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ANTIFUNGAL ACTIVITY OF ETHANOLIC LEAF EXTRACT OF BRUHAT CHAKRAMARDA (*Cassia alata linn*) ,folklore plant AN INVITROSTUDY

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Abstract
In India, numerous invaluable plants are used in ethnomedical practices as well as in *Ayurveda* and *Sidha*. One such plant is *Cassia alata Linn*.In Ayurveda it is known as Bruhat chakramarda [1]*Cassia alata Linn* is a large handsome shrub,which is found throughout the plains of India.In the developing countries, synthetic drugs are not only expensive and inadequate for treatment of fungal infections but also often will be with adulterated and side effects. So there is a need to develop newer anti-fungal drugs which are safe, effective, easily available and economic to the patients. *Cassia alata Linn* is an important medicinal plant.This plant is used as a folkmedicine for the treatment of skin diseases like Scabies, Eczema, Pruritus, Ring worm infections and other fungal infectionsetc[2,3]This study was begun to evaluate the safety and efficacy of *Cassia alata Linn* in the management of fungal infections

Key words – Ayurveda ,Cassia alata linn, antifungal activity

INTRODUCTION

Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. Antimicrobials agents are one of our most important weapons in fighting any infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antimicrobials have become less and less effective against certain illnesses, not only because many of them produce toxic reactions, but also due to emergence of drug-resistance to that particular organisms. Plants are rich in wide variety of secondary metabolites such as tannins, Terpenoids, Alkaloids, Flavonoids, Glycosides, etc., which have been found in vitro to have antimicrobial properties[4]. Herbal medicines have been known to man

for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine[5]. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population[6, 7].

Cassia alata, belonging to family caesalpiniaceae is a pantropical ornamental shrub, distributed from tropical America to India[8],[9] . It is commonly known as Ringworm senna. The leaf extracts of the plant have been reported to possess medicinal properties and used again

st ringworm, scabies, ulcers, and other skin diseases such as pruritis, eczema[10],[11].

An attempt has been made to study the antimicrobial property of

the leaf extract of three species of Cassia (Cassia alata, Cassia occidentalis

and Cassia tora) against a few Gram positive and Gram negative bacteria

as well as against a few fungi which are mostly dermatophytes causing

skin infection in human beings. It seems that C. alata is the most potent species for having signi·cant antimicrobial activity

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Demands of traditional herbal medicines are increasing day by day not only in the developing countries but also in the developed countries throughout the world. The demand is due to the increased acceptance of Ayurveda and traditional herbal medicines, because of having their minimal side effects, and as such modern people relies more on drug resources of plantorigin[12]. An attempt has been made to study the antimicrobial property of the leaf extract of Cassia alata against a few fungi which are mostly dermatophytes causing skin infection in human beings. It seems that C.alata is the most potent species for having significant antimicrobial activity.

METHODOLOGY

Source of plant Material, collection and authentication

The drug was identified and authenticated by the botanist at RegionalResearchInstituteTrivandrum, Kerala. 6 Kgs of botanically identified *Cassiaalata* leaves were collected from Odayam,varkala,Trivandrum, Kerala.

Preparation of coarse powder

Cassia alata leaves were coarsely powdered. Approximately 750.0gms of powder was obtained.The coarse powder was stored in air tight container.

Preparation of the Ethanol Leaf Extracts

The finely ground crude drug is placed in a porous bag or 'Thimble' made of strong filter paper. Which is placed in chamber E of the Soxhlet apparatus. The extracting solvent A is heated and its vapors condense in condenser D. The condensed extractant drips into the thimble contains the crude drug and extract it by contact. When the level of liquid in chamber E rises to the top of siphon tube C the liquid contents of chamber E siphon in to flask A.This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated [42]

Source of the Microorganisms

The fungal culture *Candida albicans , pencillium. SP , Aspergillus fumigatus* was procured from National Centre for Industrial microorganisms (NCIM) Pune , India.

Procedure of agar dilution method

Materials :

Chemicals

Sabouraud dextrose agar (SDA),Sabouraud dextrose broth (SDB), Peptone water and antibiotics ketoconazole (fungi) were procured from Hi-media laboratories, Mumbai, India. DMSO was procured from E.Merck Ltd., Mumbai, India.

Preparation and Standardization of Stock cultures.

Cultures on receipt were sub cultured in SDA plates and further stored in slants as stock cultures.For the experiments, stock culture was prepared by inoculating each culture fromSlants to flask in sterile SDB and incubated at 280C for 48 h. The stock culture was adjusted to 0.5 McFarland standard turbidity and used for assay. Sterile SDA plates were prepared and 0.1 ml of the inoculum from standardized culture of testorganism was spread uniformly. Wells were prepared by using a sterile borer of diameter 10mm and 100µl (To get the final concentration of 1000 and 500 µg/well) of the test substance standard antifungal were added in each well separately.A standard antibiotic, ketoconazole was tested against fungi. The plates were placed at 40C for 1 h to allow the diffusion of test solutioninto the medium and plates were incubated at a temperature optimal for the test organism and for a period of time sufficient for the growth of at least 10 to15 generations (usually48 hours at 280C). The zone of inhibition of microbial growth around the well was measured in mm.

Procedure of minimum inhibitory concentrations

materials

Chemicals:

Sodium chloride, Demineralized water, Resazurin Dye, Ketoconazole, Sabouraud Dextrose Chloramphenicol Agar

Equipments:

Weighing Balance, Autoclave, (sterilization) Autoclave (decontamination), Bacteriological Incubator – II, Biological Safety Cabinet , Micropipettes 05-50ml 2-200 ml 2-20 ml, Refrigerator , Microtitre plate 96 well, Cyclomixer, Sonicator

PROCEDURE:

Outline of themethod

The sample CL was evaluated for antifungal activity by MIC against Candida albicans and Aspergillus niger at different concentration ranging from 10mg to 0.0048mg. The MIC values of test substances were compared with the activity of standard antifungal drug.

Preparation and Standardization of Stockcultures

In 10ml of sterile saline a loopful of C. albicans culture was dissolved and total numbers of cells were adjusted to 108 CFU/ml by measuring 0.25 OD at 620nm in digital colorimeter counting.

The suspension was diluted 100folds in order to get 106 CFU/ml. The spores of A.fumigatus were adjusted to 104conidia/ml by counting under microscope using haemocytometer.

Preparation of resazurin and standard antifungal solution

The stock resazurin solution was prepared by dissolving 2.7mg in 4ml of sterile saline.

Further, working solution was prepared by dissolving 1ml of stock solution in 5ml of sterile saline.The standard antifungal drug i.e., Ketoconazole solution at 0.1% concentration was prepared in sterile distilled water.

Preparation of test samples

Test sample CL was prepared at 10% concentration by dissolving 100mg of test sample in 1ml of RPMI broth. Sample was mixed using cyclomixer for 5minutes and sonicated for 2minutes.

Determination of MIC

Experiments were performed in triplicate under aseptic conditions. A volume of 100µl sterile RPMI broth was added to all 96 wells except first three wells of the Microtitre plate A1B1C1 to which only 200µl test product was added. In first three wells (A1B1C1) of plate, 200µl of the test product was added and double diluted till A12B12C12. To the wells containing test material, 10µl of *Candidaalbicans* and Aspergillus fumigatus suspension of approximately 106 CFU/ml was added. A growth control (Fungal suspension + 100µl broth medium) from G1 to G12 and broth control (only broth medium 100µl) from H1 to H12 was kept. A positive control that

consists of the 0.1% Ketoconazole (Standard antifungaldrug) was placed in separate wells. The plates were then incubated at 32.5 ± 2.5°C for 24 to 48 hours. After incubation, 100µl of working solution of resazurin was added to all wells. The plates were wrapped with aluminum film and incubated for 1hour. The color change was then assessed visually. Any colour change from purple to pink or colorless was recorded as positive (growth). The lowest concentration at which there is no colour change occurred was taken as the MIC value.

RESULTS AND DISCUSSION

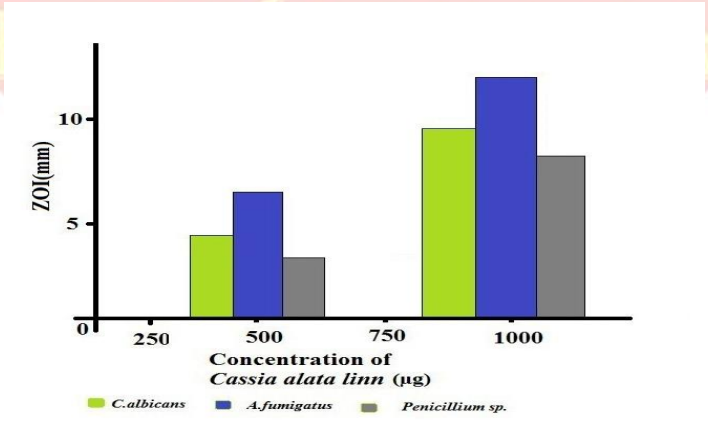
Antifungal screening of ethanolic leaf extract of c.*alata*

The result of this study showed a dose dependent antifungal activity of ethanolic leaf extract of cassia alata at 250 mg to 1000 mg on 3 selected clinical isolates of pathogenic fungi.

ZOI of CL tested against *Candida albicans, Penicillium sp and Aspergillus fumigatus*.

Table no : 1 shows values of agar dilution method

Sample Name	Sample Code	Conc. (µg)	Zone of inhibition (mm)		
			C.albicans	***A.fumigatus***	***Penicillium sp.***
CL	SB1185	1000	9.23±0.78	10.89±1.54	7.58±0.75
		500	4.12±1.23	6.35±1.47	2.96±0.54
		250	0	0	0
Ketoconazole	Standard	100	15.6	12.3±	17



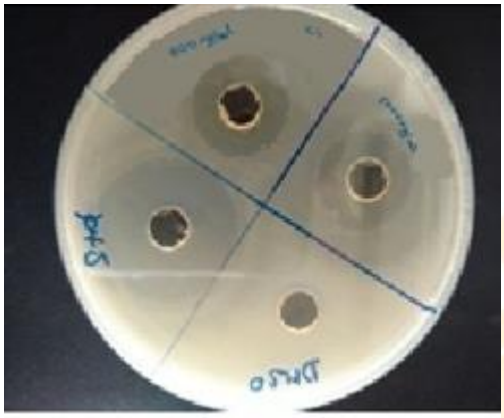


PLATE 1 –Aspergillus fumigatus

PLATE 2-Candidaalbicans

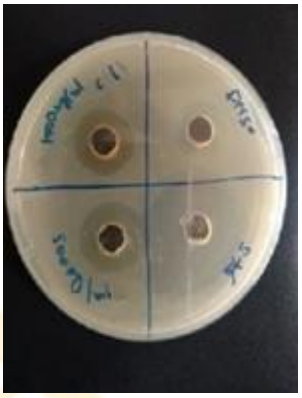


PLATE 3 –Penicillium sp.

Table no: 2 shows Minimum inhibitory concentration study report

The antifungal activity of CL tested against Candida albicans , Aspergillus fumigatus , pencillium sp. has indicated MIC value 1.25mg 1.25mg and 1.25mg respectively.

Sample Name	Sample Code	Conc. (%)	Antifungal activity MIC (mg)*		
			C.albicans	***A.fumigatus***	***Penicillium sp***
CL	RR180857	10	1.25	1.25	1.25
Ketoconazole	Standard	0.1	0.000048	0.000048	0.000048

MIC value is expressed as mean of triplicate, $n = 3$.

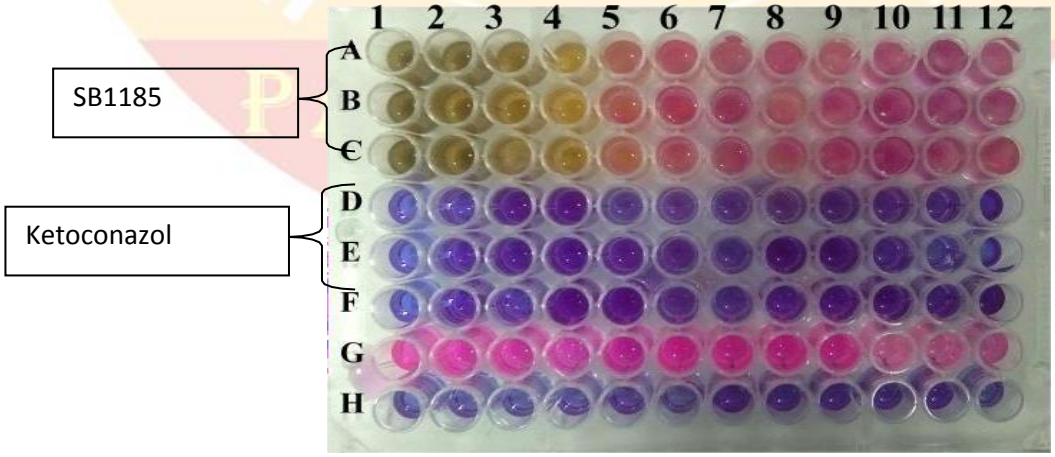


PLATE 4- ***Candidaalbicans***

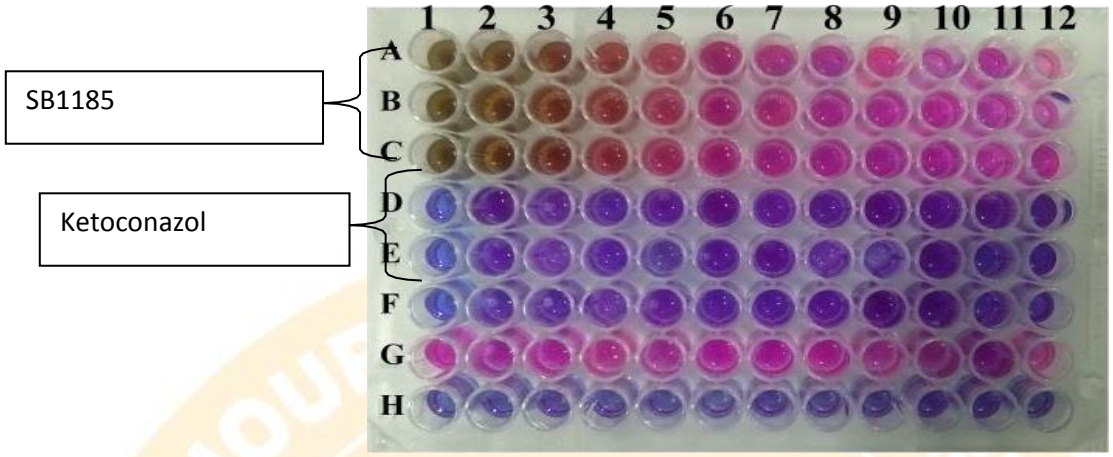


PLATE 5- ***Aspergillus fumigatus***

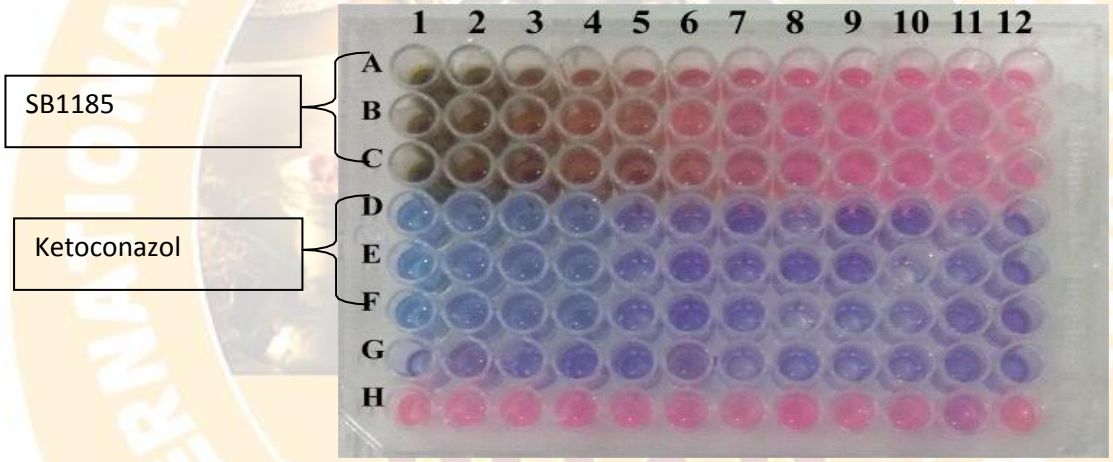


PLATE 6- *Penicillium spp*

Note: **G**:Growthcontrol **H**: Negative Control

AGAR DILUTION METHOD (ZOI)

The ethanol leaf extracts of *Cassia alata Linn* tested on *Aspergillus fumigatus, Candida albicans, Penicillium sp.* shows varied antifungal activity. Zone of inhibition test results of *Cassia alata Linn* against *Candida albicans, Penicillium sp and Aspergillus fumigatus* depicts on Table No.65 with images could be attributable to the presence of some bioactive components in the extract. The effect of ethanol leaf extract at 200 mg is statically significantly higher on *Aspergillus fumigatus* and *Candida albicans* than on *Penicillium sp.*

Generally, the ethanol leaf extract showed a higher growth inhibition than ketoconazole on *Aspergillus fumigatus*

when compared on other two organisms *Candida albicans* and *Penicillium sp.* This study showed that the ethanol extract had a dose dependent antifungal activity against *Candida albicans, Penicillium sp and Aspergillus fumigatus.* The presence of flavanoids, tannins, steroids, glycosides in the ethanolic leaf extract may be responsible for the antifungal activity.

SERIAL DILUTION:

The MIC test depicted on table no. 2 reports significant fungal activity on *Candida albicans, Penicillium sp and Aspergillus fumigatus.* The ethanol leaf extract possesses an equal range of mic value of 1.25 mg on all the tested organisms of *Candida albicans, Penicillium sp and Aspergillus fumigatus.*

CONCLUSION

Cassia alata has been found to exhibit a greater antifungal activity against some human pathogenic fungi in this study. The ethanol extract of *Cassia alata linn leaves doesnot show antifungal activity against three fungal organisms, such as Aspergillusfumigatus*, *Candidaalbicans*, *Pencillium*. S P at the dose of 250 microgram. But at the dose of 500 microgram and 1000 microgram it shows antifungal activity against the same organisms. Among these three it shows more efficacies on Aspergillus fumigatus. Therefore, further efficacy and safety studies are encouraged on this potential herb with the hope of replacing some less effective ones in clinical practice.

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