ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 07 | July - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

TO STUDY IN-VITRO ANTIFUNGAL SUSCEPTIBILITY TESTING OF INDIGENOUS ETHNOBOTANICAL EXTRACT ON CLINICAL ISOLATES OF DERMATOPHYTES AT J.L.N. MEDICAL COLLEGE AJMER

Clinical Microbiology

Dr Sarita Maholiya Department of Microbiology, J.L.N Medical college Ajmer, Rajasthan, India. 3rd Year Resident.

Dr Vijaylatha Rastogi* Department of Microbiology, J.L.N Medical college Ajmer, Rajasthan, India. H.O.D. and Senior professor *Corresponding Author

Dr Mukul Chaurasia Department of Microbiology, J.L.N Medical college Ajmer, Rajasthan, India. Senior demonstrator

Dr Abhishek Singh Department of Pathology, SMS Medical college Jaipur, Rajasthan, India 3rd Year Resident

Dr Antima Choudhary Department of Microbiology, J.L.N Medical college Ajmer, Rajasthan, India. 3rd Year Resident.

ABSTRACT

Dermatophyte were earlier reported to respond well to oral and topical antifungal agents; However an upsurge in resistance to these agents coupled with their high cost,may see the use of ethanobotanical extracts as cost effective treatment.Aim of this study is to find out the MIC range against isolated dermatophytes using extract of traditionally used ethnobotanical mixtures (Hawan samagri). **Methodology-** Identification of dermatophytes was done by direct microscopic examination using KOH mount, LPCB mount, Slide culture and biochemical tests.Essential oil of ethanobotanical [HS] extracts was extracted using steam distillation by Clevenger apparatus as standarized by V. Rastogi et.al,in 2014-2015 as part of ICMR project. Disk diffusion method and microbroth dilution (CLSI M38-A2) was performed. **Results-** out of 100 samples of clinically suspected cases of dermatophytes, 42 samples showed dermatophytic growth. Out of which 9 samples showed sensitivity to HS Oil by disk diffusion method,these samples were taken for microbroth dilution test and results obtained as-for Trichophyton mentagraphyte MIC value ranging from 2.06-8.25µg/ml.For Trichophyton schoenleinii, Trichophyton verucossum, Trichophyton tonsurans MIC value- 2.06 µg/ml.For Trichophyton rubrum MIC value-2.06-4.12 µg/ml. **Conclusion-** crude oil obtained from HS showed antifungal susceptibility to dermatophytes species, exhibited a zone of inhibition in the range of 31±4 mm with concentration 495mg/ml, and MIC range 2.06- 8.25µg/ml.

KEYWORDS

Dermatophyte , Trichophyton , Ethanobotanical extract, Microbroth dilution, MIC.

INTRODUCTION

Dermatophytes are hyaline septate moulds and these are divided into trichophyton infect skin, hair and nail, microsporon infect skin and hair, epidermophton infect skin and nail.

Dermatophytosis is a fungi infection that affects the skin, hair and nails. Dermatophytosis called as "Tinea or Ringworm" based on the infection site .Dermatophytosis of the arm,trunk and legs are referred to as Tinea corporis, on scalp are called as Tinea capitis and that of the foot Tinea pedis.[1]

Various antifungal agents like fluconazole, itraconazole, terbinafine, ketoconazole and voriconazole etc are clinically used against dermatophytes[2]. Howevevr, resistance to antifungal agents has developed nowdays, so there is need to explore alternative herbal drugs treatment.

Current problems in treatment of Dermatophytes are long duration of treatment, less patient compliance, Antifungal misuse by patient due to incomplete adherence to the course of treatment, Cost of treatment being unaffordable to many patients,treatment failure is becoming more and more common leading to emerging antifungal multidrug resistance amongst dermatophytes[3].

Seeing the above scenario there is need to identify alternative antifungals of herbal origin that can be explored for the treatment of dermatophytes as these have usually no side effects,are locally acceptable,are low cost hence more affordable and emergence of drug resistance is not known till date.

The purpose of this study is to perform invitro antifungal susceptibility by broth microdilution method [CLSI M38-A2] And find out the MIC range against isolated dermatophytes and using the extract of traditionally used ethnobotanical mixtures(Hawan samagri).

MATERIALAND METHODS-

A total of one hundred clinically diagnosed randomly selected cases of skin, hair and nail infection, of all age groups and of both sexes, attending Dermatology and Venereology outpatient department were included in the study.After taking detailed history and clinical examination as clinical diagnosis of dermatophytosis was made.The enrolled 100 cases comprised of following clinical types depending upon the site of involvement.Tinea cruris,Tinea corporis Tinea mannum, Tinea unguuim, Tinea pedis, Tinea capitis,Tinea faciei and Tinea barbae.

Study was divided into following parts that is collection and transportation of sample from clinical case, processing of sample by direct microscopic examination (KOH Mount), culture media used were Sabouraud's dextrose agar with 0.05% chloramphenicol, Sabouraud's dextrose agar with 0.05% chloramphenicol and 0.5% cycloheximide, and the two dermatophyte test medium, incubated at 25°C & 37°C for upto four weeks,Identification of growth is based on colony morphology, pigmentation, growth rate, microscopy (LPCB), slide culture, urease test, hair perforation test.

H.S. [hawan samagri] oil extraction- Essential oil of ethanobotanical [hawan samagri] extracts was extracted using steam distillation by Clevenger apparatus as standarized by V. Rastogi et.al,in 2014-2015 as part of ICMR project HS extract Disk diffusion method and microbroth dilution (CLSIM38-A2) was performed to determine sensitivity and antimicrobial testing of dermatophyte strains to H.S Extract oil.

RESULTS-

Age-total 100 cases were distributed between the ranges of 14 to 69 years. Mean age was 35.33 years. Most common age groups affected was 21 to 30 years with 31 cases (31%).

Gender- among 100 cases, the males were more commonly affected with 72 cases (72%) than females with 28 cases (28%). Male to female ratio was 2.57:1.

Culture positivity- Culture positivity was found to be 48%. Total number of dermatophyte isolates were 42 (87.50%) among culture positive isolates. In which *Trichophyton mentagrophyte* was the predominant isolate 12 (25%) followed by *T. schoenleinii* with 10 isolates (20.83%), *T. verucossum* 7 (14.58%), *T. rubrum* 7 (14.58%),

and *T. tonsurans* 6 (12.50%). Yield of Essential oil, as obtained with Clevenger apparatus on various days. Extraction of oil from Clevenger apparatus by hydrodistillation was maximum at 75°C after 4 hours. A total 2.2 ml essential oil extract was obtained from 1200 g of HS, when hydrodistillation at 75°C.

Disk Diffusion Methods-

This study shows out of 12 isolates of *Trichophyton mentagraphytes* 3 isolates (25%)showed sensitivity to HS oil, for *Trichophyton schoenleinii* out of 10 isolates 2 isolates (20%) were found to be sensitive to HS oil, For *Trichophyton rubrum* out of 7 isolates 2 isolates (28.57%) were sensitive to HS oil, For *Trichophyton verucossum* out of 7 isolates,1 isolates(14.28%) were sensitive to HS oil, For *Trichophyton tonsurans* out of 6 isolates,1 isolate(16.66%) shows sensitivity against H.S. oil.

This study indicated that the HS extract shows anti-fungal activity against isolates of dermatophytes with a mean zone of inhibition (31±4mm at a concentration of 495 mg/ml) essential oil extracted from hawan samagri.

Microbroth Dilution Method (CLSI M38A2)[4]-

Minimum inhibitory concentration by broth micro dilution (µg/ml) against H.S. oil.

TABLE: MIC value against H.S. oil.

	Minimum inhibitory concentration by broth micro dilution (µg/ml)						
ISOLATES	Drug	33	16.5	8.25	4.12	2.06	1.026
Trichophyton mentagraphytes(3)	H.S. oil			1		2	
Trichophyton schoenleinii(2)	H.S. oil					2	
Trichophyton verucossum(1)	H.S. oil					1	
Trichophyton tonsurans(1)	H.S. oil					1	
Trichophyton rubrum(2)	H.S. oil				1	1	

- Total 9 samples out of 42 dermatophytes showed sensitivity to H.S. Oil by disk diffusion method, these 9 samples were taken for microbroth dilution test (CLSI M38A2) against H.S. oil and results were obtained. *Trichophyton mentagraphyte* had MIC value ranging from 2.06-8.25µg/ml.
- *Trichophyton schoenleinii*, *Trichophyton verucossum, Trichophyton tonsurans* showed MIC values of 2.06 µg/ml.
- For *Trichophyton rubrum* MIC value were found to be ranging from 2.06-4.12 µg/ml.
- 96 well U bottom micro titre plate (After 4 days of incubation)

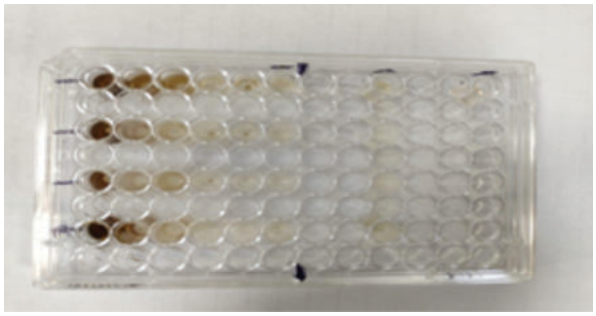
DISCUSSION-

In the present study, *T. mentagrophyte* 12 (25%) was the commonest aetiological agent in majority of clinical types followed by *T. schoenleinii* 10(20.83), followed by *T. rubrum* 7 (14.58%), *T. verrucosum* 7(14.58%) and *T.tonsurans* with 6(12.50%) cases, and the these are comparable to other studies done by Bhatia V, Sharma P et al, Himachal Pradesh.

The extraction of essential oils from Herbal fumigant (HS) by hydrodistillation under optimal operating conditions, A quantity of 100g of HS was added to 500 ml of distilled water in a 1000ml flask. Composition of the essential oil obtained from HS as ascertained by GCMS analysis was found to be: Camphor (23.9%) Camphor (23.9%), Thyme camphor (6.94%), Myristicin (2.39%), Longifolene (1.49%), Alpha Terpineol (1.80%), Alpha Copaene (1.08%), Beta- elemene (2.08%), Caryophyllene (5.87%). Muuroline (4.64%), Alpha-Himachaline (1.72%), Alpha- Curcumene (1.39%), Methyl eugenol (4.09%), Caryophyllene oxide (2.93%). Humulane (4.99%), Elemicine (3.11%), Alpha- Cadinal (1.39%), Turmerone (1.00%), Ar-Tumerone (18.78%), Asarone (0.59%), Verticol (0.63%),

In disc diffusion method this study indicated that the HS extract shows anti-fungal activity against isolates of dermatophytes with a mean zone of inhibition (31±4mm at a concentration of 495 mg/ml essential oil extracted from hawan samagree).

It was also deduced that the inhibition was concentration dependent and showed direct correlation between decreased concentration with respect to diminished susceptibility.By microbroth dilution method the MIC ranged from 2.06-8.25 µg/ml which was similar to other studies by M.S Ali-shtayeh and Suheil I Abu Ghdeib 1999, that used essential oil of Capparis spinosa and fuglans regia plants showing MIC range 0.6 to 40µg/ml by brothdilution method.



CONCLUSION-

From this study, I conclude that crude oil obtained from herbal fumigant (HS) showed antifungal susceptibility to dermatophytes species and exhibited a zone of inhibition in the range of 31±4 mm with concentration 495mg/ml.

It was also revealed that dermatophyte isolates were inhibited at MIC of 2.06- 8.25µg/ml.

I conclude that crude essential oils obtained from the formula of herbal fumigant made of natural medicinal plants and herbs have a promising role in impeding the growth of pathogenic fungi in vitro. This finding can be extrapolated to explore alternatives for treatment of fungal infections in the near future as resistance to conventional drugs is on an exponential rise.

REFERENCES:

[1] Hayette,M.P.andSacheli,R.(2015)Dermatophytosis, Trends in Epidemiology and Diagnostic Approach. Current Fungal Infection Reports, 9, 164-179.

[2] Aditya, K.G., Jennifer, E.R., Melody, C. and Elizabeth, A.C.(2005) Dermatophytosis: The Management of Fungal Infections. SKINmed: Dermatology for the Clinician, 4, 305-310

[3] Dogra, S. and Uprety, S. (2016) The Menace of Chronic and Recurrent Dermatophytosis in India: Is the Problem Deeper than We Perceive? Indian Dermatology Online Journal, 7, 73.

[4] Clinical laboratory standard institute .CLSI Document M38-A2:Refrence method for Broth Dilution Antifungal Susceptibility of testing of dermatophytes, Approved Standard, 3rd ed.; CLSI.

[5] Textbook of medical mycology-Jagdish chandra -4th edition1.

[6] Rabell G,Taplin D. Dermatophytes: Their Recognition and Identification :Florida:University of Miami Press:1974.2.

[7] Ajello, L., and L.K. Georg. 1957. In vitro hair cultures for differentiating between aypical isolates of Trichophyton mentagrophytes and Trichophyton rubrum. Mycopathol. Mycol. Appl. 8:3-17.

[8] Espinel-Ingroff A, Barchiesi F, Hazen K C, Martinez-Suarez J V, Scalise G.Stndardization of antifungal susceptibility testing and clinical relevance. Med Mycol.1998;36:68-78.

[9] Rastogi V et al. Anti tubercular minimum inhibitory concentration(MIC) and chemical characterization of ethnobotanical mixture used in the treatment of tuberculosis. Indian Journal of Microbiology Research.2019 Apr.

[10] Ana Espinel-Ingroff and Emilia Cantón, Antifungal Susceptibility Testing of Filamentous Fungi.

[11] Upadhyay RK. Essential oils: anti-microbial, antihelminthic, antiviral, anticancer and antiinsect properties. J Appl Biosci. 2010 Jun.