichols DE, McLaughlin JL. (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45, 31-34.
9. Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJC. (2003) Anti inflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. *J. Ethnopharmacol.* 84, 31-33.
10. Derardt R, Jougney S, Delevalcee F, Falhout M. (1980) Release of prostaglandins E and F in an algogenic reaction and its inhibition. *Eur. J. Pharmacol.* **51**, 17–24.
11. Anderson JE, Chang CJ, McLaughlin JL. (1988) Bioactive components of *Allamanda schottii. J. Nat. Prod.* 51, 307-308.