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## ANTIBACTERIAL EFFECT OF *CUMINUM CYMINUM* AGAINST SELECTED BACTERIAL STRAINS

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### SUMMARY

The search for novel components with antibacterial potential has recently gained increasing significance due to growing worldwide bacterial infections by antibiotic-resistant bacterial strains. Therapeutic plants are crucial to human health due to their antiseptic potential against bacterial pathogens. Botanical sources have benefitted healthcare for many years due to the various active compounds present, such as tannins, terpenoids, alkaloids, and flavonoids. This study seeks to report the antibacterial potential of *Cuminum cyminum* against *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*. The research proceeded with the use of methanolic extract of *C. cyminum* preparation using the Soxhlet extraction technique. Formulating five concentrations (150, 180, 200, 220, and 250 mg/ml) used deionized water and incurred testing at varying temperatures (40 °C, 60 °C, 80 °C, 100 °C, and 121 °C) and pH (3, 5, 7, 9, and 11 pH) ranges. The well-diffusion assay and minimum inhibitory concentration helped analyze the antimicrobial properties of *C. cyminum* methanolic extract as observed against selected bacterial strains. The results' analysis engaged the one-way ANOVA. It was evident that the maximum effect of plant extract against *E. coli*, *B. subtilis*, and *B. cereus* was at 200 mg/ml and against *Staph. aureus*, a maximum zone emerged at 180 mg/ml. At varying temperatures, the maximum inhibition of *E. coli*, *Staph. aureus*, and *B. cereus* occurred at 121 °C, with *B. subtilis* inhibited at 80 °C. For pH changes, it revealed that all strains were sensitive to acidic pH (3) at both concentrations (150 and 250 mg/ml).

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**Keywords:** *Cuminum cyminum*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*

**Key findings:** This study concluded that methanolic extracts of *C. cyminum* showed growth inhibition in all test strains. This inhibition might be due to cell membrane damages (caused by *C. cyminum* extract) leading to the restricted growth of the bacterial strain. Further studies need pursuits to validate this damage. This study also reported that *C. cyminum* methanolic extract proved effective against *B. cereus* compared with the other strains. The plant extract's characterization also appeared at various ranges of temperature and pH.

## INTRODUCTION

Medicinal plants are currently of substantial significance due to their unique attributes as a massive source of therapeutic phytochemicals that may lead to novel drug development. Most of the phytochemicals from plant sources, such as phenolics and flavonoids, had reports of positive health impacts (Garg and Astha, 2020).

The usage of non-prescribed antibiotics increases the resistance in bacteria (Tenover, 2006). Containing this emerging health problem of antibiotic resistance requires using alternative medicinal sources in time (Jabbar, 2013). Plants have enormous amounts of antioxidant properties due to their use as protective agents to cure many illnesses. Many plant antioxidant compounds help to neutralize radicals (Middleton *et al.*, 2000; Sudirga *et al.*, 2023).

Antioxidant properties of phytochemical agents have associations with various human diseases' treatments (Anderson *et al.*, 2001). In modern lifestyles, frequent encounters with people with bacterial diseases and pathogenic microorganisms also develop resistance against antibiotics (Mohammed, 2019). Reports declared various components of plants, such as alkaloids, glycosides, tannins, and flavonoids, have antimicrobial potential (Yadav and Agarwala, 2011).

Dua *et al.* (2013) reported the importance of spices concerning plant extracts. Spices also showed noticeable antibacterial activity. Spices add taste and flavor to food preparations worldwide. Additionally, these spices have become recognizable for their medicinal, antioxidant, antimicrobial, and food-stabilizing properties.

Ani *et al.* (2006) studied spices and their medicinal uses. The *C. cyminum* (cumin) is considerably an effective spice due to its nutritional importance as a nutraceutical. It is a rich source of natural iron commonly known as Jeera or zeera. Its usage is prevalent in cuisines, such as Pakistani, Indian, Mexican, and Middle Eastern, for preparing vegetarian and non-vegetarian foods. It has also treated diarrhea and other food poisoning symptoms. Fattah *et al.* (2000) reported that *C. cyminum* oil contains various constituents, such as cumin aldehyde and cymene, as prominent, along with terpenoids. Several other studies progressed on the essential oil of black cumin. All studies revealed its pharmacological properties (Burits and Bucar, 2000).

A previous study by Dua *et al.* (2013) investigated the antibacterial activities of cumin seed methanolic extract against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. pumilus*. The study reported that methanolic extract was effective against all the test strains that proceeded through an agar well-diffusion assay. The presented research hypothesizes that the methanolic extract of *C. cyminum* has noticeable antibacterial potential against *E. coli*, *Staph. aureus*, *B. subtilis*, and *B. cereus*. The plant extract's characterization also ensued at different ranges of temperatures and pH to broaden the study's viewpoint.

## MATERIALS AND METHODS

### Preparation of methanolic extract of *C. cyminum*

For extract preparation, adding 800 g of *C. cyminum* seed powder to 1200 ml of methanol

occurred. The flask containing the plant powder and methanol was placed in an orbital shaker for 8 h, with 150 rpm at 30 °C, and then filtered using the What's Man filter paper. The filtrate reached transfer in a rotary evaporator at 66 °C for 6 h to evaporate the methanol. Then, the extract incurred collection, containing a minute amount of methanol. Further methanol evaporation at room temperature took place. After complete evaporation of the solvent from the extract, 100 g powder of *C. cyminum* extract collection continued to be stored in a Pyrex jar for further use (Dua *et al.*, 2013).

### **Bacterial strains and inoculum preparation**

Four identified bacterial (*E. coli*, *B. subtilis*, *B. cereus*, and *Staph. aureus*) came from the Microbiology Lab, Department of Zoology, University of Sargodha (November 2022–April 2023). By using specific growth media, culturing bacterial strains used a nutrient agar (*Staph*, *E. coli*, and *B. subtilis*) and the MRS broth (*B. cereus*). After culturing, strains continued to incubate at 37 °C for 24 h to get a fresh culture (Mills *et al.*, 2005).

### **Inhibition zone assay**

Petri plates containing 20 ml nutrient agar medium bore inoculation with 24 h culture of bacterial strains. Forming wells of 6 mm in depth received 20 µl of the plant extract. Then, the plates sustained incubation at 37 °C for 24 h. The antibacterial activity bore assaying by measuring the diameter of the inhibition zone (using a Vernier caliper) that formed around the well (NCCLS, 2004). Each plate contains two wells to duplicate the results. Deionized water served as the negative control.

### **Minimum inhibitory concentration (MIC) assay**

After the screening test of crude plant extract (cumin powder mixed in deionized water), applying methanolic extract transpired on strains. Five concentrations of methanolic extracts (150, 180, 200, 220, and 250 mg/ml),

earlier prepared, help study the MIC using agar well-diffusion assay.

### **Temperature and antibacterial activity**

The effect of temperature on methanolic plant extracts underwent investigation by immersing test tubes containing extract material in a water bath. Temperatures varied on the plant extract continued by altering the water bath temperature. Temperatures of around 40 °C, 60 °C, 80 °C, 100 °C, and 121 °C continued before applying the extract to bacterial strains (Riaz *et al.*, 2010). The said temperature had a minimum (150 mg/ml) and maximum (250 mg/ml) concentrations used in the study plan.

### **The pH and antibacterial activity**

Effect of pH on the methanolic extract of cumin attained testing by altering the pH (3, 5, 7, 9, and 11 pH) of the extract, with minimum (150 mg/ml) and maximum (250 mg/ml) concentrations of extract obtained. Altering the pH used the solution of NaOH and HCl. The pH meter (H1 2211 pH/ORP Meter by HANNA instruments) helped to measure the pH of the solution (Riaz *et al.*, 2010). The experiment had three replicates for each methanolic extract.

### **Statistical Analysis**

The study used the IBM SPSS Statistics (IBM Corp., Armonk, NY, USA) to analyze the data. The statistical analysis employed a one-way ANOVA with a significance level of 5% (Amalia *et al.*, 2019).

## **RESULTS**

### **Antibacterial activity of *C. cyminum***

Preparing the methanolic extract of cumin using the rotary evaporator technique helped observe the set objectives of the study. Finally, a 3.5 g of methanolic extract obtained had a pH of 6.4 (acidic). Later on, changing the acidity and basicity used HCL and NaOH, respectively. Using the agar well-diffusion

method, the antibacterial activity demonstration against food-poisoning bacteria included three strains of Gram-positive bacteria (*B. cereus*, *B. subtilis*, and *S. aureus*) and one Gram-negative bacteria (*E. coli*) strain. The findings revealed that, with varying concentrations, temperatures, and pH, the plant extract was potentially effective in suppressing the microbial growth of food-poisoning bacteria.

### Variable concentrations

Methanolic extract application of *C. cyminum* at different concentrations (150, 180, 200, 220, and 250 mg/ml) occurred on bacterial strains. Zones of inhibition measured for *B. cereus* indicated *C. cyminum* showed maximum inhibitory zones (1.7 mm) at 200 mg/ml. For *B. subtilis*, the maximum inhibitory activity (1.6 mm) was evident at 200 mg/ml. *Staph. aureus* showed maximum zone (1.1 mm) at 180 mg/ml (Table 1).

### Variable temperature ranges

Methanolic extract application of *C. cyminum* at temperatures 40 °C, 60 °C, 80 °C, 100 °C, and 121 °C and a concentration of 150 mg/ml transpired on test strains. *B. cereus* showed inhibitory zones of about 0.7, 1.2, 0.6, 1.2, and 1.5 mm in diameter at 40 °C, 60 °C, 80 °C, 100 °C, and 121 °C, respectively. For *E. coli* and *Staph. aureus*, maximum zone (1.5 mm) was at 121 °C. For *B. subtilis*, the maximum zone of inhibition appeared at 80 °C.

The P value was significant for all strains (Table 2).

The results of test strains treated with a methanolic extract of cumin at different temperatures and at a concentration of 250 mg/ml are available in Table 3. The findings revealed that at 121 °C, the maximum inhibitory zones for *Bacillus cereus* measured to be 1.7 mm in diameter. Similarly, the inhibitory zones of other strains were maximum at 121 °C. At 40 °C, zones with a diameter of 1 mm manifested in both *Bacillus* species. The zone of inhibition for *E. coli* was also 1.0 mm in diameter at 40 °C and 1.8 mm at 121°C. In contrast, the *Staphylococcus aureus* showed a zone of inhibition of 1.7 mm at 121 °C. When comparing the means of the temperatures of each strain, a significant P value surfaced.

### Variable pH levels

Methanolic extract of *C. cyminum* applied on test strains had varying pH (3, 5, 7, 9, and 11). The *B. cereus* showed zones of 1.6, 1.2, 1.0, 0.8, and 0.9 mm at 3, 5, 7, 9, and 11, respectively, at 150 mg/ml. All test strains showed maximum zone at acidic pH (3) at both concentrations under study (Table 4). Changes in the pH values at 250 mg/l of *C. cyminum* extract showed a variable trend of antibacterial activity. The acidic pH effectively controls the bacterial growth of test strains by showing the maximum zone of inhibition. Results appear in Table 5.

**Table 1.** Effect of methanolic extract of *C. cyminum* different concentrations.

Bacterial Strains	Concentrations					Control group	P Value
	150 mg/ml	180 mg/ml	200 mg/ml	220 mg/ml	250 mg/ml		
<i>B. cereus</i>	1.3±0.3	1.2±0.2	1.7±0.2	1.4±0.2	1.2±0.2	0±00	0.1
<i>B. subtilis</i>	1.1±0.2	0.9±0.2	1.6±0.2	1.1±0.2	0.7±0.0	0±00	0.001*
<i>E. coli</i>	0.6±0.2	0.7±0.1	1.2±0.2	1.2±0.2	0.7±0.2	0±00	0.009*
<i>Staph. Aureus</i>	1.0±0.7	1.1±0.0	0.8±0.6	0.8±0.2	0.9±0.1	0±00	0.895

Mean ± SD (n=3). Values present in rows are variables. P values by using ANOVA and compared with significant values ( $P \leq 0.05$ ). Non-significant values are ( $P \geq 0.05$ ).

**Table 2.** Effect of different temperature ranges on antibacterial activity of *C. cyminum* extract at 150 mg/ml.

Bacterial Strains	Temperature ranges					Control group	P Value
	40 °C	60 °C	80 °C	100 °C	121 °C		
<i>B. cereus</i>	0.7±0.2	1.2±0.2	0.6±0.2	1.2±0.2	1.5±0.2	0±00	0.001*
<i>B. subtilis</i>	0.7±0.2	1.2±0.2	2.6±1.1	1.2±0.2	1.8±0.1	0±00	0.011*
<i>E. coli</i>	0.7±0.2	0.8±0.2	0.8±0.2	1.0±0.2	1.5±0.2	0±00	0.004*
<i>Staph. Aureus</i>	1.0±0.2	1.0±0.2	0.6±0.2	1.1±0.2	1.5±0.2	0±00	0.004*

Mean ± SD (n=3). Values present in rows are variables. P values by using ANOVA and compared with significant values ( $P \leq 0.05$ ). Non-significant values are ( $P \geq 0.05$ ).

**Table 3.** Effect of different temperature ranges on antibacterial activity of *C. cyminum* extract at 250 mg/ml.

Bacterial Strains	Temperature ranges					Control group	P Value
	40 °C	60 °C	80 °C	100 °C	121 °C		
<i>B. cereus</i>	1.0±0.2	1.2±0.2	0.8±0.2	1.6±0.2	1.7±0.1	0±00	0.000*
<i>B. subtilis</i>	1.0±0.2	1.2±0.2	0.8±0.2	1.6±0.2	1.6±0.2	0±00	0.002*
<i>E. coli</i>	1.0±0.2	1.0±0.2	0.8±0.2	1.5±0.2	1.8±0.1	0±00	0.000*
<i>Staph. Aureus</i>	1.5±0.2	0.7±0.2	0.9±0.1	1.5±0.2	1.7±0.1	0±00	0.000*

Mean ± SD (n=3). Values present in rows are variables. P values by using ANOVA and compared with significant value ( $P \leq 0.05$ ). Non-significant values are ( $P \geq 0.05$ ).

**Table 4.** Effect of different pH values on antibacterial activity of *C. cyminum* extract at 150 mg/ml.

Bacterial strains	pH ranges					Control group	P Value
	3 pH	5 pH	7 pH	9 pH	11 pH		
<i>B. cereus</i>	1.6±0.1	1.2±0.2	1.0±0.1	0.8±0.2	0.9±0.1	0±00	0.000*
<i>B. subtilis</i>	1.4±0.2	1.3±0.2	0.9±0.2	0.4±0.2	0.4±0.2	0±00	0.000*
<i>E. coli</i>	1.4±0.4	0.8±0.1	0.8±0.2	0.6±0.2	0.4±0.2	0±00	0.004*
<i>Staph. Aureus</i>	1.4±0.3	0.6±0.2	0.9±0.1	0.4±0.2	0.4±0.2	0±00	0.000*

Mean ± SD (n=3). Values present in rows are variables. P values by using ANOVA and compared with significant values ( $P \leq 0.05$ ). Non-significant values are ( $P \geq 0.05$ ).

**Table 5.** Effect of different pH values on antibacterial activity of *C. cyminum* at 250 mg/ml.

Bacterial Strains	pH ranges					Control group	P Value
	3 pH	5 pH	7 pH	9 pH	11 pH		
<i>B. cereus</i>	1.7±0.1	1.2±0.2	0.9±0.2	0.4±0.1	0.5±0.1	0±00	0.000*
<i>B. subtilis</i>	1.6±0.2	0.9±0.1	0.3±0.1	0.4±0.2	0.4±0.2	0±00	0.000*
<i>E. coli</i>	1.6±0.2	0.8±0.1	0.8±0.2	0.6±0.2	0.2±0.1	0±00	0.000*
<i>Staph. Aureus</i>	1.6±0.2	0.6±0.2	0.5±0.1	0.6±0.2	0.4±0.2	0±00	0.000*

Mean ± SD (n=3). Values present in rows are variables. P values by using ANOVA and compared with significant values ( $P \leq 0.05$ ). Non-significant values are ( $P \geq 0.05$ ).

## DISCUSSION

Using four bacterial strains (*E. coli*, *Staph. aureus*, *B. subtilis*, and *B. cereus*), this study assessed the antibacterial activity of *C. cyminum* methanolic extract. Herbs have been historically beneficial in treating diseases and

rebuilding and enhancing biological systems (Aslam and Ahmad, 2016). Antibiotics serve to treat infectious diseases, but doing so is expensive, risks bacterial resistance to antimicrobial drugs, and has adverse side effects that include indigestion, burning, and harm to the normal flora of the intestine. As a

result, scientists turn to using plants (Nagpal *et al.*, 2011).

Generally, plant extracts are mixtures of several substances with antioxidant and antibacterial activities, including polyphenols, terpenoids, alkaloids, and others. Methanolic extracts from various spices, including *C. cyminum*, have been shown to contain many polyphenolic components and antioxidant qualities (Yuan *et al.*, 2016). The study's use of the well-diffusion method scrutinizes the impact of the methanolic extract on bacterial growth in this study. All four test strains proved susceptible to the antibacterial effects of *C. cyminum* extract. Including the *C. cyminum* extract in the incubation mixture causes all bacterial strains to become sensitive. This investigation revealed *C. cyminum* extract effectively damages bacterial cells in Gram-positive and Gram-negative strains. Further research at the cellular level is necessary to support this claim. Previous research has shown that *C. cyminum* has promising antibacterial, antifungal, and antioxidant activity (Megraj *et al.*, 2011).

Bacterial strains' treatment with *C. cyminum* methanolic extract comprised various concentrations (150, 180, 200, 220, and 250 mg/ml), with the results measured by the inhibition zones. *C. cyminum* demonstrated maximum inhibitory zones for *Staph. aureus* at 180 mg/ml and for *B. cereus* at 200 mg/ml. The antibacterial activity of *C. cyminum* was also evident by heating the solution at different temperatures and altering pH. Results revealed that all strains under consideration showed a maximum zone at 121 °C, except for *B. subtilis*, which was inhibited more at 80 °C. Overall prevalent trend of inhibition activity revealed that increasing the temperature also increases the antibacterial potential of the extract. Gutierrez *et al.* (2008) suggested investigating the effects of methanolic plant extract on bacteria during the lag period.

Furthermore, the antibacterial activity of the methanolic extract of cumin was also distinct by altering pH. The current study reported that the activity of the extract against test strains was the maximum at three pH because of the acidic nature of the methanolic extract of cumin. The existing scientific

literature proved that altering the extract's pH could affect its antibacterial activity (Gachkar *et al.*, 2007).

According to Jabbar (2013), cumin seeds showed noticeable antibacterial activity against *E. coli*, *Staph. aureus*, and *Klebsiella sp.* His findings reported that it effectively controlled the growth of *Klebsiella sp.* The existing body of scientific literature proved that changing the pH of an extract can impact its antibacterial activity. According to Narayan *et al.* (2010), the extract showed a broader zone of inhibition at high acidic and alkaline pH.

Past studies revealed the antimicrobial properties of cumin oil and extract on bacterial strains isolated from urinary tract infections (Saeed *et al.*, 2016). The test strains were *E. coli*, *Staph. aureus*, *Staph. epidermis*, and others. They reported that both the cumin oil and extract effectively controlled the growth of test strains. Dua *et al.* (2013) found the antimicrobial properties of cumin on four enter pathogenic and food-spoiler bacterial strains. Both Gram-positive and Gram-negative bacteria have reports of susceptibility to the cumin extract. Reports of cumin extract to be effective against *E. coli*, *S. aureus*, and *B. pumilus* existed.

## CONCLUSIONS

The search for natural chemicals from medicinal plants with antibacterial and anti-virulence activities is necessary due to the rise in antibacterial drug resistance. As a result, the pertinent study examined the ethanolic extract of cumin's qualities. The latest study findings revealed that a specific plant extract could significantly lower the growth of *E. coli*, *B. subtilis*, *B. cereus*, and *Staph aureus*. In confirming the cumin extract's antibacterial capabilities, the study additionally characterizes it under various temperature and pH variations.

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