IJPSR (2012), Vol. 3, Issue 06 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 February, 2012; received in revised form 02 April, 2012; accepted 22 May, 2012

ASSESSMENT OF ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF ETHANOLIC EXTRACT OF LEAVES OF *ACALYPHA HISPIDA*

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Keywords:
Acalypha hispida, Cytoxicity, *Artemia salina*, Chloramphenicol, Antibacterial activity

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ABSTRACT

The ethanolic extract of leaves of *Acalypha hispida* was evaluated for phytochemical, cytotoxic and antibacterial activities. Phytochemical studies revealed the presence of flavonoids, saponins, glycosides, reducing sugars and steroids. Cytotoxic activity was investigated by brine shrimp (*Artemia salina*) lethality assay. The extract showed potent cytotoxic effect (LC_{50} 19.95µg/ml) which is comparable to standard cytotoxic drug chloramphenicol (LC_{50} 7µg/ml). Antibacterial activity was tested by disk diffusion method. The extract exhibited significant antibacterial activity against *Salmonella typhe* and moderate activity against *Enterococcus coli, Streptococcus saprophyticus* and *Streptococcus agalactin* whereas *Shigella dysentery* found resistant at 250µg/disc and 500µg/disc.

INTRODUCTION: The history of medicinal plants in remedy of different diseases is well established. Various species of different family of plant and other sources contribute in the development of present therapeutic processes. As a part of our ongoing research in Pharmacy Discipline, Khulna University phytochemical, cytotoxic and antimicrobial activities were studied in this experiment on *Acalypha hispida.*

A. hispida (Euphorbiaceae) is an erect, sparsely branched shrub. The cultivar, 'Alba' has creamy-white catkins. Locally it is known as Sibjhul, Sibjota, Chenille plant etc. It is native to New Guinea, the Malay Archipelago and other islands in the East Indies [1]. Phytochemical analysis of the ethanolic extract of *Acalypha hispida* indicated the presence of reducing Sugar, glycoside, steroid, flavonoid, saponin.

Traditionally leaves poultice used for leprosy [2]. Decoction of leaves and flowers taken internally as laxative, diuretic and gonorrhea. Bark root used for pulmonary problems. The decoction made from its aerial parts is used in infectious diarrhoea and dysentery.

MATERIALS AND METHODS:

Plant material collection and extraction: The leaves of *A. hispida* was collected from the Boyra, Khulna, Bangladesh in 11th July 2009 at morning and identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (Accession number-34471). A voucher specimen has been deposited in Pharmacy Discipline, Khulna University, Khulna, Bangladesh.

The collected plants were separated from undesirable materials or plants or plant parts and then were washed with water. They were shade-dried for four week. The plants were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

About 150 g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 800 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 7 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 filter paper. The filtrate (Ethanol extract) obtained was evaporated by rotary evaporator.

Microorganisms: Ten species of both gram positive and gram negative bacteria were used for antibacterial test. The bacterial strains were collected from the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The bacterial strains used for the investigation are Gram negative (*Shigella dysenteriae, Shigella sonnei, Salmonella typhi, Salmonella typhi, Shigella boydii and Shigella flexneri)* and Gram positive (*Staphylococcus saprophyticus, Streptococcus pyogenes, Streptococcus agalactiae,* and *Enterococcus faecalis*.)

Preparation of Sea Water: 20g pure NaCl and 18g table salt was weighed accurately, dissolved in distilled water to make one liter and then filtered off to get a clear solution.

Cytotoxic activity: The brine shrimp lethality bioassay was used to predict the cytotoxic activity [1-2] of the crude extracts. For the experiment, 50 mg of the extracts was dissolved in dimethylsulfoxide (DMSO) and solutions of varying concentrations (5, 10, 20, 40, 80, 160, and 320 µg/µl) were obtained by the serial dilution technique using simulated seawater. The concentration of DMSO in these test tubes did not exceed 10µl/ml. The solutions were then added to the pre-marked vials containing 10 live brine shrimp nauplii in 5 ml simulated seawater.

After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. The mortality endpoint of this bioassay was defined as the absence of controlled forward motion during 30 s of observation [3]. From this data, the percent of lethality of the brine shrimp nauplii for each concentration and control was calculated. Logarithm of concentration versus percentage of mortality [4] plotted on the graph paper and the value of LC_{50} was calculated from the graph. Chloramphenicol was used as positive control [5].

Antibacterial activity: Antibacterial activity of *A. hispida* was tested by using the disc diffusion method [6-7]. In this method-measured amount of the test samples are dissolved in definite volumes of solvent to prepare solutions of desired concentration (µg/ml). The sterile Matricel (BBL, Cocksville, USA) filter paper discs are impregnated with known amount of test substances using micropipette and dried. Disk of sample, positive control and negative control are then placed in petridishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop for anti-microbial screening .

The plates are then kept at 40^{o}C for facilitating maximum diffusion. The plates are then kept in an incubator for 12-18 hour to allow the growth of the microorganisms. If the test material has any anti-microbial activity, it will inhibit the growth of microorganism giving a clear, distinct zone called “zone of inhibition”. The antibacterial activity of the test agent is determined by measuring the diameter of the zone of inhibition in term of millimeter and compared with the standard antibiotic. The experiments are carried out duplicate manner.

RESULTS: The Ethanol extract of *A. hispida* was found to show activity against the brine shrimp nauplii (**Table 1**). The LC_{50} of the test sample and standard drug chloramphenicol were found to 19.95µg/ml and 7µg/ml respectively.

The antibacterial activity was assessed against a panel of 10 pathogenic bacterial strains (both gram positive and gram negative) at the dose of 250 and 500 µg/disc, and the results were compared with the activity of the positive control, Mecillinam (25 µg/disc) (**Table 2**). At 250 µg/disc the extract showed activity against *S. typhe* (8 mm), *E. coli* (7 mm), *S. sonnei* (9 mm), *S. boydii* (7 mm), *E. faecalis* (8 mm) , *S. agalectiae* (10 mm) and *S. saprophyticus* (6 mm). At 500 µg/ disc it showed activity against *S. typhe* (10 mm), *E. coli* (10 mm), *S. flexneri* (8 mm), *S. sonnei* (14 mm), *S. boydii* (9 mm), *E. faecalis* (10 mm) , *S. agalectiae* (13 mm), *S. pyogens* (7 mm) and *S. saprophyticus* (7 mm).

TABLE 1: BRINE SHRIMP LETHALITY BIOASSAY OF ETHANOLIC EXTRACT OF LEAVES OF *A. HISPIDA*

Test sample	Conc. (µg/ml)	Log of (Conc.)	Avg.No. of alive shrimp In Test	Avg. No. of alive shrimp in Chloramphenicol	Avg.No. of alive shrimp in Negative Control	% mortality	LC_{50} (µg/ml)
Ethanolic extract of leaves of *Acalypha hispida*	5	0.69	9	6	9	10	19.95
	10	1	7	4	10	30	
	20	1.3	5	4	9	50	
	40	1.7	4	3	10	60	
	80	1.9	2	2	10	80	
	160	2.2	0	1	8	100	
	320	2.5	0	0	10	100	

Avg.:-Average

TABLE 2: ZONE OF INHIBITION OF *ACALYPHA HISPIDA* LEAVES EXTRACTS AGAINST BACTERIAL STRAINS WITH RESPECT TO MECILLINAM

Bacterial strain	Type of Bacterial strain	Blank	Diameter of Zone of Inhibition in mm		
			Mecillinam (25 µg/disc)	Ethanolic extract of leaf of *Acalypha hispida*	
				(250µg/disc)	(500µg/disc)
Salmonella typhe	Gm(-)	-	8	8	10
Enterococcus coli	Gm(-)	-	11	7	10
Shigella flexneri	Gm(-)	-	14	-	8
Shigella sonnei	Gm(-)	-	27	9	14
Shigella boydii	Gm(-)	-	14	7	9
Shigella dysenteriae	Gm(-)	-	6	-	-
Enterococcus faecalis	Gm(+)	-	31	8	10
Streptococcus agalectiae	Gm(+)	-	32	10	13
Streptococcus pyogens	Gm(+)	-	13	-	7
Streptococcus saprophyticus	Gm(+)	-	12	6	7

Gram (-):-Gram Negative Bacteria; Gram (+):-Gram Positive Bacteria; (-):- No inhibition

DISCUSSION: The brine shrimp lethality bioassay can be recommended as a guide for the detection of antitumour and pesticidal compounds because of its simplicity and low cost. It indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc. of the compounds [7-8]. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted on the graph. The results tend to suggest its possible cytotoxic activity.

Therefore, ethanolic extract of *A. hispida* might possess a significant cytotoxic activity. However, further investigations are necessary to isolate the active compound(s) responsible for the activity. Antibacterial activity was tested by using the disc diffusion method. Disc diffusion method is widely acceptable for the preliminary screening of antibacterial activity. It is essentially a qualitative or semi qualitative test indicating the sensitivity or resistance of microorganisms to the test materials [9]. The extract was found active against both gram positive and gram negative bacteria except *Shigella dysentery* and the inhibitory effects on tested species was concentration dependent.

It is well known that plant containing various phytochemical constituents such as flavonids, saponins and steroids have antimicrobial activity [10].

Plant containing Quercetagetin-7-arabinosyl-galactoside, a flavonoid has been used extensively to treat infectious disease [11]. The flavone baicalein is reported to be largely responsible for antimicrobial effects [12]. Flavonoidrich plant extracts from species of *Hypericum* [13], *Capsella* [14] and *Chromolaena* [14] have been reported to possess antibacterial activity. Many other phytochemical preparations with high flavonoid content have also been reported to exhibit antibacterial activity [15-23]. It has been reported that sponins have potent antimicrobial activity [24].

The antibacterial activity of *A. hispida* probably due to the presence of flavonoids and saponins that revealed in phytochemical studies. The zone of inhibition varies within the ranges of 6-10 mm and 7-14 mm at the dose of 250 and 500 µg/disc respectively. The highest zone of inhibition was found against *Shigella sonnei* (14 mm) at 500 µg/disc. As it showed a moderate activity against *E. coli, E. facealis* and *S. agalectiae*, the results support the traditional use of this plant as a remedy of

infectious skin infections and gonorrhea. In conclusion, it can be suggested that the crude ethanolic extract of *A.hispida* may possess cytotoxicity and antibacterial activity, which correlates well with the traditional uses of the plant. Therefore, further researches are essential to find out the active principle(s) responsible for these activities.

ACKNOWLEDGEMENT: The authors are grateful to the authority of International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for providing the experimental mice and bacterial strains.

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