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Antimicrobial Potential of Garlic and Cinnamon Extracts Against Foodborne Pathogens

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ABSTRACT: Garlic and cinnamon had a nutritional importance and traditional medicine. They can be used to inhibit such pathogens and also to increase shelf life and safety of food products. The current study evaluated the antimicrobial activity of garlic and cinnamon extract against isolated foodborne pathogens Klebsiella spp., Salmonella typhi, Micrococcus spp., Clostridium perfringens, Bacillus cereus, Staphylococcus aureus, Penicillium spp. and Aspergillus oryzae. For cinnamon extract, Klebsiella spp. showed the highest zone of inhibition (16mm) followed by Clostridium perfringens least inhibition zone is observed in Staphylococcus aureus (8mm) and in garlic extract, Micrococcus spp. showed the highest zone of inhibition (12mm) followed by Salmonella typhi (10mm) and Clostridium perfringens showed very least zone of inhibition(7mm). Bacillus cereus showed resistance towards garlic extract. Cinnamon extract showed the antifungal activity with zone of inhibition of 12mm and 11mm against Penicillium spp. and Aspergillus oryzae respectively. While the garlic extract showed resistance against fungal species.

Key words: Klebsiella spp., Salmonella typhi, Micrococcus spp., Clostridium perfringens, Bacillus cereus, Staphylococcus aureus, Penicillium

I. INTRODUCTION:

Safety Food is a known problem worldwide and affecting hundreds of millions of people that suffer from contaminated food. Nowadays, consumers are continuously concerned with the growing number of illnesses caused by some pathogenic and spoilage microorganisms in food and also for the safety of foods containing synthetic preservatives. There is growing interest in using natural antimicrobial compounds like extracts of herbs, for food conservation [1]. Food borne disease is an increasingly serious public health problem all over the world. The main cause is determined to be microorganisms. The control of pathogens may significantly reduce the food borne disease outbreaks [2].

The high prevalence of infection and long-term exposure to antibiotics has led to the antibiotic resistance of microorganisms. Therefore, much attention has been paid to the discovery and development of new antimicrobial agents that might act against these resistant microorganisms, and spices (Cinnamon & Garlic) could be an interesting candidate [3]. Since the ancient times, they have been used for preventing food spoilage and deterioration and also for extending the shelf life of foods [4].

Garlic (Allium sativum), belongs to the Liliaceae family),mainly the bulb of garlic, has been used as a spice in cooking worldwide especially in Southeast Asia and Italy. Garlic is one of the oldest known nutritional and medicinal plants and has long been used in human pathogens treatment [5]. The great scientist Louis Pasteur was the first described the antibacterial activity of garlic juices against both Gram positive and Gram-negative bacteria [6]. Garlic is a strong antibacterial agent against both Gram-positive and Gram-negative bacteria such as Streptococcus spp., Staphylococcus aureus, E. coli, Salmonella spp., Klebsiella spp., Proteus mirabilis, Shigella dysenteriae, Pseudomonas aeruginosa and Helicobacter pylori [7].

Cinnamon belongs to the Lauraceae family. The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines. Overall, approximately 250 species have been identified among the cinnamon genus, with trees being scattered all over the world [8]. The Cinnamon barks are commonly used to treat various disorders and are known to exert antibacterial, antifungal,

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antioxidant, antidiabetic, anti-inflammatory, nematicidal, insecticidal and anticancer [9] effects. The most important phenolic compounds in cinnamon plants are cinnamaldehyde, cinnamate and cinnamic acid. Except that, the presence and concentration of each compound vary depending on the plant part, trans-cinnamaldehyde in bark was shown to be responsible for antimicrobial activity in cinnamon extracts [10]. Hence the purpose of this study is to estimate the antimicrobial activity of garlic and cinnamon extract against foodborne pathogens.

II. MATERIALS AND METHODS:

Type of Study: Prospective study
Place of study: Chennai, Tamil Nadu
Duration of study: One month

The spoiled food (Banana, Meat, Cheese, Rice) samples were collected and which were inoculated into nutrient agar (Quadrant streak) and Potato dextrose agar (Single streak) incubated at 37° for 24 hours, and 48 hours respectively. The most potent isolates were selected, gram staining was done as preliminary process. The colony characters were recorded and further identification of organisms was carried out by endospore stain and biochemical characterization methods. The microbes were sub-cultured on nutrient agar. Lactophenol Cotton Blue is a stain which is used for making semi-permanent microscopic preparation of fungi. It is the most commonly used method in the mycology laboratory to identify filamentous fungi. Identification of filamentous fungi is made by their characteristic microscopic morphology such as shape, size, arrangement of spores and hyphae.

COLLECTION OF SPICES & PREPARATION OF AQUEOUS DECOCTION [4]:

Garlic and Cinnamon were collected from the local market.The aqueous decoction of garlic and cinnamon were prepared by boiling 5g in 50ml sterile distilled water in flask for 20 minutes. Then flask was removed from heat and decoction allowed to cool. After cooling the content of flasks were filtered by Whatman filter paper to obtain clear decoction.

ANTIMICROBIAL ACTIVITY TESTING USING DISC DIFFUSION METHOD:

A simple and still widely used method is the disk diffusion test, which determines susceptibility of an organisms. Discs of 6 mm in diameter were punched out using filter paper with paper punch. Then discs were sterilized. Sterilized disc of filter paper (6 mm diameter) was soaked in decoction for 1-2 minutes then used for AST.

III. RESULTS AND DISCUSSION:

The various spoiled food samples were collected from local market of Chennai, 8 isolates have been identified. They are: Salmonella typhi,Klebsiella spp.,Clostridium perfringens,Staphylococcus aureus,Micrococcus spp., Bacillus cereus, Aspergillus oryzae and Penicillium species

MORPHOLOGICAL CHARACTERIZATION

The identified isolates were characterization the basis of colour, shape, texture, margin, arrangement and staining characteristics.

Fig 1: Gram staining shown Gram-positive bacilli:

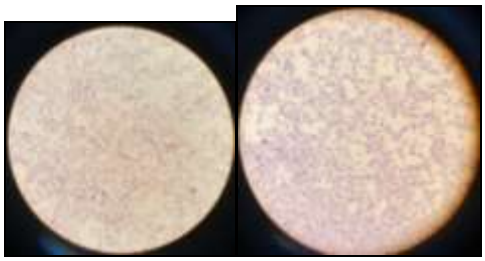
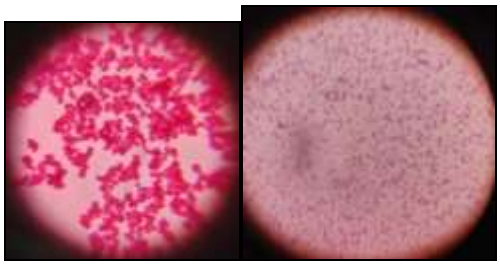


Fig 2: Gram staining shown Gram-negative bacilli:



BIOCHEMICAL CHARACTERIZATION: The six isolates were characterized on the basis of biochemical tests. Staphylococcus aureus is a Gram-positive coccus arranged in clusters, it shows positive results for catalase, coagulase, MR, VP, citrate and urease. Negative results for indole and oxidase. It produces acid slant and acid butt in TSI. Micrococcus spp. is a Gram-positive coccus arranged in irregular clusters, groups of four or of eight. It shows negative results for coagulase, indole and citrate. Positive results for catalase and oxidase. Bacillus cereus is a Gram-positive rod and

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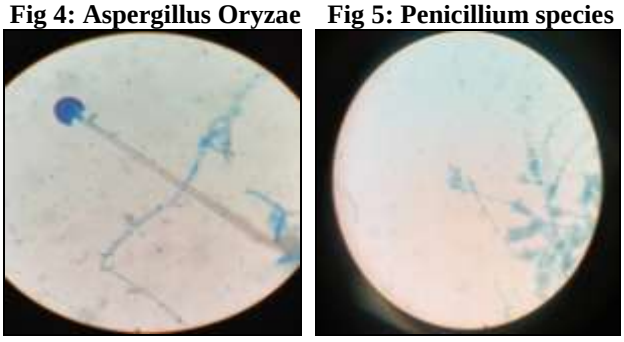
produce spores, it shows positive results for urease, catalase, voges-proskauer, oxidase and nitrate and negative results for methyl red and indole. Clostridium perfringens sis a Gram-positive rod shaped, non-motile and forms pores, it shows negative results for catalase, indole, oxidase, voges-proskauer and urease and positive results for nitrate and methyl red. Klebsiella spp. is a Gram-negative bacillus and non-motile, it shows positive results for catalase, citrate, nitrate and Voges-Proskauer. Negative results for indole, oxidase, methyl red, urease. It produces acid slant and acid butt with gas production in triple sugar iron test. Salmonella typhi is a Gram-negative bacillus, it shows negative results for indole, VP, citrate, urease and oxidase, positive results for catalase and methyl red. It produces alkaline slant and acid butt with H_2S production no gas in TSI. **(Table 1) (Fig 1-3)**

Table 1: Biochemical characterization of the isolated colony

PROPERTIES	ORGANISMS					
	Staphylococcus aureus	**Micrococcus spp.**	**Bacillus cereus**	**Clostridium perfringens**	**Klebsiella spp.**	**Salmonella typhi**
Indole	-	-	-	-	-	-
MR	+	NA	-	-	-	+
VP	+	NA	+	+	+	-
Citrate	+	-	+	+	+	-
Urease	+	+	+	-	-	-
TSI	Acid/Acid	Alkaline/ Alkaline	NA	NA	Acid/Acid with gas	Alkaline/Acid with H_2S
Catalase	+	+	+	-	+	+
Oxidase	-	+	+	-	-	-
Coagulase	+	-	NA	NA	-	-
Gram stain	+	+	+	+	-	-
Endospore stain	-	-	+	+	-	-

LACTOPHENOL COTTON BLUE STAIN: Two fungal spp., were identified by Lactophenol Cotton Blue stain. They are, Aspergillus Oryzae & Penicillium species (Fig 4 & 5).

Fig 4: Aspergillus Oryzae **Fig 5: Penicillium species**



ANTIMICROBIAL ACTIVITY OF GARLIC AND CINNAMON EXTRACTS BY AST METHOD [4]: In cinnamon extract, Klebsiella spp. showed the highest zone of inhibition (16mm) followed by Clostridium perfringens and micrococcus spp. (12mm), Salmonella typhi (11mm), bacillus cereus (9mm) and Staphylococcus aureus (8mm). In garlic extract, micrococcus spp. showed the highest zone of inhibition (12mm) followed by Salmonella typhi

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(10mm), Klebsiella spp. (8mm), Staphylococcus aureus and Clostridium perfringens (7mm). Bacillus cereus shows resistant. Cinnamon extract showed the antifungal activity with zone of inhibition of 12mm and 11mm against penicillium species and aspergillus oryzae respectively. While the garlic extract showed resistant against fungal species. Cinnamon extract shows zone of inhibition of 16mm against Klebsiella spp., which is the highest zone of inhibition in this study. **(Chart 1) (Fig 6)**

Chart 1: The antimicrobial activity of garlic and cinnamon extracts by AST method.

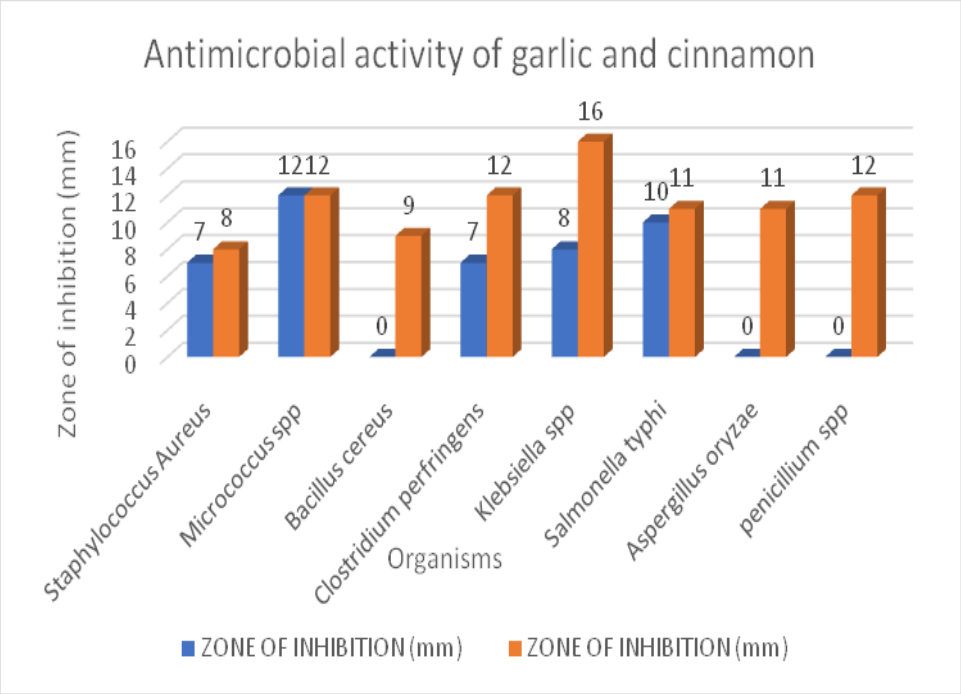

Fig 6: Antibacterial activity of garlic and cinnamon extracts against isolated pathogens:

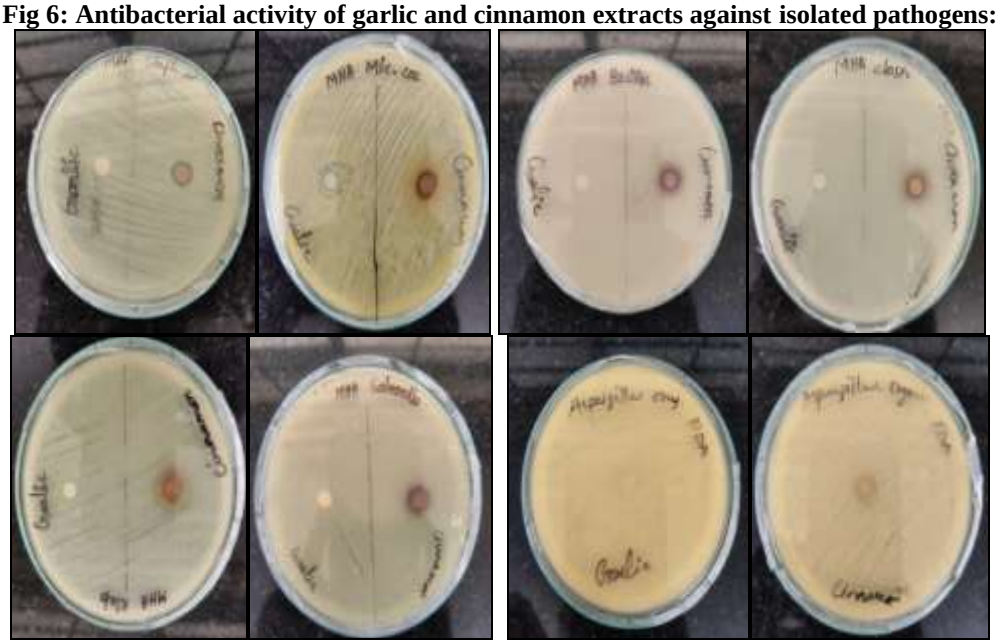

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Table 3: Biostatistical inference

ORGANISMS	GARLIC	CINNAMON
Staphylococcus aureus	6.77 ±0.20	7.57 ± 0.45
Micrococcus spp.	11.57 ± 0.30	11.33 ± 0.29
Bacillus cereus	0	8.57 ± 0.20
Clostridium Perfringens	6.50 ± 0.45	11.63 ± 0.37
Klebsiella spp.	7.47 ± 0.28	15.67 ± 0.39
Salmonella typhi	9.50 ± 0.25	10.53 ± 0.34
Aspergillus oryzae	0	10.27 ± 0.14
Penicillium species	0	11.47 ± 0.30

Values are in Mean ± Standard Deviation

In garlic extract, Micrococcus spp. shown the maximum mean value (11.57) followed by Salmonella typhi (9.50), Klebsiella spp. (7.47), Staphylococcus aureus and Clostridium perfringens. Bacillus cereus shows resistant. In cinnamon extract, Klebsiella spp. shown the maximum mean value (15.67) followed by Clostridium perfringens (11.63) and micrococcus spp. (11.33), Salmonella typhi (11mm), bacillus cereus (8.57) and Staphylococcus aureus (7.57). Cinnamon extract shown the antifungal activity with mean values of 11.47 and 10.27 against penicillium species and aspergillus oryzae respectively. While the garlic extract showed resistant against fungal species. Cinnamon extract shown mean value of 15.67 against Klebsiella spp., which is the highest mean value in this study **(Table 3)**.

The natural products are found to be more effective with least side effects as compared to commercial antibiotics so that reason they are used an alternated remedy for treatment of various infections [11]. Tung et al., showed that, highest zone of inhibition of 8mm for cinnamon, followed by garlic with the zone of inhibition of 7mm against Staphylococcus aureus [12]. Kong et al., 2007 worked on antibacterial activity of garlic, cinnamon and chitosan against food pathogens E. coli, S. typhi and S. aureus [13]. According to their study, garlic had excellent antibacterial activity followed by cinnamon and chitosan. Staphylococcus aureus was the most susceptible organism to the spices. Cinnamon extract showed the highest zone of inhibition against Escherichia coli followed by Staphylococcus aureus and Salmonella typhi [14]. In present study, cinnamon extract showed highest zone of inhibition against Klebsiella spp. (16mm) followed by Staphylococcus aureus, Clostridium perfringens, micrococcus spp., Salmonella typhi, bacillus cereus, aspergillus oryzae and penicillium spp. In this present study, garlic showed highest zone of inhibition against Micrococcus spp. followed by Salmonella typhi, Klebsiella spp., Staphylococcus aureus and Clostridium perfringens.

IV. CONCLUSION:

In this study, it is concluded that garlic and cinnamon extracts shown highest antimicrobial efficiency on most of the foodborne pathogens.

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Cinnamon extract was shown inhibitory activity on both bacterial and fungal species. While the garlic extract was shown inhibitory activity only bacterial species. Hence, it is proved that cinnamon has both antibacterial and antifungal activity while the garlic has antibacterial activity alone.

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