

## Research Article

# Antioxidant, antimicrobial activity and phytochemical analysis of the seeds extract of *Cucumis sativus* Linn

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### Abstract

The prehistoric interaction of plants and human is being strengthened by the remarkable use of plants as remedy against diseases. *Cucumis sativus* L. is extensively cultivated globally including Pakistan. The present study was carried out to find the antioxidant, antimicrobial and phytochemical analysis of *Cucumis sativus* seed extract. Phytochemical analysis of ethanolic extract revealed the presence of flavonoids, terpenoids, tannins, cardiac glycoside, phenols and carbohydrates. 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used for antioxidant activity. Crude ethanolic extract showed maximum DPPH scavenging activity of  $46.05 \pm 1.23$  at  $500 \mu\text{g/ml}$ , while ascorbic acid showed 92.5%. For Antimicrobial activity against selected bacteria and fungi by agar well diffusion method was used. In the antibacterial activity, crude ethanolic extract was most active against *Staphylococcus aureus* (21.5mm) and less against *Shigella flexneri* (17.0mm). The n-haxane fraction was highly active against *Salmonella typhi* (26mm), DCM against *E. coli* (16.25mm) and *Salmonella typhi* showed 16.0mm inhibition with ethyl acetate. Crude extract of ethanol was tested against *Alternaria*, *Acremonium*, *Verticellium*, *Pythium* and *Tricoderma* spp. *Alternaria* showed low zone of inhibition (08mm) while the rest of fungi were highly susceptible with zone of inhibition 15mm, 14mm, 17mm and 15mm respectively. The *Pythium* spp. was highly susceptible to n-haxane fraction (20.00mm), *Acremonium* to DCM fraction (20.00mm) and to ethyl acetate (16.00 mm). The results showed significant antimicrobial and antioxidant activity which might be because of flavonoids, terpenoids, tannins and phenols in there, suggesting that *C. sativus* should be used as an active nutrition to control microbial infections leading to chronic diseases.

**Keywords:** Antimicrobial activity; Antioxidant activity; *Cucumis sativus* L.; Phytochemical analysis

### Introduction

Plants are basic therapy springs for diseases that can be traced back to time immemorial. Having little or no harm effects of plant

materials, therefore herbs based medicines flourished now a days. Plants possess specific biochemical components which exerts curative effects on living organism to treat

various diseases. [1]. The concrete significance of the medicinal plants is because of antioxidants and antimicrobials properties [2, 3]. Microbes become resistant to the available sources of drugs, novel plant sources are needed to handle these resistant strains. It is needed to comprehend the genetics of the resistant microbes for developing new drugs better than the existing ones to reduce threat of infectious diseases. . Plant sources possess chemicals like alkaloids, glycosides, flavonoids, terpenoids etc. in plenty which are potential sources of biological properties [4]. There is an inborn antioxidative mechanism in the human body and many natural activities such as the anti-carcinogenic and anti-aging responses, initiate due to antioxidants. Antioxidants soothes or disable free radicals, earlier their attack to the target cells. Disease occurred due to production of free radicals in living system which damage it causing serious and long lasting diseases like diabetes, ageing, low immune system neuro-degeneration etc. The diseased body needed antioxidants in the form of proper nutritional and medicinal supplements to overcome the threat. Plant based research showed that antioxidants are there in the form of chemical components like phenols, flavonoids, proanthocynidine etc. Plants based antioxidants enhances the defense against diseases and slows down the aging process. Natural antioxidants reduces the risks of infectious and chronic diseases [5]. Medicinal plants are continuously investigated for the therapeutic properties especially for antioxidants. Natural antioxidants either in fresh form or its pure chemical ingredients are active to check the critical progressions produced by reactive oxygen species (ROS) [6]. Plants are best sources of human therapy due to its phytochemicals present there, these have resisting potential against diseases. Anti-inflammatory, anticancer, anti-malarial, inhibiting cholesterol production, antiviral

and antibacterial bioassays are important pharmacological activities of terpenoids. Alkaloids are sedatives and are mostly plants derivatives phytochemicals like alkaloids are anesthetics, terpenoids are anti-inflammatory anticanereous, anti-malarial, inhibiting cholesterol formataion and anti-microbial. These all are obtained from plat sources [7]. The *Cucumis* is an annual climbing plant and about 15-30 cm with large lamina covering the fruit. The *Cucumis sativus* (Cucurbitaceae) fruit is edible. The fruit possesses numerous activities such as antihyperglycemic activity [8], inhibitory effects on protein Kinase C (PKC) activity [9], anti-oxidant activity [10-12], amyl-lytic activity [13], anticancer activity [14], anti-clastogenic activity [15], and anti-mutagenic activity [16, 17]. *Cucumis sativus* is used in raw form fresh. Its fruit extract possess antioxidant and analgesic activity [18], used as carminative and antacid [19]. Studies showed antioxidant and anti-ulcer effects [20] Seeds are good sources of protein, fat, minerals and calcium [21]. Deficiency of antioxidants can be replaced by using fresh *Cucumis* [22, 23]. The only harmless substitute to internal antioxidants is fresh Fruits, vegetables and similar foodstuff. For preserving the normal cell activities. Fruits and vegetables offer defense against long-lasting illnesses like cancer and cardiovascular disorder due to the presence of antioxidants especially, vitamins, phenolic and flavonoids, coumarin, tannins and stilbenes [24]. Due to marvelous prospective of the components against prolonged diseases, various researches have proved the energetic character of fruits and vegetables as antioxidants to preserve physique utility in regular manner [25]. The purpose of the present project was to explore the underlying curative potentials of the plant in the form of antioxidant, antibacterial, antifungal and preliminarily phytochemical screening of seeds of *Cucumis sativus*.

## Materials and methods

### Plant Collection

Fresh seeds of *Cucumis sativus* were collected from ripened dried fruit of the plant. These were properly dried and seed coats were removed from all seeds carefully. Dried seeds were grounded with the help of pestle and mortar to get powdered material.

### Preparation of crude extract and its fractions

Extraction is the process in which desired plant tissues will be soaked in solvent for a certain time period, from which medicinally active principles are dissolved in the solvent

leaving undissolved materials. In this study 70% ethanol were used for extraction. The grounded material was soaked in 70% ethanol and kept at room temperature for 3-7 days. Filtrate was collected and was concentrated with the help of rotary evaporator. The concentrated crude extract was like a gum. This was the crude ethanolic extract and further fractionation was carried out, subjecting the crude sample to extraction with n-hexane, Dichloromethane and ethyl acetate respectively on polarity bases (Table 1).

**Table 1. Amount of different extracts of seeds of *Cucumis sativus* Linn**

Grounded material	Ethanolic extract	n-hexane fractions	DCM fraction	Ethyl acetate fraction
1kg	26g	7g	3g	3g

### Preparation of media

#### Preparing the growth-media

Required bacterial broth was dissolved in distilled water to prepare media for bacterial growth. pH was attuned as at 7.0-6.8 and autoclaved. Strains were injected and incubated at 37°C for one day. Bacterial Nutrient agar was dissolved in distilled water (for bioassay in petriplates) and autoclaved.

**Selected Strains:** *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*

**Inoculation:** from one day old bacterial broth, inoculum was selected to be used in agar poured in petriplates for activity after getting the McFarland Turbidity Standard. Bacteria from 24-hour old culture in LB broth (Miller) of selected bacterial strains were mixed with physiological normal saline solution until a McFarland turbidity standard [ $10^6$  colony forming unit (CFU) ml<sup>-1</sup>] was obtained. Then this inoculum was used to seed LB Agar Medium.

Autoclaved media was left to solidify in petriplates at room temperature. Agar well diffusion technique [26] was used. Agar plates were covered with the selected strains

by using a sterile cotton-swab then wells were made with help of a sterile cork borer.

#### Extract Concentration

20mg of dried or partially dried extract was liquefied in 1ml of dimethylsulfoxide (DMSO).

#### Zone of inhibition

75 µl of each extract was used into the wells with the help of a sterile micropipette and plates were incubated for one day at 37°C to find out the inhibition zone. Diameter of inhibiting zones were calculated all-around the wells. Activity was repeated three times and mean value with standard error was calculated by DMRT (Duncan multiple range test) through SPSS.

#### Fungal strains used

*Alternaria*, *Acremonium*, *Verticellium*, *Pythium* and *Trichoderma* were used for antifungal assessment.

#### Media preparation for fungal growth

PDA potato dextrose agar prepared in sterile water was used for culturing fungi, autoclaved and poured into autoclaved petriplates. Kept at room temperature to solidify.

### Agar -well method

Method described by Samie et al. [27] was used for activity. Using the micropipette, 100µl of different fungal cultures were spread over the agar plates with the help of inoculating loop in double distil water. Using a sterile cork borer, hole) were made in each of the culture plates. 75µl of crude and different fractions of *Cucumis sativus* extract was poured added and inoculated plates were incubated at 28°C for one day or two day showing clear inhibitions. Inhibiting zones were calculated in millimeter, indicating the activity. Each test was triplicated and standard deviation was calculated.

### Preliminary phytochemical tests

Various phytochemical methods [22, 29, 30, 36] were used to screen the crude extract of *C. sativus* seeds.

### Antioxidant assay: Scavenging activity of free radicals

Antioxidant assay of seeds of *Cucumis sativus* was calculated using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) assay following the method of Ahmad and Patel & Patel [31, 32]. 0.1 mM concentration of DPPH in methanol was arranged. 1ml of this concentration was used in 3ml of crude extract to different concentration (250 & 500µg/ml). This mixture was shaken briskly and stand at room temperature for 30 minutes. Then by using spectrophotometer, absorbance was calculated at 517 nm. Ascorbic acid was used as standard. The percent DPPH was measured by using the following equation:

DPPH scavenging effect (%) or Percent inhibition =  $A_0 - A_1 / A_0 \times 100$ .

Where  $A_0$  was the Absorbance of control reaction and  $A_1$  was the Absorbance in the presence of the test or standard sample [33].

### Results and discussion

Antimicrobial activity of the crude ethanolic extract and three fractions n-hexane, dichloromethane and ethyl acetate were studied against five bacterial and five fungal

strains. Extracts showed potent activity against all the microorganisms.

Antimicrobial potential was assessed by recording the zone of inhibition of the microbial growth in mm. The (Table 2) showed antibacterial activity and (Table 3) shows antifungal activity. It is reported that medicinal plants can be good sources of antibacterial agents [34]. In the antibacterial activity, the crude extract was the most active against *Escherichia coli*, *Staphylococcus aureus* (22.5mm) and less against *Shigella flexneri* (17.0mm). The n-hexane fraction was the most active against *Salmonella typhi* (26.0mm), DCM was 16.25mm against *Escherichia coli* and *Salmonella typhi* showed 16.0mm zone of inhibition with ethyl acetate fraction. Similarly ethanol and methanol extracts of *P. emblica* reduces the growth of bacteria [35]. The bacterial organism were susceptible to medicinal plant extracts. It is reported that as compared to gram positive, gram negative bacterial strains are very vulnerable to plant extracts [36, 37]. In antifungal activity the crude ethanolic extract was tested against *Alternaria*, *Acremonium*, *Verticellium*, *Pythium* and *Tricoderma* spp. *Alternaria* showed less zone of inhibition (08mm) while the rest of the fungal pathogens showed susceptibility with the zone of inhibition of 15mm, 14mm, 17mm, and 15mm against *Acremonium*, *Verticellium*, *Pythium* and *Tricoderma* respectively. The *Pythium* spp. was highly susceptible to n-hexane fraction showing 20.00mm, *Acremonium* to DCM fraction with 20.00mm and to ethyl acetate showing 16.00 mm zone of inhibition. *Candida albicans* showed resistance to various extract of medicinal plants of western Himalayas [38]. Fungi were also found susceptible to the extracts and fractions of *Cucumis sativus*. It is reported that the fungus *Phytium* was specifically inhibited, recorded in various reports [39-42].

The microbial susceptibility to different extract of the seeds of *Cucumis sativus*, expresses its anti-infection ability. All the solvent extracts of the seeds of *C. sativus* was found active against the tested organisms, which indicated the presence of broad spectrum antibiotic compounds. These extracts also showed active potentials against tested fungus which revealed the presence of potent antimicrobial constituents in all the solvent extracts. Regular intake of this fruit may decrease the possibilities of infections. Preliminary phytochemical analysis showed (Table 4) the presence of Alkaloids, Flavonoids, Terpenoids, Tannin, Cardiac glycosides, phenols and Carbohydrates. The ethanolic extracts of seeds of *C. sativus* was screened for different Phytochemicals like anthraquinones, alkaloids, steroids, tannin, terpenoid, flavonoids, saponin and cardiac glycosides. The presence of flavonoids, cardiac glycosides, tannin and saponin as shown in (Table 4), explain its dietary and therapeutic significance. The present study is in accordance with the previous work and the slight change may be because of soil mineral composition, cultivation and climatic differences [18, 43-45].

Antioxidants help to protect living system from being damage. Fruits, vegetables and other wild plants are good sources of these antioxidants [46, 47]. In vitro, free radical scavenging effects of *C. sativus* was evaluated by DPPH method, ascorbic acid was used as standard and a spectrophotometer was uses at 517 nm. The results showed (Table 5) that at 125 µg/ml conc. the effect was 28.1%, 250 µg/ml concentration the effect was 32.0% and at 500µg/ml, it was 46.05%. So, the extract showed having a clear effect on scavenging the free radicals. Free radicals are said to be actively involved in many chronic diseases, such as cardiac and cancer among others [18]. The antioxidants, hunt the free radicals which formed in living organisms. The antioxidative ability of natural products has been extensively studied by DPPH test. From the preliminary phytochemical screening of the seeds ethanolic extract of *Cucumis sativus* showed to consist of glycosides, steroids, carbohydrates, saponin and tannins, it is concluded that, the occurrence of flavonoids & tannins in the extract proposes that these complexes might be responsible for scavenging free radicals and said to be as an effective antioxidant [48, 49].

**Table 2. Antibacterial activity of seeds of *Cucumis sativus* Linn. Zone of inhibition in mm, concentration in µ/ml**

Sample	<i>Staphylococcus aureus</i>	<i>Shigella flexneri</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
Crude ethanolic extract	21.5±0.02	17.0±0.22	20.9±0.03	22.5±0.06	20.0±0.31
n-hexane	19.0±0.21	10.0±0.03	09.2±0.21	18.25±0.11	26.0±0.19
DCM	10.15±0.12	12.0±0.19	07.15±0.14	16.25±0.15	14.5±0.14
Ethyl acetate	11.5±0.11	13±0.09	07.5±0.12	14.5±0.13	16.0±0.15

Average of triplicate

**Table 3. Antifungal activity of seeds of *Cucumis sativus* Linn. Zone of inhibition in mm, concentration in µ/ml**

Sample	<i>Alternaria</i>	<i>Acremonium</i>	<i>Verticellium</i>	<i>Pythium</i>	<i>Trichoderma</i>
Crude ethanolic extract	8.0±0.12	15.0±0.19	1.04±0.15	17.0±0.21	15.0±0.21
n-hexane	6.0±0.19	14.0±0.12	11.0±0.09	20.0±0.09	14.0±0.13
DCM	7.0±0.19	20.00.09	14.5±0.08	10.5±0.16	14.5±0.09
Ethyl acetate	7.0±0.11	16.0±0.09	13.0±0.12	8.5±0.02	8.5±0.12

Average of triplicate

**Table 4. Preliminary phytochemical tests *Cucumis sativus* Linn**

Botanical name	<i>Cucumis sativus</i>
Parts used	seeds
Alkaloids	-
Flavonoids	+
Terpenoids	+
Tannin	+
Anthraquinones	-
Cardiac glycosides	+
phenols	+
Carbohydrates	+
Saponin	-

**Table 5. Antioxidant activity of seeds of *Cucumis sativus* Linn**

	Conc.( $\mu\text{g/ml}$ )	Inhibition %age
Crude ethanolic extract	500	46.05 $\pm$ 1.23
	250	32.0 $\pm$ 1.01
	125	28.1 $\pm$ 1.0
Ascorbic acid	500	92.55 $\pm$ 2.50
	250	62.33 $\pm$ 0.22
	125	45.2 $\pm$ 1.0

Average of triplicate

### Conclusion

The present study suggests that the use of plant *Cucumis sativus* is very beneficial in terms of enhancing immune system of the living organisms and can be good source of isolating novel drugs to improve human health.

### Authors' contributions

Experimental design and lab facility, analyze data and wrote the paper: HA Begum, Lab work and help in writing the paper: F Asad, Analyze the data: A Sadiq, Help in paper writing: S Mulk, Help in data analysis: K Ali.

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