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Anti-candidal activity and effect on relative cell surface hydrophobicity of *Pongamia pinnata*

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Pongamia pinnata is used in Sri Lankan traditional medicinal systems to cure diseases including microbial infections. Different parts of this plant were investigated for *in vitro* anti-candidal activity. Extracts of bark, roots and leaves of *P. pinnata* were examined for their activity on different *Candida* spp. using well diffusion assay. The extract with best activity was evaluated for its minimum inhibitory concentrations (MIC) using agar dilution method. Its effects on germ tube formation, germ tube elongation and relative cell surface hydrophobicity were determined. The best activity was shown by the root extract of *P. pinnata*. Neither bark nor leaf showed positive activity. MICs of the root extracts ranged from 1.6 to 12.8 mg/ml. The treatment with root extract of *P. pinnata* inhibited the germ tube formation, germ tube elongation and relative cell surface hydrophobicity in a dose dependent manner. The results reveal the significant anti-candidal activity of root extracts of *P. pinnata*.

Key words: Anti-candidal, *Pongamia pinnata*, *Candida*, germ tube, cell surface hydrophobicity.

INTRODUCTION

Plant material has played a major role in traditional medicinal systems throughout the world for centuries. Antimicrobial activity is one such medicinal property of plants in these medicinal systems. This traditional knowledge on antimicrobial activity in higher plants has been systematically proven in numerous studies throughout the world (Chea et al., 2007; More et al., 2008). Plant compounds such as flavonoids, alkaloids, essential oils, tannins and saponins are proven to have antimicrobial properties (Cowan, 1999; Arif et al., 2009; Duarte et al., 2007). Furthermore, plant derived antimicrobial products have recently come into the picture as a potential solution to counter the rising incidence of drug resistance in microbes (<http://www.who.int/mediacentre/factsheets/fs194/en/>).

The medicinal plant *Pongamia pinnata* (L.) Pierre. (Fabaceae), is used extensively in Sri Lankan traditional medicinal systems to cure diseases including microbial infections (Jayaweera, 1981). Fresh bark and leaves of *P. pinnata* have been used against acute rheumatism, rheumatoid and gonorrheal arthritis, lymphangitis and elephantiasis. Seeds of this plant are employed to cure chronic eczema, psoriasis, scabies and ringworm, while the bark is used internally for diarrhea, dyspepsia and flatulence. The root bark of *P. pinnata* has been used for the treatment of bleeding piles (Jayaweera, 1981). Furthermore, roots and twigs of *P. pinnata* have been used as a traditional tooth brush in ancient times and is still in practice in remote areas of Sri Lanka (Uragoda, 2003). The plant is also popular in Indian traditional medicinal

practices. It has been used for skin diseases such as eczema, scabies, leprosy, and for ulcers, tumors, piles, enlargement of spleen, vaginal and urinary dis-charges. Juice of root is used for closing fistulous sores and cleaning foul ulcers while flowers are used for the cure of diabetes. Powder of seeds is used for whooping and irritating coughs of children. Seed oil is used in cutaneous affections, herpes and scabies (Khare, 2007).

Candida species are present in oral cavities of more than 50% of the adult population (Zaremba et al., 2006). This relatively innocuous commensal may revert to an opportunistic pathogen when the host immunity is altered (Ellepola, 2005). Lesions caused by *Candida* spp. termed candidosis or candidiasis manifests as superficial infections (example, oral candidiasis, vaginal candidiasis and skin infections) and / or systemic infections (example, candidemia). Among different *Candida* spp. *C. albicans* has been identified as the most common species causing infections (Melo et al., 2004; Babic and Hukic, 2010). The contribution of others, which are called non-albicans (example *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. guilliermondii*, *C. krusei* etc), has recently gained prominence in the light of increasing populations with compromised immune status (Samonis et al., 2008). Moreover, clinical and laboratory studies have shown rising incidence of antifungal resistance by *Candida* spp. (Baily et al., 1994). Resistant strains of *C. glabrata*, *C. albicans*, *C. tropicalis* and *C. haemulonii* against fluconazole have been reported (Giusiano et al., 2006). Zomorodian et al. (2011) reports that the highest rates of resistance to ketoconazole were seen in *C. glabrata* (16.6%) and *C. albicans* (3.2%) among a total of 206 yeast isolates that were assessed. Under these circumstances, it is logical to look into the possible antifungal effects of selected plants. Hence the current study attempted to study the anti-candidal activity of the extracts of *P. pinnata* against selected candidal species.

MATERIALS AND METHODS

Plant materials

Voucher specimens of the plant *P. pinnata*, were prepared and identified at the National Herbarium at Peradeniya, Sri Lanka. A voucher specimen (NO:DK2009-01) was deposited in the herbarium of Department of Botany, University of Peradeniya, Sri Lanka. The fresh roots, leaves and bark of *P. pinnata*, were collected during July 2009 from the botanical garden of the Department of Botany, University of Peradeniya, Sri Lanka (latitude 7°26'; longitude 80°60'), and used for extraction on the same day.

Extraction of plant material

Roots of *P. pinnata* (20 g) were cut into 0.5 cm x 0.5 cm pieces and soaked in 75% ethanol at 85°C for 2 h (Sathishkumar and Baskar, 2008). The sample was kept in 75% ethanol overnight and extracted by the vacuum infiltration technique (Adikaram and Bandara, 1998) in which the sample was placed in a conical flask (250 ml) with a side arm connected to a vacuum pump. Solvent

infiltration was carried out under continuous stirring. The extraction procedure was repeated with bark (20 g) and leaf (20 g) samples.

The extracts were filtered using Whatman No.1 filter paper. The filtrates were evaporated to dryness *in vacuo* using a rotary evaporator (Stuart RE 300, UK) at 50 rpm and 45°C and the residue was further freeze dried at -54°C. The dried samples were stored in sterile vials at -70°C.

Anti-candidal activity

Candida species

Five standard isolates of *Candida* species: *C. albicans* (ATCC 90028), *C. parapsilosis* (ATCC 22019), *C. glabrata* (ATCC 90030), *C. krusei* (ATCC 6258) and *C. tropicalis* (ATCC 13803) were used for screening. In addition, the extracts were tested against ten clinical isolates [*C. albicans* (5 isolates), *C. rugosa*, *C. guilliermondii* (2 isolates), *C. parapsilosis* and *C. dubliniensis*] which were previously identified using Analytical Profile Index (API). The organisms were maintained on Sabouraud dextrose agar (SDA) at 37°C for 20 to 22 h.

Well diffusion assay

Anti-candidal activity was demonstrated using well diffusion bioassay (Rangama et al., 2009), with some modifications. Inocula of *Candida* were prepared by suspending 20 to 22 h old cultures in NaCl (0.85%) and adjusting their turbidity to 0.5 McFarland standards. Inocula were flood inoculated onto the surface of Petri plates containing 25 ml of Mueller Hinton Agar (MHA). Wells of 9 mm diameter were cut in the agar using a sterile cork borer. The base of the wells was sealed by adding a drop of molten agar into each well.

Aliquots (180 µl) of the extracts were added into separate wells. Sterile distilled water was used as the negative control. After incubation overnight at 37°C, the plates were examined and the diameter of zone of growth inhibition was measured. Results were expressed as mean ± SD.

Determination of the Minimum Inhibitory Concentration

The extracts which showed activity against most of the *Candida* isolates in the well diffusion bioassay were further tested to determine the MIC (Andrews, 2001). Plant extracts were incorporated in different concentrations to prepare dilution plates of MHA. The inocula (2 µl) adjusted to 0.5 McFarland were spotted on the dilution plates. The presence or absence of the growth of each isolate on the dilution plates was observed after incubating the plates overnight at 37°C. The lowest concentration of the extract that inhibited the growth of an isolate was considered as its MIC of the plant extract. MIC of fluconazole used as the positive control was also determined.

Effect of root extract of *P. pinnata* on germ tube formation by *C. albicans*

C. albicans (ATCC 90028) was harvested from overnight cultures maintained in SDA at 37°C, suspended in human blood serum and the turbidity was adjusted to 0.5 McFarland standard. The extract equivalent to the MIC was incorporated into 1 ml of prepared inoculum and vortexed for ten seconds. The mixture was further incubated at 37°C for 2 h re-vortexed and examined microscopically. The percentage of germ tube formation was enumerated by counting 100 cells. A cell was considered to have germinated when

the germ tube has elongated to the length of a blastospore (Pinto et al., 2009). Length of 30 germ tubes was measured using an ocular micrometer. The test was repeated at varying concentrations (MIC, 2, 3 and 4 MIC) of the root extract of *P. pinnata*. In addition clinical isolates of *C. albicans* were subjected to the above procedure. All experiments were repeated three times with each isolate.

Effect of root extract of *P. pinnata* on relative cell surface hydrophobicity (RCSH) of *Candida* spp.

The method given by Sweet et al. (1987) was used for the hydrophobicity assay. A 5 ml portion each of a suspension of *C. albicans* (ATCC 90028) (OD \pm 0.02; 520 nm) was added to two glass tubes (tube A and B). Thereafter, 1 ml of xylene was added to tube B. Both tubes were placed in a water bath at 37°C for 10 min to equilibrate, and vortexed for 30 s and returned to the water bath for a further 45 min to allow the immiscible xylene and aqueous phases to separate. The lower aqueous phase was carefully pipetted out and transferred to a clean test tube. The absorbance of the tube A and B was measured at 520 nm. The hydrophobicity was expressed as the percentage reduction in optical density (OD) of the tube B compared to tube A.

$$\% \text{ Reduction in (OD)} = \frac{OD_A - OD_B}{OD_A}$$

The entire procedure was repeated incorporating the different concentrations of the freeze dried extract of *P. pinnata* (MIC, 1/2 MIC, 1/4 MIC, and 1/8 MIC) to the inoculum using the above procedure.

The percentage reduction of the OD of this test experiment was compared with that of previously mentioned control experiment. The RCSH was repeated with five selected isolates which were sensitive to the root extract of *P. pinnata*.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan multiple range test at $P < 0.05$ significant levels using the statistical package SigmaPlot Version 12.0.

Preliminary phytochemical screening

Fifty microliters of crude extract of roots of *P. pinnata* (100 mg/ml) was spotted on TLC plates (glass 10x20 cm², Silica gel GF 254 [silica containing UV fluorescent indicator] thickness, 0.5 mm). The plates were developed in CHCl₃:MeOH (96:4, v/v). Subsequently they were air-dried allowing the developing solvent to evaporate. Finally, plates were subjected to the following:

- i). Inspection under visible, ultraviolet light (254 and 365 nm) (Andersen and Markham, 2006).
- ii). Detection of flavonoids; TLC plates were sprayed with 1% ethanolic aluminium chloride and observed under ultraviolet light (365 nm) (Purkayastha and Dahiya, 2012).
- iii). Detection of phenols; TLC plates were sprayed with 5% ferric chloride and observed under ultraviolet light (Andersen and Markham, 2006).
- iv). Detection of antifungal compounds (Sawaya et al., 2004). A thin layer (1 to 2 mm) of SDA inoculated with *C. albicans* (ATCC 90028) was applied on TLC plates. The inoculated plates were incubated for 48 h at 37°C. Subsequently, the plates were sprayed with 1% TTC (2,3,5 - triphenyltetrazolium chloride) and incubated at for 24 h at 37°C.

RESULTS

Root extract of *P. pinnata* was active against all standard and clinical isolates of *C. albicans* investigated. Among four standard non-albicans strains subjected to the assay, only *C. tropicalis* (ATCC 13803) was sensitive to the root extract of *P. pinnata*. Majority of the clinical non-albicans isolates were sensitive to the root extract. Extracts of bark and leaf did not show any inhibitory activity (Table 1).

The MICs of *P. pinnata* against the tested organisms varied between 1.6 and 12.8 mg/ml. *C. guilliermondii* (2) had the lowest and a clinical isolate of *C. albicans* had the highest MIC. The extract was inactive against *C. tropicalis* (ATCC 13803) even at the highest concentration tested. Comparing the MIC values for the plant extract with fluconazole, the plant extract had relatively higher MIC values for all the isolates (Table 2).

A 2 h exposure of *C. albicans* isolates to the root extract of *P. pinnata*, resulted in a reduction in their germ tube formation (Figure 1) and elongation (Figure 2) in a concentration dependent manner. Except *C. albicans* (3), the other four isolates showed a significant reduction ($P < 0.05$) in the germ tube elongation when treated with their MIC values. *C. albicans* (3) achieved a significant reduction in its germ tube length when treated with 2 x MIC (Figure 2).

Five different isolates were selected to determine the effect of the root extract of *P. pinnata*, on RCSH. RCSH was reduced greatly by exposing the organisms to sub-inhibitory concentrations of the extract in a concentration dependent manner (Figure 3). Three isolates, including *C. albicans* (ATCC 90028) showed a significant reduction ($P < 0.05$) in their RCSH when treated with 1/8 MIC, which was the lowest concentration used in the study. However, 1/4 MIC value was needed to obtain a significant reduction in the RCSH of the other two isolates.

When the root extract was subjected to thin layer chromatography and observed under UV light - 254 nm, a dark spot (Rf = 0.8) against a fluorescent green background was observed (Figure 4b). Under UV light 365 nm, a blue fluorescence was observed at Rf = 0.88 (Figure 4c). Spraying with ethanolic AlCl₃ resulted to a yellow spot at Rf = 0.9 (Figure 4d) and the spot fluoresced in green colour when observed under UV - 365 nm (Figure 4e). There was no blue colouration when the TLC plate was sprayed with FeCl₃ (Figure 4f). An inhibition zone was visualized as a clear area (Rf value of 0.92) against a red coloured background, on the TLC plate subjected to bio-autography (Figure 4g).

DISCUSSION

The traditional knowledge based drug development reduces the time and cost of the development process (Patwardhan et al., 2004). The production of an enormous number of bio-active compounds in the plant has

Table 1. Average zone of inhibition obtained for extracts of *P. pinnata* (25 mg/ml), against standard and clinical isolates of *Candida* spp. in the well diffusion bio-assay.

Name of the isolate	Average zones of inhibition with extract of <i>P. pinnata</i> (mm) \pm SD (n=6)		
	Root	Leaf	Bark
<i>C. albicans</i> (ATCC 90028)	5.33 \pm 0.52	ND	ND
<i>C. parapsilosis</i> (ATCC 22019)	0	ND	ND
<i>C. glabrata</i> (ATCC 90030)	0	ND	ND
<i>C. krusei</i> (ATCC 6258)	0	ND	ND
<i>C. tropicalis</i> (ATCC 13803)	3.00 \pm 0.63	ND	ND
<i>C. albicans</i> (1)	12.69 \pm 2.08	0	0
<i>C. albicans</i> (2)	13.33 \pm 1.51	ND	ND
<i>C. albicans</i> (3)	8 \pm 0.89	ND	ND
<i>C. albicans</i> (4)	10.17 \pm 1.72	ND	ND
<i>C. dubliniensis</i>	11.83 \pm 0.75	0	0
<i>C. guilliermondii</i> (1)	4.5 \pm 0.55	0	0
<i>C. guilliermondii</i> (2)	11.17 \pm 1.17	ND	ND
<i>C. rugosa</i>	0	0	0
<i>C. parapsilosis</i>	20.83 \pm 0.75	0	0

*ND, Not Determined

Table 2. MIC values of *P. pinnata* and the positive control obtained from agar dilution method.

Name of the isolate	MIC value (mg/ml)	
	<i>P. pinnata</i>	Fluconazole
Standard isolates		
<i>C. albicans</i> (ATCC 90028)	6.4	0.128
<i>C. tropicalis</i> (ATCC 13803)	>12.8	>0.128
Clinical isolates		
<i>C. albicans</i> (1)	6.4	>0.128
<i>C. albicans</i> (2)	6.4	>0.128
<i>C. albicans</i> (3)	12.8	>0.128
<i>C. albicans</i> (4)	6.4	>0.128
<i>C. guilliermondii</i> (1)	6.4	0.032
<i>C. guilliermondii</i> (2)	1.6	>0.128
<i>C. dubliniensis</i>	3.2	0.032
<i>C. parapsilosis</i>	3.2	0.032

led to its versatile applications in the pharmaceutical industry (Fabricant and Farnsworth, 2001a, 2001b). Hence in the present study an attempt was made to identify the potential of *P. pinnata* as a source of new anti-candidal agents.

Among different parts of the plant, only the root extract exhibited anti-candidal activity during the current assay. However, Sharma et al. (2011) have reported the ability of leaf extract of *P. pinnata* in inhibiting *C. albicans*. The reason for this difference in the two observations may be the fact that Sharma et al. (2011) have used the purified flavonoid fraction of leaf extract of *P. pinnata* while in the current study the crude extracts have been used.

According to reports on the chemical composition of this plant, furanoflavonoids have been identified as the main component (Tanaka et al., 1992; Pavanaram and Ramachandra Row, 1955). In 2008, Sathishkumar and colleagues have shown that maximum extraction of flavonoids from a plant material can be achieved by using 75% ethanol as the solvent. Hence the furanoflavonoids could be the main active compound contributing to the positive activity of ethanolic root extract. Even though, previous reports have shown the antimicrobial property of different parts of *P. pinnata* (Baswa et al., 2001; Wagh et al., 2007), this is the first record of anti-candidal activity of roots of this plant. Although MIC values shown by the

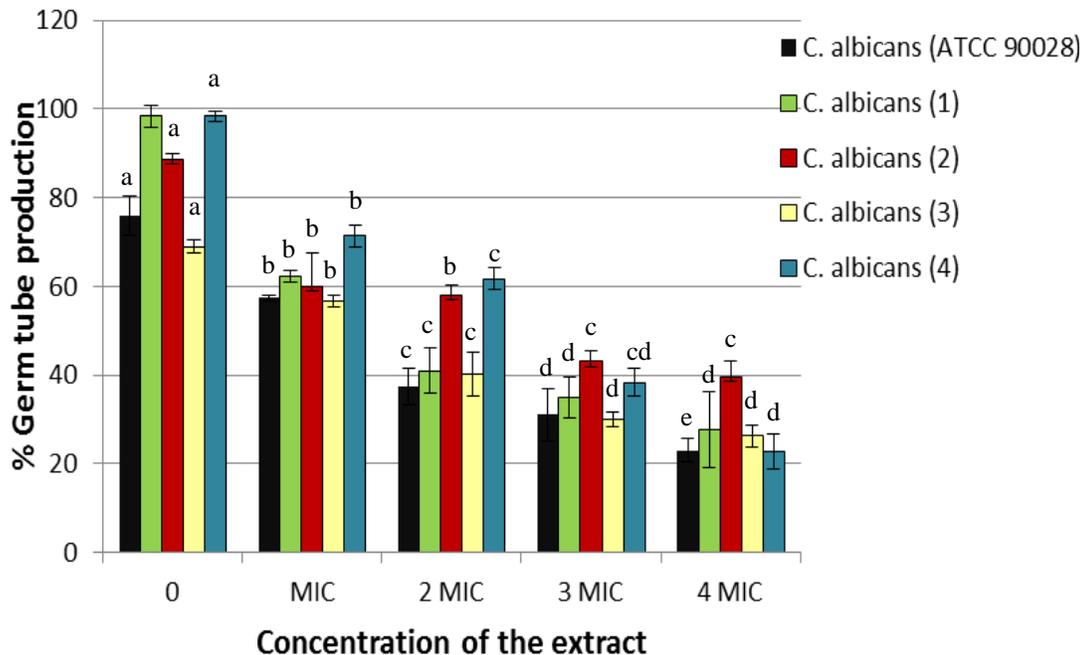


Figure 1. Effect of different concentrations of root extract of *P. pinnata* on the percentage germ tube production of clinical isolates of *C. albicans*, expressed as the mean \pm SEM (n=3). Bars with the same letter among concentrations are not significantly different (P > 0.05).

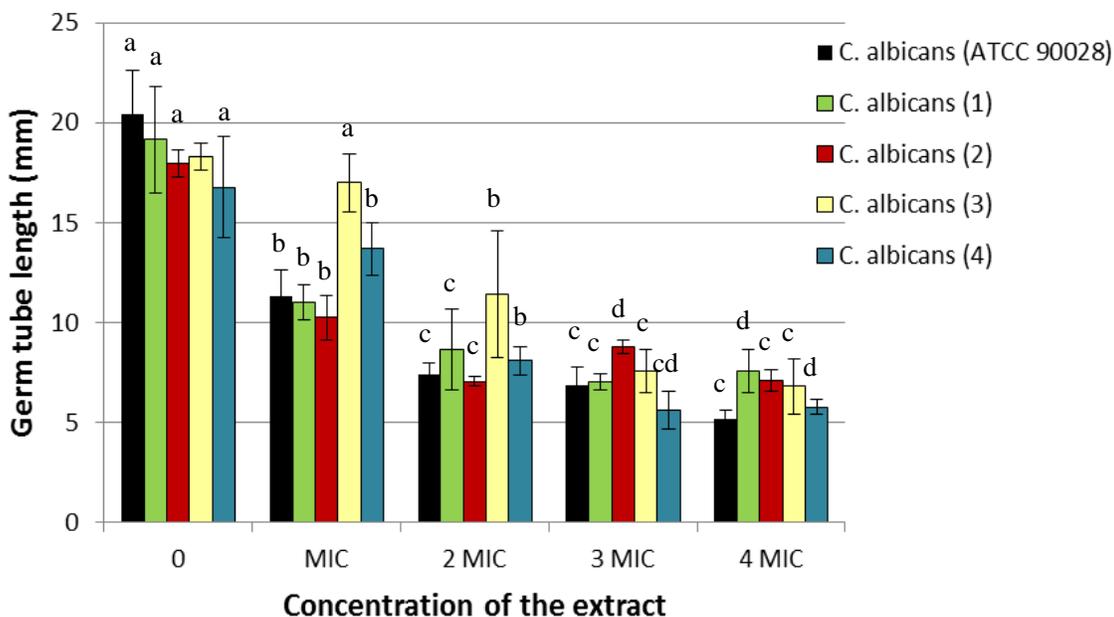


Figure 2. Effect of different concentration of root extract of *P. pinnata* on the length of the germ tubes of clinical isolates of *C. albicans*, expressed as the mean \pm SEM (n=30 X 3). Bars with the same letter among concentrations are not significantly different (P > 0.05).

crude root extract was always higher than that of fluconazole, if the active compound of *P.pinnata* root is used the MIC values will be lower.

Since the germ tube formation by *C. albicans* supports the organism to adhere to the host tissue and initiate infections (Kimura and Pearsall, 1980) it is considered as

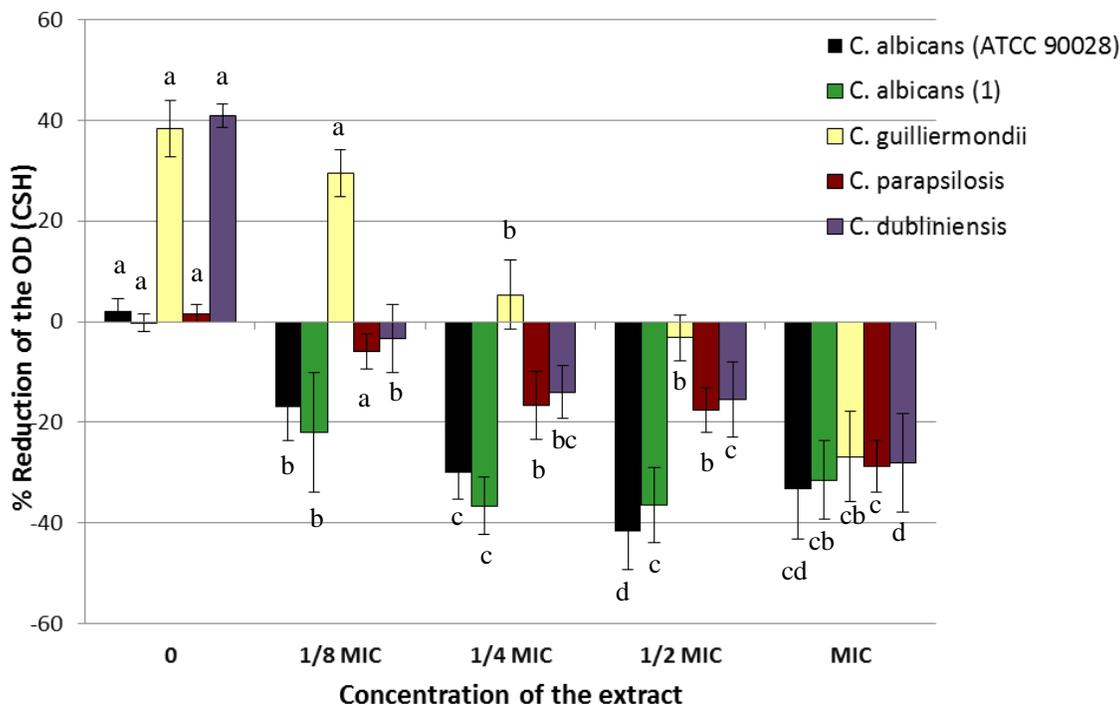


Figure 3. CSH, after the addition of root extract, of *P. pinnata* at 1/8 MIC, 1/4 MIC, 1/2 MIC and MIC values. Values plotted are means of percentage reduction of the absorbance \pm standard deviations of six determinations. Bars with the same letter among concentrations are not significantly different ($P > 0.05$).

an important feature that contributes to the pathogenicity of *C. albicans*. The ability of the essential oil extracted from *Syzygium aromaticum* to inhibit the germ tube production by *C. albicans* has been previously shown by Pinto et al. (2009b). *Melaleuca alternifolia*, *Aloe vera* and *Streblus asper* are some other plants with a potential of controlling germ tube production by *C. albicans* (Hammer et al., 2008; Bernardes et al., 2012; Taweechaisupapong et al., 2005). Controlling the germ tube formation will make the organism less adherent to host tissues.

Relative cell surface hydrophobicity (RCSH) has been identified as one of the key factors for adhesion of the candidal cell to the host tissue (Hazen et al., 1991). The ability of root extract of *P. pinnata* to influence the RCSH could be due to its potential in altering the surface characteristics of the organism. The ability of the root extract of *P. pinnata* to make a significant reduction in the RCSH at the sub-inhibitory concentrations will assist the control of the pathogen instead of its complete inhibition. Since *Candida* is a commensal fungus, usage of sub-inhibitory concentrations will help to maintain the balance of the host microflora while controlling pathogenicity of the organism. The ability of different plant extracts to influence the RCSH of pathogenic microbes has been identified by previous scientists. The ability of *Piper betle* and *Psidium guajava* to reduce the CSH of oral pathogenic bacteria was shown by Razak and Rahim (2003). However, an increment in the cell surface hydro-

phobicity of *C. albicans*, when they were treated with the aqueous extract of *Azadirachta indica* have also been shown (Polaquini et al., 2005).

It is possible that despite not showing significant anti-candidal activity in the well diffusion bio assay, extracts of leaf and bark of *P. pinnata* could have an effect on germ tube formation, elongation and cell surface hydrophobicity in *Candida* spp. Further investigations should be carried out to identify such effects of bark and leaves of the plant.

During bioautography, since similar Rf values were obtained for spots identified as flavonoids and inhibitory spots with *Candida* sp., it can be assumed that the compound/s responsible for the anti-candidal activity of roots of *P. pinnata* could be flavonoids. Similarly, earlier researchers have identified flavonoids as the main constituent of roots (Tanaka et al., 1992). In addition, flavonoids have already proven to possess antimicrobial activity against a wide range of microbes including fungi, bacteria and virus. *Eysenhardtia texana* is one such plant consisting of Flavanones. viz: 4',5,7-trihydroxy-8-methyl-6-(3-methyl-[2-butenyl])-(2S)-flavanone, 4',5,7-trihydroxy-6-methyl-8-(3-methyl-[2-butenyl])-(2S)-flavanone and pathogens (Dall'Agnol et al., 2003; El-Abyad et al., 1990).

Considering the cytotoxicity of *P. pinnata*, Sharma et al. (2011) have shown that purified flavonoid fraction of the leaf extract of *P. pinnata* have no cellular toxicity to fresh 4',5-dihydroxy-7-methoxy-6-(3-methyl-[2-butenyl])-(2S)-

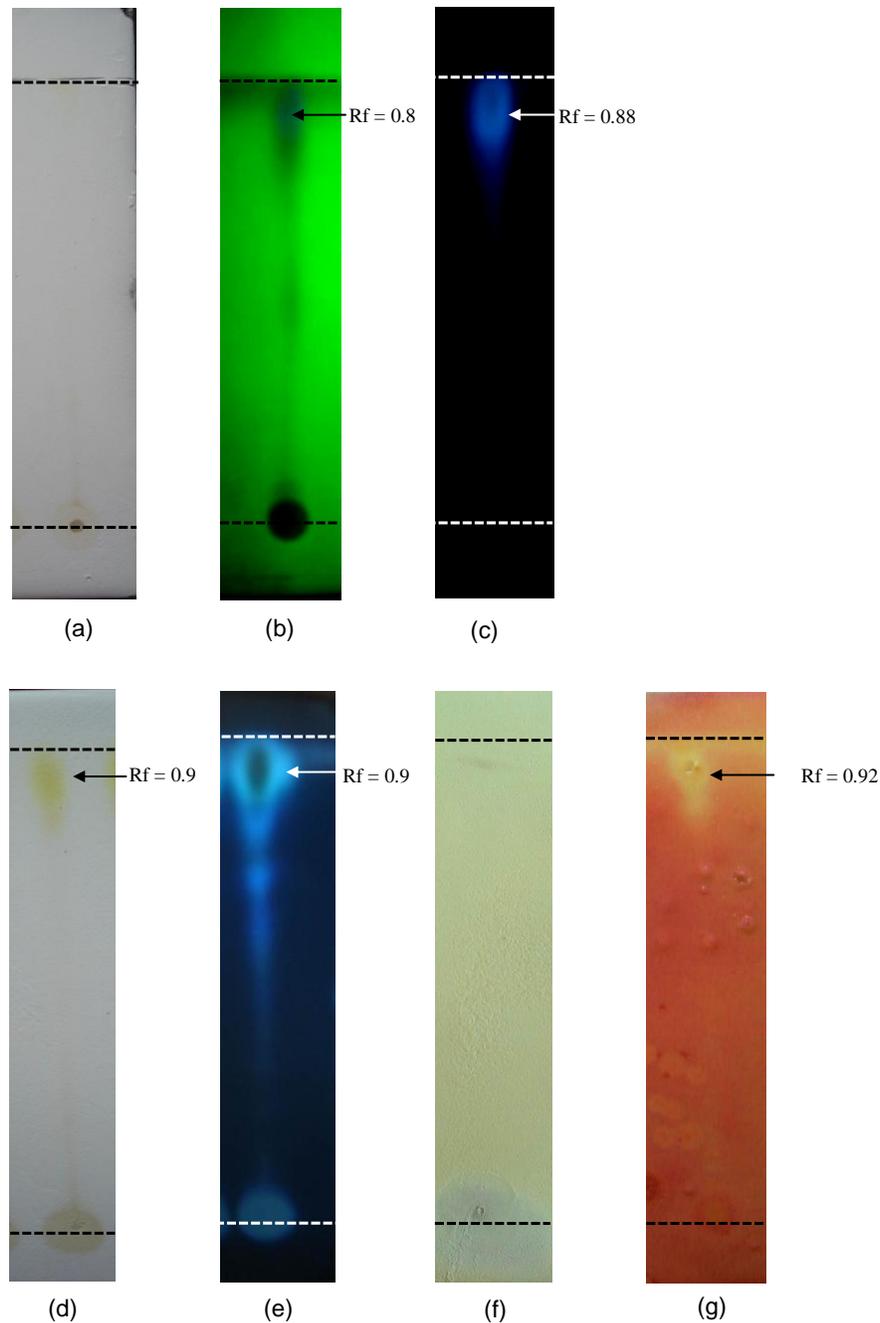


Figure 4. Thin layer chromatograms of ethanolic root extract of *P. pinnata* (a) under day light (b) under UV light - 254 nm (c) under UV light - 365 nm (d) sprayed with AlCl_3 and observed under day light (e) sprayed with AlCl_3 , observed under UV - 365 nm (f) sprayed with FeCl_3 and (g) subjected to bio-autography.

flavanone, possessing significant antifungal activity (Wächtera et al., 1999). In addition, the activity specifically against *Candida* spp have been recorded by plant extracts which constitute flavonoids. Fruits of *Terminalia bellerica* contain flavonoid compounds named as termilignan, thannilignan, 7-hydroxy-3', 4'-(methylenedioxy) flavan and anolignan B which can inhibit *C. albicans*

(Valsaraj et al., 1997). It has been proven that *Apulian propolis* can inhibit *C. albicans* as well as other fungal pathogens causing dermatosis (Cafarchia et al., 1999). Flavonoids isolated from *Hypericum* spp., *Capsella* spp. and *Cromolaena* spp. are also known to bear inhibitory activity against bacterial human erythrocytes at a concentration of 100 $\mu\text{g/ml}$. However, cytotoxicity assays have to

be carried out to identify the toxicity effects of crude extract of *P. pinnata* against human cells.

In conclusion, the results of our study reveal that roots of *P. pinnata* possess a significant anti-candidal activity. In addition, the results showed the ability of the root extract of *P. pinnata* in reducing the germ tube formation, germ tube elongation and the relative cell surface hydrophobicity *in vitro*. Moreover, the study suggests that the active component of root extract of *P. pinnata* could be compound/s belonging to the group of flavonoids.

Hence this study could lead to the establishment of some compounds that could be used to form novel anti-candidal drugs of plant origin. However, further phytochemical studies are required to identify the compound(s) responsible for the anti-candidal activity of this plant extract.

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REFERENCES

- Adikaram NKB, Bandara BMR (1998). Methodology for studying defense mechanisms against fungal pathogens: an overview. In: JOHNSON, G. I., HIGHLEY, E. & JOYEE, D. C., (eds) Disease resistance in fruits, Canberra, Australia. pp. 177-185.
- Andersen QM, Markham KR (eds.) (2006). Flavonoids, Chemistry, Biochemistry and Applications, New York: CRC Press.
- Andrews J (2001). Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemoth.* 48:5-16.
- Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, Dabur R (2009). Natural products--antifungal agents derived from plants. *J. Asian. Nat. Prod. Res.* 11:621-638.
- Babic M, Hukic M (2010). *Candida albicans* and non-albicans species as etiological agent of vaginitis in pregnant and non-pregnant women. *Bosnian. J. Basic. Med. Sci.* 10:89-97.
- Baily GG, Perry FM, Denning DW, Mandal BK (1994). Fluconazole-resistant candidosis in an HIV cohort. *AIDS* 8:787-92.
- Baswa M, Rath CC, Dash SK, Mishra RK (2001). Antibacterial activity of Karanj (*Pongamia pinnata*) and Neem (*Azadirachta indica*) seed oil: a preliminary report. *Microbios* 105:183-189.
- Bernardes I, Rodrigues MPF, Bacelli BK, Munin E, Alves LP, Costa MS (2012). Aloe vera extract reduces both growth and germ tube formation by *Candida albicans*. *Mycoses* 55:257-261.
- Cafarchia C, De Laurentis N, Miliillo MA, Losacco V, Puccini V (1999). Antifungal activity of Apulia region propolis. *Parassitologia* 41:587-90.
- Chea A, Jonville MC, Bun SS, Laget M, Elias R, Dumenil G, Balansard G (2007). *In vitro* antimicrobial activity of plants used in Cambodian traditional medicine. *Am. J. Chinese. Med.* 35:867-873.
- Cowan MM (1999). Plant Products as Antimicrobial Agents. *Clin. Microbiol. Rev.* 12:564-582.
- Dall'agnol R, Ferraz A, Bernardi AP, Albring D, Nor C, Sarmento L, Lamb L, Hass M, Von Poser G, Schapoval EE (2003). Antimicrobial activity of some Hypericum species. *Phytomedicine* 10:511-6.
- Duarte MC, Leme EE, Delarmelina C, Soares AA, Figueira GM, Sartoratto A (2007). Activity of essential oils from Brazilian medicinal plants on *Escherichia coli*. *J. Ethnopharmacol.* 111:197-201.
- EI-Abyad MS, Morsi NM, Zaki DA, Shaaban MT (1990). Preliminary screening of some Egyptian weeds for antimicrobial activity. *Microbios* 62:47-57.
- Ellepola ANB (2005). Oral Candidosis: a brief overview. *Bulletin of the Kuwait Institute for Medical specialization* 4:17-24.
- Fabricant DS, Farnsworth NR (2001a). The Value of Plants Used in Traditional Medicine for Drug Discovery. *Environmental Health Perspectives* 109:69-75.
- Fabricant DS, Farnsworth NR (2001b). The Value of Plants Used in Traditional Medicine for Drug Discovery. *Environ. Health Persp.* 109:69-75.
- Giusiano G, Mangiaterra M, Garcia Saito V, Rojas F, Gomez V, Diaz MC (2006). Fluconazole and itraconazole resistance of yeasts isolated from the bloodstream and catheters of hospitalized pediatric patients. *Chemotherapy* 52:254-259.
- Hammer KA, Carson CF, Riley TV (2008). *Melaleuca alternifolia* (tea tree) oil inhibits germ tube formation by *Candida albicans*. *Med. Mycol.* 38:355-362.
- Hazen KC, Brawner DL, Riesselman MH, M.A. J, Cutler JE (1991). Differential adherence of hydrophobic and hydrophilic *Candida albicans* yeast cells to mouse tissues. *Infect. Immun.* 59:907-912.
- Jayaweera DMA (1981). Medicinal Plants Used in Ceylon, Colombo, Sri Lanka, National Science Council of Sri Lanka.
- Khare CP (2007). Indian Medicinal Plants. An Illustrated Dictionary, New York, Springer Science.
- Kimura LH, Pearsall NN (1980). Relationship between germination of *Candida albicans* and increased adherence to human buccal epithelial cells. *Infect. Immun.* 28:464-468.
- Melo NR, Taguchi H, Jorge J, Pedro RJ, Almeida OP, Fukushima K, Nishimura K, Miyaji M (2004). Oral Candida flora from Brazilian human immunodeficiency virus-infected patients in the highly active antiretroviral therapy era. *Mem. I. Oswaldo Cruz.* 99:425-31.
- More G, Tshikalange TE, Lall N, Botha F, Meyer JJM (2008). Antimicrobial activity of medicinal plants against oral microorganisms. *J. Ethnopharm.* 119:473-477.
- Patwardhan B, Vaidya ADB, Chorghade B (2004). Ayurveda and natural products drug discovery. *Curr. Sci. India* 86:789-799.
- Pavanaram SK, Ramachandra Row L (1955). New Flavones from *Pongamia pinnata* (L.) Merr.: Identification of Compound D. *Nature* 176:1177.
- Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L (2009). Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and dermatophyte species. *J. Med. Microbiol.* 58:1454-1462.
- Polaquini SRB, Svidzinski TIE, Kemmelmeier C, Gasparetto A (2005). Effect of aqueous extract from Neem (*Azadirachta indica* A. Juss) on hydrophobicity, biofilm formation and adhesion in composite resin by *Candida albicans*. *Arch. Oral Biol.* 51:482-490.
- Purkayastha S, Dahiya P (2012). Phytochemical Analysis and Antibacterial Efficacy of Babchi Oil (*Psoralea corylifolia*) Against Multi-drug Resistant Clinical Isolates. International Conference on Bioscience, Biochemistry and Bioinformatics. Singapore: IACSIT Press.
- Rangama BNLD, Abayasekera CL, Panagoda GJ, Senanayake MRDM (2009). Antimicrobial activity of *Tephrosia purpurea* (Linn.) Pers. and *Mimusops elengi* (Linn.) against some clinical bacterial isolates. *J. Nat. Sci. Found.* 37:139-145.
- Razak FA, Rahim ZHA (2003). The anti-adherence effect of *Piper betle* and *Psidium guajava* extracts on the adhesion of early settlers in dental plaque to saliva-coated glass surfaces. *J. Oral Sci.* 45:201-206.
- Samonis G, Kofteridis DP, Saloustros E, Giannopoulou KP, Ntziora F, Christidou A, Maraki S, Falagas ME (2008). *Candida albicans* versus non-albicans bloodstream infection in patients in a tertiary hospital: an analysis of microbiological data. *Scand. J. Infect. Dis.* 40:414-9.
- Sathishkumar T, Baskar R (2008). Optimization of flavanoids extraction from the leaves of *Tabernaemontana heyneana* Wall. Using L 16 orthogonal design. *Nat. Sci.* 6:10-21.
- Sawaya A, C. H. F., Souza KS, Marcucci MC, Cunha IBS, Shimizu MT (2004). Analysis of the composition of Brazilian propolis extracts by chromatography and evaluation of their *in vitro* activity against gram-positive bacteria. *Braz. J. Microbiol.* p. 35.
- Sweet SP, Macfarlane TW, Samaranyake LP (1987). Determination of the cell-surface-hydrophobicity of oral bacteria using a modified hydrocarbon adherence method. *FEMS Microbiol. Lett.* 48:159-163.

- Tanaka T, Linuma M, Yuki K, Fujii Y, Mizuno M (1992). Flavonoids in root bark of *Pongamia pinnata*. *Phytochemistry* 31:993-998.
- Taweechaisupapong S, Choopan T, Singhara S, Chatrchaiwiwatana S, Wongkham. (2005). *In vitro* inhibitory effect of *Streblus asper* leaf-extract on adhesion of *Candida albicans* to human buccal epithelial cells. *J. Ethnopharmacol.* 96:221-226.
- Uragoda CG (2003). Some Sri Lankan traditions on health and disease discovered as scientifically valid. *Ayurveda Sameeksha* 1:232-240.
- Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A, Christensen SB, Sittie A, Nyman U, Nielsen C, Olsen CE (1997). New anti-HIV-1, antimalarial, and antifungal compounds from *Terminalia bellerica*. *J. Nat. Prod.* 60:739-742.
- Wächtera GA, Hoffmannb JJ, Furbacherb T, Blakec ME, Timmermann BN (1999). Antibacterial and antifungal flavanones from *Eysenhardtia texana*. *Phytochemistry* 52:1469-1471.
- Wagh P, Rai M, Deshmukh SK, Durate MCT (2007). Bio-activity of oils of *Trigonella foenum-graecum* and *Pongamia pinnata*. *Afr. J. of Biotechnol.* 6:1592-1596.
- Zaremba ML, Daniluk T, Rozkiewicz D, Cylwik-Rokicka D, Kierklo A, Tokajuk G, Dabrowska E, Pawinska M, Klimiuk A, Stokowska W, Abdelrazek S (2006). Incidence rate of *Candida* species in the oral cavity of middle-aged and elderly subjects. *Adv. Med. Sci.* 51:233-236.
- Zomorodian K, Rahimi MJ, Pakshir K, Motamedi M, Ghiasi MR, Rezashah H (2011). Determination of Antifungal Susceptibility Patterns Among the Clinical Isolates of *Candida* Species. *J. Glob. Infect. Dis.* 3:357-360.