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Study of inhibition action of some plant extracts against some dermatophyte

Yahya Hossin Mohammed Khabrani

Faisal Fahad Ghazzai Almutairi

Fahad Saad Fahad Alothaymin

Abdullah Mohammed Haif Alotaibi

Abdullah Mohammed Mohammed Alqahani

Abdulaziz Atallah Turays Almutairi.

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ABSTRACT

In folk medicine, plants like Rosmarinus officinalis and Olea europaea are used to cure illnesses like infectious infections and skin conditions. Examined the antifungal properties of hydro alcoholic snippets from Rosmarinus officinalis and Olea europaea against strains of these three fungi. By using the micro dilution method and microscopy, dried leaves from Rosmarinus officinalis and Olea europaea hydro alcoholic extracts were tested against dermatophyte species. Based on the lowest inhibitory concentration (MIC) and minimal fungicidal concentration (MFC), R. officinalis and O. europaea which the most effective against dermatophytes. In order to explore how the two extracts inhibited hyphal development, fluorescence microscopy and scanning electron microscopy were utilized. The results revealed a substantial inhibition and an uneven growth pattern. Both extracts effectively combatted dermatophytes by preventing fungal growth and altering their hyphae. R. officinalis and O. europaea are therefore likely sources of novel chemicals for the creation of antifungal medications.

Keywords: *Plant extract, antifungal and dermatophytosis.*

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Introduction

The keratin-eating dermatophyte fungi that infect the epidermis and appendages produce dermatophytoses, which are infections. The three genera that these fungi belong to are Epidermophyton, Trichophyton, and Microsporum (Johnson,2003). Occasionally, superficial dermatophyte-related fungal infections are some of the common human infectious diseases that recorded significant familial, health, and financial repercussions (Vishnu et al., 2015). Even though there are several antifungals on the market, there is a need for safer and more efficient medications due to adverse effects, drug interactions, and the presence of resistant organisms (Smith et al,2015). Additionally, dermatophytosis therapies are typically costly and need repeated applications over extended periods of time. Since plants create a wide range of chemicals to defend themselves against a wide range of pathogens, plant Substances demonstrated for being a promising substitute for discovering new psychoactive chemical (Uganda,2016).

Plants have long served as the principal source of basic medical care, particularly in impoverished nations. Rosemary, *Rosmarinus officinalis*, offers a wide range of therapeutic benefits and is excellent in treating yeasts and bacteria linked to dermatological conditions (Melo et al.,2015). Additionally, it inhibits the growth of *Aspergillus niger* and *Candida albicans* (Hoog et al.,2017). This study assessed the anti-dermatophytic efficacy of hydro alcoholic extracts from *R. officinalis* and *O. europaea*

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Some of bacteria and fungi strains have been demonstrated to be inhibited in growth by phenolic chemicals extracted from *O. europaea* (olive leaves). 1,2 Oleuropein, an acrid iridioid glycoside isolated from olive leaves, demonstrated to prevent Bacillus cereus development (Tassou and Nychas,1991).

A metabolite of oleuropein called hydroxytyrosol has recently been shown successful towards *Moraxella catarrhalis, Salmonella typhi, Vibrio parahaemolyticus*, and *Haemophilus influenzae* acute pathogens genotypes. Antimicrobial resistances, widespread use of immunosuppressants, and an increase in all underscore the need to discover and create novel antimicrobial agents in fungal diseases (Bisignano et al.,1999).

We wanted to identify and describe the broad array of pathogens fungus which resistant to the activities of rosemary and olive leaves in our investigation.

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Methodology

Varieties of plants materials

Rosmarinus officinalis (rosmarey leaves) *Olea europaea* (olive leaves) Plant samples were collected, identified, and dried in the shade for reduce the deterioration of active compounds. Using a crusher and pestle, those being pounded into a fine powder, sieved, and kept in an airtight container.

Fig.1 Rosmarinus *officinalis* (rosemary leaves) Olea *europaea* (olive leaves)

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Extraction preparation

In a 40 °C circulating-air oven, leaves were dried and crushed. They were then submerged in 90/10% (v/v)

The technique Esazah et al. (2015) provided for the crude ethanol extraction process was slightly altered. 200 grammes were soaking in alcohol (95percent of the total v/v) for 24 hours while being agitated occasionally in a 2 L conical flask. After the extract was filtered out using Whatman No. 1 filter paper, it was concentrated (ethanol was allowed to drip off) inside a hot-air oven at 45°C. The container holding the concentrated crude extracts was appropriately labeled, kept in the refrigerator at 4°C, and kept at room temperature. Yielding (%, w/w) powder form leaf crude extract was calculated using equation output (%) = (W1 x 100)/W2, whereby W2 would be the quantity of a powder before dryness and W1 is the amount of the leaf extracts.

Fungicide effects

For each of the three plant extracts, the MIC and MFC were calculated. The MIC range for *R. officinalis* and *O. europaea* activity against dermatophytes was 75 to 275 g/mL. The fungicidal concentrations (in brackets) were either identical to or just one two-fold dilution above the inhibitory concentration.

This study's goal was to determine the prevalence of dermatophytosis as a reflect to antifungal efficacy of *Rosmarinus officinalis* and *Olea europaea* crude leaf extract in ethanol when applied to dermatophytes that were isolated from patients. 100 scrapings of skin and nails were obtained, and they were examined using culture techniques and conventional microscopy (KOH). The conventional extraction procedure was used to collect and treat the leaves of *Rosmarinus officinalis* and *Olea europaea* using 95% ethanol. Using a modified agar well diffusion technique, the dermatophytes Trichophyton tonsurans, T. mentagrophyte, and Microsporum audouinii were tested for resistance towards the pure leaves extract.

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Broth tube dilution and culture, respectively, leaf extracts 's minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were also determined. 100 samples were obtained, and 59 of them had acceptable microscopic tests. Dermatophytosis Frequency observed significantly (p0.001) linked to participant catagories, having higher viral loads, with 75.0% each, in those between the ages of 10 and 20 and 30 and 40. 59 items were tested positive by microscopy and 28 of those samples also positively identified.

Antidermatophytic Activity Testing

On newly made, sterile Sabouraud Dextrose Agar, *T. tonsurans, T. mentagrophyte, and M. audouinii*, the testing pathogens, being autoclavable injected first at middle of multiple dishes. Three wells top the agar plates were carefully drilled glass cork borers that are sterile (6 mm) at a distance of 0.05 cm from the inoculated test organism. As a stock solution, 2 ml of DMSO was used to dissolve 2 grammes of the crude extract from T. riparia leaves (Esazah et al.,2015). The remaining wells were filled with 10% DMSO, terbinafine 40 g/ml as the positive control as well as the control group, respectively. The 1 g/ml extract, 100 in each well (Das and Godbole ,2015)

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Minimum Inhibitory Concentration (MIC)

Optical density from the sporangial suspension of *T. tonsurans*, *T. mentagrophyte*, and *M. audouinii* was calibrated using a spectrophotometer at 530 nm to 0.014, or 1.0x105 inoculated. Using the tube dilution technique, the MIC of plant extract of *R. officinalis* and *O. europaea* leaves was ascertained. Ten tubes each held one millilitre (1ml) of 0.5% Agar and Brain Heart Infused Mixture. Each organism's initial vial received one millilitre of standard solutions (2 g/ml), and subsequent test tubes underwent twofold serial dilutions until quantities of less than 0.0156 g/ml were attained. Terbinafine group (2 mg/ml) was placed into a second test tube set and pipetted twice using the same procedure as above. Inoculum preparation of each fungus, 20 microlitres (20 l), was then added to the aforementioned serially diluted tubes. As a negative control, one millilitre (1 ml) of 10% DMSO was employed. The test tubes were then incubated for 4 to 7 days at 28 to 30°C. The MIC was determined by looking at the test tubes that has lower level and un apparent increase (optical clarity) (Khan et al.,2006**)**.

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Fungicidal Minimum Concentration

After the MIC is determined using tube dilution procedure, the MFC was estimated by inoculating 20 of a colony from each negatives well (with no visible roughness) of the extracted as well as positive control. When the pans were incubated at 28 to 30°C during 3-5 days, the lowest dose that resulted in negative subcultures (no growth) was referred to as MFC. (Kummar et al.,2016).

Analysis of Data

The results were put into a Microsoft Excel spreadsheet. Dermatophytosis frequent, age groups' average and standard deviation of members were determined using descriptive analysis. Using SPSS software (version 16), Chi-square was utilized to contrast demographic features of study participants of the prevalence of dermatophytosis, and p0.05 was judged significant.

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Results

Active components of crude leaves extract

1,8-cineole, Limonene, and -pinene were the active ingredients in rosemary leaves, and the total phenolic content was 9.25 g. The qualitative composition of the generated volatile oil is influenced by variations in the total phenol content and volatile oil content of rosemary leaves according to climate conditions, harvest time, and style of distillation (Umit et al. ,2011).

Oleuropein, Caffeic acid, and Luteolin-7-o-were found to be the primary active ingredients in olive leaf, whereas complete contents phenolic, tannins and flavonoids presented in Table 1.

Table1. Active components of crude leaves extract

Components%	***Rosmarinus officinalis***	***Olea europaea***
Total phenolic	9.25	19.7
Flavonoids	-	0.29
Tannins	-	0.52
1.8 cineole	25.43	-
Limonene	12.87	-
β - pinene	11.18	-
comphene	3.72	-
p-cymene	2.96	-
β-caryophyllene	3.89	-
Quercetin	-	0.027
Apigenine-7-*O*-glucoside	-	0.07
Luteolin-7-*O*-glucoside	-	0.02
Caffeic acid	-	0.04
Oleuropein	-	24.5

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The demographics of the participants

A total of one hundred (100) patients were recruited in this study. 40 women and 60 men. Participants in the study varied in age from 1 to 49. Participants in the research were 35 years old on average.

According to microscopy data on dermatophytosis prevalence by gender, male participants had the highest prevalence, with 35/53 (58.33%) compared to their female counterparts' 18/53 (45.00%). Using the chi-square test, the individuals' gender was not significantly related to the dermatophytosis is frequent ($p< 0.990$) (Table 2 and Fig.1). This was in line with the conclusions of Leiva-Salinas et al. (2015), found that among schoolchildren lowland Ethiopia, Boys are more often than girls to have dermatophytosis, at 42.2% versus 30.5%, accordingly. This was in contrast to the results of of Dogo et al. (2016), noted that among schoolchildren; dermatophytosis was more common in girls than in boys, with respective prevalence rates of 51.4% and 41.5%.

According to microscopy, the age groups of >1 year had the highest prevalence of dermatophytosis, each with a prevalence of 88.89%, while the age groups of >40 years had the lowest prevalence, each with a prevalence of 25.00%. However, using chi-square tests, the participants' age group was significantly linked to frequent dermatophytosis and microscopic inspection ($p < 0.001$). (Table 3 and Fig.2). Young children are impacted by dermatophytosis greater than the elderly, according to the literature (Ferguson and Fuller,2017). But Moto et al., (2015) observed about 68.0% among school-age children are impacted by dermatophytosis. The frequency obtained in this study was greater than the prevalence reported by Leiva-Salinas, (2015) of 36.5%children and 45.0%older.

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Table 2. Based on a microscopic examination (KOH), the rate of patients' dermatophytosis gender.

Samples		(KOH)		Culture		
		Positive	**Negative**	**Positive**	**Negative**	**Total**
Male	60	35 (58.33%)	25 (41.67%)	27 (77.14%)	8 (44.44%)	35 (66.04%)
Female	40	18 (45.00%)	22 (55.00%)	8 (22.86)	10 (55.56%)	18 (33.96%)
Total	*100*	*53 (100%)*	*47(100%)*	*35(66.04%)*	*18(33.96%)*	*53(100)*

chi-square, p = 0.990.

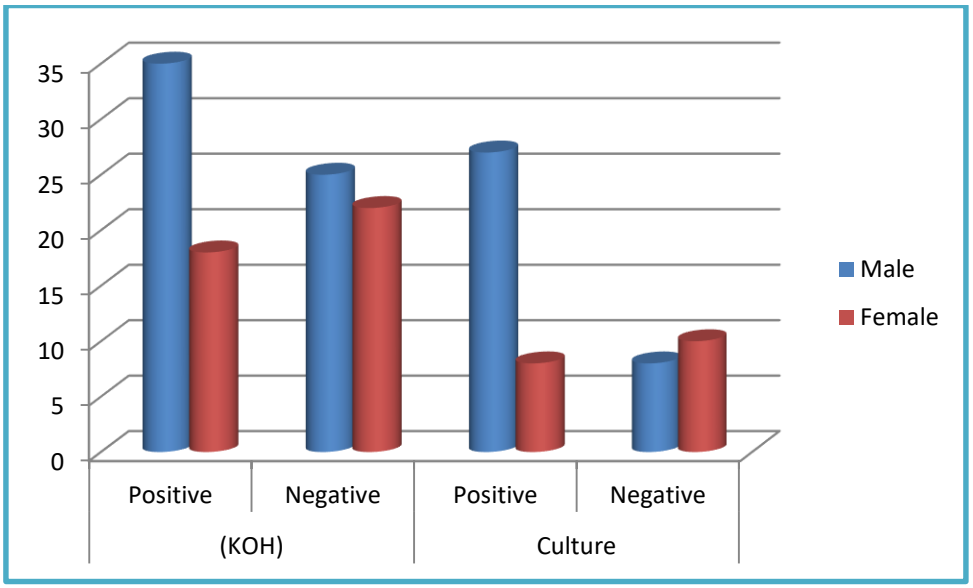

Fig.1 the rate of patients' dermatophytosis vary with gender.

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Table3. Based on a microscopic examination (KOH), the rate of patients' dermatophytosis varies with age.

age/ year	Samples	(KOH)		Culture	
		Positive	**Negative**	**Positive**	**Negative**
>1	9	8 (88.89%)	1 (11.11%)	6 (75.00%)	2 (25.00%)
>10	21	17 (80.95%)	4 (19.05%)	7 (41.18%)	10 (58.82%)
>20	28	18 (64.29%)	10 (35.71%)	8 (44.44%)	10 (55.56%)
>30	30	15 (50.00%)	15 (50.00%)	7 (46.67%)	8 (53.33%)
>40	12	3 (25.00%)	9 (25.00%)	0 (00.00%)	3 (100.00%)
Total	*100*	*53 (100%)*	*47(100%)*	*35(66.04%)*	*18(33.96%)*

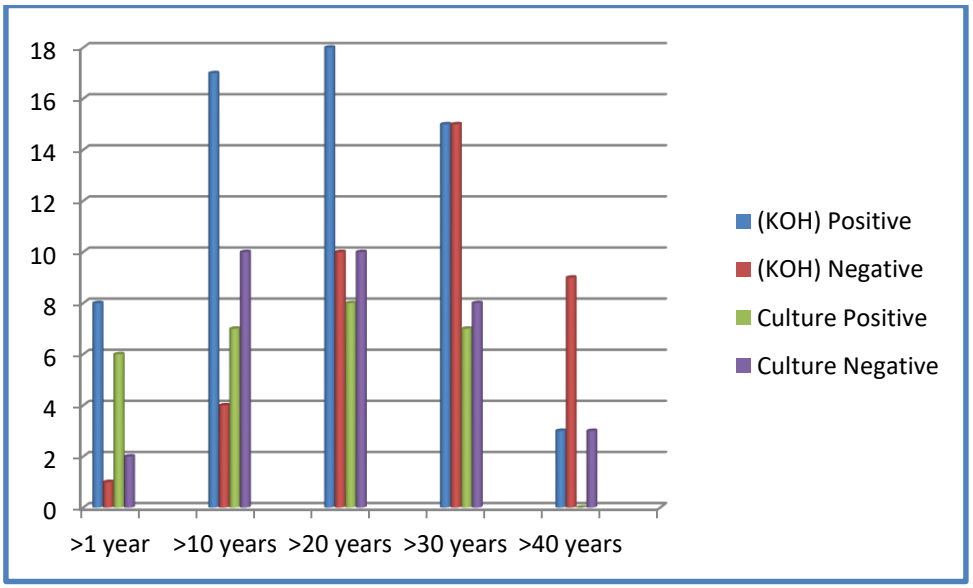

Fig.2 the rate of patients' dermatophytosis varies with age.

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Minimum Fungicidal Concentration and an inhibitor's minimal effective level

The extract of *R. officinalis* leaves level of MIC varied from 65 to 260 mg/ml. MIC values for O. europaea were leaves varied from 72.5 to 290 mg/ml, terbinafine utilized as a control, was successful in preventing Wiegand et al. (2016) observed success rates of 82.6% (n = 115) and 87.8% (n = 101) for the Blankophor and culture procedures, correspondingly. To demonstrate MFC at a dosage of 156 mg/ml, the MFC level of the extract of *R. officinalis* leaves even against pathogens spp. tested ranged 130 - 520 mg/ml. It could mean that this particular dermatophyte was less responsive to the antifungal medication. (Table 4 , Fig.3 and 4).

Different species responded differently to terbinafine and the extract, *T. tonsurans* being was sensitive greater than *T. mentagrophyte* and *M. audouinii which* less sensitive to extract. Slower growth of *T. tonsurans* and *T. mentagrophyte*, which take 8–12 days and 7–10 days, respectively, in comparison to *M. audouinii*, it develops in 5–10 days, may explain higher vulnerability to these organisms (Santos and Hamdan2005); According to Ahmad and Aqil (Ahmad and Aqil,2007), crude extracts may contain certain molecules with antagonistic action toward other bioactive chemicals, which would explain the poor effectiveness against the tested organism.

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Table 4. Minimum fungicidal concentrations as well as minimal inhibitory concentrations.

	Testing microbes(mg/ml)					
	T. tonsurans		***T. mentagrophyte***		***M. audouini***	
	MIC	MFC	MIC	MFC	MIC	MFC
R. officinalis extract	65.00	130	130	260	260	520
O. europaea extract	72.50	145	145	290	290	580
Terbinafine	**78.00**	**156**	**178**	**313**	**313**	**625**

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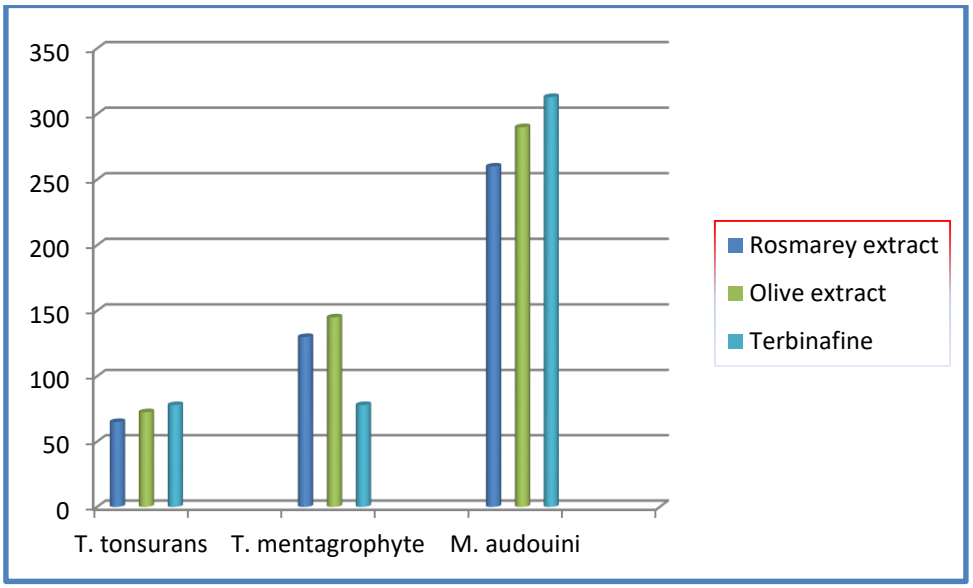

Fig.4 minimal inhibitory concentrations.

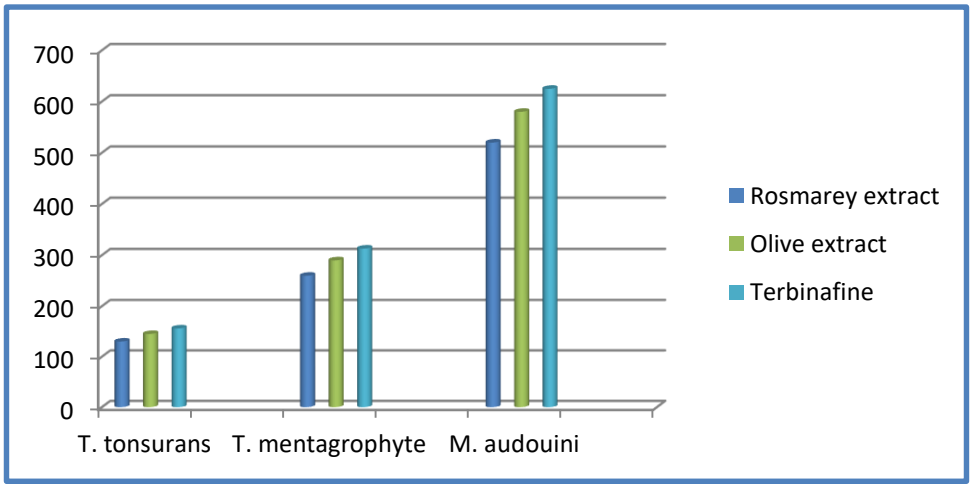

Fig5. Minimum Fungicidal Concentration

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Conclusion

This study suggests that the leaves of R. officinalis and O. europaea contain bioactive compounds that are antifungal. The hydrsoalcoholic extracts of both species were effective in suppressing the growth of dermatophytes and modifying the shape of their hyphae. This is in favor of its used for treatment of skin pathogens in conventional medicine. These species might be used to develop antifungal treatment strategies.

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ISSN: 2616-9185
www.mecsj.com

References

Ahmad, I., & Aqil, F. (2007) In vitro efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. Microbiological Research.; 162(3):264–275.

Bisignano, G., Tomaino, A., LoCascio, R., Crisafi, G., Uccella, N., & Saija A. (1999) On the in-vitro activity of oleuropein and hydroxytyrosol. J Pharm Pharmacol; 51: 971–4.

Das, L., & Godbole, S. (2015) Antifungal and phytochemical analysis of lantana camara, citrus limonum (lemon, azadirachta indica (neem) and hibiscus rosasinensis (china rose). Journal of Pharmacy Research.; 9(7):476–479

Dogo, J., Afegbua, S., & Dung, E. (2016) Prevalence of Tinea capitis among school children in Nok community of Kaduna state. Prevalence of Tinea capitis among school children in Nok community of Kaduna state:10 –1155.

Esazah, K., Fredric, A., Jasper, O., & Godwin, A. (2015) Phytochemical analysis and screening of ugandan medicinal plants for antifungal activity against candida albicans. International Journal of Tropical Disease & Health.;9 (1):1–8.

Ferguson, L., & Fuller, L. (2017) Spectrum and burden of dermatophytes in children. Infection.;74: S54–S60.

Hoog de, G., Dukik, K., & Monod, M. (2017) Toward a Novel Multilocus Phylogenetic Taxonomy for the Dermatophytes. Mycopathologia.;182(1-2):5–31.

Johnson, L. (2003) Dermatophytes-the skin eaters. Mycologist.;17(4):147–149.

Khan, S., Singhal,S., Mathur, T., Upadhyay ,D., & Rattan, A.(2006) Antifungal susceptibility testing method for resource constrained laboratories. Indian Journal of Medical Microbiology.;24 (3): p. 171.

Kumar, P., Kumar, J., Kumar, R., & Dubey, R. (2016) Studies on phytochemical constituents and antimicrobial activities of leaves, fruits and stems of Solanum nigrum L. AJPSKY.;6(4):57–68.

Leiva-Salinas, M., Marin-Cabanas, I., & Betlloch. I. (2015) Tinea capitis in schoolchildren in a rural area in southern Ethiopia. International Journal of Dermatology.;54 (7):800–805

Leiva-Salinas, M., Marin-Cabanas, I., Betlloch, I. (2015). Tinea capitis in schoolchildren in a rural area in southern Ethiopia. International Journal of Dermatology.;54 (7):800–805.

Melo de, N., Carvalho de, C., & Fracarolli, L. (2015). Antimicrobial activity of the essential oil of Tetradenia riparia (Hochst.) Codd. (Lamiaceae) against cariogenic bacteria. Brazilian Journal of Microbiology.;46 (2):519–525.

Multi-Knowledge Electronic Comprehensive Journal For Education And Science Publications (MECSJ)
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ISSN: 2616-9185
www.mecsj.com

Moto, J., Maingi, J., & Nyamache, A. (2015) Prevalence of Tinea capitis in school going children from Mathare, informal settlement in Nairobi, Kenya. BMC Research Notes.;8 (1): p. 274.

Santos, D., & Hamdan, J. (2005) Evaluation of Broth Microdilution Antifungal Susceptibility Testing Conditions for Trichophyton rubrum. Journal of Clinical Microbiology.;43(4):1917–1920. doi: 10.1128/JCM.43.4.1917-1920.2005.

Smith, K., Achan, B., &Hullsiek, K. (2015) Increased Antifungal Drug Resistance in Clinical Isolates of Cryptococcus neoformans in Uganda. Antimicrobial Agents and Chemotherapy.;59 (12):7197–7204.

Tassou, C., &Nychas, G. (1991) Effect of phenolic compounds and oleuropein on the germination of Bacillus Cereus T spores. Biotechnol Appl Biochem; 13: 231–7.

Uganda, M., (2016) Uganda Clinical Guidelines. Fungal skin infections; pp. 935–938.

Umit P., Derya, Y., & Mustafa, E. (2011) Serum biochemical profile of broiler chickens fed diets containing rosemary and rosemary volatile oil. J. Biol. Environ. Sci., 5(13), 23-30.

Vishnu, S., Tarun, K., Anima, S., Ruchi, S., & Subhash, C. (2015) Dermatophytes: Diagnosis of dermatophytosis and its treatment. African Journal of Microbiology Research.;9(19):1286–1293.

Wiegand, C., Mugisham, P., Mulyowa, G., (2016) Identification of the causative dermatophyte of tinea capitis in children attending Mbarara Regional Referral Hospital in Uganda by PCR-ELISA and comparison with conventional mycological diagnostic methods. Medical Mycology.;55 (6):660–668.