Chemical characterisation and antifungal activity of methanolic extract of *Cinnamomum verum* J. Presl bark against *Malassezia* spp.

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**A R T I C L E   I N F O**

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**A B S T R A C T**

*Context:* *Malassezia* spp. are the organisms causing skin infections like dandruff, pityriasis versicolor, seborrhoeic dermatitis etc. in human and in animals. Using natural ingredients for treating these diseases could be a better task.

*Aim:* To investigate the phytochemical constituents and antifungal efficacy of *Cinnamomum verum* (CV) bark methanolic extract against dandruff, pityriasis versicolor, seborrhoeic dermatitis causing dimorphic fungi belonging to the genus *Malassezia*.

*Materials and methods:* Cultures of *Malassezia globosa*, *Malassezia sympodialis*, and *Malassezia furfur* of clinical origin were prepared by adjusting inoculum size to 10⁵ cfu/ml. Minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) for methanolic extract of CV bark were done to find out the lowest concentration of extract required to inhibit the growth of *Malassezia* spp. and the preliminary phytochemical and GC–MS analysis was carried out to identify the phytoconstituents.

*Results:* Methanolic extract of bark of CV showed MIC value ranging from 0.5 to 2 mg/ml against *Malassezia* spp. The major chemical constituents were identified as trans-Cinnamaldehyde (20.28%), (E)-3-(2-Methoxyphenyl)-2-propenoic acid (40.41%), 4-Vinyl benzoic acid (10.54%) and coumarin (8.47%) by GC–MS.

*Conclusions:* *C. verum* bark methanolic extract has potential antifungal activity and can be exploited against skin infections caused by *M. globosa*, *M. sympodialis*, and *M. furfur*.

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**1. Introduction**

Dandruff, pityriasis versicolor, seborrhoeic dermatitis are common clinical conditions of the scalp and skin caused by *Malassezia* species which are major concern to people all over the world. *Malassezia* species are dimorphic fungi that exist both in yeast and mycelial phases. Among the *Malassezia* species, *Malassezia globosa* and *Malassezia restricta* have been most closely associated with dandruff in humans. *Malassezia* spp. may stimulate cytokine production by keratinocytes (epidermal cells that synthesize keratin), further contributing to the inflammatory component of seborrhoeic dermatitis and dandruff.

*Cinnamomum verum* Presl (CV) (syn: *Cinnamomum zeylanicum* Blume) belongs to the family Lauraceae, is a medicinal plant widely used in traditional systems of medicine. The bark is widely used as spice, condiment and flavouring agent. The bark is acrid, bitter, sweet, aromatic, astringent, aphrodisiac, deodorant, stimulating, astringent, expectorant, febrifuge, diuretic and carminative. It is useful in bronchitis, asthma, cephalalgia, odontalgia, cardiac diseases, diarrhoea, urethritis, nausea and vomiting, flatulence, fever, halitosis and restoring normal skin colour on the face. The cinnamon bark possesses anti-oxidant, anti-ulcer, antimicrobial, anti-diabetic, hypoglycemic, hypolipidemic and anti-inflammatory activity.

The earlier reports revealed the antifungal activity of cinnamon bark extracts and oil against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium notatum*, *Alternaria solani* and *Curvularia lunata*, Rhizomucor, and ringworm causing organisms *Microsporum canis*, *Malassezia gypseum*, *Malassezia audouini*,...
Ketoconazole, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Cryptococcus neoformans*.\(^{5–10}\) Gupta et al.\(^7\) also studied the antibacterial property of cinnamon oil against food borne pathogens *viz.* *Bacillus* spp., *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella* spp. Ferhout et al.\(^{10}\) reported the antifungal activity of cinnamon oil, cinnamaldehyde and carvacrol against *Malassezia furfur* and *Candida albicans*. Recent literature survey revealed that there is paucity of reports on antifungal activity of CV bark against *Malassezia* spp. Hence, the present study was undertaken to evaluate the methanolic extract of CV bark against clinical isolates of *M. globosa*, *Malassezia sympodialis* and *M. furfur* by MIC and agar well diffusion assay and screening the phytoconstituents.

### 2. Materials and methods

#### 2.1. Test organism

*M. globosa*, *M. sympodialis*, and *M. furfur* of clinical origin received from the Department of Microbiology, The New College, Chennai were used for the study. The cultures were subcultured and maintained on Sabouraud’s dextrose agar (SDA) (Himedia) slants overlaid with sterile olive oil (commercial) and stored in refrigerator at 4 °C.

#### 2.2. Inoculum preparation

Inoculum of the above cultures was prepared by inoculating in 5 ml of Sabouraud’s dextrose broth (Himedia) with 2 drops of olive oil and incubated at 30 °C for 2 days. The inoculum size was adjusted to 10⁷ cfu/ml.\(^7\)

#### 2.3. Preparation of the extracts

Barks of *C. verum* were purchased from the local market, Chennai and authenticated and the voucher specimen (CPL R&D/ VS/#002) was preserved at R&D Centre, Cholayil Private Limited, Chennai, India. Sample was powdered using mixer grinder and 500 g of powdered sample was extracted with 2.5 l of methanol (Merck) using soxhlet apparatus at 80 °C for 6 h. Then the extract was filtered and concentrated on water-bath at 50 °C. The final extract residue was re-dissolved in methanol and made up to required quantity and used for the screening of MIC, ZOI and phytoconstituents. GC–MS study was carried out to find out the major components in the extract.

#### 2.4. Minimum inhibitory concentration (MIC) test

MIC was determined by incorporating various concentration of the extract ranging from 0.5 to 5 mg/ml in 10 ml of SDA. Culture medium and extract were mixed thoroughly and allowed to solidify. A drop of olive oil was overlaid and 100 μl of the inoculum was inoculated on each plate. The plates were incubated at 30 °C for 5 days. Negative control was maintained with solvent. The concentrations which completely inhibit the growth of organisms considered as MIC.\(^{11}\)

#### 2.5. Agar well diffusion method

SDA plates were overlaid with olive oil and inoculated with respective cultures by spreading on the surface of the media. A well was made in the centre of the medium and the extract (10 μg/μl) was loaded in the well. Ketoconazole (10 μg) (Himedia) was used as positive control. The plates were incubated at 32 °C for 5 days. The antifungal activity was assessed by measuring the diameter of the ZOI in mm. All the analyses were carried out in triplicates and data values are expressed in mean ± SD.\(^{12}\)

#### 2.6. Phytochemical analysis

Phytochemical analysis was carried out to find the presence of various groups of compounds *viz.* phenolics, flavonoids, terpenoids, alkaloids, tannins and saponins.\(^{13}\)

#### 2.7. Gas chromatography–mass spectrum analysis (GC–MS)

GC–MS technique was used to identify the phytocomponents present in the bark methanolic extract. GC–MS–5975C [AGILENT] fitted with electron impact (EI) mode and equipped with column DB-5sms Agilent with length: 30 m, diameter: 0.25 mm, film thickness: 0.25 μm. For GC–MS detection, an electron ionization energy system with ionization energy of 70 eV was used. The

### Table 1

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Phytochemicals</th>
<th>Methanolic extract</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Phenols/Polyphenols</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
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<td>Terpenoids</td>
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</tr>
<tr>
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<tr>
<td>6</td>
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</table>

Fig. 1. Minimum inhibitory concentration of methanolic extract of CV against *Malassezia* spp.

Fig. 2. Zone of inhibition of methanolic extract of CV against *Malassezia* spp.
helium (99.995%) was used as the carrier gas at a flow rate of 1.51 ml/min and an injection volume of 1 μl was employed (split ratio: 10). Injector temperature 240 °C; ion source temperature 200 °C. The oven temperature was programmed from 70 °C for 2 min then increased to 300 °C at the rate of 10 min. Mass spectra were taken at 70 eV; a scan interval of 0.5 s with scan range of 40–1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

2.8. Identification of components

Interpretation of mass spectrum GC–MS was conducted using the database of NIST-11 (National Institute of Standards and Technology). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST-11. The name, peak, RT, area and area (%) of the component of the test material were ascertained.

3. Results

In the present study the methanolic extract of CV bark was showing inhibition against *M. furfur*, *M. globosa* and *M. sympodialis*. The result shows MIC of the extract ranges from 0.5 to 2 mg/ml. The values of MIC of the extract for above organisms were presented in Fig. 1. Among the three organisms *M. globosa* shows MIC at low concentration (0.75 mg/ml) when compared to others. The results of well diffusion method are presented in Fig. 2. The extract exhibited the ZOI ranging from 11 to 26 mm in diameter and synthetic ingredient ketoconazole shows 30–39 mm in diameter. The bark extract shows less activity when compared to ketoconazole. The results exhibit *M. furfur* has more sensitivity when compared to others.

The preliminary phytochemical analysis shows the presence of phenolics, flavonoids, terpenoids and tannins (Table 1). GC–MS analysis revealed the presence of (E)-3-(2-Methoxyphenyl)-2-propenoic acid (40.41%), trans-Cinnamaldehyde (20.28%), 4-Vinyl benzoic acid (10.54%) and coumarin (8.47%) as major compounds in the methanolic extract of bark (Figs. 3 and 4, Table 2).

4. Discussion

Cinnamon has been widely used as spice and condiments and traditionally used for various ailments in the indigenous systems of medicine. It is rich in essential oil which contains cinnamic acid, cinnamaldehyde, cinnamate, trans-cinnamaldehyde, caryophyllene oxide, l-borneol, l-bornyl acetate, eugenol, β-caryophyllene, E-nerolidol, cinnamyl acetate, terpinolene, α-terpineol, α-cubebene, α-thujene. Several studies show that CV bark has broad spectrum of antimicrobial properties against various organisms. The present investigation also exhibit that the methanolic extract has potential antifungal activity against *M. furfur*, *M. globosa* and *M. sympodialis*. Ferhout et al, 1999, reported that *M. furfur* has more sensitivity to cinnamaldehyde and cinnamon oil with MIC at the range of 1–1000 μg/ml. But our experiment with bark methanolic extract shows MIC at 1 mg/ml against *M. furfur*. Though it is a crude extract, the result is comparable to cinnamaldehyde and cinnamon oil. The ZOI also shows 26 mm diameter against *M. furfur*.

The inhibitory activity of cinnamon is due to the presence of aromatic aldehydes that inhibit the amino acid enzymatic activities and has been proven to be active against pathogenic bacteria.
Cinnamaldehyde is a highly electro-negative compound which interferes in the biological process involving electron transfer and reacts with nitrogen-containing components like protein and nucleic acid and also the mechanisms of fungicidal activity involved cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular enzymes. These biological events could take place separately or concomitantly, culminating the growth inhibition.7,13,14

Our GC–MS analysis of methanolic extract also shows the major compounds such as (E)-3-(2-Methoxyphenyl)-2-propenoic acid (40.41%), trans-Cinnamaldehyde (20.28%) and these compounds may be responsible for the antifungal activity.

5. Conclusion

From the present study, we conclude that the methanolic extract of cinnamon bark has potential antifungal activity against M. globosa, M. sympodialis and M. furfur and can be exploited in the development of herbal formulations like shampoos, hair gels, hair/skin creams etc. for the management of clinical conditions like dandruff, seborrhoeic dermatitis, pityriasis versicolor. Even though the extract shows less activity than Ketoconazole, it has its own value or influence for its antimicrobial properties as a natural ingredient from plant source.

Fig. 4. Mass spectrum (m/z) of CV bark extract.
Fig. 4. (continued).
Fig. 4. (continued).
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All authors have none to declare.

Acknowledgement

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References


Table 2

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