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Oriental Pharmacy and Experimental Medicine 2007 7(2), 157-161

Pharmacological profiles of *Holarhena antidysenterica* (L.) Wall bark

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SUMMARY

We undertook the present study to evaluate different pharmacological as antioxidant, antibacterial and cytotoxic activities of the crude ethanolic extract of the stem bark of *Holarhena antidysenterica* (Family: Apocynaceae). The antioxidant property of the extract was assessed by 1, 1-diphenyl -2-picryl hydrazyl free radical scavenging assay. The extract showed antioxidant activity (IC_{50} about ~08 μg/ml), which was comparable to standard drug ascorbic acid (IC_{50} about ~10 μg/ml). The extract showed a broad spectrum of antibacterial activity against all tested gram positive and gram negative bacteria most prominent against Enterococci, *Staphylococcus pyogenase* and *Shigella sonnie*. And the zones of inhibitions were ranging from 10 - 21 mm for all the tested bacteria. Its cytotoxic property was evaluated by brine shrimp lethality bioassay. The extract showed significant lethality and the LC_{50}, LC_{90} values were 80 μg/ml, 320 μg/ml respectively. The results tend to suggest that the extract might possess chemical constituent(s) that are responsible for antioxidant, antibacterial and cytotoxic activities.

Key words: *Holarhena antidysenterica*; Antioxidant activity; 1, 1-diphenyl-2-picryl hydrazyl; Antibacterial activity; Cytotoxic activity

INTRODUCTION

Holarhena antidysenterica (L.) Wall (Family: Apocynaceae) locally known as 'kurchi' (Beng) is a small tree, the bark is strongly antidysenteric, astringent, stomachic, antidiarrhoeal, anthelmintic and used as febrifuge, in amoebic dysentery, abdominal and glandular tumours (IF, 1964; Lloydia, 1967; Gani, 2003). Ethanolic extract of the bark possesses hypotensive properties (IJEB, 1968). Aqueous and ethanolic bark extracts were effective against enterohaemorrhagic *Escherichia coli* (Voravuthikunchai *et al.*, 2004), and ethanolic bark extract against beta-lactamase producing methicillin resistant *staphylococcus aureus* (Aqil *et al.*, 2005) and methanolic bark extract against multi-drug resistant *salmonella typhi* (Rani *et al.*, 2004). Alkaloids of ethanolic seed extract shows antibacterial and antidiarrhoeal activities (Kavitha *et al.*, 2004). Bark is rich in conessine, nor-conessine, conessinine, conessidine, kurchine, holarrhenine, holarrhine, conkurchin, kurchicine etc (Gani, 2003). Also presence of pyrrolizidine alkaloids was proved (Arseculeratne *et al*, 1981). It has got different traditional uses in different localities of Bangladesh. Considering traditional uses and researches done before on it, we intended to investigate antioxidant, antibacterial on some others and cytotoxic activities of the bark.

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MATERIALS AND METHODS

Plant material and extraction

The bark of the *Holarhena antidysenterica* (L.) Wall was collected from Carmichael University College, Rangpur campus in February, 2005 and was identified by the experts of Bangladesh National Herbarium

where a voucher specimen had been submitted for future reference (Voucher specimen no. DACB-30925). The bark was dried for 21 days without the direct contact of sunrays. Then the dried bark was finally ground and about 400 g of it was extracted by maceration over 20 days with 1,200 ml of 80% ethanol. The extract was filtered off. The solvent was evaporated at room temperature with an electric fan to get the dried extract (approx. yield value 13.4%). The crude extract was subjected to preliminary phytochemical screening for the detection of major chemical groups (Evans, 1989). The extract showed the presence of alkaloids, reducing sugars, gums, steroids, tannins, saponins etc. In each test 10% (w/v) solution of the extract in solvent was taken unless otherwise mentioned in individual test. This extract was used for pharmacological screening.

Antioxidant activity

Free radical scavenging or antioxidant activity of the extract was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl -2-picryl hydrazyl (DPPH) free radical.

Qualitative assay

Suitably diluted stock solution was spotted on pre-coated Silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted (Sadhu *et al.*, 2003).

Quantitative assay

Quantitative assay was performed on the basis of the modified method of Gupta *et al.* (2003). Stock solution (10 mg/ml) of the extract was prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 mg/ml. Then 2 ml from each diluted solution was added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm for each concentration and from these values corresponding percentages of inhibition were calculated. Then using % inhibitions and respective concentrations a graph was constructed and from it IC_{50} was calculated. The experiment was performed in duplicate and average absorbance was noted for each concentration. Ascorbic acid was used as positive control (Gupta *et al.*, 2003).

Antibacterial activity

Antibacterial activity of the crude extract was determined by disk diffusion method (Bauer *et al.*, 1966; Ahmed *et al.*, 2001).

Preparation of disks

Three types of disk were used for antibacterial screening.

Sample disks

Sterile filter paper disks (5 mm in diameter) were taken in a Petri dish. 6 μl of sample solution (prepared by dissolving 1 g of the extract in 10 ml of methanol) of the desired concentration (100 μg/μl) was applied on the disks with the help of a micropipette in an aseptic condition. These disks were left for few min in aseptic condition for complete removal of solvent.

Standard disks

In this investigation standard amikacin disks (30 μg/disk, Oxoid, U.K.) 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 sample.

Blank disks

These were used as negative control. 6 μl of methanol was applied on the sterile filter paper disk with the help of a micropipette and left for few min for complete removal of solvent.

Table 1. Antioxidant activity of *Holarhena antidysenterica* bark

Sample	Concentration (μg/ml)	% inhibition	IC_{50} (μg/ml)
Et. extract of *Holarhena antidysenterica* (stem bark)	1	19	~08
	5	32	
	10	58	
	50	78	
	100	81	
	500	84	
Ascorbic acid	1	16	~10
	5	29	
	10	51	
	50	92	
	100	97	
	500	97	

Table 2. Antibacterial activity of *Holarhena antidysenterica* bark

Bacteria	Zone of inhibition (mm)	
	Methanol Extract (600 μg/disk)	Amikacin (30 μg/disk)
Gram Positive		
Staphylococcus aureus	11	22
Staphylococcus epidermis	10	30
Gram Negative		
Shigella dysenteriae	12	30
Shigella flexneri	10	25
Shigella sonnei	15	30
Plesiomonas	12	31
Streptococcus pyogenase	21	26
Proteus spp	10	27
Enterococci	21	24
Salmonella typhi	10	31
pseudomonus	14	31

(-): No inhibition.

Preparation of media

14 g of dried Nutrient Agar Media (Oxoid, U.K.) 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.

Selection of the test organisms

Following bacteria were used as test organisms for the antibacterial activity test (Table 2).

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 then 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 experiment was carried out more than twice and the mean of the reading was recorded.

Brine shrimp

The investigation was done on *Artemia salina* (Brine shrimp). One spoon of cyst were hatched for 48 h in saline water, prepared by dissolving 20 g pure NaCl and 18 g normal edible NaCl into 1 l water. The cyst became nauplii.

Lethality bioassay

Solution of different concentrations was prepared with the extract by using dimethyl sulfoxide (DMSO) as solvent. Eight test tubes were used, in each test tube 10 shrimps were taken and solution of different

Table 3. Result of Brine Shrimp lethality bioassay of *Holarhena antidysenterica* bark

Sample	Conc. of extract (μg/ml)	Number of shrimps taken	Number of shrimps alive	Number of shrimps died	% Mortality
Ethanolic extract of *Holarhena antidysenterica* (stem bark)	5	10	10	00	0
	10	10	10	00	0
	20	10	09	01	10
	40	10	08	02	20
	80	10	05	05	50
	160	10	03	07	70
	320	10	01	09	90
	640	10	00	10	100
Chloramphenicol	200 μg/ml	10	0	10	100

concentrations applied on it. Finally volume of liquid was adjusted by saline water. The test tubes were kept for 24 h. For blank control, a test tube with saline water was kept for observation with 10 shrimps under the same condition with the test sample. For positive control, in another test tube 10 shrimps were taken with saline water. A known drug chloramphenicol as standard was introduced in the test tube with a concentration of 200 μg/ml (Table 3). The percent of mortality of the brine shrimp nauplii was calculated for every concentration to determine LC_{50} (lethal concentration).

RESULTS

In the preliminary phytochemical screening the extract showed the presence of alkaloids, reducing sugars, gums, steroids, tannins, saponins etc.

Antioxidant activity of the extract was performed on the basis of the modified method of Gupta *et al.*, (2003). The extract showed comparable antioxidant activity (IC_{50} about ~ 08 μg/ml) against DPPH free radical to that of standard drug ascorbic acid (IC_{50} about ~ 10 mg/ml) (Table 1). The extract showed antibacterial activity against all the tested gram positive and gram negative bacteria (Table 2). Most prominent against Enterococci, *Streptococcus pyogenase*, Pseudomonus, *Shigella sonnei* where the Zones of inhibitions were from 14 - 21 mm. The extract showed a mild activity against others where the zones of inhibitions were from 10 - 12 mm compare to standard antibiotic Amikacine. The bark extract was found to show lethal activity against the brine shrimp nauplii and LC_{50} was found at 80 mg/ml,) and the LC_{90} value was 320 μg/ml respectively (Table 3).

DISCUSSION

Both in vitro and in vivo systems have been therefore developed to evaluate antioxidant activity of drugs which are useful in free radical-induced different types of diseases. In this study % inhibition of free radical scavenging activity was increased with the increase concentrations of both crude extract and ascorbic acid (Fig. 1). The result might partially support the traditional uses of it for different tumours. Further studies as lipid per-oxidation inhibition, xanthin oxidase inhibition, erythrocyte membrane stability and other studies are essential to characterize them as biological antioxidants. The extract also showed antibacterial activity against all the tested bacteria. On some of it activities were prominent whereas on others activities were mild. Some of them might be the causative agents for diarrhea, dysentery and other diseases. So the results partially support the traditional use of this plant as a remedy of diarrhoea and dysentery. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor etc.

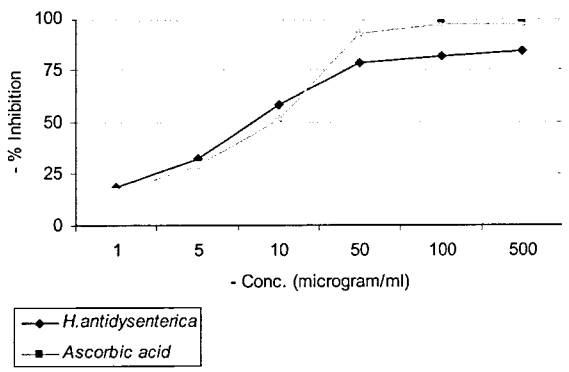

Fig. 1. Graphical representation of % inhibition of DPPH vs. concentration.

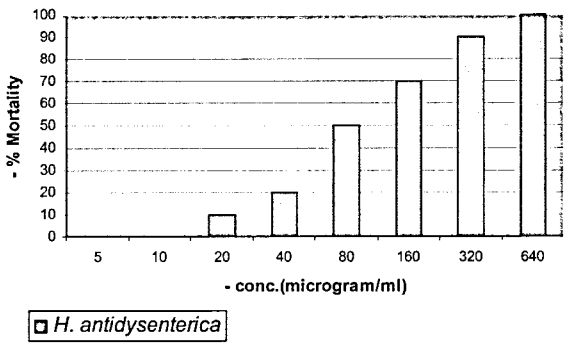

Fig. 2. Graphical representation of lethality bioassay.

activity of the compounds (Meyer, 1982; McLaughlin *et al.*, 1988). The extract was found to show significant lethality to Brine Shrimp nauplii. The rate of mortality was found to be increased with the increase of concentrations which indicated a linear relationship between them (Fig. 2). Further investigations using carcinoma cell line or others are necessary and to isolate the compounds responsible.

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