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INVESTIGATION OF PHYTOCHEMICAL SCREENING AND ANALGESIC ACTIVITY OF DIFFERENT EXTRACTS OF *CUSCUTA CHITTAGONGENSIS* LEAVES

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ABSTRACT

The purpose of this study was to investigate the presence of phytochemical and determine the analgesic activity of *Cuscuta chittagongensis*. In Phytochemical screening, leaves contain different types of compound such as glycosides, alkaloids, flavonoids, tannin etc. Acetic acid and formalin induced method were performed to investigate the analgesic effect of leaves extracted with different solvent system. Diclofenac Na was used as standard. *Cuscuta chittagongensis* extracts were given orally to the 4 groups of experimental mice. Among these fractions the most potent activity was found in ethyl acetate extract which showed highest % of inhibition (69.15) at 200 mg/kg where standard (Diclofenac-Na) showed 80.44. From this result, it is clear that all the extracts of *cuscuta chittagongensis* contain analgesic activity. The inhibition increased as the dose of extract was increased.

KEYWORDS: *Cuscuta chittagongensis,* acetic acid induced and formalin induced methods, analgesic activity.

INTRODUCTION

The mankind has been a victim of diseases since the very beginning of their existence. But Mother nature provided us with the remedy of those menacing diseases. Despite of the immense advances in modern medical science, still most of the people all around the world rely on medicinal plants for the purpose of treatment. According to WHO, about 80% of the world population, particularly the people living in the developing countries use various

traditional medicines which rely on plants as sources of drugs.[1] Surprisingly, less than 15% of the plants are known to have been investigated pharmacologically out of the estimated 500,000 species of higher plants growing on earth.[2] Thus plants are considered as one of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drug candidates. Bangladesh has a rich and prestigious heritage of herbal medicines among the South Asian countries. About 500 species of medicinal plants that are estimated as growing in Bangladesh also about 250 species of them are used for the preparation of traditional medicines. However, the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compounds.[3] Traditional records and ecological diversity indicate that Bangladeshi plants which represent an exciting resource for possible lead structures in drug design.[4] It is absolutely imperative to ensure that these medicinal plants or their products really possess the claimed properties and exert the desired therapeutic effects. In an effort to substantiate the validity of claimed therapeutic effects of medicinal plants, they must be subjected to extensive scientific study. Attempts must be made to exclude the useless plants those are misleadingly claimed to be medicinal. Medicinal plants can exhibit unwanted side effects due to the presence of some additional toxic constituents when used in the crude from.[5] So, the purpose of extensive phytochemical and pharmacological work is to isolate the active constituents in the pure form to avoid adverse effects and to ensure safe use of herbal drugs.

Pain is an unpleasant feeling which is caused by with tissue damage. Tissue injury is the immediate cause of pain is tissue injury and it occurs due to release of different chemical mediators like prostaglandins, bradykinins, substance P which act on the nociceptors causing this sensation. It is often classified as chronic and acute. Acute pain may be characterized by its quick onset and short duration, lasting for hours. On the other hand, chronic pain is often associated with persistent pain over a large period of time.[6,7] The drugs which can reduce pain are called analgesic drugs. Currently used analgesic drugs in most cases are either steroidal like corticosteroids or non-steroidal like NSAIDs. Most of them cause more or less adverse effects such as renal failure, allergic reactions, hearing loss or affecting platelet function. Some plant derived medicines has been used from centuries without any serious adverse effects. So, more researches should be conducted to develop new pain management medicines with plant based origin.[8,9] *Cuscuta chittagongensis,* a plant of *c*onvolvulaceae family is distributed in Bangladesh at the place of Bandarban, Rangamati and Sylhet.[10] No

extensive works are done on this plant till now. The purpose of this study was to investigate the presence of Phytochemical compounds and determine the analgesic activity of *Cuscuta chittagongensis*.

MATERIALS AND METHODS

Collection, identification and authentication of selected plant

The fresh leaves of were collected at February-2015 from Bandharban district, Bangladesh this plant leaves was identified by expert taxonomist. It was authenticated at Bangladesh National Herbarium, where a voucher specimen (No. DACB-41674) for had been deposited.

Extraction of plants

Plants were washed properly to remove dirty materials and shade dried for several days with sun drying. These were dried in an oven for 24 hours at considerably low temperature for better grinding. The dried plants were ground into coarse powder by a grinding machine in the department of Pharmacy, Southeast University. Powdered plant materials that having a weight of about 350 gm were taken in three amber colored reagent bottle and soaked in 1 liter of three reagents like ethyl acetate, pet-ether and chloroform respectively. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper and was concentrated with a rotary evaporator under reduced pressure at 50°C temperature to afford crude extracts. The extracts obtained are Ethyl acetate extract (EAE), Pet Ether Extract (PEE), Choloroform Extract (CLFE).

Phytochemical Screening Methods

Test for Glycosides: 2 ml solution of the extract was taken into a test tube. 1 ml mixture of Fehling solution was added into the test tube. The tube was placed in a water-bath at 60°C. If brick red color forms that shows the presence of glycosides.

Test for Alkaloids: In testing for Alkaloids, about 0.5g of extract will be stirred with 5 ml of 1 percent aqueous hydrochloric acid on a water bath; 1 ml of the filtrate is to be treated with a few drops of mayer's reagent and a second 1 ml portion is to be treated the same way with Dragendorff's reagent. Presence of orange-red color indicates the presence of alkaloid.

Test for Flavonoids: A small quantity of test residue was dissolved in 5 ml of ethanol (95% v/v) and treated with few drops of concentrated hydrochloric acid and 0.5 g of magnesium

metal. If the pink, crimson or magenta color is developed within a minute or two that mean flavonoids are present.

Test for Tannins: About 5 g of each portion of plant extract will be stirred with 10 ml distilled water, filtered and ferric chloride reagent will then be added to the filtrate. If dark green or deep blue color is obtained, it means tannins are present.

Test for Saponins: A few mg of the test residue was taken in a test tube and shaken vigorously with small amount of sodium bicarbonate and water. If stable, characteristic honeycomb like froth is obtained, it means saponins are present.[11,13]

Drugs and chemicals

Diclofenac Na, acetic acid were obtained as gift sample from Square pharmaceuticals Ltd. All other chemicals used in this study were obtained commercially and were of analytical grade.

Experimental animals

Four-five week-old Swiss albino mice were (25-30g) were collected from purchased from Animal Resource Branch, ICDDR'B, Mohakhali, Dhaka, Bangladesh and were housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hours light: 12 hours dark cycle). The mice were feed with standard pellet diet. The animals used in this study were cared in accordance with the ethical guidelines on animal experimentation of Department Pharmacy, Southeast University, Banani-1213, Dhaka. The mice were selected and divided into 4 groups, containing 5 mice in each group. Each group received a particular treatment i.e. control, standard and the dose of the extracts of the plant respectively.

• Group 1- Saline water 0.9% were given orally as control solution
• Group II - Standard (Diclofenac-Na) drug 10 mg/Kg. B.W. orally.
• Group III - *cuscuta chittagongensis* 100 mg/mice orally.
• Group IV- *cuscuta chittagongensis* 200 mg/mice orally.

Analgesic activity evaluation by acetic acid induced writhing method

In this method**,** acetic acid is administered intra peritoneally to the experimental animals to create pain sensation.[14] As a result, the animals squirms their body at regular interval out of pain. This squirm or contraction of the body is termed as "writhing". As long as the animals

feel pain, they continue to give writhing. Each writhing is counted and taken as an indication of pain sensation. Any substance that has got analgesic activity is supposed to lessen the number of writhing of animals within in a given time frame and with respect to the control group. The writhing inhibition of positive control was taken as standard and compared with test samples and control. In the present study, Diclofenac-Na was used to serve the purpose. At zero hour; test samples, saline water (Group I), Diclofenac Na (Group II) and test sample (Group III to VI) were administered orally by a feeding needle. After 30 minutes acetic acid (1%) was administered intra peritoneal to each of the animals of all the groups (from Group I to VI). Five minutes after the administration of acetic acid, number of writhing were counted for each mouse for thirty minutes.

Analgesic activity evaluation by formalin test

The antinociceptive activity of the drugs was determined using the formalin test described by Control group received 5% formalin. 20 µl of 5% formalin was injected into the dorsal surface of the right hind paw 60 min after administration of test sample and 60 min after administration of Diclofenac Na (10 mg/kg, B.W).[15] The mice were observed for 30 min after the injection of formalin and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 and 30.

RESULTS

Phytochemicals obtained in *cuscuta chittagongensis*

No	Phytochemicals	Presence
01	Glycosides	++
02	Alkaloids	+++
03	Flavonoids	+++
04	Taninis	-
05	Saponins	-

Table-1: Evaluation of analgesic activity of plants by acetic acid solution induced method.

Groups	Treatment	Dose	Avg. no. of Writhing	%inhibition
01	Control (Saline)	10ml/kg	26.75 ± 1.72	-
02	Diclofenac-Na	10mg/kg	5.23 ± 1.25	80.44
03	Ethyl Acetate extract	100	13.52 ± 2.54	49.45
04		200	8.25 ±2.29	69.15
05	Chloroform	100	11.12 ± 1.62	58.42
06		200	9.73 ± 2.09	63.62
07	Pet-ether Fraction	100	12.53 ± 1.31	53.15
08		200	10.63 ± 1.43	60.26

Figure-1: Evaluation of analgesic activity of different extract of *cuscuta chittagongensis* by acetic acid induced writhing method.

Among these fractions the most potent activity was also found in ethyl acetate extract show highest % of inhibition (69.15) at 200 mg/kg where standard (Diclofenac-Na) showed 80.44. From this result, it is clear that all the extracts of *cuscuta chittagongensis* contain analgesic activity.

Table-2: Evaluation of analgesic activity of different extract of *cuscuta chittagongensis* by Formalin induced writhing method (Early Phase).

Groups	Treatment	Dose	Avg. no. of Writhing	% inhibition
01	Control (Saline)	10ml/kg	22.89. ± 1.30	-
02	Diclofenac-Na	10mg/kg	5.53 ± 1.29	75.84
03	Ethyl acetate extract	100	10.6 ±1.55	53.69
04		200	9.53 ± 1.55	58.36
05	Pet-ether extract	100	12.17 ± 1.76	46.83
06		200	10.71 ± 1.64	53.21
07	Chloroform extract	100	9.4 ± 1.51	58.52
08		200	8.2 ± 1.51	64.17

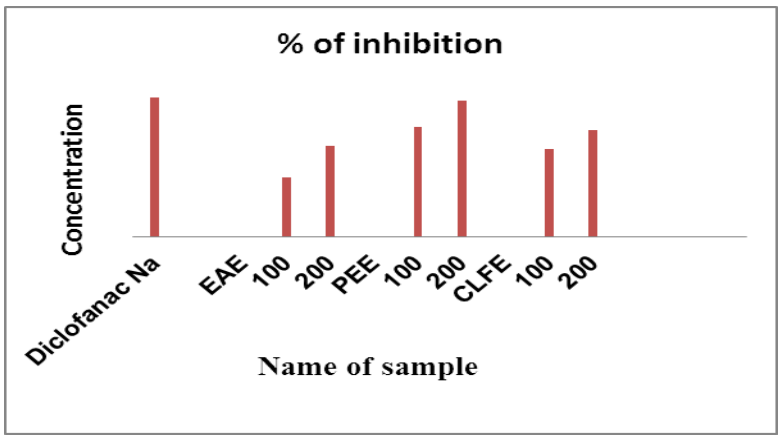

Figure-2: Evaluation of analgesic activity of extract & different fraction of *cuscuta chittagongensis* by formalin induced writhing method (Early Phase).

Table-3: Evaluation of analgesic activity of different extract of *cuscuta chittagongensis* by Formalin induced writhing method (Late phase).

Groups	Treatment	Dose	Avg. no. of Writhing	% inhibition
01	Control (Saline)	10ml/kg	13.63 ± 1.30	-
02	Diclofenac-Na	10mg/kg	4.20 ± 1.43	69.18
03	Ethyl Acetate Fraction	100	5.23 ±1.26	61.62
04		200	4.83 ± 1.14	64.56
05	Pet-ether Fraction	100	4.59 ± 1.15	66.32
06		200	4.25 ± 1.72	68.81
07	Chloroform Fraction	100	4.87 ± 1.06	64.26
08		200	4.63 ± 0.66	66.03

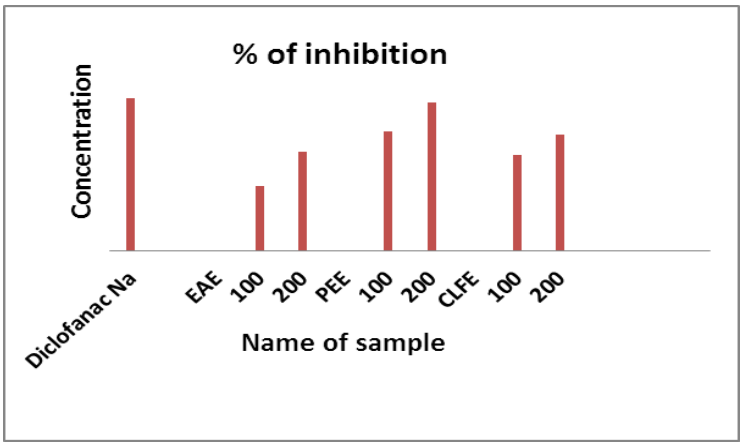

Figure-3: Evaluation of analgesic activity of extract & different fraction of *cuscuta chittagongensis* by formalin induced writhing method (Late Phase).

DISCUSSION

The analgesic activity was performed using acetic acid-induced writhing and formalin induced paw licking method. The inhibition increased as the dose of extract was increased. Preliminary qualitative phytochemical screening reveals the presence of alkaloids, flavonoids, steroids and tannins in *cuscuta chittagongensis.* Flavonods, tannins and alkaloids have been reported to have a role in analgesic activity primarily by targeting prostaglandins.[16] Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response.[17,18] Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipid via cyclooxygenase & prostaglandin biosynthesis.[19] The studied plant (*cuscuta chittagongensis*) showed good pain inhibition compared to control group. In formalin induce paw licking method, *cuscuta chittagongensis* reduces inflammatory pain 0-5 minutes and 20-30 minutes compared to control group. So the

result found in the present study demonstrated that the leaves extracts of *cuscuta chittagongensis* has good analgesic effect.

CONLUSION

Based on the results of our study it can be concluded that the different solvent system of pet-ether, ethyl acetate, chloroform extract of *C. chittagongensis* possess significant analgesic effects in two methods (Acetic acid-induced writhing method, Formalin induced paw licking method) done for analgesic activity. All of the experiments were performed in multiple dose and further studies have to be carried out to identify the phyto-constituent responsible for the exact and detailed mechanism of action responsible for this activity.

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