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RESEARCH ARTICLE

Antibacterial Potential of *Cinnamomum Tamala* Extracts and its Chemical Analysis by GC-MS

Chaudhary P[*1], Singh P[1]

[1]*Department of Microbiology, Kanya Gurukul Campus, Gurukul Kangri University, Haridwar-249407.*

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ABSTRACT

Spices and herbs are used in foods mainly for their flavour and aroma, but in addition to imparting flavour, certain spices prolong the storage life of food to which they are added. Inhibitory activity of spices and derivatives on the growth of bacteria, yeasts, fungi, and microbial toxins synthesis has been well reported. Being natural foodstuff, they appeal to consumers who tend to question the safety of synthetic food additives. This study was aimed to evaluate the antibacterial potential of *Cinnamomum tamala* against two food borne and spoilage bacteria (isolated from spice mixes), *E.coli* and *Bacillus sp*. by Kirby-Bauer disc diffusion method. The extracts showed good antibacterial activity against both the food borne bacteria. The best diameter of the inhibition zone was obtained by methanolic extract of *Cinnamomum tamala* against *Bacillus sp*. (12.6 mm), which was comparable to standard food preservative, Sodium propionate. Active extracts thus obtained were subjected to determine their minimum inhibitory concentration(s) (MIC) followed by their chemical analysis with the aid of GC-MS. This study shows the potential for replacement of synthetic preservatives by the use of natural extracts which also represents an inexpensive source of food preserving agents.

KEYWORDS

Cinnamomum tamala, Kirby –Bauer, Spices, MIC, GC-MS

INTRODUCTION

The problem of food spoilage has plagued humans since ancient times and since antiquity, man has used plants to treat common infectious diseases. Concern over pathogenic and spoilage micro-organisms in foods is increasing due to the increase in outbreaks of food borne diseases. Currently there is a growing interest to use natural antibacterial compounds like essential oils and extract of various species of edible and medicinal plants, herbs and spices which have long been used as natural agents for food preservation, in food and beverages due to the presence of antimicrobial compounds[1].

***Address for Correspondence:**
Priyanka Chaudhary
Department of Microbiology, Kanya Gurukul Campus, Gurukul Kangri University, Haridwar-249407, India.
E-Mail Id: priyanka.chaudhary817@gmail.com

Furthermore, with increase of bacterial resistance to antibiotics, there is considerable interest to investigate the antimicrobial effects of essential oils and different extracts against a wide range of bacteria, to develop other classes of natural antimicrobials useful for food preservation.

The genus *Cinnamomum* contains over 300 species[2], distributed in tropical and subtropical regions of North America, Central America, South America, Asia and Australia. *Cinnamomum tamala*, Indian bay leaf also known as 'Tejpat', a native species of India, can grow upto 20 m (66 ft) tall. It has aromatic leaves which are used fr culinary and medicinal purpose. They are commonly used as spice and are reported to possess antitoxigenic,

antibacterial and antifungal properties. Apart from this *Cinnamomum tamala* is the sole species cultivated for its Tejpat leaves in the whole region of Uttarakhand for the production of spices and related products[3]. Essential oil constituents of leaves have been extensively investigated and four chemotypes present in essential oil i.e., eugenol type, cinnamaldehyde type, linalool type and transsabinene hydrate type reported from different region[4-7]. A little investigation has been reported about the chemical constituents of *Cinnamomum tamala* leaves extracts. So, the present study is done to assess the antibacterial potential of extracts of *Cinnamomum tamala* leaves and to determine the chemical constituents present in it.

MATERIALS AND METHOD

Plant Material

Leaves of *Cinnamomum 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% $HgCl_2$. Spice material was dried in hot air oven at 35-40^{0}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 at least 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 volume[8].

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 volume[8]. It was stored at 4^{0}C in airtight bottles for further study.

Test Microorganisms

Two bacterial strains *Bacillus sp.S10* (Gram positive) and *E.coli* strain *DLF.3* (Gram negative), isolated from various spices and spice mix were selected for the present study. The species were confirmed through 16 S rRNA sequencing (Xcelris Genomics, Ahmedabad).

Antibacterial Susceptibility Assay

Antibacterial screening of the extracts was done by disc diffusion method (Kirby Bauer, 1966)[9]. Filter paper discs of 6mm diameter were prepared and sterilized by dipping them in 95% ethanol using sterile forceps. 100 mg/ml concentration of each extract was applied on each disc and placed over Muller Hinton agar plates seeded with respective pathogens. The plates were incubated in an upright position at 37^{0}C for 24-48 hrs.

The experiment was performed in triplicates under strict aseptic condition to ensure consistency of all findings. The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period.

Organic solvents used in preparation of extracts were also used as negative controls during the study. Sodium propionate (5mg/ml) was used as standard food preservative (i.e., positive control) in the present study.

Assessment of Minimum Inhibitory Concentration

MIC (Minimum Inhibitory Concentration) of active extracts thus obtained were further examined by standard two fold microdilution broth methodology (NCCLS, 1997)[10]. A stock

solution of each active extract was serially diluted in 96 wells microtitre plate with Muller Hinton Broth to obtain a decreasing concentration ranging from 100mg/ml to 0.195 mg/ml. A standardized inoculums for each bacterial strain was prepared so as to give an inoculums size of ≥ 0.1 O.D at 620 nm. Microtitre plates were then kept at 35 ± 2^0C for an overnight incubation. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain.

Gas Chromatography / Mass Spectrometry Analysis

GC-MS analysis of the extract was performed using Shimadzu QP-2010 plus with thermal desorption system TD-20 equipped with a split-splitless injector (split ratio 1:10) data handling system. The column was an Rtx-5MS (30mm × 0.25mm i.d ×0.25 µm film thickness) and temperature programme was used as follows: initial temp. Of 60^0C (hold: 2 min), then 250^0C (hold: 5 min) and finally to 310^0C (hold: 14 min) at the rate of 5^0C/min. Helium was the carrier gas at a flow rate of1.21ml/min. The mass spectra were generally recorded over 40-650 amu that revealed the total ion current (TIC) chromatograms.

Compounds Identification

Identification of the individual components was made by matching their recorded mass spectra with the library (NIST) provided by the instrument software and by comparing calculated retention indices with literature value.

RESULTS AND DISCUSSION

Leaf extracts of *Cinnamomum tamala* were evaluated for *in vitro* antibacterial potential by disc diffusion assay, the result of which has been mentioned in Table 1.

All the three extracts showed variable degree of inhibition zones against both the bacterial species. The maximum zone of inhibition was obtained against *Bacillus sp.S10* (12.6 mm) with the methanolic extract followed by ethanolic extract. Aqueous extract was found effective only against *E.coli* with zone size of 8.00 mm.

Inhibitory concentrations of *Cinnamomum tamala* as evaluated by microbroth dilution are shown in Table 2.

MIC of methanol and ethanol extract was found to be 3.125 mg/ml and 6.25 mg/ml against both *E.coli* strain *DLF.3* and *Bacillus sp.S10* respectively.

Table 1: Antibacterial activity of Aqueous and Organic extracts of *Cinnamomum tamala* leaves.

Type of extract	Zone of inhibition* (in mm)	
	Bacillus sp.S10	***E.coli DLF.3***
Aqueous	4.3 ± 0.57	8.6 ± 0.57
Ethanol	11 ± 0.0	10 ± 1.0
Methanol	12.6 ± 0.57	8.3 ± 2.51
Sodium propionate+	13.33 ± 0.577	12 ± 0.00

*Assay was performed in triplicate and results are the mean of three values ± Standard deviation. + Sodium propionate (5 mg/ml) was used as positive control.

Chemical Composition of Methanolic (Active) Extract of *Cinnamomum Tamala* Leaves

Over all ten major compounds representing Eugenol (58.99%), Spathulenol (12.42%), Caryophyllene (3.1%), Isogermacrene D (2.6%), Pyranone (2.53%), Muurolene (1.2%), Linalool (1.12%), Phytol (0.98%), Lenoleic acid (0.78%), Terpeneol (0.68%) etc. were identified with the aid of GC- MS. A study conducted in North East region of Himalaya showed that the leaf oil of *Cinnamomum tamala* was rich in Eugenol[4]. Similar results were reported with market samples from Dehradun region[5].

Table 2: Minimum Inhibitory Concentration of Active Extracts of *Cinnamomum Tamala* Leaves

Active Extract	Test Micro-organisms	Concentration of Extracts (in mg/ml)										MIC (mg/ml)
		100	50	25	12.5	6.25	3.125	1.56	0.781	0.390	0.195	
Ethanol	*Bacillus sp.S10*	-	-	-	-	-	+	+	+	+	+	6.25
Ethanol	*E.coli DLF.3*	-	-	-	-	-	+	+	+	+	+	6.25
Methanol	*Bacillus sp.S10*	-	-	-	-	-	-	+	+	+	+	3.125
Methanol	*E.coli DLF.3*	-	-	-	-	-	-	+	+	+	+	3.125

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

CONCLUSION

The study demonstrated that methanol extracts of *Cinnamomum tamala* possess significant activity against both the Gram positive and Gram negative bacteria. It confirmed that some antimicrobial substances could only be extracted by organic solvents, suggesting that organic solvents are clearly better solvents of antimicrobial agents[11].

Also the activity was explained due to the presence of Eugenol in high percentage[12]. So the current study supports the traditional advantages of the studied plant and suggests that it possess good antimicrobial compounds that can be highly inhibitory to select pathogenic and spoilage microorganisms and may provide better alternative to the conventional antibacterial or antifungal additives in foods.

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