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# A COMPARATIVE STUDY OF ANTIMICROBIAL EFFICACY OF CINNAMOMUM ZEYLANICUM LEAF OIL AND CINNAMOMUM TAMALA LEAF OIL

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## ABSTRACT:

Cinnamomum has a wide range of medicinal properties and has been used from ancient times due to its medicinal properties. Cinnamomum is an aromatic plant that belongs to the family Lauraceae and has antimicrobial, antioxidants, antidiabetics, anti-inflammatory, and antiseptics properties. This comparative study is conducted to evaluate the antimicrobial efficacy of essential leaf oil of C.zeylanicum and C.tamala against a broad range of tested bacteria. Hydrodistillation is used to produce the essential leaf oil by using the Clevenger apparatus. The antibacterial activity was tested against V.cholerae, Alcaligenes xylosoxidans, Staphylococcus aureus, Rhizobium trifolii, Klebsiella pneumoniae, Proteus vulgaris, Shigella dysenteriae, and Streptomyces cinerochromogenes by agar diffusion plate method and determining the anti-microbial activity of essential leaf oil by observing the inhibition zone. Results indicated that C.zeylanicum and C.tamala leaf extract have a potential antibacterial property and thus could be used in wide applications after evaluating the potential efficacy of essential leaf oil for human use.

## INTRODUCTION:

The increased use of modern medicine in the last three decades has put tremendous pressure on pharmacological industries (1). Modern medicine has wider applications ranging from personal care, cosmetics, food industry to treating various infections and ailments. The major concern for the consumer for using modern medicine is because of their high cost and development of antimicrobial resistance (2). The disease-causing microorganism in the food industry has also caused a major concern for the consumer which directly affects the food quality and its standard (3). Recently there has been increased interest in herbal plants, whether the extract of plants and essential oils are rich in a wide variety of effective compounds such as tannins, terpenoids, flavonoids, alkaloids, and polyphenols present in different quantities depending on the part of the plant (4) imparting a wide range of medicinal properties which are cost-effective and possessing lesser side effects (5).

In a previous study, it was shown that major world populations are using herbal medicine because of their effectiveness in treating various infections with lesser side effects (6). India has recorded and possesses knowledge of herbal medicine and is used by tribals from ancient times, Ayurveda which is the oldest system of medicine is widely practiced in India as well as in other countries as they are effective in treating various infections and diseases. Herb extract possesses wider applications ranging from medicinal syrups, wines, and tinctures and is used in liquid form, powdered and in dried form. Microorganisms such as bacteria are found in air, soil, and water and even in harsh conditions such as acidic hot springe and radioactive waste and causes a major threat to human health causing diseases and infections such as skin disease, digestive problems, diarrhea, vomiting, fever, abdominal cramps and many more infections(6).

Due to the development of drug-resistant towards antibiotics has paved the way to search for herbal plants as the therapeutic agent which could be effective against these bacteria(7).In ancient times, secondary metabolites from medicinal plants and aromatic plants have widely used as a taste enhancer, used in perfume industries and flavor in the food industry(8). Cinnamomum belongs to the family Lauraceae and is distributed in the tropical and subtropical regions of Australia, Central, and North America, and South Asia (9). Cinnamomum possesses medicinal properties such as antiseptics, antioxidants, carminative, antiviral and also possessing blood purifying properties and are used by tribals of the developed and developing countries(10)(11)(12).In the present work, some aromatic plants of notable ethnic importance are chosen. Cinnamomum zeylanicum and Cinnamomum tamala have been considered for their efficacy as antimicrobial agents. In the previous study, C.zeylanicum has shown efficacy in vivo and invitro (11) (13). Essential oils from C.zeylanicum and C.tamala possess the antibacterial, antifungal, and anti-plasmodial activity and are currently investigated for a better understanding of natural food preservative (15). Essential oils are versatile and effective in treating many common problems, it has a broad spectrum of activity, inhibiting bacteria, fungi, and yeast. Through literature review, it is evident that there is little work reported on the antimicrobial efficacy of C.tamala leaf oil and C.zeylanicum leaf oil against some pathogenic

microorganisms (11) (15) (16). Keeping in view the ever-rising trend in herbal research, this study aims to investigate the antimicrobial activity of essential oil from leaves of C.zeylanicum and C.tamala:
In the present work, experiments were designed to achieve the following objectives:
1. Extraction of essential oil from leaves of C.zeylanicum and C.tamala.
2. Purification of essential oil.
3. Inhibition studies of the above test sample on a spectrum of pathogenic/non-pathogenic bacterial culture namely-V.cholera, Alcaligenes xylosoxidans, Staphylococcus aureus, Rhizobium trifolii, Klebsiella pneumoniae, Proteus vulgaris, Shigella dysenteriae, and Streptomyces cinerochromogenes.

## MATERIALS AND METHODS:

### Collection of Plants Material:
Every plant part possesses antibacterial property, so we selected leaves of Cinnamomum Zeylanicum and Cinnamomum tamala were collected from the FRI botanical garden and Chota Nagpur region of Bihar respectively, and are brought to the laboratory.
The fresh plant samples (leaves) were washed thoroughly with sterile distilled water and dried in shade for 3-5days and weighed before calculating the yield.

### Extraction of essential oils and its purification:
In this study, Essential oils from plant samples were extracted from the Clevenger apparatus. It is round bottom flask apparatus and consists of Clevenger's main unit and condenser. Bumping of hot water occurs in the process to avoid bumping of hot water porcelain chip is added to the round bottom flask of Clevenger.
The Hydrodistillation method was used in this study where essential oils are extracted from plant samples under atmospheric pressure at the boiling temperature of about 100oC.
, and then within 10-15 minutes oil starts coming and the temperature of the heating mantle is lowered between 65-70oC, and this oil and water(emulsion) are collected in a conical flask and allowed to cool at room temperature. This essential oil is recovered by ether by using a separating funnel. Two phases are distinguishable, and this mixture of ether and oil was separated by filtration after 24hours and afterward, ether was evaporated on a water bath.

### Preparation of culture media:
Fresh Nutrient agar culture media was prepared and sterilized at 121$^{o}$C and 15lbs for 15-20min.
Composition for Nutrient Agar media

| Chemicals | Amount(gm/1000ml) |
|---|---|
| Peptone | 5.10gm |
| Beaf extract | 3.0gm |
| Nacl | 5.0gm |
| Agar | 15gm |

Dissolved all these components in 1000ml of distilled water and maintained a pH of 7.

### Source of Test microorganisms:
The Test microorganism used in this study were: Vibrio cholerae, Alcaligenes xylosoxidans, Staphylococcus aureus, Rhizobium trifolii, Klebsiella pneumoniae, Proteus vulgaris, Shigella dysenteriae, and Streptomyces cinerochromogenes. All these test microorganisms were obtained from departmental culture, and microorganism was subcultured on Nutrient agar culture media in different agar slants and stock culture was maintained at 37 oC.

**Whole plate diffusion method:**
Sterile agar plates of Nutrient agar were prepared under aseptic conditions. After solidification, 100ml of different microbial suspension was spread by spreader on each agar plates, a cork borer was used to make bore on the surface.

**Preparation of Microbial suspension:**
The solution of dilution concentration of oil (25%) was prepared with 0.1% tween 20 (Dissolved in sterile distilled water).
Four wells were made on each agar plate on which organisms are already spread by borer of 8mm diameter. Dilutions of 25% of essential oil at the rate of 35ml was loaded at the center of each well.
Plates were incubated at 37oC for 1-2 days. This assay was done in 3 replicates to ensure consistency.

**Measurement of Inhibition zone:**
After suitable incubation, the Inhibition zone was measured in millimeters by using vernier calipers.

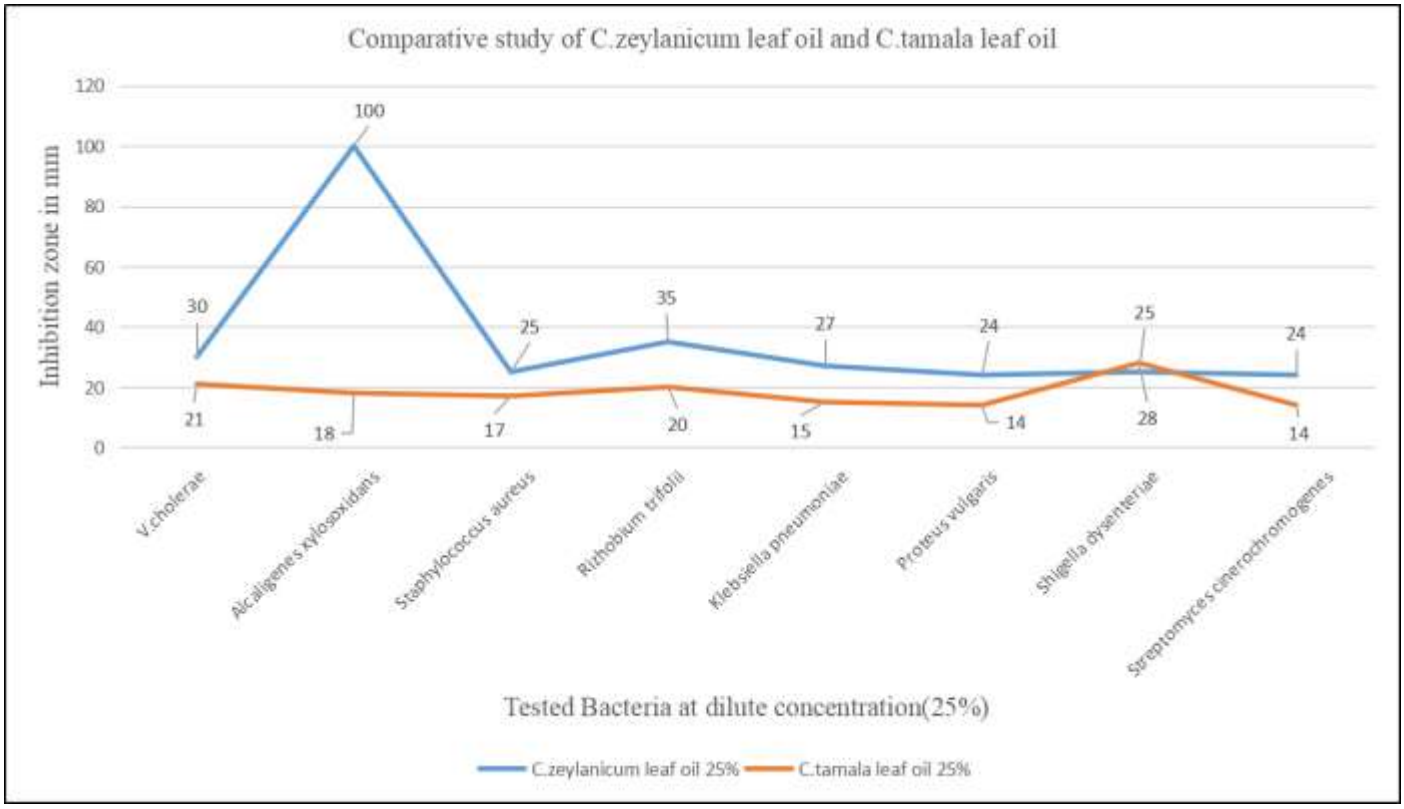
**Table1: Bacterial growth inhibition assay with C.zeylanicum leaf oil and C.tamala leaf oil.Data represents an average of 3 replicates.**

| **Essential Oil Organisms** | **C.zeylanicum leaf oil** | **C.tamala leaf oil** |
|---|---|---|
| | **25%** | **25%** |
| **V.cholerae** | 30 | 21 |
| **Alcaligenes xylosoxidans** | Plate Clearance | 18 |
| **Staphylococcus aureus** | 25 | 17 |
| **Rizhobium trifolii** | 35 | 20 |
| **Klebsiella pneumonia** | 27 | 15 |
| **Proteus vulgaris** | 24 | 14 |
| **Shigella dysenteriae** | 25 | 28 |
| **Streptomyces cinerochromogenes** | 24 | 14 |

Zone diameter in mm:
15mm and below-low activity
15-25mm-moderate activity
25mm above - high activity

*Inhibition zone of 100mm - Showing the plate clearance

**Figure 1: Comparative study showing the inhibition zone of C.zeylanicum leaf oil and C.tamala leaf oil**




Inhibition zone of 100mm - Showing the plate clearance

**RESULT:**

**Effects on different organisms are described below:**

**Vibrio cholerae**: Leaf oil of C.zeylanicum proved to be most efficacious against V.cholerae exhibiting inhibition zone up to 30mm (Table 1).
At 25% concentration C.tamala leaf oil proved to be moderate effective producing 21mm of inhibition zone. This inhibition potential of the leaf oil of C.tamala can be correlated with a high concentration of eugenol present in essential oil (Shown in Table 1 and Figure 1).

**Alcaligenes xylosoxidans**: C.zeylanicum leaf oil showed an overwhelming response at 25% concentration against Alcaligenes xylosoxidans showing plate clearance which proved to be an effective antimicrobial against Alcaligenes xylosoxidans on the contrary C.tamala leaf oil showed less inhibition compared to leaf oil of C.zeylanicum giving a zone of 18m (Shown in Table1 and Figure 1).

**Staphylococcus aureus**: C.zeylanicum leaf oil exhibited moderated result at concentration 25% against Staphylococcus aureus, exhibiting an inhibition zone of 25mm, on the contrary, and C.tamala leaf oil showed the inhibition zone of 17mm at a concentration of 25 %( Shown in Table1 and Figure 1).

**Rhizobium trifolii**: Leaf oil of C.zeylanicum proved to be most efficacious against Rizhobium trifolii exhibiting inhibition zones up to 35mm.
At 25% concentration C.tamala leaf oil proved to be moderate effective producing 20mm of inhibition zone (Shown in Table 1 and Figure 1).

**Klebsiella pneumoniae**: Leaf oil of C.zeylanicum showed potent result at concentration 25% against Rhizopus stolonifer, producing a range of 27mm inhibition zone. At 25% concentration C.tamala leaf oil-producing 15mm of inhibition zone (Shown in Table 1 and Figure 1).

**Proteus vulgaris**: Leaf oil of C.zeylanicum showed a moderate response showing the inhibition zone of 24mm on the contrary C.tamala leaf oil showed the inhibition zone of 14mm at a concentration of 25 %( shown in Table 1 and Figure 1).

**Shigella dysenteriae**: C.zeylanicum leaf oil exhibited high activity results against Shigella dysenteriae showing an inhibition zone of 25mm While C.tamala showed the inhibition zone of 28mm at a concentration of 25 %( Shown in Table1 and Figure 1).

**Streptomyces cinerochromogenes**: C.zelanicum leaf oil exhibited a moderate response exhibiting a zone of 24mm,on the contrary, C.tamala leaf oil showed the inhibition zone of 14mm at the lowest concentration(25%)(Shown in Table 1 and Figure 1).

## DISCUSSION:

Herbs and spices have played a major role in lifestyle from traditional times having various applications in food preservation, aromas, food additives, alternative medicines, and natural therapies and pharmaceuticals. The increasing incidence of infections by bacteria and poor efficacy of available drugs and the development of antibacterial resistance (2)(16) has to lead to draw our attention towards herbal products as antimicrobial agents that show better efficacy in a previous study(7)(10). The present study investigated the antimicrobial effect of the leaf oil of C.zeylanicum and C.tamala which possess active secondary metabolites showing antimicrobial activity against some tested bacteria (Table-1).In a previous study, bark oil of C.zeylanicum has been reported for its antimicrobial properties (10). C.zeylanicum has a high amount of cinnamaldehyde (60-70% w/w) and eugenol which is highly electronegative which interferes in biological processes that directly inhibit the growth of the microorganisms (17) (20) (21).C.tamala leaf oil contains a major portion of eugenol, cinnamaldehyde, cinnamyl alcohol, cinnamyl acetate and cinnamic acid (18) (22) may thus imparting the antibacterial properties. Different concentrations of essential oil may impart different inhibiting activity against tested microorganism (Table-1). From the scanning of data (Table 1), it is inferred that the overall performance of C.zeylanicum has shown the best result. In the case of Alcaligene xylasoxidans the C.zeylanicum essential leaf oil has shown an outstanding effect of plate clearance.C.tamala leaf oil though not tested on higher concentration in present work, has also shown promising results with a few bacteria. Our findings in this study showed that the leaf essential oil of C.zeylanicum and C.tamala inhibiting tested microorganisms. The result of this study also indicates that herbal extract like cinnamon may act as a natural antimicrobial agent. The result is in accordance with (10) who showed that hydrophobicity plays an important role in antimicrobial effects. The size of the inhibition zone also depends on the solubility of essential oils in the agar well diffusion plate and as well as diffusion characteristics of essential oils (20). However, these inhibition zones are visible, essential oils show the efficacy against the tested bacteria. From the above discussion, it may be concluded that herbal plant extracts and essential oils could pave the way to numerous useful drugs with less cost and fewer side effects.

## CONCLUSION:

The result of this comparative study showed that essential leaf oil of C...zeylanicum and C.tamala are rich in bioactive compounds and possess antibacterial activity. Pharmacological studies are necessary to investigate

the active compounds in these extracts and essential oils for better efficacy and evaluating potential safety for human health. However, further research is required to unravel the mode of action of essential leaf oil against the bacteria and their effect on large scale applications.

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