Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 2442 - 2449
Received 15 April 2021; Accepted 05 May 2021.

Antifungal activity of Cinnamomum Spp: A comparative study of Aromatic Oil

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Abstract:

Emergence of new fungal diseases and due to the development of resistance towards available antibiotics and due to side effects imparted by modern antibiotics, studies are directed towards the development of new antimicrobial agents that are plant-based which impart fewer side effects. Herbal medicine has a wide range of medicinal properties and shows the potential biological activity ranging from antimicrobial, antifungal, antioxidant has paved our way to curing diseases and conditions by natural herbs.
The Objective of this study is to compare the efficacy of antifungal potential of essential bark oil C.zeylanicum and bark oil of C.camphora. Essential oil was extracted from the bark of C.zeylanicum by using the Clevenger apparatus and the agar well diffusion method was used to study and screened for antifungal activity by determining the inhibition zone(in mm)by using vernier calipers.
Our results obtained from this comparative study suggest that C.zeylanicum bark oil and its constituents could be promising treatment strategies against these fungal strain. The further study requires to understand the mechanism behind the mode of action and inhibiting potential for human use.

Keywords:Antifungal,essentialoil,Cinnamomumzeylanicum,Cinnamomumcamphora,aromatic oils

Introduction:

A large number of new antibiotics are produced in the last three decades by pharmacological industries and possess wider applications ranging from personal care to treating various disorders[1].With major production of antibiotics in developing and developed countries major concern arises due to the development of resistance to particular drugs and causing side effects[2].Interest toward Herbal products and their compounds have also increased due to effectiveness and possessing antibacterial, antiviral, and antifungal potential and showing less side effects[3]. These Herbal plants are used by people from ancient times in treating various disorders. Extract from herbal plants consist of various phytochemicals around (30–60)at

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different concentrations and possessing different properties[3]. The most common Phytocompounds are polyphenols, terpenes, aromatic and aliphatic compounds. According to the survey of the World Health Organization (WHO), more than 65% of the world population has to include medicinal plants for treating diseases in general healthcare[4]. Use of Herbal products in the Asian Countries traditional medicines are frequently used from ancient times. A survey revealed that about 80% of Asians regularly use medicinal plants[9,19].
Phytocompounds present in medicinal plants possess antibacterial agents that have the potential to fight against microorganism[5]. Bacterial and fungal infections cause much discomfort and sickness and affect men around the globe due to the presence of biologically active compounds such as mycotoxins which are toxic to several plants and animals[6]. Essential oils derived from herbal extract has gained preference over synthetic chemicals due to awareness and due to fewer side effects. In the present study, the essential oils from Cinnamomum zeylanicum and Cinnamomum camphora were investigated for antifungal activities.

Cinnamomum belongs to the family Lauraceae and is distributed in the tropical and subtropical regions of Australia, Central, and North America, and South Asia[7]. The essential oil from Cinnamomum bark and leaf oil possesses antibacterial, antifungal, and anti-plasmodial activity and have a pharmacological effect on various tissue and are currently being studied to procure a better understanding of their potential in natural food preservative[8]. Polyphenols and volatile phenols are two main classes that are isolated from Cinnamomum zeylanicum[10].Selected Fungi affect a wide range including from food crop disease such as smut disease in maize to foodborne illness such as skin disease, digestive problems, diarrhea, vomiting, fever, abdominal cramps[11].A previous study has examined the role of C.zeylanicum about a large number of bacteria[8,9]. Whereas Cinnamomum camphora also belongs to the same family Lauraceae and is frequently cultivated in China and distributed in subtropical areas of Southeast Asia and East Asia[11]. C.camphora essential oil also imparts various potential in treating diseases such as rheumatic conditions, inflammation bronchitis and also act as antibacterial agents against E.coli, Staphylococcus aureus, etc showed in previous study[16,17]. Due to the presence of a different concentration of compounds such as camphor,alpha-terpineol, linalool, safrol, etc C.camphora is also used in wide applications[12,16].

Keeping in view the ever-rising trend in herbal research, this study aims to investigate the antifungal efficacy of essential oil from the bark of C.zeylanicum and C.camphora:
In the present work, experiments were designed to achieve the following objectives:
1. Extraction of essential oil from the bark of C.zeylanicum and C.camphora.
2. Purification of essential oil.
3. Inhibition studies of the above test sample on a spectrum of pathogenic/non-pathogenic Fungal culture namely-Aspergillus niger, Aspergillus flavus, Trichoderma viridae, Fusarium sp., Rhizopus stolonifer, Candida sp., Penicillium chryosogenum

2. MATERIALS AND METHODS

2.1 Collection and Preprocessing of plant sample

The bark of C.zeylanicum and bark of C.camphora plant samples were collected from F.R.I botanical garden and evaluated for their antifungal activity against nine fungi. The Bark plant samples were cleaned and washed thoroughly under running tap water and then with sterile

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distilled water to remove any soil impurities and dust and dried in shade for 4-6 days. After that bark sample was crushed and weighed before calculating the yield.

2.2 Extraction of essential oils and its purification

In this study, essential oil from the bark of C.zeylanicum and C.camphora were obtained by using the Clevenger apparatus. The Hydrodistillation method was used in the extraction of essential oils. Clevenger apparatus is a round bottom flask consisting of a main unit and condenser. In the process, bumping of hot water occurs, to avoid this porcelain chip is added. The extraction of essential oils is carried out under atmospheric pressure at a boiling temperature about 100oC, the oil starts coming within 20minutes, then the temperature of the heating mantle is reduced to 55-70oC and this essential oil and water (called as an emulsion) are collected in a conical flask and allowed to cool at room temperature. Two distinguishable phases are seen and this mixture was separated by filtration method after 24 hours for evaluating antifungal activities against test fungal strain.

2.3 Preparation of culture media

Potato Dextrose Agar (PDA) commercial media was used at a rate of 39gm in 1000ml. pH was maintained to 4.8 and then sterilized at 121oC and 15lbs for 20min in an autoclave.

2.4 Source of Test microorganisms

The seven test microorganisms namely: Aspergillus niger, Aspergillus flavus, Trichoderma viridae, Fusarium sp., Rhizopus stolonifer, Penicillium chryosogenum, and Candida sp. All these fungal strains were obtained from departmental culture, and fungal strains were subcultured on PDA culture media in different slants and incubated at 25-28^{o}C for 6-7days and a regular check for optimal growth during the incubation period.

2.5 Bore plate diffusion method

Sterile agar plates of Potato Dextrose agar were prepared under aseptic conditions(Laminar Air Flow). After solidification, 100µl of different fungal suspension was spread by spreader on each agar plate (in triplicates).

2.6 Preparation of Microbial suspension

A 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

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already spread by borer of 8mm diameter. Dilutions of 25%of essential oil of C.zeylanicum and C.camphora separately at a rate of 35µl was poured at the center of each well. Plates were incubated at 25-28oC for 7-8days. This assay was done in triplicate to ensure consistency.

2.7 Measurement of Inhibition zone

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

Result:

Effects on different organisms are described below:

Aspergillus niger: Bark oil of C.zeylanicum proved to be most efficacious against Aspergillus niger exhibiting inhibition zone up to 57mm (Table 1).
At 25% concentration C.camphora bark oil also proved to be effective producing 32mm of inhibition zone. This inhibition potential of the bark oil of C.camphora can be correlated with a high concentration of camphor present in essential oil(Shown in Table 1 and Figure 1).

Aspergillus flavus: Bark oil of C.zeylanicum showed a moderate response at concentration 25% against Aspergillus flavus, producing a range of 18mm inhibition zone. At 25% concentration, C.camphora bark oil-producing showed 20mm of inhibition zone(Shown in Table 1 and Figure 1).

Trichoderma viridae: Bark oil of C.zeylanicum showed a moderate response showing the inhibition zone of 19mm on the contrary C.camphora bark oil showed the inhibition zone of 21mm at a concentration of 25%(shown in Table 1 and Figure 1).

Fusarium sp.: C.zeylanicum bark oil showed an overwhelming response at 25% concentration against Fusarium sp. showing plate clearance which proved to be an effective antifungal against Fusarium sp. on the contrary C.camphora bark oil showed no inhibition against Fusarium sp.(Shown in Table1 and Figure 1).

Rhizopus stolonifer: Bark oil of C.zeylanicum showed a moderate response showing the inhibition zone of 22mm on the contrary C.camphora bark oil showed the inhibition zone of 10mm at a concentration of 25%(shown in Table 1 and Figure 1).

Candida sp.:C.zeylanicum bark oil showed an overwhelming response at 25% concentration against Candida sp. showing plate clearance which proved to be an effective antifungal against Candida sp. on the contrary C.camphora bark oil showed an inhibition zone of 27mm against Candida sp.(Shown in Table1 and Figure 1).

Penicillium chryosogenum:C.zeylanicum bark oil showed no inhibition at 25% concentration against Penicillium chryosogenum,On the contrary, C.camphora bark oil

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showed high activity and showed an inhibition zone of 38mm against Penicillium chryosogenum.(Shown in Table1 and Figure 1).

Discussion:

The recent development of widespread antibiotic-resistant microorganisms and due to undesirable side effects associated with modern synthetic drugs have influenced the scientist to find the alternative therapeutics majorly plant-based compounds to treat various bacterial and fungal diseases[13]. The bioactivity of aromatic plants is contributed by its phytocompounds. Synthetic fungicide is used widely as a control method but they have developed drug resistance and thus causing the risk to health and the environment. The present study investigated a comparison of the antifungal effect of the bark oil of C.zeylanicum and C.camphora which possess active secondary metabolites showing antifungal efficacy against some tested fungal strain(Table-1). From the literature review, the effect of 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[10].Several studies have been reported for their antifungal activities of essential oils of some Pakistani spices[18]. Chemical characterization of C.camphora was done in a previous study which showed the presence of various secondary metabolites such as Camphor(in major proportion), cineole, linalool, terpineol,alpha-terpineol etc[17]. In a previous study, It was shown that plant which is rich in secondary metabolites have antimicrobial potential due to their characteristic property that allows constituents to react with bacterial proteins to form complex and thus directly damaging the cell membrane and killing the bacteria[14]. From the scanning of data(Table 1), it is inferred that the overall performance of C.zeylanicum bark oil at 25% concentration has shown the best result. In the case of Fusarium sp. and Candida sp the C.zeylanicum essential bark oil has shown an outstanding effect of plate clearance. Bark oil of both species though not tested on higher concentration in the present work has also shown promising results with a few fungal strain. Our findings in this study showed that the bark essential oil of C.zeylanicum and C.camphora showed inhibiting potential against tested fungal strain. The result of this study also indicates that herbal extract like cinnamon may act as a natural antifungal agent as well. 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[15]. However, these inhibition zones are visible, essential oils show the efficacy against the tested fungal strain. 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:

In conclusion, our result of this comparative study showed the potential of essential bark oil of C.zeylanicum and C.camphora both are rich in bioactive compounds and thus show the potential to inhibit the tested fungal strain. The zone of inhibition varied suggest that phytochemicals present in C.zeylanicum and C.camphora have a different mode of action on different organisms. Pharmacological studies are necessary to investigate the active compounds in these extracts and the mode of action of these essential oils for better efficacy and evaluating potential safety for human health. However, further research is required to

isolate and characterize the essential bark oil against the fungal strain and their effect on large scale applications.

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Essential Oils	C.zeylanicum bark oil	C.camphora bark oil
Organisms	25%	25%
Aspergillus niger	57	32
Aspergillus flavus	18	20
Trichoderma viridae	19	21
Fusarium sp.	Plate clearance	No inhibition
Rhizopus stolonifer	22	10
Candida sp.	Plate Clearance	28
Penicillium chryosogenum	No inhibition	38

Table 1. Mycelial growth inhibition assay with C.zeylanicum bark oil and C.camphora bark oil.

Zone diameter in mm:

15mm and below-low activity;

15-25mm-moderate activity;

25mm above-high activity

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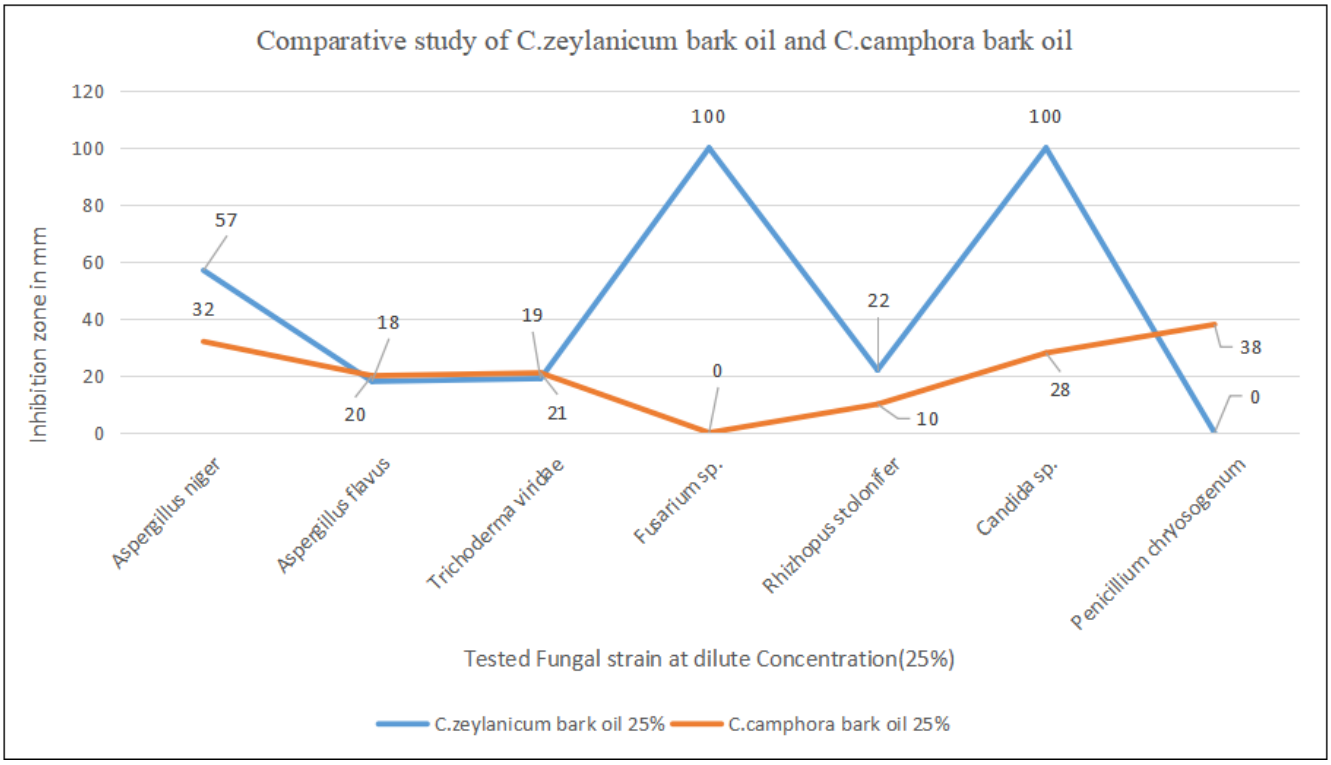

We consider

*Inhibition zone of 100mm - Showing the plate clearance

Inhibition zone of 0mm - Showing No inhibition

Figure 1. Comparative study showing the inhibition zone of C.zeylanicum bark oil and C.camphora bark oil against tested fungal strain.