

Phytochemical and pharmacological investigation of *Viscum monoicum* (whole plant)

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Abstract

The ethanol extract of the whole plant, *Viscum monoicum* (Family- Loranthaceae) was investigated for analgesic, antidiarrheal and antibacterial activities. Phytochemical analysis of the extract indicated the presence of reducing sugar, alkaloids, gum, tannins and saponins. The ethanolic extract of with oral doses showed potential ($P < 0.05$) analgesic activity by tail immersion method in Swiss Albino mice at the doses of 200 and 400 mg/kg-body weight as compared to the standard drug pentazocine at the dose 10 mg/kg-body weight which was in dose dependent manner. In antimicrobial activity of the crude extracts performed by disc diffusion method, the extract showed activity against the bacterial strains including *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Staphylococcus epidermis* at the dose of 250 μ g/disc and 500 μ g/disc in comparison with standard drug kanamycin (30 μ g/disc). The results exhibited that some phytochemicals of the crude extracts might be responsible for analgesic, antidiarrhoeal & antibacterial activities.

Key words: *Viscum monoicum*, tail immersion, pentazocine, disc diffusion, kanamycin

Introduction

Viscum monoicum (Family-Loranthaceae), local name: Shamu lota (Bangla), is a species of mangrove. It is an evergreen shrubby, rarely herbaceous, stem-parasite of trees, shrubs. It is locally used as medicinal plant in the treatment of various major and minor ailments. It is widely distributed in different countries, namely, Bangladesh, India, Sri Lanka, Myanmar, Bhutan, Nepal, Philippines, China and Africa (1, 2).

Folkloric, whole of *Viscum monoicum* considered poisonous; in India used as a substitute for nuxvomica, used for pustular itches. Leaves are burned to ashes which are then mixed with sulphur and coconut oil, and rubbed on the body. It is also used in the treatment of rheumatism, menses, neuritis, hematemesi(3).

In this project work, an attempt was made to justify the traditional uses as per scientific experiments. Moreover, using various standard qualitative chemical tests, the presence of reported compounds were detected. Upon sufficient literature survey, it is found that a little work has been performed to evaluate the rationale for the uses of this plant in traditional medicine of Bangladesh (4). In the present study, we therefore tried to evaluate the analgesic, antidiarrheal and antibacterial activity of the ethanol extract of the whole plant *V. monoicum*.

Materials and Methods

Sample collection and extraction

The whole plant of *v. monoicum* was collected from the mangrove forest sundorban. The time of collection was October, 2011 at the daytime. The fresh whole plant was collected from the healthy host plants. During collection, any type of adulteration was strictly prohibited. The plants were mounted on paper and the sample was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession No. 36543).

The plant was shade dried. After sufficient drying,

the plant cut into small pieces, and then slashed to coarse powder with the help of mechanical grinder. The powder was stored in a suitable container to avoid any possible fungal attack.

The whole plant was subjected for shade drying and the dried plant was cut into small pieces by knife. Then the small pieces crushed to coarse powder with the help of mechanical grinder. After that the powder was stored in a suitable air-tight container. The powdered plant material was extracted by maceration for 14 days with 750ml of ethanol in a glass container accompanying regular shaking and stirring. Then plant extract filtered through the clear cotton plug. The filtrate (ethanol extract) was evaporated at room temperature with an electric fan to get the dried extract. Then dried extract was taken in a small air-tight glass container, kept in a cool, dark, and dry place for further use. The yield of the ethanol extract of dried plant material was 3.42%.

Test Animals and Pathogens

Healthy, Swiss-Albino mice of either sex weighing between 25-32 gm were purchased from the animal research branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) and used for the current experiment. The animals were maintained under standard nutritional and environmental conditions throughout the experiment (ICDDR, B formulated). The animals were housed in animal Laboratory, Pharmacy Discipline, Khulna University under the standard laboratory condition (relative humidity 55-60%, room temperature $25\pm 2^{\circ}$ C, and 12 hours light: dark cycle) to this laboratory condition for a period of 10 days prior to performing the experiments. The animals were fasted overnight before the experiments.

Antibacterial activity was performed by using both Gram-negative and Gram-positive bacteria like *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus* and *Staphylococcus epidermis* (5). These pathogens

were supplied by ICCDR, B.

Chemicals, Reagents and Standard Drugs

Castor oil was purchased from Sigma-Aldrich [(St. Louis, MO, USA)], distilled water, ethanol provided by pharmacy discipline, khulna University, Loba Chemie Pvt Ltd, India supplied Tween-80. All solvents and chemicals were the analytical grade.

Pentazocine and loperamide were also purchased from Square Pharmaceuticals Ltd, Bangladesh.

Phytochemical Screening

Phytochemical Tests

Phytochemical screening of the crude extracts was performed using the following chemicals & reagents for example, Reducing sugars with Fehling's reagent, alkaloids with Dragendorff's reagent, flavonoids with the use of concentrated hydrochloric acid, tannins with ferric chloride and potassium dichromate solutions, gums with Molish reagent & concentrated sulfuric acid, tannins with ferric chloride & potassium dichromate solution and saponins with ability to produce suds(6, 7).

Analgesic Activity

Tail immersion method

The analgesic activity of the sample was determined by the tail immersion test method described by Sewell and Spencer (8). Experimental animals were randomly selected, and divided into four groups denoted as control, positive control, and test group I and II consisting of five mice in each group. Control group received 1% Tween-80 in distilled water (10 ml/kg body weight), and positive control group received pentazocine(10 mg/kg body weight). Test group I and II were treated with the test sample at the doses of 200 and 400 mg/kg body weight. The control, positive control(standard drug) and test samples were given to the mice by gastric tube(9). After administration, the basal reaction

time was measured after in a regular interval of 30 minutes (for 30, 60, 90 & 120 minutes) by immersing the tail tips of the mice (Last 1-2 cm) in hot water heated at temperature (52 ± 1) °C. The actual flick responses of mice i.e. time taken in second to withdrawn it's tail from hot water was calculated(10).

Anti-diarrhoeal Activity

Castor Oil Induced Diarrhoea

The castor oil induced diarrhea in mice was carried out according to the method described by Uddin SN, 2008. Experimental mice were selected based on their sensitivity to castor oil-induced diarrhoea and divided into four groups ($n = 5$). Test groups were treated with the bark extract (250 and 500 mg/kg, p.o.) and positive control group was provided with loperamide (3 mg/kg, p.o.) in suspension form. Control group was treated with 1% tween-80 in distilled water (10 ml/kg, p.o.). Each mouse was provided with 0.5 mL of castor oil in oral route after the interval of 60 min for inducing diarrhoea. Each mouse was housed in individual plastic transparent cage and floor was lined with clean white blotting paper which was changed in every hour throughout the observation period of 4 h. Onset of diarrhoea and the number of stool for each mouse was counted. For the assessment of anti-diarrhoeal activity, onset of diarrhoea and percent inhibition of defecation were compared with the control group (11).

Antibacterial Activity

Disc Diffusion Assay

Antibacterial activity of the ethanolic extract of the whole plant *V. monoicum* was assessed by disc diffusion assay against a number of Gram-positive and Gram-negative strains including *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus*, and *Staphylococcus epidermis* (12). Sterile blank discs (BBL, Cocksville, USA) were saturated with the test

extract at the doses of 250 and 500 µg/disc using micropipette. Test extract was prepared using ethanol at the desired concentrations. Control discs (blank) were prepared using ethanol. Both sample and control discs were dried. Sample containing discs, standard antibiotic discs (Kanamycin 30 µg/disc, Oxoid Ltd, UK) and control discs were placed in Petri dishes containing nutrient agar medium seeded with the test pathogens using sterile forceps. Then Petri dishes were transferred into incubator and incubated at 37 °C for 16 h. After incubation, zone of inhibition was measured using digital slide calipers (5, 12).

Statistical Analysis

Results were presented as mean ± SEM. The statistical analysis of the results was done using ANOVA followed by Dunnett's test and the results (differences between groups) were considered significant at a level of $p < 0.05$.

Results

Phytochemical Tests

The ethanol extract was subjected to different qualitative phytochemical tests for detection of different biologically active chemical groups and the results are summarized in the Table 1.

Analgesic activity

Hot water tail immersion method was applied for the investigation of analgesic activity of extract of whole plant *V. Monoicum* for its central activity. The crude extract showed inhibition of thermal stimulus (5.05 ± 0.43 seconds) and (5.81 ± 0.23 seconds) at a dose 200 mg/kg and 400 mg/kg respectively as compared to standard drug pentazocine (6.29 ± 0.28 seconds) at a dose 10 mg/kg ($P > 0.05$) after 120 minutes (13).

See Table 2 & Figure 1.

Antidiarrheal activity

The crude extracts of the whole plant of *V. Monoicum* exhibited good antidiarrheal activity by castor oil induced diarrhoeal method. In this method, increases the latent period of diarrhoea and decreases the frequency of defecation at dose dependent manner. The inhibition of defecation was 34.34 % and 52.02 % at the doses of 250 & 500 mg/kg respectively as compared to standard drug loperamide at dose of 3 mg/kg showed 81.31 %.

See Table 3.

Antibacterial Activity in Disc Diffusion Assay

The antibacterial activity of the whole plant of *V. monoicum* was performed by measuring the diameter of zone of inhibition i.e. millimeter with a calibrated scale after careful incubation. The ethanol extract revealed antibacterial activity with the zone of inhibition ranging from 6.35 to 9.10 mm and 10.25 to 13.00 mm against all the tested pathogens including *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Staphylococcus epidermis* at the doses of 250 µg/disc and 500 µg/disc, respectively (14).

See Table 4.

Discussion

The phytochemical evaluation of the extract of whole plant of *V. monoicum* demonstrated the presence of reducing sugar, alkaloids, gum, tannins and saponins.

Hot water tail immersion method was applied for the investigation of analgesic activity of the whole plant of *V. monoicum* which acts centrally. Central analgesia is produced by the drugs for the inhibition of transmission of pain sensation that acts spinal cord level. The standard drug (opioid analgesics such as morphine, pentazocine, pethidine etc) bind with pre and post synaptic membrane receptor including μ , κ , and δ and acts in spinal cord level. It is adequate

tely observed that steroidal drugs such as narcotics and local anesthetics are the higher efficacious analgesia where as nonsteroidal drugs don't intensify pain threshold (15).

It is well established that non-steroidal drugs do not increase pain threshold while steroidal drugs like narcotics and local anesthetics are of strong potential analgesia. The ethanol extract exhibited centrally acting analgesia by potential increase in pain threshold analgesia which was in dose dependent manner that was highly comparable with standard drug (pentazocine).

Evaluation of *in vivo* anti-diarrhoeal activity in mice by Castor oil induced diarrhoeal model is very sensible for the involvement of prostaglandins in formation of diarrhoea by castor oil because of ricinoleic acid secretion which is responsible for the irritation of intestinal mucosa and consequently enhances bowel movement and poor absorption and ultimately watery diarrhoeal stools.

Several mechanisms are already established to identify the reasons of castor oil induced diarrhoea including inhibition of intestinal Na^+ , K^+ -ATPase activity, stimulation of prostaglandins formation through irritation of the intestinal mucosa, involvement of nitric oxide and activation of adenylate cyclase mediated active secretion

Based on its folkloric uses in the skin diseases, antibacterial activity of the ethanol extract of the whole of plant *V. monoicum* was evaluated using disk diffusion assay against all the tested bacterial strains namely *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Staphylococcus epidermis* (16). The phytochemicals such as alkaloids obtained by phytochemical test might be responsible for antibacterial activities.

The investigations were carried out using crude extract of the plant. Separation and isolation of pure compounds of the extract might be proved responsible for these pharmacological effects.

Conclusion

Our present experiments demonstrate that ethanol extracts of whole plant of *V. monoicum* contain significant analgesic, antidiarrhoeal & antibacterial activity. The ethanol extract of the whole plant of *V. monoicum* demonstrated potential analgesic, antidiarrhoeal and antibacterial activities which clarify its uses in folkloric, and detailed study is required in order to identify the active compounds responsible for bioactivities as well as its mechanism.

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Phytochemical groups	Results
Reducing sugars	+
Glycosides	-
Tannins	+
Saponins	+
Gums	+
Flavonoids	-
Alkaloids	+

Table 1: Phytochemical tests of *V. monoicum*

Treatment	Dose(per kg b.w)	Tail flick latency in seconds ($\bar{X} \pm \text{SEM}$)				
		0 min	30 min	60 min	90 min	120 min
Control	10ml	2.29 \pm 0.14	2.98 \pm 0.29	2.88 \pm 0.19	3.36 \pm 0.15	2.58 \pm 0.20
Pentazocine	10mg	1.98 \pm 0.038	4.25 \pm 0.24	5.45 \pm 0.47*	6.06 \pm 0.24*	6.29 \pm 0.28*
Test samples	200 mg	2.11 \pm 0.13	3.04 \pm 0.31	4.19 \pm 0.38 *	4.82 \pm 0.41 *	5.05 \pm 0.43*
	400mg	2.06 \pm 0.06	3.56 \pm 0.18	4.67 \pm 0.39*	5.20 \pm 0.087*	5.81 \pm 0.23*

Table 2: Analgesic activity of the whole plant of *V. monoicum* on tail immersion method

Values are expressed as mean \pm SEM (Standard Error Mean); n = 5 in each group. *indicates P<0.05; The results were analyzed by ANOVA followed by Dunnett's; b.w.:body weight

Treatment n=5	Dose (per kg b.w.)	Onset of diarrhoea (min)	No. of stools after 4 h	% Inhibition of defecation
Control	10ml	45±2.47	19.80±1.50	---
Loperamide	3mg	172.94±1.90*	3.70±0.80*	81.31
Test samples	250mg	68.45±1.65*	13.00±0.75*	34.34
	500mg	95.80±1.58*	9.50±0.65*	52.02

Table 3: Antidiarrhoeal activity of the whole plant *V. monoicum* in castor oil induced diarrhoea

Values are expressed as mean±SEM (Standard Error Mean);
The results were analyzed by ANOVA followed by Dunnett's test; b.w.:body weight.

Bacterial strains	Type of bacteria	Diameter of zone of inhibition (mm)			
		Blank	Kanamycin (30 µg/disc)	Extract (250 µg/disc)	Extract (500 µg/disc)
<i>Escherichia coli</i>	Gram (-)	-	23.00	6.37	10.25
<i>Shigella dysenteriae</i>	Gram (-)	-	25.54	9.10	11.19
<i>Salmonella typhi</i>	Gram (-)	-	26.25	8.68	13.00
<i>Salmonella paratyphi</i>	Gram (-)	-	24.58	7.56	13.60
<i>Staphylococcus aureus</i>	Gram (+)	-	27.50	7.00	12.54
<i>Staphylococcus epidermis</i>	Gram (+)	-	26.85	6.35	11.65

Table 4: Antibacterial activity of the whole plant *V. Monoicum* in disk diffusion assay

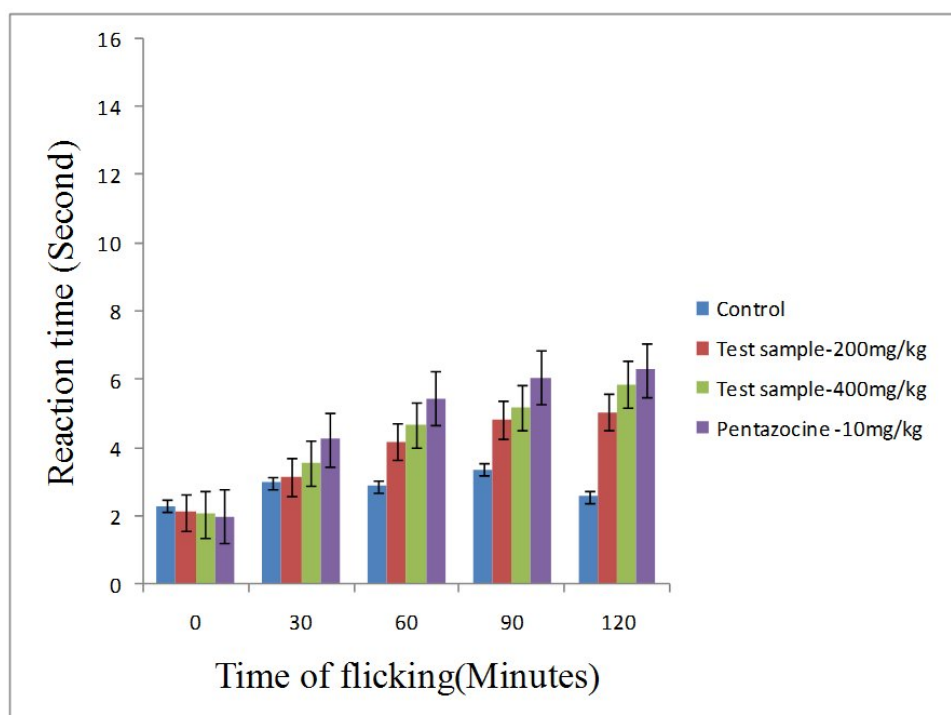


Figure1: Effect of ethanolic extract of whole plant of *V.monoicum* on tail immersion