



**ANTIBACTERIAL EFFECTS OF CERTAIN SPICE EXTRACTS ON PATHOGENS  
ISOLATED FROM A FRESHWATER CRAB, *OZIOTELPHUSA SENEX SENEX***

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**ABSTRACT**

Indian spices play an important role in traditional cooking and possess high medicinal value. The present study evaluates the antimicrobial activity of six Indian spice extracts, which are used in Indian cooking for centuries namely Cumin seed (*Cuminum cyminum*), Curry leaf (*Murraya koenigii*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Pepper (*Piper nigrum*) and Turmeric (*Curcuma longa*). These extracts were tested against the pathogens such as *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella paratyphi A*, *Salmonella paratyphi B* and *Salmonella typhi* isolated from the freshwater crab, *Oziotelphusa senex senex*. The antimicrobial activity was investigated by disc diffusion method. Certain bacteria showed sensitivity towards certain spice extracts. Therefore, from the present study it is evident that the extracts of Pepper (*Piper nigrum*) and Turmeric (*Curcuma longa*) showed highest antibacterial activity against the pathogens.

**KEYWORDS:** Crabs, Bacterial Isolate, Spices, Antimicrobial Properties.

**INTRODUCTION**

International Committee for Microbiological Food Safety (ICMFS) has conceived tolerable counts for various pathogens in different food products. In all the parts of the world the microbiological value of seafood has significant health hazards and cause economic fatalities (Soundarapandian and Sowmiya, 2013). The United States Environmental Protection Agency (USEPA) recommends that the bacterium *Escherichia coli* is used as an indicator of faecal contamination in freshwaters as it is present in high quantities in the gastrointestinal tract of healthy animals and humans (Dufour, 1984; USEPA, 1986; McLellan and Salmore, 2003). Since the freshwater crabs are available round the year, consumers prefer these crabs as a food source. Consumers have little or no awareness about the bacterial contamination in the crabs. The crabs that are consumed have loads of microorganisms which get transmitted effortlessly from one living being to another, and they can remain in the same host causing various food borne problems (Varadharajan et al., 2012). Indian spices have an essential role both in the health care system and food industry. Early literature has a treasure of information related to Indian spices and health care which has been well-thought-out for the present study (Sofia et al., 2007). The food borne pathogens isolated from the crabs are sensitive to various extracts of spices (Arora and Kaur, 1999). The natural compounds originate in various

spices possess antimicrobial property and guards us from foodborne diseases (Hatha et al., 2006 and Pandey et al., 2014). The aim of the present study is to investigate the antibacterial effects of certain Indian spices against the pathogenic bacteria isolated from the freshwater crab, *Oziotelphusa senex senex*.

**METHODOLOGY**

**Collection of Samples**

The most common edible freshwater crab, *Oziotelphusa senex senex* was collected from the local market in Porur, Chennai. The crabs were immediately transferred to the laboratory within 24 hours. The crab was dissected to obtain different tissues and these tissues were subjected to bacterial analysis.

**Isolation and confirmation of pathogens**

The isolated bacteria were confirmed by using standard parameters in accordance with Bergery's manual (Garrity et al., 2005) followed by microscopic examination and biochemical characterization (Cappuccino and Sherman, 1999). The isolates were inoculated on selective media like Eosin-Methylene blue agar, *Pseudomonas* agar (APHA, 1998), blood agar, *Aeromonas* Agar with ampicillin, starch hydrolysis agar, mannitol salt agar, *Salmonella-Shigella* agar, luminescent agar and TCBS agar to confirm the bacteria isolates.

### Preparation of certain Spices extracts

For the present study the extracts were prepared from the Indian spices namely Cumin seed (*Cuminum cyminum*), Curry leaf (*Murraya koenigii*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Pepper (*Piper nigrum*) and Turmeric (*Curcuma longa*). The extracts of each spice was prepared by taking 1g of dry spice in 10ml acetone. After 48hrs the extracts were used to test its antibacterial effects.

### Antibacterial sensitivity Test by Paper disc diffusion method (Kirby Bauer Technique)

The discs with various concentrations of spice extracts such as Cumin seed (*Cuminum cyminum*), Curry leaf (*Murraya koenigii*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Pepper (*Piper nigrum*) and Turmeric (*Curcuma longa*) were placed on the surface of inoculated Muller Hinton agar plates. After 24hrs of incubation at 37°C the diameter of the zones of inhibition was recorded. Control assay discs impregnated with sterile distilled water, without spices, were used. All the analyses were done in triplicates (Black, 2002).

### RESULTS AND DISCUSSION

Indian spices used in traditional cooking contain high amount of secondary metabolites due to these metabolites they have high antimicrobial activity and it can be used as good preservative and it can also be used for medicinal purpose (Pandey and Khan, 2013). In the present study the Indian spices such as Cumin seed (*Cuminum cyminum*), Curry leaf (*Murraya koenigii*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Pepper (*Piper nigrum*) and Turmeric (*Curcuma longa*) are measured to check its antibacterial effect against pathogens.

Bacteria isolated from the hemolymph of normal blue crabs were found to be predominantly *Vibrio* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Bacillus* sp., *Flavobacterium* sp., and coliforms. *Vibrio parahaemolyticus*, a cosmopolitan facultative pathogen broadly implicated in outbreaks of gastroenteritis related to the consumption of inappropriately processed seafood, was present in crabs collected between the months of May to November and was identified in up to 21% of the hemolymph sampled (Sizemore et al., 1975). The food borne pathogens present in the edible freshwater crab,

*Ozotetelphusa senex senex* can be a causative agent for various foodborne diseases. The foodborne pathogens isolated from the edible freshwater crab, *Ozotetelphusa senex senex* are tabulated in Table 1. The results confirmed that the crab contained high pathogenic bacterial load which are made-up to be threat to food safety generating food borne diseases (Saima et al., 2012).

Antibiotic toxicity and multi drug resistant pathogens are the two greatest challenges being faced by today's medical world (Pandey and Khan, 2013). In the present study, the antimicrobial activity of spices has been explored as an alternative to antibiotics in order to challenge these hazards.

In the present study, a total of six extracts of certain spices namely Cumin seed (*Cuminum cyminum*), Curry leaf (*Murraya koenigii*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Pepper (*Piper nigrum*) and Turmeric (*Curcuma longa*) were evaluated for their antibacterial activity. All the extracts showed antibacterial activity against all the bacterial isolates (Table 2). The extracts of Turmeric (*Curcuma longa*) and Pepper (*Piper nigrum*) showed maximum zone of inhibition against the isolated pathogens from freshwater crab, *Ozotetelphusa senex senex*. Based on the findings, these extracts may be substitute to chemical preservatives and can be used as natural antimicrobial preservatives to reclaim the shelf-life of food (Pundir and Jain, 2010).

Thus from the present study, the amount of high counts of pathogens in crustacean flesh may cause zoonotic diseases; especially in individuals who consume fish food raw, lightly or insufficiently cooked. Thus, it is sensible to avoid consuming uncooked aquatic feed. The current research mentions continuous monitoring of the total microbial load before the crustacean is commercially sold. These spices act through their natural inhibitory mechanisms either by inhibiting or killing the pathogens completely. With the increasing awareness of people towards natural food and natural therapies, spices might act as the most obvious alternative. In developing countries like India, where spices are produced and used as food additives, their use as antimicrobial agents and potential preservatives can be extremely useful.

**TABLE: 1. MICROBIAL DIVERSITY OF OZOTETELPHUSA SENEX SENEX**

CRABS	HEMOLYMPH	GILLS	MUSCLE	GUT	OVARY/ TESTIS
<i>Ozotetelphusa senex senex</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Bacillus subtilis</i>	<i>Bacillus subtilis</i>
	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
	<i>Salmonella paratyphi B</i>	<i>Salmonella paratyphi B</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Salmonella paratyphi A</i>
	<i>Salmonella typhi</i>	<i>Salmonella typhi</i>		<i>Salmonella typhi</i>	<i>Salmonella paratyphi B</i> <i>Salmonella typhi</i>

**TABLE: 2. DISC DIFFUSION (mm) ANTIBACTERIAL ACTIVITY OF SPICES**

S.NO.	BACTERIA	CONTROL	CUMIN	CURRY LEAF	GARLIC	GINGER	PEPPER	TURMERIC
1	Bacillus subtilis	16(S)	16(S)	16(S)	19(S)	17(S)	14(S)	19(S)
2	Escherichia coli	23(S)	12(I)	09(I)	7 (R)	11(I)	20(S)	21(S)
3	Klebsiella pneumoniae	20(S)	11(I)	19(S)	12(I)	12(I)	16(S)	19(S)
4	Pseudomonas aeruginosa	17(S)	08(R)	16(S)	10(I)	16(S)	13(S)	18(S)
5	Salmonella paratyphi A	21(S)	12(I)	12(I)	12(I)	09(I)	15(S)	10(I)
6	Salmonella paratyphi B	23(S)	15(S)	14(S)	17(S)	13(S)	19(S)	16(S)
7	Salmonella typhi	17(S)	11(I)	12(S)	19(S)	17(S)	15(S)	20(S)

(S) – sensitive ; (I) – intermediate; (R) - resistance

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