



## PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION OF SEEDS OF *Hyptis suaveolens*

M.A. Islam\*, S. Parvin, M. M. Rahman, M.I. Ahmed, N.N. Biswas,  
U.K. Karmakar and K. Naher

Pharmacy Discipline, Khulna University, Khulna 9208, Bangladesh

KUS: 10/11-010610

Manuscript received: June 01, 2010; Accepted : April 27, 2011

**Abstract:** The crude ethanolic extract of the seeds of *Hyptis suaveolens* was evaluated for its analgesic, antidiarrhoeal and cytotoxic activities. The extract significantly and dose dependently inhibited the acetic acid induced writhing in mice (69.7%,  $P < 0.001$  and 60.6%,  $P < 0.001$  for 500 and 250 mg/kg body weight, respectively), comparable to the standard drug diclofenac sodium. The extract also decreased the frequency of defecation and increased mean latent period significantly ( $P < 0.05$ ) at the doses of 250 and 500 mg/kg body weight respectively compared with the control group. Moreover, it displayed considerable general toxicity towards brine shrimps. The  $LC_{50}$  value of the extract was 80  $\mu$ g/ml which was comparable to that of standard drug chloramphenicol (43  $\mu$ g/ml). All the results tend to justify the traditional uses of the seeds of *Hyptis suaveolens*.

**Keywords:** *Hyptis suaveolens*, analgesic activity, antidiarrhoeal activity, cytotoxic activity

### Introduction

*Hyptis suaveolens* (Family- Labiatae) locally known as Tokma, Ganja Tulsi, Bilati Tulsi, etc. is an annual herb grows in Southeast Asia, Western Australia, Northern Territory, Queensland, alien to Western Australia. In Bangladesh, it grows in all parts of the country especially in Jessore, Khulna, Bogra and Lakshmipur (Ghani, 2003). The plant is used as emetic, tonic, diuretic, and for pains in the joints. Flowers are used for treating lumbago, neuralgia, burns scalds. The flavonoids and anthraquinones isolated from the petals possess marked antibiotic activity against some pathogenic fungi and bacteria and show anti-anaphylactic effects. Powdered seeds are given in difficult labour. Ethanolic extract of the plant is reported to possess anticancer and wound healing properties (Ghani, 2003; Shirwaikar *et al.*, 2003). Literature review revealed that the leaves of *H. suaveolens* contain  $\alpha$ -Pinene,  $\beta$ -pinene  $\alpha$ -terpinene, sabinene, p-cymene and terpinen-4-ol, 1,8-cineole (Eshilokun *et al.*, 2003). The objective of our present study was to investigate the analgesic, antidiarrhoeal and cytotoxic activities of the ethanolic extract of seeds of *H. suaveolens*.

### Materials and Method

**Plant material collection and extraction:** The seeds of *H. suaveolens* were collected from Khulna, Bangladesh and were identified by the experts at Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. DACB- 33879). About 100 gm of powdered material was taken in a clean, flat bottomed glass container and drenched in 500 ml of 90% ethanol. The container with

\*Corresponding author: < maislam26@gmail.com >

DOI: <https://doi.org/10.53808/KUS.2010.10.1&2.1011-L>

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 thus obtained was evaporated in open air to get a viscous mass. The viscous mass was then dried to get crude ethanolic extract (yield: 10% w/w). The extract thus obtained was used for experimental purposes.

**Drugs:** Diclofenac sodium (Drug International Ltd.), Loperamide (Drug International Ltd.) and Chloramphenicol (Square Pharmaceuticals Ltd.).

**Preliminary phytochemical screening:** The ethanolic extract of the seeds was subjected to preliminary phytochemical test for the detection of major chemical groups. In each test 10% (w/v) solution of extract in ethanol was taken unless otherwise mentioned in individual test (Evans, 1989; Wagner *et al.*, 1984, Ghani, 2003).

**Animals:** The experimental animals, Swiss-albino mice of either sex (weighing 20-25 g) were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). 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  $21.0 \pm 2.0$  °C and 12 h light/dark cycle) and fed with standard diets and had free access to tap water.

**Analgesic activity:** Analgesic activity of the ethanolic extract of the seeds of *H. suaveolens* was tested using the model of acetic acid induced writhing in mice (Whittle, 1964; Ahmed *et al.*, 2004). Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group- IV consisting of 5 mice in each group. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at a dose of 10 mg/kg of body weight; group II was treated as 'positive control' and given the standard drug diclofenac sodium at a dose of 25mg/kg of body weight; group III and group IV were test groups and treated with the extract at the doses of 250 and 500 mg/kg of body weight respectively. Control vehicle, standard drug and extract were administered orally, 30 minutes prior to acetic acid (0.7%) intraperitoneal injection. After an interval of 5 minutes, the number of wriths (squirms) was counted for 15 minutes.

**Antidiarrhoeal Activity:** Antidiarrhoeal activity of *H. suaveolens* was tested by using the model of castor oil induced diarrhoea in mice (Chatterjee, 1993). 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 three groups having five mice in each. Group-I was kept as 'control' and received only distilled water containing 1% Tween-80; group-II was treated as 'positive control' and received standard antitomotility drug loperamide at a dose of 50 mg/ kg; group III was 'test group' and treated with EtOH extract at the dose of 500 mg/kg. Control vehicle, standard drug and the extract were administered orally, 1 h prior to the oral administration of castor oil at a dose of 0.5 ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in 5 h study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment (5 h). The latent period of each mouse was also counted. At the beginning of each hour new papers were placed for the old ones.

**Cytotoxic activity:** The method of Meyer *et al.* (1982) with some modifications was adapted to study the general toxicity of seeds of *H. suaveolens*. The brine shrimp eggs were hatched in a conical flask containing brine shrimp medium (300 ml). The flasks were well aerated with the aid of an air pump, and kept in a water bath at 29 – 30 °C. A bright light was left on. The nauplii hatched within 48 h. The extract was dissolved in brine shrimp medium with addition of few drops of 5% DMSO to obtain a concentration of 5, 10, 20, 40, 80 and 160 µg/ml. Each preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. For

Islam, M.A., Parvin, S., Rahman, M. M., Ahmed, M.I., Biswas, N.N., Karmakar, U.K. and Naher, K. 2010. Phytochemical and biological investigation of seeds of *Hypsis suaveolens*. *Khulna University Studies* 10 (1&2): 185-190

control, same procedure was followed except test samples. A series of the same concentration as of the sample was prepared for positive control, chloramphenicol (Rahman *et al.*, 2007). After marking the test tubes properly, 10 living shrimps were added to each of the test tubes with the help of a Pasteur pipette. The test tubes containing the sample, control and positive control were then incubated at 29°C for 24 h in a water bath, after which each tube was examined and the surviving brine shrimps counted and recorded. From this, the percentage of mortality was calculated at each concentration. The LC<sub>50</sub> values were calculated with best fit line by using Microsoft Excel 2007.

## Results

**Preliminary phytochemical screening:** Phytochemical studies showed the presence of alkaloids, glycoside, tannins, saponins & flavonoids in the extract (Table 1).

Table 1. Results of preliminary phytochemical analysis

Plant Extract	Alkaloids	Glycosides	Steroids	Gums	tannins	saponins	flavonoids
Ethanollic extract of <i>H. suaveolens</i>	+	+	-	-	+	+	+
+ = Presence		- = Absence					

**Analgesic activity:** Table 2 showed the effect of the ethanolic extract of *H. suaveolens* on acetic acid-induced writhing model in mice. It produced about 60% and 69% ( $P < 0.001$ ) writhing inhibition at dose of 250 and 500 mg/kg respectively, which was comparable to the standard drug diclofenac sodium where the inhibition was about 80% ( $P < 0.001$ ) at the dose of 25 mg/kg.

Table 2. Effect of *H. suaveolens* on acetic acid induced writhing in mice

Animal Group	Treatment	Writhing Count (%Writhing)	%Writhing Inhibition
Group I n=5	1% tween-80 solution in water	13.2 ± 0.59* (100)	0
Group II n=5	Diclofenac sodium 25mg/kg	2.6 ± 0.51* (19.70)	80.30
Group III n=5	Et. Extract (250mg/kg)	5.2 ± 0.37* (39.40)	60.60
Group IV n=5	Et. Extract (500mg/kg)	4.0 ± 0.59* (30.30)	69.70

Values are expressed as mean±SEM, SEM=Standard error of Mean, n=No. of mice, Et.=Ethanolic, \*  $P < 0.001$  vs. control

**Antidiarrhoeal Activity:** Antidiarrhoeal activity of the ethanolic extract of *H. suaveolens* was tested by castor oil induced diarrhoea in mice. It caused an increase in latent period (1.58 h) i.e. delayed the onset of diarrhoeal episode at the dose of 500 mg/kg body of weight significantly ( $P<0.05$ ) which was comparable to the standard drug loperamide at the dose of 50 mg/kg body weight in which the value was 1.57 h ( $P<0.05$ ) (Table 3a). The extract also decreased the frequency of defecation at the same dose where 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> h of study were 0.6, 1.2, 1.4, 0.6 and 0.4 respectively and in standard drug the values were 0.4, 1.6, 2.2, 0.8 and 0.4 respectively (Table 3b).

Table 3a. Effect of *H. suaveolens* on castor oil induced diarrhoea in mice (Latent period)

Animal Group/ Treatment	Dose (/kg, p.o.)	Latent period (h)
Group-I (Control) (1% tween-80 solution in water)	10 ml	0.766±0.128
Group-II (Positive control) Loperamide.	50 mg	1.57±0.191*
Group - III <i>Et. Extract</i>	500 mg	1.58±0.330*

Values are expressed as mean ± SEM (n=5); \* indicates  $P < 0.05$  vs. control; Et., ethanol p.o., per oral.

Table 3b. Effect of *H. suaveolens* on castor oil induced diarrhoea in mice (Number of stools)

Animal Group/ Treatment	Dose (/kg, p.o.)	Period of study (h)	Total number of stool
<i>Group-I (Control)</i> (1% tween-80 solution in water)	10 ml	1	1.8±0.374
		2	2.8±0.800
		3	3.0±0.447
		4	2.6±0.510
		5	1.8±0.374
<i>Group-II</i> (Positive control) Loperamide	50 mg	1	0.4±0.400
		2	1.6±0.678
		3	2.2±.969
		4	0.8±0.583
		5	0.4±0.250*
<i>Group -III</i> <i>Et. Extract</i>	500 mg	1	0.6±0.247
		2	1.2±0.450
		3	1.4±0.230*
		4	0.6±0.230*
		5	0.4±0.240*

Values are expressed as mean ± SEM (n=5); \* indicates  $P < 0.05$  vs. control; Et., ethanol p.o., per oral.

Islam, M.A., Parvin, S., Rahman, M. M., Ahmed, M.I., Biswas, N.N., Karmakar, U.K. and Naher, K. 2010. Phytochemical and biological investigation of seeds of *Hyptis suaveolens*. *Khulna University Studies* 10 (1&2): 185-190

**Cytotoxic activity:** Table 4 illustrated the results of activity of the extract against brine shrimp nauplii. The LC<sub>50</sub> of the test sample and standard drug chloramphenicol were found to 80 µg/ml and 43 µg/ml, respectively.

Table 4. Effect of *H. suaveolens* on Brine shrimp lethality bioassay

Test Sample	Conc. of test sample (µg/ml)	No. of alive shrimps	% mortality	LC <sub>50</sub> (µg/ml)
Ethanol extract of HS	5	10	0	80
	10	9	10	
	20	8	20	
	40	7	30	
	80	5	50	
	160	0	100	
Chloramphenicol	5	9	10	43
	10	7	30	
	20	6	40	
	40	4	60	
	80	1	90	
	160	0	100	

#### Discussion

Analgesic activity of the ethanol extract of *H. suaveolens* 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 algisia 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 (Derardt *et al.*, 1980). The ethanol extract of seeds of *H. suaveolens* produced significant writhing inhibition comparable to the standard drug diclofenac sodium. On the basis of this result it can be concluded that the ethanol extract of *H. suaveolens* possesses analgesic activity.

Preliminary phytochemical screening showed the presence of various classes of constituents, such as alkaloids, glycoside, tannins, saponins and flavonoids. Since several flavonoids and tannins isolated from medicinal plants have been discovered for their significant antinociceptive and/or anti-inflammatory activity (Pathak *et al.*, 1991; Bittar *et al.*, 2000; Duke, 1992), it is, therefore, possible that the antinociceptive effects observed with this extract in the present study may be attributing to its flavonoids and tannins component.

Antidiarrhoeal activity of the ethanol extract of *H. suaveolens* was tested by using the model of castor oil induced diarrhoea in mice. Castor oil, which is used to induce diarrhoea in mice, mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti absorptive or secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that ricinoleate salts stimulates the intestinal epithelial cell's adenylyl cyclase (Racusen *et al.*, 1979) or release prostaglandin (Beubler *et al.*, 1979). The extract caused an increase in latent period (1.58 h) i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation as well as the number of stool. On the basis of the result of castor oil induced diarrhoea, it can be concluded that the ethanol extract of *H. suaveolens* possesses antidiarrhoeal activity.

The brine shrimp lethality bioassay can be recommended as a guide for the detection and evaluation of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc. of the

compounds (Meyer *et al.*, 1982; McLaughlin *et al.*, 1988). 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. Eshilokun *et al.*, (2003) reported the presence of essential oil as a major component in ethanolic extract of *H. suaveolens*. Therefore, the essential oil might involve in the mechanism of cytotoxic activity. However, further investigations are necessary to isolate the active compound(s) responsible for the activity.

### Conclusion

The results obtained in this study indicate that the ethanolic extract of *H. suaveolens* possess considerable analgesic, antidiarrhoeal and cytotoxic activity at the investigated doses on the experimental laboratory animal. This could provide a rationale for traditional uses of this plant and suggests for further investigation and isolation of biologically active constituents responsible for the activity.

### Acknowledgement

The authors are grateful to Professor Dr. Samir Kumar Sadhu, Head, Pharmacy Discipline and Professor Dr. Ayaz Hasan Chisty, Fisheries and Marine Resources Technology Discipline, Khulna University, Khulna, Bangladesh for providing technical support and valuable advice.

### References

- Ahmed, F., Selim, M.S.T., Das, A.K. and Choudhuri, M.S.K. 2004. Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. *Pharmazie* 59: 329-333
- 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. *J. Pharm. Pharmacol.* 31: 681-685
- Bittar, M., De Souza, M.M., Yunes, R.A., Lento, R., Delle Monache, F. and Cechinel, F.V. 2000. Antinociceptive activity of I3,II8-binarigenin, a biflavonoid present in plants of the Guttiferae. *Planta Medica* 66 (1): 84-86
- Chatterjee, T.K. 1993. *Handbook of laboratory Mice and Rats*. Jadavpur University, India
- Derardt, R., Jougney, S., Delevalcee, F. and Falhout, M. 1980. Release of prostaglandins E and F in an 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
- Eshilokun, A.O., Kasali, A.A. and Giwa-Ajeniya, A.O. 2003. Chemical composition of essential oils of *Hyptis suaveolens* (Lin.) poit leaves from Nigeria. *Flavour and Fragrance Journal* 45: 211-321
- Evans, W.C. 1989. *Trease and Evan's Pharmacognosy*. Cambridge University Press, London
- Ghani, A. 2003. *Medicinal Plants of Bangladesh*. The Asiatic Society of Bangladesh
- McLaughlin, J.L., Anderson, J.E. and Chang, C.J. 1988. Bioactive components of *Allamanda schottii*. *Journal of Natural Products* 51(2): 307-308
- Meyer, S. 1982. *Phytochemical methods* (a guide to modern techniques to plant analysis). Champan and Hall, USA
- Pathak, D., Pathak, K. and Singla, A.K. 1991. *Flavonoids as medicinal agents: recent advances*. *Fitoterapia* 62(5): 371-389
- Racusen, L.C. and Binder, H.J. 1979. Ricinoleic acid stimulation of active anion secretion in colonic mucosa of the rat. *Journal of Clin. Invest* 63: 743-749
- Rahman, S., Ali, E. and Haque, R. 2007. Pharmacological profiles of *Holarhena antidysenterica* (L.) wall bark. *Ori. Pharm. and Exper. Med.* 7(2): 157-161
- Shirwaikar, A., Shenoy, R., Udupa, A.L., Udupa, S.L. and Shetty, S. 2003. Wound healing property of ethanolic extract of leaves of *Hyptis suaveolens* with supportive role of antioxidant enzymes. *Indian Journal of Experimental Biology* 34: 523-526
- Taesotikul, T., Panthong, A., Kanjanapothi, D., Verpoorte, R. and Scheffer, J.J.C. 2003. Antiinflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. *Journal of Ethnopharmacology* 84: 31-35
- Wagner H., Bladt, S. and Zgainski E.M. 1984. *Plant drug analysis*. Springer, Berlin
- Whittle, B.A. 1964. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesic. *British Journal of Pharmacological Chemotherapy* 22: 246