



Research Article

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ANTIMICROBIAL PROPERTIES OF METHANOLIC EXTRACT OF CUMIN (*CUMINUM CYMINUM*) SEEDS

Anita Dua^{1*}, Garg Gaurav², Singh Balkar³, Ritu Mahajan⁴

¹Department of Biochemistry, University College, Kurukshetra University, Kurukshetra, India

²Department of Biotechnology, JMIT, Radaur, India

³Department of Botany and Biotechnology, Arya P.G.College, Panipat, India

⁴Department of Biotechnology, Kurukshetra University, Kurukshetra, India

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*Corresponding author

E-mail: anitadua2012@gmail.com

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ABSTRACT

Antimicrobial properties of methanolic extract of cumin (*Cuminum cyminum*) seeds on four enteropathogenic and food-spoiler bacterial strains have been investigated. The cumin extract has been found to be effective against both gram positive and gram negative bacteria. Increase in absorbance between 260-280 nm indicates that incubation of bacterial cultures with cumin extract causes damage to their cell membranes and release of intracellular nucleotides and proteinaceous materials from the cells. The growth inhibition zones observed by agar well diffusion method were 10.7 to 14.0 mm in diameter in presence of cumin extract. Minimum concentrations of cumin extract effective against *E.coli*, *P.aeruginosa*, *S.aureus* and *B.pumilus* were found to be 12.5, 6.25, 25.0 and 6.25 mg dry weight per ml respectively.

Keywords: Antimicrobial property, cumin, cell damage, growth inhibition, MIC

INTRODUCTION

Nature has been a source of medicinal agents for many centuries and the use of medicinal plants, especially in traditional medicine, is well acknowledged and established¹. In the last two decades, antibiotic resistance is an emerging problem worldwide. Resistance of pathogens to different drugs is very common, which is a major concern in treatment of various diseases. Inappropriate use of readily available antibiotics, prolonged hospitalization and poor implementation of infection control measures are the main causes of drug resistance. This has led to the search for new safe and effective antimicrobial agents from alternative natural resources like plant products. Extraction of different bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity². Furthermore, the active components of herbal products have the advantage of being combined with other substances that appear to be inactive and these complementary components give the plant as a whole safety and efficacy, much superior to that of its isolated and pure active components³. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments^{4,5}. In addition, food industry is also looking for natural preservatives or additives, which are being preferred as these are safer, flavor enhancer and without any side effects as compared to the synthetic or chemical additives⁶.

Spices, added to food preparations worldwide for their taste and flavor are being recognized for their medicinal, antioxidant, antimicrobial and food stabilizing properties. Various medicinal plants and spices are reported to inhibit

the growth of microorganisms⁵⁻⁸. Cumin (*Cuminum cyminum*) is a widely used spice condiment in India, South East Asia and Arabia. Cumin commonly known as jeera or Kashmiri jeera belongs to the family Apiaceae. Cumin is known for its carminative, stimulant, diuretic, emmanogogic, antispasmodic and astringent properties. The aqueous extract of cumin is reported to inhibit the growth of many pathogens including *Escherichia coli*, *Staphylococcus aureus*⁸, *Salmonella species*⁹, *Bacillus cereus* and *Aspergillus niger*¹⁰. Composition, concentration of the constituents and extraction procedure are some factors which affect the efficiency of the extract. In the present studies, antibacterial activity of the aqueous methanolic extract of cumin against four pathogens, two gram negative bacteria and two gram positive bacteria were examined for possible cell damage and growth inhibition. Minimum inhibitory concentration for the growth of these pathogens were also determined.

MATERIALS AND METHODS

The cumin seeds were procured from the local market, identified and authenticated at Department of Botany, Kurukshetra University, Kurukshetra, India.

Extraction

Cumin seeds were dried at 60°C in hot air oven till constant weight was attained. Finely powdered cumin seeds were extracted with 80% methanol (1g/10ml) in a shaker at room temperature for 4 h. Residue was again extracted with 80% methanol for 2 h. Collected extract was filtered through double layered muslin followed by centrifugation at 5000 rpm for 5 min in order to get clear supernatant. Extract was concentrated in a vacuum evaporator and stored at -20°C for further use. The extract was diluted appropriately for different experiments.

Test Organisms

Four enteropathogenic and food-spoiler bacterial strains [two gram negative bacteria i.e. *Escherichia coli* (MTCC 119), *Pseudomonas aeruginosa* (MTCC 741) and two gram positive bacteria i.e. *Staphylococcus aureus* (MTCC 96) and *Bacillus pumilus* (MTCC 7411)] were obtained from MTCC, IMTECH, Chandigarh, India. All bacterial cultures were maintained and subcultured regularly on Nutrient agar media (NAM) containing peptone 5 g; beef extract 3 g; sodium chloride 5 g and agar 2% in a final volume of 1l.

The size of inoculum was adjusted to approximately 10^8 colony-forming units per ml by suspending the culture in sterile distilled water. Petridishes containing nearly 25 ml of nutrient agar medium were seeded with 100 μ l culture of the respective bacterial strains and kept for 15 min for the absorption of culture.

Bacterial Cell Damage

Bacterial cell cultures incubated in presence and absence of cumin extract for 30 min at 37°C were analyzed spectrophotometrically to estimate cell damage¹¹. The culture was grown in nutrient broth up to the log phase of the culture. The cultured broth was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was discarded and the pellet was suspended in 20 mM phosphate buffer of pH 7.0. Culture was washed again and turbidity of each suspension was adjusted to 0.5 McFarland units by suspending the cultures in sterile phosphate buffer. To check the amount of cell damage due to cumin extract, 100 μ l of bacterial suspension was incubated with 100 μ l of cumin extract (equivalent to 1.25mg dry seeds per ml extract) at 37 °C for 30 min in water bath. The incubation mixtures were diluted appropriately to observe spectra at 230 – 340 nm before and after incubation for 30 min against methanol blank.

Well Diffusion Assay

Using a sterile cork borer, nearly 8mm diameter wells were bored in the seeded agar plates and a 100 μ l volume of cumin seed extract (equivalent to 33.33 mg dry seeds) diluted in 10 % methanol was added into the wells. All the plates were incubated at 37°C for 24 h. Antibacterial activity was determined by measuring the zone of growth inhibition around the well using agar well diffusion assay technique¹². The antimicrobial activity of the cumin extract was compared against the standard drugs, ampicillin and chloramphenicol (concentration 25 μ g/ml) (negative control) and 10% methanol (positive control). These tests were performed in triplicate and the mean of inhibition diameter was taken.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of cumin was determined by the agar well diffusion method. Petridishes containing 25 ml nutrient agar medium were swabbed with the 100 μ l culture of inoculum containing approximately 10^8 colony-forming units per ml. Two fold serial dilutions ranging from 200 – 3.125 mg/ml concentrations of cumin seed extract were made in 10% methanol and used to determine the minimum concentration of cumin inhibiting the growth of bacteria under study.

RESULTS AND DISCUSSION

Antibiotics are generally an efficient means of treating bacterial infections. Treatment with antibiotics is not only expensive but the risk of bacterial resistance to antimicrobial agents and side effects such as acidity, burning sensation and damage to natural fauna of intestine are also involved¹³. The polyphenolics have evolved in plants as antioxidant and antimicrobial agents against environmental stress due to a variety of oxidizing and potentially harmful free radicals. The antimicrobial potential of the methanolic extract of cumin seeds against pathogenic bacteria *E.coli*, *P.aeruginosa* causing gastroenteritis or urinary tract infections, *B.pumilus* causing stomach cramps, food poisoning and *S.aureus* causing pneumonia, food poisoning and toxic shock syndrome (TSS) were examined.

Cumin extract has exhibited antimicrobial activity against all the four bacteria tested. Results in Figure 1 show that all the bacterial strains are sensitive to the presence of cumin extract in the incubation mixture. Increased absorbance between 260 to 280 nm indicates the damage to the cell membranes and leakage of intracellular nucleotides and proteinaceous materials respectively into the growth medium. *B.pumilus* culture has shown maximum increase in absorbance from 0.35 to 1.1 at 260 nm and 0.27 to 0.99 at 280 nm on incubation with cumin extract, indicating the highest damage to the membranes of these bacteria (Figure 1.d). Incubation of, *P.aeruginosa* and *S.aureus* cells with cumin extract resulted in an increase of absorbance by 0.30 - 0.44, whereas *E.coli* culture has exhibited an increase up to 0.285 in absorbance between 260- 280 nm. The results showed that cumin extract is effectively inducing cell damage in both gram negative as well as gram positive bacteria, although the effect is more pronounced against *B.pumilus*. Similar inhibitory effect on the growth of *B.subtilis* and *E.coli* by parsley and cilantro leaves and stem extracts has been reported¹¹.

A variety of plant- and spice-based antimicrobials are used by the food industry as natural agents for extending the shelf life of foods by reducing or eliminating pathogenic bacteria and increasing the overall quality of food products. Aromatic and volatile oily liquids from flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots of plants have antimicrobial activities. Essential oils from plants, such as oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, sage, vanillin^{5-7, 13} have antimicrobial properties. Extracts of plants generally are mixtures of several components such as polyphenols, terpenoids, alkaloids etc. and have both antioxidant and antimicrobial characteristics. Methanolic extracts of various spices including cumin are reported to have various polyphenolic compounds and antioxidant activities¹⁴. Methanolic extract, in the present study, was examined for inhibition of bacterial growth by well diffusion method (Figure 2, Table 1).

Table 1: In vitro antibacterial activity of cumin extract and standard antibiotics by agar well diffusion method

Compound	Concentration of the compound	Diameter of growth of inhibition zone (mm)			
		<i>E.coli</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>B.pumilus</i>
Cumin extract	33.3mg seeds/ml	10.7±0.20	13.1±0.31	14.0±0.25	12.8±0.15
Chloramphenicol	25µg/ml	17.5±0.25	24.7±0.15	23.7±0.16	26.3±0.31
Ampicillin	25µg/ml	35.7±0.21	44.1±0.26	41.4±0.20	38.6±0.31
10% methanol		0	0	0	0

The values represent mean of sample ± SD for n = 3. Diameter of inhibition zone was measured as the clear area centered on the agar well containing the sample. Wells with non-inhibition zone were recorded 0.

Table 2: Minimum inhibitory concentration (MIC) of cumin extract and standard antibiotics

Compound	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>B.pumilus</i>
Cumin extract	12.5	6.25	25.0	6.25
Chloramphenicol	0.2	0.4	0.4	0.4
Ampicillin	0.5	0.5	0.8	0.5

The values for cumin are in mg dry wt of seeds/ ml and for standard antibiotics are in µg /ml.

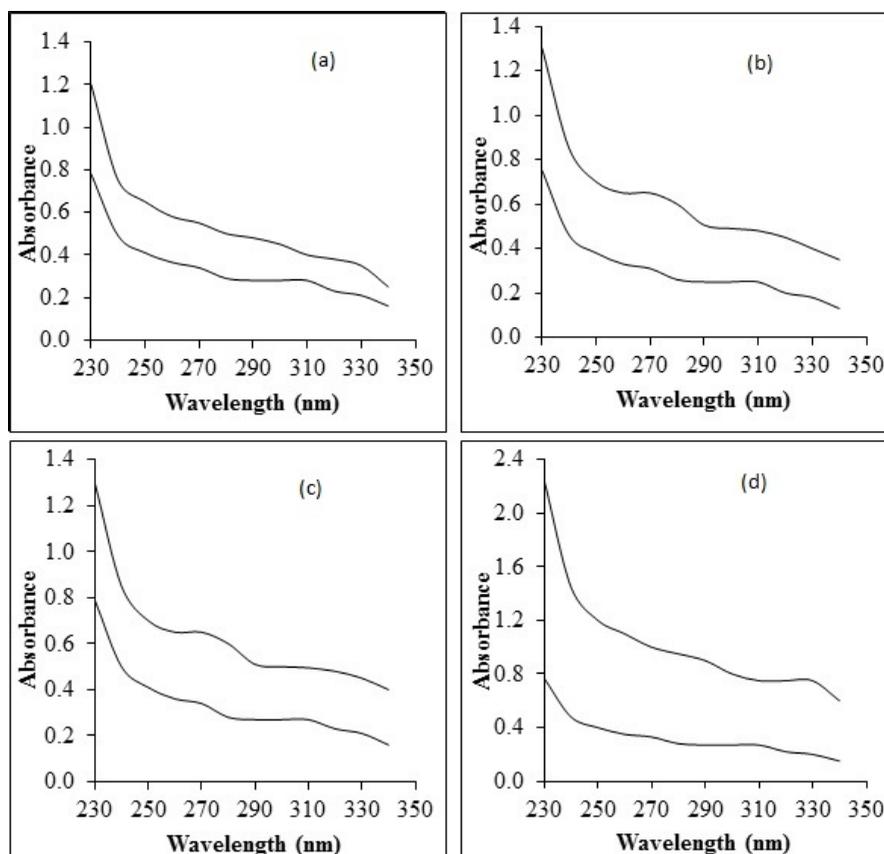


Figure 1: Bacterial cell damage induced by methanolic cumin extract

(a) *E.coli*; (b) *P.aeruginosa*; (c) *S.aureus*; (d) *B.pumilus*. Increased absorbance between 260nm and 280 nm indicates leakage of cellular materials (DNA, proteins and enzymes) into growth medium

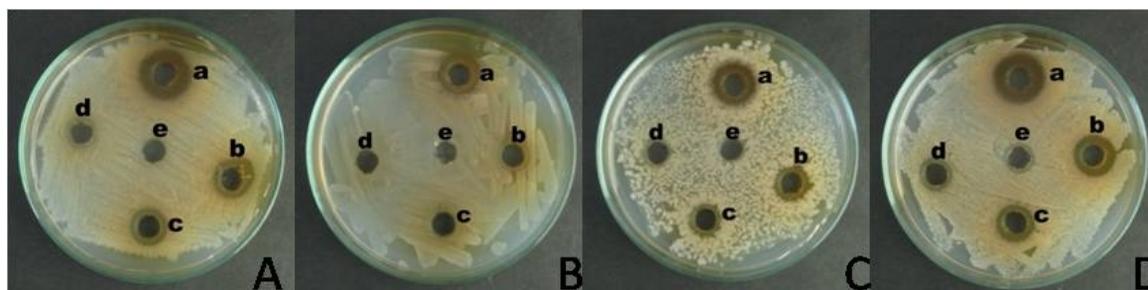


Figure 2: Bacterial growth inhibition by methanolic cumin extract

A: *E.coli*; a-e concentrations are 100, 50, 25, 12.5, 6.25 mg/ml respectively. B: *P.aeruginosa* and D: *B.pumilus*; a-e concentrations are 50, 25, 12.5, 6.25, 3.125 mg/ml respectively. C: *S.aureus*; a-e concentrations are 200, 100, 50, 25, 12.5 mg/ml respectively.

The growth inhibition zone in presence of extract equivalent to 33.33 mg cumin seeds, were 10.7mm for *E.coli*, 13.1 mm for *P.aeruginosa* and 12.8 mm for *B.pumilus*. Growth of *S.aureus* was inhibited up to 14.0 mm. These values are lower than 18 mm - 26 mm for chloramphenicol (25µg /ml) and 36-44 mm for ampicillin (25µg /ml) standards used. Aqueous extract of cumin is reported to have antimicrobial activity with inhibitory zone of 18mm for *E.coli* and 10 mm for *P.aeruginosa*⁹. Methanolic extract of cumin inhibits the growth of *E.coli* upto 10mm and that of *S.aureus* up to 11 mm¹⁰. Aqueous extract of cumin is reported to have antimicrobial activity against many gram negative pathogens including *E.coli*, except *P.aeruginosa* but not active against gram positive bacteria except *S.aureus*⁹. However methanol extract has exhibited antimicrobial activity against both gram positive and gram negative pathogens examined in this study. Minimum concentration of the cumin seeds, presence of which can inhibit the growth of microbes i.e. MIC for *P.aeruginosa* and *B.pumilus* determined by well diffusion method was 6.25 mg/ml, whereas inhibition of *E.coli* and *S.aureus* growth was achieved in presence of extract containing 12.5 and 25.0 mg/ml cumin seeds as compared to 0.2-0.8 µg/ml for standard antibacterial compounds tested (Table 2). The antimicrobial properties of aqueous extracts of various medicinal plant species against *E.coli* with an MIC value ranging from 0.09-6.25 mg/ml has been reported^{5,7}. MIC of cumin seeds observed here falls somewhere in between the reported extremities for *P.aeruginosa* and *B.pumilus*. However MIC observed for *E. coli* and *S.aureus* is higher than this range. Das et al 2012 have also reported high MIC of 10-15 mg /ml for *E.coli* and 15-20mg/ml for *S.aureus* when extracted with water or ethanol. Different antimicrobial activity of herbs against bacterial strains may be due to different bioreactive compounds in the extracts prepared by different methods. Growth of bacteria is sensitive to the redox potential of the media. Moderately reducing environment of the growth medium can contribute in part to the growth inhibition of various bacteria¹¹. Methanolic extracts of spices contain phytochemicals including polyphenols and are reported to exhibit considerably high free radical scavenging and peroxide inhibition activity indicating its reducing character, which may in part explain the inhibition of bacterial growth. Metal ion chelating property of the polyphenols in the extracts of the spices may also be contributing to the antimicrobial properties by leading to the deficiency of essential metal ions in the growth medium.

The knowledge about the cell damage and inhibition of growth of various pathogens by methanolic extract of cumin can be extended for future investigation and application into the field of pharmacology, phytochemistry or food chemistry for the development of better medicinal or preservative preparation.

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