

Full Length Research Paper

Screening of *Pogostemon parviflorus* Benth. for anti-*Candida* activity

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Pogostemon parviflorus, an aromatic woody herbaceous plant, is found in high rainfall localities. The medicinal properties of this plant show that leaves are used for anxiety, cellulite, depression, eczema and wounds. Also, they are used for thrush, mycotic enteritis and vaginitis. The aim of the present study is to assay the anti-*Candida* activity of the ethanolic and methanolic extracts of *Pogostemon parviflorus* leaf against opportunistic mycosis causing pathogenic fungi, such as *Candida albicans* (5), *Candida glabrata* (2), *Candida tropicalis* (2) and *Candida dubliensis* (1), by agar well diffusion method. Antifungal activity of the ethanolic and methanolic extracts of *Pogostemon parviflorus* leaf was characterized by ranges of inhibition zones from 8 to 15 mm and 8 to 20 mm, and minimum inhibitory concentration (MIC) by ranges of 2.5 to 20 mg ml⁻¹ and 2.5 to 10 mg ml⁻¹, respectively. The ethanolic extract of tested plant has more anti-*Candida* effect at 5.7 mg ml⁻¹ when compared to the methanolic extract at 6.6 mg ml⁻¹. The results prove *Pogostemon parviflorus* leaf as a potent source of natural anti-*Candida* compounds.

Key words: Anti-*Candida* activity, *Pogostemon parviflorus*.

INTRODUCTION

Candida is a thin-walled small yeast that is usually an opportunistic pathogen and can cause disease in humans, especially in immunocompromised patients. Virtually 150 species of *Candida* have been identified; out of them, *Candida albicans* is one of the most pathogenic species and it cause candidiasis. *C. krusei*, *C. tropicalis*, *C. dubliniensis*, *C. parapsilosis*, etc. are some other pathogenic species from genus *Candida*. Most *Candida* infections can be treated with topical antifungal drugs, such as clotrimazole, miconazole, nystatin and tioconazole, or oral drugs, such as fluconazole and amphotericin B. Widespread and overuse of these drugs lead microbes to resistance mechanism against those particular drugs. To overcome this problem, the study searched for the isolation of new potential therapeutic compounds from plants (Gaurav et al., 2010). Although, plants are vital sources, safety and lower side effects of

many herbal extracts have also suggested them as sources of new pharmaceuticals (Atai et al., 2009). Traditional healers claim that their medicine is cheaper and more effective than modern medicine.

In developing countries, the people with low income, such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infections (Veeramuthu et al., 2006). Therefore, *Pogostemon parviflorus* leaf, used in folk medicine, was chosen to determine their anti-*Candida* activity. *Pogostemon parviflorus* Benth belongs to Labiatae family and it has a strong odor (Figure 1). It grows in areas with high annual rainfall. This plant has antiseptic activity and it is useful in the treatment of enteritis, eczema and mycotic enteritis (Sadeghi and Deokule, 2010). The leaf of *Pogostemon parviflorus* contained saponins, reducing sugars, tannins, phenols and proteins, but it did not have any glycosides, anthraquinones, alkaloids or flavonoids. However, the high performance thin layer chromatography (HPTLC) study indicated that the ethyl acetate extract of *Pogostemon parviflorus* leaves included triterpenes (Sadeghi and Deokule, 2010). The

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Figure 1. *Pogostemon parviflorus* Benth.

screening of medicinal plants for antimicrobial activities and phytochemicals is important in finding potential new compounds for therapeutic use. This paper reported the results of a survey that was done based on folk uses, by tribal people, with bioassay test for anti-*Candida* activity.

MATERIALS AND METHODS

Plant material and extraction

The material used in the present investigation is the leaf of *Pogostemon parviflorus* collected from Mulshi District Pune of Maharashtra State, India. The botanical identification of the collected plant was done by an associate Professor, S.S. Deokule, Botany department of Pune University. Avoucher specimen (No. S2/05) has been deposited at the herbarium of Botany department of Pune University, India. The leaves of plant were washed with water and cut into small pieces, and then they were dried in shade to avoid decomposition of chemical constituents and stored in clean and dry airtight containers for extraction of bioactive compounds.

Preparation of plant extracts

Ten gram of the powdered plant material was extracted with 100 ml of methanol and ethanol (80%) in conical flask separately for maceration. The flask was plugged with cotton and kept on a rotary shaker for 24 h. Thereafter, it was filtered and the supernatant was collected and evaporated to dryness in room temperature to give the crude dried extract. Finally, one gram of dryness extract was dissolved in five milliliter dimethyl sulfoxide (DMSO, 100%) and a final concentration of this extract was adjusted to 200 mg ml⁻¹.

Candida spp. isolates and fungal inoculums preparation

In the present investigation, ten species of *Candida* such as *Candida albicans* (5), *Candida glabrata* (2), *Candida tropicalis* (2) and *Candida dubliensis* (1) were obtained from periodontitis and the

gingivitis patients were referred to the School of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. *Candida* species were identified by *Candida* (CHROMagar Company, Paris, France), in a germ tube test and Clamyoconidia formation (Corn meal agar plus Tween 80, medium). The isolates were maintained on Sabouraud's dextrose agar (SDA; Hi Media, India) and incubated at 37°C for 24 h. *Candida* species colonies from SDA plates were suspended in sterile distilled water and adjusted to 10⁶ cells with colony-forming units (CFU)/ml (0.5 McFarland standard).

Antifungal drug

The antifungal commercial, such as ketoconazole (Janssen Pharmaceutica, Beerse, Belgium), was dissolved in dimethyl sulfoxide (DMSO) and stored as 2 mg/ml at -20°C.

Determination of minimum inhibition concentration (MIC)

The ethanolic and methanolic extracts of *Pogostemon parviflorus* leaf were screened for anti-*Candida* activity using an agar well diffusion technique. One hundred microliters (100 µl) inoculum (10⁶ CFU/ml) of each test *Candida* was spread with the help of sterile bent glass rod onto SDA medium. The plates were allowed to dry and a sterile cork borer (6 mm diameter) was used to bore wells in the agar medium. Subsequently, 100 µl of the methanolic and ethanolic extracts with different concentrations (0.625 to 20 mg ml⁻¹) were poured in wells of the agar plates. Sterile DMSO (100%) was used as a negative control, and the plates were incubated at 37°C for 24 h. The standard antifungal drug, such as ketoconazole, was used as positive control. The minimum inhibition concentration (MIC) value was defined as the lowest extract concentration that inhibited the growth for 24 h. The lowest concentration of plant extract showed a clear zone of inhibition that was considered as the MIC.

RESULTS AND DISCUSSION

In this study, the anti-*Candida* activity of the ethanolic and methanolic extracts of *P. parviflorus* leaf was evaluated. The results of the anti-*Candida* activity of the ethanolic and methanolic extracts of the tested plant, by the use of agar well diffusion technique, were represented in Table 1 and Figure 2. In this study, it was demonstrated that the ethanolic and methanolic extracts of *P. parviflorus* leaf had inhibitory effects against *Candida* strains. The diameters of growth inhibition zone of the ethanolic and methanolic extracts were between the 8 and 15 mm and 8 and 20 mm inhibition zone, respectively. The inhibition zones for ketoconazole were determined between the 8 and 20 mm inhibition zone. However, the MIC values confirmed the existence of inhibitory effects on *Candida* spp. tested in this study, with MIC values ranging from 2.5 to 20 mg ml⁻¹. The mean of MICs of methanolic extracts against 10 isolates of *Candida* was for 5 isolates of *C. albicans* (6.5 mg ml⁻¹), *C. glabrata* (10 mg ml⁻¹), *C. dubliensis* (5 mg ml⁻¹) and *C. tropicalis* (5 mg ml⁻¹). Likewise, the mean of MICs of ethanolic extracts against 10 isolates of *Candida* was for 5 isolates of *C. albicans* (8 mg ml⁻¹), *C. glabrata* (7.5 mg ml⁻¹), *C. dubliensis* (2.5 mg ml⁻¹) and *C. tropicalis*

Table 1. Minimal inhibitory concentration (MIC) in mg/ml and inhibition zone in mm of the ethanolic and methanolic extracts of *Pogostemon parviflorus* leaf against *Candida* spp.

Pathogenic yeast	MIC (mg ml ⁻¹) (mm inhibition zone diameter)		
	Methanolic extract mg ml ⁻¹ (mm)	Ethanolic extract mg ml ⁻¹ (mm)	Ketoconazole mg ml ⁻¹ (mm)
<i>C. albicans</i> C ₁	5.0 (12)	10 (12)	0.125 (18)
<i>C. albicans</i> C ₂	5.0 (11)	20 (15)	0.0625 (12)
<i>C. albicans</i> C ₃	2.5 (8)	2.5 (14)	0.125 (12)
<i>C. albicans</i> C ₄	10 (18)	2.5 (13)	0.0625 (20)
<i>C. albicans</i> C ₅	10 (10)	5 (8)	0.015 (12)
<i>C. dubliensis</i> D ₀	5.0 (8)	2.5 (8)	0.015 (10)
<i>C. glabrata</i> G ₁	10 (10)	5.0 (9)	0.0039 (18)
<i>C. glabrata</i> G ₂	10 (20)	10 (15)	0.0039 (12)
<i>C. tropicalis</i> T ₁	5.0 (15)	5.0 (11)	0.0625 (18)
<i>C. tropicalis</i> T ₂	5.0 (14)	5.0 (12)	0.25 (10)

Tests were done in triplicate. Tested concentrations: extracts=20 mg ml⁻¹; Positive Control: Ketoconazole (2 mg ml⁻¹).

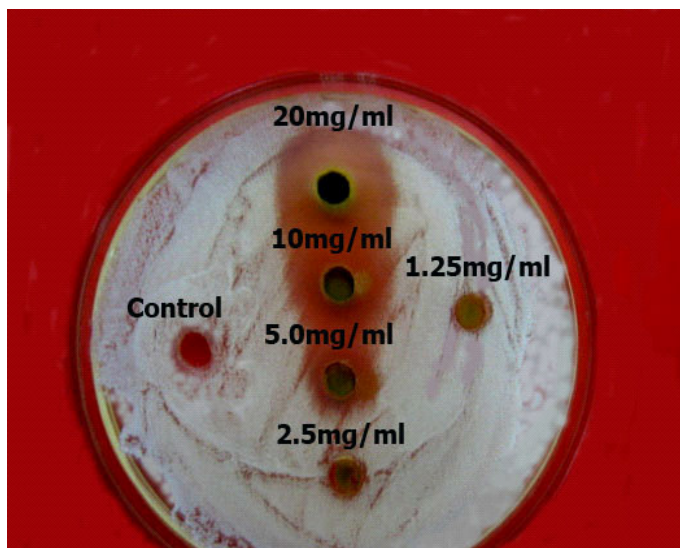


Figure 2. Inhibitory effects of ethanolic extract of *Pogostemon parviflorus* leaf on the growth of *Candida albicans* by dilution method. Decreasing dilution ranging from 0.625 to 20 mg ml⁻¹; MIC = 5.0 mg ml⁻¹.

(5 mg ml⁻¹). Thus, it is concluded that the ethanolic extract of the tested plant has more anti-*Candida* effect at 5.7 mg ml⁻¹ when compared to the methanolic extract at 6.6 mg ml⁻¹. In this study, the lowest concentration of the tested plant showed good antifungal activity against *C. dubliensis*, while the highest concentration showed an inhibitory effect against *C. albicans* and *C. glabrata*.

In the present investigation, this work has been carried out for the first time because anti-*Candida* effects of *P. parviflorus* leaf have not been reported so far. *Candida* species are harmless commensal yeast-like fungi in healthy humans, which can cause superficial as well as,

life-threatening systemic infections under immune-compromised situations (Demet et al., 2008). Furthermore, long-term treatments with commonly used antifungal drugs, such as polyenes and azoles, have toxic effects and results in strain resistance (Singh et al., 2006). However, azole antifungal agents dominate as drugs of choice against *Candida* infections, and as topical applications or as oral drugs (Mukherjee et al., 2003). Briefly, based on the results of this study, the methanolic and ethanolic extracts of *P. parviflorus* leaf can be considered as a new source for developing local antifungal agents. Further studies are needed to determine the efficacy of the active chemical constituent of this plant extract. Nonetheless, toxicological studies must also be performed to ensure the safety of the extract (Naeini et al., 2009).

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