Antimicrobial Activity of Some of the Indian Spices Against Food Borne Pathogens

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ABSTRACT: Antimicrobial activity of 25, 50, 75 & 100 % alcohol extract of *Cinnamomum verum* and *Syzygium aromaticum* has been evaluated against bacterial strains of *Pseudomonas lundensis*, *Bacillus cereus*, and fungal strains of *Aspergillus flavus*, *Aspergillus niger*. Extracts from both *Cinnamomum verum* and *Syzygium aromaticum*, showed excellent antimicrobial activity against all the test organisms. In 25 and 50% concentration, *Cinnamomum verum* showed the highest of 19 mm and 16 mm antibacterial zone against *Bacillus cereus* and *Pseudomonas lundensis* respectively. In 75 and 100 % concentration, *Syzygium aromaticum* showed the highest of 15 mm and 21 mm of antimicrobial zone against *Aspergillus niger* and *Bacillus cereus* respectively. Among all the results obtained, the maximum antimicrobial zone formation with minimal concentration was recorded with 100 % extract of *Syzygium aromaticum* against *Bacillus cereus* with 21 mm of antibacterial zone and with 100 % extract of *Syzygium aromaticum*, with 18 mm of antifungal zone against *Aspergillus niger*. It was concluded that the herbals *Cinnamomum verum* and *Syzygium aromaticum* has resistivity against food borne pathogens.
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Keywords: Antimicrobial activity, *Pseudomonas lundensis*, *Bacillus cereus*, *Aspergillus niger* and *Aspergillus flavus.*

INTRODUCTION

Food borne pathogens such as *Pseudomonas lundensis*, *Bacillus cereus*, *Aspergillus niger* and *Aspergillus flavus* are widely distributed in nature, causing considerable mortality and morbidity in the population. It is well known that pseudomonads are ubiquitous bacteria in nature. Due to their ability to utilize a wide range of organic compounds, they occupy an important ecological position in the carbon cycle. Therefore, the ecology of pseudomonads in the biosphere has been a matter of interest.

Bacillus cereus has been recognized as an agent of food poisoning since 1955. Between 1972 and 1986, 52 outbreaks of food-borne disease associated with *B. cereus* were reported[1]. Bacillus food poisoning strains from 39 outbreaks were identified. *B. cereus* in 23 outbreaks, *B. thuringiensis* in 4, *B. mycoides* in 1 and mixed strains of Bacillus in 11 outbreaks[2]. *A. flavus* produce aflatoxin, which can cause acute hepatitis, immunosuppression, and hepatocellular carcinoma[3]. The absence of any regulation of screening for the fungus also has a high prevalence of viral hepatitis, highly increases the risk of hepatocellular carcinoma[4].

Aspergillus niger, if inhaled with large amounts of spores, causes a serious lung disease, aspergillosis. *A. niger* is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss and, in severe cases, damage to the ear canal and tympanic membrane.

These bacteria have broad host range and have often been isolated from humans with diarrhea[5]. Since the introduction of antibiotics there has been tremendous increase in the resistance of diverse bacterial pathogens[6,7]. This shift in susceptibility greatly affects our ability to successfully treat patients empirically. Plant derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80 % of the world population rely on botanical preparations as medicines to meet their health needs. Spices are generally considered safe and proved to be effective against certain ailments[8]. They are also extensively used, particularly, in many Asian, African and other countries. In recent years, in view of their beneficial effects, use of spices has been gradually increasing in developed countries also.

In the present study, we have evaluated the antibacterial effect of the extracts of two widely used spices in India, such as *Cinnamomum verum* and *Syzygium aromaticum* has been evaluated against two bacterial strains of food borne pathogens such as *Pseudomonas lundensis*, *Bacillus cereus*, and two fungal strains of food borne pathogens such as

Aspergillus niger and *Aspergillus flavus*, and the results are discussed.

MATERIALS AND METHODS

Microorganisms

Pseudomonas lundensis, *Bacillus cereus*, *Aspergillus niger* and *Aspergillus flavus* were the pathogenic microorganisms included in the study. All the cultures were obtained in pure form from the culture collection of Institute of Microbial Technology (IMTECH), Chandigarh, India.

Preparation of Spices Extracts

The fresh spices were obtained from the local market. The spices were cleaned, descaled when necessary, and washed in sterile distilled water. In order to obtain the spice's extracts, about 100g of washed spice were crushed with mortar and pestle. The extracts were sieved through a fine mesh cloth and sterilized using membrane filter (0.45 - micron sterile filter). This extract was considered as the 100 % concentration of the extract.

The concentrations, 75, 50 and 25 % were made by diluting the concentrated extract with appropriate volumes of sterile distilled water.

Antimicrobial sensitivity testing using filter paper method

Filer paper discs of 7 mm diameter were prepared and sterilized. Using an ethanol dipped and flamed forceps, these discs were aseptically placed over nutrient agar plates seeded with the respective test organisms[9]. One hundred microlitres of the various spices' extract (100, 75, 50, 25 %) were aseptically transferred to these discs. The plates were incubated in an upright position at 37 °C for 24 hours. The diameter of inhibition zones were measured in mm and the results were recorded. Inhibition zones with diameter less than 12 mm were considered as having no antimicrobial activity. Diameters between 12 and 16 mm were considered moderately active, and these with > 16 mm were considered highly active.

All the media used in the present investigation were obtained from Hi-Media Laboratories Ltd., Mumbai, India.

RESULTS AND DISCUSSION

Among the two spices tested, against four food borne pathogens, both the spices tested, showed excellent antimicrobial activity. The results of the antimicrobial activity against the tested pathogens are given in Table 1.

Table 1: Antibacterial activity of different concentrations of Spices' Extract

Pathogenic Organisms	Diameter of Inhibition Zone in mm against various concentrations of Spices Extract							
	Cinnamomum verum				*Syzygium aromaticum*			
	25 %	50 %	75 %	100 %	25 %	50 %	75 %	100 %
Pseudomonas lundensis	2 mm	16 mm	-	8 mm	3 mm	-	3 mm	11 mm
Bacillus cereus	19 mm	5 mm	2 mm	-	3 mm	11 mm	1 mm	21 mm
Aspergillus niger	2 mm	15 mm	11 mm	16 mm	10 mm	12 mm	10 mm	18 mm
Aspergillus flavus	14 mm	15 mm	2 mm	10 mm	13 mm	14 mm	15 mm	10 mm

In 25 % concentration, *Cinnamomum verum and Syzygium aromaticum* showed the highest of 19 mm, 14 mm and 11 mm and 14 mm antimicrobial zone against *Bacillus cereus* and *Aspergillus flavus*, respectively. In 50 % concentration, *Cinnamomum verum* showed the highest of 16 mm antibacterial zone against *Pseudomonas lundensis* and a maximum of 15 mm antifungal zone against *Aspergillus niger* and *Aspergillus flavus*, where *Syzygium aromaticum* showed the highest antibacterial zone of 11 mm against *Bacillus cereus* and the highest of 14 mm antifungal zone against *Aspergillus niger*. In 75 % concentration, antibacterial zone was not found so significant, whereas *Cinnamomum verum* showed prominent antifungal zone against *Aspergillus niger*, where antifungal zone was prominent for *Cinnamomum verum* with 11 mm against *Aspergillus niger* and 15 mm against *Aspergillus flavus*. In 100 % concentration, antibacterial zone was found significant only with *Syzygium aromaticum* against *Bacillus cereus*, where antifungal zone was prominently found with 16mm against *Aspergillus niger*, *Syzygium aromaticum* with 21 mm against *Bacillus cereus*. Among all the results obtained, the

maximum antimicrobial zone formation with minimal concentration was recorded with 100 % extract of *Syzygium aromaticum* against *Bacillus cereus* with 21 mm of antibacterial zone and with 100 % extract of *Syzygium aromaticum*, with 18mm of antifungal zone against *Aspergillus niger.*

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