European Journal of Molecular & Clinical Medicine, *2022,*
Volume 9, Issue 7, Pages 6648-6663

Article type: Original article

Efficacy of herbal extracts on drug-resistant dermatophytes isolated from clinical samples

Running title: Herbal extracts on drug-resistant dermatophytes

Authors

Uma Arasu T[1], Arjunan S[2], Thirunavukkarasu D[3]

1. **Uma Arasu T**

Assistant Professor, Department of Microbiology, Swamy Vivekanandha Medical College Hospital & Research Institute, Elayampalayam, Tamil Nadu, India.

2. **Arjunan S**

Professor, Department of Microbiology, Mahendra Arts & Science College, Kalipatti, Tamil Nadu, India, India.

3. **Thirunavukkarasu D**

Assistant Professor, Department of Microbiology, Government Mohan kumaramangalam Medical College, Salem, TamilNadu, India.

Corresponding Author:

Thirunavukkarasu D

Assistant Professor, Department of Microbiology, Government Mohan kumaramangalam Medical College, Salem, TamilNadu, India.

E-mail: rajammicrocare@gmail.com

ABSTRACT

Introduction*:* Superficial mycoses are common skin diseases affecting millions of people worldwide.

Objectives*:* This study aimed at examining the drug-resistant dermatophytes from clinical samples and the efficacy of herbal extracts on them.

Materials and methods*:* 150 clinical samples (skin, hair, and nail) were collected from suspected cases in Government Hospital, Erode, Tamil Nadu, India for a period of one year. Overall 112 (74.67%) positive cultures were identified. The dermatophytes isolates include *Trichophyton* species (34.82%), *Epidermophyton* species (13.39%), *Microsporum* species (37.5%) and *Aspergillus* species(14.28%).Age and sex-wise perspectives of dermatophytes infections indicated that females (64%) and adults between 15-30 years were highly infected by superficial mycoses.

Results*:* The overall percentage of multidrug resistance was higher than 98%. The efficiency of herbal extracts revealed that *Aloevera* gel, leaf extracts of *Annona reticulata* and *Acalyphaindica* were found to be an effective while, *Achyranthusaspera, Azadiractaindica and pine* were ineffective.

Conclusion*:* Medicinal plants are good sources of new pharmaceuticals. From these findings, the use of herbal medicines will reduce the side effects of synthetic antifungal drugs and the development of drug resistance.

Keywords: Antifungal agents, Drug resistance, Herbal extracts, Superficial mycoses

INTRODUCTION

Superficial mycoses are common skin diseases affecting millions of people worldwide. These infections are caused by dermatophytes fungi and it is estimated as of 10-20% are acquiring lifetime risk. [1] Dermatophytic fungi are the predominant pathogens of

superficial mycoses which include the members of genera *Trichophyton*, *Epidermophyton*, and *Microsporum* that tend to grow in moist parts of the body where skin comes together such as fingers, toes, under the breast and in the genital area causing superficial mycoses. [2] According to World Health Organization (WHO), dermatophytes affect 25% of the world's population. The epidemiology of these superficial infections varies significantly from country to country and region to region. In developing countries like India, the incidence of this infection ranges from 3-36%. [3] In Tamilnadu1-4%, the superficial infections are only documented. [4]

Several therapeutic agents such as griseofulvin, terbinafine, nystatin and zole compounds namely ketoconazole, itraconazole, fluconazole, clotrimazole, miconazoleare commonly used to treat superficial mycoses. These agents cause various side effects and need long-term treatment. [5] The worldwide emergence of drug resistance in superficial mycoses to multiple clinically relevant agents is a serious health problem. Moreover, the trends in antibiograms have been changing from time to time. This givesan alarm signal for the development of new measures to control drug resistance. Potential alternative therapies include natural systems that are important in health care and new pharmaceuticals. Active compoundsof plant origin has been found to have antifungal activity. [6] Considering these aspects, to control the spread of these infections and to find effective therapy the present study was done to examine drug-resistant dermatophytes from clinical patients and the efficiency of herbal extracts on them.

MATERIALS AND METHODS

All chemicals and reagents used in this present study were procured from Hi Media Laboratories (Mumbai, India).

Sample size

Total 150 participants with suspected of dermatophytosis and were selected from the Dermatology outpatient department.

Study group

The study population included 150 patients who were suspected of dermatophytosis and were selected from the Dermatology outpatient department, Government hospital, Erode city, Tamil Nadu, India.

Study design and procedure

All the investigation was done by a single trained investigator after obtaining consent from participants. The procedures follow the guidelines laid down in the Declaration of Helsinki". The specimens were collected from each participant by scrapping the edge of the affected skin, nail scrapping, and clipping the infected hair. Data related to the patents sex, age, and socioeconomic status was collected in a predesigned proforma. Collected samples were transported to the lab within one hour and processed without delay.

Microbiological investigation

The samples were inoculated on Sabouraud Dextrose Agar and further subcultured on Potato Dextrose Agar, Rose Bengal Agar, and dermatophytes test media. Morphological identification of the dermatophytic fungi was done by lactophenol cotton blue mount [7] and the differentiation was made by Urease test on Christensen's agar medium. [8]

Antibiogram of the isolated fungi was done by disc diffusion method [9] using commercially available drugs such as nystatin, clotrimazole, ketoconazole, miconazole, itraconazole, fluconazole, and amphotericin B (Hi Media) and the results were recorded based on CLSI guidelines. Fresh leaf materials were collected from different plants namely *Acalyphaindica*, *Achyranthusaspera*, *Azadiractaindica*, *Annona reticulate*, and *Aloe vera* gel. The selected materials were shade dried and powdered. Aqueous extracts were prepared

by soaking1.0 gof powder in 100ml sterile distilled water for 24 h. The filtrate was dried at 50 °C. 100mg of the extract was mixed with 1 ml of sterile distilled water. The efficiency of these plant extracts were assessed by well diffusion method.

Statistical evaluation : The obtained data was tabulated and statistically evaluated using IBM, SPSS software version 21.0, Chicago, USA using Chi square test and one way ANOVA test with $p<0.05$.

RESULTS

Dermatophytic fungal examinations clearly indicated thatthe overall prevalence of superficial infection was 112/150 (74.66%). Dermatophytes were isolated in 96 cases (85.71%) and non-dermatophytic fungi in 16 cases (14.29%) while 38 samples (25.33%) showed no growth. Species-wise occurrence of dermatophytes and non-dermatophytic fungi is tabulated (Table 1). The prevalence rate of isolated pathogens emphasized that a high rate of incidence was exhibited by *Microsporum* species (37.5%) and *Trichophyton* species (34.82%) followed by *Epidermophyton*species (13.39%). Among the non-dermatophytic mold, *Aspergillus* species has shown 14.29% of occurrence.

Sex-wise distribution of superficial infection (Figure 1) showed that males were more highly affected (64%) than females (36%). This occurrence may be due to the physical work of individuals in the study area where many textile and dye industries are located. The depictioage-wise wise occurrence is shown in Table 2. The result showed that the incidence of superficial infection mycoses is higher between 16-60 years than in children and old aged people. This incidence may be due to the frequent exposure of adults in the working area. The depiction the of the socioeconomic status of the infected patients are tabulated (Table 3). The result showed that low (48 cases) and middle-class (52 cases) people were highly affected and high-class (12 cases) people were less affected. This documentation is due to poor hygienic practices and also, they are not taking care of their health.

The drug sensitivity of the isolated dermatophytic fungal strains is shown in Table 4. *Trichophyton tonsurans* demonstrated a high degree of antibiotic sensitivity towards clotrimazole (65%) and resistance towards ketoconazole (100%) and itraconazole (82%). Similar kind of results was noticed among *Trichophyton rubrum* and *Trichophyton mentagrophytes*. *Epidermophytonfloccosum* was found to be resistant towards clotrimazole (73%), fluconazole (73%), amphotericin B (100%), ketoconazole (100%) and miconazole (100%). *Microsporum* species exhibited sensitivity against clotrimazole and nystatin and resistance against amphotericin B, ketoconazole, miconazole and itraconazole. *Microsporum gypsum* exhibited 100% resistance towards fluconazole. These results emphasized that clotrimazole and nystatin are highly effective and amphotericin B, miconazole,fluconazole, itraconazole and ketoconazole are found to be less effective against the isolates.

Many strains exhibited resistance to multiple drugs. Table 5 shows the occurrence of multidrug resistance among the isolates. *Trichophyton rubrum, Trichophyton mentagrophytes, and Microsporum gypsum* exhibited 100% resistance. In *Trichophyton tonsurans and Epidermophytonfloccosum*, the rate of multi-drug resistance is 76% and 87% respectively.

Overall results indicated that herbal extracts such as *Aloe Vera, Annona reticulata* were effective against MDR isolates of superficial fungi. *Acalyphaindica* was moderate and the remaining extracts were ineffective.

DISCUSSION

Worldwide millions of people (about 25%) are been commonly affected by superficial fungal infections. Causative agents include dermatophytes, yeasts and non-dermatophytic fungi. Dermatophytes are responsible for most superficial mycoses.[1] There are approximately 40 different species of dermatophytes belonging to the genera *Trichophyton*, *Epidermophyton* and *Microsporum*. In India, the incidence of superficial mycoses ranges

from 3-36%. The occurrence of dermatophytosis has been documented in New Delhi as 45% [10] and in Himachal Pradesh as 36.6%. [11] In South India, 78% dermatophytosis has been documented in and around Tiruchirapalli. *Trichophyton rubrum* has been the predominant pathogen followed by *Trichophyton mentagrophytes*.[4] In the present investigation, prevalence rate of superficial mycoses was 74.66% which ascertain the earlier published reports. [4] The rate of incidence showed that *Microsporum* species (37.5%) and *Trichophyton* species (34.52) caused infection predominantly.

In a previous study, it was reported that the occurrence of dermatophytes was 29.5%, non-dermatophytes 13.6%, *Candida* species (5.6%) and 51.1% yielded no growth. [12] Contra indication was found in the present study where Dermatophytes grown in 85.7% of cases and non-dermatophytes in 14.29 while 25.33% yielded no growth. Factors such as climate, social practices, migration and individual characters are responsible for the outcome of superficial mycoses. Superficial mycosis was more common in males below 30 years followed by the age group of 31-45 years. [13, 14] Similar findings were noticed in the present study.

In a study, the efficacy of antifungal drugs was measured as ketoconazole (77.5%), griseofulvin (7.5%), miconazole (90%) and cotrimoxazole (97.5%). Overall results revealed that clotrimazole and terbinafine were effective antifungal drugs and fluconazole exhibited poor sensitivity. [15]The present work emphasized that clotrimazole and nystatin are highly effective and amphotericin B, miconazole, fluconazole, itraconazole and ketoconazole are found to be less effective antifungal drugs. It is also indicated that the rate of multiple drug resistance has increased. 100% resistance was exhibited by *Trichophyton rubrum, Trichophyton mentagrophytes* and *Microsporum gypsum*. In *Trichophyton tonsurans and Epidermophytonfloccosum*, the rate of multi-drug resistance is 76% and 87% respectively. Superficial fungal infections are treated by various drugs namely griseofulvin, terbinafine, nystatin and zole compounds. Rise in antifungal resistance has been documented worldwide.

Therapeutic efficiency of natural systems has been described in traditional medicine. Bioactive compounds of plant origin have been identified with antimycotic activity. [6] Herbs and their extracts such as Tannins, Tea oil, Aloe vera, Honey, Marigold, Hibiscus, Neem etc., are used as medication for dermatophytosis. [16] Sharma et al from the review article observed that, adoption of the remedial approach (herbal and herbonanoconjugate) can be recommended after preclinical trials' approval as a safe treatment. [17] Moreno-Sabater et al from their study concluded that, Terbinafine resistance is present in France and the dermatophyte epidemiology is changing. Efficient systems must be implemented to survey the evolution of newly introduced species and to identify TerR isolates. [18]

In the present study, extracts obtained from seven different plant sources such as *Acalyphaindica, Achyranthesaspera, Azadirachtaindica, Aloe vera, Palm, Pine and Annona reticulate* have been treated for antifungal activity against multidrug resistant isolates. Results revealed that the extracts of *Aloe vera* and *Annona reticulata* exhibited effective antimycotic activity and *Acalyphaindica* was moderately effective.

CONCLUSION

Drugs used for the treatment of dermatophytosis cause side effects and need long-term exposure. The development of antifungal resistance is a threatening problem in the present therapeutic scenario. Dermatophytes continue to evolve resistance against newer drugs. Trends in antibiograms have been changing from time to time. Medicinal plants are good sources of new pharmaceuticals. From these findings, the use of herbal medicines will reduce the side effects of synthetic antifungal drugs and the development of drug resistance.

Conflict of interest: Nil

Source of funding: self

REFERENCES

1. Grag J, Tilak R, Garg A, Prakash P, Gulati AK, Nath G. Rapid detection of dermatophytes from skin and hair. BMC Research Notes. 2009; 2:60.
2. Tan HH. Superficial fungal infections seen at the National Skin Centre, Singapore. Jpn J Med Mycol. 2005; 46: 77-80.
3. Bannerjee U, Sethi M, Pasricha JS. Study of onychomycosis in India. Mycoses. 1990; 33:411-5.
4. Balakumar S, Rajan S, Thirunalasundari T, Jeeve S. Epidemiology of dermatophytosis in and around Tiruchirapalli, Tamil Nadu, India. Asian Pacific J. Trop. Dis. 2012; 2:286-89.
5. Deepika T, Lakshmipathy and Krishnan K, Review on dermatomycosis: Pathogenesis and Treatment. Nat Sci. 2010; 2: 726-731.
6. Natarajan V, Venugopal P.V, Menon, T. Effect of *Azadirachtaindica* (Neem) on the growth pattern of dermatophytes. Ind J Med Micro. 2003; 21: 98-101.
7. Robert R and Pihet M. Conventional methods for the diagnosis of dermatophycosis. Mycopathologia. 2008; 166: 295-306.
8. Philpot C. The differentiation of *Trichophyton mentagrophytes* from *T. rubrum* by a simple Urease test. Sabouraudia 1967; 5:189-193.
9. Bauer AW, Kirby WM Sherris JC, Turck M. Antibiotic susceptibility testing by standard single disk method. Am J ClinPathol. 1966; 45: 493-496.
10. Kaur R, Kashyap B, Makkar R. Evaluation of clinicomycological aspects of onychomycosis. Indian J Dermatol. 2008;53:174–8
11. Bhatia VK, Sharma PC. Epidemiological studies on Dermatophytosis in human patients in Himachal Pradesh, India. Springerplus. 2014; 3: 134.

12. Veer, P, Patwardhan NS Damle AS. Study of Onchymycosis: Prevailing fungi and pattern of infection. Ind J Med Micro. 2007; 25: 53-56.
13. Bindu V, Pavithran K. Clinico - mycological study of dermatophytosis in Calicut. Indian J Dermatol VenereolLeprol 2002; 68: 259-261
14. Peerapur BV, Inamdar AC, Pushpa PV, Srikant B. Mycological study of Dermatophytosis in Bijapur. Ind J Dermatol VenereolLeprol. 2004; 22: 273-74.
15. Pakshir K, Bahaedinie L, Razaei Z, Sodaifi M, Zomorodian K. In vitro activity of six antifungal drugs against clinically important dermatophytes. Jun.J.Microbiol. 2009; 2: 158-63.
16. Monica K, Bedi MD, Philip D, Shenefelt, MD. Herbal therapy in dermatology. Arch Dermatol. 2002; 138:232-42.
17. Sharma A, Gupta S. Protective manifestation of herbonanoceuticals as antifungals: A possible drug candidate for dermatophytic infection. Health Sci Rep. 2022;5(5):e775. 10.1002/hsr2.775
18. Moreno-Sabater A, Normand AC, Bidaud AL, Cremer G, Foulet F, Brun S , et al. Terbinafine Resistance in Dermatophytes: A French Multicenter Prospective Study. J. Fungi 2022, *8*(3): 1-6. 10.3390/jof8030220

Fig. 1: Sex wise distribution of skin infection

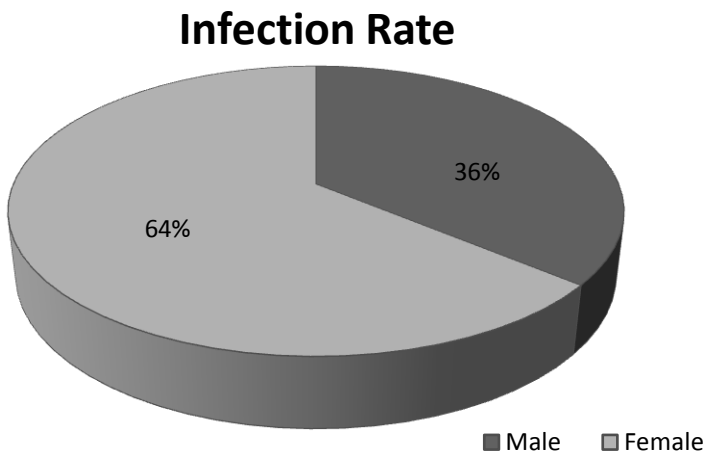

Table 1: Prevalence of superficial fungal pathogens

S.No	Name of the Fungal Pathogen	Number of isolates	Prevalence Rate (%)
1	*Trichphytontonsurans*	17	15.18
2	*Trichophyton rubrum*	16	14.29
3	*Trichophyton mmentagrophytes*	05	4.46
4	*Trichophyton terrestre*	01	0.89
5	*Epidermophytonfloccosum*	15	13.39
6	*Microsporumgypseum*	14	12.5
7	*Microsporumaudouinii*	14	12.5
8	*Microsporumcanis*	14	12.5
9	*Aspergillus flavus*	07	6.25
10	*Aspergillus fumigatus*	02	1.79
11	*Aspergillus niger*	02	1.79
12	*Aspergillus clavatus*	02	1.79
13	*Aspergillus calidoustus*	03	2.67

Table 2: Age wise distribution of superficial mycoses

S.No.	Age Group (Years)	Frequency
1.	5-15	03
2.	16-30	36
3.	31-45	31
4.	46-60	31
5.	61-75	08
6.	76-90	03

Table 3: Relationship between Socio-economic status and superficial mycoses

S.No.	**Socio-economic status**	**No. of Patients**
1.	Low	48
2.	Middle	52
3.	High	12

Table 4: Multidrug resistance among the superficial fungal isolates

S.No.	**Name of the isolate**	**No. of MDR**	**MDR in %**
1	*Trichphytontonsurans*	13/17	76%
2	*Trichophyton rubrum*	16/16	100%
3	*Trichophyton mentagrophytes*	05/05	100%
4	*Trichophyton terrestre*	01/01	100%
5	*Epidermophytonfloccosum*	13/15	87%
6	*Microsporumgypseum*	14/14	100%
7	*Microsporumaudouinii*	12/14	86%
8	*Microsporumcanis*	13/14	93%
9	*Aspergillus flavus*	07/02	100%
10	*Aspergillus fumigatus*	02/02	100%
11	*Aspergillus niger*	02/02	100%
12	*Aspergillus clavatus*	02/02	100%
13	*Aspergillus calidoustus*	03/03	100%

Isolates	**Antibiotics (Conc.)**													
	Clotrimazole (10µg)		**Flucanozole (10µg)**		**Amphotericin B (20µg)**		**Ketoconazole (30µg)**		**Miconozole (30µg)**		**Itraconozole (30µg)**		**Nystatin (50µg)**	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Trichphytontonsurans	11 (65%)	6 (35%)	6 (35%)	11 (65%)	4 (24%)	13 (76%)	0 (0%)	17 (100%)	0 (0%)	17 (100%)	3 (18%)	14 (82%)	10 (59%)	7 (41%)
Trichophyton rubrum)	11 (69%)	5 (31%)	1 (6%)	15 (94%)	3 (19%)	13 (81%)	0 (0%)	16 (100%)	0 (0%)	16 (100%)	1 (6%)	15 (94%)	8 (50%)	8 (50%)
Trichophyt entagrophyt	4 (80%)	1 (20%)	0 (0%)	5 (100%)	1 (20%)	4 (80%)	0 (0%)	5 (100%)	0 (0%)	5 (100%)	0 (0%)	5 (100%)	3 (60%)	2 (0%)
Trichophyton terrestre	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Epidermophytonfloccosum	4 (27%)	11 (73%)	4 (27%)	11 (73%)	0 (0%)	15 (100%)	0 (0%)	15 (100%)	0 (0%)	15 (100%)	8 (53%)	7 (47%)	8 (53%)	7 (47%)
Microsporumgypseum	9 (64%)	5 (36%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	4 (29%)	10 (71%)	9 (64%)	5 (36%)
Microsporumaudouinii	8 (57%)	6 (43%)	3 (21%)	11 (79%)	0 (0%)	13 (93%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	3 (21%)	11 (79%)	11 (79%)	3 (21%)
Microsporumcanis	8 (57%)	6 (43%)	3 (21%)	11 (79%)	1 (7%)	7 (100%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	11 (79%)	3 (21%)

Table 5: Antibiogram of the dermatophyticfungal isolates

Table 6: Effect of herbal extracts on MDR isolates of *Trichophyton tonsurans*

Isolate No.	**Herbal Extract/ Zone of inhibition in mm diameter**						
	Acalypha indica	*Achyranthes aspera*	*Azadirachtaindica*	*Aloe vera*	*Panai*	*Pine*	*Annona reticulata*
TT02	S (21mm)	R (13mm)	R (8mm)	S (22mm)	R (16mm)	S (24mm)	S (26mm)
TT04	R (11mm)	R (No Zone)	R (No Zone)	R (15mm)	R (18mm)	R (10mm)	S (28mm)
TT05	R (12mm)	R (16mm)	R (9mm)	S (23mm)	R (19mm)	R (14mm)	S (29mm)
TT06	S (24mm)	S (23mm)	R (8mm)	S (24mm)	R (15mm)	S (21mm)	R (18mm)
TT07	S (23mm)	R (17mm)	R (9mm)	S (23mm)	R (19mm)	R (17mm)	R (18mm)
TT08	S (24mm)	R (13mm)	R (8mm)	S (24mm)	R (18mm)	R (16mm)	S (27mm)
TT11	R (11mm)	R (10mm)	R (8mm)	R (18mm)	R (15mm)	R (11mm)	R (16mm)
TT12	R (10mm)	R (11mm)	R (9mm)	S (21mm)	R (16mm)	R (12mm)	R (15mm)
TT13	S (19mm)	R (10mm)	R (9mm)	R (17mm)	R (19mm)	S (21mm)	S (30mm)
TT14	R (11mm)	R (14mm)	R (8mm)	R (16mm)	R (14mm)	R (12mm)	S (24mm)
TT15	R (11mm)	R (15mm)	R (8mm)	S (23mm)	R (17mm)	R (15mm)	S (29mm)
TT16	R (10mm)	R (17mm)	R (9mm)	S (26mm)	R (15mm)	R (11mm)	S (27mm)
TT17	R (11mm)	R (16mm)	R (7mm)	S (25mm)	R (16mm)	R (12mm)	S (28mm)
Average	15.23mm	13.46mm	7.69mm	21.3mm	16.69mm	15.07mm	24.23mm
	S-8 R-5	S-1 R-12	S-0 R-13	S-9 R-4	S-0 R-13	S-3 R-10	S-9 R-4

***S- Sensitive** *** R- Resistant**

Table 6 shows the results of antifungal effect of various herbal extracts on multidrug resistant isolates of *T.tonsurans. Annona reticulata* (9/13), *Aloe Vera* (9/13) were measured as effective, *Acalyphaindica* was moderately effective and the rest were ineffective against *T.tonsurans*. The average zone of inhibition was higher in *Annona reticulta* (24.23mm) and *Aloe Vera* (21.3mm).

Table 7: Effect of herbal extracts on MDR isolates of *Epidermophytonfloccosum*

Isolate No.	**Herbal Extract/ Zone of inhibition in mm diameter**						
	Acalypha indica	*Achyranthes aspera*	*Azadirachtaindica*	*Aloe vera*	*Panai*	*Pine*	*Annona reticulata*
EP01	R (11mm)	R (17mm)	R (9mm)	S (25mm)	R (17mm)	S (14mm)	S (34mm)
EP02	S (19mm)	R (16mm)	R (8mm)	S (26mm)	R (18mm)	R (15mm)	S (35mm)
EP03	R (NoZone)	R (NoZone)	R (NoZone)	R (NoZone)	R (NoZone)	R (NoZone)	R (NoZone)
EP04	R (10mm)	R (17mm)	R (9mm)	S (25mm)	R (17mm)	R (15mm)	S (34mm)
EP05	R (NoZone)	R (NoZone)	R (NoZone)	R (NoZone)	R (NoZone)	R (NoZone)	R (NoZone)
EP06	S (25mm)	R (17mm)	R (NoZone)	S (25mm)	R (19mm)	S (23mm)	R (NoZone)
EP08	R (10mm)	S (30mm)	R (7mm)	S (24mm)	R (16mm)	S (28mm)	S (30mm)
EP10	R (11mm)	R (NoZone)	R (NoZone)	R (19mm)	R (16mm)	R (15mm)	S (30mm)
EP11	R (10mm)	R (NoZone)	R (NoZone)	R (19mm)	R (16mm)	S (25mm)	R (18mm)
EP12	R (11mm)	R (14mm)	R (NoZone)	R (17mm)	R (19mm)	R (NoZone)	R (NoZone)
EP13	R (9mm)	R (NoZone)	R (NoZone)	R (19mm)	R (NoZone)	S (25mm)	R (17mm)
EP14	R (10mm)	S (30mm)	R (7mm)	S (24mm)	R (19mm)	S (28mm)	S (30mm)
EP15	R (11mm)	R (14mm)	R (8mm)	R (17mm)	R (16mm)	S (25mm)	R (18mm)
Average	10.53mm	11.92mm	3.69mm	18.46mm	13.30mm	16.38mm	18.92mm
	S- 2 R-11	S-2 R-11	S-0 R-13	S-6 R-7	S-0 R-13	S-7 R-6	S-6 R-7

***S- Sensitive** *** R- Resistant**

Table 7 describes the antibiogrampattern of herbal extracts on multidrug resistant isolates of *E.floccosum*. The results revealed that *Annona reticulata* (6/13), *Aloe Vera* (6/13) were found to be effective and the rest of herbs were ineffective. The average Zone of Inhibition was measured as 18.92 for *Annona reticulata* and 18.46 for *Aloe Vera.*

Table 8: Effect of herbal extracts on MDR isolates of *Microsporumgypseum*

Isolate No.	**Herbal Extract/ Zone of inhibition in mm diameter**						
	Acalypha indica	*Achyranthes aspera*	*Azadirachtaindica*	*Aloe vera*	*Panai*	*Pine*	*Annona reticulata*
MG01	S (20mm)	R (NoZone)	R (NoZone)	S (29mm)	R (19mm)	S (23mm)	R (12mm)
MG02	R (19mm)	R (NoZone)	R (NoZone)	S (30mm)	R (18mm)	S (24mm)	S (26mm)
MG03	R (19mm)	R (9mm)	R (NoZone)	R (18mm)	R (13mm)	S (36mm)	S (24mm)
MG04	S (20mm)	R (10mm)	R (NoZone)	S (21mm)	R (14mm)	S (37mm)	S (25mm)
MG05	R (11mm)	R (NoZone)	R (NoZone)	S (20mm)	R (12mm)	R (11mm)	S (26mm)
MG06	R (10mm)	R (16mm)	R (7mm)	S (28mm)	R (17mm)	R (17mm)	S (27mm)
MG07	R (9mm)	R (16mm)	R (8mm)	S (20mm)	R (12mm)	R (17mm)	R (14mm)
MG08	R (11mm)	R (9mm)	R (9mm)	R (11mm)	R (13mm)	R (11mm)	R (18mm)
MG09	S (20mm)	R (NoZone)	R (NoZone)	S (22mm)	R (13mm)	R (14mm)	R (16mm)
MG10	R (11mm)	R (NoZone)	R (NoZone)	R (12mm)	R (14mm)	R (13mm)	R (17mm)
MG11	S (22mm)	R (11mm)	R (9mm)	S (21mm)	R (19mm)	R (15mm)	S (25mm)
MG12	S (23mm)	R (12mm)	R (6mm)	S (22mm)	R (15mm)	R (15mm)	S (26mm)
MG13	S (26mm)	R (14mm)	R (NoZone)	S (25mm)	R (18mm)	R (16mm)	S (29mm)
MG14	S (25mm)	R (16mm)	R (NoZone)	S (27mm)	R (12mm)	R (17mm)	S (27mm)
Average	18.92mm	8.69mm	3.0mm	23.53mm	15.07mm	20.46mm	24.0mm
	S-7 R-7	S-0 R-14	S-0 R-14	S-11 R-3	S-0 R-14	S-4 R-10	S-9 R-5

***S- Sensitive** *** R- Resistant**

Table 8 describes the effect of herbal extracts on multidrug resistant isolates of *M.gypseum*. Aloe Vera (11/14), *Annona reticulate* (9/14) and *Acalyphaindica*(7/14) were effective and the rest of the extracts were ineffective. The average Zone of Inhibition was measured as 24mm for *Annona reticulata*, 23.53mm for *Aloe Vera* and 18.92 for *Acalyphaindica*.