

## ANTIMICROBIAL AND CYTOTOXIC ACTIVITY OF ETHYL ACETATE, CHLOROFORM AND N-HEXANE EXTRACTS OF CUCUMIS SATIVUS LEAVES.

### ABSTRACT

Plants are the important source of diverse range of bioactive principles. A large number of plants are constantly being screened for their possible medicinal value. The present study was designed to investigate the antimicrobial and cytotoxic activity of ethyl acetate (EAE), chloroform (CE) and n-hexane (HE) extracts of leaves of *Cucumis sativus*. Antimicrobial activity of EAE, CE and HE was tested against eleven important pathogenic bacteria including both gram positive and gram negative bacteria and three fungi by disc diffusion technique. Here kanamycin disc (30µg /disc) was used as standard for antibacterial study. Cytotoxicity test of EAE, CE and HE was studied by brine shrimp lethality bioassay and compare with LC<sub>50</sub> values of standard vincristin sulphate as a positive control. The tested extracts (EAE, CE and HE) showed almost same antimicrobial potentiality against most of the bacterial strains with an average zone of inhibition of 9-20mm. These extracts also showed moderate to good antifungal activity with an average 9 -16 mm zone of inhibition. The results of cytotoxic study of these, illustrated significant potentiality against *A. salina*, with LC<sub>50</sub> 7.17µg/ml, 12.29µg/ml and 15.03µg/ml for EAE, CE and HE, respectively. LC<sub>50</sub> value of 2.32µg/ml was found for vincristin sulphate. Further investigations are, however, necessary to explore mechanism(s) of action involved in these pharmacological activities

**Keywords:** *Cucumis sativus* , antimicrobial activity, zone of inhibition, LC<sub>50</sub>

### INTRODUCTION

*Cucumis sativus* Linn. (Family: Cucurbitaceae) is an annual, rather coarse, fleshy, prostrate or climbing vine widely distributed all over the world particularly in Asia, Africa and South America<sup>1</sup>. Traditionally, this plant is used for headaches; the seeds used as cooling and diuretic, the fruit juice is used as a nutritive and as a demulcent in anti-acne lotions; Juice of leaves used as an emetic in acute indigestion in children<sup>5</sup>. Several investigations revealed antidiabetic<sup>2</sup>, antiulcer<sup>3</sup>, moisturizing<sup>4</sup>, antioxidant and analgesic property<sup>5</sup> of the fruit extracts. The seed extracts were found efficient on controlling weight loss of diabetic rats<sup>1</sup> and against tapeworms<sup>6</sup>. Leaves and stems extract have been reported for cytotoxic, antifungal<sup>11</sup> and antibacterial activity<sup>7</sup>. Identified phytochemicals from its leaves and seeds were 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<sup>10</sup>, cucurbitasides B, C and ferredoxin<sup>8,9</sup> and α- and β-amyrin, sitosterols and cucurbitasides<sup>10</sup>. The aim of the present study was to explore antibacterial, antifungal and cytotoxic potentiality of different solvent extract of leaves of *Cucumis sativus*

### EXPERIMENTAL

#### Plant material

The leaves of plant *Cucumis sativus* were collected from Gazipur in the month of March, 2012 and authenticated at Bangladesh National Herbarium, where a voucher specimen no-34479 has been deposited.

#### Preparation of the extracts

The leaves were first washed with water to remove adhering dirt and then dried at 45°C for 36 h in an electric oven, then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The dried powdered material (1kg) was taken in a clean, flat bottomed glass container and soaked in methanol for seven days. The whole mixture then underwent a coarse filtration by a piece of

clean, white cotton material. The extracts were filtered and concentrated under vacuum to obtain a crude methanol extract (ME) of leaves. The extract was fractionated by the modified Kupchan partitioning method<sup>11</sup> into ethyl acetate, chloroform and n-hexane. The fractionated extracts were concentrated under vacuum to obtain solid ethyl acetate (EAE) chloroform (CE) and n-hexane (HE) extract.

## Drugs and chemicals

The active drugs Loperamide, Atropine sulphate were the generous gift samples from Square Pharmaceuticals Ltd., Bangladesh. Tween-80 and DMSO was obtained from BDH Chemicals, UK. Castor oil, acacia, was purchased from CDH, India. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. All chemicals used were of analytical reagent grade.

## Antimicrobial assay

### Microorganisms

Antimicrobial activity was tested against *B. megaterium*, *B. subtilis*, *Staphylococcus aureus*, *Sarcina lutea*, *Escherichia coli*, *Salmonella paratyphi*, *S. typhi*, *Shigella boydii*, *S. dysenteriae*, *Vibrio mimicus* and *V. parahemolyticus*, *Saccharomyces cerevaceae*, *Candida albicans* and *Aspergillus niger*. These microbial strains were isolated from clinical samples and obtained as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

### Determination of the diameters of inhibition zone

The leaves crude extracts were tested in vitro for antimicrobial activity by the standard disc diffusion method<sup>12</sup> against the selected bacteria and fungi. 50 mg of the samples was dissolved in 1 ml of solvents to prepare a concentration of 500µg/ 10µl of the test samples. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances (500µg/ disc) using micropipette and the enduring solvents was allowed to evaporate completely. These Discs (containing the test materials) were placed on to nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4°C) for 16 hours to allow maximum diffusion of the test materials and kanamycin after that were incubated at 37°C for 12 hours to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms by exerting a clear, distinct zone of inhibition surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The experiment was carried out in triplicate and the mean value was taken.

## Cytotoxicity screening

Brine shrimp lethality bioassay is a very convenient method to determine cytotoxic activity of extracts<sup>13,14</sup>. This method detects the lethality of crude extracts on *Artemia salina*, used as a convenient monitor for the screening. The eggs of brine shrimp (*A. salina*) were hatched in a tank in artificial seawater (3.8% NaCl solution) at a temperature around 37°C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. For the experiment, the test samples (extracts) were prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) and sea water to attain concentrations of 20, 40, 60, 80 and 100 µg/ml. A vial containing 50 µl DMSO diluted to 5ml was used as a control. Standard vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. Each test tube contained about 5 ml of seawater and 10 shrimp nauplii. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. The rate of mortality of nauplii was found to be increased in concentration of each of the samples. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

### Lethality concentration determination

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. Lethal median dose of the extracts ( $LC_{50}$ ) were calculated from the best-fit line plotted concentration verses percentage lethality.

### Statistical analysis

The data obtained in the animal experiments was subjected to statistical analysis. All values are expressed as Mean  $\pm$  S.E.M (Standard Error of Mean).

## RESULTS

### Antimicrobial activity

The different solvent extracts of *C. stivus* leaves were screened against eleven human pathogenic bacteria to check antibacterial activities by disc diffusion method. The extracts showed variable zone of inhibition against tested pathogenic bacteria which was shown in table 1.

All the extracts showed significant antimicrobial activity. EAE showed very good antibacterial activity against all tested pathogenic bacteria. The maximum zone of inhibition exhibited against *Sarcina lutea* ( $17.9 \pm 0.31$  mm). The minimum zone of inhibition showed around 11 mm against *Salmonella paratyphii* and *Pseudomonas aeruginosa*.

CE showed moderate to good antibacterial activity against all the tested pathogenic bacteria with an average zone of inhibition of 9-17 mm. This extract exhibited very good antimicrobial activity against *Staphylococcus aureus* ( $17.1 \pm 0.39$  mm) and lowest activity against *S. paratyphii* ( $8.5 \pm 0.6$  mm)

HE showed no activity against *S. dysenteria* and *P. aeruginosa*. The maximum zone of inhibition exhibited against *Staphylococcus aureus* ( $20.21 \pm 0.21$  mm). The minimum zone of inhibition showed  $11.4 \pm 0.3$  mm against *Shigella boydii*.

All the extracts showed moderate to good activity against the fungus. The exhibited diameter of zone of inhibitions were  $12.1 \pm 0.8$  mm,  $8.2 \pm 0.7$  mm,  $13 \pm 0.3$  mm against *Aspergillus niger*,  $15.7 \pm 0.3$  mm,  $13.5 \pm 0.4$  mm,  $12 \pm 0.7$  mm against *Sacharomyces cerevaceae* and  $9 \pm 0.4$  mm,  $13.6 \pm 0.3$  mm,  $11 \pm 0.5$  mm against *Candida albicans* for EAE, CE and HE extracts, respectively.

### Cytotoxic activity

The results of the different extracts of *C. stivus* (% mortality at different concentrations and  $LC_{50}$  values) were shown in Fig. 1. The percent mortality increased with an increase in concentration. The crude EAE, CE, HE extract significant cytotoxicity with  $LC_{50}$  values of  $7.17 \mu\text{g/ml}$ ,  $12.29 \mu\text{g/ml}$  and  $15.03 \mu\text{g/ml}$ , respectively.  $LC_{50}$  value of  $2.32 \mu\text{g/ml}$  was found for vincristine sulphate.

## DISCUSSION

Present study was conducted to elucidate antimicrobial and cytotoxic activity of different solvent extract of *C. sativus* leaves. Bacteria are becoming increasingly resistant to conventional antibiotics<sup>15</sup> and resistance is emerging at alarming rate. Thus the alarming increase in resistance to antibacterial has created desperate need for the search of new antibacterial<sup>16,17</sup>. The disc diffusion method had shown that all the three solvent extracts of the leaves of *C. sativus* was found potential against the tested organisms. No significant difference was found for the activity of these extracts to gram positive and gram negative bacteria, which indicated the presence of broad

spectrum antibiotic compounds. These extracts also showed same potentiality against tested fungus which revealed presence of potent antimicrobial constituents in all the three solvent extracts.

Brine shrimp lethality bioassay is the assay procedure of bioactive compounds, which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal, AIDS, etc.) of the compounds<sup>18</sup>. The assay is considered as a useful tool for preliminary assessment of toxicity<sup>19</sup> and it has been used for the detection of fungal toxins<sup>20</sup>, plant extract toxin<sup>21</sup>, heavy metal toxin<sup>22</sup>, cyanobacterial toxins<sup>23</sup>, pesticides<sup>24</sup> and cytotoxicity testing of dental materials<sup>25</sup>. All the extracts exhibited a remarkable cytotoxic effect. The inhibitory effect of the extract might be due to the toxic compounds present in the active fraction that possess ovicidal and larvicidal properties. The metabolites either affected the embryonic development or slay the eggs<sup>26</sup>. Among them ethanolic extract showed highest cytotoxic activity ( $LC_{50} = 7.17 \mu\text{g/ml}$ ) than chloroform and n-hexane extract which attributed effect of polar fractions on exhibited cytotoxicity of extracts. The cytotoxic effects of the plant extracts enunciate that it can be selected for further cell line assay because there is a correlation between cytotoxicity and activity against the brine shrimp nauplii using extracts.

## CONCLUSION

Based on the results of the present study, we conclude that Ethanolic, chloroform and n-hexane extracts of *Cucumis sativus* leaves showed significant antibacterial, antifungal and cytotoxic activity. However, further studies are indispensable to examine underlying mechanisms of such pharmacological effects and to isolate the active compounds responsible for these pharmacological activities.

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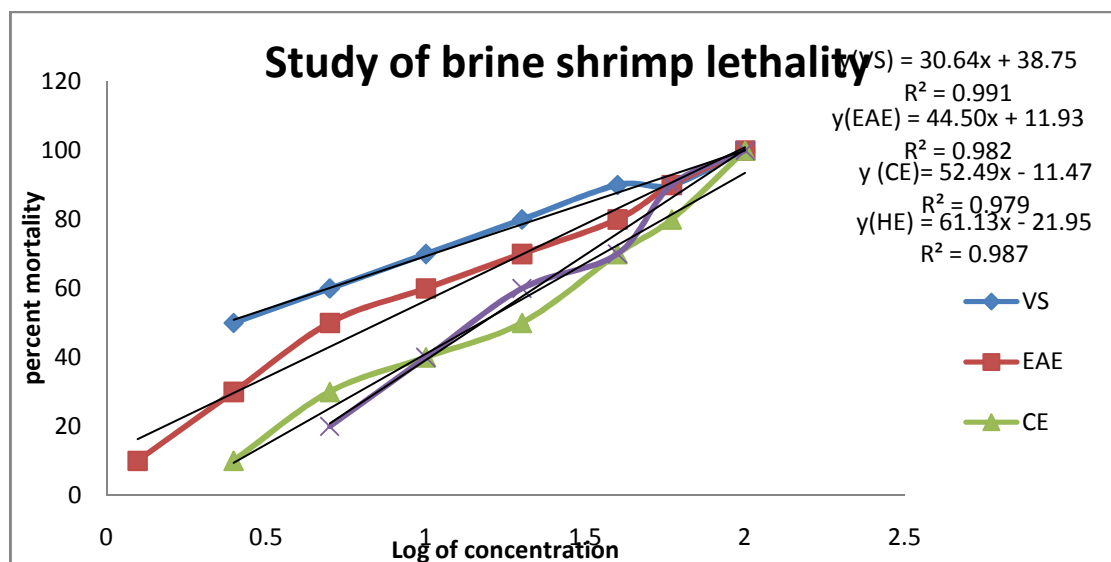
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	Diameter of zone of inhibition (in mm)			
Test organisms	Ethyl acetate extract (500µg/disc)	chloroform extract (500µg/disc)	N-hexane extract (500µg/disc)	Kanamycin (30µg/disc)
<b>Gram (+)ve bacteria</b>				
Bacillus subtilis	15.14±0.86	14.23±0.57	15.67±0.33	32
Bacillus cereus	13.92±0.38	15.55±0.45	17±1.09	26
Bacillus megaterium	11.77±0.23	14.9±.87	15.5±0.58	28
Sercina lutia	17.9±0.31	16.00±0.69	13.33±0.73	35
Staphylococcus aureus	12±0.11	17.1±0.39	20.21±0.21	30
<b>Gram (-)ve bacteria</b>				
E.coli	15.6±0.57	16.9±0.12	17.43±0.12	35
Vibrio mimicus	14±00	11.22±0.78	14±1.2	30
Vibrio parahemolyticus	13.5±0.47	14.1±0.77	11.5±0.43	32
Salmonella paratyphii	11±0.3	08.5±0.6	17±0.2	33
Shigella bodii	12.8±0.2	13.67±0.34	11.4±0.3	27
Shigella dysenteria	13.6±0.4	12.56±0.	-	26
Pseudomonus aeruginosa	10±1.1	14.7±0.2	-	26
<b>Fungus</b>				
Aspergillus niger	12.1±0.8	8.2±0.7	13±0.3	30
Candida albicans	9±0.4	13.6±0.3	11±0.5	26
sacharomyces cerevaceae	15.7±0.3	13.5±0.4	12±0.7	31

**Table 1: In vitro antimicrobial activity of different extracts of Cucumis sativus Leaves.**

Results are presented as mean  $\pm$  SEM, (n=3)



**Fig 1: Determination of  $LC_{50}$  values for standard and crude ethyl acetate, chloroform and n-hexane extracts of *Cucumis sativus* leaves from linear correlation between logarithms of concentration versus percentage of mortality**

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