Anti-Candidal Activity of Three Medicinal Plants with Special Reference to Anti-Virulent and Anti-Biofilm Properties of *Pongamia Pinnata*

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*Candida* spp. is a commensal fungus found in human oral, gastrointestinal, vaginal and cutaneous surfaces. Immunocompromised individuals are prone to this organism resulting in superficial and systemic infections. The infective ability of *Candida* is triggered by virulent features such as germ tube production, cell surface hydrophobicity (CSH), extracellular enzyme production etc. Treating candidal infections have become a challenge due to the emergence of resistance to currently available antifungal drugs. Colonization of this opportunistic pathogen, by the production of biofilms further increases resistance thereby resulting in a therapeutic dilemma. In overcoming this situation, plants have been recognized as important reservoirs of natural products, which can be utilized as antifungal agents. In this study, solvent extracts of different plant parts of selected three medicinal plants (*viz.* bark, roots and leaves of *Pongamia pinnata*, flower buds of *Syzygium aromaticum* and fruits of *Piper nigrum*) were screened for their anti-candidal activity.

Flower buds of *S. aromaticum* showed the best anti-candidal activity and its minimum inhibitory concentrations (MIC) ranged from 0.4 to 1.6 mg/ml. Roots of *P. pinnata* showed positive activity while neither bark nor leaf showed positive activity. MICs of the root extracts ranged from 1.6 to 12.8 mg/ml. Fruits of *P. nigrum* did not show inhibitory potential against any of the candidal isolates.

Detailed studies on anti-virulent properties of *P. pinnata* were carried out when the organism was in contact with the extract and when the organism was pre-exposed to the extract. Long term pre-exposure (18 h) of the isolates to the concentrations less than MIC values resulted in a significant reduction in the germ tube formation and elongation (*P* < 0.05). The isolates which were in contact with the extract achieved a significant reduction (*P* < 0.05) in germ tube production and elongation when they were provided with extracts with concentrations higher than MICs. It was revealed that reduced hyphal formation as well as hyphal elongation could be achieved by either long-term pre-exposure of the *C. albicans* isolates to the sub-inhibitory concentrations or in the presence of the extract in concentrations higher than MIC for a time period as short as 2 h. However, the pre-exposure did not affect the CSH (*P* > 0.05). *C. guilliermondii* and *C. dubliniensis* when in contact with the extract for a short time period (in sub-inhibitory concentrations; 1.5 h), a drastic reduction of CSH was observed (*P* < 0.05). Above results imply that the presence of the extract was required to achieve a reduced CSH of *Candida* spp. as the pre-exposure did not affect the hydrophobic property of candidal isolates. However, the extracellular phospholipase production of the organism was not affected by both the presence and pre-exposure of *Candida* sp. to the extract.
Root extracts of *P. pinnata* were further tested for anti-biofilm property. The adhesion of two candidal isolates, which is the very first step of biofilm production, was significantly reduced by sub-inhibitory concentrations of the extract. However, results indicated a considerable increase in the level of biofilms when already initiated biofilms and matured biofilms were treated with even very high concentrations of the extract.

These findings suggest the possibility of using *S. aromaticum* and *P. pinnata* in medicated products to treat candidal infections. This is the first report on the anti-candidal activity and anti-virulent potential of root extract of *P. pinnata*. However, the variability shown by the effects of *P. pinnata* on different growth phases and features of *Candida* spp. highlights the need of further investigations to explore possible applications of the extract in the pharmaceutical field.