



## ASSESSMENT OF PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES OF THE ETHANOLIC EXTRACTS OF *Hygrophila spinosa*

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**Abstract:** Ethanollic extract of *Hygrophila spinosa* plant was prepared and tested for its phytochemical and pharmacological investigation. The extract showed the presence of steroid, alkaloid, reducing sugar, tannin, flavonoids, saponin and glycoside. The extract significantly and dose dependently inhibited the acetic acid induced writhing in mice (58.8%,  $P<0.001$  and 25% at the dose of 500 and 250 mg/kg body weight respectively) and it is comparable to the activity of the standard analgesic drug diclofenac sodium (72.5%,  $P<0.001$  at the dose of 25 mg/kg body weight). The extract of *H. spinosa* offered about 1.20 hr and 1.8 hr ( $P<0.001$ ) of the mean latent period for diarrhoeal episode at the doses of 250 and 500 mg/kg body weight which is comparable to the standard antidiarrhoeal drug loperamide. The results tend to suggest that the plant possess analgesic and antidiarrhoeal activities or active constituent(s) responsible for the mentioned activities and thus supported its traditional uses.

**Keywords:** *Hygrophila spinosa*, analgesic activity, antidiarrhoeal activity, loperamide, diclofenac sodium

### Introduction

The plant, *Hygrophila spinosa* (Family: Acanthaceae), commonly known as 'Kulekhara or Kulfi sag' is an erect, branching and covered densely with stellate (star shaped) hairs and is rather twiggy with tough stringy bark. It is found very common throughout Bangladesh, Australia, India, Sri Lanka, Burma, Malaysia, and Nepal. Especially it grows in moist places on banks of tanks, ditches and paddy fields in all areas of Bangladesh. It is found in paddocks, gardens, waste places, disturbed forests and roadsides (Ghani, 2003).

The plant is having low molluscicidal activity against *Bulinus truncatus* and antitumor activity (Mazumder *et al.*, 1997). The leaf, root and seed of this plant are being traditionally used for the treatment of rheumatism, inflammation, jaundice, hepatic obstruction, urinary infection, oedema, gout, diabetes, bacterial infection etc. (Ghani, 2003). Based on the traditional uses the objective of the present study was to investigate the analgesic and antidiarrhoeal activities, which are not reported yet of the crude extract of *H. spinosa*.

### Materials and methods

**Sample collection and identification:** The plant *Hygrophila spinosa* was collected from Bankra, Jessore, Bangladesh during June 2009. After collection the plants were washed by water to remove soils and dusts, identified by Bangladesh National Herbarium, Mirpur; Dhaka DACB Accession No: 34474 and a voucher specimen also deposited there.

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**Preparation of extract:** The collected plants were shade-dried for four week. The plants were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

About 150 gm 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. The filtrate obtain is then evaporated by rotary evaporator.

**Animals:** Mice of random sex (*Swiss-webstar* strain, 20-25 g body weight) collected from animal resources branch of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) were used for the experiments. The animals were kept at animal house (Pharmacy Discipline, Khulna University, Khulna) for adaptation after collection under standard laboratory conditions (relative humidity 55-65%, room temperature  $25 \pm 2$  °C and 12 hour light: dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water.

**Chemicals:** Diclofenac-Na and loperamide were provided by Square Pharmaceuticals Ltd. Bangladesh. Molisch's, Mayer's, Dragendroff's, Fehling's and Benedict's reagent.

**Phytochemical tests:** Small amount of dried extract was appropriately treated to prepare sample solution and then subjected to the specific phytochemical tests (Ghani, 2003). Libermann-Burchard test was performed to identify steroids. Mayer's reagent and Dragendroff's reagent test was performed to identify alkaloids. Ferric chloride test was performed to identify tannin. Molisch's test, Fehling's test and Benedict's test were performed to investigate the presence of reducing sugar. For saponin, flavonoid and glycosides general identifying test were performed (Evans, 1989).

**Determination of analgesic activity:** The method of Whittle (1964) and Ahmed *et al.* (2004) was adopted with minor modification. The experimental animals were randomly divided into four groups, each consisting of five 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 the dose of 25 mg/kg of body weight; group III and group IV were test groups and were treated with the extract of *Hygrophila spinosa* at the doses of 250 and 500 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally, 30 minutes prior to the intraperitoneal injection of 0.7% acetic acid; after an interval of 15 minutes, the number of writhes (squirms) was counted for 5 minutes. The number of writhings in the control was taken as 100% and percent inhibition was calculated as follow:

$$\% \text{ Inhibition of writhing} = 100 - (\text{treated mean} / \text{control mean}) \times 100$$

**Antidiarrhoeal Activity:** The method of Chatterjee (1993) with minor modification was adapted to study the Antidiarrhoeal activity of the ethanolic extract of *Hygrophila spinosa* using castor oil induced diarrhoea in mice. The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into four groups having five mice in each. Group-I was kept as control and received 1% Tween-80 at the dose of 10 mL/kg of body weight; group-II was 'positive control' and was treated with standard ant motility drug loperamide at the dose of 50 mg/kg of body weight. Group III and Group IV were 'test group' and treated with the extract at the doses of 250 and 500 mg /kg of body weight respectively. Control vehicle and the extract were administered orally, 40 min prior to the oral administration of castor oil. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the

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presence of diarrhoea every hour in five hours study after the castor oil administration. Number of stools and any fluid material that stained the adsorbent paper were counted at each successive hour during the experiment (5 hour). The latent period of each mouse was also counted. At the beginning of each hour new papers were placed for the old ones.

### Results and Discussion

The ethanol extract of *H. spinosa* was subjected to different qualitative phytochemical tests for detection of different classes of biologically active chemical compounds and the results are summarized in the Table 1. It shows that the ethanolic extract of *H. spinosa* contains steroid, alkaloids, reducing sugars, tannins, flavonoids, glycosides and saponins. These compounds are supposed to be responsible for biological activities of *H. spinosa*.

Table 1. Results of Phytochemical group tests

Extract	Steroids	Alkaloids	Reducing Sugars	Tannins	Flavonoids	Saponins	Glycosides
Ethanolic extract of <i>Hygrophila spinosa</i>	+	+	+	+	+	+	+

Analgesic activity of the extract of *H. spinosa* 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 algnesia by liberation of endogenous substances, which in turn excite the pain nerve endings (Taesotikul *et al.*, 2003). Increased levels of PGE<sub>2</sub> and PGF<sub>2α</sub> in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Deraedt *et al.*, 1980). In acetic acid induced writhing test, the ethanolic extract of *H. spinosa* significantly and dose dependently suppressed the frequency of acetic acid induced writhing in mice. It showed 25% and 58.8% ( $P < 0.001$ ) writhing inhibition at the dose of 250 and 500 mg/kg body weight respectively, while the standard drug diclofenac-Na showed 72.5% writhing inhibition (Table 2). These results showed that the analgesic effect of *H. spinosa* was significant at 500 mg/kg body weight. Several flavonoids isolated from medicinal plants have been discovered to possess significant anti-nociceptive and/or anti-inflammatory effects (Duke, 1992). Systemic (i.p. or p.o.) administration of the flavonoid myricitrin, at doses that did not produce any important motor dysfunction, alterations in basal temperature, or any other obvious side effects induced a dose-dependent inhibition of acetic acid-induced visceral nociceptive response in mice. (Meotti *et al.*, 2006). The Gi/o protein dependent mechanism is involved on antinociception caused by flavonoid myricitrin. The opening of voltage and small conductance calcium-gated K<sup>+</sup> channels and the reduction of calcium influx led to the antinociceptive of flavonoid myricitrin (Meotti *et al.*, 2007). It is, therefore, possible that the anti-nociceptive effects observed with this extract may be attributable to its flavonoid component, shown to be present during phytochemical analysis. On the basis of the result of acetic acid induced writhing test, it can be concluded that the dried ethanolic extract of whole plant of *H. spinosa* possess analgesic activity and the mechanism to

suppress the nociception is probably the opening of voltage- and small-conductance calcium-gated K(+) channels and the reduction of calcium influx.

Table 2. Effect of *Hygrophila spinosa* extract on acetic acid induced writhing in mice

	Dose (mg/kg, p.o.)	No. of Writhes (% writhing)	Inhibition (%)
Control (1% tween-80 solution in water, 10 mL/kg, p.o.)	-	16 ± 1.41 (100)	
Positive Control (Diclofenac sodium)	25	4.4 ± 2.65 (27.5)	72.5*
Ethanollic extract of HS	250	12 ± 2.17 (75)	25
Ethanollic extract of HS	500	6.6 ± 3.27 (41.3)	58.8*

HS = *Hygrophila spinosa*; values are expressed as mean ± SEM (Standard Error of Mean); (n=5) n= number of mice; \*, p< 0.001 vs. control; p.o. = per oral

Table 3. Effect of *Hygrophila spinosa* on castor oil induced diarrhoea in mice

Treatment	Dose (mg/kg, p.o.)	Latent period (hr)	Period of study (hr)	Total number of stool
Control (1% tween-80 solution in water, 10 mL/kg, p.o.)	-	0.77±0.002	1	8±1.07
			2	10±0.65
			3	6±0.98
			4	12±0.75
			5	7±0.80
Positive Control (Loperamide)	50	2.60±0.44	1	0.6±0.45 <sup>b</sup>
			2	1.8±0.90 <sup>c</sup>
			3	3.4±0.98 <sup>c</sup>
			4	1.4±0.97 <sup>d</sup>
			5	0.6±0.45 <sup>d</sup>
<i>Test group-I</i> Et. Extract	250	1.20±0.007	1	1.2±0.5 <sup>a</sup>
			2	3.4±1.85
			3	4.2±1.86
			4	3.6±2.11
			5	2.2±0.89
<i>Test group-II</i> Et. Extract	500	1.8±0.01	1	1.1±0.97 <sup>a</sup>
			2	4.2±0.74
			3	5.4±1.71
			4	3.2±1.39
			5	1.4±1.09

Values are expressed as mean ± SEM (Standard Error of Mean) (n=5); <sup>a</sup>P< 0.01; <sup>b</sup>P<0.001; <sup>c</sup>P<0.02; <sup>d</sup>P< 0.05 vs. control; p.o (per oral), Et.= Ethanollic

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Antidiarrhoeal activity of crude extract of *H. spinosa* was tested by castor oil induced diarrhoea in mice (Chatterjee, 1993). Castor oil is believed to link with the liberation of ricinoleate salts, which stimulates the intestinal epithelial cell's adenylyl cyclase (Racusen *et al.*, 1979) or release prostaglandin (Beubler *et al.*, 1979). The results (Table 3) showed that the extract offered about 1.20 hr and 3.70 hr of the mean latent period for diarrhoeal episode to ensue at the dose of 250 and 500mg/ kg of body weight, respectively. The mean numbers of stool at the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> hour of study were 1.2, 3.4, 4.2, 3.6 and 2.2 for 250mg/ kg and 1.1, 4.2, 5.4, 3.2 and 1.4 for 500mg/ kg of body weight, respectively. The extract caused an increase in latent period i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation in castor oil induced test in mice at both doses as compared to the standard antidiarrhoeal agent loperamide. The mean latent period for control and standard were 1.2 and 1.8, respectively. Thus it can be suggested that *H. spinosa* possess antidiarrhoeal activity.

### Conclusion

Ethanolic extract of *Hygrophila spinosa* contains steroid, alkaloids, reducing sugars, tannins, flavonoids and saponins and it i.e. the extract possess analgesic and antidiarrhoeal activities. Results of the experiment tend to suggest that the plant could be a good source of alternative medicine for rheumatism, inflammation, abdominal pain and in diarrhoea and bacterial infection. Further researches are essential to find out the active principle(s) and explore the mechanisms involved for these activities.

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### References

- Ahmed, F., Selim, M.S.T., Das, A.K., Choudhuri, M.S.K. 2004. Anti-inflammatory and antinociceptive activities of *Lipia nodiaflora* Linn. *Pharmazie* 59: 329-330
- Beubler, E. and Juan, H. 1979. Effect of Ricinoleic acid and other Laxatives in Net Water Flux and Prostaglandin E release by the Rat Colon, *Journal of Pharmacy and Pharmacology* 31: 681-685
- Chatterjee, T.K. 1993. *Handbook of laboratory Mice and Rats*. Jadavpur University, India
- Deraedt, R., Jouquey, S., Delevallée, F. and Flahaut, M. 1980. Release of Prostaglandins E and F in algogenic reaction and its inhibition. *European Journal of pharmacology* 51:17-24
- Duke, J.A. 1992. *Handbook of Biologically Active Phytochemicals and Their Activities*. CRC Press, Boca, Raton, FL
- Evans, W.C. 1989. *Trease and Evan's Text book of Pharmacognosy*, 13<sup>th</sup> edition, Cambridge University Press, London. 546p.
- Flavia, C.M., Luiz, A.P., Pizzolatti, M.G., Cândida, A.L., Kassuya, J.B., Adair, R. S. and Santos, A.R. 2006. Analysis of the antinociceptive effect of the Flavonoid Myricitrin: evidence for a role of the L-arginine-nitric oxide and protein kinase C pathways. *The Journal of Pharmacology and Experimental Therapeutics* 316:789-796
- Ghani, A. 2003. *Medicinal Plants of Bangladesh: Chemical constituents and uses*. Asiatic Society of Bangladesh, Dhaka

- Mazumdar, U.K., Gupta, M., Maiti, S. and Mukherjee, D. 1997. Antitumor activity of *Hygrophila spinosa* on Ehrlich ascites carcinoma and sarcoma-180 induced mice. *Indian Journal of Experimental Biology* 35(5): 473-477
- Meotti F.C., Fachinetto R., Maffi LC, Missau F.C., Pizzolatti M.G., Rocha J.B. and Santos A.R. 2007. Antinociceptive action of myricitrin: involvement of the K<sup>+</sup> and Ca<sup>2+</sup> channels. *European Journal of Pharmacology* 19:567(3):198-205
- Racusen, L.C. and Binder, H.J. 1979. Ricinoleic acid stimulation of active anion secretion in colonic mucosa of the rat. *Journal of Clinical Investigation* 63: 743-749
- Taesotikul, T., Panthong, A., Kanjanapothi, D., Verpoorte, R. and Scheffer, J. J. C. 2003. Anti-inflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. *Journal of Ethnopharmacology* 84(1): 31-35
- Whittle B.A. 1964. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. *British Journal of Pharmacology and Chemotherapy* 22: 246-253