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Screening of biological activities of *Cucumis sativus* leaf, growing in Bangladesh

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Abstract
The crude methanol extract of leaves of *Cucumis sativus* as well as its hexane, carbon tetrachloride and chloroform soluble partitionates were subjected to screening for antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities. In DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, the hexane soluble fraction demonstrated the highest free radical scavenging activity (IC_{50}= 3.11±0.45 µg/ml). The carbon tetrachloride soluble fraction revealed the highest cytotoxic activity with LC_{50} value of 1.84±0.31 µg/ml in brine shrimp lethality bioassay. In thrombolytic activity assay, the hexane soluble fraction showed 8.53±0.68% of clot lysis as compared to 66.77% clot lysis by standard streptokinase. The carbon tetrachloride soluble fraction at 1.0 mg/ml concentration inhibited 49.24±0.41% of haemolysis of RBCs induced by heat as compared to 42.12% by acetyl salicylic acid, respectively. In disc diffusion assay, none of the extractives of *C. sativus* revealed any zone of inhibition against the test organisms.

Keywords: *Cucumis sativus*, free radical scavenging assay, brine shrimp lethality, thrombolytic activity, haemolysis, zone of inhibition

1. Introduction
Estimations of WHO come to the finding that more than 80% people of developing countries depend on traditional medicines for their primary health needs [1, 2]. Use of herbal medicines is quite common among rural population due to high cost or unavailability of western medicines [2]. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway [3]. The high demand for drugs from plant sources therefore requires systematic evaluation of plants used in traditional medicine for various ailments. Hence, it is necessary to evaluate medicinal plants for promising biological activity [4]. Between 1983 and 1994, 78% of the new drugs approved by the FDA correspond to those derived from unmodified natural products or drugs semi-synthetically obtained from natural sources [5].

Cucumis sativus L. (Synonym: *Cucumis muricatus* Willd., *Cucumis esculentus* Salisb.), commonly known as Cucumber in English and Shosha or khira in Bengali, belongs to the family Cucurbitaceae. About 30 Cucumis species are found in Asia and Africa. The plant is native to the tropics. Cucumber grows widely throughout Bangladesh [6]. Being an annual climber, cucumber plant grows to a height of 15-30 cm. It has large leaves. Cucumber is the edible fruit of the cucumber plant *C. sativus*. Different parts of this plant are traditionally used in headache, as cooling and diuretic, nutritive and demulcent and emetic in acute indigestion in children [7]. Cucumber fruit is helpful in constipation and indigestion. It is taken as a cooling food in summer season. Cucumber seeds are cooling, tonic, diuretic and anthelmintic [8]. The fruit has been reported to have several activities such as anti-hyperglycemic [9], antioxidant [10, 11, 12], amylolytic [13], anti-cancer [14], anti-clastogenic [15], anti-mutagenic [16, 17] and protein kinase C inhibitory [18] activities. Phytochemical screenings have revealed the presence of cucurbitasides B, C and ferredoxin and flavone glycosides such as isovitexin, saponarin and various acylated flavone C-glycosides in leaves [19 20, 21]. Antiulcer 9-beta-methyl-19-norlanosta-5-ene type glycosides have been isolated from seeds [22].

As part of our ongoing investigations on medicinal plants of Bangladesh [23, 24], an attempt was made to evaluate the crude methanol extract of leaves of *C. sativus* growing in Bangladesh as well as its organic and aqueous soluble fractions for antioxidant potential in terms of total phenolic content and free radical scavenging property; cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities and we, here in, report the results of our preliminary investigations.

2. Materials and Methods

2.1 *Plant materials*

The leaves of *C. sativus* were collected from Dhaka, Bangladesh, in May 2012. A voucher specimen (DUSH - 10772) for this plant has been maintained in Dhaka University Salar Khan Herbarium for future reference.

The sun dried and powdered leaves (500 g) were macerated in 1.5 L of methanol for 7 days. The extract was filtered through fresh cotton bed and finally with Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (5 g) of the concentrated methanol extract was fractionated by modified Kupchan [25] partition protocol and the resultant partitionates were evaporated to dryness with rotary evaporator to yield hexane (HXSF, 1.5 g), carbon tetrachloride (CTCSF, 1.5 g), chloroform (CSF, 1 g) and aqueous (AQSF, 0.08 g) soluble materials. The residues were then stored in the refrigerator until further use.

2.2 Total phenolic content

The total phenolic content of the extractives was determined with Folin-Ciocalteau reagent using the method developed by Harbertson and Spayd (2006) [26].

2.3 DPPH free radical scavenging assay

Following the method developed by Brand-Williams *et al*. (1995) [27], the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid as positive controls.

2.4 Brine shrimp lethality bioassay

This technique was applied for the determination of general toxic properties of the dimethylsulfoxide (DMSO) solutions of plant extractives against *Artemia salina* in a single day in vivo assay [28]. Vincristine sulphate was used as positive control.

2.5 Thrombolytic activity

The thrombolytic activity was evaluated by the method developed by Prasad *et al*. (2006) [29] by using streptokinase (SK) as positive control.

2.6 Membrane stabilizing activity

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution and heat induced haemolysis of human erythrocytes following the method developed by Omale *et al*. (2008) [30].

2.7 Antimicrobial screening

Antimicrobial activity was determined by disc diffusion method [31].

2.8 Statistical analysis

For all bioassays, three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD.

3. Results and Discussion

The present study was undertaken to evaluate the antioxidant potential in terms of total phenolic content and free radical scavenging property; cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities of different organic and aqueous soluble materials of the crude methanol extract of *C. sativus*.

In DPPH free radical scavenging assay, different extractives of *C. sativus* demonstrated highly significant free radical scavenging potential with IC_{50} values ranging from 3.11 to 57.50 µg/ml. The highest free radical scavenging activity was demonstrated by the hexane soluble fraction (IC_{50}= 3.11±0.45 µg/ml) followed by the crude methanol extract with an IC_{50} value of 5.80±0.58 µg/ml (Table 1).

Table 1: Total phenolic content, free radical scavenging and cytotoxic activitis of *C. sativus*

Samples/ Standards	Total phenolic content (mg of GAE/ gm of dried extract)	Free radical scavenging activity IC_{50} (µg/ml)	Brine shrimp lethality bioassay LC_{50} (µg/ml)
ME	2.19±0.11	5.80±0.58	12.65±0.78
HXSF	0.46±0.21	3.11±0.45	11.75±0.12
CTCSF	8.24±0.39	37.38±0.38	1.84±0.31
CSF	8.42±0.28	57.50±0.19	55.18±0.29
VS	-	-	0.45±0.04
BHT	-	27.50±0.54	-
Ascorbic acid	-	5.80±0.21	-

ME= Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; VS= Vincristine sulfate; BHT= Butylated hydroxytoluene

In case of brine shrimp lethality bioassay, all the fractions demonstrated significant cytotoxic potential against *A. salina* with LC_{50} values ranging from 1.84 to 55.18 µg/ml. The carbon tetrachloride soluble fraction revealed the highest cytotoxic activity with an LC_{50} value of 1.84±0.31µg/ml as compared to 0.45 µg/ml for the standard Vincristine sulphate (Table 1).

In thrombolytic activity assay, all the extractives showed very weak thrombolytic activity compared to the standard streptokinase (66.77% clot lysis). Among the extractives, the highest thrombolytic activity was revealed by the hexane soluble fraction (8.53±0.68 % of clot lysis) (Table 2).

Table 2: Thrombolytic activity of *C. sativus*

Samples/ Standard	% of lysis of RBCs
ME	1.35±0.23
HXSF	8.53±0.68
CTCSF	5.68±0.32
CSF	3.44±0.29
Water	3.79±0.55
Streptokinase	66.77±0.36

ME = Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction

At concentration 1.0 mg/ml, the extractives of *C. sativus* significantly protected the haemolysis of RBC induced by heat but none of the samples produced any promising result against hypotonic solution induced haemolysis. The carbon tetrachloride soluble fraction inhibited 49.24±0.41d% and 8.69±0.46% of haemolysis of RBCs induced by heat and hypotonic solution and as compared to 42.12% and 71.90% by the standard acetyl salicylic acid (0.10 mg/ml), respectively (Table 3).

Table 3: Effect of different extractives of leaf of *C. sativus* on heat and hypotonic solution-induced haemolysis of erythrocyte membrane

Samples/ Standard	% Inhibition of haemolysis	
	Heat induced	Hypotonic solution induced
Hypotonic medium	--	--
ME	48.58±0.22	7.88±0.12
HXSF	38.31±0.28	3.67±0.14
CTCSF	49.24±0.41	8.69±0.46
Acetyl salicylic acid	42.12±0.38	71.90±0.78

ME = Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction

C. sativus test samples were evaluated against five gram positive and eight gram negative bacteria and three fungi and the results were compared with standard antibiotic, ciprofloxacin. But, none of the test samples of *C. sativus* revealed any antimicrobial activity against those organisms.

4. Conclusion

It is clearly evident from the above findings that the test samples of *C. sativus* possess different types of bioactivities. Therefore, the plant is a good candidate for further systematic, chemical and biological studies to isolate the active principles.

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