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

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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* anbacterial 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 LC_{50} 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 IC_{50} 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], antihypergycemic [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 curcurbita-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 $^{13}C_3$-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, glycitein, 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, 300 µg *β*-sitostanol/ 100g 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. 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. 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. 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 megaterium, Bacillus subtilis, Staphylococcus aureus and Sarcina lutea)* and Gram-negative *(Salmonella paratyphi, S. typhi, Vibrio parahaemolyticus, V. mimicus, Escherichia coli, Shigella dysenteriae, S. boydii* and *Pseudomonas 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 50 mg/ml. 10 µl of such solution was applied on sterile disc (5 mm diameter, filter paper) and allowed to dry off the solvent in an aseptic hood. Thus, such discs contain 500 µg of crude extracts. To compare the activity with standard antibiotics, Kanamycin (30 µg/disc) was used.

Cytotoxicity Screening

Brine shrimp Lethality Bioassay

Brine shrimp lethality bioassay [17]; [18] was used for probable cytotoxic activity. The eggs of Brine Shrimp (*Artemia salina*) was collected from local pet shops and hatched in a tank at a temperature around 37 ºC with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solution of the sample was prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO) to attain concentrations 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. 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. 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-

% DPPH radical scavenging = [1 – (As/ Ac)] × 100

Here, Ac = absorbance of control, 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. sativus* extractives 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–

ciocalteu (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

LC_{50} 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 LC_{50} value 8.844 µg/ml. Among them chloroform fraction of the plant exhibited the potent cytotoxic activity (Fig: 1).

DPPH radical scavenging activity

Qualitative assay

The color changes (yellow on purple background) on the TLC plates were observed due to the bleaching of DPPH by the resolved bands.

Quantitative assay

n-hexane, chloroform, ethyl acetate extracts of the plant showed significant antioxidant activity with the IC_{50} value of 75.62 µg/ml, 54.06 µg/ml, 67.07 µg/ ml respectively compared with the standard ascorbic acid with IC_{50} value of 45.47 µg/ml (Fig: 2), the fractions exhibited a concentration dependant DPPH radical scavenging activity.

Total phenolic content

The phenolic content of plant fractions was determined using the Folin–Ciocalteu assay and was expressed as gallic acid equivalents (GAE). The phenolic contents of n-Hexane, chloroform and ethyl acetate soluble fractions of *C. sativus* were 16.08 mg/g, 19.04 mg/g and 46.01 mg/g of the dry weight.

Conclusion

The present study indicates that the n-hexane, chloroform and ethyl acetate extracts of the different fractions of *C. sativus* exhibited mild to moderate antibacterial, profound antioxidant, total phenolic content and cytotoxic activities. The chloroform extract of the plant showed moderate antibacterial activity. So, the studied plant may have clinical and therapeutic proposition in the most life threaten diseases like tumor or cancer, various infectious diseases and the aging process of human being. Therefore, further investigation should be necessary for the development of novel lead compound.

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

Test organism	Diameter of zone of inhibition (mm)			
	Chloroform extract (500µg/disc)	Ethyl acetate extract (500µg/disc)	N-Hexane extract (500µg/disc)	Kanamycin(30µg/ disc)
Gram positive bacteria				
B. subtilis	**8**	**0**	**8**	**25**
B.megaterium	**9**	**0**	**8**	**23**
S.aureus	**7**	**0**	**7**	**21**
S. lutea	**0**	**0**	**7**	**19**
Gram negative bacteria				
V. mimicus	**8**	**6**	**9**	**29**
S. boydii	**8**	**7**	**8**	**28**
P.aeruginosa	**8**	**0**	**8**	**25**
S.typhi	**7**	**0**	**8**	**24**
S.paratyphi	**12**	**0**	**8**	**31**
V.parahemolyticus	**11**	**0**	**8**	**30**
S.dysenteriae	**7**	**0**	**7**	**11**
E.coli	**7**	**0**	**0**	**15**

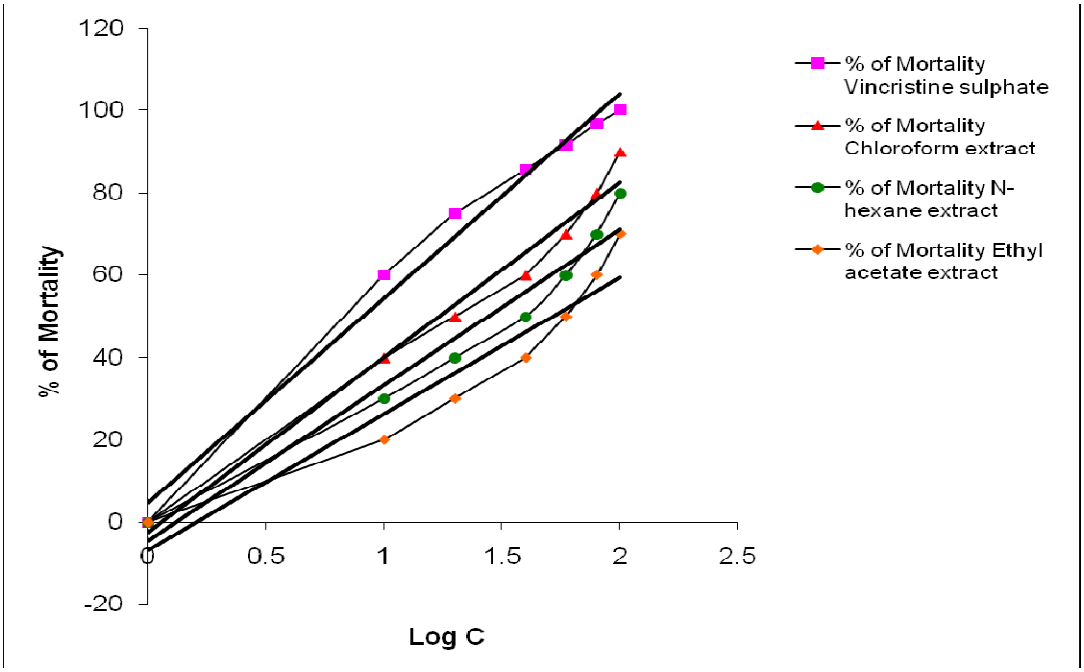

Figure 1: Determination of LC_{50} values for standard and crude chloroform, n-hexane, and ethyl acetate extracts of *C. sativus* from linear correlation between logarithms of concentration versus percentage of mortality

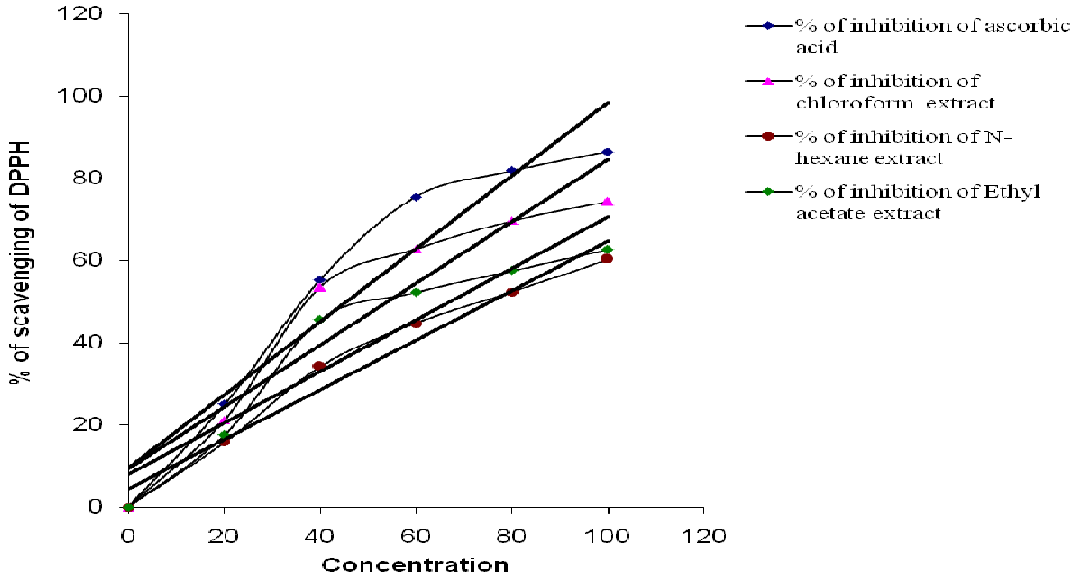

Figure 2: Determination of IC_{50} value for standard and crude chloroform, N-hexane and ethyl acetate extracts of leaves of *C. sativus* from linear correlation between concentrations (µg/ml) versus percentage of scavenging of DPPH.

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