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Antimicrobial activity and chemical profiling of *Cinnamoum tamala* essential oil from Uttarakhand region of India

Radha Tyagi

Abstract
Nowadays, antimicrobial resistance is one of the major problem thus this study was made to finding the chemical composition and antimicrobial effects of essential oil of *Cinnamomum tamala* for the development of natural antimicrobial drugs without any side effects. *Cinnamomum tamala* (Indian Bay Leaf) or Nees Ebern, is an important traditional medicinal plant used for the treatment of infectious disease, mentioned in various ancient literatures such as Ayurveda. The Essential oil (EO), (volatile oil) is a concentrated hydrophobic liquid containing volatile aroma compounds, which obtained from the air dried leaves of *C. tamala* was analysed by capillary GC-MS and contained 15 components. In which, Linalool (36.71%), Cinnamaldehyde (30.71%) and DL-Limonene (7.39%) were major components used to determine the antimicrobial activity. EO from air dried leaves were highly effective against *Staphlococcusaures*, *Bacillus cereus* associated with bacterial disease and *Yeast*, *Candia albicans* associated with fungal disease. The antimicrobial activity of EO was determined by disc diffusion method, broth dilution method and assessed by the determination of MIC and MBC. This paper of reviews the literature published over the last 10-15 years regarding the antimicrobial effects of essential of *C. tamala*. In addition, a brief summary of the history, traditional uses, antimicrobial effects and clinical impact of *C. tamala* is provided. From the present work it can be concluded that *C. tamla* (Tejpat) can prove to be good source of herbal drugs there by aiding to explore an antimicrobial lead that is helpful in combating the disease cause by pathogenic bacterial and fungal species.

Keywords: Antimicrobial activity, essential oil, chemical composition, disc diffusion method, broth dilution method, ayurveda, *Cinnamomum tamala*, GC-MS, bacterial and fungal species, MIC and MBC

Introduction
Cinnamomum tamala, known as Indian Bay Leaf or Indian cassia (*Tejpat)* and belongs to *Lauraceae* family, it is native to India, China, Japan, Nepal and Bhutan, cultivated in India. Atree is distributed in tropical and subtropical Himalayas at the altitudes of 1000-2400m [1]. On an average, a tree produces 10-25 kg of dry leaves, 0.2- 0.4% oil can be extracted from leaves. Timely collection of leaf is important since early and late collection may result in poor quality of the leaves of essential oil [2]. The leaves of *C. tamala* have also been used extensively as spice in the home kitchen and foods industry due to its special aroma [3]. An essential oilis a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile oils, etherealoils, aetheroleaoil from the plant [4]. A hydro-distilledoilobtained from the leaves of *C. tamala* was analysed by capillary GC and GC–MS. The leaf volatile oil that contained 40 constituents, of which a high proportion were monoterpenes (65.6%). The predominant monoterpenes were trans-sabinene hydrate (29.8%), (*Z*)-*β*-ocimene (17.9%), myrcene (4.6%), *α*-pinene (3.1%) and *β*-sabinene (2.3%). Among 21 sesquiterpenes (32.9%), the major ones were germacrene A (11.3%) and *α*-gurjunene (4.7%), Methanol oleoresin: eugenol (69.3%), spathulenol (1.5%), methyl linoleate (1.3%), linoleic acid (1.0%) [5]. Antimicrobial potential of cinnamon extracts, essential oils and their components is the antibacterial activity against Gram-positive and Gram-negative bacteria and antifungal activity against fungal strains responsible for human infectious diseases. The antibacterial activity was expressed as both diameters of inhibition and minimum inhibitory concentration (MIC) values at different times of incubation.

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C. tamala has also been used as a health-promoting agent for the treatment of diseases such as inflammation, gastrointestinal disorders and urinary infection etc. [6]. It has also been used as a neuro protective agent [7] and for the treatment of diabetes [8]. Another potential medical use of *C. tamala* would be with regards to its microbial properties, especially antibacterial activity. It is well known that infection is one of the leading causes of morbidity and mortality worldwide. According to WHO reports, in 2011, there were more than 55 million deaths worldwide with infection belong responsible for one-third of all deaths [9]. The leaves of *C. tamala* is a brain tonic, antihelminthic, diuretic, is good for liver and spleen and its bark is useful for the treatment of Gonorrhea [10]. The essential oil from *C. tamala* exhibits antidermatophyt [11], antibacterial [12], antifungal, antihyperglycaemic and antihypercholestrolanemic effects [13]. *Staphylococcus aureus* is a gram positive, round-shaped, bacterium. It is a notorious suppurative (pus forming) pathogen, a major cause of hospital acquired (nosocomial) wound infection, urinary tract infection, and food poisoning [14]. The emergence of antibiotic-resistance strains of *S.aureus* such as methicillin-resistance *S. aureus* (MRSA) is a worldwide problem in clinical medicine. *Bacillus cereus* is a gram positive, rod shaped, beta-hemolyticandone of the food-borne disease causing bacteria. *B. cereus*causes two different types of food poisoning the diarrheal type and the emetic type [15] and also causing endocarditis and endopthalmitis [16, 17]. *Yeast* are eukaryotic, unicellular organisms which evolved from multicellular ancestors [18]. Some species of yeast are opportunistic pathogens that can cause infection in people with compromised immune systems. *Cryptococcus neoformans* and *Cryptococcus gattii* are significant pathogens of immunocompromised people. They are the species primarily responsible for cryptococcosis, a fungal disease that occurs in about one million HIV/AIDS patients, causing over 600,000 deaths annually [19]. Thegenus *Candida*, another group of opportunistic pathogens, cause oral and vaginal infections in humans, known as candidiasis [20]. *C. albicans* infections faces a number of problems including limited number of effective antifungal agents, toxicity of the available antifungal agents, resistance of *Candida* to commonly used antifungals, relapse of Candida infections and non-cost effective antifungal agents [21]. Around 75% of adult women have at least one episode of vulvovaginal candidiasis (VVC) during their life, with prevalence of *C. albicans* in 70–90% [22]. In order to alleviate the problem of reduced availability of drugs needed to treat candidiasis, traditional medicine derived fromplants are still being used [23]. This prompted the search for novel and active anti- *C. albicans* agents from plant sources.

Synthetic antibiotics are the most popular antimicrobial agents during their discovery. However, the emergence of multiple drug resistant pathogenic bacteria has a major cause of failure of the antibacterial drug [24] and synthetic drugs are more effective but have side effects. That is why, it is very essential to investigate newer drugs with less resistance as which are cheap, easily available and eco-friendly. The investigation is going to describe the beneficial and essential values of "Antimicrobial activity and chemical profiling of *C. tamala* EO from Uttarakhand region of India.

Material and Methods

Extraction of extraction oil: Air dried leaves of *C. tamala* chopped and ground into small pieces. The known amounts of processed samples were placed in a round bottom flask together with distilled water and hydro-distillation was performed using Clevenger apparatus. Essential oil will be collected, dehydrated using sodium sulfate and dispensed into dark bottles. Oil will be stored at 4 ^{0}C until used. T-ween 20 (0.1%) was used as dispersing agent.

Physical characteristics of essential oil

Yield: - the yield of essential oil was calculated as:-

$$\% \text{ yield} = \frac{\text{Amount of oil (in ml)}}{\text{Weight of leaves (in gram)}} \times 100$$

$$= \frac{3.5}{760} \text{x} 100 = 4.6\%$$

Color: - The color of oil was pale yellow noted.

GC-MS Analysis of Essential Oil: Essential oil was analyzed by Hewlett – Packard GC/MS (model 7890 series II) operating at 70e V ionization energy, equipped with a HP–5 capillary column phenyl methyl siloxane (30m, 0.25mm, 0.25µm film thickness) with helium as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Dool method (Van den dool and Kratz, 1963), using n–alkanes as standard. The compounds were identified by comparison of retention indices (RRI–HP–5) with those reported in the literature and by comparison of their mass spectra with the Wiley and Mass finder 3 libraries or with the published mass spectra (Adams, 2001).

Preparation of stock solution: A stock solution (16%) of essential oil was prepared by diluting the essential oil in 10% DMSO and 0.5% T-80 in the aseptic conditions (stored at 4 ^{0}C in dark).

Procurement of culture: Bacterial cultures *Bacillus cereus* and *Staphylococcus aureus* and fungal culture *Yeast* and *Candida albicans* were used. The cultures were obtained from microbial type culture collection, IM-Tech, Chandigarh. The routine sub culturing will be done on Nutrient Agar and Glucose Yeast Extract Agar.

Preparation of inoculum: Bacterial inoculums; two to three colonies of bacterial test organisms were inoculated in nutrient broth and incubated at 37^0 C broth. Until the turbidity of broth matches with McFarland (10^{6}cfu/ ml).

Fungal inoculums; two to three colonies of fungal test organism were inoculated in sabouraud dextrose broth and incubated at 28 ^{0}C broth. Until the turbidity of broth matches with McFarland (10^{6}cfu/ ml).

Screening of antimicrobial activity of essential oils against Bacteria and Fungus by Disc Diffusion method

- The test cultures were spread uniformly on Muller Hinton Agar and Sabouraud Dextrose Agar plates using a sterile spreader. The plates were dried for 15 minutes. Sterile discs were placed at equal distance.
- With the help of micropipette placed 20µl of different concentrations of test material or control on the sterile discs.
- 10% DMSO and Streptomycin and Nystatin were used as control. DMSO as negative control & Streptomycin and

Nystatin as positive control respectively.

- The plates were incubated at ±37 ^{0}C for 24h and ±28 ^{0}C for 24h respectively.
- After the incubation three readings were noted for the zone of inhibition (diameter). The final result will be expressed as average of three readings ± SD.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of essential oil by broth dilution method

1. Minimum inhibitory concentration (MIC)

- 95µl of double stranded Nutrient Broth and Sabouraud Dextrose Broth were added to the wells of the micro-titre plate.
- 5µl inoculums were added to each well except the first control.
- 100µl of essential oil were poured in the first control and in the second well. The oil were serially diluted in wells of micro-titre plates. The plates were incubated at ±37 ^{0}C for 18h and ±28 ^{0}C for 18h, respectively.
- After incubation, 20µl resazurin dye was added to every well.
- Now it was incubated for 2h, the color was noted.
- Pink/violet color was observed indicating presence/absence of bacteria and fungus in the medium.

2. Minimum Bactericidal Concentration

- A loopful of solution was taken from each well of the micro titre plate with the help of nichrome loop, streaked over the MHA plates.
- Now the plates was incubated at 37^0 C for 18h to 24h in the incubator.
- After this appearance of growth were observed on the plates.
- The last concentration, in which there was absence of growth, was noted as MBC value of that oil.

Results

Collection of *C. Tamala* (Tejpat) Leaves

Fresh Leaves

Dry Leaves

Physical Characterisation of Essential Oil

Yield: The yield of essential oil was calculated as:-

$$\% \text{ yield} = \frac{\text{Amount of oil (in ml)}}{\text{Weight of leaves (in gram)}} \times 100$$

$$\% \text{ yield} = \frac{3.5}{760} \times 100 = 0.46\%.$$

- It was calculated in the following manner and was calculated to be 0.46%.

Colour and fragrance: The colour of oil extracted from leaves was pale-yellow and having a good fragrance.

Fig: Essential oil collected from *C. tamala* leaves.

GC-MS analysis of essential oil

The gas chromatography – mass spectrometry (GC-MS) analysis of the oil was performed with a Perkin Elmer Clarus 500 gas-chromatography equipped with a split/splitless injector (split ratio 50:1) data handling system. Helium (He) was the carrier gas at a flow rate 1.0ml/min.

Table 1: Chemical composition of C. *tamala* essential oil.

S. No.	Components	%
1.	α-pinene	2.27
2.	Benzaldehyde	0.92
3.	β-pinene	1.77
4.	β-myrcene	0.60
5.	L-phellandrene	0.56
6.	δ-3 carene	0.24
7.	Cymene	2.08
8.	DL-limonene	7.39
9.	1,8 cineole	1.13
10.	Linalool	36.71
11.	Benzenepropanal	0.44
12.	α-terpineol	0.97
13.	Cinnamaldehyde	30.71
14.	Bornyl acetate	1.31
15.	Cinnamyl acetate	2.97

On the basis of GC- MS, Linalool (36.71%), Cinnamaldehyde (30.71%) and DL-Limonene were major component.

Antimicrobial Activity

Antimicrobial activity of *Cinnamomum tamala* essential oils was determined against bacteria and fungus namely, *S. aureus* (MTCC87), *B. cereus* (MTCC268) and *Yeast*(MTCC36), *C.albicans* (MTCC3017) respectively by disc diffusion method. The antimicrobial activity of essential oil was assessed by determination of MIC & MBC.

Screening of Antimicrobial activity of Essential oil of *C. tamala*againstdifferent bacteria.

Treated with neat oil **Control set** **Treated with different conc.**

Fig 1: Antimicrobial activity of *C. tamala*essential oil against *B. cereus.*

Treated with neat oil **Control set** **Treated with different conc.**

Fig 2: Antimicrobial activity of *C. tamala* essential oil against *S. aureus.*

Screening of Antimicrobial activity of Essential Oil of *C. tamala* against different fungus.

Treated with neat oil **Control set** **Treated with different conc.**

Fig 3: Antimicrobial activity of *C. tamala* essential oil against *Yeast.*

Treated with neat oil **Control set** **Treated with different conc.**

Fig 4: Antimicrobial activity of *C. tamala* essential oil against *C. albicans.*

Table 2: Inhibition of bacteria by *C. tamala* essential oil.

Organisms	Zone of inhibition (mm)							
	Treated with different conc.					Neat oil	Control	
	0.5	0.25	0.12	0.06	0.03		Streptomycin (positive control)	DMSO
B. cereus	13±0	9±0	9±0	R	R	26±0	24.6±0.057	R
S. aureus	8.6±0.057	7±0	7±0	7±0	R	33±0	25.6±0.057	R

*R = Resistant

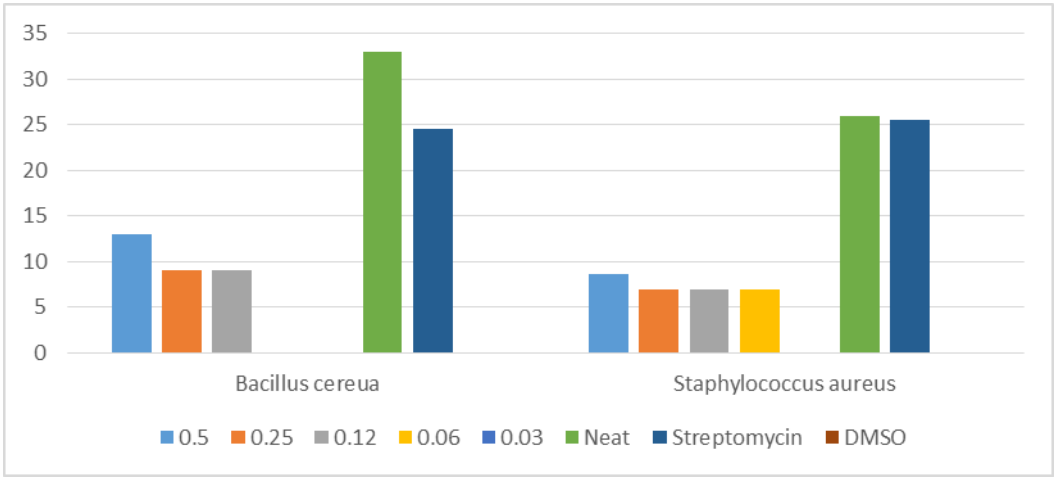
- The readings represent the average of three reading was ±SD.

Table 3: Inhibition of fungus by *C. tamala* essential oil.

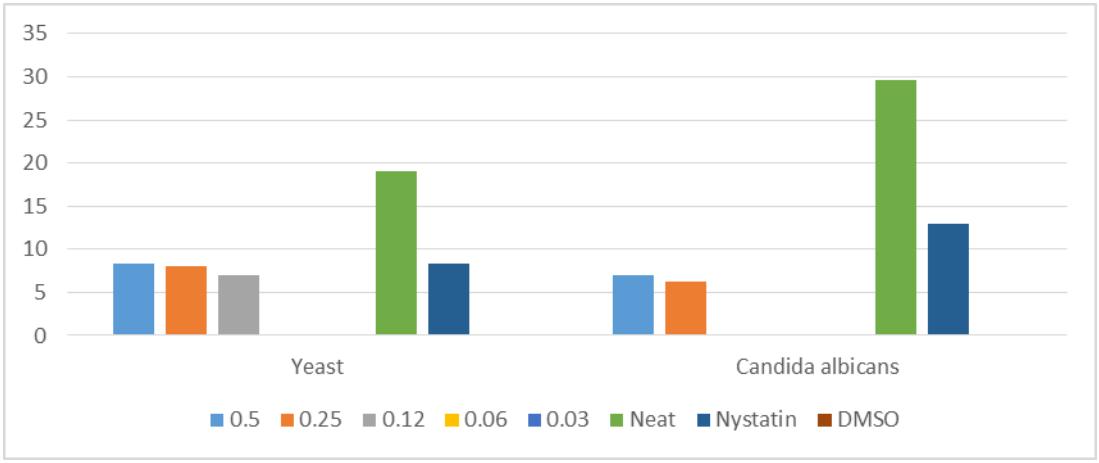
Organisms	Zone of inhibition (mm)							
	Treated with different conc.					Neat oil	Control	
	0.5	0.25	0.12	0.06	0.03		Nystatin (positive control)	DMSO
Yeast	8.3±0.057	8±0.1	7±0	R	R	19±0	8.3±0.057	R
C. albicans	7±0	6.3±0.057	R	R	R	29.6±0.1	13±0	R

*R = Resistant

- The readings represent the average of three reading was ±SD.



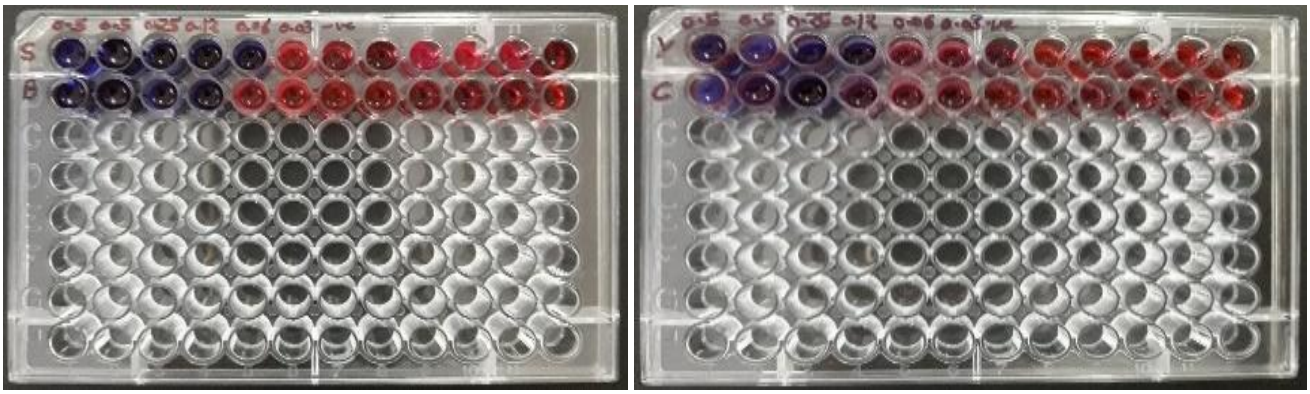
Graph 1: Comparison of antimicrobial activity of *C. tamala* essential oil against bacteria.



Graph 2: Comparison of antimicrobial activity of *C.tamala* essential oil against fungus.

Minimum Inhibitory Concentration of *C. tamala* essential oil.

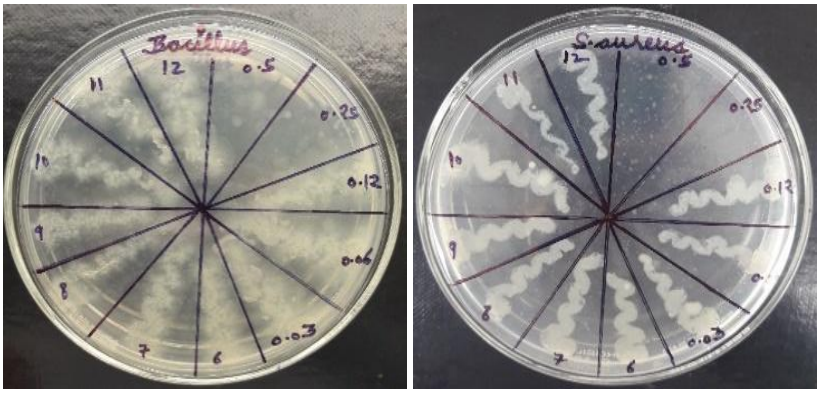
Minimum inhibitory concentration of *C. tamala* essential oil were determined against bacterial isolates and fungal isolates. MIC were determined against *B. cereus*, *S. aureus* and *Yeast*and *C.albicans*.



S = *S. aureus*Y = *Yeast*
B = *B. cereus*C = *C.albicans*

Fig 6: Determination of MIC*C. tamala* essential oil against Bacteria and fungus.

Minimum Bactericidal Concentration of *C. tamala* essential oil.

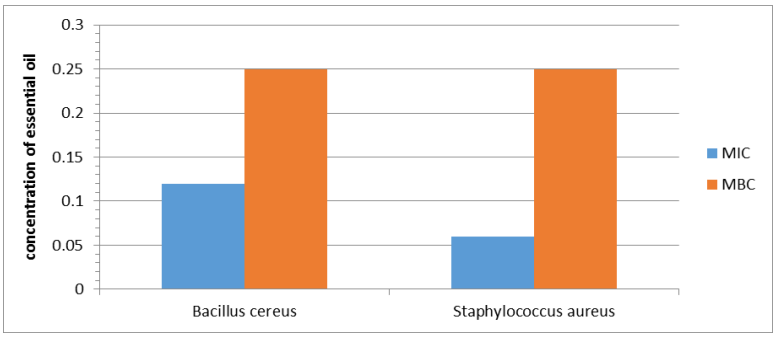


A} MBC of *B. cereus* **B} MBC of *S. aureus***

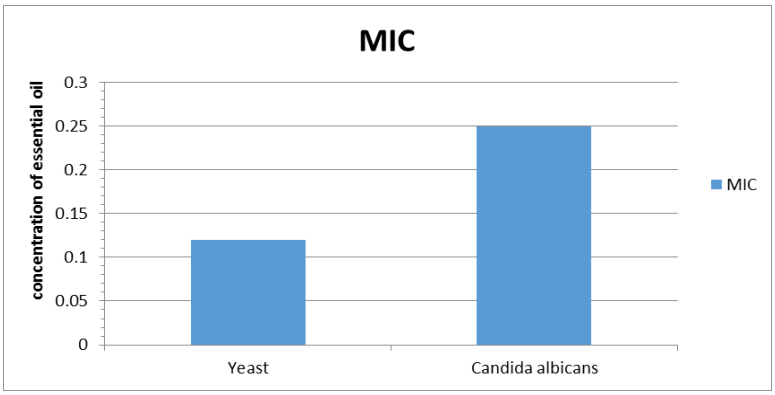
Table 4: Minimum inhibitory concentration and Minimum Bactericidal Concentration of *C. tamala* essential oil:

Organisms	MIC		MBC	
	%	(µl/ml)	%	(µl/ml)
B. cereus	0.12	150	0.12	150
S. sureus	0.06	75	0.12	150
Yeast	0.12	150	-	-
C. albicans	0.25	310	-	-

- µl/ml of Essential Oil was taken from 8% of stock solution.



Graph 3: Comparison between MIC and MBC of different Bacteria.



Graph 4: Comparison between MIC of different Fungus.

Discussion

Essential Oil of *C. tamala*is a volatile aromatic concenterated hydrophobic oily liquid obtained from leaves. *C. tamala* belonging to the *Lauraceae* family which is a highly valued medically important spice.In the present study, an attempt was to determine the chemical profiling of *Cinnamomum tamala*, obtained 15 components and the major component of the EO are Linalool (36.71%), Cinnamaldehyde (30.71%) and DL-Limonene (7.39%). However, the chemical profiling of *C. tamala* has been previously explored by a number of researchers and determined that monoterpenes were in high proportion [5]. The zone of inhibition by *C. tamala* EO was determined to be in ranges from 17-25mm against *Aspergillusniger*, *A. fumigatus*, *Candida albicans*, *Rhizopusstolonifer* and *Penicillium spp* in agar diffusion assay [25].

According to the present study, *C. tamala* showed excellent antibacterial and antifungal activity, completely inhibit growth at 0.12% of essential oil against *B. cereus*; 0.06% of essential oil against *S. aureus*; 0.12% of essential oil against *Yeast* and 0.25% of essential oil against *C. albicans*. MIC of *C. tamala* was determined to 0.12% against *B. cereus*, 0.06% against *S. aureus*, 0.12% against *Yeast* and 0.25% against *C.albicans*. MBC of C. *tamala* was determined to be 0.12%

against both *B. cereus* and *S. aureus*.

In our study, the extracts of *C. tamala* leaves were tested for their antimicrobial efficacy and were found equally effective against bacteria (*B. cereuss, S. aureus*) and fungus *(Yeast, C. albicans).* The EO showed their best activity against *B. cereus, S. aureus, Yeast, C. albicans* and showed inhibition zone measuring 26mm, 33mm, 19mm, 29.6mm.

Based on the above discussion it may be concluded that nature is the best combinatorial chemist and possibly has answers to all diseases. Till now, natural products and compounds discovered from medicinal plants have provided numerous clinically useful drugs. In spite of the various challenges encountered in the medicinal plant-based drug discovery, natural products isolated from plants will still remain an essential component in the search for new medicines. The results have established the intense antimicrobial potential of *C. tamala* EO, respectively. This indicates that the phytoconstituents present in test plant have considerable potential to prevent spoilage of food and diseases caused by fungi and bacteria as well as the diseases resulting from over production of radicals. So the natural phytochemicals derived from *C. tamala* could be regarded as promising alternative to synthetic antifungal and antibacterial preparations for future use [25].

Conclusions

According to the present study entitled as "Anti-microbial activity and chemical profiling of essential oil of *C. tamala* from Uttarakhandreigon of Inda" we concluded that the anti-bacterial and anti-fungal activity of *C. tamala* oil against bacteria (*B. cereus* & *S. aureus*) and fungus (*Yeast* &*C. albicans*) was done by Disc-Diffusion Method and Broth-Dilution Method.

The best anti-bacterial and anti-fungal activity was observed at different concentration (ranging from 0.03%, 0.06%, 0.12%, 0.25% and 0.5%) against *B. cereus, S. aureus, Yeast* and *C. albicans*. MIC at the concentration 0.12% against *B. cereus*, 0.06% against *S. aureus*, 0.12% against *Yeast* and 0.25% against *C. albicans* was observed. And the concentration 0.12% is the MBC against both *B. cereus* and *S. aureus*. The present study indicated the inhibitory action of *C. tamala* (tejpat) were highly effective against *B. cereus* and *S. aureus* associated with bacterial disease, *Yeast* and *C. albicans* associated with fungal disease.

Results from present study demonstrate the intense antimicrobial potential of *C. tamala* leaves extracts against bacteria and a fungus. The findings suggest that the plant could be regarded as promising alternative for development of efficient and effective drug from natural source that can be used for therapy of infectious diseases. The most active extracts could be subjected for further pharmacological evaluation by isolation of the therapeutic antimicrobials and further research on this plant can specify its pharmaceutical application.

From the present work it can be concluded that *C. tamala* (Tejpat) can prove to be good source of herbal drug there by aiding to explore an antimicrobial lead that is helpful in combating the disease cause by pathogenic bacterial and fungal species.

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