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ISSN: 2161-0509
Journal of Nutritional Disorders & Therapy

Research Article

In-vitro Antibacterial Efficacy and Phytochemical Profiles of *Cinnamomum Tamala* Leaf Extracts against Some Pathogenic Bacteria

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ABSTRACT

The present investigation was carried out to explore the antibacterial activity, phytochemical constituents and elemental composition of the crude methanolic extract of *Cinnamomum tamala* leaves. The antibacterial activity of methanol and aqueous extract of *Cinnamomum tamala* leaves were tested on different bacterial pathogens. The evaluated extracts showed a variable degree of inhibition zones against all tested microbes. In the methanolic zone of inhibition ranged from 6 mm-30 mm with maximum activity against *Staphylococcus aureus, Pseudomonas aeruginosa, E.coli, Citrobacter* and *Klebsiella pneumonia*. The methanol extract showed inhibition range with zone sizes from 07.00 mm to 29.10 mm. Aqueous extract showed inhibition in the range of 1.62 mm to 28.66 mm slightly lower than methanolic extract with maximum activity against *Staphylococcus aureus, Pseudomonas aeruginosa, E.coli, Citrobacter* and *Klebsiella pneumonia*. It was found that methanolic extract was more effective than the aqueous extract. The result showed that extracts of *Cinnamomum tamala* leaf possess potent antibacterial activity. GC/MS analysis showed the major components of *Cinnamomum tamala* essential oil as cinnamaldehyde (42.898%), tans-cinnamyl acetate (24.327%), ascabin (14.249%), hydro cinnamyl acetate (3.384%), beta-caryophyllene (1.669%) and alpha-copaene (1.414%).

Keywords: Antibacterial activity; *Cinnamomum tamala*; Mineral elements; Phytochemicals

Abbreviations: GC: Gas Chromatography; MS: Mass Spectroscopy

INTRODUCTION

Evolution of new strains of disease-causing agents and spread of antibiotic resistance and are of great concern to the global health community. Medicinal plant of India have been found of immense global importance in treatment because of an adverse effect of synthetic drug had created varied types of complicated diseases, besides causing resistance to synthetic drugs. Thus, global attention has been shifted towards finding new sources, specifically herbal extracts, for the development of therapeutic drugs. There is need to develop a Nobel herbal antibacterial formulation to get rid of resistance and also to investigate newer drugs with less resistance as well as cheap, easily available and eco-friendly. The pictorial organisms over a period of time change their antibiotic sensitivity pattern and develop resistance against commonly used therapeutic agents. A feasible way to overcome the problem of microbial resistance is the development of new antibacterial agents for substitution with ineffective ones. Herbal medicines have been used from centuries to cure human diseases, theses medicinal properties are due to the presence of phytochemicals in the plants. Various health related effects such as anticarcinogenic, antibacterial, antifungal, antimutagenic, antithrombotic, anticarcinogenic and vasodilatory activities, are possed by phytochemicals.

A wide variety of plants have been found to possess antibacterial activity. Tejpat (*Cinnamomum tamala*, Buch-Ham) belongs to *Lauraceae* family and is used in Indian system of traditional medicines in various ayurvedic formulations. It has been used in traditional medicines as an astringent, stimulant and carminative. The leaves are used as a spice but can be employed

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Received: 02-Jul-2020, Manuscript No. JNDT-24-5278; **Editor assigned:** 07-Jul-2020, PreQC No. JNDT-24-5278 (PQ); **Reviewed:** 21-Jul-2020, QC No. JNDT-24-5278; **Revised:** 16-Aug-2024, Manuscript No. JNDT-24-5278 (R); **Published:** 13-Sep-2024, DOI: 10.35248/2161-0509.24.14.300

Citation: Chandel RK, Khan Z, Gambhir S (2024) *In-vitro* Antibacterial Efficacy and Phytochemical Profiles of *Cinnamomum Tamala* Leaf Extracts against Some Pathogenic Bacteria. J Nutr Disord Ther. 14:300.

with myrobalans during dyeing and in the manufacture of vinegar. Its leaves are widely used as an important spice throughout the world since ancient times [1]. Leaves and bark have aromatic, astringent, stimulant and carminative qualities and used in rheumatism, colic, diarrhoea, nausea and vomiting. Essential oil extracted from the leaves known as Tejpat oil is medicinally important and used as a carminative and various heart and kidney disorders. It is also used in pharmaceutical preparation because of its hypoglycemic, stimulant and carminative properties. The essential oil from *Cinnamomum tamala* exhibits antidermatophytic, antibacterial, antifungal, antihyperglycaemic and (antihypercholesterolaemic) effects. Thus the present study was carried out to examine the antibacterial effects of aqueous and methanolic leaf extracts of *Cinnamomum tamala* against major pathogenic bacteria.

MATERIALS AND METHODS

Plant material

Leaves of *C. tamala* were collected from the local market of Delhi, India.

Extract preparation

The collected plant material was washed and surface sterilized with 0.1% $HgCl_2$. Spice material was dried out in hot air oven at 35°C-40°C for 2-3 days and was powdered using a grinder mixer and extracts were prepared following the methodology given below:

Aqueous extraction

10 g of powdered plant part was mixed thouroughly with 100 ml distilled water. The solution was kept at room temperature for at least 24 hr and then filtered using a muslin cloth. The filtrate was again filtered using Whatman's filter paper no.1 under strict aseptic conditions [2]. Finally the filtrate was concentrated by evaporation in a water bath to make the final volume $1/10^{th}$ of the original volume.

Methanolic extraction

Powdered plant material was macerated in 80% methanol to obtain the hydro-alcoholic crude extract at room temperature. After 3 days (72 hr), the filtrate was separated by Whatman No. 1. insoluble materials obtained after extraction were re-macerated. The extract was concentrated by heating on the dry oven at 40°C to evaporate the alcohol from the filtrate. Finally the extract was stored at 4°C until the actual experiment is done.

Gas Chromatography-Mass Spectroscopy (GC/MS) analysis

The separation and identification of compounds in *C. tamala* were done by using Shimadzu QP 2010 Ultra GC-MS equipped with Rtx-5ms column measuring 30 × 0.25 mm and NIST14 library. Helium with flow rate 1 ml/min was used as a carrier gas. 1 µl volume of each sample was utilized. The injection temperature was maintained at 250°C. The oven temperature programme was set with an initial temperature of 50°C and then it was increased to 250°C with 4°C/min ramping rate. The temperature for ion source was maintained at 200°C and the interface at 250°C.

Test microorganisms

The test bacterial cultures were obtained from a microbial type culture collection, IM-Tech, Chandigarh. Stock culture of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli*, *Citrobacter* and *Klebsiella pneumonia* were grown in nutrient broth at 37°C and were subcultured and maintained in nutrient broth at 4°C.

Antibacterial activity by disc diffusion method

Screening for antibacterial activity of extract was done following disc diffusion method. Inoculation of the plate with the test organisms by streaking the swab in a back and forth motion, or by streaking (zigzag) method, manual placement of discs on the nutrient agar plate by forceps and measurement of the zone of inhibition was done in mm [3].

Phytochemical analyses

Total phenol was determined by Folin-Ciocalteau reagent, following the protocol proposed by Ramamoorthy and Bono. Saponin content was determined following Obdona and Ocheko. Alkaloid content was determined by the protocol given by Helrich. Flavonoids were determined by Shinoda test, steroids and triterpenoids were quantified by Salkowski test.

RESULTS

The study of antibacterial properties of *Cinnamomum tamala* against various pathogens was conducted. The antibacterial activities of the plant were studied against five bacterial strains *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli*, *Citrobacter* and *Klebsiella pneumonia*. Evaluated aqueous and methanolic extracts showed a variable degree of inhibition zones against different bacterial species. The methanolic extract was found comparatively more effective with zone sizes ranging from 07.00 mm to 29.10 mm (Table 1) [4]. The aqueous extract showed inhibition in the range of 1.62 mm to 28.66 mm, slightly lower than that of methanolic extract. The result showed that both extracts (methanol and aqueous) possess antibacterial activity against tested microorganisms *i.e.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli*, *Citrobacter* and *Klebsiella pneumonia*. The methanolic extract of the leaf of *Cinnamomum tamala* showed pronounced inhibition against all the test microorganisms *i.e.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli*, *Citrobacter* and *Klebsiella pneumonia*. The order of inhibition (in mm) was *S. aureus* and *E.coli*> *K. pneumoniae*> *C. bacter*> *P. aeruginosa*. Zone of inhibition for aqueous extract of the leaf of *Cinnamomum tamala* followed the order *K. pneumoniae*>*S. aureus*>*P. aeruginosa*>*C. bacter*>*E.coli*.

Phytochemical analysis of different fraction

Phytochemical screening of methanol and aqueous extract of *Cinnamomum tamala* leaves was done using different organic solvent was reported in Table 1. The methanol and aqueous extract of *Cinnamomum tamala* leaves showed a positive result for the presence of alkaloids, triterpenoids, glycosides and tannin and the results were negative for carbohydrate and saponin (Tables 2 and 3) (Figures 1-5) [5].

Table 1: Antibacterial activity of methanolic extract of *Cinnamomum tamala* against different pathogens (Mean ± SE).

Pathogens	Zone of inhibition (mm)							
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	Drug
S. aureus	20.33 ± 0.51	16.08 ± 0.89	16.08 ± 0.34	14.16 ± 0.49	13.66 ± 0.86	11.75 ± 0.27	8.75 ± 0.12	29.10 ± 0.54
P. aerruginosa	18.50 ± 0.23	16.66 ± 0.65	14.00 ± 0.48	10.91 ± 0.21	9.33 ± 0.65	9.16 ± 0.10	7.00 ± 0.54	23.00 ± 0.36
E. coli	21.50 ± 0.56	18.58 ± 0.89	15.41 ± 0.49	13.16 ± 0.24	11.33 ± 0.81	9.16 ± 0.25	8.03 ± 0.65	21.00 ± 0.59
Citrobacter	17.50 ± 0.87	16.00 ± 0.52	14.85 ± 0.44	12.75 ± 0.28	11.25 ± 0.79	10.91 ± 0.25	9.00 ± 0.21	26.00 ± 0.69
K. pneumoniae	21.16 ± 0.26	17.33 ± 0.54	14.83 ± 0.15	12.66 ± 0.65	11.16 ± 0.49	9.58 ± 0.57	7.58 ± 0.68	24.80 ± 0.48

Table 2: Antibacterial activity of aqueous extract of *Cinnamomum tamala* against different pathogens (Mean ± SE).

Pathogens	Zone of inhibition (mm)							
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	Drug
S. aureus	21.00 ± 0.59	17.50 ± 0.57	15.25 ± 0.36	13.62 ± 0.12	13.37 ± 0.26	11.12 ± 0.29	10.25 ± 0.47	27.53 ± 0.65
P. aerruginosa	16.87 ± 0.06	14.37 ± 0.29	14.50 ± 0.69	13.25 ± 0.50	8.50 ± 0.62	8.50 ± 0.55	6.75 ± 0.88	23.37 ± 0.59
E. coli	11.75 ± 0.59	11.25 ± 0.25	9.75 ± 0.55	8.37 ± 0.71	5.75 ± 0.69	3.62 ± 0.25	2.50 ± 0.39	25.62 ± 0.54
Citrobacter	13.75 ± 0.82	13.25 ± 0.45	11.75 ± 0.11	8.50 ± 0.44	7.00 ± 0.25	5.25 ± 0.35	2.50 ± 0.64	14.50 ± 0.21
K. pneumoniae	18.75 ± 0.19	15.75 ± 0.63	13.25 ± 0.45	12.00 ± 0.18	8.00 ± 0.54	6.25 ± 0.27	1.62 ± 0.69	28.66 ± 0.51

Table 3: Phytochemical analysis of methanol and aqueous leaves fractions of *Cinnamomum tamala*.

Plant	Extract	Alkaloids	Glycoside	Carbohydrate	Tanin	Flavonides	Terpenoids	Saponins
C. tamala (Leaf extract)	Aqueous	+	+	-	-	+	-	-
	Methanol	+	+	-	+	+	+	-

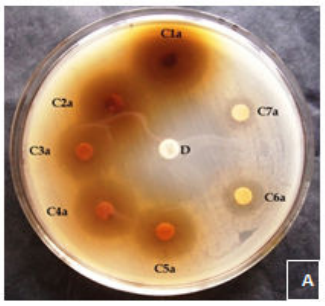

Figure 1: Antimicrobial activity of *C. tamala* extract against *Staphylococcus aureus*.

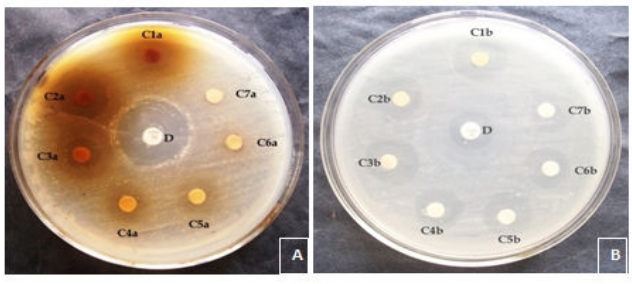

Figure 2: Antimicrobial activity of *C. tamala* extract against *Pseudomonas aerruginosa*.

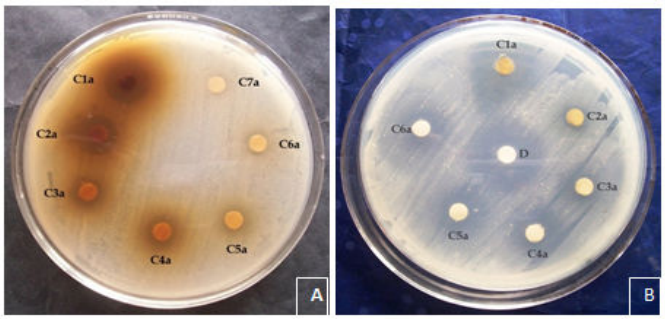

Figure 3: Antimicrobial activity of *C. tamala* extract against *Escherichia coli*.

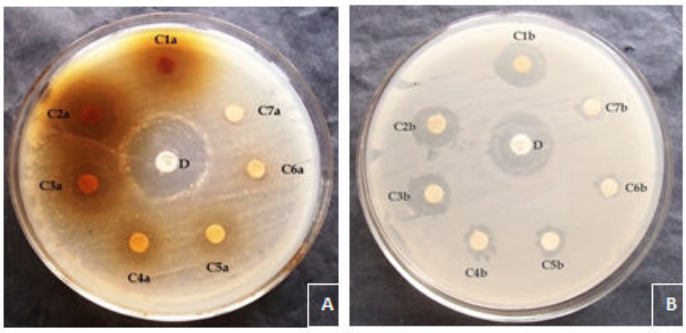

Figure 4: Antimicrobial activity of *C. tamala* extract against *Citrobacter*.

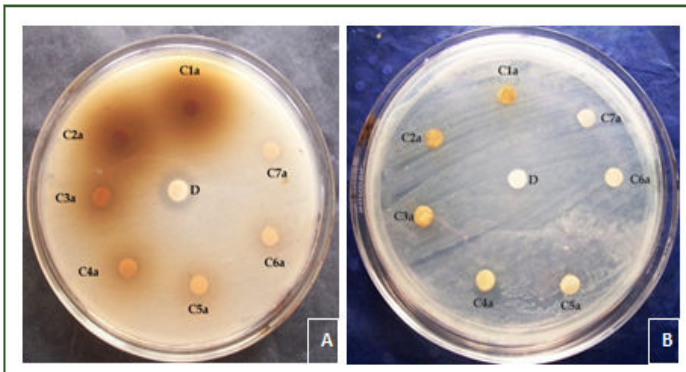

Figure 5: Antimicrobial activity of *C. tamala* extract against *Klebsiella pneumoniae*.

Chemical composition of *Cinnamomum tamala* essential oil

Thirty-one compounds were identified in the *Cinnamomum tamala* essential oil extract. These include cinnamaldehyde (42.898%), tans-cinnamyl acetate (24.327%), ascabin (14.249%), hydro cinnamyl acetate (3.384%), beta-caryophyllene (1.669%) and alpha-copaene (1.414%) as major components [6]. Chemical composition of essential oils of different eucalyptus species have earlier been elucidated. Among the chemical constituents of *Cinnamomum tamala* oil, cinnamaldehyde (42.898%), tans-cinnamyl acetate (24.327%), ascabin (14.249%) were identified as major components (Table 4) [7].

Table 4: Chemical compounds identified in chronological order using GCMS analysis of *Cinnamomum tamala* essential oil (major compounds are marked in the bold form).

Peak	Compound	*RT(Min.)	Area (%)
1	Alpha-pinene	3.621	0.095%
2	Camphene	3.864	0.037%
3	Benzaldehyde	4.05	1.222%
4	Beta-pinene	4.312	0.034%
5	p-cymene	5.16	0.065%
6	Beta-Phellandrene	5.276	0.106%
7	Acetophenone	6.15	0.044%
8	Linalool	6.853	0.442%
9	Beta-phenylpropionaldehyde	8.608	0.856%
10	Phenetol	8.775	1.109%
11	Benzofuran	9.006	0.185%
12	Acrolein	10.285	0.286%
13	Cinnamaldehyde	10.886	42.898%

14	2-propenal, 3-phenyl cinnamaldehyde	13.249	1.087%
15	Eugenol	14.473	0.078%
16	Hydro cinnamyl acetate	14.836	3.384%
17	Alpha-copaene	14.98	1.414%
18	Pivalic acid	15.34	0.129%
19	Cinnamyl acetate	15.45	0.091%
20	Methyl eugenol	15.909	0.107%
21	Beta-caryophyllene	16.329	1.669%
22	Valecene	16.91	1.089%
23	Tans-cinnamyl acetate	17.172	24.327%
24	Alpha-humulene	17.365	1.636%
25	Bicyclogermacrene-lepdozene	18.658	0.192%
26	Naphthalene	19.455	0.066%
27	Spathulenol	21.108	0.780%
28	Caryophyllene oxide	21.23	0.135%
29	Alpha-patchoulene	21.547	1.090%
30	Humulene oxide	22.993	1.097%
31	Ascabin	24.584	14.249%

Note: *RT: Retention time obtained by chromatogram

DISCUSSION

The inhibition zone in five bacterial strains *Staphylococcus aureus*, *Pseudomonas aeruginosa, E.coli, Citrobacter* and *Klebsiella pneumoniae* by the aqueous extract was less as compared to other organic fractions [8]. This may be because the same active substances were present in aqueous extracts, but in low concentration, substances were soluble in organic solvents and therefore, not present in aqueous extracts [9].

Methanolic extract of *Cinnamomum tamala* was found more effective against all test pathogen as compared to aqueous it was confirmed from the study of Mishra et al. *Cinnamomum tamala* extract can be considered as a potential antimicrobial agent for the treatment of various infectious diseases [10]. Methanolic extract of *Cinnamomum tamala* shows maximum antibacterial activity against *Staphylococcus aureus* and *E.coli* followed by *Pseudomonas aeruginosa, Citrobacter* and *Klebsiella pneumoniae*.

Aqueous extract of *Cinnamomum tamala* shows maximum antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumonia* followed by *Pseudomonas aeruginosa, E.coli* and *Citrobacter*. The results of the present investigation also encourage the use of organic solvents in the preparation of plant extracts as compared to aqueous extracts. The polarity of antibacterial compounds make them more readily extracted by organic solvents In the current investigation phytochemical screening of *Cinnamomum* leaf extract revealed that it possesses at least three to four of the following classes of secondary metabolites: Phenols, alkaloids, flavonoids, terpenoids, tannins and saponins [11]. Phenols are the aromatic compounds having hydroxyl groups, which occur almost in all parts of plants and are widespread among the plant kingdom and offer resistance to diseases and pests in plants.

There are also studies showing that alkaloids and flavonoids are the responsible compounds for the antibacterial activities in higher plants.

The antibacterial effects of *C. tamala* cinnamaldehyde (42.898%), tans-cinnamyl acetate (24.327%), ascabin (14.249%), hydro cinnamyl acetate (3.384%) have been demonstrated by other researchers too. Some researchers found cinnamaldehyde (42.898%), to be highly toxic to bacteria. While, in some of the studies indicate that tans-cinnamyl acetate (24.327%), ascabin (14.249%), to possess potent antibacterial and toxic effects. Among various compounds of *Cinnamomum tamala* essential oil, cinnamaldehyde (42.898%), tans-cinnamyl acetate (24.327%) and ascabin (14.249%) was found as major ones [12]. The above findings suggest that acute toxicity of *C. tamala* essential oil and its constituent compounds especially include cinnamaldehyde (42.898%) and tans-cinnamyl acetate (24.327%) are quite promising and have antibacterial effects against gram-negative and gram-positive bacteria.

The possible mechanism of action may be due to the blockage of protein synthesis by these compounds either at transcriptional or at the translational level and in turn peptidoglycan synthesis inhibition at membrane level. Presence of these phytochemicals justifies the observed antibacterial activities in the current study The result suggests that the plant extract could be used as a potential alternative for the development of an efficient and effective drug from natural sources that can be used for the treatment of infectious diseases and will help in combating the diseases caused by pathogenic bacterial strains.

CONCLUSION

It can be concluded that leaf extracts of the plant under study have great potential as antibacterial compounds against bacteria. And they can be used in the treatment of infectious disease caused by resistant bacteria. Such screening of various natural organic compounds and identification of active agents in one need of the hour because the successful prediction of lead molecule and drug discovery will pay off late in drug development. The present study reveals that the selected plants would exert several beneficial effects by antibacterial activity could be harnessed as drug formulation. The obtained results provide support for the use of these plants in medicine and suggest their further advance investigation.

Further investigations are needed to more clearly understand the therapeutic values and curative effects of this plant.

CONFLICT OF INTEREST

There is no conflict of interest among the authors or anybody else.

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