

Research Article

Evaluation of few bioactive components of spice origin for their antimicrobial potential towards microbes commonly implicated in food spoilage and foodborne pathogenesis

Mamta Bhatia*

Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar-125001 (Haryana), India

Alka Sharma

Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar-125001 (Haryana), India

*Corresponding author. Email: bhatiamamta09@gmail.com

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Abstract

Natural components isolated from spices are gaining the attention of food researchers to prevent the growth of microbes associated with food spoilage and foodborne pathogenesis to meet the ever-increasing consumers' demand for safe and wholesome food free from harmful synthetic preservatives. In the present study, five bioactive components of spice origin, namely, allyl-isothiocyanate (AITC), cinnamic aldehyde (CIA), cuminic aldehyde (CUA), eugenol (EU), and menthol (MT) were evaluated for their antimicrobial potential towards six bacterial strains (*Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas alcaligenes*, *Shigella sonnei*, *Staphylococcus aureus*) and eleven fungal strains (*Alternaria solani*, *Aspergillus niger*, *Botrytis cinerea*, *Cladosporium herbarum*, *Fusarium oxysporum*, *Geotrichum candidum*, *Penicillium citrinum*, *Penicillium expansum*, *Phoma exigua*, *Rhizopus arrhizus* and *Rhizopus stolonifer*), by opting agar well diffusion assay, impregnated paper disc method and broth dilution technique. All these seventeen microbes pose deleterious effects on food and human health. Among the bioactive compounds, CIA and CUA turned out to be the most potent inhibitors of microorganisms, whereas MT was found to be the least effective. Lower concentrations of bioactive components, ranging from 1.95 $\mu\text{L/mL}$ to 15.62 $\mu\text{L/mL}$, were needed to inhibit fungi, while higher concentrations ranging from 15.62 $\mu\text{L/mL}$ to 1000 $\mu\text{L/mL}$ were needed to inhibit bacterial strains. Among the bacterial strains tested, gram-negative bacteria were inhibited at higher component concentration levels (31.25 $\mu\text{L/mL}$ -1000 $\mu\text{L/mL}$) compared to gram-positive bacteria. The present study updates the existing information on the antimicrobial potency of natural substances, paving the way to further research on establishing spice bioactive components as 'natural additives'.

Keywords: Antimicrobial, Bioactive components, Essential oils, Pathogens, Spices

INTRODUCTION

Food spoilage and foodborne pathogenesis are serious burdens on public health and the economy, as diseases caused by food contaminated with microorganisms are an important cause of morbidity and mortality worldwide. Therefore, food preservation is paramount to attaining food safety and security. In the current scenario, pressure is being posed to the food industry either to remove or to reduce the use of conventional chemical preservatives from food products due to their possible carcinogenicity, teratogenicity, high and acute neurotoxicity, long degradation periods and environmental pollution (Embuscado, 2015). Hence, food re-

searchers are prospecting for novel natural agents which may delay the onset of food spoilage in order to maintain or extend product's shelf life and simultaneously prevent the growth of pathogens to meet consumers' demand for safe and wholesome food with regard to nutritional and sensory aspects (Myszka *et al.*, 2019).

Innumerable substances of animal, plant and microbial origin have been identified and studied for their efficacies as food preservatives in great detail, but spice extracts, particularly their volatile essential oils (EOs), have widely gained the attention of researchers due to their long history of usage in domestic culinary practices and as therapeutics, rendering them safe (Bekuma

and Ahmed, 2018; Papadochristopoulos *et al.*, 2021; Lee and Paik, 2016). Crude extracts, EOs of various spices, e.g. caraway, clove, cinnamon, cumin, garlic, ginger mustard, onion, turmeric, etc., and some bioactive components such as allicin, cinnamic aldehyde, curcumin, eugenol have been reported to possess antimicrobial activities against infectious bacteria, fungi, yeast and viruses (Hetta *et al.*, 2020; De-Montizo-Prieto *et al.*, 2021; Chouhan *et al.*, 2017).

It is also demonstrated that EOs of spices are a treasure trove of components, viz., aldehydes (cinnamic aldehyde, cuminic aldehyde), phenols (curcumin, eugenol, gingerols, shogaols), terpenoids (eucalyptol, menthol, thymol) and thiols (allicin, allylthiocyanate), preferentially known as bioactive components (Sharifi-Rad *et al.*, 2020; Martínez-Pabón and Ortega-Cuadros 2020; Gutierrez-del-Rio *et al.*, 2018). These bioactive components serve as arsenals of spices' natural defense from microbes and insects, and scientists are exploiting this knowledge to ward off microorganisms affecting human health adversely. In this context, the present study aimed to evaluate the antimicrobial potentials of five bioactive components of spice origin towards seventeen microbes of food spoilage and health significance.

MATERIALS AND METHODS

Bioactive components

In their liquid forms, allyl isothiocyanate, cuminic aldehyde, and menthol were purchased from Pioneer Chemical Company, India. Cinnamic aldehyde and eugenol in liquid states were supplied by Central Drug House Pvt. Ltd. (CDH), India. Names of tested bioactive components and their spices of origin are enlisted in Table 1. Companies assured the purity of bioactive components as 99.999%.

Microbial strains

Bacterial strains

Pure cultures of two gram-positive bacterial strains (*Bacillus cereus* MTCC 430 and *Staphylococcus aureus* MTCC 5021) and four gram-negative bacterial strains (*Escherichia coli* MTCC 1687, *Pseudomonas aeruginosa* MTCC 1688, *Pseudomonas alcaligenes* MTCC 405 and *Shigella sonnei* MTCC 2957), were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. All the six bacterial strains under consideration are responsible for the spoilage of a wide array of food commodities and put human health at risk due to their grave pathogenesis.

Fungal strains

Pure cultures of *Alternaria solani* NCIM 887, *Aspergillus niger* NCIM 456, *Cladosporium herbarum* NCIM 1112, *Geotrichum candidum* NCIM 980, *Phoma exigua*

NCIM 1237, *Rhizopus arrhizus* NCIM 877 and *Rhizopus stolonifer* NCIM 1139, were procured from National Collection of Industrial Microorganisms (NCIM), Pune, India, and that of *Botrytis cinerea* MTCC 359, *Fusarium oxysporum* MTCC 284, *Penicillium citrinum* MTCC 2553 and *Penicillium expansum* MTCC 2006, were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India.

Selected eleven fungal strains are primarily associated with the spoilage of fruits and vegetables and derived food products, making them unfit for human consumption and resulting in substantial losses.

The growth conditions of microbes, as per the recommendations of NCIM and MTCC, are presented in Table 2. All the microbial cultures were renewed by subculturing them bimonthly to maintain their viability and were stored on slants at $4\pm 1^\circ\text{C}$.

Chemicals

Agar powder, ethyl violet azide dextrose broth, MacConkey broth, nutrient broth, potato dextrose broth were supplied by Hi-Media Pvt. Ltd., India. Dimethylsulphoxide (DMSO), sodium chloride and Tween-80 were purchased from CDH, India.

Preparation of microbial strains inoculum

Bacterial strains inoculum

Bacterial cultures grown for 24 h in their respective broths (Table 2) were adjusted to McFarland standard 0.5, equivalent to 1.5×10^8 CFU/mL and were further diluted with broth to get 1×10^7 CFU/mL.

Fungal strains inoculum

To prepare fungal inoculum, spores of fungal strains were harvested from their pure PDA culture slants (15 days old) by adding 10 mL of sterilized distilled water and Tween 80 (0.05%) under aseptic conditions. Harvested spores were quantified by a hemocytometer to adjust at 1×10^7 spores/mL.

Determination of antibacterial and antifungal activities of bioactive components

Agar-well diffusion assay

Agar-well diffusion assay (Iroegbu and Nkere, 2005) was used for the determination of antibacterial activities of bioactive components. Sterile cork borer (diameter: 8 mm) was used to bore wells in the solidified media plates previously seeded with bacterial inoculum (100 μL). Subsequently, 10 μL of each bioactive component (liquid, concentration: 99.999%) was introduced independently in the wells of agar plates. Sterile DMSO, instead of bioactive components, served as the negative control. After the incubation, zones of inhibition formed around the wells were measured, and the results were expressed as the net zone of inhibition (mm), which represented the subtraction of the diame-

ter of the well from the measured zone.

Impregnated paper disc method

The impregnated paper disc method (Kim *et al.*, 2004) was followed to screen bioactive components' antifungal activities. Sterilized filter paper discs (6 mm) moistened with 5 μ L of bioactive components (liquid, concentration: 99.999%) were placed on the surface of solidified plates previously seeded with 100 μ L of fungal inoculum. Paper discs moistened with DMSO were used as a negative control. After the incubation period, inhibitory zones formed around the discs and were measured in mm, and the results were expressed as the net zone of inhibition (mm), which represented the subtraction of the diameter of the paper disc from the measured zone.

Broth dilution technique

The broth dilution technique determined all five bioactive components' minimum inhibitory concentrations (MICs) (Kim *et al.*, 2004). Samples were prepared from the procured bioactive components (liquid, concentration: 99.999%) by two-fold serial dilution in sterile broth (v/v), to give 16 different dilutions of 2000, 1000, 500, 250, 125, 62.50, 31.25, 15.62, 7.81, 3.90, 1.95, 0.97, 0.48, 0.24, 0.12, 0.06 μ L/mL. Sterile DMSO, instead of tested samples, was considered a negative control. Freshly prepared inoculum (100 μ L) of each microbial strain was added to the diluted solutions. These mixtures were incubated at suitable incubation temperatures for microbes. After the incubation, 100 μ L of the above mixture was evenly spread on the surface of solidified media petri plates with the help of a sterile bent glass rod. Seeded petri plates were incubated for 48 h (bacterial strains) and 72 h (fungal strains) to observe the lowest concentration (MIC) of bioactive components, in which there was no visible growth of tested microbes.

Statistical analysis

All the experiments were run in triplicates. Results of zone inhibition assays were analyzed using statistical analysis software SPSS version 7.5 and are represented as the Mean \pm SD.

RESULTS AND DISCUSSION

Antibacterial potentials of bioactive components

Data obtained from the results of agar well diffusion assay (Table 3) show that allyl isothiocyanate (AITC), cinnamic aldehyde (CIA), cuminic aldehyde (CUA), eugenol (EU) and menthol (MT), at a volume of 10 μ L/well, exhibited distinct growth inhibitory zones towards all the bacterial strains under observation. Tested substances exhibited wider inhibitory zones towards gram-positive bacteria (*B. cereus*, *S. aureus*) than gram-

negative bacteria (*E. coli*, *P. aeruginosa*, *P. alcaligenes*, *S. sonnei*). MT gave the widest zone towards *B. cereus* (18.00 mm) followed by *S. aureus* (16.20 mm), while AITC, CIA, CUA and EU produced the widest inhibitory zones against *S. aureus* followed by *B. cereus*. EU and MT exhibited the narrowest zones, measuring diameters of 12.10 mm and 8.00 mm, respectively, towards the gram-negative bacterial strain *E. faecalis*. On the other hand, AITC, CIA and CUA displayed the smallest inhibition zones with diameters of 25.00 mm, 22.00 mm and 18.50 mm, respectively, against gram-negative *E. coli*.

The present results are in agreement with some previous studies in which it has been reported that AITC exhibited a bactericidal effect against anaerobic foodborne bacteria *Campylobacter jejuni* and *Clostridium perfringens* (El Fayoumy, 2021) and EU was found to have an antibacterial effect against some pathogenic bacteria, wherein gram-positive bacterial strains (*Staphylococcus aureus* and Methicillin-resistant *S. aureus* (MRSA)) displayed wider inhibitory zones compared to gram-negative bacterial strains (*Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Serratia marcescens*) (Jayapal, 2021). Similarly, CIA, CUA and MT have shown inhibitory activities against *Aeromonas hydrophila*, *Bacillus* spp., verotoxin-producing *E. coli*, *Lactobacillus*, *Listeria monocytogenes*, *Salmonella*, *Shigella*, *S. aureus* and *Streptococcus* (Martínez-Pabón and Ortega-Cuadros, 2020; Doyale and Stephens, 2019; Aljaafari *et al.*, 2022; Quinto *et al.*, 2019; Monteiro-Neto *et al.*, 2020).

From the results of the broth dilution method (Table 4), it is pretty obvious that at a concentration level of 15.62 μ L/mL, CIA inhibited two bacterial strains (*B. cereus*, *S. aureus*) and CUA inhibited one bacterial strain (*B. cereus*), while AITC, EU and MT were not able to arrest the visible growth of bacterial strains (*B. cereus*, *S. aureus*) at referred concentration level.

Among all the pure components tested, the MIC values of CIA were lowest (15.62 μ L/mL - 62.50 μ L/mL), followed by CUA (15.62 μ L/mL - 125.00 μ L/mL), and the highest values were of MT (125.00 μ L/mL - 1000 μ L/mL). It is worth mentioning that test components inhibited gram-positive bacterial strains at lower concentration levels than gram-negative bacteria.

The greater susceptibility of gram-positive bacterial strains towards components during agar well diffusion assay and broth dilution method may be due to the absence of an outer membrane in their cell membrane, which makes them more sensitive to external environmental changes such as temperature, pH and other antimicrobial substances (Angane *et al.*, 2022).

As per present observations, antibacterial potency of bioactive components, based on the number of bacterial strains inhibited at a particular concentration level and in terms of increasing MIC values towards bacterial

Table 1. Tested bioactive components and their source spices

Bioactive components	Botanical names of source spices	Common names of source spices	Indian names of source spices
Allyl isothiocyanate	<i>Brassica juncea</i>	Brown Mustard	Sarson
	<i>Brassica nigra</i>	Black Mustard	Sarson
Cinnamic aldehyde	<i>Cinnamomum cassia</i>	Cassia	Daalchini
	<i>Cinnamomum zeylanicum</i>	Cinnamon	Daalchini
Cuminic aldehyde	<i>Cuminum cyminum</i>	Cumin	Jeera
Eugenol	<i>Ocimum sanctum</i>	Holy Basil	Tulsi
	<i>Syzygium aromaticum</i>	Clove	Laung
Menthol	<i>Mentha piperita</i>	Peppermint	Paudina

Table 2. Growth conditions of tested microorganisms

Tested microorganisms	Media used	Temperature of incubation	Duration of incubation
Bacterial strains			
<i>B. cereus</i> MTCC 430	Nutrient agar, Nutrient broth	30°C -32°C	24 h
<i>P. aeruginosa</i> MTCC 1688			
<i>P. alcaligenes</i> MTCC 405			
<i>S. sonnei</i> MTCC 2957			
<i>E. coli</i> MTCC 1687	MacConkey agar, MacConkey broth	45°C	24 h
<i>S. aureus</i> MTCC 5021	Nutrient agar, Nutrient broth	37°C	24 h
Fungal strains			
Eleven fungal strains	Potato Dextrose agar, Potato Dextrose broth	25°C - 28°C	48 h

MTCC: Microbial Type Culture Collection, Chandigarh, India.

Table 3. Inhibitory zones exhibited by bioactive components towards bacterial strains

Bacterial strains	Zones of inhibition (mm)					
	AITC (10 µL)	CIA (10 µL)	CUA (10 µL)	EU (10 µL)	MT (10 µL)	DMSO (10 µL)
<i>B. cereus</i>	33.80±0.22	34.00±0.54	30.00±0.22	30.20±0.17	18.00±0.81	ND
<i>E. faecalis</i>	30.00±0.47	27.50±0.21	21.50±0.21	12.10±0.25	8.00±0.34	ND
<i>E. coli</i>	25.00±0.20	22.00±0.26	18.50±0.15	18.20±0.47	9.10±0.31	ND
<i>P. aeruginosa</i>	30.20±0.17	31.00±0.23	24.00±0.20	30.00±1.71	15.80±0.41	ND
<i>P. alcaligenes</i>	30.00±0.39	28.50±0.33	20.00±0.36	20.10±0.23	9.20±0.21	ND
<i>S. sonnei</i>	30.20±0.64	32.00±0.41	24.00±0.44	26.00±0.33	15.70±0.24	ND
<i>S. aureus</i>	41.00±0.62	44.10±0.27	35.00±0.29	38.80±0.65	16.20±0.26	ND

AITC: Allyl isothiocyanate, CIA: Cinnamic aldehyde, CUA: Cuminic aldehyde, EU: Eugenol, MT: Menthol, DMSO: Dimethylsulphoxide.

Table 4. Minimum inhibitory concentrations of bioactive components towards bacterial strains

Bacterial strains	MICs (µL/mL)					
	AITC	CIA	CUA	EU	MT	DMSO
<i>B. cereus</i>	31.25	15.62	15.62	31.25	250.00	ND
<i>E. faecalis</i>	125.00	62.50	125.00	250.00	500.00	ND
<i>E. coli</i>	125.00	62.50	62.50	125.00	1000.00	ND
<i>P. aeruginosa</i>	250.00	62.50	62.50	125.00	1000.00	ND
<i>P. alcaligenes</i>	250.00	31.25	125.00	125.00	1000.00	ND
<i>S. sonnei</i>	125.00	31.25	62.50	250.00	500.00	ND
<i>S. aureus</i>	31.25	15.62	31.25	31.25	125.00	ND

AITC: Allyl isothiocyanate, CIA: Cinnamic aldehyde, CUA: Cuminic aldehyde, EU: Eugenol, MT: Menthol, DMSO: Dimethylsulphoxide.

strains, can be put in the order: CIA> CUA> AITC= EU> MT. This differential effect of bioactive components may be attributed to their different chemical structures, relative permeability through cell wall/ cell membrane and precise mode of action (Angane *et al.*, 2022). AITC is a non-phenolic organosulfur compound (Romeo *et al.*, 2018), CIA and CUA are aldehydes

(Doyale and Stephens, 2019; Aljaafari *et al.*, 2022; Aquil *et al.*, 2021), EU is phenolic in nature (Walsh *et al.*, 2019; Devi *et al.*, 2013), while MT is a terpenoid (Martínez-Pabón and Ortega-Cuadros, 2020; Pfuchtová *et al.*, 2018) (Fig. 1). The exact mechanisms of antimicrobial actions of referred components at molecular levels are not yet much understood and would re-

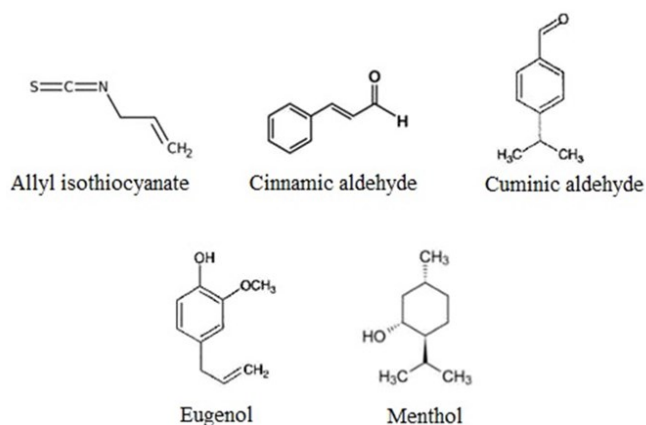


Fig. 1. Structures of tested bioactive components

main a line of further research. However, according to some previous research, it is most likely that spice EOs and their components affect microbial cells differently through various mechanisms, *i.e.*, either by attacking the cell wall, damaging the cell membrane, disrupting the protein synthesis and enzyme systems, coagulating cytoplasm, depleting proton motive force and compromising the genetic material of microbe (Vasconcelos *et al.*, 2018; La Stora *et al.*, 2011; Rattanachai-kunsopon *et al.*, 2010). Other related factors for different efficacies of referred substances may include their different molecular weight, pH, volatility, diffusion in growth medium and type of microorganism implicated in the study (Vasconcelos *et al.*, 2018; La Stora *et al.*, 2011; Rattanachai-kunsopon *et al.*, 2010).

Antifungal potentials of bioactive components

Results of impregnated paper disc method (Table 5) indicate that bioactive components at a volume of 5 μ L/disc, exhibited distinct growth inhibitory zones towards all the eleven fungal strains under investigation. The diameter of inhibitory zones (mm) varied with the type of fungal strain and bioactive component implicated in the study. AITC and CIA produced the widest zones

measuring diameters 50.20 mm and 53.00 mm, respectively, against *A. niger*, whereas CUA displayed widest inhibitory zones towards *P. exigua* (53.00 mm). On the other hand, EU and MT displayed widest zones with diameters 55.00 mm and 30.60 mm, respectively, against *C. herbarum*. *R. stolonifer* was the most resistant fungal strain by showing the narrowest zones towards four bioactive components, *i.e.*, AITC, CIA, EU and MT. It is also important to highlight that MT produced the smallest inhibitory zones against all the tested fungi. Antifungal activities of EU and CIA towards fungi (*Aspergillus* spp. and *Penicillium* spp.) and yeasts (*Candida* spp. and *Saccharomyces cerevisiae*) have been reported (Quinto *et al.*, 2019).

In the present study, while evaluating the MICs of bioactive components towards fungal strains (Table 6), it was noted that at a concentration level of 1.95 μ L/mL, CIA and CUA inhibited seven fungal strains (*A. solani*, *A. niger*, *C. herbarum*, *G. candidum*, *P. citrinum*, *P. expansum*, *P. exigua*), AITC inhibited six fungal strains (*A. solani*, *A. niger*, *B. cinerea*, *C. herbarum*, *G. candidum*, *F. oxysporum*), EU also inhibited six fungal strains (*A. niger*, *C. herbarum*, *G. candidum*, *P. citrinum*, *P. exigua*, *R. arrhizus*), while MT at the aforementioned level did not produce any antifungal effect. MIC values of CIA, CUA and EU were low and ranged from 1.95 μ L/mL - 7.81 μ L/mL. AITC up to 7.81 μ L/mL inhibited all the fungi under observation except *P. expansum*, which was inhibited at 15.62 μ L/mL. MIC values of MT towards tested fungi were highest and varied from 3.90 μ L/mL - 31.25 μ L/mL. Based on the number of fungal strains, the antifungal potential of bioactive components inhibited at a particular concentration level and in terms of increasing MIC, followed the sequence as: CIA= CUA > EU > AITC > MT. The reasons for the higher antifungal effectiveness of CIA and CUA and the lower effectiveness of MT against fungal strains are similar to those mentioned in the previous section (antibacterial potential of bioactive components) of this

Table 5. Inhibitory zones exhibited by bioactive components towards fungal strains

Fungal strains	Zones of inhibition (mm)					
	AITC (5 μ L)	CIA (5 μ L)	CUA (5 μ L)	EU (5 μ L)	MT (5 μ L)	DMSO(5 μ L)
<i>A. solani</i>	40.90 \pm 1.06	45.00 \pm 0.85	43.00 \pm 0.59	40.00 \pm 0.56	20.00 \pm 0.17	ND
<i>A. niger</i>	50.20 \pm 0.15	53.50 \pm 0.36	50.00 \pm 1.00	30.00 \pm 1.13	26.00 \pm 0.22	ND
<i>B. cinerea</i>	35.00 \pm 0.87	30.00 \pm 0.65	45.00 \pm 0.38	35.00 \pm 1.16	30.00 \pm 0.30	ND
<i>C. herbarum</i>	40.00 \pm 0.24	45.00 \pm 0.88	50.00 \pm 0.77	55.00 \pm 1.02	30.60 \pm 0.04	ND
<i>G. candidum</i>	33.50 \pm 0.08	41.50 \pm 0.43	39.00 \pm 0.81	40.50 \pm 0.78	22.00 \pm 0.31	ND
<i>F. oxysporum</i>	45.00 \pm 0.44	36.30 \pm 0.49	40.00 \pm 0.35	53.00 \pm 0.70	11.20 \pm 0.20	ND
<i>P. citrinum</i>	41.50 \pm 0.09	45.00 \pm 0.84	43.00 \pm 0.59	43.10 \pm 0.20	12.40 \pm 0.60	ND
<i>P. expansum</i>	34.00 \pm 0.12	22.50 \pm 0.34	42.00 \pm 0.78	37.00 \pm 0.78	14.00 \pm 0.32	ND
<i>P. exigua</i>	34.00 \pm 0.23	43.00 \pm 0.21	53.00 \pm 1.14	36.50 \pm 0.56	20.10 \pm 0.21	ND
<i>R. arrhizus</i>	33.10 \pm 0.19	26.50 \pm 0.41	47.00 \pm 0.95	28.00 \pm 1.41	18.00 \pm 0.00	ND
<i>R. stolonifer</i>	26.00 \pm 0.54	18.00 \pm 0.88	38.00 \pm 0.26	26.00 \pm 0.28	11.00 \pm 0.25	ND

AITC: Allyl isothiocyanate, CIA: Cinnamic aldehyde, CUA: Cumenic aldehyde, EU: Eugenol, MT: Menthol, DMSO: Dimethylsulphoxide.

Table 6. Minimum inhibitory concentrations of bioactive components towards fungal strains

Fungal strains	MICs ($\mu\text{L/mL}$)					
	AITC	CIA	CUA	EU	MT	DMSO
<i>A. solani</i>	1.95	1.95	1.95	3.90	3.90	ND
<i>A. niger</i>	1.95	1.95	1.95	1.95	15.62	ND
<i>B. cinerea</i>	1.95	3.90	3.90	7.81	3.90	ND
<i>C. herbarum</i>	1.95	1.95	3.90	1.95	7.81	ND
<i>G. candidum</i>	1.95	1.95	1.95	1.95	7.81	ND
<i>F. oxysporum</i>	1.95	3.90	3.90	3.90	7.81	ND
<i>P. citrinum</i>	7.81	1.95	1.95	1.95	7.81	ND
<i>P. expansum</i>	15.62	1.95	1.95	3.90	7.81	ND
<i>P. exigua</i>	7.81	1.95	7.81	1.95	15.62	ND
<i>R. arrhizus</i>	7.81	3.90	1.95	1.95	15.62	ND
<i>R. stolonifer</i>	7.81	3.90	7.81	3.90	15.62	ND

AITC: Allyl isothiocyanate, CIA: Cinnamic aldehyde, CUA: Cumenic aldehyde, EU: Eugenol, MT: Menthol, DMSO: Dimethylsulphoxide.

research paper (Vasconcelos *et al.*, 2018; La Storia *et al.*, 2011; Rattanachaikunsopon *et al.*, 2010). The greater susceptibility of fungal strains as compared to bacterial strains towards bioactive components in the present study may be due to the presence of sterols in the membranes of fungi (Mehta *et al.*, 2023; Garcia-Rubio *et al.*, 2020; Lima *et al.*, 2019; Sant *et al.*, 2016).

Conclusion

In conclusion, the present study indicated that allyl-isothiocyanate (AITC), cinnamic aldehyde (CIA), cuminic aldehyde (CUA), eugenol (EU), and menthol (MT) inhibited the growth of six bacterial strains (*B. cereus*, *E. coli*, *P. aeruginosa*, *P. alcaligenes*, *S. sonnei*, *S. aureus*) and eleven fungal strains (*A. solani*, *A. niger*, *B. cinerea*, *C. herbarum*, *F. oxysporum*, *G. candidum*, *P. citrinum*, *P. expansum*, *P. exigua*, *R. arrhizus*, *R. stolonifer*). CIA and CUA were most effective in inhibiting microbes, followed by EU and AITC, whereas MT proved least efficient. Furthermore, fungal strains were more susceptible towards tested components than bacterial strains. Among bacterial strains, gram-positive bacteria (*B. cereus*, *S. aureus*) were found to be more sensitive towards bioactive components than gram-negative bacteria (*E. coli*, *P. aeruginosa*, *P. alcaligenes*, *S. sonnei*). Thus, the present findings are encouraging and will update the existing information on antimicrobial potencies of natural substances, which would pave the way to further studies for establishing bioactive components of spice origin as 'green additives', to achieve a particular antimicrobial effect for food safety and health purposes. However, detailed studies involving interactions between bioactive components and other food ingredients are required; also, possible allergic effects of bioactive components on humans need to be addressed.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

- Aljaafari, M.N., Alkhoori, M.A., Hag-Ali, M., Cheng, W.H., Lim, S.H.E., Loh, J.Y. & Lai, K.S. (2022). Contribution of aldehydes and their derivatives to antimicrobial and immunomodulatory activities. *Molecules*. 27(11), 01-12. DOI: 10.3390/molecules27113589.
- Angane, M., Swift, S., Huang, K., Butts, C.A. & Quek, S.Y. (2022). Essential oils and their major components: an updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. *Foods*. 11 (3), 01-26. DOI: 10.3390/foods11030464.
- Aquil, F., Jeyabalan, J., Munagala, R., Ahmad, I., Schultz, D.J. & Gupta R.C. (2021). Cumin prevents 17 β -estradiol-associated breast cancer in ACI rats. *International Journal of Molecular Sciences*. 22(12), 01-17. DOI: 10.3390/ijms22126194.
- Bekuma, A. & Ahmed, W.M. (2018). Biopreservation, promising strategies to improve the safety and shelf-life of foods: A review. *International Journal of Microbiological Research*. 9(3), 76-80. DOI: 10.5829/idosi.ijmr.2018.76.80
- Chouhan, S., Sharma, K. & Guleria S. (2017). Antimicrobial activity of some essential oils-present status and future perspectives. *Medicines*. 4(3), 01-21. DOI: 10.3390/medicines4030058.
- De-Montizo-Prieto, S., Razola-Diaz, M.C., Gomez-Caravaca, A.M., Guerra-Haenandez, E.J., Jimenez-Valera, M., Garcia-Villanova, B., Ruiz-Bravo, A. & Verado V. (2021). Essential oils from fruits and vegetables, aro-

- matic herbs and spices : composition, antioxidant and antimicrobial activities. *Biology*. 10(11), 01-21. DOI: 10.3390/biology10111091.
7. Devi, K.P., Sakthivel, R., Nisha, S.A., Suganthy, N. & Pandian SK. (2013). Eugenol alters the integrity of cell membrane and acts against the nosocomial pathogen. *Proteus mirabilis*. *Archives of Pharmacal Research*. 36(3), 282-292. DOI: 10.1007/s12272-013-0028-3.
 8. Doyale, A.A. & Stephens, J.C. (2019). A review of cinnamaldehyde and its derivatives as antibacterial agents. *Fitoterapia*. 139, 01-18. DOI: 10.1016/j.fitote.2019.104405.
 9. Embuscado, M.E. (2015). Spices and herbs: Natural sources of antioxidants-A mini review. *Journal of Functional Foods*. 18, 811-819. DOI: 10.1016/j.jff.2015.03.005.
 10. El Fayoumy, R.A. (2021). Natural food preservation system as allyl isothiocyanate and edible brown seaweed *Laminaria japonica* against selected anaerobic foodborne bacteria: *Clostridium perfringens* and *Campylobacter jejuni*. *Journal of Microbiology, Biotechnology and Food Science*. 10(5), 01-04. DOI: 10.15414/jmbfs.1923.
 11. Garcia-Rubio, R., de Oliveira, H.C., Rivera, J. & Trevijano-Contador, N. (2020). The fungal cell wall: *Candida*, *Cryptococcus*, and *Aspergillus* Species. *Frontiers in Microbiology*. 10, 01-13. DOI: 10.3389/fmicb.2019.02993.
 12. Gutierrez-del-Rio, I., Fernandez, J. & Lombo F. (2018). Plant nutraceuticals as antimicrobial agents in food preservation: terpenoids, polyphenols and thiols. *International Journal of Antimicrobial Agents*. 52(3), 309-315. DOI: 10.1016/j.ijantimicag.2018.04.024.
 13. Hetta, H.F., Meshaal, A.K., Algammal, A.M., Yahia, R., Makhariha, R.R., Marraiki, N., Shah, M.A., Hassan, H.A.M. & Batiha GE. (2020). *In-vitro* antimicrobial activity of essential oils and spices, powder of some medicinal plants against *Bacillus* species isolated from raw and processed Meat. *Infection and Drug Resistance*. 13, 4367-4378. DOI: 10.2147/IDR.S277295.
 14. Iroegbu, C.U. & Nkere, C.K. (2005). Evaluation of the antibacterial properties of *Picralima nitida* stem bark extracts. *International Journal of Molecular Medicine and Advance Sciences*. 1, 182-189.
 15. Jayapal, V. (2021). Antimicrobial activity of eugenol against human pathogenic bacteria by minimal inhibitory concentration, minimal bactericidal concentration and disc-diffusion methods. *International Journal of Pharmaceutical Sciences and Research*. 12(1), 330-335. DOI: 10.13040/IJPSR.0975-8232.12(1).330-35.
 16. Kim, H.O., Park, S.W. & Park HD. (2004). Inactivation of *Escherichia coli* O157:H7 by cinnamic aldehyde purified from *Cinnamomum cassia* shoot. *Journal of Food Microbiology*. 21, 105-110. DOI:10.1016/S0740-0020(03)00010-8.
 17. La Stora, A., Ercolini, D., Marinello, F., di Pasqua, R., Villani, F. & Mauriello G. (2011). Atomic force microscopy analysis shows surface structure changes in carvacrol-treated bacterial cells. *Research in Microbiology*. 162(2), 164-172. DOI: 10.1016/j.resmic.2010.11.006.
 18. Lee, N.K. & Paik, H.D. (2016). Status, antimicrobial mechanism, and regulation of natural preservatives in livestock food systems. *Korean Journal for Food Sciences of Animal Resources*. 36(4), 547-557. DOI: 10.5851/kosfa.2016.36.4.547.
 19. Lima, L.S., Colombo, A.L. & de Almeida, J.N. (2019). Fungal cell wall: Emerging antifungals and drug resistance. *Frontiers in Microbiology*. 10, 01-09. DOI: 10.3389/fmicb.2019.02573.
 20. Martínez-Pabón, M.C. & Ortega-Cuadros, M. (2020). Thymol, menthol and eucalyptol as agents for microbiological control in the oral cavity: A scoping review. *Revista Colombiana de Ciencias Químico-Farmacéuticas*. 49(1), 44-69. DOI: 10.15446/rcciquifa.v49n1.87006.
 21. Mehta, D., Saini, V. & Bajaj A. (2023). Recent developments in membrane targeting antifungal agents to mitigate antifungal resistance. *RSC Medicinal Chemistry*. 14, 1603-1628. DOI: 10.1039/D3MD00151B.
 22. Monteiro-Neto, V., de Souza, C.D., Gonzaga, L.F., da Silveira, B.C., Sousa, N.C.F. & Pontes, J.P. (2020). Cuminaldehyde potentiates the antimicrobial actions of iprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. *Plos One*. 15(5), 01-14. DOI: 10.1371/journal.pone.0232987.
 23. Myszka, K., Leja, K. & Majcher, M.A. (2019). A current opinion on the antimicrobial importance of popular pepper essential and its application in food industry. *Journal of Essential oil Research*. 31(1), 01-18. DOI: 10.1080/10412905.2018.1511482.
 24. Papadochristopoulos, A., Kerry, J.P., Fegan, N., Burgess, C.M. & Duffy, G. (2021). Natural anti-microbials for enhanced microbial safety and shelf-life of processed packaged meat. *Foods*. 10(7), 01-42. DOI: 10.3390/foods10071598.
 25. Pfluchtová, M., Gervasib, T., Benameur, Q., Pellizzerib, V., Grul'ová, D., Camponed, L., Sedláke, V. & Cicerob N. (2018). Antimicrobial Activity of two *Mentha* species essential oil and its dependence on different origin and chemical diversity. *Natural Products Communications*. 13 (8), 1051-1054. DOI: 10.1177/1934578X1801300832.
 26. Quinto, E.J., Caro, I., Villalobos-Delgado, L.H., Mateo, J., De-Mateo-Silleras, B. & Redondo-Del-Río, M.P. (2019). *Food Safety through Natural Antimicrobials*. *Antibiotics*. 8(4), 01-30. DOI: 10.3390/antibiotics8040208.
 27. Rattanachaiakunsopon, P. & Phumkhachorn, P. (2010). Assessment of factors influencing antimicrobial activity of carvacrol and cymene against *Vibrio cholerae* in food. *Journal of Bioscience and Bioengineering*. 110(5), 614-619. DOI: 10.1016/j.jbiosc.2010.06.010.
 28. Romeo, L., Iori, R., Rollin, P., Bramanti, P. & Mazzon E. (2018). Isothiocyanates : An overview of their antimicrobial activity against human infections. *Molecules*. 23(3), 01-18. DOI: 10.3390/molecules23030624.
 29. Sant, D.G., Tupe, S.G., Ramana, C.V. & Deshpande, M.V. (2016). Fungal cell membrane-promising drug target for antifungal therapy. *Journal of Applied Microbiology*. 121(6), 1498-1510. DOI: 10.1111/jam.13301.
 30. Sharifi-Rad, J., El Rayess, Y., Abi Rizk, A., Sadaka, C., Zgheib, R. & Zam, W. (2020). Turmeric and its major compound curcumin on health: Bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. *Frontiers in Pharmacology*. 11, 01-23. DOI: 10.3389/fphar.2020.01021.
 31. Vasconcelos, N.G., Croda, J. & Simionatto, S. (2018). Antibacterial mechanisms of cinnamon and its constituents: A review. *Microbial Pathogenesis*. 120, 198-203. DOI:10.1016/micpath2018.04.036.
 32. Walsh, D.J., Livinghouse, T., Goeres, D.M., Mettler, M. & Stewart, P.S. (2019). Antimicrobial activity of naturally occurring phenols and derivatives against biofilm and planktonic bacteria. *Frontiers in chemistry*. 7, 01-13. DOI: 10.3389/fchem.2019.00653.