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Phytochemical, Antibacterial and Antinociceptive Studies of *Hoya parasitica*

[1]M.S.H. Reza, [1]C. Mandal, [1]K.A. Alam, [1]A. Salam, [1]M.A. Rahman, [1]M.R. Amin, [1]M.N. Huda, [1]N.C. Ghosh, [1]M.R. Ali and [2]F. Ahmed
[1]Biotechnology and Genetic Engineering Discipline,
Khulna University, Khulna-9208, Bangladesh
[2]Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh

Abstract: The ethanol extract of leaves of *Hoya parasitica* was tested for its phytochemical groups, antibacterial and antinociceptive activities. The ethanol extract showed the presence of flavonoids, reducing sugars, tannins, gums and saponins. The extract showed moderate antibacterial activity against both gram-positive and gram-negative bacteria. It also produced significant ($p<0.01$) writhing inhibition in Swiss albino mice at oral dose of 500 mg kg^{-1} body weight comparable to the standard drug diclofenac sodium.

Key words: *Hoya parasitica*, apocynaceae, phytochemical analysis, antibacterial activity, antinociceptive activity

INTRODUCTION

Hoya parasitica Roxb. (Apocynaceae), (synonym- *Hoya acuta*), commonly known as waxvine, waxflower or simply *Hoya*, porcelain flower or *Bayupriya*, is an evergreen tropical perennial shrub native to tropical wet forests and humid climate of southern Asia (India east to southern China and southward), Australia and Polynesia. The plant is traditionally used as antirheumatic and in acute renal failure (Ahmed, 1997). Mukherjee *et al.* (1986) isolated triterpenic 3,4-seco acid 3,4-secolup-20 (29)-en-3-oic acid, along with lupeol and lupenone from *Hoya parasitica*. However, no biological activity has been reported so far on this plant. The objective of the present study was to investigate the phytochemical groups and antibacterial and antinociceptive activities of the ethanol extract of leaves of *Hoya parasitica*.

MATERIALS AND METHODS

Preparation of Plant Extract

H. parasitica was collected from the district of Satkhira, Bangladesh in October 2005 before its flowering stage and was taxonomically identified by the experts at Bangladesh National Herbarium (Voucher No. 30224). About 500 g of powdered leaves were taken in a clean, flat-bottomed glass container (4 L) and soaked in 2200 mL of 90% ethanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring state. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material followed by a filtration through Whatmann filter paper and the filtrate thus obtained was air-dried to get the crude extract.

Preliminary Phytochemical Screening

The crude extract was subjected to preliminary phytochemical testing for the detection of phytochemical compounds such as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins (Evans, 1989; Ghani, 1998).

Corresponding Author: M.S.H. Reza, Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh Tel: +88-01716537640

Microorganisms

Both gram positive and gram-negative bacterial species were used for antibacterial test. The bacterial strains used for the investigation are shown in Table 2. These bacterial species were collected from the Microbiology Laboratory of Square Pharmaceutical Limited, Pabna, Bangladesh.

Animals

Young Swiss-albino mice of either sex, weighing 20-25 g, purchased from the Animal Research Branch of International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for antinociceptive activity. 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°C and 12 h light: dark cycle), fed with standard diets (ICDDR, B formulated) and had free access to tap water.

Antibacterial Activity

Antibacterial activity of *H. parasitica* was tested by using agar diffusion method (Rios *et al*., 1988). Measured amount of the test samples were dissolved in definite volumes of solvent to give solutions of known concentration (10 μg mL^{-1}). Three wells were made through the media of the seeded plates (for the sample, blank and the standard Gentamycin) by using sterile cork borer (4 mm). Solution of sample, blank and Gentamycin were poured through the holes by using micropipette with desired amount. All the plates were kept in refrigerator (4°C) for 2 h to ensure proper diffusion. Finally the plates were incubated upside down at 37°C for 18-24 h. After proper incubation, the antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition (millimeter) with a slide calipers.

Antinociceptive Activity

Antinociceptive activity of the ethanol extract of *H. parasitica* was tested using the model of acetic acid induced writhing in mice (Whittle, 1964; Ahmed *et al*., 2004). The experimental animals were randomly divided into three groups, each consisting of five animals. Group I was treated as 'control group' and received 1% (v/v) Tween-80 in water at the dose of 10 mL kg^{-1} of body weight; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at the dose of 25 mg kg^{-1} of body weight; group III was test group and was treated with ethanol extract of *H. parasitica* at the dose of 500 mg kg^{-1} of body weight. Control vehicle, standard drug and extract were administered orally 30 min prior to the intraperitoneal injection of 0.7% acetic acid (10 mL kg^{-1} body wt.); after interval of 15 min, the number of writhes (squirms) were counted for 5 min.

Statistical Analysis

Student's t-test was used to determine a significant difference between the control group and experimental groups. A p-value of <0.01 and <0.001 were considered statistically significant.

RESULTS

Phytochemical Screening

The ethanolic extract of *H. parasitica* revealed the presence of flavonoids, reducing sugars, tannins, gums and saponins (Table 1).

Table1: Phytochemical screening of ethanolic leaves extract of *H. parasitica*

Plant extract	Steroids	Alkaloids	Reducing sugars	Tannins	Gums	Flavonoids	Saponins
Ethanol extract of *H. parasitica*	-	-	+	+	+	+	+

+: Indicates positive result; -: Indicates negative result

Table 2: Antibacterial activity of ethanolic leaves extract of *H. parasitica*

	Diameter of zone of inhibition (mm)	
Bacterial species	Gentamycin (30 μg well^{-1})	Ethanol extract (500 μg well^{-1})
Staphylococcus aureus	44	23
Proteus sp.	43	19
Hafinia sp.	33	9
Escherichia coli	34	10
Enterococci sp.	39	15
Shigella sonnei	35	0
Shigella flexneri	35	8
Shigella dysenteriae	32	20

Values greater than 6 mm indicates some activity

Table 3: Effect of ethanolic leaves extract of *H. parasitica* on acetic acid induced writhing in albino mice

Animal group/Treatment	No. of writhes (% writhing)	Inhibition (%)
Group-I		
(Control) 1% tween-80 10 mL kg^{-1}, p.o.	20±2.3 (100)	-
Group-II		
(Positive control) Diclofenac sodium, 25 mg kg^{-1}, p.o.	6.2±0.8** (31)	69
Group-III		
Ethanol extract, 500 mg kg^{-1}, p.o.	10±0.6* (50)	50

Values are expressed as mean±SEM (No. of animals, n = 05); *: Indicates $p<0.01$, **: Indicates $p<0.001$, vs. control; po: per os (orally)

Antibacterial Activity

The ethanolic extract of the plant demonstrated strong antibacterial activity against *Staphylococcus aureus*, *Proteus* sp., *Enterococci* sp. and *Shigella dysenteriae* with zone of inhibition of 23, 19, 15 and 20 mm, respectively. This was comparable to the values of Gentamycin 44, 43, 39 and 32 mm, respectively. The extract produced moderate antibacterial activity against *Hafinia* sp., *E. coli* and *Shigella flexneri* where the zone of inhibition were 9, 10 and 8 mm, respectively (Table 2).

Antinociceptive Activity

At the oral dose of 500 mg kg^{-1} of body weight, the extract produced 50% writhing inhibition in test animals. The result was statistically significant ($p<0.01$) and was comparable to the standard drug diclofenac sodium, which showed 69% writhing inhibition at the dose of 25 mg kg^{-1} of body weight (Table 3).

DISCUSSION

Antibacterial activity of *H. parasitica* was tested by using the agar diffusion method. Agar diffusion method is widely acceptable for the preliminary screening of antimicrobial activity. It is essentially a qualitative or semi qualitative test indicating the sensitivity or resistance of microorganisms to the test materials (Pelczar *et al*., 1993). The extract showed strong antibacterial activity against all the test microorganisms with exception of *Shigella sonnei*. As the extract showed antibacterial activity, this plant extract may be used in remedy for different microbial diseases such as diarrhea, dysentery and skin diseases. The antibacterial activity of the plant may be attributable to the presence of the phytochemical constituents observed. The presence of tannins, flavonoids, saponins and reducing sugars has earlier been associated with antibacterial activity (Hostettman and Nakanishi, 1979; Sodipo *et al*., 1991; Okwute and Mann, 1999).

Antinociceptive activity of the ethanol extract of *H. parasitica* 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 (Taesotikul *et al*., 2003). 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 (Derardt *et al*., 1980). The ethanol extract of *H. parasitica* produced significant writhing inhibition comparable to the standard drug diclofenac sodium. On the basis of this result it can be concluded that the extract of *H. parasitica* possesses antinociceptive activity and this may be due to the phytochemical constituents.

In conclusion, it can be suggested that the ethanol extract of *H. parasitica* possess antibacterial and antinociceptive effects which support the traditional uses of this plant as antirheumatic. Therefore, further research is essential to find out the active principles responsible for these activities.

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