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STUDYING THE EFFECT OF ROSEMARY EXTRACT ON TYPES OF DERMATOPHYTE THAT EFFECT IN HAIR SCALP

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Abstract:

Plants such as Rosmarinus officinalis are used in folk medicine to treat skin problems and infections. investigated the antifungal effects of Rosmarinus officinalis hydroalcoholic extracts against isolates of these three fungi. Dried Rosmarinus officinalis leaves and hydroalcoholic extracts were evaluated against dermatophyte species using the microdilution technique and microscopy. R. officinalis is the most efficient against dermatophytes based on the lowest inhibitory concentration (MIC) and minimal fungicidal concentration (MFC). The mechanisms by which this extract impeded hyphal growth were investigated using scanning electron and fluorescence microscopy. The findings showed a marked inhibition as well as an irregular growth pattern. Through its modification of fungal hyphae and prevention of fungal development, this extract efficiently combats dermatophytes. Therefore, R. officinalis is probably a source of new compounds for the synthesis of antifungal drugs.

Keywords: Plant extract, antifungal and dermatophytosis.

Introduction

Dermatophytoses are infections caused by the keratin-eating dermatophyte fungus that infects the epidermis and appendages. These fungi are classified into three genera: Microsporum, Trichophyton, and Epidermophyton. (Johnson,2003). On occasion, fungal infections associated with superficial dermatophytes are among the prevalent infectious disorders that have serious health, economic, and familial consequences. (Vishnu et al., 2015). Even though there are many antifungals available, side effects, drug interactions, and the existence of resistant organisms make safer and more effective treatments necessary. (Smith et al.,2015). Furthermore, dermatophytosis treatments are usually expensive and need long-term, regular administration. Plants produce an extensive array of compounds to protect themselves from various infections. As a result, plant substances have shown promise as a viable alternative to the discovery of novel psychoactive chemicals . (Uganda,2016)

Traditionally, plants have been the primary source of basic medical care, especially in developing countries. Rosemary, Rosmarinus officinalis, has several medicinal uses and is particularly effective in treating germs and yeasts associated with skin disorders.(Melo et al.,2015) Furthermore, it prevents Aspergillus niger and Candida albicans from growing. (Hoog et al.,2017). This investigation evaluated the anti-dermatophytic properties of hydroalcoholic extracts derived from R. officinalis.

Our goal in conducting this experiment was to discover and characterize the wide range of fungi and diseases that are resistant to the effects of rosemary leaves.

Methodology

Varieties of plants materials

Rosmarinus officinalis or leaves of rosemary Samples of plants were gathered, identified, and then dried in the shade to prevent the active chemicals from deteriorating. They are ground into a fine powder using a pestle crushed, sieved, and stored in an airtight container.

Fig.1 Rosmarinus officinalis (rosemary leaves)

Extraction preparation

The leaves were dried and crushed in a circulating-air oven set at 40 °C. After that, they were immersed in 90/10% (v/v)

A modest modification was made to the crude ethanol extraction method described by Esazah et al. (2015). In a 2 L conical flask, 200 grams were immersed in alcohol (95 percent of the entire volume by volume) for a whole day, with periodic stirring. The extract was concentrated (ethanol was allowed to drop out) inside a hot-air oven at 45°C after being filtered out using Whatman No. 1 filter paper. The concentrated crude extracts were stored in a container with the proper label. both at room temperature and in the refrigerator (at 4°C). The formula output (%) = (W1 x 100)/W2, where W2 is the quantity of a powder before drying and W1 is the

amount of leaf extracts, was used to determine the yielding (%, w/w) powder form leaf crude extract.

Effects of fungicides

The MIC and MFC were determined for extracts made from rosemary leaves. For R. officinalis to be active against dermatophytes, the MIC range was 75–275 g/mL. The inhibitory concentration was either the same as the fungicidal concentrations (in brackets) or one two-fold dilution above it.

The purpose of this study was to ascertain the frequency of dermatophytosis in relation to the antifungal activity of crude leaf extract in ethanol from Rosmarinus officinalis when administered to dermatophytes that were isolated from patients. After obtaining 100 skin and nail scrapings, the samples were analyzed by conventional microscopy (KOH) and culture methods. The leave of Rosmarinus officinalis were collected and treated with 95% ethanol using the traditional extraction method. The dermatophytes Trichophyton tonsurans, T. mentagrophyte, and Microsporum audouinii were examined for resistance to the pure leaf extract using a modified agar well diffusion method.

The minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC) of leaf extracts were also ascertained by broth tube dilution and culture, respectively. Thirty of the 80 samples that were collected had satisfactory microscopic testing. Dermatophytosis Frequency was found to be substantially (p0.05) correlated with participant categories, with higher virus loads of 75.0% in each age group between 10, 20 and 30 years old. Microscopy testing confirmed the positive results, and 28 of those samples were also successfully identified.

Testing for Anti dermatophytic Activity

The testing pathogens, T. tonsurans, T. mentagrophyte, and M. audouinii, were autoclavable and injected first amid numerous plates over freshly manufactured, sterile Sabouraud Dextrose Agar. Carefully drilled glass cork borers, measuring 6 mm and positioned 0.05 cm apart from the inoculated test organism, were positioned to create three wells on top of the agar plates. Two grams of the crude extract from T. riparia leaves were dissolved in two milliliters of DMSO to create a stock solution (Esazah et al., 2015). 10% DMSO and 40 g/ml of terbinafine were added to the remaining wells to serve as the positive control and control groups, respectively. Each well contained 100 of the 1 g/ml extract (Das and Godbole, 2015).

The MIC, or minimum inhibitory concentration

A spectrophotometer was used to calibrate the optical density of the sporangial suspension of T. tonsurans, T. mentagrophyte, and M. audouinii at 530 nm to 0.014, or 1.0x105 infected. The MIC of a plant extract made from the leaves of R. officinalis was determined using the tube dilution technique. One milliliter (1 ml) of Brain Heart Infused Mixture and 0.5% Agar were included in each of the ten tubes. One milliliter of standard solutions (2 g/ml) was added to each organism's first vial. Subsequent test tubes were serially diluted twice until the final

concentration was less than 0.0156 g/ml. Using the same method as before, the terbinafine group (2 mg/ml) was added to a second test tube set and pipetted twice. The previously indicated serially diluted tubes were then filled with 20 microliters (20 l) of the inoculum solution for each fungus. One milliliter (1 ml) of 10% DMSO was used as a negative control. After that, the test tubes were incubated at 28 to 30°C for four to seven days. The test tubes with a lower level and an undetectable improvement in optical clarity were used to calculate the minimum inhibitory concentration (MIC) reference (Khan et al., 2006).

Minimum Fungicidal Concentration

Following the tube dilution process to calculate the MIC, 20 colonies from each negative well (without any apparent roughness) of the extracted and positive control were inoculated to estimate the MFC. The lowest dose that produced negative subcultures (no growth) when the pans were incubated at 28 to 30°C for three to five days was called MFC. (Kummar and others, 2016).

Analysis of Data

Constituents in crude leaf extract that are active

The active components of rosemary leaves were 1,8-cineole, limonene, and pinene; the total phenolic content of the leaves was 8.5 g. Variations in the total phenol and volatile oil content of rosemary leaves depending on the environment, when the leaves are harvested, and the distillation method all affect the qualitative makeup of the produced volatile oil (Umit et al.,2011).

Table1. Active of Rosmarinus officinalis crude leaves extract

Components%	*Rosmarinus officinalis*
Total phenolic	9.25
Flavonoids	-
Tannins	-
1.8 cineole	
Limonene $_{\beta}$	25.43
- pinene	12.87
comphene	11.18
p-cymene	3.72
$_{\beta}$-caryophyllene	2.96
Quercetin	3.89
Apigenine-7-*O*-glucoside	
Luteolin-7-*O*-glucoside	-
Caffeic acid	-
Oleuropein	-
	-

The demographics of the participants

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All in all, 75 patients were enrolled in this investigation. 40 males and 35 women. The age range of the research participants ranged from 1 to 30. The average age of the research participants was 25 years old.

Microscopy data on the prevalence of dermatophytosis by gender showed that, among the subjects, men had the highest prevalence, with 28/45 (62.22%) compared to 17/45 (37.7%) for females. The gender of the subjects did not significantly correlate with the frequency of dermatophytosis ($p<0.899$) according to the chi-square test (Table 2 and Fig. 1). This was consistent with the findings of Leiva-Salinas et al. (2015), who discovered that among schools in lowland Ethiopia, boys experience dermatophytosis at a rate of 42.2% vs. 30.5%, respectively. This was in contrast to the findings of Dogo et al. (2016), who reported that among students, females had a higher prevalence of dermatophytosis than boys did (51.4% versus 41.5%).

Microscopy revealed that the age groups older than one year had the highest frequency of dermatophytosis, with a prevalence of 72.7%, while the age groups older than twenty years had the lowest frequency, with 62.7%. On the other hand, chi-square tests revealed a strong correlation between the age group of the subjects with both microscopic examination and frequent dermatophytosis ($p < 0.05$). (Figure 2 and Table 3). The literature indicates that young children are more affected by dermatophytosis than the elderly (Ferguson and Fuller, 2017).

However, Moto et al. (2015) found that dermatophytosis affects around 68.0% of school-age children. The frequency found in this investigation exceeded the prevalence of 36.5% of youngsters and 45.0% of older people reported by Leiva-Salinas (2015).

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

	Samples	(KOH)		Culture			X^2	P value
		Positive	Negative	Positive	Negative	Total		
Male	40	28 (62.22%)	12 40%	20 71.4%	8 28.5%	28	0.016	0.899(NS)
Female	35	17 (37.77%)	18 60%	7 41%	10 58%	17	0.252	0.616(NS)
Total	*75*	*45(100%)*	*30(100%)*	*27(60%)*	*18(60%)*	*45(100%)*	*4.51*	*0.034(S)*
X^2		2.60		4.03				
P value		0.107(NS)		0.045(S)				

NS: No significant difference at $P<0.05$, S: Significant difference at $P<0.05$

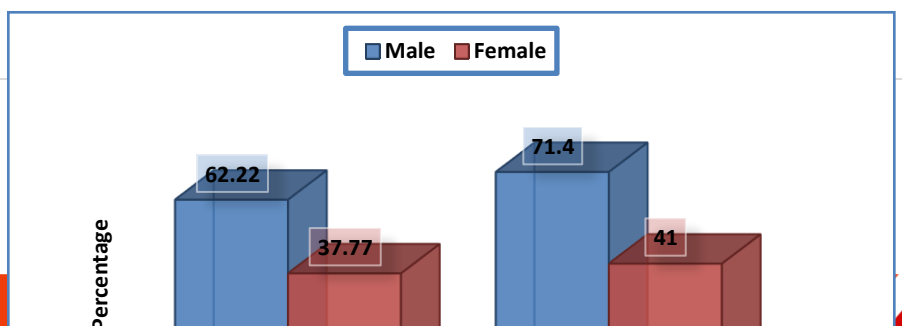

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Fig.2 the rate of patients' dermatophytosis vary with gender.

Table 3 Based on a microscopic examination (KOH), the rate of patients' dermatophytosis varies with age.

age/ year	Samples	(KOH)		Culture		X^2	P value
		Positive	Negative	Positive	Negative		
>1	11	8 (72.7%)	3 (27.2%)	6 (22.25%	2 (11.1%	0.012	0.912(NS)
>10	21	10 (47.6%)	11 (52.3%)	7 (25.9%	3 16.6%	1.37	0.242(NS)
>20	43	27 (62.7%)	16 (37.20%)	14 (51.85%	13 (72.2%	0.818	0.366(NS)
Total	75	45 (100%)	30(100%)	27	18	0	1(NS)
X^2		2.22		1.91			
P value		0.329(NS)		0.384(NS)			

NS: No significant difference at P<0.05, S: Significant difference at P<0.05

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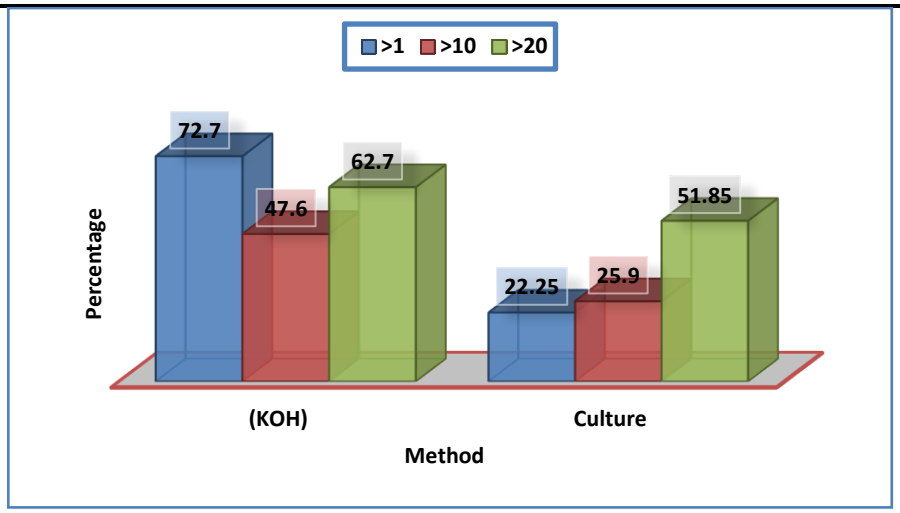

Fig.3 the rate of patients' dermatophytosis varies with age.

The smallest effective amount of an inhibitor and the minimum fungicidal concentration
The MIC range for the R. officinalis leaf extract was 61–255mg/mL.. Wiegand et al. (2016) reported success rates of 82.6% (n = 115) and 87.8% (n = 101) for the Blankophor and culture techniques, respectively, with terbinafine used as a control. The MFC level of the R. officinalis leaf extract, even against pathogen spp. tested, varied between 130 and 520 mg/ml, demonstrating MFC at a dose of 156 mg/ml. It can indicate that the antifungal drug had less of an effect on this specific dermatophyte. (Figs. 3, Table 4).

	Testing microbes(mg/ml)					
	T. tonsurans		*T. mentagrophyte*		*M. audouini*	
	MIC	MFC	MIC	MFC	MIC	MFC
R. officinalis extract	61	128	128	255	255	515
Terbinafine	**78**	**156**	**178**	**313**	**313**	**625**

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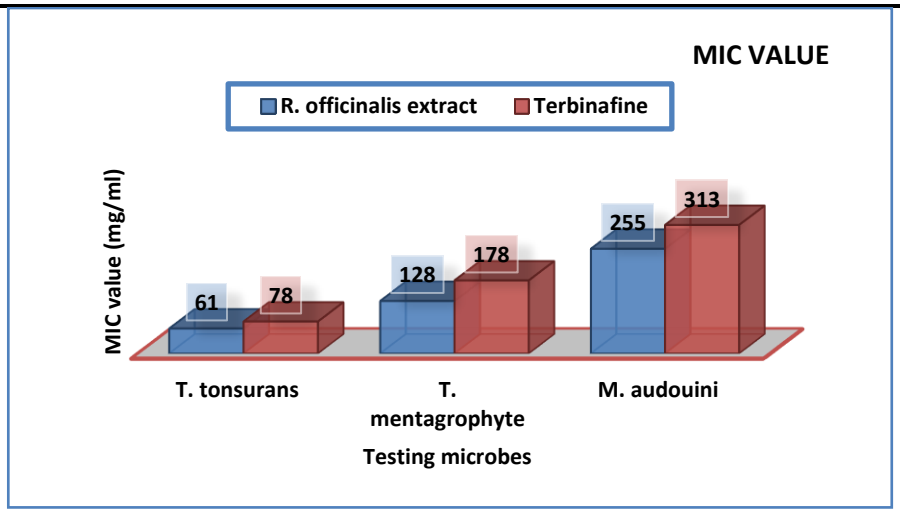

Fig.4 minimal inhibitory concentrations.

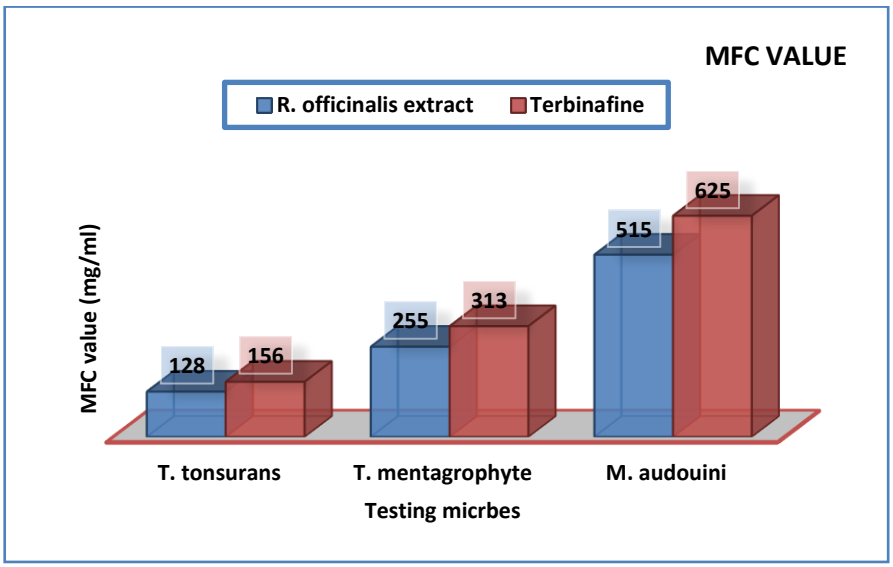

Fig5. Minimum Fungicidal Concentration

Conclusion

According to this study, R. officinalis leaves contain bioactive chemicals with antifungal properties. The hydrsoalcoholic extracts of R. officinalis were successful in changing the hypha's form and inhibiting the development of dermatophytes. This supports the use of it in traditional medicine to treat skin infections. It may be possible to create antifungal treatment plans using these species

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