

Antifungal activity of *Salacia oblonga* extracts Against Human Pathogen *Candida albicans*

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Abstract: *Salacia Oblonga* shrub belonging to the family 'Celestraceae'. It is found in India and Srilanka. We have collected the plant extract from Western Ghats, India. In traditional medicines it is used for the treatment of diabetes and can be exploited to treat many chronic and infectious diseases. The plant extracts of *S.oblonga* has been used on the treatment of diabetes, polyuria, gonorrhoea, rheumatism, asthma and fever. We have undertaken present study to evaluate the antifungal activity of *S.oblonga* aerial and root extracts against human pathogen *Candida albicans*. Antifungal activity of methanol extract (1 mg/ml) and ethyl acetate extracts were tested. Extracts have shown good growth inhibition against *Candida albicans*. Identified the minimum inhibitory concentration of the plant extract 40µg/ml comparison with synthetic drug Clotrimazole 60µg/ml. Separation of the phytochemical from the crude extracts by thin layer chromatography and identified the active fraction. Our study highlighted the antifungal activity of *Salacia Oblonga* extracts.

Keywords: *Salacia oblonga*, Antifungal activity, *Candida albicans* and Chromatography

I. INTRODUCTION

Medicinal plants have ability to synthesize the wide variety of chemical compounds that are used to perform important biological functions and to defend against pathogens [1]. Plant based medicines are employed for curing many diseases owing to their bioactive secondary metabolites of therapeutic importance [2]. Such plants are used to treat respiratory disorders, chronic fever, cold, cough, malaria, dysentery, skin diseases etc. traditional uses of plants have been led to investigating their bioactive compounds, which have resulted in detection of a significant number of therapeutic properties. To treat different fungal infections, many synthetic drugs like amphotericin, β nystatin, clotrimazole are used, resulting short term side effects like headache, nausea, vomiting. In order to reduce the side effects, medicinal plants have been used to treat several fungal infections [3-5].

Salacia Oblongais a strangling shrub native to India and Srilanka. It belongs to 'Celestraceae' family, commonly known as 'Saptarangi' in telugu. In traditional medicine it is used for the treatment of chronic and infectious diseases [6-

8]. Only limited knowledge is available regarding the antifungal activity of the plant. *Candida albicans* a fungus that is normally present on skin and mucus membrane such as vagina, mouth and rectum and most of the common fungal infections are caused by this organism. *Candida albicans* is found worldwide but generally compromises immune compromised individuals diagnosed with diseases like HIV and cancer. These are common group of organism that cause hospital acquired infection [9]. *C. albicans* is the top source of fungal infections in critical ill or immune compromised patients. These patients mainly develop oropharyngeal or thrush candidiasis, which can lead to malnutrition and interfere with the absorption of medication [9&10]. Therefore the present study was designed to evaluate the antifungal activity of *Salacia oblonga* extracts against clinical isolate of *Candida albicans*.

II. METHODOLOGY

Extract preparation:

Salacia oblonga plants were collected from the Western Ghats, India. The shade dried plants were separated into

aerial, root extracts and ground into a fine powder, using an electric blender. The phytochemicals were extracted in methanol with the help of soxhlet apparatus. The extracts were concentrated, using a rotavapor and were stored at -20°C for further use.

Culture collection:

Clinical isolates of *Candida albicans* were procured from NICE hospital, Mehadipatnam Hyderabad India. The fungus were cultured on potato dextrose agar (PDA) and activated in PD broth at 30°C , 20 hours before experimentation. The fungal culture is maintained on PD agar and sub culture every fortnight. Glycerol stocks of *C. albicans* were prepared and stored at -20°C for further use.

Antifungal assay:

The antifungal activity of plant extracts were investigated by agar well diffusion method [11]. The potato dextrose agar was poured onto the petriplates with an inoculum of $10\mu\text{l}$ (PDB) of *Candida*. The wells were made in the PDA plates with the help of borer. The extracts at a concentration of 1 mg/ml were used for evaluating the antifungal activity. A standard antifungal agent, clotrimazole at a concentration of 1mg/ml was used as positive control, whereas the solvent served as negative control. The plates were incubated overnight at 30°C for allowing fungal growth. After incubation, the zones of inhibition were observed around the wells (including the well diameter) and measured.

MIC:

Minimum inhibitory concentration (MIC) was determined by broth dilution method [12], two fold serial dilutions of crude extracts, with appropriate antifungal agent (clotrimazole mg/ml) as positive control were prepared in PDB. A direct suspension of fungal organism from a 24 hours old suspension of PDB is taken. For broth dilution test, $20\mu\text{l}$ of standardized suspension of *Candida* was added to each tube at a final concentration of extracts $20\mu\text{g/ml}$ - $100\mu\text{g/ml}$ and incubated at 37°C .

Thin layer chromatography:

Thin layer chromatography was carried out by 10mm-210mm mesh size silica gel to elute individual components from *Salacia oblonga* methanol extracts, exhibiting maximum antifungal activity. Compounds were eluted using a mobile phase of Hexane, Chloroform, Toulene and Ethyl

acetate (3: 1: 1: 1). The resulting fractions were collected separately, which were analyzed for the antifungal activity.

III. RESULTS AND DISCUSSION

Antifungal assay:

In the present study antifungal activity of *Salacia oblonga* wall extracts evaluated by agar well diffusion method against human pathogen *Candida albicans*. Different solvents of *S. Oblonga* extracts were evaluated for antifungal activity, methanol extracts have shown superior activity compared to the other solvent. The aerial and root parts were separated and analyzed for activity. Extracts displayed good antifungal activity against human pathogen *Candida*. Aerial & Root extracts displayed growth inhibition 16mm and 14mm respectively. Aerial extracts (A) have shown better growth inhibition against *Candida* compared to root extracts (B) as shown below Figure (1).

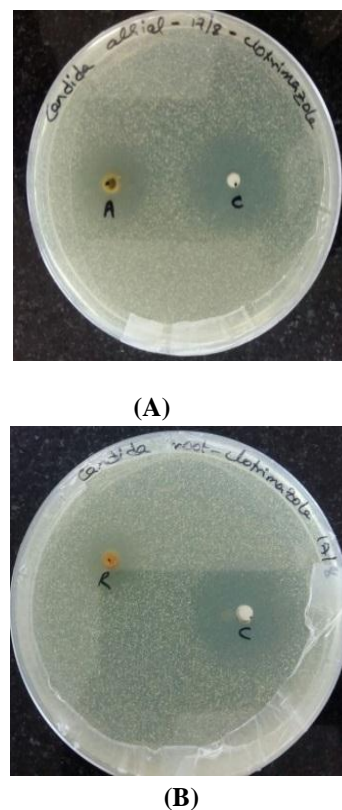


Figure 1: The growth inhibition of aerial and root extracts and clotrimazole against *Candida albicans*

MIC:

The methanol extracts exhibited exceptionally low values of MIC tested in range of 20µg/ml to 100µg/ml. *Salacia oblonga* aerial root extracts displayed MIC values 40 µg/ml, root extracts displayed 40µg/ml against *Candida*. Aerial & Root extracts exhibited similar MIC values where as antifungal agent standard clotrimazole exhibited MIC values 60µg/ml. MIC values were mentioned in table 1.

Table 1: MIC values of aerial and root extracts and clotrimazole

Pathogen	Aerial extract (µg/ml)	Root extract (µg/ml)	Clotrimazole(µg/ml)
<i>Candida albicans</i>	40µg/ml	40 µg/ml	60 µg/ml

Thin layer chromatography:

In order to obtain active components present in the crude extracts of *Salacia Oblonga*, various chromatographic techniques were applied. TLC was used initially for separation of compounds and mobile phase was optimized for better resolution. Methanol plant extracts exhibiting maximum antifungal activity were subjected to silica gel chromatography. The extracts were introduced onto the TLC plates and separate into different fractions with the help of mobile phase containing Hexane, Chloroform, Toulene, and Ethyl Acetate. Once the fractions were obtained, they were pooled together based on the similar thin layer chromatography patterns, the activity of each fraction were checked independently. The obtained fractions evaluated for their activity against *Candida albicans*. Among the tested fractions, few fractions exhibited excellent antifungal activity against *Candida albicans*.

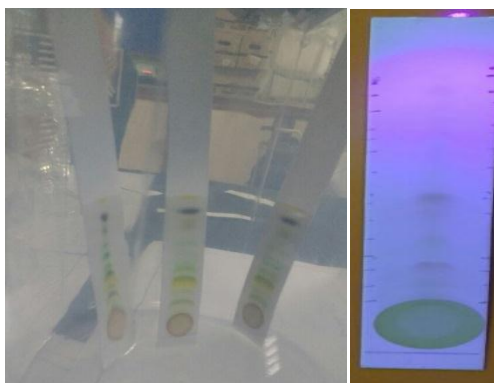


Figure 2: Separation of the plant extracts by TLC



Figure 3: Anti fungal activity by the active fractions of aerial extracts against *Candida albicans*

Candidiasis is acute or chronic, superficial or deep infection with a wide clinical spectrum. High heterozygosity is one of the most important features of *C. albicans* genome. This heterozygosity lies the occurrence of numeric and structural chromosomal rearrangements and changes as means of generating genetic diversity. These karyotypic alterations lead to modify the phenotype, which is an adaptation strategy of this fungus [13]. *S. oblonga* extracts exhibited excellent antimicrobial activity against this fungal pathogen. Bioactive natural compounds extracted from species with significant antifungal activity such as *Aloe vera*, *Glycyrrhiza glabra* and *Allium sativum* [14]. Our extract *S. oblonga* exhibited excellent antifungal activity against *C. albicans* fungal pathogen. Plant extracts contain a spectrum of secondary metabolites such as alkaloids, quinones, flavonoids, glycosides, saponins, tannins and terpenoids and shown various biological activities [14& 15]. *S. oblonga* extracts might contain these secondary metabolites and shown biological activity towards *C. albicans* fungal pathogen.

IV. CONCLUSION

Present study confirms the Antifungal activity of *Salacia oblonga* extracts against human pathogen *Candida albicans*. However, further investigations are needed to identify the active compounds from the active fractions of the extract.

CONFLICT OF INTEREST

Author has no conflict of interest.

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