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Antimicrobial and peripherally acting analgesic activity of *Senna siamea*

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Abstract

The present study was carried out to evaluate the analgesic and antimicrobial potential of *Senna siamea* leaf. For these purposes the 80% concentrated ethanolic extract of the leaf was used. Analgesic activity of the ethanolic extract of *Senna siamea* was tested using the model of acetic acid induced writhing in mice. The test consists of injecting the 0.7% acetic acid solution and then observing the animal for specific contraction of body referred as 'writhing'. Diclofenac sodium is used as reference standard drug for the analgesic activity test. Antibacterial activity of plant extract was carried out using disc diffusion method with thirteen pathogenic bacteria. The acetic acid induced writhing test showed that ethanol extract of *Senna siamea* at the dose of 500 mg/kg exhibit significant ($P < 0.001$) inhibition of writhing reflex by 61.98% while the standard drug diclofenac Na inhibition was found to be 85.95 % at a dose of 25 mg/kg body weight. The extracts were not active to the microorganisms at the concentration levels of 100µg/disc and 200µg/disc; however the extract showed activity with *Pseudomonas aeruginosa* at concentration of 500 µg/disc in comparison with standard kanamycin. The zone of inhibition of the extract was 10 mm.

Keywords: *Senna siamea*; Analgesic; Antimicrobial; Bangladesh; Ethanolic extract; Diclofenac sodium.

Introduction

To prevent and cure several ailments, the use of medicinal plants as herbal remedies differs from community to community (Sharif and Banik, 2006). Plants are considered as a cheapest and safer alternative source of antimicrobials (Doughari *et al*., 2007). Medicinal plants possess beneficial pharmacological effects on the animal body (Ghani, 2003) and about 25% of prescribed drugs in the world are of plant origin (Rates, 2001). For the primary health care needs about 80% people rely on traditional plant based medicines in developing countries (FAO: Trade in medicinal plants, 2001). Only small amounts are investigated from

the abundant number of medicinal plants for its biological and pharmacological activities. There is a wide range of medicinal plant parts used as powerful raw drug possessing a variety of pharmacological activities. From medicinal plants discovery of new pharmaceutical agents can combat the drastic increase in infectious diseases in rural areas of many countries and it has been used as an economic reason as well. The current widespread interest of plant origin drugs reflects its recognition of the validity of many traditional claims regarding the value of natural products in health care (Nair *et al*., 2005). Therefore, in order to determine the potential use of herbal medicine, it is essential to emphasize the study of medicinal plants that finds place in folklore (Nair *et al*., 2005; Awadh *et al*., 2001).

Senna siamea is a tree in the subfamily Caesalpinoideae of the family Leguminosae. *Senna* is an Arabian name and the herb was first brought into use by the Arabian physicians Serapion and Mesue (Bernard, 2005). It has many regional names but it is commonly called Thailand shower, minjiri or kassod (Forestry/ Fuel wood Research and Development Project, 1994). Including Bangladesh It has been widely planted in many Southeast Asian countries (Khan and Alam, 1996) and is naturalized in many locations (Gutteridge, 1997). *S. siamea* is a medium sized evergreen tree attaining 5 meter height in and conditions. It rarely exceeds 20 m height and 50 cm diameter at breast height (Jensen, 1995). The traditional use of *S. siamea* is for the treatment of typhoid fever, jaundice, abdominal pain, menstrual pain, and is also used to reduce sugar level in the blood. Ethno medicinally *S. siamea* is used as laxative, blood cleaning agent, cure for digestive system and genitourinary disorders, herpes and rhinitis (Aliyu, 2006). When decocted *S. siamea* leaves are locally used as anti-malaria drug (Lose *et al*., 2000). The fruit is used to charm away intestinal worms and to prevent convulsion in children in traditional medicine (Alli Smith, 2009). Previous studies on *S. siamea* extracts have confirmed some of the traditional uses: anti plasmodial activity (Gbeassor *et al*., 1989; Nsonde-Ntandou *et al*., 2005; Mbatchi *et al*., 2006) antioxidant and antihypertensive activity (Kaur *et al*., 2006), laxative activity(Elujoba *et al*., 1989), sedative activity (Thongsaard *et al*., 2001; Sukma *et al*., 2002) have been validated by pharmacological studies. However, there are as yet no published studies concerning the analgesic activity of *S. siamea* leaf extract, though it has been noted that the plant has anxiolytic and CNS inhibitory effects (Sukma *et al*., 2002). Besides this there are also insufficient studies on antimicrobial effects of *S. siamea* leaf extract and studies must be conducted to determine its activity as medicinal plants. The present study was carried out on rats to investigate analgesic activity of the ethanol extracts of *S. siamea* leaf and also antimicrobial potential.

Materials and Methods

Collection and Identification of Plant material

The *Senna siamea* leaf was collected from South-Western region of Bangladesh in 2007. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka.

Preparation of the Plant material

The collected plant parts (leaves) were separated from undesirable materials or plants or plant parts. They were shade-dried for four weeks. The plant parts were ground into a coa-

rse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of Plant Extract

About 300 gm of powered leaf was taken in a clean, flat-bottomed glass container and soaked in 1000 ml of 80% ethanol. The container with its contents were sealed and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate (ethanol extract) obtained was evaporated under ceiling fan until dried. It rendered a greenish black. The concentrated part of leaf was designated as crude extract or ethanolic extract.

Experimental animal

Swiss-albino mice aged 4-5 weeks, average weight 20-28 gm were used for the experiment. The mice were purchased from the Animal Research Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB). They were kept standard environmental condition for one week for adaptation after their purchase and fed ICDDRB formulated rodent food and water. The ethical rules published by International Association for the Study of Pain (Zimmermann, 1983) were respected.

Chemicals and Reagents

All the chemicals used are of analytical reagent grade. Acetic Acid, Tween-80 was obtained from Sigma Chemical Co. USA. Kanamycin and Diclofenac-Na was collected from local market.

Analgesic Activity

Analgesic activity of the ethanolic extract of *S. siamea* was tested using the model of acetic acid induced writhing in mice (Rang and Dale, 1993). The test consists of injecting the 0.7% acetic acid solution and then observing the animal for specific contraction of body referred as 'writhing'. Diclofenac is used as reference standard drug. Experimental animals were randomly selected and divided into three groups consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and the 1 dose of the extract. Each mouse was weighed properly and the dose of the test samples (500 mg) and control materials (10 ml and 25 mg) were adjusted on the basis of per kg of body weight.

Preparation of test samples and control materials

For sample preparation 0.5 g of the sample was measured. The extracts were triturated in unidirectional manner by the addition of small amount of Tween-80. After proper mixing of extract and Tween-80 the distilled water was slowly added. The final volume of the suspension was made 10 ml. For positive control 0.025 gm of Diclofenac-Na was taken and a suspension of 10 ml was made. 2 drops of Tween-80 was added with distilled water to 10 ml to prepare control solution.

Determination of analgesic activity

Test samples, control and Diclofenac-Na were given orally by means of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%, 10 ml/kg) was administered intraperitoneally to each of the animals of a group. After an interval of five minutes, which was given for absorption of acetic acid, number of writhing was counted for 15 minutes. Two halves writhing were counted as a full writhing. The percent inhibition (% analgesic activity) was calculated by,

$$\% \text{ inhibition} = \{(A-B)/A\} \times 100$$

Where, A= Average number of writhing of the control group; B= Average number of writhing of the test group (Emran *et al*., 2012).

Antimicrobial Potential

The antimicrobial assay was performed by using the disc diffusion method (Bauer *et al*., 1996; Barry, 1980). Thirteen pathogenic bacteria were used as test organisms for antibacterial activity of sample extract. The bacterial strains were collected from BCSIR Dhaka, Bangladesh. 0.1 mg/disc of the sample extract were used to observe the antimicrobial activity of the plant extract and compared with the standard kanamycin (0.03µg/disc). The test organisms were inoculated on 10 ml previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile petri dish in an aseptic condition using a sterile loop. Prepared sample and standard solutions were applied to the corresponding petri dish. The plates were incubated for overnight at 37^0 C. After proper incubation, clear zone of inhibition around the point of application of sample solution were measured which is expressed in millimeter (mm).

Phytochemical screening

Phytochemical tests have been performed according to the literature by Nayak and Pereira (Nayak, 2006).

Test for saponins

300 mg of extract was boiled with 5 ml water for two minutes. The mixture was cooled and mixed vigorously and left for three minutes. The formation of frothing indicated the presence of saponins.

Test for tannins

To an aliquot of the extract, sodium chloride is added to make to 2% strength. Then it is filtered and mixed with 1% gelatin solution. Precipitation indicated the presence of tannins.

Test for Triterpenes

300 mg of extract was mixed with 5 ml chloroform and warmed for 30 minutes. The chloroform solution was then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red color indicated the presence of triterpenes.

Test for alkaloids

300 mg of extract was digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature, and examined the alcoholic layer for the pink color which indicated the presence of alkaloids.

Test for flavonoids

The presence of flavonoids was determined using 1% aluminium chloride solution in methanol, concentrated HCl, magnesium turnings, and potassium hydroxide solution.

Test for glycosides

A small amount of alcoholic extract of sample was dissolved in 1.0 ml of water and then aqueous solution of sodium hydroxide was added. Formation of a yellow color indicates the presence of glycosides.

Results

Analgesic activity

Figure 1 shows the result of statistical evaluation of the writhing effect of Ethanolic extract of *S. siamea* on acetic acid induced writhing in mice. The result of the test showed th-

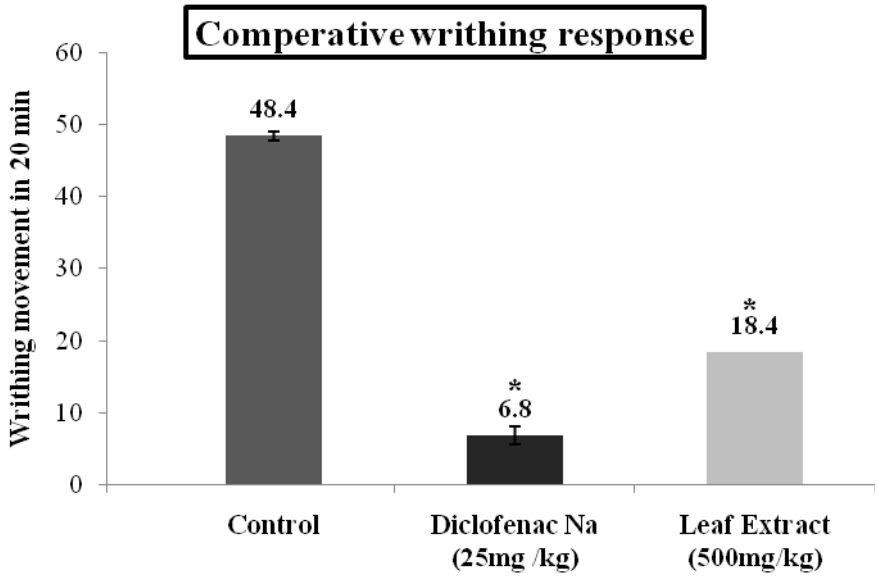

Figure 1. Effect of *S. siamea* extract on acetic acid induced wreathing response. *P<0.05 refers to significant compared to control.

Table 1. Anti-microbial activity of ethanolic extract of *Senna siamea* leaf.

S. No.	Microorganism	Diameter of zone inhibition (in mm)			
		Sample extract			Kanamycin standard 30µg/disc
		100 µg/disc	200 µg/disc	500 µg/disc	
1.	*Staphylococcus aureus*	NS	NS	NS	25.8
2.	*Staphylococcus epidermidis*	NS	NS	NS	25.8
3.	*Staphylococcus pyrogenous*	NS	NS	NS	22
4.	*Shigella boydii*	NS	NS	NS	32.4
5.	*Salmonella typhi*	NS	NS	NS	33.34
6.	*Proteus Spp.*	NS	NS	NS	33.1
7.	*Shigella dyst-1*	NS	NS	NS	24.22
8.	*Plesiomonas*	NS	NS	NS	31
9.	*Shigella sonnie*	NS	NS	NS	31.92
10.	*Hafnia*	NS	NS	NS	33.04
11.	*Enterococci*	NS	NS	NS	33.8
12.	*Pseudomonas aeroginosa*	NS	NS	10	30

at ethanol extract of *S. siamea* at the dose of 500 mg/kg exhibit significant ($P < 0.001$) inhibition of writhing reflex by 61.98% while the standard drug Diclofenac-Na inhibition was found to be 85.95 % at a dose of 25 mg/kg body weight.

Antimicrobial potentials

Antibacterial activities of the extract were tested against thirteen pathogenic bacteria and were compared with the standard antibiotic kanamycin by measuring the zone of inhibition diameter and expressed in millimeter (mm), (Figure 1). Result shows the weak antimicrobial activity of the ethanolic extracts of *S. siamea* (leaves) on *Pseudomonas aeruginosa*. All the organisms were resistant to the extracts at 100 and 200µg/disc concentrations, with anti-pseudomonal activity only exhibited at 500µg/disc. The zone diameter of inhibitions of 10 mm for Ethanolic extracts reveals a dose dependent anti-pseudomonal activity.

Phytochemical Screening

According to results, saponins, tannins, triterpenes, alkaloids, flavonoids and glycosides were detected using the corresponding Phytochemical tests.

Discussion

The ethanol extract was evaluated in the acetic acid-induced writhing test for its analgesic activity. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesic. The response is thought to be mediated by the prostaglandin pathways, peritoneal mast cells and acid sensing ion channels (Hossain *et al*., 2006; Ronaldo *et al*., 2000; Voilley, 2004). Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic mediators like PGE_2 and $PGF_{2\alpha}$ and their levels increase in the peritoneal fluid of the acetic acid induced mice (Deraedt *et al*., 1980). The abdominal constrictions produced after administration of acetic acid is related to sensitization of nociceptive receptors to prostaglandins. It is therefore possible that the extract exerts its analgesic effect by inhibiting the synthesis or action of prostaglandins which may be due to phytochemicals

present in the extract. Thermally induced nociception indicates narcotic involvement (Besra *et al*., 1996). The centrally acting analgesics generally elevate the pain threshold of mice towards heat. The extract significantly delayed the response time to thermal pain sensation in tail flick method indicating narcotic involvements. Moreover, since the extract inhibited both peripheral and central mechanisms of pain, it is possible that the extract acted on opioid receptor (Elisabetsky *et al*., 1995; Pal *et al*., 1999). Therefore, the significant pain reduction of the plant extract may be due to the presence of analgesic principles acting with the prostaglandin pathways or interfering with other mediators responsible for peripheral pain. Antibacterial activity of *Senna siamea* leaf extract (100, 200 and 500 µg/disc) was studied on thirteen pathogenic bacteria and were compared with the standard antibiotic kanamycin (30 µg/disc).

Senna siamea leaf extract showed no activity (resistance) against bacteria like *Staphylococcus saprophyticu, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus pyrogenous, Shigella boydii, Salmonella typhi, Proteus Spp., Shigella dyst-1, Plesiomonas, Shigella sonnie, Hafnia, Enterococci and Pseudomonas aeroginosa* at 100 and 200 µg/disc concentrations. But at extract concentration 500µg/disc the diameter of inhibitions was found 10 mm against *Pseudomonas aeruginosa*, reveals a dose dependent anti-pseudomonal activity. On the other hand, standard antibiotic kanamycin (30 µg/disc) showed significant antibacterial activity against all tested Gram (+)ve and Gram (-)ve bacteria. In case of standard antibiotic kanamycin, the highest zone diameter of inhibitions (33.80 mm) was found against *Enterococci*

It was observed from the present study that the ethanolic extract of *S. siamea* posses analgesic activity. The extract showed no activity against the twelve test organisms, only antipseudomonal activity exhibited at 500µg/disc. It could be concluded that the leaf extract of *S. siamea* could be suitable candidate in the preparation of drugs for the treatment of infections caused by *Pseudomonas aeruginosa*, But further pharmacological studies are required to be undertaken to understand the underlying possible mechanisms of the observed activities as well as need to isolate, purify, characterize active phytochemicals responsible for these bioactivities.

Conflict of interest

The Authors have declared that there no conflict of competing interest.

References

Aliyu B.S. (2006). West African Ethnomedicinal Plants. *Triumph Publishing Company*; Kano, Nigeria.

Alli Smith. (2009). Determination of Chemical Composition of *Senna siamea* (*Cassia* leaves). *Pak J Nutr* 8 (2):119 – 121.

Awadh Ali N.A., Juelich W.D., Kusnick C. and Lindequist U. (2001). Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J Ethnopharmacol* 74: 173-9.

Barry A.L. (1980). Procedures for testing antimicrobial agents in agar media. Antibiotics in laboratory medicine; (V. Lorian Ed.); Willams and Wilkins Company; Baltimore; USA; pp. 1-23

Bauer A.W., Kirbey W.M.M., Sherries J.C. and Truck M. (1996). Antibiotic susceptibility testing by standardized single disc method. *Am J Clin Pathol* 45: 493-496.

Bernard M.L.(2005). The Phytochemical analysis and Antibacterial Screening of extracts of *Tetracaptatum consphorum*. *J Chem Soc Nig* 6, 53 – 55.

Besra S.E., Sharma R.M. and Gomes A. (1996). Anti-inflammatory effect of petroleum ether extract of leaves of *Litchi chinensis* Gaertn (Sapinadaceae). J *Ethnopharmacology* 54, 1-6.

Deraedt R., Joughney S., Delevakee F. and Falhour M. (1980). Release of prostaglandin E and F in an algogenic reaction and its inhibition. *Eur J Pharmacol* 51, 17-24.

Doughari, J.H., El-mahmood, A.M. and Manzara, S. 2007. Studies on the antibacterial activity of root extracts of *Carica papaya* L. *Afr J Microbiol* 037- 041.

Elisabetsky E., Amador T.A., Albuquerque R.R., Nunes D.S. and Ado C.C. (1995). Analgesic activity of *Psychotria colorata* (Willd. ex R. and S.). Muell. Arg. alkaloids. *J Ethnopharm-acol* 48, 77-83.

Elujoba, A.A., Ajulo, O.O. and Iweibo, G.O. (1989). Chemical and biological analyses of Nigerian Cassia species for laxative activity. *J Pharm Biomed Analysis* 7, 1453–1457.

Emran T.B., Rahman M.A., Hosen S.M.Z., Rahman M.M., Islam A.M.T., Chowdhury M.A.U. and Uddin M.E. (2012). Analgesic activity of *Leea indica* (Burm. f.) Merr. *Phytopharmacol* 3, 150-157.

FAO (2004). Trade in medicinal plants. In: Economic and Social Department, Food and Agriculture Organization of the United Nations, Rome. 2–3.

Forestry/ Fuel wood Research and Development Project (F/FRED) (1994). Growing Multipurpose Trees on Small Farms, Module 9: Species Fact Sheets (2nd ed.), Bangkok, Thailand: Winrock Intemational. 127 p.

Gbeassor M., Kossou Y., Amegbo K., De Souza, C., Koumaglo K. and Denke A. (1989). Antimalarial effects of eight African medicinal plants. *J Ethnopharmacol* 25, 115–118.

Ghani A. (2003). Medicinal Plants of Bangladesh: Chemical constituents & uses. Asiatic Society of Bangladesh.2nd edition pp; 1-16,138.

Gutteridge R.C.(1997). *Senna siarnea* (Larnk) Irwin & Bameby. In: Faridah Hanum, I. & van der Maesen, L.J.G. (Editors): Plant Resources of South-East Asia No. I 1. Auxiliary plants. Backhuys Publishers, Leiden, the Netherlands. p. 232-36.

Hossain MM, Ali M.S., Saha A. and Alimuzzaman M. (2006). Antinociceptive activity of whole plant extracts of *Paederia foetida*. *Dhaka Uni J Pharm Sci* 5, 67-69.

Jensen M. (1995) Trees Commonly Cultivated in Southeast Asia - an illustrated field guide; RAP Publication: 1995/38, FAO, Bangkok, Thailand. p. 93.

Kaur G., Alam M., Jabbar Z., Javed K. and Athar M. (2006). Evaluation of antioxidant activity of Cassia siamea flowers. *J Ethnopharmacol* 108, 340–348.

Khan M.S. and Alam M.K. (1996). Homestead flora of Bangladesh. Bangladesh Agricultural Research Council, Farmgate, Dhaka, Bangladesh. p. 275.

Lose GA, Bernard SJ, Leihner DE. (2000). Studies on agro forestry hedgerow system with Senna siamea rooting patterns and competition effects. *Journal of Ethnopharmacology* 38, 57–60.

Mbatchi S.F., Mbatchi B., Banzouzi J.T., Bansimba T., Nsonde-Ntandou G.F., Ouamba J.-M., Berry, A. and Benoit-Vica L.F. (2006). *In vitro* antiplasmodial activity of 18 plants used in Congo Br-azzaville traditional medicine. *J Ethnopharmacol* 104, 168–174.

Nair R., Kalariya T. and Chanda S. (2005) Antibacterial activity of some selected Indian medicinal flora. *Turk J Biol* 29, 1-7.

Nayak B.S. and Pinto Pereira L.M. (2006). *BMC Complement Altern Med* 6, 41-47.

Nsonde-Ntandou G.F., Ndounga M., Ouamba, J.M., Gbeassor M., Etou-Ossibi A., Ntoumi F. and Abena A.A. (2005). Ethnobotanical survey, chemical screening and effective treatment of certain plants used in traditional medicine to treat malaria in Brazzaville. *Phytotherapia*, 1, 13–18.

Pal S., Sen T. and Chaudhuri AK. (1999). Neuropsychopharmacological profile of the methanolic fraction of *Bryophyllum pinnatum* leaf extract. *J Pharm Pharmacol* 51, 313-318.

Rang H.P. and Dale M.M. (1993). Pharmacology, second edition, Churchill Livingstone Publisher,

UK, pp.706-711.

Rates S.M.K. (2001). Plants as source of drugs. *Toxicon,* 39, 603–613

Ronaldo A.R., Mariana L.V., Sara M.T., Adriana B.P.P., Steve P., Ferreira S.H. and Fernando Q.C. (2000). Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *Eur J Pharmacol* 387, 111- 118.

Sharif M.D.M. and Banik G.R. (2006). Status and Utilization of Medicinal Plants in Rangamati of Bangladesh. *J Agri Sci Biol* 2, 268-273.

Sukma M., Chaichantipyuth C., Murakami Y., Tohda M., Matsumoto K. and Watanabe H. (2002). CNS inhibitory effects of barakol, a constituent of *Cassia siamea* Lamk. *J Ethnopharmacol* 83, 87–94.

Thongsaard W., Chainakul S., Bennett G.W. and Marsden C.A. (2001). Determination of barakol extracted from *Cassia siamea* by HPLC with electrochemical detection. *J Pharm Biomed Analysis*, 25, 853–859.

Voilley N. (2004). Acid-Sensing Ion Channels (ASICs): New targets for the analgesic effects of Non-Steroid Anti- inflammatory Drugs (NSAIDs). *Curr Drug Targets-Inflammation Allergy* 3, 71-79.

Zimmermann M. (1983). Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 16, 109–110.

