Section A-Research paper
ISSN 2063-5346

Evaluation Of Antifungal Activity Of Novel Herbal Drug Emulsion On Different Fungi Species: An *In Vitro* Study

Gauri Jadhav[1] B.K.Sevatkar[2] Reetu Sharma[3] Gaurav Sharma[4]

[1-] MD Scholar, Department of Roga Nidana Evum Vikriti Vigyana, NIA Jaipur.

[2-] Professor, Department of Roga Nidana Evum Vikriti Vigyana, NIA Jaipur.

[3-] Associate Professor, Department of Roga Nidana Evum Vikriti Vigyana, NIA Jaipur.

[4-] Pharmacologist, Drug Discovery and Development Unit, NIA Jaipur.

Email id- jadhavgauri730@gmail.com

DOI:10.48047/ecb/2023.12.si4.1551

Abstract-

Background: *Candida albicans, Trichophyton rubrum, Microsporum canis, Epidermophyton floccosum* are the most important species responsible for infections with invasive fungal disease. Resistance of these fungi species to antifungal drugs has led scientists to pay more attention to traditional medicine herbs. Due to the limitations in the treatment of fungal diseases such as shortages, high prices and drug resistance or reduced susceptibility to fungal drugs we decided to study the antifungal effect of novel herbal emulsion. **Aim**: The purpose of this study was to evaluate the antifungal activity of novel herbal drug emulsion against most common fungal strains by implementing modern diagnostic technique. **Methodology**: Novel herbal drug emulsion made by two different methods named as sample A and sample B (decoction method) using different concentrations (50% 75% and 100 %) The antifungal activity was determined by using agar well diffusion method. Zone of inhibition of samples were compared with that of standard drug Ketoconazole 5 % w/v (Positive control) **Result**: Drug showed significant biological activity against all fungal strains. **Conclusion**: *In vitro* findings of this study confirmed antifungal activity of Novel herbal drug emulsion on common fungal pathogens.

Key words- *In vitro* study, antifungal activity, *dadru kushtha*, fungal infections.

Introduction-

A fungal infection viz. also known as Tinea is very common condition found in people due to unhygienic condition, fungal infection to the skin. This can be treated with anti-fungal drugs with Local anti-fungal ointment. In Ayurveda this condition is explained as *dadru Kushtha*. Acharya Charak mentioned *dadru* into *kshudra kushtha*[1] but Acharya Sushrut explained it into *maha kushtha* [2]*Kandu*(itching), *pidika* (eruption), *raag* (redness), and *utsana mandal* (elevated patches in round shape) are the symptoms of *dadru*. The samprapti of *Dadru* mainly involves vitiation of *Pitta-kapha doshas* and *dushti* of *rasa* and *raktavaha strotas*.[3]All the

symptoms of *dadru* are similar to Tinea corporis in modern medicine. So, we can correlate *dadru* as tinea corporis.[4]

Trichophyton rubrum is a dermatophytic fungus in the phylum Ascomycota. It is an exclusively clonal, anthropophilic saprotroph that colonizes the upper layers of dead skin, and is the most common cause of athlete's foot, fungal infection of nail, jock itch, and ringworm worldwide.[5] ***Microsporum canis*** has been identified as a causal agent of a ringworm infection, tinea capitis and tinea corporis in humans, children in particular.[6] ***Candida albicans*** is an opportunistic pathogenic yeast that is a common member of the human gut flora. It is one of the few species of the genus *Candida* that causes the human infection candidiasis, which results from an overgrowth of the fungus.[7]***Epidermophyton floccosum*** is a filamentous fungus that causes skin and nail infections in humans.[8] This anthropophilic dermatophyte can lead to diseases such as tinea pedis (athlete's foot), tinea cruris, tinea corporis and onychomycosis.[9] The infection typically stays within the nonliving conidified layer of host epidermis, since the fungus cannot pierce through living tissues of individuals with normal immunity. However, it has been found to cause invasive infections in immunocompromised patients, demonstrating severe onychomycosis, skin lesions, and subcutaneous nodules.[10]
In modern science, all medicines undergo through the drug discovery and development process like preclinical, clinical research, FDA reviews, etc. where Ayurvedic herbal formulations needs more attention in preclinical research like In- vitro study of drugs so that antibacterial, antifungal properties of the drugs will be substantiate.

Material And Method-

Pathogens (*Trichophyton rubrum, Microsporum canis,Epidermophyton floccosum,Candida albicans*) distilled water, beaker , glass rod , hot plate , SDA (sabouraud dextrose agar), MHA (Muller hinton agar),loop, conical flask, spirit lamp, hot air oven , incubator , mask gloves , vials for preserving extract , 10ul pipette, weighting machine

Test sample –

The Drugs was Prepared in Drug Discovery and Development Unit (DDDU), NIA

PART A (AQUEOUS PART)

DRUGS	BOTENICAL NAME	PART	QUANTITY
Ral	*Shore robusta*	1 part	4 gm
Khurasani Ajvain	*Hyoseyamus niger*	1 part	4 gm
Chakramarda beej	*Cassia tora*	2 part	8 gm
Tankan	Borax	1 part	4 gm
Gandhak	Sulfur	1 part	4 gm
Saindhav lavan	Rock salt	½ part	2 gm

Distilled water			49ml
Steric acid			5gm

PART B (LIPID PART)

Til tail	10 ml
Cetyl alcohol	5 gm
Triethanolomine	5 ml

Method 1- (Sample A)

Weight of all ingredients was taken according to table and divided in two parts separately (Aqueous and Lipid part) and placed on the hot plate at 60 °c until cetyl alcohol in Lipid part and steric acid in Aqueous part was melted. After that both parts were mixed, stirred until it concerted into semisolid state.

Method 2 – (Sample B)

Chakramarda beej churna and *khurasani ajwain* taken according to table. 14 parts water was added and boiled till 1/4th water was remaining and then solution was filtered. Then remaining drugs i.e *ral, tankan, gandhak* were added according to table. In 49 ml water, 5 gm steric acid was added and boiled until it melted. Then both aqueous and lipid part was mixed, stirred until it concerted into semisolid state.

Media: SDA (Sabourad dextrose agar) Growing media, MHA (Muller hinton agar) for drug sensitivity.

Dose: 50 %, 75%, 100% for each group

Selection of pathogenes - All fungi strain were purchased from MTCC (Microbial type culture collection & gene bank, chandigarh)

- *Trichophyton rubrum* (MTCC No-296)
- *Microsporum canis* (MTCC No- 2820)
- *Epidermophyton floccosum* (MTCC No-7880)
- *Candida albicans* (MTCC No-227)

Methodology: well diffusion

Observation: zone of inhibition

Group design –

- Negative control – ointment base

- Positive control- ketoconazole 5% w/v

Determination of zone of inhibition –

Each plate was inspected after incubation for antifungal activity. The diameter of the well as well as the diameter of the zones of absolute inhibition were measured and recorded to the nearest whole millimetre. average diameter of the zone of inhibition was measured in millimeters by the help of the standardised software and then mean was calculated.

Determination of the activity index-

The activity index was calculated from the mean of the three measurements by using formula as follows

Activity index (AI) =

$$\frac{\text{Zone of inhibition of the sample drug}}{\text{Zone of inhibition obtained for standard antifungal drug}}$$

Result & Observation-

Observation –
Table No. 1: Mean of ZOI (in mm) of two samples against *C.albicans, M.canis, E.flocossum, T. rubrum* with negative and positive control

Zone of Inhibition (mm)					
Sample	**Concentration (%)**	**Candida albicans (MTCC 227)**	**Microsporum Canis (MTCC 2820)**	**Epidermophyton floccosum (MTCC 7880)**	**Trichophyton rubrum (MTCC 296)**
Negative control	--	0	0	0	0
Sample A	50 %	13.2	19.9	20.9	24.1
	75 %	15.2	21.0	27.5	30.9
	100 %	17.7	22.8	27.4	28.3
Sample B	50 %	18.0	18.2	19.7	24.5
	75 %	19.2	19.9	20.6	24.5
	100 %	17.3	22.1	23.4	24.9
Positive Control	5 %	10.9	26.3	23.2	36.9

Table No. 2 : Activity index of two samples against trial pathogens in comparison to positive control

Activity Index					
Sample	**Concentration (%)**	**Candida albicans (MTCC 227)**	**Microsporum Canis (MTCC 2820)**	**Epidermophyton floccosum (MTCC 7880)**	**Trichophyton rubrum (MTCC 296)**
Negative	--	0	0	0	0

control					
Sample A	50 %	1.21	0.76	0.90	0.65
	75 %	1.39	0.80	1.19	0.84
	100 %	1.62	0.87	1.18	0.77
Sample B	50 %	1.65	0.69	0.85	0.66
	75 %	1.76	0.76	0.89	0.66
	100 %	1.59	0.84	1.01	0.67

Sample A and B both found effective against all pathogens in all concentration.

Sample B showed significant biological activity against *C. albicans* at 75% conc, Sample A was found to be biologically active against *M. canis, E. floccosum, T. rubrum* at 100% 75% and75% conc. Respectively.

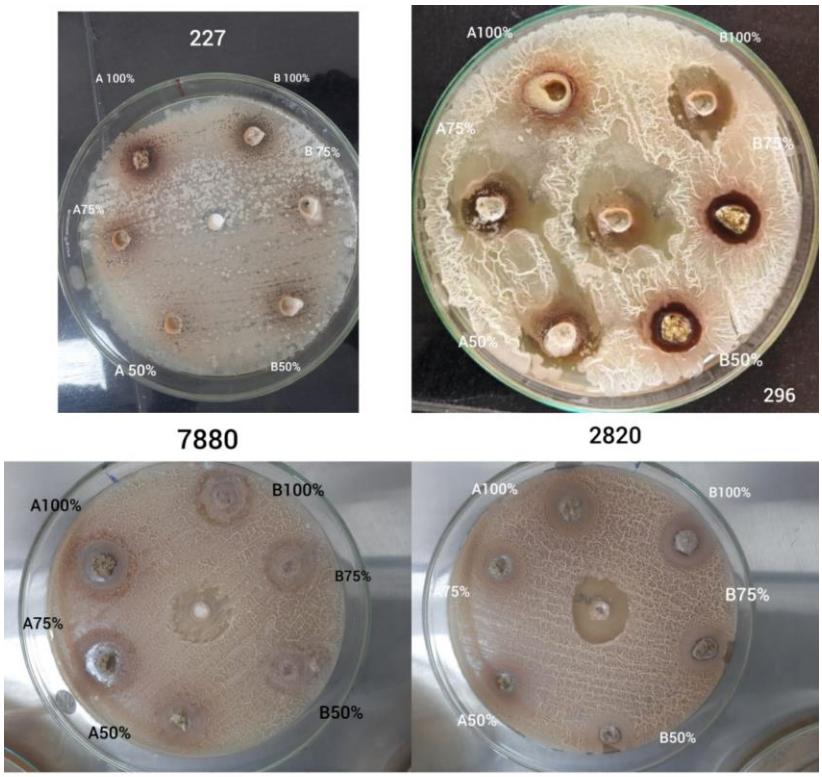

Fig1. Zone of inhibition of drug against all pathogenes

Discussion-

Probable mode of action of drugs

1. *Ral* is gum extract from sal tree. It consists Bergenin, shoreaphenol, chalcone, 4′-hydroxychalcone-4-O-β-D-glucopyranoside, 12αhydroxy-3-oxo-olenano-28. Bergenin, isolated from Bergenia ligulata is a potent antioxidant and antilithiatic agent.[11] Antifungal activity of shoreaphenol against Alternaria solani by MTT assay was proven[12].

In ayurveda, *Ral* has been mentioned having *Kandughna* (Itching), *Krimighna* (Helminthiasis), *Kushthaghna* (Leprosy / diseases of skin) effect.[13]

2. *Khurasani ajwain* consists of the seed of Hyoscyamus niger. It consists Tropane alkaloids hyoscyamine.[14] Antifungal activity of Tropane alkaloids hyoscyamine was studied.[15] In ayurveda, *khurasani ajwain* has been mentioned having *krimighna* effect. Here we can consider fungus as a *krimi. Madaka* and *nidrakara* are most important *gunas* explained in ayurveda[16] and *kandu* (Itching) is the main symptom of *dadru kushtha* which may be subside due to *madaka* and *nidrakara gunas* of *khurasani ajwain*.
3. *Chakramarda beej* consists of dried seed of Cassia tora consist of Anthraquinones. There have already been many reports about antifungal activities of the anthraquinones against *C.albicans* [17] In ayurveda, *chakramarda* is a principle drug of *dadru* so called *dadrughna* in Sanskrit. It has *Kushthaghna, Dadrughna,Krmighna, Kandughna* effect.[18]
4. *Tankan* is Borax mineral (Na2B4O7, 10 H2O), also known as Tincal.[19] In ayurveda, it has *kaphanissaraka* property due to which it is used as a *vrana ropak* (Wound healing)[20]
5. *Saindhav* is considered the purest salt among all types of salts. In ayurveda it has *vishada, kledhara guna*. It cures *vrana* (wound infection)[21]
6. *Gandhaka* is sulphur (S), a non-metallic solid element. It shows antifungal activity against fluconazole resistance *C.albicans*.[22] In ayurveda, Therapeutic Uses of *śuddha Gandhaka* is *Kandughna*(itching); *Kushthaghna* (diseases of the skin); *Dadrughna* (taeniasis)[23]

All the drugs included in novel herbal drug emulsion is used in skin diseases separately hence mixture of these drugs was found effective in fungal infection. In ayurveda In-vitro study has some limitation like inadequacy of standard drug disk with different concentration, standardization for measuring zone of inhibition of drugs.

Conclusion-

In vitro findings of this study confirmed antifungal activity of Novel herbal drug emulsion on common fungal pathogens. As compared with standard drug, the results revealed that both samples possess antifungal activity but sample A was found to have maximum activity against all pathogens taken in this study. Also, maximum antifungal activity was reported against *Candida albicans* by two samples. This is the success story by implementing modern diagnostic technique in ayurveda practice.

Acknowledgement-

The author would like to acknowledge Mr. Gaurav Sharma, Pharmacologist, DDDU, NIA, Jaipur, for his help in vitro part of this study.

References-

1. Sharma PV, Charaka Samhita of Agnivesa with English Translation,1st Edition-Reprint, Chaukhambha Orientalia,Varanasi, vol-2,2008:184
2. Jadavaji Trikamji, Choukambha Orientalia, Sushruta Samhita (Commentary of Dalhanacharya) and the Nyaya Chandrika, Varanasi. 5th edition; 2005:37

3. Shukla Vidyadhar and Ravi Dutt Tripathi Charak Samhita of Agnivesha revised by Charaka Redacted By Drudbala with vaidya manorama' hindi commentary,Chaukambha Sanskrit pratishthan, Delhi reprint-2011 chapter- 7 chikitsasthanshloka29-30 pp.185
4. Burns, Breathnach, Cox, Griffiths, Rook's textbook of dermatology, Blackwell publishers, 7th edition2004, vol- 2, chpte
5. Zaugg, C; Monod, M; Weber, J; Harshman, K; Pradervand, S; Thomas, J; Bueno, M; Giddey, K; Staib, P (2009). "Gene expression profiling in the human pathogenic dermatophyte Trichophyton rubrum during growth on proteins". *Eukaryotic Cell*. **8** (2): 241–50. doi:10.1128/EC.00208-08. PMC 2643602. PMID 19098130.
6. Kane, Julius; Summerbell, Richard; Sigler, Lynne; Krajden, Sigmund.; Land, Geoffrey (1997). *Laboratory handbook of dermatophytes: a clinical guide and laboratory handbook of dermatophytes and other filamentous fungi from skin, hair, and nails*. Belmont, CA: Star Pub. ISBN 978-0-89863-157-9.
7. Martins N, Ferreira IC, Barros L, Silva S, Henriques M (June 2014). "Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment". *Mycopathologia*. **177** (5–6): 223–240. doi:10.1007/s11046-014-9749-1. hdl:10198/10147. PMID 24789109. S2CID 795450
8. Guy., St-Germain (1996). *Identifying filamentous fungi : a clinical laboratory handbook*. Summerbell, Richard Charles, 1956-. Belmont, Calif.: Star Pub. ISBN 978-0898631777. OCLC 34711609
9. *Fungal infections*. Diamond, Richard D., 1942-. Philadelphia: Current Medicine. 2000. ISBN 978-1573401364. OCLC 42862084.
10. Seddon, M. E.; Thomas, M. G. (July 1997). "Invasive disease due to Epidermophyton floccosum in an immunocompromised patient with Behçet's syndrome". *Clinical Infectious Diseases*. **25** (1): 153–154. doi:10.1086/516887. ISSN 1058-4838. PMID 9243051
11. Ayurvedic Pharmacopoeia of India (API) part I, volume VI, Edition 1st page no. CLXXII
12. AID 380255 - Antifungal activity against Alternaria solani by MTT assay - PubChem. pubchem.ncbi.nlm.nih.gov. https://pubchem.ncbi.nlm.nih.gov/bioassay/380255 modified on 25th Oct 2018, Accessed on 16th May 2023
13. Ayurvedic Pharmacopoeia of India (API) part I, volume VI, Edition 1st page no. CLXXII
14. Ayurvedic Pharmacopoeia of India (API) part I, volume V, page no. 153
15. F F Abdel-Motaal Responses of fungi to tropane alkaloids produced by a medicinal plant Hyoscyamus muticus (Egyptian henbane) Publication Date: 2009-01-01 PubChem (nih.gov) site visit 16th May 2023 5.35 pm
16. Ayurvedic Pharmacopoeia of India (API) part I, volume V, page no. 153
17. AID 623564 - Antifungal activity against Candida albicans CGMCC 2.2086 after 24 hrs by turbidimetric method - PubChem (nih.gov) 17th May 2023 5 pm
18. Ayurvedic Pharmacopoeia of India (API) part I, volume III, page no. 155
19. Ayurvedic Pharmacopoeia of India (API) part I, volume VII, Edition 1st page no. 42
20. Ayurvedic Pharmacopoeia of India (API) part I, volume VII, Edition 1st page no.44

21. Acharya Charaka, Charaka Samhita, Ayurveda Deepika Vyakhya by Sri Chakrapanidatta, Krishnadas Academy, Varanasi, 1998, sutra sthan 27/ 300,p.561
22. AID 1179571 - Antifungal activity against fluconazole-resistant Candida albicans at 100 ug/well after 24 hrs by well diffusion assay - PubChem (nih.gov) 18th may 5.10pm
23. Ayurvedic Pharmacopoeia of India (API) part I, volume VII, Edition 1st page no.10