Evaluation of antifungal efficacy of some medicinal plants on Candida spp. causing vulvovaginitis

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The opportunistic yeast pathogen Candida albicans and the non albicans Candida spp. cause life threatening infections in patients leading to increased mortality rate. High toxicity of synthetic drugs on host tissues and multidrug resistance of organisms aggravates the problem. Medicinal plants are rich source of secondary metabolites with potential therapeutic effects. In this study, we tested ethanolic extracts of four different plants, namely Terminalia chebula, Ventilago maderaspatana, Clerodendrum serratum and Curcuma longa for their antifungal activity against three different Candida spp. The MIC value of T. chebula was 25 μg/mL and that of C. serratum was 50 μg/mL, whereas C. longa and V. maderaspatana showed activity at 12.5 μg/mL against C. albicans. The MIC values against C. tropicalis were reported as 50 μg/mL for T. chebula and V. maderaspatana, 25 μg/mL for C. longa and 100 μg/mL for C. serratum. In a similar way antifungal activity for C. glabrata were reported as 25 μg/mL for C. longa and T. chebula and finally 50 μg/mL for C. serratum and V. maderaspatana.

Keywords: Bharangi, Black myrobalan, Clerodendrum serratum, Curcuma longa, Glorybower, Red creeper, Terminalia chebula, Turmeric, Ventilago maderaspatana

Vulvovaginal candidiasis (VVC) predisposes to the overgrowth of Candida species in genital area particularly when the immune system is suppressed; it also affects up to 75% of women at least once in their lifetime¹. Risk factors for vulvovaginal candidiasis include sexual activity, recurrent antibiotic use, pregnancy, and immunosuppression from conditions, such as poorly controlled HIV infection or diabetes². During pregnancy there is an increased secretion of estrogen which results in increased amounts of glycogen in the vagina that acts as a source of carbon which is needed for the growth and germination of Candida³. Vulvovaginal candidiasis is most often caused by Candida albicans. However, the incidences of other species of Candida viz. Candida glabrata, C. parapsilosis and C. tropicalis are also being reported recently¹². Usually, 10-20% VVC is caused by non-albicans especially C. glabrata⁴. Hence in our study, we have included two clinical strains of Candida, namely C. glabrata and C. tropicalis and an ATCC strain of C. albicans.

Treatment of Candida infections is complicated by several factors including availability of limited number of effective antifungal drugs, toxicity of the available antifungal agents, resistance of Candida to commonly used antifungals, relapse of Candida infections, and expensive antifungal agents⁵. In order to alleviate the above mentioned problems traditional medicine derived from plants are still being used⁶,⁷. Antifungal properties of certain medicinal plants are reported in traditional medicines and a few attempts were made to utilise this knowledge into useful products. Some Chinese herbal medicines including Areca cathechu, Centella asiatica, Cymbopogon citratus (DC) Stapf (Gramineae), Euphorbia hirta L. and Syngonanthus nitens, etc. have shown beneficial role in slowing the progression of VVC⁸.

Medicinal plants are rich source of antimicrobial agents and are used as a source of many potent and powerful drugs⁹. Terminalia chebula Retz. belongs to the family “Combretaceae”, commonly known as black myrobalan is routinely used in traditional systems of medicine to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, UTI and wound infections¹⁰. The powdered stem bark of Ventilago maderaspatana Gaertn. (red creeper) from the family Rhamnaceae, is mixed with sesame oil and applied externally for skin diseases and itch¹¹. Clerodendrum serratum (L.) Moon belonging to the family Verbenaceae is commonly known as glorybower (bharangi in traditional medicines) and is reported to have antioxidant, antibacterial, anti-inflammatory and antipyretic activity¹². Curcuma longa or common turmeric is a rhizomatous herbaceous perennial plant of the family, Zingiberaceae with a long history of therapeutic uses. It has a wide spectrum of biological

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activities such as antibacterial, antifungal, antioxidant, anti-inflammatory, anticaner and antidiabetic activities. Based on the available data we selected four plants *T. chebula*, *C. longa*, *C. serratum* and *V. maderaspatana* for a detailed study to understand their activity against *Candida* spp. and the results can be used for the development of antifungal therapeutics for vaginal candidiasis.

**Materials and Methods**

**Plant material**

Dry fruits of *Terminalia chebula*, stem bark of *Ventilago maderaspatana*, rhizome of *Curcuma longa* and leaves of *Clerodendrum serratum* were obtained from the Ayurvedic Research Institute, Thiruvananthapuram, Kerala. Voucher specimen is deposited at the Corporate Research and Development Centre, HLL Lifecare Ltd., Thiruvananthapuram.

**Preparation of extracts**

Plant materials were cleaned, dried under shade and powdered, and 24 g of the powder was subjected to pressurized sequential extraction using Accelerated Solvent Extractor (ASE 150, Dionex Inc., USA). The materials were mixed with diatomaceous earth at 4:1 ratio. The mixture was placed into a sample cell (100 mL) and loaded onto the ASE 150 system. Extraction was performed using 80% ethanol under pressure (1500 psi) at 60°C with a flush volume of 60% using 2 static cycles. The solvent was then evaporated in a rotary evaporator (Buchi, Switzerland). The dried extracts were weighed and dissolved in DMSO to obtain the following concentrations 12.5, 25, 50, 100 and 200 μg/mL and were assayed for antifungal activity.

**Antifungal activity analysis**

**Microorganisms tested**

The in vitro antifungal activity was evaluated against *C. albicans* (ATCC 90028), *C. tropicalis* and *C. glabrata*. The isolates- *C. tropicalis* and *C. glabrata* were recovered from blood samples of the patients attending Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala. Both the isolates were identified using VITEK 2 YST identification card AST Y607 (Biomerieux, France) with >99% accuracy.

**Preparation of test organisms**

Antifungal activity of plant extracts were tested by Macro Broth Dilution Method as per Clinical Laboratory Standards Institute (CLSI) M-27 A-3 (2008): reference method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard; 3rd Edition. The incubation temperature throughout was 35°C. The inoculum was prepared by picking five colonies ~1mm in diameter from 24 h old cultures of *Candida* species. The colonies were suspended in 5 mL of sterile 0.145 mol/L saline (8.5 g/NaCl; 0.85% saline). The resulting suspension was vortexed for 15 s and the cell density adjusted with spectrophotometer by adding sufficient sterile saline to increase the transmittance to that produced by a 0.5 McFarland standard at 530 nm wavelength. This procedure yielded a yeast stock suspension of 1×10⁶ to 5×10⁶ cells/mL. A working suspension was made by a 1:100 dilution followed by a 1:20 dilution of the stock suspension with RPMI 1640 broth medium, which is equivalent to 5.0×10⁵ to 2.5×10³ cells/mL.

**Antifungal assay by broth macro dilution method**

Before adjusting the inoculum, 1.0 mL of the various antifungal concentrations was placed in sterile test tubes. The growth control received 1.0 mL of drug diluent without antifungal agent. Within 15 min after the inoculum had been standardized 9 mL of the adjusted inoculum was added to each tube in the dilution series and were mixed. This resulted in a 1:10 dilution of each antifungal concentration and a 10% dilution of the inoculum. A tube containing RPMI 1640 medium without antifungal agents (but with nonaqueous solvent where necessary) for organism tested was used as a growth control tube. Fluconazole was taken as standard drug. The drug concentration was taken as 4 to 64 μg/mL. The inoculated macro dilution tubes were incubated at 35±2°C for 48 h in an ambient air incubator.

**Results and Discussion**

Approximately, three quarters of all women experience at least one episode of vaginal candidiasis (VVC) during their lifetime and nearly half of them suffer from multiple episodes. Recurrent Vulvovaginal candidiasis (RVVC) is defined as four or more episodes of VVC in a period of 12 months. The physical, psychological and monetary burden of VVC and RVVC in the population is high and the prevalence varies by age. Although majority of cases of vulvovaginal candidiasis are caused by *Candida albicans*, episodes due to non-albicans species of *Candida* also appear to be increasing. *C. glabrata* and *C. tropicalis* are non albicans species
that are frequently isolated from the vaginal, oral and gastrointestinal samples. Most non-albicans Candida species have higher Minimum inhibitory concentrations (MIC) to theazole antifungal agents; also the infections caused by them are often difficult to treat. Presently, in modern medicine there are variety of effective treatments for candidiasis, such as polyenes, fluoropyrimidine analogues, azoles and echinocandins. Among these amphotericin B, fluconosine, miconazole, fluconazole, itraconazole and voriconazole are some of the synthetic fungicides. But these drugs exhibit certain adverse side effects in terms of antifungal spectra of activity, toxicity, or resistance. Amphotericin B was the only systemic antifungal agent for the treatment of invasive candidiasis for the last so many years. In the 1990’s the advent of the triazoles and lipid amphotericin B formulations provided alternative therapeutic options. However, renal toxicity remains a major drawback of amphotericin B formulations, while drug interactions, hepatotoxicity and limitations to use in renal failure are primary concerns with newer generation azoles.

Monotherapy with fluconosine is barely tolerated and hence not used today and increasing use of fluconazole and miconazole for treatment of Candida infections has resulted in the development of azole resistance among candida species. Moreover, the current cost of most of these chemotherapeutic agents is unbearable to the public especially in developing countries like India. Therefore attempts must be directed towards the development of effective, natural, nontoxic drug for treatment of Candida infections, both albicans and non albicans.

Therefore, present work was undertaken to explore the antifungal property of medicinal plants, such as C. longa, V. maderaspatana, C. serratum and T. chebula against three Candida species (C. albicans, C. tropicalis and C. glabrata) that are suspected to cause vulvovaginal candidiasis. The antifungal activity of the four plant extracts were checked using Macro Broth Dilution method at various concentrations along with the standard antifungal agent fluconazole. Fluconazole has been used widely for treating skin disease caused by C. albicans. The growth of inhibition was measured at different concentrations (12.5, 25, 50, 100 and 200 µg/mL) in DMSO. The results are tabulated in the Table 1.

The ethanolic extract of T. chebula was found to be active at a concentration of 25 µg/mL and C. serratum showed significant activity at 50 µg/mL whereas C. longa and V. maderaspatana showed activity at 12.5 µg/mL against C. albicans. The MIC values of the plant extracts against C. tropicalis were reported as 50 µg/mL for T. chebula and V. maderaspatana, 25 µg/mL for C. longa and 100 µg/mL for C. serratum. Similarly antifungal activity for C. glabrata was also examined and was reported as 25 µg/mL for C. longa and T. chebula and 50 µg/mL for C. serratum and V. maderaspatana. The growth control showed significant growth of the organisms.

Sharma et al., who studied the crude extract of T. chebula fruit and seed has demonstrated its activity against C. albicans, with the zone of inhibition of 9 mm and activity index of 0.28 mm. Literature supports that the presence of ellagic acid, gallic acid and other glycosides in T. chebula fruits are responsible for the antifungal activity. In another study, a zone of inhibition of 15 mm and an inhibitory response of 82.43% against C. albicans was reported. In our study, we got an MIC of 25 µg/mL for the ethanolic extract of T. chebula against C. albicans, 12.5 µg/mL against C. tropicalis and 25 µg/mL against C. glabrata.

The antifungal activity of C. serratum is attributed to flavonoid compounds viz. apigenin-7-glucoside, quercetin, saponnins, phenolics and steroids. Kajaria et al. have worked on antimicrobial and anti-inflammatory effect on indigenous Ayurvedic drug-Bharangyadi. Bharangyadi compound consists of three herbal drugs namely Bharangi (C. serratum), Sati (Hedychium spicatum) and Kantakari (Solanthum xanthocarpum). The MIC obtained for the three plant combination; against C. albicans and Kantakari (Solanthum xanthocarpum). The MIC obtained for the three plant combination; against C. albicans in their study was found to be 12.5 mg/mL, whereas in our study the MIC obtained for the ethanolic extract of C. serratum against C. albicans is 50 µg/mL, C. tropicalis is 100 µg/mL and against C. glabrata is 50 µg/mL. Cikrikci et al. who studied efficacy of curcuminoids isolated from C. longa reported MIC of

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Organisms tested</th>
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<tbody>
<tr>
<td></td>
<td>C. albicans</td>
</tr>
<tr>
<td>Ventilago madaraspatana</td>
<td>≤12.5</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>≤12.5</td>
</tr>
<tr>
<td>Clerodendrum serratum</td>
<td>50</td>
</tr>
<tr>
<td>Terminalia chebula</td>
<td>25</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>≤4</td>
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<tr>
<td>Growth control</td>
<td>0</td>
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</tbody>
</table>

“0” implies no inhibition

C. longa and V. maderaspatana showed activity at 12.5 µg/mL against C. albicans. The MIC values of the plant extracts against C. tropicalis were reported as 50 µg/mL for T. chebula and V. maderaspatana, 25 µg/mL for C. longa and 100 µg/mL for C. serratum. Similarly antifungal activity for C. glabrata was also examined and was reported as 25 µg/mL for C. longa and T. chebula and 50 µg/mL for C. serratum and V. maderaspatana. The growth control showed significant growth of the organisms.

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512 µg/mL against *C. albicans* using tube dilution method while Martins *et al.*29, reported MIC of 64 mg/mL. In another study, the methanolic extract of *curcuma longa* exhibited antifungal activity against *C.albicans* with an MIC of 256 µg/mL30. The isolated curcumin, bis demethoxy curcumin and demethoxy curcumin on antifungal activity test against *C. albicans* showed zone of inhibition at 19, 11 and 8 mm, respectively13. Curcumin, an yellow bioactive pigment is the major components of turmeric which possess a wide spectrum of biological activities, such as antifungal, antidiabetic, antioxidant, anti-inflammatory, anticancer, anti-allergic, anti-protozoal and antibacterial. The MIC obtained for *C. longa* in our study against *C. albicans* is found to be 12.5 and 25 µg/mL against both *C. tropicalis* and *C. glabrata*.

Reports indicate that *V. maderaspatana* has been used for thousands of years for its medicinal properties, such as antigout31, antihyperlipidemic, antidiabetic, antibacterial and antioxidant32. The aqueous extracts of stem bark of *V. maderaspatana* showed no antimicrobial and antifungal activity against *C. albicans*, whereas the methanolic extracts of plant showed significant activity against the fungus. The zone of inhibition obtained for methanolic extracts were 11.0±0.3 and 14±0.3 mm, respectively13. Rajesh *et al.*34 carried out *in vitro* biological activity of aromadendrin-4′methyl ether isolated from the root extract of *V. maderaspatana* Gaertn with relevance to MIC 12.8±0.6 mm against *C. albicans* using two-fold dilution method34. In our study, we have got an MIC value 12.5 µg/mL for the Ethanolic extract of *V. maderaspatana* and 50 µg/mL against both *C. tropicalis* and *C. glabrata*.

We studied the antifungal activities of four plant extracts against three different species of *Candida*, which are responsible for development of vaginal candidiasis. All the four plant extracts showed significant antifungal activity against all species studied. These plant extracts can be used in the development of effective therapeutics for controlling vaginal candidiasis from both *albicans* and *non albicans* spp.

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**References**


