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# *In vitro*-antibacterial activity and phytochemical profiles of *Cinnamomum tamala* (Tejpat) leaf extracts and oil

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**Abstract**

Plant extracts and essential oils have been known since antiquity to possess notable biological activity, including antibacterial, antifungal and antiviral properties. There is a growing interest in the use of natural products in the human food and animal feed industries as consumer resistance to synthetic additives increases. A base for the development of a medicine, a natural blue print for the development of new drugs or phytomedicine to be used for the treatment of disease. Plant extracts have great potential as antimicrobial compounds against several pathogenic microorganism which cause infectious disease and resistant towards synthetic drugs. The aim of this study was to examine the antibacterial effects of leaf extracts and oil of *Cinnamomum tamala* against major pathogenic bacteria. Phytochemical analysis has shown the presence of various antimicrobial components as natural antibiotics. The antibacterial and Minimum Inhibitory Disc Concentration were evaluated by the Kirby Baure Paper Method. Minimum Inhibitory Disc Concentration and Minimum Inhibitory Concentration of oil and extracts against various bacteria range from 0.90-2.25 µg/disc and 0.60-2.40 mg/ml. Acetone and aqueous fraction of extracts showed strongest inhibitory potential. According to results *Cinnamomum tamala* (*Ct*) leaf oil and extracts can be considered as potential antimicrobial agent for the treatment of various infectious diseases.

**Keywords:** *Cinnamomum tamala*; Essential oil; Leaf extract; MIC/MBC; Phytochemicals.

**Abbreviations:** *A. Solani_Alternaria solani*; AC_Acetone; AQ_Aqueous; BZ_Benzene; *C. lunata_Curvularia lunata*; *Cz_Cinnamomum zeylanicum*; CH_Chloroform; Ct_*Cinnamomum tamala*; *E. Coli_Escherichia coli*; EA_Ethyl acetate; ET_Ethanol; *K. pneumoniae_Klebsiella pneumoniae*; MBC_Minimum Bactericidal Concentration; MIC_Minimum Inhibitory Concentration; MIDC_Minimum Inhibitory Disc Concentration; *P. aeruginosa_Pseudomonas aeruginosa*; *P. vulgaris_Proteus vulgaris*; PE_ Petroleum Ether; *S. aureus_Staphylococcus aureus*; *S. Pneumoniae_Streptococcus pneumonia*; ZOI_Zone of Inhibition

## Introduction

Many plants have been used for centuries as remedies for human disease and the medicinal values of plants lies in the some chemical substances that produce definite physiological action on the human body. Drugs used in medicine today are obtained from nature or of synthetic origin. The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases[1]. World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants which are widely used as medicine constitute a major source of natural organic compounds. Plants have an almost limitless ability to synthesize aromatic substances most of which are phenols or their oxygen derivatives. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties[2,3]. Some oils have been used in cancer treatment[4]. Some other oils have been used in food preservation[5], aromatherapy[6] and fragrance industries. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils[7]. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential[8]. Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production. An estimated 3000 essential oils are known, of which 300 are comer cially-important in fragrance market[9]. Essential oils are complex mixtures comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of these constituents contri- butes to the beneficial or adverse effects. The bark and leaves of *Cinnamomum* spp are commonly used as spice in the home kitchen and their distilled essential oils or synthetic analogs are used as flavouring agents in the food and beverage industry[7], The antimould potential of *C. zeylanicum* extracts against *A. solani* and *C. lunata*, shows it as promising alternative antimicrobial preparation to be inserted in pharm-aceutical formulations used to treat mycoses of different clinical severities and the plant diseases, particularly, those caused by dematiaceous moulds[8]. Several members of entero-bacteriaceae are responsible for causing severe infections[10].

**Table 1.** Phytochemical analysis of C. *tamala* leaf extract

| Phytochemicals | Extracts | | |
|---|---|---|---|
| | PE | AC | AQ |
| Terpenoids | + | + | + |
| Tannins | - | + | + |
| Phenol/Polyphenols | + | + | + |
| Flavonoids | - | + | + |
| Alkaloids | + | - | + |
| Saponin | + | + | + |

Phytochemical analysis of different extracts of *Ct*- leaves was done as shown in method section. PE = Petroleum Ether; AC = Acetone; AQ = Water; (+) present; (-) not detected.

Several reports have been published in recent years on the antimicrobial activity of some essential oils and crude extracts derived from plants against etiological agents of infectious diseases and food-borne pathogens[11-15]. Spices are some of the most commonly used natural antimicrobial agents in foods. Addition of spices in foods not only imparts flavour and pungent stimuli but also provides antimicrobial property[16]. Natural antimicrobial compounds in spices were found to possess antimicrobial activity[17]. In traditional popular medicine, essential oils and other plant products have been used traditionally to treat respiratory tract infections. Inhalation of essential oils has been used to treat pharyngitis, bronchitis and sinusitis. Recent scientific studies have shown that essential oils of *Cinnamomum* plants *C. cassia*, *C. camphora*, *C. iners*, *C. osmophloe*, *C. zeylanicum* and *C. porrectum* have antimicrobial activity[18-21]. The research on the medicinal plants should be extended with the identification of the active principles in the plants. These types of research activities could also lead to the development of new drug as in the past. *Cinnamomum tamala* L. (*Lauraceae*) having the Tejpat as the local Indian spice name was evaluated for antibacterial efficacy and phytochemical screening. In the present work we have evaluated the antimicrobial activity of leaf essential oil and extracts of *Ct* and the antibiotics against *E. coli*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, *S. aureus* and *S. Pneumoniae*, the etiological agents of several infections including nosocomical infections and other types of respiratory tract infections.

## Materials and methods

### *Plant leaves collection*

The leaves of *Cinnamomum tamala (Lauraceae)* were collected from Ranchi (India) October/November 2007. Freshly collected plant parts were shade-dried at room temperature for 10–15 days. Dried leaves samples were separately crushed and ground into fine powder with mortar and pestle.

### *Preparation of Extract*

Powdered plant materials were sequentially extracted with different solvents in a Soxhlet apparatus for 8 h. The solvents used for extraction included petroleum ether (PE), benzene (BZ), chloroform (CH), ethyl acetate (EA), acetone (AC), ethanol (ET) and water (AQ). The respective extracts were filtered and dried under reduced pressure using rotary evaporator to yield solid/semisolid residues. The residues were lyophilized to get dry solid mass.

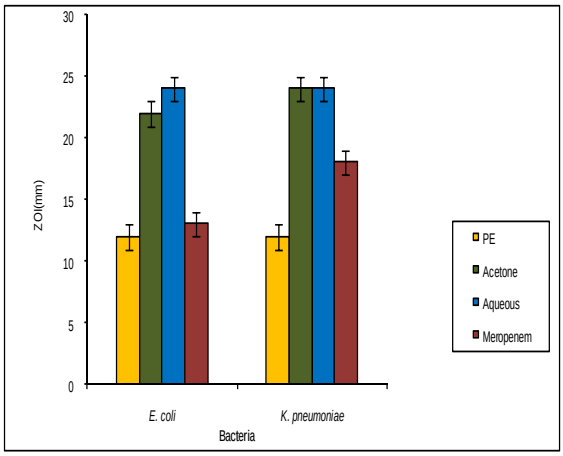


**Fig 1.** ZOI (mm) of *Ct*-leaf extracts against *E. Coli and Klebsiella pneumonae.*The *Ct*-leaf extracts were prepared in (1) Petroleum ether (PE), (2) Acetone (AC) (3) Water (AQ) as discussed in method section. Loaded disc contain 5 mg of PE, AC and AQ extracts.

### *Isolation of essential oil*

Shaded dried leaves (50 g) were ground and subjected to hydrodistillation for 3 h in 500 ml water, using a Clevenger-type apparatus.

### *Chemicals*

$FeCl_3$ and acetic anhydride, Sulphuric acid ($H_2SO_4$), Tween-20, DMSO, Sodium carbonate, ethanol, chloroform, acetone, petroleum ether, ethyl acetate were purchased from Merck Chemical Supplies (India) and Folin-ciocalteau reagent. Nutrient agar, MH Agar and Maconkey agar (Hi media). All the other chemicals used including the solvents were of G.R. grade.

### *Test Organism*

Pathogenic microorganisms were obtained from the Department of Microbiology, MLN Medical College, Allahabad, India. Four strains of gram-negative human pathogenic bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. vulgaris*) and two strains of gram-positive human pathogenic bacteria (*S. Pneumoniae* and *S. aureus)* were used. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

### *Phytochemical Analysis*

Qualitative phytochemical analysis of *Ct*-leaf extracts were done as follows:

### *Tannins*

20 mg powder was dissolved in 2 ml distilled water and filtered. 2 ml $FeCl_3$ was added to the filtrate, blue-black precipitate indicated the presence of tannins[8].

### *Alkaloids*

20 mg extract was dissolved in 2 ml distilled water and filtered. To the filtrate, 2–4 drops of 1% HCl was added and steam was passed through it. To the 1 ml of this solution 6

**Table 2.** Minimum Inhibitory Concentration (MIC)/Minimum bactericidal Concentration (MBC) of *Ct*-leaf extract (mg/ml)

| Bacteria | Ct-leaf extracts | | | | | |
|---|---|---|---|---|---|---|
| | PE | | AC | | AQ | |
| | MBC | MIC | MBC | MIC | MBC | MIC |
| *E. coli* | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 |
| *K. pneumoniae* | 2.40 | 1.20 | 1.20 | 0.60 | 2.40 | 1.20 |
| *P. vulgaris* | 2.40 | 1.20 | 1.20 | 0.60 | 1.20 | 1.20 |
| *P. aeruginosa* | 2.40 | 1.20 | 2.40 | 1.20 | 1.20 | 1.20 |
| *S. pneumoniae* | 2.40 | 1.20 | 2.40 | 1.20 | 1.20 | 2.40 |
| *S. aureus* | 0.60 | 0.60 | 2.40 | 1.20 | 2.40 | 2.40 |

MIC/MBC (mg/ml) of *Ct*-leaf extracts against *E.coli, K.pneumonae, Proteus vulgaris, Pseudomonas aeruginosa, Streptococcus pneumonae and staphylococcus aureus.* PE = Petroleum Ether; AC = Acetone; AQ = Water

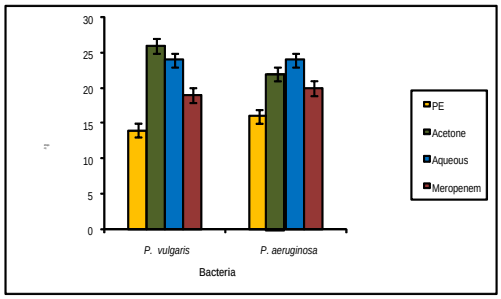


**Fig 2.** ZOI (mm) of *Ct*-leaf extracts against *P. vulgaris and Pseudomonas aeruginosa*. The Ct leaf extracts were prepared in (1) Petroleum ether (PE), (2) Acetone (AC) (3) Water (AQ) as discussed in method section. Loaded disc contain 5 mg of PE, AC and AQ extracts.

drops of Wagner's reagent was added. Brownish-red precipitate indicated the presence of alkaloids[8].

***Saponins***

To 0.5 ml of the filtrate obtained in alkaloids test 5 ml distilled water was added. Frothing persistence indicated the presence of saponins[8].

***Flavonoids***

20 mg extract was dissolved in 10 ml ethanol and filtered. 0.5 ml conc. HCl and magnesium ribbon were added to 2 ml filtrate. Development of pink-tomato red color indicated the presence of flavonoids[8].

***Terpenoids***

Salkovski test was performed using a small amount of extract solution. To this solution 5 drops of conc. $H_2SO_4$ and 1 ml Chloroform were added. Change of yellow colour into red indicated the presence of terpenoids[22].

***Phenols/polyphenols***

A small amount of material was extracted in Methanol and evaporated to dryness. Residue was dissolved in distilled water and 0.5 ml Folin-ciocalteau reagent was added followed by 2 ml 20% $Na_2CO_3$ solution. Development of bluish colour indicated the presence of phenols[23].

***Bio-activity testing***

***MIC/MBC Assay***

The MIC were determined as the lowest concentration of extract inhibiting the complete growth of each organism in growth tubes, while MBC minimum amount of substance which inhibit the visible growth of provided microorganism. Brath dilution assay determines the MIC/MBC (Minimum Inhibitory Concentration) against various pathogenic bacteria. Tubes were prepared in peptone water at 37° C for 18h. Experiments were carried out in triplicate. MIC of each extract ranging from 0.60-2.40 mg/ml. were observed 100 mg/ml extracts was taken initially then serial diluted again and again, tubes were dried in oven for 3 hour prior to inoculation. Finally we observed 0.60 to 2.40 mg/ml of extract inhibit the complete growth against *E. coli, K. pneumoniae* and 2.40 mg/ml in *S. Pneumoniae* and *S. aureus*. Inhibitions of bacterial growth in the tubes containing test extracts were judged by comparison with growth in blank control tubes. Table 2 shows range of MBC 0.60-2.40 mg/ml against *E.coli, Klebsiella, Proteus, Streptococcus* and *Staphylococcus*. The best result of MBC against *S. aureus* showed 0.60 mg/ml.

***MIDC (Minimum Inhibitory Disc Concentration) assay***

A series of two fold dilution of each oil sample was prepared in 0.1% Tween (20) to enhance its solubility and infusibility in agar medium (Muller Hinton). Disc loaded with 20 µl of diluted oil samples (containing decreasing oil concentration) were placed on MHA plates inoculated with pathogenic bacterial strains 0.1% Tween (20) was used as vehicle control. Plates were incubated at 37° C for 18h. Determination of MIDC was recorded as the lowest concentration of oil that inhibits visible growth of organism around disk[24].

**Results**

***Phytochemical Analysis***

*Cinnamomum tamala* L-leaf extract contained terpenoid, phenol/polyphenols and Alkaloids invariably present in all the three extract fractions however tannin only in acetone and aqueous fraction. Flavonoid well marked only in acctone part of *Ct*-leaf extract. Results of phytochemical analysis of *Ct*-leaf extract are given in (Table-1).

***Antibacterial assay***

Analysis of essential oil and extracts (*Ct*-leaf) for antibacterial activity was done by Kirby Baure paper method.[25] It was performed using an 18 h cultures at 37°C in 10ml of Muller Hinton Broth (Hi Media). Five hundred microliters of suspension were spread over the plates. 500 mg/ml of each extract dissolved in pure 1ml DMSO. After centrifugation complete dissolved extracts soaken in ten 6 mm disk each contain 5 mg of the extract. In case of oil sample re-prepared in Tween (20) to enhance infusibility, so each disks loaded with 20 µl of oil sample- paper disc moistened with 0.1% Tween (20) and DMSO was placed on seeded petriplates as a vehicle control. Standard antibiotic

**Table 3.** MIDC of Ct leaf essential oil against various pathogenic bacteria

| Bacteria | MIDC (µg/disc) |
|---|---|
| *E.coli* | 2.25 |
| *K.pneumoniae* | 0.90 |
| *P. vulgaris* | 1.35 |
| *P. aeruginosa* | 0.90 |
| *S. Pneumoniae* | 2.25 |
| *S. aureus* | 2.25 |

MIDC value of *Ct*-leaf oil against *E.Coli, Klebsiella pneumonae, P.vulgaris, Pseudomonas aeruginosa, Streptococcus pneumoniae* and *Staphylococcus aureus.*

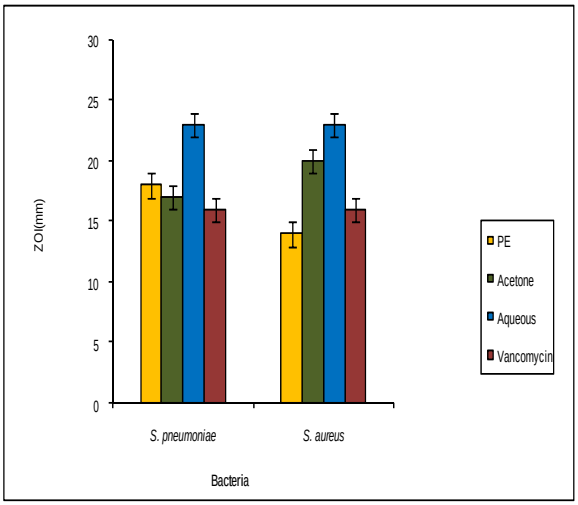


**Fig 3.** ZOI (mm) of *Ct*-leaf extracts against *Streptococcus pneumonae* and *Staphylococcus aureus*. The *Ct* leaf extracts were prepared in (1) Petroleum ether (PE), (2) Acetone (AC) (3) Water (AQ) as discussed in method section. Loaded disc contain 5 mg of PE, AC and AQ extracts.

disc containing meropenem (10µg/disc against gram-negative bacteria) and Vancomycin (30 µg/disc against gram-positive bacteria) were used reference control. All petridishes were sealed with sterile laboratory parafilm to avoid eventual exoporation of test samples-plates were left for 18 h at 37°C for incubation. After the incubation period the zone of inhibition (ZOI) was measured with a Callipers presented in fig. 1-4. Studies were performed in triplicate and mean values was calculated in SD±1.

***Inhibition of bacterial growth by different Ct-leaf extracts***

The antibacterial activity of different leaf extracts have done but three extracts showed visible killing potential against six bacterial species, is summarized in Fig.1, Fig.2 and Fig.3. Different experiments showed antibacterial efficacy with varying magnitudes. The ZOI above 8 mm in diameter was taken as positive result. Fig-1 shows potential effect of PE, AC and AQ extracts of *Ct*-Leaf against *E.coli* and *K. pneumoniae* ZOI range from (12-23 mm). In acetone and aqueous fraction against *K. pneumoniae* showed high inhibitory potential with comparison to standard antibiotic meropenem. However in Fig-2 ZOI range between 14-26mm against *P. vulgaris* and *pseudomonas*. Acetone fraction against *P. vulgaris* has maximum inhibitory potential. Inhibitory potential of *Ct*-leaf extract fraction have well illustrated in Fig-3 against gram-positive bacteria (*S. Pneumoniae* and *S. aureus*). Aqueous fraction in both case have same ZOI-24 mm followed acetone fraction. There was no inhibition of growth in the vehicle control DMSO. Amongst all the extracts of *Ct* leaf, acetone and aqueous extracts at the concentration 5 mg/disk exhibited maximum antibacterial efficacy against gram-positive and gram-negative bacteria.

***Inhibition of bacterial growth by Ct- leaf Oil***

The antibacterial efficacy of leaf essential oil against six bacterial strains is summarized in Fig- 4. The ZOI range from 23-33 mm, best result observed against gram positive bacteria (*streptococcus* and *staphylococcus*) followed *K. pneumoniae*, *Pseudomonas* and *E. coli.* Minimum inhibitory disc concentration (MIDC) towards six bacterial strains range from 0.90-2.25 µg/disc (Table 3). Study revealed that *Ct*-oil against *K. pneumoniae and pseudomonas* have strongest killing potential at lowest concentration.

**Discussion**

Plants extracts and essential oil have been used for many thousands of years[23]. In food preservation, pharmaceuticals alternative medicine and natural therapies, Antibacterial susceptibility testing remains an area of intense interest. Susceptibility testing can be used for drug discovery and epidemiology. Number of reports is available showing efficacy of *Cz* essential oils as antimicrobial agents[19]. In vitro studies in this work showed the essential oils and extracts inhibited bacterial growth but their effectiveness varied. In our study, *Ct*-leaf essential oil and extract exhibited strong activity against selected bacterial strains. Our study showed least inhibitory activity of petroleum ether extract fraction. Among these extracts analyzed in this work the essential oil and extracts were the most effective as an antibacterial agent. The antibacterial role of these fraction due to active constituents, oil contain mainly eugenol and cinnamic acid[26] and extracts richly cinnamaldehyde. An Important charact-eristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structure and rendering them more permeable[27]. Extensive leakages from bacterial cells or exist of critical molecules and ions will lead to death[28] Gram-positive bacteria not easily killed than gram-negative by the essential oil. Our study has indicated the antibacterial potential of plant extracts, as the *Ct*-leaf extracts displayed complete inhibitory effect as AC and AQ fraction. The organic and aqueous extracts and oils of *Ct*-leaves studied in the current work showed marked antibacterial activities against six strains of pathogenic bacteria responsible for causing diseases in humans. However, the extracts differ significantly in their activity against the above strains. The differences observed in the bioactivity assays suggest the susceptibility of these species to various secondary metabolites present in this endemic plant. The composition of these secondary metabolites in turn varies from species to species and also on climatic conditions and the physiological state of develop-pments of the plants[29]. The relative antibacterial activity of *Ct*-leaf extracts may not be easily correlated with any individual component but with a mixture of compounds present in these extracts. There are reports showing that alkaloids and flavonoids are the responsible compounds for the antibacterial activities in higher plants[30]. Moreover, secondary metabolites such as tannins and other compounds of phenolic nature are also classified as active antimicrobial compounds. Phenols, the aromatic compounds with hydroxyl groups are widespread in plant kingdom. They occur in all parts of plants. Phenols are said to offer resistance to diseases and pests in plants. Interestingly, phytochemical screening of

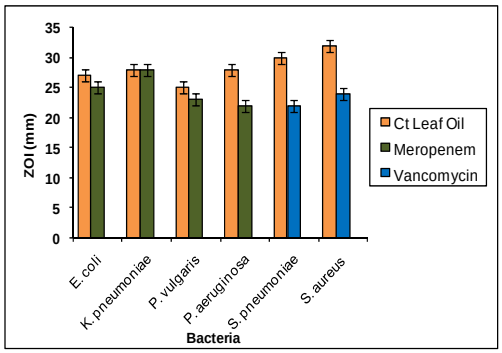


**Fig 4.** ZOI (mm) of *Ct*-leaf oil against *E.Coli, Klebsiella pneumonae, P.vulgaris, Pseudomonas aeruginosa (*Meropenem) *Streptococcus pneumonae* and *Staphylococcus aureus* (Vancomycin) 20µl of disc loaded with Ct leaf oil against each bacteria.

the current investigation has revealed that extracts from *Ct*-leaf possess at least three to four of the following classes of secondary metabolites: phenols, flavonoids, terpenoids, tannins, alkaloids and saponins. Therefore, the presence of these phytochemicals could to some extent justify the observed antibacterial activities in the current study.

**Conclusion**

Our results have established the intense antibacterial potential of *Ct*-leaf oil and extracts against gram-positive and gram-negative bacteria. It could be regarded as promising alternative antimicrobial preparation to be inserted in pharmaceutical formulation used to treat nosocomical infection. The aforesaid Indian spice plant contains phytochemicals to be developed as prospective antibacterial agents.

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