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ANTIFUNGAL ACTIVITIES OF HERBS AGAINST CANDIDA ALBICANS

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Abstract: *Lagenaria siceraria, Momordica charantia* and *Chenopodium ambrosioides* were investigated separately for their comparative in-vitro anti-fungal activity. The physio chemical studies and preliminary phyto chemical screening was done to assure purity of drugs. Anti-fungal activity of extracts against *Candida albicans* was evaluated as it causes 90% of skin infection. Disc diffusion assay, Micro dilution assay, Agar well (Lawn and Pour) method and mythelne reductase test was done against *Candida albicans*. The significant zone of inhibition (ZOI) and minimum inhibitory concentration (*MIC*) was found against the *Candida albicans*. The comparison of drugs as well as anti-fungal bioassay was done and disc diffusion showed the best result among applied method. Fluconazole was kept as standard. Ethanol and chloroform extracts of drugs at concentration 100 µ/ml and Fluconazole inhibited the growth of ATCC strains of *Candida albicans*. *Chenopodium ambrosioides* showed maximum anti-fungal activity as compared with *Momordica charantia* and *Lagenaria siceraria*. The extract would be used in future to formulate internal and external dosage form individually or in combination.

Keywords: *Candida albicans, Anti-fungal activity, Minimum inhibitory concentration*

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INTRODUCTION

Candida, yeast like fungus, is commonly part of normal flora of human skin, mouth, intestine and vagina etc. and most likely to cause disease in the human. The other *Candida species* includes *C. krusei, C. tropicalis, C. parapsilosis, C. guillecrmandii* . Although, *Candida* is a commonsal flora of human skin (Scalp) but when and why it turns to pathogen is not clearly known and causes dandruff.

Candidiasis is the opportunistic fungal infection. It can be of two types superficial and systemic. *Lagenaria siceraria, Momordica charantia* and *Chenopodium ambrosioides* were chosen due to their folk use regarding anti dandruff activity in Gujarat & Malwa region. In the light of anti dandruff activity. In the light of anti dandruff activity of plants the present study was done to know the best antifungal and to formulate topical dosage form for dandruff in future.

Lagenaria siceraria and *Momordica charantia* fruits and the *Chenopodium leaves* were used. The physico chemical and phyto chemicals screening of drugs were done to assure presence of certain effective chemical constituents.

MATERIAL AND METHODS

Plant material – Extraction preparation *Lagenaria siceraria, Momordica charantia* and *Chenopodium ambrosioides* were collected from local market Ujjain (MP) and washed with sterilized double distilled water, dried at room temperature, powdered, sieved and weighed accurately subjected to extraction in soxhlet apparatus at room temperature using petroleum ether, chloroform, ethanol and water.

Micro organism Used

Fungal strain – *Candida albicans*

The fungal strain of ATCC No. were used in this study which was obtained from R.D. Gardi Medical College, Ujjain (MP)

Preparation of inoculum

Suspension of organisms was prepared as per Macfarland's standard which contains $1x10^3$ cells per ml. The Fungi inoculum was prepared from 7 days old culture. Suspension of organism was made in the sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted such that it contains approx. 1×10^3 cells per ml.

Preparation of culture media

The dihydrated SDA was used.

Anti fungal activity

Evaluation of activity was carried out by agar wall method (Pour and lawn), disc diffusion method, macro broth dilution and methylene reductase test. Anti fungal activity was measured in terms of zone of inhibition and minimum inhibitory concentration.

I. **Agar well method** (**lawn method**) – Preliminary antifungal activity was studied by agar well method by slide modifications on the solidified agar. Wells of 6 mm diameter were punched with sterile borer. Stock culture well prepared by taking $1x10^5$ colonies of 24 hours old culture into 5 ml normal saline. After 24 hours incubation these were firmly swept over the agar plate using sterile cotton swab to make uniform culture lawns. By extracts (10,50,100,200,300, µ/ ml.) were poured in wells and incubated to 72 hours. Next day these plates were obtained for clear zone around the wells if any. Amikicin and Gentamicin were used as a standard.

% inhibition =AIC –AIT / AIC x 100

Where,

AIC - Area of inhibition of control

AIT – Area of inhibition of extracts.

II. **Agar well method** (**Pour method**) - The medium was prepared by dehydrated media but dissolving in distilled water and subjected to sterilization in autoclave at 121° for 15 minutes. The petri plates were washed thoroughly and sterilized in hot air over at 160° C for two hours. 30 ml of sterile molten agar media was seeded by organism (about 2 ml according to Mcf. Standard) in semi hot condition was poured aseptically in sterile petri plates and allowed to solidify at RT.

Bores of 6 mm diameter were made on medium using sterile borer and extracts were added to respective bores. Amikacin 30 mg and Gentamicin 10 mg were taken as standards. The petri plates seeded with organism containing extracts and the standard were kept in room temperature for one hour to facilitate the diffusion of extracts and the standards into the media. After diffusion the plates were incubated at 37°C (±1) for 72 hours incubation and zone of inhibition was observed.

II. **Disc diffusion method** – A comparison of extract was done with the standard in terms of zone of inhibition. 10,50,100,200,300 µ/ ml solutions of respective extracts were prepared by using DMSO. Paper disc of 6 mm diameter (Wattman Filter paper No. 1) were sterilized impregnated with extracts, were dried and placed on the surface of inoculated petri plates for SDA plates were prepared for fungal isolation and sterilized Plates were inoculated using corresponding broth culture of fungi and are pressed down gently to ensure even contact. Disc of Flucanazole 150 mg. was used as anti fungal standard. The plates were kept in refrigerator

for two hours to allow diffusion followed by incubation for 72 hours (37°C± 1°C) for fungi. Diameter of zone of inhibition was measured using scale and recorded in table. All above tests were carried out in aseptic environment and average values were recorded.

III. **Micro dilution technique** – Dilutions of 10,50,100,200,300 µ/ml of each extract was prepared by dissolving in DMSO and .5 ml of each solution was taken in the test tube. All the test tubes were shaken well to mix. 0.5 ml sterile broth was added to each followed by inoculums using 50 mcl. of overnight broth cultures of yeast type fungal. On test tube containing inoculated broth with solvent as positive control. One without inoculums as negative control was used. All test tubes well incubated and 25°C± 2°C for 72 hours. The test tubes were observed for appearance turbidity in comparison to both positive and negative control. The minimum concentration showing no turbidity (no growth of microorganism) was considered as MIC for confirmation, respective solutions of each test tubes were sub cultured on respective sterile agar plates and incubated as mentioned above. Plates showing no growth with respect to lowest concentration confirms the MIC values.

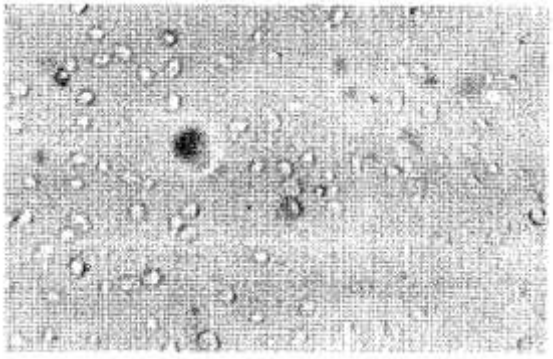
Assessment of anti fungal activity by macro dilution method

Fungal strain	Extracts	1 ml/50 mg	1 ml / 500 mg	1 ml/1 gm
Candida albicans	*Chenopodium ethanol*	130	98	50
	Chenopodium pet.ether	119	86	77
	Chenopodium Chloroform	121	82	45
	Chenopodium water	>150	>150	140
	L.siceraria ethanol	110	92	48
	L.siceraria pet. ether	115	105	62
	L.siceraria chloroform	95	84	55
	L.siceraria water	>150	>150	>150
	M.charantia ethanol	145	140	120
	M.charantia pet. Ether	130	72	54
	M.charantia Chloroform	98	91	86
	M.charantia water	145	120	125

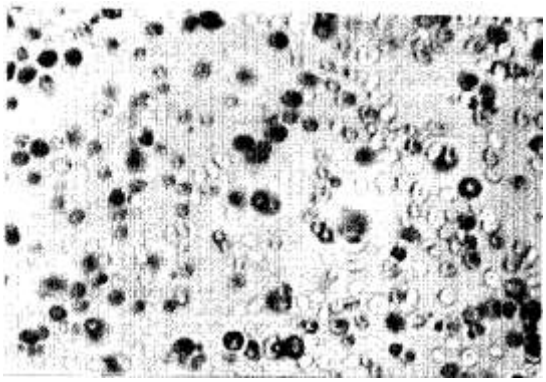
* D/W 1 ml – Candida suspension 1 drop (0.5 MAC), Control >150 colonies

IV. Methylene reductase test[25] – The test was done to establish the effect of *Candida albicans* at MIC level. The extracts were incorporated into distilled water. Those extracts showing same degree of antifungal activity by inhibiting the growth of inoculated *Candida albicans,* the extracts were tested by methylene reductase test as follows.

Sub MIC of these extracts were prepared in SDA in which *Candida albicans* strain was inoculated and incubated for 48 hours. After incubation the organism were scooped from the surface of medium stained with methylene blue were examined under microscope. As a dead *Candida cells* take up the stains and appear blue in color were as a life cells appear colorless due to presence of enzyme methylene blue reductase that reduced the dye. The number of stain and unstained cells were noted which would reflect the killing effect of extract at sub MIC level.



Live Cells of *Candida albicans* (untreated, control)



Dead Cells of *Candida albicans* stained by methylene blue after treatment with herbal extract.

Assessment of antifungal activity in terms of zone of inhibition

Fungal strains	Zone of inhibition (mm)											
	L.siceraria (100µ/ml)				*M.charantia* (100µ/ml)				***Chenopodium*** (100µ/ml)			
	PE	Ch	Et	Aq	PE	Ch	Et	Aq	PE	Ch	Et	**Aq**
Candida albicans	-	13	18	-	-	10	21	-	-	18	21	-
Flucanazole	←					37			→			

* (-) shows no inhibition, mean value of 6 independent experiments, flucanazole is used for positive control.

Assessment of anti fungal activity in terms of Minimum inhibitory concentration (MIC)

Fungal Strain	Conc. µ/ml	Diameter of zone in mm											
		L.siceraria				*M.charantia*				*Chenopodium*			
		PE	Ch	Et	Aq	PE	Ch	Et	Aq	PE	Ch	Et	Aq
Candida albicans	10	-	-	-	-	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	13	16	-	-	10	9	-	-	7	8	-
	200	-	17	18	-	-	14	11	-	-	15	16	-
	300	-	23	21	-	-	19	14	-	-	19	20	-
Flucan azole	←						37		→				

* (-) shows no inhibition, mean value of 6 independent experiments, flucanazole is used for positive control.

Comparison of the disc diffusion, pour method and lawn method

Fungal Strain	Conc. of extracts (100µ/ml)	Disc diffusion	Agar well method	
			Pour method	Lawn method
Candida albicans	*Chenopodium ethanol*	2	1.4	1.2
	Chenopodium pet.ether	-	-	-
	Chenopodium Chloroform	1.8	1.6	1.3
	Chenopodium water	-	-	-
	L.siceraria ethanol	2.1	1.8	1.6
	L.siceraria pet. ether	-	-	-
	L.siceraria chloroform	1.4	1.3	1.2
	L.siceraria water	-	-	-
	M.charantia	2	1.9	1.4

	ethanol			
	M.charantia pet. Ether	-	-	-
	M.charantia Chloroform	2	0.9	1.3
	M.charantia water	-	-	-
	Flucanazole	3.7	3.5	3.2

* (-) shows no inhibition, mean value of 6 independent experiments, flucanazole is used as positive control.

Disc diffusion is the most effective method; in the agar well procedure pour method is effective as compared with lawn method.

RESULT AND DISCUSSION

The physico chemical and preliminary phyto chemical screening and anti fungal activities of pet. ether, chloroform, ethanol and water extracts of *Lagenaria siceraria, Momordica charantia* and *Chenopodium ambrosioides* was done. The ethanol and chloroform extracts showed significant ZOI and MIC. Disc diffusion method gave the most satisfactory results and *Chenopodium* showed the maximum ZOI.

The result of study revealed that extracts of ethanol and chloroform exhibited the anti fungal activity against *Candida albicans* and can be used to formulate suitable formulation for dandruff in future.

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