Antibacterial, Cytotoxic and Antioxidant Activities of n-Hexane, Chloroform and Ethyl Acetate extracts of *Cucumis sativus* leaves.

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Date Received: 11-Mar-2014  
Date of Accepted: 25-Mar-2014  
Date Published: 08-Apr-2014

Abstract:
The main aim of this study was to find out the antibacterial, cytotoxic and antioxidant activities of n-hexane, chloroform and ethyl acetate extracts of *C. sativus* (Cucurbitaceae). Disc diffusion technique was used for *in vitro* antibacterial screening against gram positive, gram negative human pathogenic bacteria. Here kanamycin disc (30 µg/disc) was used as standard. The chloroform and the n-hexane extract of *C. sativus* showed moderate antibacterial activity with the average zone of inhibition 7-12 mm and 7-9 mm respectively. The brine shrimp lethality bioassay method was used to determine the cytotoxic activity and vincristine sulphate was used as positive control. Among the extractives the chloroform soluble fraction demonstrated the highest cytotoxic activity with LC₅₀ 16.89 µg/ml which indicates the compounds present in the chloroform extract are promisingly cytotoxic. Antioxidant activity test of the crude extracts were assessed by means of DPPH free radical scavenging method where ascorbic acid was used as standard. The chloroform fraction of *C. sativus* showed strongest antioxidant activity with IC₅₀ value of 54.06 µg/ml. In case of phenolic content, the n-hexane, chloroform and ethyl acetate extracts of *C. sativus* revealed 16.08 mg/g, 19.04 mg/g and 46.01 mg/g of extractives, respectively.

Keywords:  
*C. sativus*, antibacterial, antioxidant, cytotoxic.

Introduction

Cucumber (*Cucumis sativus*) is a widely cultivated plant in the gourd family Cucurbitaceae. It is a creeping vine that bears cylindrical fruits that are used as culinary vegetables. *C. sativus* have been evaluated for a wide spectrum of activity including diuretic [1], antihyperglycemic [2], antioxidant [3], amylolytic [4], anticancer [5] and analgesic [6] using various *in vitro* and *in vivo* models. The leaf juice is emetic; it is used to treat dyspepsia in children [7]. Cucumber fruit is composed mostly of water; more than 96% of edible unpeeled fruit is water [8]. Other constituents of *C. sativus* L are vitamins, minerals, amino acids, phytosterols, phenolic acids, fatty acids, and cucurbitacins [9]. According to another source, traces of essential oil, amino acids, pectins, starch, sugars, vitamin C, and cucurbita-cin are found in cucumbers [10]. Glycosides, steroids, flavonoids, carbohydrates, terpenoids, and tannins were identified in an aqueous extract of the cucumber fruit [11]. Liquid chromatography–mass spectrometry that incorporated ¹³C₃-labelled standards determined that cucumber contained 12-13 µg phytoestrogens/100 g wet wt cucumber [12]. In the breakdown of the phytoestrogen composition, the content was primarily the lignan secoisolariciresinol; the lignan matairesinol, the isoflavones daidzein, genistein, glycine, biochanin A and for-mononetin, and coumestrol, comprised <1 µg/100 g wet wt of the fruit. Another source reports the following phytosterols in cucumber fruit: 3800 µg β-sitosterol/100 g edible portion, 200 µg campesterol/100 g edible portion, 2900 µg stigmasterol/100 g edible portion, 100 µg campestanol/100 g edible portion, and 100 µg campestanol/100 g edible portion, giving a total plant sterol content of 7300 µg/100 g edible portion [13]. The major fatty acids in cucumbers are palmitic acid (23.6-27.5%), linoleic acid (22.7-26.3%) and linolenic acid (40-46%) [14]; [15].
The objective of the present study was to investigate the antibacterial, cytotoxic and antioxidant activity of the different fractions of \( C. \text{ sativus} \). Therefore, systematic research with medicinal plants may open the door of many therapeutic choices.

**Materials and methods**

**Plant material**
The leaves of the plant \( C. \text{ sativus} \) were collected during the month of July 2010 from the area of Moynertak, Tongi, Dhaka.

**Plant materials extraction and fractionation**
The fresh leaf was collected, sun dried for seven days and ground. The dried powder of \( C. \text{ sativus} \) leaf (200 gm) was soaked in 600 ml of ethanol for 7 days and filtered through a cotton plug followed by Whatman filter paper number 1. The concentrated ethanolic extract of leaf was fractionated by the modified Kupchan partitioning method [16] into n-hexane, chloroform and ethyl acetate. The subsequent evaporation of solvents afforded n-hexane (450 mg), chloroform (700 mg) and ethyl acetate (350 mg) from leaf extract.

**Antibacterial assay**
In our present study, the antibacterial activity of n-hexane, chloroform and ethyl acetate fractions of the plant were investigated in comparison with standard kanamycin (30 µg/disc) against a number of pathogenic Gram-positive (\( Bacillus \text{ megaterium}, Bacillus \text{ subtilis}, Staphylococcus \text{ aureus} \) and \( Sarcina \text{ lutea} \)) and Gram-negative (\( Salmonella \text{ paratyphi}, S. \text{ typhi}, Vibrio \text{ parahaemolyticus}, V. \text{ mimicus}, Escherichia \text{ coli}, Shigella \text{ dysenteriae}, S. \text{ boydii} \) and \( Pseudomonas \text{ aeruginosa} \)) bacteria. The microorganisms were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The sample solution of the material to be tested was prepared by dissolving a definite amount of material in methanol to attain a concentration of 20, 40, 60, 80 and 100 µg/ml. With the help of a pasteur pipette nauplii were exposed to different concentrations of the extracts.

**DPPH radical scavenging activity**
Antioxidant activity of n-hexane, chloroform and ethyl acetate of leaf extracts of \( C. \text{ sativus} \) was determined on the basis of their scavenging potential of the stable DPPH free radical in both qualitative and quantitative assay.

**Qualitative analysis**
A suitably diluted stock solutions were spotted on precoated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extracts. The plates were dried at room temperature and were sprayed with 0.02% DPPH in methanol. Bleaching of DPPH by the resolved band was observed for 10 minutes and the color changes (yellow on purple background) were noted [19].

**Quantitative analysis**
The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams et al., (1995) [20]. During this experiment the test samples of n-hexane, chloroform and ethyl acetate extracts of \( C. \text{ sativus} \) at different concentrations were mixed with 3.0 ml of DPPH methanol solution. The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extracts as compared to that of ascorbic acid by UV spectrophotometer (UV–1501PC SHIMADZU, Japan) at 517 nm. Ascorbic acid was used as a positive control. Percent scavenging of the DPPH free radical was measured using the following equation-

\[
\% \text{ DPPH radical scavenging} = \left[ 1 - \frac{\text{As}}{\text{Ac}} \right] \times 100
\]

Here, \( \text{Ac} \) = absorbance of control, \( \text{As} \) = absorbance of sample solution.

Then % inhibitions were plotted against respective concentrations used and from the graph \( IC_{50} \) was calculated. The lower \( IC_{50} \) indicates higher radical scavenging activity and vice versa.

**Assay for Total Phenolics**
Total phenolic content of different parts of \( C. \text{ sativus} \) extracts was measured employing the method as described by Skerget et al., 2005 [21] involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard [22]. 0.5 ml of diluted plant extract and standard of different concentrations solution were taken in the test tube followed by adding 2.5 ml of Folin–
Ciacaltec (Diluted 10 fold with water) & 2 ml of Sodium carbonate (1 M) respectively. Solutions were then incubated for 20 minutes at 45ºC in the water bath. The absorbance was measured colorimetrically at 760 nm to determine the total phenol contents by using standard curve prepared from gallic acid solution with different concentration.

Results and discussion

Antibacterial activity
Different extractives of C. sativus were screened against human pathogenic organisms to evaluate antibacterial activities by disc diffusion method. The chloroform fraction possesses the zone of inhibition value ranged from 7-12 mm (Table: 1). Among different fractions tested, chloroform fraction of the plant exhibited moderate inhibitory activity followed by n-hexane fraction (7-9 mm) whereas ethyl acetate fraction showed little or no activity on the tested microorganisms. The most sensitivity was observed in S. paratyphi (12 mm) and V. parahaemolyticus (11 mm) by chloroform fraction of the plant.

Cytotoxicity screening
LC50 value of chloroform, n-hexane and ethyl acetate fractions found with the value of 16.89 µg/ml, 27.71 µg/ml and 51.52 µg/ml respectively in comparison with vincristine sulphate as standard whose LC50 value 8.844 µg/ml. Among them chloroform fraction of the plant exhibited the potent cytotoxic activity (Fig: 1).

Table 1: In vitro antibacterial activity of the extracts of C. sativus (leaves) and kanamycin discs

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Chloroform extract (500µg/disc)</th>
<th>Ethyl acetate extract (500µg/disc)</th>
<th>N-Hexane extract (500µg/disc)</th>
<th>Kanamycin(30µg/ disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. subtilis</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>B.megaterium</td>
<td>9</td>
<td>0</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>S.aureus</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>S. lutea</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>Gram negative bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. mimicus</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>29</td>
</tr>
<tr>
<td>S. boydii</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>28</td>
</tr>
<tr>
<td>P.aeruginosa</td>
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<td>0</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>S.typhi</td>
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<td>8</td>
<td>24</td>
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<tr>
<td>S.paratyphi</td>
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<td>0</td>
<td>8</td>
<td>31</td>
</tr>
<tr>
<td>V.parahemolyticus</td>
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<td>8</td>
<td>30</td>
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<tr>
<td>S.dysenteriae</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>E.coli</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
</tbody>
</table>
Figure 1: Determination of LC$_{50}$ values for standard and crude chloroform, n-hexane, and ethyl acetate extracts of $C. sativus$ from linear correlation between logarithms of concentration versus percentage of mortality.

Figure 2: Determination of IC$_{50}$ value for standard and crude chloroform, N-hexane and ethyl acetate extracts of leaves of $C. sativus$ from linear correlation between concentrations ($\mu$g/ml) versus percentage of scavenging of DPPH.

References:


