



Research Article

FUNGICIDAL POTENCY OF *CINNAMOMUM TAMALA* LEAVES AGAINST COMMON FOOD BORNE PATHOGENS

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ABSTRACT

Due to residual toxicity and tremendous increase in antimicrobial resistant pathogens, there has been increasing concern of the consumers for alternative and efficient compounds for food conservation, aiming a partial or total replacement of antimicrobial chemical additives. Antifungal activity of *Cinnamomum tamala* leaves was evaluated by Food Poisoned Technique (FPT) and Agar Well diffusion assay against *Aspergillus flavus* and *A.niger* isolated from spices. Extracts of *C.tamala* leaves were prepared by Soxhlet extraction method using ethanol, methanol and distilled water. The active extracts thus obtained were subjected to determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Methanolic extract of *C.tamala* showed maximum zone of inhibition against *A.flavus* (18mm) and *A.niger* (16.33mm) respectively with the lowest MIC. The results of the present study are quite encouraging and the study opens up the possibility for replacement of synthetic preservatives by natural extracts to check the growth of microorganisms present in spices and other food products.

Keywords: Food borne; *Cinnamomum tamala*; FPT; MIC; *Aspergillus flavus*; *Aspergillus niger*.

INTRODUCTION

Food borne diseases are a severe health problem in the world, even in well developed nations. The consumption of food contaminated with food borne micro-organisms can pose a serious threat to human health. The existence of microorganisms causes spoilage and results in reduction of the quality and quantity of processed foods.

Plants and natural products have their intrinsic ability to resist pathogenic microorganisms and thus have led the researchers to investigate their mechanisms of action and isolation of active compounds¹. Plant based spices, represent a vast untapped source of pharmaceuticals and are one of the most commonly used natural antimicrobial agent in foods and have been used traditionally for thousands of years by many cultures for preserving foods as food additives and to enhance aroma and flavor².

Cinnamomum tamala, a medium sized tree distributed in tropical and subtropical Himalayas at the altitude of 1000-2400m. It represents about 3000 species worldwide. In India, it is represented by 20 species commonly occurs in North western Himalaya, Sikkim, Assam, Mizoram and Meghalaya region. Apart from this, *C. tamala* is the sole species cultivated for its 'Tejpat' leaves in the whole region of Uttarakhand for the production of spices and related products³. Essential oil constituents of leaves have been extensively investigated but a little investigation has been done on the antimicrobial effectiveness of *C. tamala* extracts. So, the present study is done to assess the antifungal potency of extracts of *C. tamala* leaves against food borne fungi, *A. flavus* and *A. niger*.

MATERIALS AND METHODS

Plant material

Leaves of *C. tamala* were collected from the local market of Haridwar, India. The material was taken to the laboratory and was

authenticated by referring taxonomic literature available in the University library.

Extract preparation

The collected plant material was washed and surface sterilized with 0.1% HgCl₂. Spice material was dried in hot air oven at 35-40°C for 2-3 days and was powdered using a grinder mixer. Plant material was then extracted using the following methodology:

Aqueous extraction

10 g of plant part was thoroughly mixed with 100 ml distilled water. The solution was kept at room temperature for atleast 24 hr and then filtered using muslin cloth. The filtrate was again filtered using Whatman's filter paper no.1 under strict aseptic conditions. The filtrate was then concentrated by evaporation of solvent in water bath to make the final volume 1/10th of the original volume⁴.

Extraction using organic solvents (Soxhlet Extraction method)

In order to obtain the spice extract, cold extraction method was used, in which about 50 gm of the sample (solid material) was placed inside a thimble made from thick filter paper which is loaded into the main chamber of the Soxhlet Extractor. Then 500 ml of the solvent (ethanol and methanol) was added and the apparatus was kept undisturbed for 12-24 hrs to obtain the extract. Then it was concentrated to make final volume 1/10th of the original volume⁴. It was stored at 4°C in airtight bottles for further study.

Test microorganisms

Two fungal strains *Aspergillus flavus* and *A.niger*, isolated from various spices and spice mix were selected for the present study.

Antifungal Assay

The assessment of fungitoxicity was done by Poisoned Food Technique^{5,6} and Agar well diffusion Technique⁷.

1. Poisoned Food Technique: A volume of 0.5 ml of each extract (100 mg/ml) was aseptically poured into the petriplate followed by the addition of 9.5 ml of melted PDA and was swirled gently to achieve thorough mixing of the contents. After the solidification of the media, seven day old fungal culture disk of 6mm diameter was aseptically inoculated at the center of the petriplate. All the plates were incubated at $25 \pm 2^\circ\text{C}$ and radial growth of colony was measured on the 7th day of incubation.

2. Agar well diffusion method: 1 ml of standardized inoculum (10^6 spores/ml) was poured into sterilized petriplates followed by pouring of sterilized Sabouraud Dextrose Agar medium. A well of 8mm in diameter was made with a sterile cork borer aseptically in the middle of the plate. 100 μl of each extract having 100mg/ml of concentration was then introduced into the bored agar well and incubated at $25 \pm 2^\circ\text{C}$ for 5-7 days.

Ethanol was used as negative control while Sodium propionate (5 mg/ml) was served as positive control. The zone of inhibition was measured and expressed in millimeters⁷. The diameter of inhibition zone <9mm was considered as inactive; 9-12 mm, partially active; 13-18 mm, active; > 18 mm very active⁸.

Assessment of Minimum Inhibitory Concentration

MIC (Minimum Inhibitory Concentration) of active extracts thus obtained were further examined by standard two fold microdilution broth methodology⁹. A stock solution of each active extract was serially diluted in 96 wells microtitre plate with Sabouraud Dextrose Broth so as to obtain a decreasing concentration ranging from 4096 $\mu\text{g/ml}$ to 08 $\mu\text{g/ml}$. A standardized inoculum for fungal strain was prepared so as to give an inoculum size of ≥ 0.4 O.D at 530nm. Microtitre plates were then incubated at $25 \pm 2^\circ\text{C}$ for 5-7 days. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial and fungal strain.

Statistical analysis: All the experiments were done in triplicates and results are the mean of three independent values $\pm$ Standard Deviation. The comparison of values obtained was done by ANOVA through web based Richard Lowry method.

RESULT AND DISCUSSION

The antifungal activity was evaluated against *A. flavus* and *A. niger* by Food Poisoned Technique (Table 1; Figure 2 & 3) and Agar Well Diffusion Assay (Table 2; Figure 4 & 5). In FPT, no growth was observed with ethanolic and methanolic extract of *C. tamala* leaves against both *A. flavus* and *A. niger* showing 100% inhibition at 100mg/ml concentration. In Agar Well Diffusion assay also, the methanolic extract of *C. tamala* exhibited maximum zone of inhibition of 18mm and 16.33mm for *Asp. flavus* and *Asp. niger* respectively which was quite high as compared with standard food preservative, Sodium propionate.

Inhibitory concentrations of *C. tamala* as evaluated by microbroth dilution are shown in Table 3. MIC of methanol and ethanol extract was found to be 64 $\mu\text{g/ml}$ and 128 $\mu\text{g/ml}$ against both *A. flavus* and *A. niger* respectively.

Food poisoning is still a concern for both consumers and the food industry despite of the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and spoilage micro-organisms in food. The consumption of food contaminated with food borne microorganisms can pose a serious threat to human health. The existence of micro-organisms causes spoilage and reduction of the quality and quantity of the processed foods. In the United States, an estimated 46 million foodborne infections occur each year, along with 2,50,000 hospitalizations and 3000 deaths^{10,11}. So food safety is one of the major issues in our world today and the World Health Organization calls for more systematic aggressive steps to be taken to significantly reduce the risk of food borne diseases. Antimicrobial properties of spices have been documented in recent years^{12,13}. Still little information is available emphasizing the preservative and antimicrobial role of spices in the preservation of food against microbial action¹⁴. So, here antifungal potency of *C. tamala* was evaluated by two different techniques- Food Poisoned Technique and Agar Well Diffusion Assay and both of them showed strong antifungal potential of *C. tamala*. Jain et al. (2011)¹⁵ also examined the efficacy of methanolic extract of *C. tamala* leaves against five bacteria and two fungi and found it more potent than standard antibiotics (Ampicillin and Flucanazole) compared. The possible explanation for the strong antimicrobial activity was due to the higher percentage of phenolic compounds including Eugenol^{16,17}.

Table 1 Antifungal activity of Aqueous and Organic extracts of *C. tamala* leaves by FPT

Type of extract	Radial growth	
	<i>A. flavus</i>	<i>A. niger</i>
Aqueous	++++	++++
Ethanol	-	-
Methanol	-	-
Sodium propionate	+	-
[#] Ethanol	++++	++++

Sodium propionate (Standard food preservative, 5 mg/ml) – positive control; # negative control. (-) represents 'No growth'; (+) represents 'limited growth'; (++) represents 'Growth'; (+++) represents 'Abundant growth'; (++++ represents 'Maximum growth'.

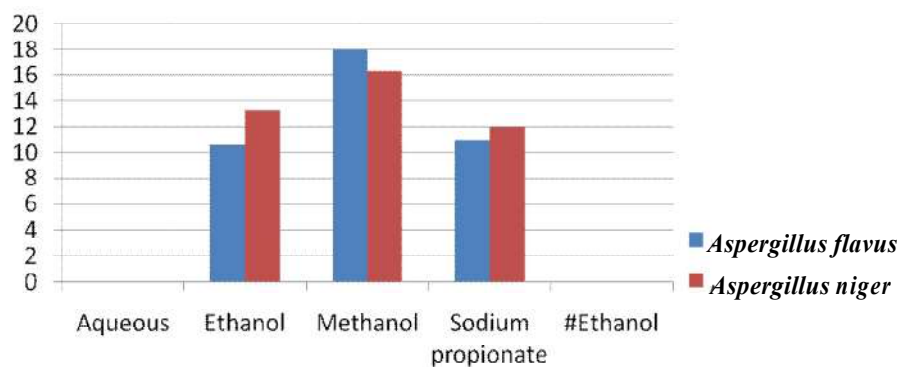
Table 2 Antifungal activity of Aqueous and Organic extracts of *C. tamala* leaves by Agar Well Diffusion Assay

Type of extract	Zone of inhibition (in mm) $\pm$ S.D.	
	<i>A. flavus</i>	<i>A. niger</i>
Aqueous	-	-
Ethanol	10.66 $\pm$ 3.78	13.33 $\pm$ 3.055
Methanol	18.0 $\pm$ 1.00	16.33 $\pm$ 1.527
Sodium propionate	11.00 $\pm$ 1.00	12.00 $\pm$ 0.00
[#] Ethanol	-	-

ANOVA analysis:
HSD[.05]=5.67; HSD[.01]=8.25
HSD[.05]=4.95; HSD[.01]=7.2
M1 vs M2 P<.01
M1 vs M2 P<.01
M1 vs M3 P<.01
M1 vs M3 P<.01
M2 vs M3 P<.05
M2 vs M3 nonsignificant

*Mean of three values $\pm$ Standard deviation; (-) 'No zone of inhibition observed'

Zone of inhibition (in mm)



(-) represents 'No zone of inhibition'.

Sodium propionate – positive control; # negative control.

Figure 1 Antifungal activity Aqueous and Organic extracts of *C.tamala* leaves

Table 3 Minimum Inhibitory Concentration of Active Extracts of *C.tamala* leaves against test fungi

Active Extract	Test Microorganism	Concentration of Extracts (in $\mu\text{g/ml}$)										MIC/ MFC ($\mu\text{g/ml}$)
		4096	2048	1024	512	256	128	64	32	16	08	
Ethanol	<i>A.flavus.</i>	-	-	-	-	-	-	+	+	+	+	128/256
Ethanol	<i>A.niger.</i>	-	-	-	-	-	-	+	+	+	+	128/256
Methanol	<i>A.flavus</i>	-	-	-	-	-	-	-	+	+	+	64/128
Methanol	<i>A.niger</i>	-	-	-	-	-	-	-	+	+	+	64/128

(-) 'No growth'; (+) represents 'growth'.

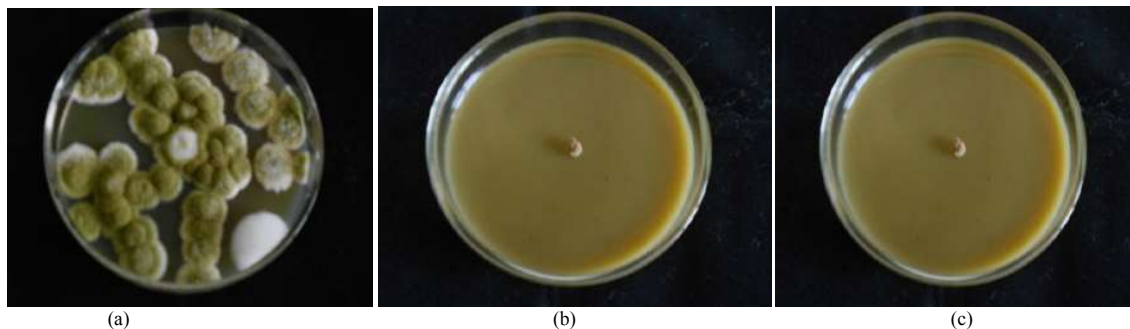


Figure 2 Antifungal activity of *C.tamala* a) aqueous b) ethanolic and c) methanolic extract against *A.flavus* (FPT).

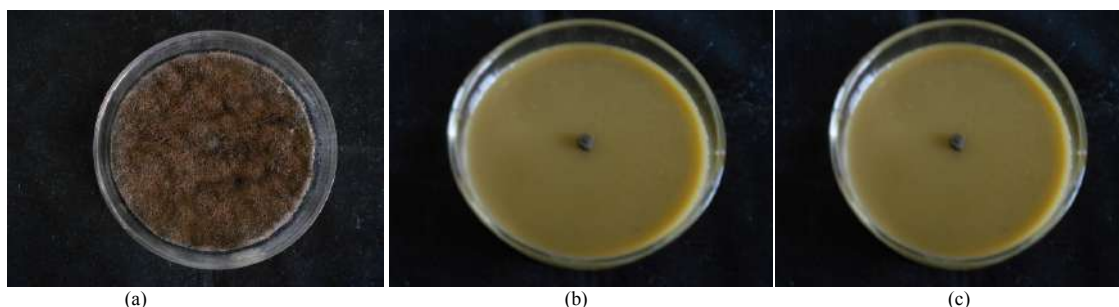


Figure 3. Antifungal activity of *C. tamala* a)aqueous b)ethanolic and c)methanolic extract against *A. niger* (FPT)

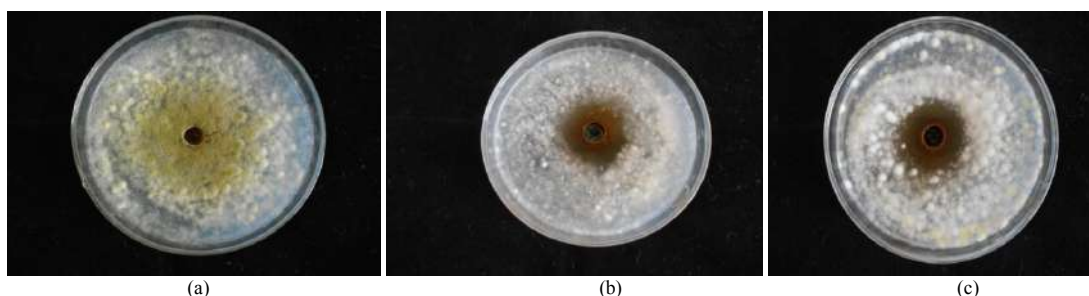


Figure 4. Antifungal activity of *C. tamala* a)aqueous b)ethanolic and c)methanolic extract against *A. flavus* (Agar well diffusion assay)

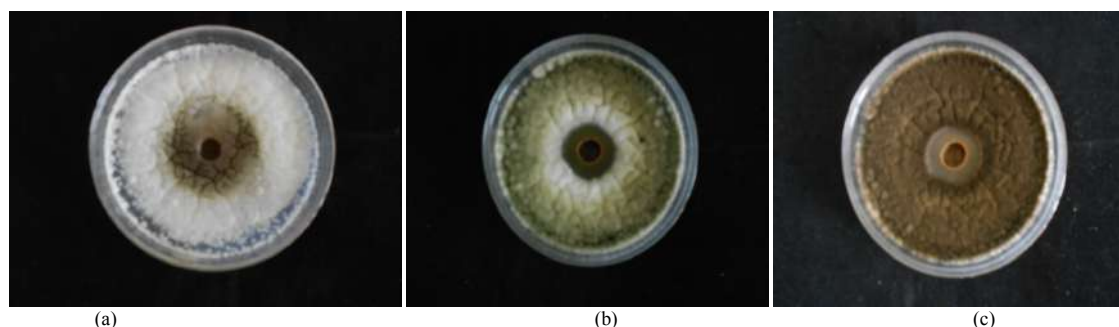


Figure 5. Antifungal activity of *C. tamala* a)aqueous b)ethanolic and c)methanolic extract against *A. niger* (Agar well diffusion assay)

CONCLUSION

The study demonstrated that methanol extract of *C. tamala* possess significant activity against both the fungi, *A. flavus* and *A. niger*. It confirmed that some antimicrobial substances could only be extracted by organic solvents, suggesting that organic solvents are clearly better solvents of antimicrobial agents. The active potency of this plant confirmed that it possessed good antimicrobial compounds that can be highly inhibitory to selected pathogenic and spoilage microorganisms and may provide better alternative to the conventional antibacterial and antifungal additives in food.

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