P: ISSN NO.: 2394-0344 RNI No.UPBIL/2016/67980 VOL-I* ISSUE- VIII* November- 2016
E: ISSN NO.: 2455-0817 *Remarking An Analisation*

A Study of Hair Perforation Ability of *Microsporum qypseum* and Effect of Some Plant Extracts on It's Growth

Sakshi Walker
Assistant Professor,
Deptt.of Botany,
St.John's College,
Agra

Abstract

Microsporum gypseum is a dermatophyte causing potential infections of the skin.The hair perforation ability of this dermatophyte was studied using four of it's isolates.Hair from various animals were used. Certain plant extracts were assessed to ascertain their antifungal activity.This assessment was carried out as herbal antifungal alternatives are desireable.

Keywords: Keratin, Perforating Organs

Introduction

Keratin is a resistant scleroprotein,the structure of which is stabilised by disulphide and hydrogen bonds,salt linkages and other crosslinks.

Keratin digestion is the source of deriving nutrition for dermatophytes during the course of superficial skin infections.The phenomenon of digestion of this very resistant substrate forms the central problem of physiology of keratinized molds.A number of workers have reported the ability of these organisms to digest keratin and have put forth different views (Page 1950;Vanbreuseghem 1952b;Raubitschek 1961 ;Octenasek and Dvorak 1964;Bohme and Ziegler 1967;English 1969) .According to Yu *et.al*(1969) cell bound keratinolytic enzymes have been found to be released at the time of keratin digestion.Takinchi and Higuchi(1977),Takinchi *et.al*.,(1984)have isolated the hair degrading enzymes from *Microsporum gypseum,Microsporum canis*.

The study of hair segments attacked by keratinophilic fungi has been carried out by several workers(Davidson and Gregory 1934,Daniels 1953,Barlow and Chattaway 1955,Ajello and Georg 1957,Page 1950,Lu 1962,Friedrich 1964;Padhye *et.al.,*1980,Baxter and Mann1969).However few studies have been carried out on the perforating organs of *Microsporum gypseum*.These were termed as "intrusions" by Page(1950).

There fore an attempt was made to test the ability of Microsporum gypseum to perforate and degrade different types of hair.

Earlier workers have proved that the allopathic drugs are still found effective against dermatomycoses.But these drugs could not be accepted as a routine treatment for every case,as they are expensive and require long treatments.It is almost inaffordable by middle and lower class people.Methanolic acid and flavonoid extracts proved to be good antidermatophytic plant extracts(Seema Bhadouria,Padma Kumar 2012).Plant extracts of garlic,ginger,onion, ocimum and neem have been variously used in the past few years as anti-microbial agents.These are quite effective,soothing and without any side effects.The sensitivity of *Microsporum gypseum* to various doses of plant extracts obtained from these medicinal plants was therefore determined.

Materials and Methods

The test procedure followed was that of Ajello and Georg(1957)and involved the use of different types of hair.The test fungus was *Microsporum gypseum* as this has shown highest perforating ability during earlier studies.This fungus was isolated from soil by hair baiting technique.Sterilised hair segments were placed in petridishes to which 25 ml sterile distilled water and 2-3 drops of yeast extract was added.The hair segments were then inoculated with test fungus.Inoculated dishes were incubated at 28±2ºC in the dark.All experiments were carried out in triplicate with hair and fungal controls.The observations were made after each 48hrs of incubation upto 8 days.The hair segments were considered to be completely lysed when they could not be picked up with forcep.

For the study of antifungal activity of plant extracts, garlic *Allium sativum* Linn.. Onion *Allium cepa* Linn. and ginger *Zingiber officinale* Roso were dehulled, chopped into small pieces, the leaves of neem *Azadiracta indica* A.Juss., ocimum *Ocimum sanctum* Linn. and yellow oleander *Thevetia nerofolia* Juss. were cut into small pieces. Hundred grams of samples were blended in a Waring blender with 100ml of water. Extract was obtained after vaccum filtration and added to Sabouraud's dextrose agar to prepare different concentrations. In this way four sets of dishes were prepared as follows

1. Extract 20ml per 100 ml medium.
2. Extract 80ml per 100 ml medium
3. Extract mixture (equal quantity of all the 6 types of extracts) 80 ml per 100 ml medium.
4. Basal medium 100 ml as control.

The medium with extract was sterilized at 15 lbs pressure for 10 minutes and then plated in petridishes. After sufficient cooling the dishes were inoculated in the centre by an agar disc obtained from 4 days old fungal colony. Dishes were incubated in dark at 28 ± 1ºC for 8 days. Results were obtained by measuring the colony diameter. All the experiments were performed in triplicate.

Results and Discussion

Dog and goat hair were colonised in 1-3 days and 100 percent perforation was observed in isolate 1,2,3 and 4.Colonization on cow and human white hair was observed in 3 days in isolate 1 and 3 and 2 days in isolate 2 and 4.The perforation was 100 percent in all the four isolates.Human black hair took 5 days in isolate 1 and 3 and 6 days for isolate 2 and 4 for colonisation and 100 percent perforation is noticed in all four isolates,while buffalo and horse hair took 9 days for colonisation in isolates 2 and 4 took 8 and 7 days for buffalo and horse respectively.The perforation could not be observed even for 20 days in all the four isolates.

Table 1
Hair Perforation Ability of *Microsporum gypseum*

Hair	***Microsporum gypseum* Isolate 1**					***Microsporum gypseum* Isolate 2**				
	Time taken for Colonization In days		Hair perforation (%) Days			Time taken for Colonization In days		Hair perforation (%) Days		
	2	4	6	8		2	4	6	8	
Buffalo	9	0	0	0	0	8	0	0	0	0
Cow	3	5	100	100	100	2	3	100	100	100
Dog	1	100	100	100	100	2	100	100	100	100
Goat	2	70	100	100	100	3	71	100	100	100
Horse	9	0	0	0	0	7	0	0	0	0
Human(black)	5	0	70	100	100	6	0	72	100	100
Human (white)	3	0	90	100	100	2	0	92	100	100

Hair	***Microsporum gypseum* Isolate 3**					***Microsporum gypseum* Isolate 4**				
	Time taken for Colonization In days		Hair perforation (%) Days			Time taken for Colonization In days		Hair perforation (%) Days		
	2	4	6	8		2	4	6	8	
Buffalo	9	0	0	0	0	8	0	0	0	0
Cow	3	4	100	100	100	2	5	100	100	100
Dog	1	100	100	100	100	2	100	100	100	100
Goat	2	75	100	100	100	3	70	100	100	100
Horse	9	0	0	0	0	7	0	0	0	0
Human (black)	5	0	71	100	100	6	0	77	100	100
Human (white)	3	0	93	100	100	2	0	93	100	100

Broad perforating organs were observed in cow and goat hair.In dog hair undulation of cuticle, projections from medulla and narrow perforating organs were observed.Cuticle lifting ,undulation of cuticle,narrow perforating organs along with decolouration were also observed in human black hair.Some micromor phological changes were observed for human white hair except that there was no decolouration.Complete digestion of cow,dog and human white hair was observed.Grading of perforation was also carried out depending upon the perforating ability of *Microsporum gypseum* on the respective hair.

Table 2
Grading of Perforation

Hair	A	B	C	D	E
Buffalo					√
Cow				√	
Goat				√	
Dog				√	
Horse					√
Human(black)				√	
Human(white)				√	

1. A 0-25%perforation
2. B-25.1-50%perforation
3. C-50.1-75%perforation

4. D-75.1-100%perforation
5. E-nil perforation.

All the extracts showed inhibition of the fungus. Garlic, ginger and neem extracts clearly prevented the growth of the fungus at higher as well as lower concentrations. Tansey and Appleton (1975) also reported the toxic effect of garlic extracts against some dermatophytic molds in *Microsporum gypseum*.

At higher concentrations ocimum and onion allowed growth of the isolates of *Microsporum gypseum*. The isolates were found quite resistant at a lower dose of onion extract. A slight accelerating activity was recorded when extract was provided at a higher dose of 80 ml/100 ml medium. Yellow oleander did not show any remarkable inhibition in any of the isolates. The isolates did not grow well in the mixture of extracts.

Table 3
Bioassay of Plant Extracts for Antifungal Activity

Extracts	Set of Dishes	*Microsporum gypseum* Isolate 1	*Microsporum gypseum* Isolate 2	*Microsporum gypseum* Isolate 3	*Microsporum gypseum* Isolate 4
Garlic	I	38	35	30	31
	II	24	26	20	22
Ginger	I	42	40	45	43
	II	26	21	27	29
Neem	I	44	45	49	46
	II	40	41	39	42
Ocimum	I	40	44	42	46
	II	70	73	75	72
Onion	I	46	45	42	41
	II	60	65	62	61
Yellow oleander	I	54	51	55	56
	II	50	52	53	54
Mixture	III	42	41	45	43
Control	IV	50	50	50	50

Mycelial Growth in Terms of Colony Diameter in Mm is an Average of three Independent Determinations.

The source of keratin degraded by dermatophytes in test in vitro is most often human and guniea pig hair.Bird feathers have been used only by kushwaha(1984b) and krystyna *et.al*(1987).Wool has been used by Safranek and Goos(1982) as keratinous bait in their study.The types of hair used in the study have been grouped under two heads perforating group(dog,cow,goat and human hair)and non-perforating group(buffalo and horse hair).In buffalo and horse hair nil perforation(no perforating organs) was found.While in other types of hair perforating organs were found.

Due to their high cystine content the above two types of hair are resistant to keratinolytic activity.

The ultimate disposal of different types of hair is of great economical and ecological value,as these are discharged in large numbers from tanneries and pose a potential threat to the environment.

However a number of workers have worked out the effect of various factors on hair perforation by *Microsporum gypseum*.Excellent hair perforation was observed at pH 7.

Biosynthesis of silver nanoparticles as antifungal drugs has been investigated recently (L.Pereira *et.al*.,2014).Aqueous plant extracts of *Fragaria virginiana* and *Epilobium angustifolium* and *Pontentilla simplex* demonstrated strong antifungal potential(Duncan *et.al.,*2008).

Antifungal effect of garlic extract has been claimed to be due to the presence of diallyl sulphide, unstable sulfur in alkylpolysulphides, (Kitagawa and Amano, 1936) unsaturated aldehydes or due to phytoncides.The growth of both *Aspergillus niger* and *Candida albicans* was inhibited by ajoene,derived from garlic(S.Yoshid *et.al.,*1987) The growth inhibitory action of garlic extract may be due to its direct effect and suppression of enzymatic activity Sehgal (1961).

The slight stimulation in the growth of the isolates may be due to the separate batches of onion extracts, processed in the same manner, as it is also put forth by Tansey and Appleton (1975) in the case of garlic. The antimicrobial factor of onion is known as cycloalliin, C_6 H_{11} O_3 NS.HCL. Onion contains from 1.4-3.2 grams of cycloalliin hydrochloride per kg from all parts of onion. Isolated sulphoxides from chilled onions and these sulphoxides readily convert in the corresponding thiosulphates under the influence of enzymes liberated on crushing the onion, and these are thought to be responsible for their strong antimicrobial action.

Thus as clear from the study *Microsporum gypseum* is readily able to perforate human hair and thus cause infections of the skin.Plant extracts are

therefore,as assessed,can prove to be an important ingredient of ointments and drugs.These are inexpensive alternatives and effective too.Therefore they prove to be a relief for the people affected in the lower strata of the society.

Aim of the study

Microsporum gypseum is a soil inhabiting dermatophyte causing infections of the skin in people living in unhygienic conditions.A study of the hair perforation ability of this dermatophyte gave a clear idea of the degree of invasion on different organisms.This helps to ascertain the extent of risk.

Study of plant extracts was carried out as they serve as desireable alternatives in terms of being cost effective and providing relief in a shorter span of time.

References

1. Ajello, L.; 1959 A new *Microsporum* and its occurrence in soil and on animals. Mycologia, 51:69-76
2. Barlow, A.J.E. and Chattaway, F.W.; 1955 The attack of chemically modified keratin by certain dermatophytes. J. Invest. Dermatol. 24:65-75.
3. Baxter, M. and Mann, P.R.; 1969 Electron microscopic studies of the invasion of human hair in vitro by three keratinophilic fungi. Sabouraudia 7:33-37.
4. Bohme, H. and Ziegler, H.; 1967 Keratin abban durch Pilze Arch. Microbiol. 67:95-110.
5. Daniels, G.; 1953 The digestion of human hair keratin by *Microsporum canis.* J. Gen. Microbiol., 8:289-294.
6. Davidson, A.M. and Gregory, P.H.; 1934 In situ cultures of dermatophytes. Can. J. Res. 10:373-393.
7. Duncan Webster et.al.;2008 Journal of Ethnopharmacology Volume 115,Issue 1,140-146.
8. English. M.P.; 1969 Destruction of hair by *Chrysosporim keratinophilum*. Trans. Br. Mycol. Soc. 52 : 247-255.
9. Friedrich, E.; 1964 Perforation Von Frauenhaar durch Dermatophyten. Mykosen, 7:53-63.
10. Kitagawa, M. and Amano, C.A.; 1936. Bul. Sci. Fakult. Terk. Tjusu Imp. Univ., 30:3019
11. Kushwaha, R.K.S.; 1984b morphological studies of some dermatophytes and keratinophilic fungi. Bull. Bot. Soc., 30&31:48-51
12. Krystyna, W.; Obarezewski, J. and Walski, T.; 1987 Intercellular keratinase of *Trichophyton gallinae*. J. Med. Vet. Mycology. 25:261-268.
13. L.Pereira et.al.; 2014 Journal of Applied Microbiology,Volume 117,Issue 6,1601-1613
14. Lu, Y.C.; 1962 A new method for the study of hair digestion by dermatophytes. Mycopathol. Mycol. Appl., 17:225-235.
15. Otcenasek, M. and Dvorak, J.; 1964 the isolation of *Chrysosporium keratinophilum* (frey) Carmichael and similar fungi from Czechoslovakian soil. Mycopath. Mycol. Appl. 23:121-124.
16. Padhye, A.A.; Young, C.N. and Ajello, L.; 1980 Hair perforation as a diagnostic criterion in the identification of *Epidermophyton, Microsporum* and *Trichophyton* species. In superficial, cutaneous and subcutaneous infection. Pan American Health Organization, Sci. Publ. No. 396, Washington, D.C. pp. 115-120.
17. Page, R.M.; 1950 Observation on keratin digestion by *Microsporum gypseum.* Mycologia. 42:591-602.
18. Raubitschek, F.; 1961 Mechanical Versus chemical keratinolysis by dermatophytes. Sabouraudia, 1:87.
19. Safranek, W.W. and Goos, R.D.; 1982 Degradation of wool by saprophytic fungi. Can. J. Microbiol. 28:138-140.
20. S.Yoshid et.al.; 1987 Journal of Ethnopharmacology Volume 53,Issue 3, 615-617.
21. Seema Bhadouria, Padma Kumar.; 2012 Mycoses,Volume 55,Issue 4,July 2012 339-343.
22. Sehgal, J.M.; 1961. Antimicrobial substances from flowering plants. Hindustan Antibiot. Bull., 4:3-29.
23. Takiuchi, J. and Higuchi, D.; 1977 Isolation purification and biochemical properties of keratinase elaborated from *Microsporum gypseum* Jap. J. derm. 87:303-309.
24. Takiuchi, I,; Higuchi, D.; Sei, Y. and Koga, M.; 1984 Partial characterization of the extracellular proteinase (keratinase) from *Microsporum canis*. Sabouraudia 22:219-224.
25. Tansey, M.R. and Appleton, J.A.; 1975. Inhibition of fungal growth by garlic extracts. Mycologia, 67:409-413.
26. Vanbreuseghem, R.; 1952b keratin digestion by dermatophytes : a specific diagnostic method. Mycologia, 44 : 176-182.
27. Yu, R. J.; Harmon, S.R. and Blank, F.; 1969 Hair digestion by keratinase of *Trichophyton mentagrophytes* J. Invest. Dermatol. 53:166-170.