

Antifungal Activity of *Pongamia pinnata* on *Candida albicans* Isolated from Antenatal Women

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ABSTRACT

Traditional system of medicine in India had herbal plant parts for curing many diseases. In traditional system of medicines, such as ayurveda and Unani, the *Pongamia pinnata* plant is used for anti-inflammatory, anti-plasmodial, anti-ulcer, CNS depressant activity and antioxidant activity. A preliminary study was carried out to study the antifungal activity of *Candida albicans* in vagina among antenatal women. Isolation and characterization of *Candida albicans* from the vaginal samples of 50 antenatal women were done. Aqueous and ethanolic extracts of *Pongamia pinnata* seeds were used to find out the efficacy of the *in vitro* susceptibility against standard strain and Clinical isolate. Prevalence of Candidiasis among the antenatal women in age, diabetic and semester wise was also carried out. The observed results indicated that the crude aqueous extracts exhibited an appreciable antifungal activity. This has to be confirmed in further analysis using more number of isolates.

Keywords: Antifungal activity, Antenatal women, *Pongamia pinnata*, *Candida albicans*

INTRODUCTION

Candida albicans is one of the causative organisms for the common fungal infection vaginal candidiasis. It is the most common vaginal infection in women during reproductive age group, also affecting pregnant women (Horowitz [1], Cassone [2] and Dolatabadi [3]). It occurs in 1-14% of all women of reproductive age throughout the world and its prevalence in India is estimated to be 30% Thulkar [4]. Prenatal clinical check-up is of prime importance in the prevention of the disease of both the mother and fetus and for the prevention of abortion as certain species of *Candida* (*Candida tropicalis*) results in abortion prenatally. Herbal medicines are good alternative for synthetic medicine as the latter have various side effects.

METHODOLOGY

Sample collection

Vaginal Swab samples were collected from 50 volunteers of Antenatal women in triplicates. The age wise distribution among the 50 women was 21-25, 26-30 and 31-32 with the number of antenatal cases of 13, 31 and 6, respectively. Clinical history of the volunteers was also recorded in a Performa sheet. Collected swabs were grown on SDA (Sabouraud Dextrose Agar) media plates at 37°C and confirmed by germ tube test.

Plant collection

Dried seeds of *Pongamia pinnata* were collected and surface sterilization was done. Seeds and water are mixed (1:5) and ground and filtered using Seitz filter (pore size – 0.2 µm) with a pH of 6.2.

Test microorganisms

The fungal strain *Candida albicans* (ATCC 90029) used was obtained from Hi MEDIA laboratory and clinical isolates were obtained from a gynecological hospital. Clinical isolates from individuals were plated onto Sabouraud Dextrose Agar plates and incubated for 48 h at 25°C. Colonies were streaked and identified by standard methods Warren and Shadomy [5] Standard antifungal drugs used in this study are Ampotericin-B and Clotrimazole.

Antifungal susceptibility test methods

Each isolate was tested by reference microdilution methods

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following NCCLS guidelines for inoculums preparation, media formulation and incubation (NCCLS [6]). Plant extracts used were lyophilized aqueous extracts and ethanolic extracts of *Pongamia pinnata* seeds. It is used to find out the efficacy of the *in vitro* susceptibility against standard strain and clinical isolates using microdilution and disc diffusion method. The experiment was done three times and the mean values are presented.

RESULTS AND DISCUSSION

In present study samples (vaginal swabs) were being collected from 50 volunteers prenatal women attending a private gynecological clinic located at Chetpet, Chennai.

From the study carried among the 50 volunteers within the age group of 21-32 those which showed positive and negative results for Candidiasis were listed in the **Table 1**. It was found that large number of positivity to vaginal Candidiasis was in the age group 26-30.

Table 1. Total number of antenatal cases with Candidiasis.

Age Group	No. of Antenatal cases	Candidiasis	
		Positive	Negative
21-25	13	07	06
26-30	31	15	16
31-32	6	04	02

From the positivity of neonatal women to vaginal candidiasis was found to be 71.4% in the 2nd semester of age group 21-25 followed by 67% and 50 % in 2nd and 3rd

semester of age groups 26-30 and 31-32, respectively (**Table 2**).

Table 2. Distribution of antenatal cases age wise, during pregnancy.

Age Group	Total No. of Antenatal cases	Semester of Pregnancy					
		Antenatal cases			positive to Candidiasis		
		I	II	III	I	II	III
21-25	13	2	8	3	1	4	2
26-30	31	5	19	7	3	10	2
31-32	6	1	3	2	1	1	2

It was also found that prenatal women with diabetics to be high positivity than non-diabetic (**Table 3**). The results coincide with other investigators who have suggested that

vulvovaginal candidiasis occurs more frequently in diabetics [7], Zdolsek [8] and Bohannon [9].

Table 3. Distribution of diabetic and non-diabetic antenatal cases.

Age Group	Diabetic		Non-Diabetic	
	Total	Positive	Total	Positive
21-25	06	04	07	03
26-30	10	08	21	07
31-32	03	03	03	01

Out of the 50 clinical samples 26 showed positivity to isolation by In vitro cultivation on SDA plates. Subsequent characterization by Gram staining, Germ tube formation and its morphology was observed and all the 26 isolates were proved to be positive for *Candida albicans*.

The 26 isolates were subjected to *in vitro* susceptibility test to 6 antifungal drugs (Amphotericin, Clotrimazole, Itraconazole, Fluconazole, Ketoconazole and Nystatin) (**Table 4**). Standard strain (ATCC) and the 26 isolates were not susceptible to Amphotericin B and Clotrimazole. This

study also focuses for the prevalence of Multi Drug Resistance among *Candida albicans* isolates from vaginal Candidiasis this has been supported by observation in *in vitro* susceptibility test by Micro Dilution Assay in which turbidity is seen for the resistance reported through Disc Diffusion Assay. Study done by Srinivasan et al. [10]

revealed that Sertaconazole was more effective than Clotrimazole. The alarming increase in infections with multidrug resistant bacteria is due to overuse of a broad spectrum antimicrobials, which leads to over growth of *Candida* spp., thus, enhancing its opportunity to cause the disease [11].

Table 4. Antibiotic susceptible pattern of positive cases in antenatal women.

No. of Antenatal Cases	Antibiotic Discs					
	ApB	Cc	Fu	It	Kt	Ns
1	R	S	R	R	R	S
2	S	R	S	R	R	S
3	S	S	R	S	R	R
4	R	S	R	S	R	R
5	R	R	S	S	S	R
6	S	S	R	R	S	R
7	R	R	S	S	S	R
8	S	S	R	S	R	R
9	R	S	S	S	R	R
0	R	R	S	S	S	R
11	R	R	S	R	S	S
12	R	R	S	R	S	S
13	S	S	S	R	R	S
14	S	S	R	S	R	R
15	R	R	S	S	S	R
16	R	S	R	R	S	S
17	S	S	R	S	R	R
18	R	S	S	S	R	R
19	R	R	S	S	S	R
20	S	S	R	R	S	R
21	R	R	R	R	S	S
22	S	R	S	R	R	S
23	S	R	S	R	R	S
24	S	S	R	R	R	R
25	S	S	R	S	R	R
26	S	S	R	S	R	R

R=Resistant; S=Sensitive

ApB: Amphotericin B; **Cc:** Clotrimazole; **It:** Itraconazole; **Fu:** Fluconazole; **Kt:** Ketoconazole; **Ns:** Nystatin

Among the aqueous and ethanolic seed extracts of *Pongamia pinnata* used along with the drugs aqueous extracts exhibited appreciable antifungal activity which has to be verified by

further studies with increased number of clinical isolates (Table 5).

Table 5. *In vitro* susceptibility of standard Candidal strain and 1 multi drug resistance clinical isolate (21st sample).

S. No	Dilution	ATCC Strain of <i>Candida albicans</i>				Clinical Sample of <i>Candida albicans</i>			
		Clotrimazole	Amphotericin B	Aqueous Extract <i>P. pinnata</i>	Ethanolic Extract <i>P. pinnata</i>	Clotrimazole	Amphotericin B	Aqueous Extract <i>P. pinnata</i>	Ethanolic Extract <i>P. pinnata</i>
1	200 mcg/ml	+	+	+	+	+	+	Highly Marked Inhibition (-)	+
2	100 mcg/ml	+	+	+	+	+	+	+	+
3	50 mcg/ml	+	+	+	+	+	+	+	+
4	25 mcg/ml	+	+	+	+	+	+	+	+
5	12.5 mcg/ml	+	+	+	+	+	+	+	+
6	6.25 mcg/ml	+	+	+	+	+	+	+	+
7	3.75 mcg/ml	+	+	+	+	+	+	+	+
8	1.87 mcg/ml	+	+	+	+	+	+	+	+

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