ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(3):555-560

ORIGINAL RESEARCH ARTICLE

Phytochemical Screening and *In-vitro* Evaluation of Reducing Power, Cytotoxicity and Anti-Fungal Activities of Ethanol Extracts of *Cucumis sativus*

Jony Mallik*, Roksana Akhter

Department of Pharmacy, BGC Trust University, Bangladesh

Received 28 Feb 2012; Revised 24 May 2012; Accepted 02 Jun 2012

ABSTRACT

The present study was aimed to investigate phytochemical screening and to evaluate reducing power, cytotoxicity and antifungal activities of the ethanol extracts of peels of *Cucumis sativus* Linn (Cucurbitaceae). The qualitative phytochemical tests showed the presence of alkaloids, glycosides, saponins, flavonoids, steroids and tannins in the ethanol extract of *Cucumis sativus* Linn while gums and reducing sugar were absent in the ethanol extract 3. It was observed that this extracts of the studied plant possessed cytotoxic activity. In brine shrimp lethality bioassay, the LC_{50} (µg/ml) and LC_{90} (µg/ml) of the ethanol extract of were 75µg/ml & LC_{90}: 250µg/ml respectively. Moreover, the extracts showed significant reducing power. It was also identified that the ethanol extracts showed antifungal activity. The clinical fungi that are used in this test are *Aspergillus niger*, *Blastomyces dermatittidis*, *Candida albicans*, *Pityrosporum ovale*, *Trichophyton spp.*,*Microsporum spp*. The obtained results provide a support for the use of this plant in traditional medicine. The plant studied can be a potential source of biologically active compounds as antifungal, anticancer agent and pesticide.

Key words: *Cucumis sativus* , *Aspergillus niger*, *Blastomyces dermatittidis*, *Candida albicans*, *Pityrosporum ovale*, *Trichophyton spp.*, *Microsporum spp.*

INTRODUCTION

Traditional medicine (also known as indigenous or folk medicine) comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as: "the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being" (World Health Organization. 2008-12-01)

The cucumber (Cucumis sativus) is a widely cultivated plant in the gourd family Cucurbitaceae, which includes squash, and in the same genus as the muskmelon. The plant is a creeping vine which bears cylindrical edible fruit. There are three main varieties of cucumber: "slicing", "pickling", and "burpless" **5**.Within these varieties, several different cultivars have emerged. The cucumber is originally from India, but is now grown on most continents. Many different varieties are traded on the global market.

The cucumber is a creeping vine that roots in the ground and grows up trellises or other supporting frames, wrapping around ribbing with thin, spiraling tendrils. The plant has large leaves that form a canopy over the fruit. The fruit of the cucumber is roughly cylindrical, elongated with tapered ends, and may be as large as 60 centimeters (24 in) long and 10 centimeters (3.9 in) in diameter. Having an enclosed seed and developing from a flower, botanically speaking, cucumbers are classified as fruits. However, much like tomatoes and squash they are often perceived, prepared and eaten as vegetables. (www.wikipedia.com)

Fig 1: Flower, Leaves & Fruits of *Cucumis sativus*

***Corresponding Author:** Jony Mallik**, Email:** jonymallik@ymail.com, **Phone No:** +8801830144424

MATERIALS AND METHODS

Plant materials collection

The leaves and stem of *Cucumis sativus* were selected for this project work, which was collected from Pahartolly, Anderkilla (in Chittagong town) and Satkania (in Satkania Thana), Bangladesh in January, 2011 at day time. The plant is available in winter season and the leaves and stems were collected from fresh plants.

Preparation of crude extract by Ethanol

Drying and grinding

The collected plant parts were separated from undesirable materials or plant parts. Then the leaves and the stems were washed and cutted into very small pieces and kept in the open air under shadow for 15 days. Then the plant parts (leaves & stems) were grinded into a coarse 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.

Cold extraction (Ethanol extraction)

In this project work we used the cold extraction process with the help of Ethanol. About 100 gm of powdered materials was taken in a clean, flat bottomed plastic container and soaked in 100ml ethanol. The container with its contents was sealed and kept for a period of 21 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of clean, white cotton materials.

Evaporation of solvent

The filtrate (ethanol extract) obtained was evaporated under ceiling fan and in a tray until dried. It rendered a gummy concentrate of reddish black color and the gummy concentrate was designated as crude extract ethanol.

Phytochemical screening of cucumis sativus

Reagents used for the identification of different phytochemicls

The following reagents were used for the phytochemical screening of *Cucumis sativus* (Trease 1983,Ali 1998, Dev,2002).

Mayer's reagent
Dragendroff's reagent
Fehling's solution A
Fehling's solution B
Benedict's reagent
Molish reagent

Test procedure for the identification of different phytochemicals

The following tests were performed for identifying different chemical groups (Trease 1983, Ali 1998, Dev 2002).

Test for alkaloids

(a) Mayer's test: 2ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added.

(b) Dragendroff's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendroff's reagent was added.

Test for Glycosides

(a) A small amount of an alcoholic extract of the fresh or dried plant material was taken in 1 ml of water. Then a few drops of aqueous sodium hydroxide were added.

(b) A small amount of an alcoholic extract of the plant material was taken in water and alcohol and boiled with Fehling's solution.

(c) Another portion of the extract was dissolved in water and alcohol and boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution and boiled with Fehling's solution.

Test for Steroids

Sulphuric acid test

1 ml solution of chloroform extract was taken and then added 1 ml Sulphuric acid. Red colour indicates the presence of steroid.

Test for gums

5 ml solution of the extract was taken and then Molish reagent & Sulphuric acid were added. Red violet ring were produced at the junction of two liquids indicates in the presence of gums and carbohydrate.

Test for reducing sugar

(a) Benedict's test

0.5 ml of an aqueous extract of the plant material was taken in a test tube. 5 ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously.

(b) Fehling's test (Standard test)

2 ml of an aqueous extract of the plant material was added to 1 ml of a mixture of equal volumes of Fehling's solutions A & B and was boiled for a few minutes.

Test for tannins

(a) Ferric chloride test

5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added.

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate

development of a red colour indicates the presence of Flavonoids.

Test for Saponins

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. One centimeter layer of foam indicates the presence of Saponins.

Investigation of antifungal activity of cucumis sativus

Culture media

Potato dextrose agar (PDA) medium was used to perform the antifungal activity and for subculture of the test organisms.

Table 1: Composition of the Potato dextrose agar (PDA) medium

Potato dextrose agar (PDA) medium (1000 ml)	
Ingredients	**Amount**
Dextrose	20gm
Bacterial agar medium	20gm
Distilled water	1000ml

Preparation of the medium

The weight amount of potato slice was boiled with a little amount of distilled water for 30 minutes and applied for course filtration by the help of cotton. The required amount of dextrose and bacterial agar medium were properly mixed in a conical flask. Finally the constituents of two flasks were mixed thoroughly after the adjustment of volume by the distilled water the medium was sterilized in an autoclave.

Placement of discs, diffusion and incubation

Preparation of the test plates, preparation of the discs, preparation of the test sample, placement of the discs diffusion and incubation. Preparation of the test plates, disc, test sample, placement of the discs, diffusion and incubation process were almost same of the antibacterial activity screening. Here, only the incubation period was replaced by 72 hours at 25°c temperature.

Preparation of 1000µg/ml, 500µg/ml, 250µg/ml, 125 µg/ml, 50µg/ml,

25 µg/ml, sample and standard

Preparation of sample

0.1 gm of sample was taken in 100 ml volumetric flasks and adjusted the volume up to 100 ml with water. Then 1000 µg/ml, 500 µg/ml, 250µg/ml, 125 µg/ml, 50 µg/ml, 25 µg/ml, of sample was made consequently.

Preparation of standard

0.1 gm of ascorbic acid was taken in 100 ml volumetric flasks and the other part was filled with distilled water. Then preparation of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 50 µg/ml, 25 µg/ml, of standard solution as per procedure of sample.

***In-vitro* Cytotoxicity Bioassay**

Brine shrimp lethality bioassay is a recent development in the bioassay for the bioactive compounds. In this method natural product extracts and pure compounds can be tested for their bioactivity. Bioactive compounds are almost toxic in high doses. Pharmacology is simple toxicology at a lower dose, or toxicology is simply pharmacology at a higher dose. Thus, in-vivo lethality of a simple zoologic organism can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products (Hui et a.l, 1990). Here the simple zoologic organism is brine shrimp nauplii. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as anti microbial, pesticidal, antitumor etc. activity of the compounds (Meyer, 1982; Mclaughlin, 1988.)

The brine shrimp bioassay has several advantages such as rapid in process (24 hours), inexpensive, and simple (no aseptic technique is required). It easily utilizes a large number of organisms for statistical validation and requires no special equipment and relatively small amount of sample is sufficient. Furthermore, it does not require animal serum as it is needed for determination of cytotoxicities.

Preparation of sea water (brine)

19g NaCl(with iodine) and 19 g NaCl(without iodine) salt were dissolved in 1000 ml distilled water and then filtered to prepare sea water.

Hatching of Brine shrimp

Sea water was taken in the small tank and shrimp eggs were added to the one side of the divided tank and the side was covered. The shrimps were allowed for 36 hrs to hatch and mature as nauplii. During this period constant oxygen supply and temperature (around 37°c) was maintained. The hatched shrimps were attracted to the lamp through the perforations in the dam and they were taken for bioassay.

***In-vitro* brine shrimp lethality bioassay**

In this bioassay, the crude extract showed lethality indicating the biological activity as the test sample showed different mortality rates at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase order of concentrations and plot of percent mortality versus the concentration of crude extract used on the graph paper produced an approximate linear correlation between them. From the graph the concentration at which 50% & 90% mortality

(LC_{50} & LC_{90}) of brine shrimp nauplii occurred were obtained by extrapolation.

RESULTS & DISCUSSION

Preliminary phytochemical analysis

Phytochemical study showed that alkaloid, glycoside, saponins, steroid and tannins were present and gums, flavonoid and reducing sugar are absent in the extract of cucumis sativus. (**Table 2)** showed the results of the qualitative analysis of the ethanol extract of cucumis sativus Linn.

Table 2: Results of phytochemical analysis of the ethanol extracts of cucumis sativus Linn.

Extracts of *cucumis sativus*	Alkaloid	Glycoside	Steroid	Gum	Flavonoid	Saponin	Reducing sugars	Tannin
Ethanol extract	+	+	+	--	--	+	--	+

+ = presence and – = absence

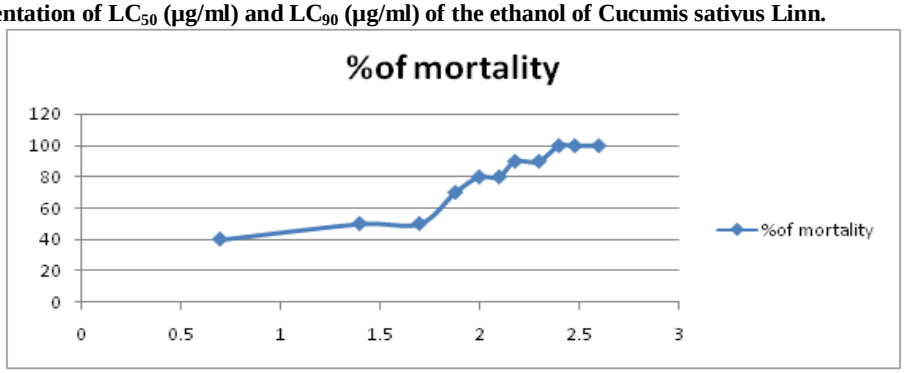
Cytotoxic activity

In brine shrimp lethality bioassay, the Ethanol extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations **(Table 3).** From the plot of percent mortality versus log concentration on the graph paper **(Fig 2)**, LC_{50} (µg/ml) and LC_{90} (µg/ml) of the ethanol extract of *Cucumis sativus* Linn. were deduced respectively.

Table 3: Brine shrimp lethality bioassay of ethanol extract of cucumis sativus Linn.

Conc. (µg/µl)	Log (Conc.)	No. of alive shrimp	%of mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
5	0.70	6	40		
25	1.40	5	50		
50	1.70	5	50		
75	1.88	3	70		
100	2.00	2	80		
125	2.10	2	80	75	250
150	2.18	1	90		
200	2.30	1	90		
250	2.40	0	100		
300	2.48	0	100		
400	2.60	0	100		
Blank	0.00	8	20		

Fig 2: Graphical presentation of LC_{50} (µg/ml) and LC_{90} (µg/ml) of the ethanol of Cucumis sativus Linn.



Antifungal activity

(Table 4) showed the results of antifungal test. The antifungal potentials of the ethanol extract of cucumis sativus Linn. (30µg/disc) were assessed against six fungus. The results (diameter of zone of inhibition) were compared with the activity of the standard drug, Griseofulvin (30µg/disc). At 80µg/disc, the ethanol extract of *Cucumis sativus* Linn.

Table 4: Anti-fungal activity of the ethanol extract of *Cucumis sativus* Linn., standard and blank

Tested Fungi	Zone of inhibition (mm) by the crude ethanol extract of *Cucumis sativus* (80µg/disc)	Standard drug, Griseofulvin (30µg/disc)	Blank
Aspergillus niger	3.45	5.5	-
Blastomyces dermatitides	2.15	3.25	-
Candida albicans	1.75	3.95	-
Pityrosporum ovale	2.25	4.5	-
Trichophyton spp	2	3.9	-
Microsporum spp	1.5	4	-

Reducing power

(Table 5) represents the reductive capabilities of the plant extracts compared to ascorbic acid. An increase in absorbance in the reducing power method implies that both of the crude extracts are capable of donating hydrogen atoms in a dose dependent manner.

Table 5: The absorbance of ethanol extract with standard ascorbic acid at 700nm

Concentration(µg/ml)	Standard	Ethanol extract
25	0.16	0.017
50	0.21	0.018
125	0.33	0.020
250	0.40	0.023
500	0.55	0.028
1000	0.70	0.026

DISCUSSION

Cucumis sativus (Family: Cucurbitaceae) is a medicinal plant which has many therapeutic effect. The whole plants were collected and the leaves were separated, washed, dried, crushed and then socked with ethanol. After some days the solvent was evaporated and concentrated extract were collected. Phytochemical study showed that alkaloid, glycoside, saponins, steroid, and tannins were present and gums ,flavonoid and reducing sugar are absent in the extract of *Cucumis sativus* . The extracts have less antifungul effect in compare with standard. Cytotoxic activity was tested found that the extract has moderate cytotoxic effect on the brine shrimp nauplii. The extracts have antioxidant effect in compare with standard.

CONCLUSION

From the present study, it was observed that ethanol extracts of the peel possess cytotoxic and antifungal activities. The plant extracts also showed reducing power. It was also observed that extracts of the studied plant possessed cytotoxic activity. In brine shrimp lethality bioassay, the LC_{50} (µg/ml) and LC_{90} (µg/ml) of the ethanol extract of *Cucumis sativus* Linn. were 75µg/ml& LC_{90}: 250µg/ml respectively Further investigation is required to isolate pure compounds for establishing its mechanism of action.

ACKNOWLEDGEMENT

The authors are grateful to the Bangladesh Council of Scientific & Industrial Research (BCSIR) for providing the laboratory support.

REFERENCES

1. Abdul Ghoni, 2003, Medicinal plants of Bangladesh, second edition, PP- 1-40.
2. Abdul Ghoni, 1990, Traditional Medicine, PP- 14-40.
3. Aboyami Sofoqara, 1982, Medicinal plant and traditional medicine in African trop, Vol. 55, No. 3: 225-229, doi: 10.1089.
4. Bauer A. W., Kirby WM, Sherries J.C. Truck, 1966, Antibiotic susceptible testing by standard drug Borges by single disc method, 45(4):493-6. PMID: 5325707.
5. B. Rossi-Bergmann, S. S. Costa , M. B. S. Borges , S. A. da Silva , G. R. Noleto , M. L. M. Souza , V. L. G. Moraes Phytotherapy Research,Volume 8 Issue 7, Pages 399 – 402, Doi: 10.1002/ptr.2650080704.
6. Dev S. 2002, Phytochemical and pharmacological studies on Albizzia lebbeck Benth, Journal of Ethnopharmacology, Volume 125, Issue 2, 7 September 2009, Pages 356-360, Doi:10.1016/j.jep.2009.02.041.
7. Farnsworth NR, Akerele O, Bingol AS, Guo ZA, (Medicinal plants in therapy), Cellular and Molecular Life Sciences, IssueVolume 46, Number 3 / March, 1990 Category Research Articles DOI10.1007/BF01951779 Pages324-327.
8. Gaurav Vijay Harlalka, Chandragauda Raosaheb Patil, Mahesh Ramu Patil, Indian journal of pharmacology, Year: 2007, Volume: 39, Issue: 4, P: 201-205, DOI: 10.4103/0253-7613.36540.
9. Hui YH, Chang KJ, Smith Dl, MacLaughin JL, 1990, 16α-hydroxy (-) kauronic acid: a selectively cytotoxic diterpine from Annona Bullata Pharm, Pharmaceutical Research, ISSN0724-8741 (Print) 1573-904X (Online) Issue Volume 7, Number 4 / April, 1990 DOI10.1023/A:1015819422479 P-376-378.
10. K. N. Gaind, R. L. Gupta, Georg Thieme Verlag Stuttgart, planta Med 1974; PP-193-197, 149-153, 368-373, DOI: 10.1055/s-0028-1097931.
11. Li-Song Chen, Qin Lin and Akihiro Nose, JournalBiologia Plantarum, ISSN0006-3134 (Print) 1573-8264 (Online) IssueVolume 52, 2008, DOI10.1007/s10535-008-0008-5 Pages59-65.
12. Mayaral M. L. and Medina E, 1985 14C translocation in Kalanchoe pinnata at two different stages of development PP- 1405-1413.
13. Michelle F. Muzitano, Luzineide W. Tinoco, Catherine Guette, Carlos R. Kaiser, Bartira Rossi-Bergmann and Sônia S. Costa, Phytochemistry Volume 67, Issue 18, September 2006, Pages 2071-

2077, doi:10.1016/j.phytochem.2006.06.027.

14. Q. Lin, Y. M. Wang, S. Agarie, H. T. K. hong, Journal Biologia Plantarum, 2008, ISSN 0006-3134 (Print) 1573-8264 (Online), Volume 52, P- 59-65 8DOI10.1007/s10535-008-0008-5.
15. Unang Supratman and Tomoyuki Fujita, Kohki Akiyama and Hideo Hayashi, Phytochemistry Volume 58, Issue 2, September 2001, P- 311-314, doi:10.1016/S0031-9422(01)00199-6.