

Phytochemical Screening and Evaluation of Thrombolytic, Analgesic and Antidiarrhoeal Activity of the Leaves of *Cucumis sativus* Linn. (Cucurbitaceae) of Methanolic Extracts

Aklima Akter^{1*}, Md. Zamshed Alam Begh¹, Fahadul Islam¹, Tahmina Afroz¹,
Md. Shakhawat Hossain¹, Md. Faysal¹, Md. Mominur Rahman¹

¹Department of pharmacy, Faculty of Allied Health Sciences, Daffodil International University,
Shukrabad, Dhaka-1207, Bangladesh.

Abstract

Aims: The present study was performed to evaluate the phytochemical screening of compounds, thrombolytic activity, analgesic activity and antidiarrhoeal activity of the methanolic extract of *Cucumis sativus* Linn. (Cucurbitaceae) leaves.

Background: *Cucumis sativus* Linn. is generally disseminated all through the world especially in Asia, Africa and South America. Locally the plant is utilized for basic role of cerebral pain, the seed is utilized for diuretic and cooling purposes. The leaf sap is emetic, it is utilized for treating dyspepsia in children.

Methods: The thrombolytic activity was evaluated by using clot lysis method. Analgesic activity was investigated using writhing method in mice and anti-diarrhoeal activity test was performed castor oil-induced diarrhoea in mice as well.

Result: Significant thrombolytic activity was found for *Cucumis sativus* Linn 100mg plant leaves extract (29.33%) clot lysis in respect of positive control streptokinase (30000 IU) was shown 65.26% clot lysis. Analgesic activity was investigated using writhing method at the doses of 250/kg and 500 mg/ kg body weight and found 54.72 % and 55.66 % of inhibition respectively compared to standard Diclofenac Na (76.41 %) writhing inhibition. Anti-diarrhoeal activity test was performed at the dose of 500 mg/kg body weight (PO) against castor oil-induced diarrhoea and % of inhibition of feces was found about 62.5% respectively.

Conclusion: *Cucumis sativus* Linn. was shown significant pharmacological potentiality in various study model which will helpful for further studies.

Keywords: *Cucumis sativus* Linn., Phytochemical screening, Streptokinase, Diclofenac sodium

BACKGROUND

Plants produce wide arrangement of bioactive principles and constitute an abundance source of medicines. Many developing countries traditional medicine are used as primary purpose of their health care systems [1]. *Cucumis sativus* Linn. is the botanical name of the cucumber which belongs to family of Cucurbitaceae. It is widely distributed throughout the world particularly in Asia, Africa and South America [2]. The cucumber plant is a delicate yearly with a harsh, succulent, trailing stem. The bushy leaves have 3 to 5 pointed flaps just as the stem bears expanded extension by which the plant can be prepared to bolsters [3]. It grows up to two meters tall and five meters long and bald cylindrical fruits are warty, yellow to green as well as up to 50cm long [4]. Locally the plant is used for primary purpose of headache, the seed is used for diuretic and cooling purposes [5]. The leaf sap is emetic, it is used for treating dyspepsia in children. The fruit has special properties such as depurative, diuretic, emollient, purgative, resolvent and fresh and green fruit is used internally for treating of blemished skin, heat rash and as a cosmetic for softening the skin as well [6]. Cardiovascular disorder was caused by clotting blood that is known as thrombus formation, is one among the most detrimental disorders which are rising at minacious rate in the present times [7]. Thrombolysis may be correlated with an increased risk of complications in patients who are pregnant or at an increased age, and in people with other conditions [8]. The breakdown or lysis of blood clot is named as thrombolysis is caused by tissue plasminogen

activator (tPA). Intravenous heparin utilized as the main line treatment though of its security profile as well as activity [9,10]. A lot of medications have been modified with the advancement of current pharmaceutical study like anistreplase, alteplase, urokinase, streptokinase as well as tissue plasminogen (TPA) [11,12]. Analgesics are administrators that explicitly relieving torment by acting in the Central Nervous System (CNS) and periphery torment center individuals without advancing mindfulness. The study of pain in animals raises ethical, philosophical, and technical problems [13]. Analgesics minimize the levels of chemical mediators (prostaglandins) produced by inflammation, relieve pain, swelling as well as redness. Cyclo-oxygenase (COX 2) enzyme is inhibited by them which is integral in the synthesis of prostaglandins [14]. A lot of synthetic analgesic drug such as paracetamol, aspirin, ibuprofen, codeine, morphine etc. which has greater side or adverse effect like reduced concentration or confusion, vomiting, constipation, renal failure, vertigo etc. [15]. Diarrhoea is the prime concern especially developing countries [16]. Looseness of the bowels is recognized by expanded recurrence of defecation, watery stool just as stomach torment. Different national and worldwide associations are attempting to control this ailment yet the rate of rate is still high, roughly 7.1 million every year. A ton of engineered synthetic substances are accessible for the treatment of looseness of the bowels yet they have some significant symptoms [17,18].

The present study was investigated the thrombolytic activity, analgesic activity and anti-diarrheal activity *Cucumis sativus* Linn. of the leaves extract.

MATERIALS AND METHODS

Chemicals and reagents

Diclofenac sodium, streptokinase and distilled water were used.

Plant Materials

The leaves part of the plant of *Cucumis sativus* Linn. were collected from near Jahangirnagar University fields, Dhaka, Bangladesh. The identification of the plant material was confirmed by the specialists of Bangladesh National Herbarium, Mirpur, Dhaka and also by the authorities of Botanical Garden, Mirpur, Dhaka.

Drying and grinding

The collected leaves were separated from unwanted components. Then these were dried in for ten days in the sunlight and these were cutting into small pieces. The leaves were converted into coarse powder by using a suitable grinder. The powder was stored in an airtight vessel and placed in a cool, gloomy and dry place until analysis introduced.

Preparation of methanol extract

At first, a clean flat-bottomed glass vessel was taken and added approximately 400 gm of powdered sample into the vessel. Then 1500 ml of 90% methanol take away into the vessel and soaked the powder into the methanol solvent. Afterwards, the vessel was sealed with its contents and kept for a period of 10 days accompanying occasional shaking and stirring. After that, the coarse part of the leaves was separated from the mixture by using white cotton. Then the liquid portion was also filtered three times with the help of white cotton. Then again, it was filtered through whatman filter paper. Then the filtrate was placed in Rotary evaporator machine which separates solvent methanol and wanted crude extract was obtained.

Experimental animals

Swice albino mice (22-25g) were purchased from Jahangirnagar University, Dhaka, Bangladesh and their ages five to six weeks and were kept in animal's cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr. light: 12 hr. dark cycle). The mice were feed with standard pellet diet taken from, Jahangirnagar University, Dhaka. The animals used in this study were cared according to the guidelines on animal experimentation of our institute.

Phytochemical screening

Phytochemical screening of *Cucumis sativus* Linn. was identified the functional groups as narrated [19,20].

Thrombolytic activity test

Thrombolytic test was done by percentage of clot lysis method. Blood was taken from healthy volunteers (n=3) without a history of oral contraceptive or anticoagulant therapy [21].

Analgesic activity

For analgesic test all mice were divided into four groups. Each group has 4 mice. Control group taken 0.5% methyl cellulose per oral, Standard Group taken Diclofenac-

Na10mg/ kg intraperitoneally, group III and IV were taken with methanolic leaves extract of *Cucumis sativus* Linn. at the doses of 250 and 500 mg per kg of body weight, respectively. The analgesic activity of the samples was carried out using acetic acid- induced writhing model in mice. Extracts and vehicle were administered orally 30 mins before intraperitoneal administration 10ml/kg of 0.7% acetic acid but Diclofenac-Na was administered intraperitoneally 15 minutes before the acetic acid injection. The mice were observed for fixed contraction of body referred to as "writhing" for the next 10minutes [22]. Percentage protection of acetic acid induced writhing was determined by the following formula. Percentage protection = $(Wc-Wt)/Wc \times 100$; Where, wc is the mean values of control group and Wt is the mean values of treated group.

Castor oil-induced diarrhoea

12 mice were allowed to fast for 18 h and divided into three groups of four animals each. All groups taken castor oil at a dose of 1 ml/animal orally (p.o.). 30 min after castor oil administration, group I (control group) received vehicle (1% CMC in distilled water), Group III orally received the methanol extract at 500 mg/kg doses. Group II received the reference drug, loperamide (3 mg/kg p.o.). Then the animals were placed separately in cages with filter papers underneath, which was changed every hour. The severity of diarrhea was assessed each hour for 4 h and the characteristic diarrheal droppings were recorded [23].

Statistical Analysis

The results are presented as Mean \pm SEM. Data were analyzed by one-way ANOVA followed by Dunnet's test and P values <0.001 were considered statistically significant.

RESULTS AND DISCUSSIONS

Table 1. Chemical group tests of *Cucumis sativus* Linn.

Tested groups	Methanol extract
Tannins	-
Steroid	+
Flavonoids	+
Saponins	+
Carbohydrate	+
Gum	-
Alkaloids	+
Glycosides	+

(+) Indicates presence, (-) Indicates absence.

Results of the phytochemical screening of the methanol extract of *Cucumis sativus* Linn. (Leaves) has shown in the table 1.

Table-1 was shown the results of the phytochemical screening of the methanol extract of *Cucumis sativus* Linn. The founding results indicated the presence of steroid, flavonoids, saponins, carbohydrate, alkaloids and glycosides in methanol extract.

Table 2. Thrombolytic activity test.

Sample	Wt. of Blank tube (g)	1st clot + tube (g)	1st clot	2nd clot + tube (g)	2nd clot	Lysis weight (g)	% of lysis
SK	0.83±0.01	1.78±0.01	0.95±0.02	1.26±0.01	0.33±0.02	0.62±0.02	65.26
DW	0.83±0.01	1.48±0.06	0.65±0.05	1.42±0.04	0.60±0.03	0.05±0.03	7.69
ME	0.82±0.01	1.64±0.01	0.75±0.01	1.35±0.02	0.53±0.02	0.22±0.01	29.33

SK=Streptokinase (Standard), DW=Distill Water (control), ME= Methanol extract. Values are represented as Mean±SD.

Table 3. Results of Analgesic effect of *Cucumis sativus* Linn. of leaves methanol (ME) extract on acetic acid-induced writhing in mice.

Treatment	Writhing counting(Mean±SEM)	% of Inhibition
Control	26.5±0.77	-
Diclofenac sodium(10mg/kg)	6.25±0.227***	76.41%
ME 250mg	12±0.37***	54.72%
ME 500mg	11.75±0.42***	55.66%

Values are presented as Mean ± SEM (n = 4), P < 0.001, which is significant compared with the control group (one-way ANOVA followed by Dunnett's test). ***Indicates the significance of the result.

Table-2 indicates the results in which 100 µl SK, a positive control (30,000 I.U.), was used for comparison. Methanolic extract of *Cucumis sativus* Linn. was shown 29.33% clot lysis, compared to control. The reference standard streptokinase was shown 65.26 % clot lysis as well. Platelets play a vital role in the method of formation of thrombus by adhering to be injured regions of the endothelial surface. The activated platelets form platelets to platelets bonds and further bind to the leucocytes and bring off them into a perplex method of plaque formation as well as growth [24]. Streptokinase forms a 1:1 stoichiometric complex with plasminogen which is capable of converting additional plasminogen to plasmin [25].

Cucumis sativus Linn. inhibited 54.72 % and 55.66 % writhing of methanol leaves extracts at 250 and 500 mg per kg of the body weight doses, respectively, compared to standard drug Diclofenac Na inhibited 76.41 % writhing (Shown in Table-3).

Cyclooxygenase (COX) inhibiting and the resulting inhibition of prostaglandin and other eicosanoid synthesis mitigate pain, fever, as well as inflammation [26]. The constitutive isoform of COX, COX-1, has vivid physiological acts. Its activation leads, for example, to the production of prostacyclin which, when released by the endothelium, is anti-thrombogenic [27].

Table 4. Effect of methanol(ME) extract of the leaves of *Cucumis sativus* Linn.on castor oil-induced diarrhoea in mice.

Treatment	No. of faecal droppings in 4h (Mean±SEM)	% Inhibition of defaecation
Control (0.35ml/mouse)	4±0.083	-
Loperamide (3mg/kg)	0.0±0.0***	100
ME 500mg	1.5±0.0***	62.5

Values are presented as Mean ± SEM (n = 4), P < 0.001, which is significant compared with the control group (one-way ANOVA followed by Dunnett's test). ***Indicates the significance of the result.

At dose (500 mg/kg) of the methanol leaves extract, significant inhibition 62.5% of characteristic diarrhoeal feces was observed. The active component of castor oil is the ricinoleic acid, which is liberated from the action of lipases on castor oil. The ricinoleic acid produces irritating and inflammatory actions on the intestinal mucosa leading to the release of prostaglandins [28] which stimulates peristaltic activity in the small intestine that gives permeability of intestinal mucosa [29].

CONCLUSIONS

This study was revealed that extracts *Cucumis sativus* Linn. of the could be used as a source for analgesia as well as clot lysis activities. This species could be prompted for the large scale cultivation as well as marketing for the benefit of the local communities.

Acknowledgement

The authors are grateful to Department of Pharmacy, Daffodil International University to give permission and all sorts of supports to conduct the research.

Compliance with Ethical Standards: The handling and use of animals were in accordance with the National Institute for Health Guide for the Care and Use of Laboratory Animals. Our study was approved by a Research Ethics Committee for animal house of department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University.

Conflict of Interest: The authors declared that they have no conflict of interest.

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