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Cytotoxicity and Antifungal Activities of Ethanolic and Chloroform Extracts of *Cucumis sativus* Linn (Cucurbitaceae) Leaves and Stems

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ABSTRACT

Cucumis sativus Linn. (Family: Cucurbitaceae) is commonly known as cucumber which has medicinal use in the entire world. The main objective of the present study was to examine the ethanolic and chloroform extracts of leaves and stems of the medicinal plant for cytotoxicity, antifungal activities and phytoconstituents. To determine the cytotoxicity and antifungal activities, brine shrimp lethality bioassay and agar disc diffusion methods were used, respectively. The extracts were subjected to *in vitro* cytotoxicity studies at 5, 25, 50, 100, 125, 150, 200, 250, 300, 350 and 400 μg mL^{-1} and antifungal study at the dose of 80 μg $disc^{-1}$. The antifungal activity of the extracts was compared with standard drug, Griseofulvin at 30 μg $disc^{-1}$. In brine shrimp lethality bioassay, the LC_{50} (μg mL^{-1}) and LC_{90} (μg mL^{-1}) of the ethanolic extract of *Cucumis sativus* were 35.48 and 141.25 μg mL^{-1}, respectively. The chloroform extract of the cucumber also showed lethality against the brine shrimp nauplii (LC_{50}: 75.86 and LC_{90}: 151.36 μg mL^{-1}). Moreover, the ethanolic extract and chloroform extracts of *Cucumis sativus* showed moderate antifungal activities against all tested organisms used in this study with zones of inhibition ranging from 4.40±0.18 to 1.67±0.08 mm and 3.45±0.04 to 1.50±0.12 mm, respectively. Thus, the ethanolic extracts of *Cucumis sativus* showed more potent cytotoxicity and *Aspergillus niger* was the most susceptible fungal strain to ethanolic extract of *Cucumis sativus*. The presence of different phytoconstituents e.g., alkaloid, glycoside, steroid, saponin, flavonoid and tannin as confirmed by preliminary phytochemical screening suggested that the identified compounds might play an important role for cytotoxicity and antifungal effects. Finally, it was concluded that ethanolic and chloroform extracts of leaves and stems of *Cucumis sativus* possessed cytotoxicity and antifungal activities.

Key words: *Cucumis sativus,* phytochemical screening, brine shrimp, lethality, cytotoxicity, antifungal activities

INTRODUCTION

Cucumis sativus Linn. (Family: Cucurbitaceae) is widely distributed all over the world particularly in Asia, Africa and South America (Minaiyan *et al*., 2011). Acylated flavone C-glycosides such as isovitexin 2"-O-(6'"-(E)-p-coumaroyl) glucoside, isovitexin 2"-O-(6'"-(E)-p-coumaroyl)glucoside-4'-O-glucoside, isovitexin 2"-O-(6'"-(E)-feruloyl) glucoside-4'-O-glucoside and isoscoparin 2"-O-(6'"-(E)-p-coumaroyl) glucoside were identified from the leaves of *Cucumis*

sativus plants (Abou-Zaid *et al.*, 2001). The ethanolic extract of the seeds of the plant showed a significant activity against tapeworms and the results were comparable to the effect of piperazine citrate (Elisha *et al.*, 1987). The ethanol extracts of powder fruit of *Cucumis sativus* produced significant antidiabetic and hyperlipidemic effects in streptozotocin induced rats (Karthiyayini *et al.*, 2009). The hydroalcoholic and buthanolic extracts of *Cucumis sativus* seeds were also effective on controlling the loss of body weight in diabetic rats compared to controls (Minaiyan *et al.*, 2011). Moreover, sphingolipids isolated from the crude methanol extract of cucumber (*Cucumis sativus*) stems showed the antibacterial activity (Tang *et al.*, 2010). The crude extracts of *Cucumis sativus* fruits possessed moisturizing property due to the presence of cucurbitacins (Prashant *et al.*, 2005). Abiodun and Adeleke (2010) reported that the seeds of the plant served as good sources of proteins, fat and minerals and the calcium content in the seeds of *Cucumis sativus* was estimated to 2.03%. In addition to this, the preliminary phytochemical screening of the aqueous extract of *Cucumis sativus* fruits showed the presence of flavonoids and tannins which were responsible for antioxidant and analgesic activities (Kumar *et al.*, 2010). In the present study, the cytotoxic and antifungal activities of the ethanolic and chloroform extracts of *Cucumis sativus* leaves and stems were investigated. A preliminary phytochemical screening was also carried out to identify the presence of various types of phytoconstituents present in the extracts.

MATERIALS AND METHODS

Collection of plant materials: The leaves and stems of *Cucumis sativus* were collected from Chandanaish in Chittagong of Bangladesh in the month of July 2011 at day time. Fresh leaves and stems were cleaned with running tap water and dried under the sun light. Then the plant parts were cut into small pieces and homogenized to fine powder and preserved in airtight containers.

Preparation of plant extracts: The powdered materials were macerated with ethanol. Then the mixture was filtered and the filtrate was evaporated under reduced pressure in a rotary evaporator to obtain the crude ethanolic extract of *Cucumis sativus*. The same method was also employed to give the crude chloroform extract of the plant using chloroform as a solvent.

Fungal strains: The antifungal activity of the plant extracts were investigated against six fungal strains such as *Aspergillus niger, Blastomyces dermatitides, Candida albicans, Pityrosporum ovale, Trichophyton* spp. and *Microsporum* spp. All the fungal strains were collected from Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong, Bangladesh.

Phytochemical screening: Qualitative phytochemical analysis of the ethanolic and chloroform extracts of the leaves and stems of *Cucumis sativus* was carried out to identify the presence of various phytoconstituents (Trease and Evans, 1989).

Cytotoxicity study and antifungal assay: The cytotoxic activities of the ethanolic and chloroform extracts of *Cucumis sativus* were carried out by brine shrimp lethality bioassay (Meyer *et al.*, 1982). On the other hand, agar disc diffusion method was used for antifungal test of the crude extracts where potato dextrose agar medium was used to perform the antifungal activity (Bauer *et al.*, 1966). Their antifungal activities were investigated against six fungal strains and the results were compared with the standard drug, Griseofulvin (30 μg $disc^{-1}$).

Statistical analysis: Three replicates of each sample were used for statistical analysis and the values were reported as Mean±SD (Standard Deviation). Online calculator was also used for the calculation of standard deviation.

RESULTS

Preliminary phytochemical analysis: Ethanolic extract of the leaves and stems of *Cucumis sativus* showed the presence of alkaloid, glycoside, steroid, saponin and tannin. Gum, flavonoid and reducing sugars were not found in ethanolic extract of *Cucumis sativus*. On the other hand the chloroform extract of the plant revealed the presence of alkaloid, glycoside, steroid, flavonoid, saponin and tannin except gum and reducing sugars. Table 1 showed the results of phytochemical analysis of the ethanolic and chloroform extracts of *Cucumis sativus*.

Cytotoxic assay: The cytotoxicity activity of the ethanolic and chloroform extracts obtained from the plant against the brine shrimp nauplii under brine shrimp lethality bioassay was shown in Table 2 and 3. The LC_{50} (μg mL^{-1}) and LC_{90} (μg mL^{-1}) of the ethanolic and chloroform extracts of

Table 1: Results of phytochemical analysis of the ethanolic and chloroform extracts of *Cucumis sativus*

	Name of the extracts	
Name of the phytoconstituents	Ethanolic extract of *Cucumis sativus*	Chloroform extract of *Cucumis sativus*
Alkaloid	+	+
Glycoside	+	+
Steroid	+	+
Gum	-	-
Flavonoid	-	+
Saponin	+	+
Reducing sugars	-	-
Tannin	+	+

+: Indicates the presence and -: Indicates the absence of the phytoconstituents

Table 2: Brine shrimp lethality bioassay of ethanolic extract of *Cucumis sativus*

		No. of alive shrimp						
Couc. (μg μL^{-1})	Log Conc.	Test-1	Test-2	Test-3	Avg.	% of mortality	LC_{50} (μg mL^{-1})	LC_{90} (μg mL^{-1})
5	0.70	6	6	5	5.67	43.33	35.48	141.25
25	1.40	5	5	6	5.33	46.67		
50	1.70	5	3	5	4.33	56.67		
75	1.88	3	3	2	2.67	73.33		
100	2.00	2	3	2	2.33	76.67		
125	2.10	2	1	1	1.33	86.67		
150	2.18	1	1	1	1.00	90.00		
200	2.30	1	1	0	0.67	93.33		
250	2.40	0	0	0	0.00	100.00		
300	2.48	0	0	0	0.00	100.00		
350	2.54	0	0	0	0.00	100.00		
400	2.60	0	0	0	0.00	100.00		
Blank	0.00	8	9	10	9.00	10.00		

Table 3: Brine shrimp lethality bioassay of chloroform extract of *Cucumis sativus*

		No. of alive shrimp						
Conc. (µg µL^{-1})	Log Conc.	Test-1	Test-2	Test-3	Avg.	% of mortality	LC_{50} (µg mL^{-1})	LC_{90} (µg mL^{-1})
5	0.70	9	8	10	9.00	10.00	75.86	151.36
25	1.40	8	8	9	8.33	16.67		
50	1.70	7	6	7	6.67	33.33		
75	1.88	5	5	6	5.33	46.67		
100	2.00	4	5	5	4.67	53.33		
125	2.10	2	3	1	2.00	80.00		
150	2.18	1	1	1	1.00	90.00		
200	2.30	1	1	0	0.67	93.33		
250	2.40	0	1	0	0.33	96.67		
300	2.48	0	0	0	0.00	100.00		
350	2.54	0	0	0	0.00	100.00		
400	2.60	0	0	0	0.00	100.00		
Blank	0.00	10	10	10	10.0	0.00		

Table 4: Screening of the ethanolic and chloroform extract of *Cucumis sativus* for antifungal activity

	Zone of inhibition in millimeter (mm)			
Tested fungal strains	Ethanolic extract of *Cucumis sativus* (80 µg disc^{-1})	Chloroform extract of *Cucumis sativus* (80 µg disc^{-1})	Positive control [standard drug, griseofulvin (30 µg disc^{-1})]	Negative control (Blank)
Aspergillus niger	4.40±0.18	3.45±0.04	10.97±0.16	-
Blastomyces dermatitides	3.20±0.15	2.15±0.12	12.07±0.06	-
Candida albicans	2.03±0.05	1.75±0.20	12.27±0.36	-
Pityrosporum ovale	2.45±0.22	2.25±0.08	14.00±0.12	-
Trichophyton spp.	2.13±0.02	2.00±0.04	12.97±0.65	-
Microsporum spp.	1.67±0.08	1.50±0.12	11.42±0.30	-

Data were represented as Mean±SD of triplicate determination. -: No inhibition, SD: Standard deviation

Cucumis sativus were deduced respectively (LC_{50}: 35.48 and LC_{90}: 141.25 µg mL^{-1}; LC_{50}: 75.86 and LC_{90}: 151.36 µg mL^{-1}). The best-fit line slope was used for calculation of LC_{50} (µg mL^{-1}) and LC_{90} (µg mL^{-1}).

Antifungal assay: The results of antifungal screening assay of the ethanolic and chloroform extracts of the leaves and stems of *Cucumis sativus* against the tested strains were shown in Table 4. The ethanolic extract of *Cucumis sativus* showed the antifungal activity with zone of inhibition ranging from 4.40±0.18 to 1.67±0.08 mm. On the other hand, antifungal activity of chloroform extract of the plant was also observed with moderate zone of inhibition ranging from 3.45±0.04 to 1.50±0.12 mm. Finally, it was concluded that the crude ethanolic extract showed more antifungal activity than that of chloroform extract.

DISCUSSION

No report was published earlier on the cytotoxicity, antifungal potentials and phytochemical screening of the ethanolic and chloroform extracts of leaves and stems of *Cucumis sativus*. The present study is the first report about the cytotoxicity, antifungal potentials and phytochemical

screening of the ethanolic and chloroform extracts of leaves and stems of *Cucumis sativus*. In the present study, the ethanolic extract of leaves and stems of *Cucumis sativus* showed moderate antifungal action against all tested organisms used in this study. The antifungal activity profile of the plant extracts against all tested strains indicated that *Aspergillus niger* was the most susceptible fungus while *Microsporum* spp. was the least sensitive strain of all the tested fungus in this study. This indicated that both ethanolic and chloroform extracts of leaves and stems of the medicinal plant have antifungal components. The preliminary phytochemical screening of the ethanolic extract of leaves and stems *Cucumis sativus* possessed phytoconstituents such as alkaloid, glycoside, steroid, saponin and tannin except gum, flavonoid and reducing sugars while alkaloid, glycoside, steroid, flavonoid, saponin and tannin were found in the crude chloroform extract. Kumar *et al.* (2010) reported that the aqueous extract of *Cucumis sativus* fruits revealed the presence of glycosides, steroids, flavonoids, carbohydrates and tannins. Alkaloids and saponins were not identified in the aqueous extract of fruits of the plant. The most common phytochemicals found among the ethanolic, chloroform extracts of leaves and stems and aqueous extract of fruits of the plant were glycoside, steroid and flavonoid. The present study also confirmed the cytotoxic potentials of the plant. LC_{50} values were 35.48 and 75.86 μg mL^{-1} while LC_{90} values were 141.25 and 151.36 μg mL^{-1} of the ethanolic and chloroform extracts of the plant, respectively (Table 3). Thus, it was concluded that the more potent cytotoxicity was exhibited by the ethanolic extracts of leaves and stems of *Cucumis sativus*.

CONCLUSION

It was concluded that the ethanolic and chloroform extracts of *Cucumis sativus* leaves and stems had cytotoxic and antifungal activities. These results claimed further investigation to isolate the main phytochemical compounds. The present study of *in vitro* cytotoxicity and antifungal evaluation of the plant was a preliminary investigation for future research work. So, further phytochemical and pharmacological studies on *Cucumis sativus* are strongly recommended to elucidate the exact chemical compounds and mechanisms involved.

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