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Anti-Fungal Activity of Arka Tail

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Abstract:
Fungal infections are increasing due to immuno-deficient states like Diabetes Mellitus, HIV, wide spread use of broad spectrum antibiotics, steroids etc. The aim of the study is to assess the antifungal activity & determine the zone of inhibition of *Arka Tail* on fungal strains of *Candida Albicans* and *Aspergillus Niger*. *Candida Albicans* causes candidiasis, which commonly occurs in mucous membrane in mouth or vagina due to lack of hygiene & weakened immune system. *Aspergillus Niger* is an air-borne pathogen commonly causes otomycosis which is a ear disorder. Antifungal activity on these two strains was seen using Agar Cup Diffusion Method and Minimum Inhibitory Concentration (MIC) was determined using Tetrazolium Salt Method on fungal strains. The drug was effective and exhibited a significant activity against *C. albicans* and *A.Niger*.

Keywords: Antifungal, *Aspergillus Niger*, *Candida Albicans*, *Arka Tail*, Fungal infection

Introduction:

Fungal infections have become very common. It is increasing due to immune-deficient states like Diabetes Mellitus, HIV, wide spread use of broad spectrum antibiotics, steroids etc. Fungi reproduce by spreading microscopic spores. These spores are often present in the air and soil, where they can be inhaled or come into contact with the surfaces of the body, primarily the skin. Consequently, fungal infections usually begin in the lungs or on the skin. Of the wide variety of spores that land on the skin or are inhaled into the lungs, most types do not cause infection. A few types cause infection only in people who have one of the following: 1. A weakened immune system. 2. Foreign material, including medical devices (such as an artificial joint or heart valve), in their body. (1)

Aspergillus Niger is a common food contaminant which grows as a black mould on food & vegetables and is an air-borne pathogen commonly causes otomycosis which is a ear disorder which may damage tympanic membrane & ear canal and Aspergillosis which is serious lung disease which is frequent in horticultural workers. (2)

Candida Albicans is naturally present in Human gut-flora detectable in GI tract in 40% of healthy adults. It causes candidiasis, which commonly occurs in mucous membrane in mouth or vagina due to lack of hygiene & weakened immune system. (2)

Arka Tail (AT) is an herbal oil preparation which can be used for fungal infections, skin diseases etc.

Aims & Objectives: To assess the anti-fungal activity & minimum inhibitory concentration of *Arka Tail* on two fungal strains 1. *Candida Albicans* and 2. *Aspergillus Niger*

Materials & Methods:

Preparation of Arka Tail:

Reference: Preparation of *Arka Tail* was performed as described in *Chakradatta/Kushtha Chikitsa/159.*

Ingredients: *Arka Tail* consists of only 3 ingredients:

Arka leaves: Leaves of *Arka* i.e *Calotropis Gigantea* are used in skin diseases. It is said to be Vata Kapha hara. (3)

Turmeric Powder: *Cucurma longa* is a widely used spice in India & has numerous health benefits. It acts as an anti-inflammatory & also possesses anti-fungal properties. (3)

Mustard Oil: Mustard (*Brassica Nigra*) Oil improves blood circulation. It has anti-bacterial & anti-fungal properties. (3) (4)

Procedure:

One part of *Arka swaras* (obtained from *Arka* leaves) was mixed with $1/4^{th}$ part of Mustard Oil and $1/16^{th}$ part of Turmeric paste. Mix all the ingredients properly in exact proportions. Heat it on manda agni (low flame) till all the *siddhi lakshans of sneha Kalpana* are obtained. The oil prepared is *Arka Tail*. (4)

200ml Arka swaras + 50ml Mustard oil + 12.5gms Turmeric paste $\xrightarrow{\triangle}$ Arka Tail

Properties of Arka Tail: *Arka Taila* has *Katu* and *Tikta Rasa, Ruksha, Laghu, Tikshna Guna, Ushna Veerya, Katu Vipaka,* and *Kapha-Vatashamaka* properties. *Arka Tail* is indicated in *Kustha*. (4)

Evaluation Technique:

Protocol: Antifungal activity was seen using agar cup diffusion method and MIC was determined using tetrazolium salt method.

Medium: Sabouraud's Agar

The test organisms were grown in Sabouraud's broth for 48 hour and used for the study. The optical densities of the culture were fixed using 0.5 Mcfarlands standard.

Minimum inhibitory concentration:

Preparation of Samples: 100 µl of sample was diluted in DMSO and further used for analysis.

Preparation of Inoculums:-the loop culture was grown in Sabouraud's broth for 48 hour. The culture OD was adjusted was to McFarland standard 0.5 in order to get 1.5×10^8 CFU/ml. The microbial cell suspension was mixed to homogeneity to give a final density of 1×10^6 CFU/ml.

The minimum inhibitory concentration (**MIC**) of *A.niger* was determined by using tetrazolium microplate assay. This assay was performed using flat bottom 96-well clear microtitre plates. The wells in first row of each column were filled with Sabouraud's broth which serves as blank; the second row was filled with 100 µl of Sabouraud's broth containing standard antibiotics while third row was filled with 100 µl of Sabouraud's broth containing diluents. Fourth row was filled with 100 µl 2X Sabouraud's broth and 100 µl of samples was added in each column. Then 5^{th} row onwards each wall were filled with Sabouraud's broth. An identical two-fold serial dilution were made from 4^{th} row to the 12^{th} row.Lastly, 100µl of Fungal inoculum were added in all the wells from 2^{nd} row to 12^{th} row and mixed thoroughly to give final concentrations ranging from 0.5mg/ml- 1.953125 µg/ml with 5×10^5 CFU/ml. The cultured microplates were sealed with lid and incubated at 37°C for 48h. The MIC of samples was detected following addition

(40µl) of 0.2mg/ml p-iodonitrotetrazolium chloride in all the wells and incubated at 37°C for 30 min. Microbial growth were determined by observing the change of color p-iodonitrotetrazolium chloride (INT) in the microplate wells (pinkish-red formazan when there is growth and clear solution when there is no growth). MIC was defined as the lowest sample concentration showing no color change (clear) and exhibited complete inhibition of fungal growth. (1)

The minimum inhibitory concentration (MIC) of *A.niger* & *C.albicans* was determined by using tetrazolium microplate assay.

Results & Discussion:

Zone of inhibition (ZOI)

The result of anti-fungal activity of Arka Tail in presented in Table No.1. The zone of inhibition measured is 15-18mm on *Candida Albicans* and 11-12mm on *Aspergillus Niger*. This result is compared with control group. The results reveal that Arka Tail showed anti-fungal activity against *Candida Albicans* and *Aspergillus Niger*.

Minimum of Concentration (MIC)

The effect of *Arka Tail* was seen at 5th fold concentration i.e 5% on *C.albicans* and 3rd fold concentration i.e 20% on *A. Niger* as presented in Table No2.

Table No1: Zone of inhibition(ZOI) of Arka Tail

Organisms	AT (neat)	AT (diluted)	Control
Candida Albicans	18mm	15mm	10mm
Apergillus Niger	12mm	11mm	10mm

Table No2: Minimum of Concentration(MIC) of Arka Tail

Organisms	MIC
Candida Albicans	5th fold
Apergillus Niger	3rd fold

Conclusion:

Arka Tail has shown significant fungicidal activity on *Candida Albicans* & *Apergillus Niger* strains with minimum inhibitory concentration of 5% & 20% respectively. *Arka tail* can be used externally for candidiasis & aspergillus niger infection.

Image 1. Ingredients of *Arka Tail*

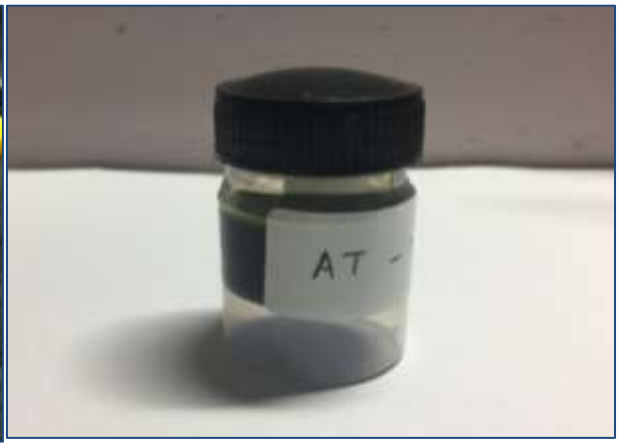

Image 2. Prepared *Arka Tail*

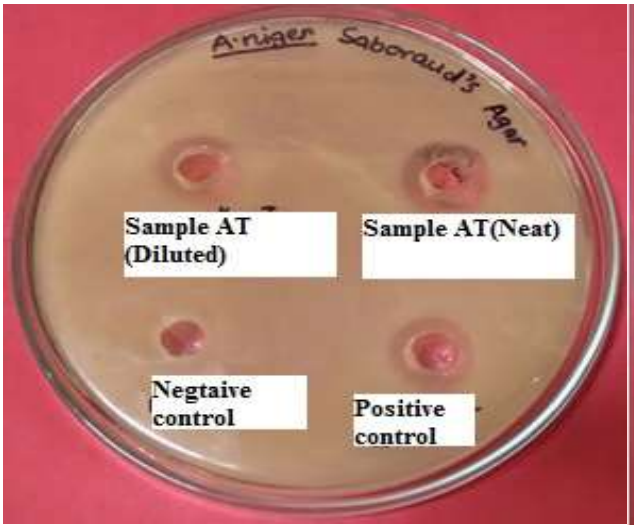

Image 3. Zone of inhibition of AT on *A.Niger*

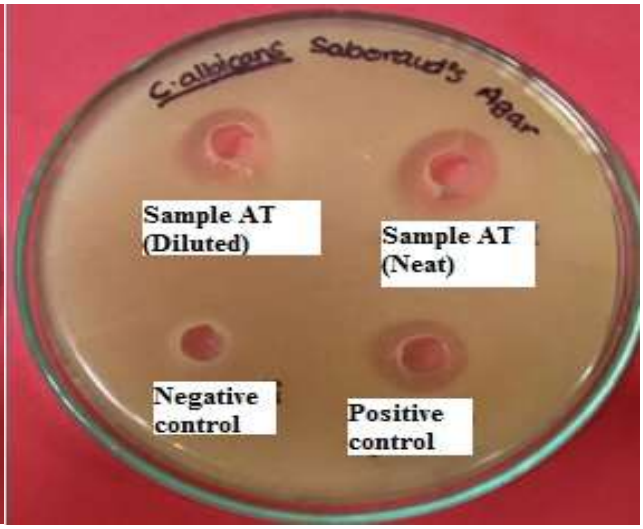

Image 4. Zone of inhibition of AT on *C. Albicans*

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