Research article

In vitro diagnosis of superficial dermatophytes isolated from primary school children in Mukalla city of Yemen and effect of some plant extracts

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ABSTRACT

Introduction and Aim: Dermatophyton fungi are a group of fungi that can attack the keratinous tissues of humans and animals such as hair, nails, and skin. Dermatophytons are skin fungi, and include three genera: *Trichophyton, Microsporium* and *Epidermophyton*. The present study is aimed to diagnose and isolate dermatophytes from primary school children in Mukalla city and prepare herbal extract composition to demonstrate the antifungal activity.

Materials and Methods: During this study, a total 105 samples were collected, including 31 samples from ear, 14 from hair, 13 samples from the hand scrapings and 47 samples from the leg scrapings from the children of the primary stage in the schools of Mukalla city. Plant extracts from *Lawsonia inermis, Curcuma longa, Frangula alnus, Olea europaea, Salvia rosmarinus Sesamum indica and Nigella sativa* were used.

Results: The results showed that the number of the positive samples was 60.95%, while the negative samples were 39.04%, where the most infected areas of the body from which the fungi were isolated were the leg scrapings with a rate of 50.8% at the probability level (*P-value* = 0.649). The most affected age group is (11-15 years) with a rate of 87.7%. The highest percentage of isolated fungi is *Epidermophyton floccosum* (21.5%), followed by *Candida albicans*, *Trichophyton violaceum, Trichophyton mentagrophytes, Trichophyton verrucosm, Microsporum canis*, then the least isolated fungus was *Trichophyton rubrum.*

Conclusion: The inhibitory effectiveness of plant extracts against isolated fungi depended on the type of plants used, its concentration and the type of fungus, as the inhibitory effectiveness was higher for *Lawsonia inermis* extract against the growth of *T. mentagrophytes*. As for the effect of the oils used in this study, the *Nigella sativa* oil had a higher inhibitory effect compared to the other oils, as it had an inhibitory effect against five of the dermatophytes isolated.

Keywords: Skin infection; inhibitory effect; antibiotics; *Lawsonia inermis*; *Curcuma longa*; *Nigella sativa*.

INTRODUCTION

Dermatophytosis is a common fungal infection worldwide, causing morbidity and posing a public health problem, especially in tropical regions like Hadhramout (Mukalla city) due to the hot and humid climate. Skin or nail infections can result from non-dermatophyte fungi and yeast-like fungi, which may also indicate the presence of systemic mycosis (1).

Human skin is a protective tissue structure made of keratin protein. It has several defense mechanisms like dryness, low pH, and fatty secretions. However, vulnerability arises from factors such as immunosuppression, diabetes, burns, and wounds. Skin can be infected by microorganisms that secrete enzymes like keratinase (2). Over the years, there has been a rise in the recognition of non-dermatophyte filamentous fungi as agents of skin and nail infections in humans, producing clinically similar lesions to those caused by dermatophytes (3). Dermatophytes are classified into three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*. They are further grouped into anthropophilic (human-to-human transmission), zoophilic (animal-to-human transmission), and geophilic (transmitted through soil) species (4).

Zoophilic and geophilic organisms elicit a more potent inflammatory response in humans than anthropophilic organisms (5). Tinea infections, commonly caused by dermatophytes, are contagious fungal skin infections that can be transmitted through direct contact, clothing, shower surfaces, and even pets. Practicing good personal hygiene, using antifungal treatments, and avoiding sharing personal items can help prevent and manage Tinea infections (5). The estimated

lifetime risk of acquiring a dermatophyte infection is between 10-20%, and these common fungal infections primarily affect the skin, hair, and nails, causing symptoms such as itching and redness (6). Recognition and appropriate treatment of dermatophytosis, including the use of antifungal medications and herbal therapies, can reduce morbidity, discomfort, and transmission of the infection (7). Treating chronic fungal infections can be challenging, as it requires costly and sometimes ineffective antimycotic drugs (7). Patient non-compliance has been identified as a significant issue in clinical practice, leading to drug therapy failures and poor disease control (8). Main reasons for noncompliance in treatment of superficial mycoses:

- High cost of medicines
- Long duration of therapy (several weeks)
- Patients discontinue application when symptoms improve. This leads to the recurrence of the disease due to interrupted treatment (9).

Since (*Lawsonia inermis, Frangula alnus* and *Curcuma longa*) aqueous plant extracts and oil plant extracts like (*Sesamum indicum, Salvia rosmarinus, Nigella sativa, Olea europaea*) affordable antifungal treatments enable patients to overcome cost barriers and effectively eliminate fungal infections. The present study aimed to diagnose and isolate dermatophytes from primary school children in Mukalla city and prepared herbal extract composition to demonstrate the antifungal activity of them.

MATERIALS AND METHODS

Study design

An experimental analytical study was conducted at the Laboratory of Science Faculty, Biology Department, Hadhramout University, Hadhramaut Governorate, Yemen, during March-June 2023, focusing on a specific research objective.

Study samples

One hundred and five clinical samples were collected including (hand skin scabs - hair samples - ear samples and leg scale samples) from different age groups of primary school students, during the period March 2023 to June 2023, and a questionnaire was assigned to each student.

Collection of samples

Samples were collected from students by sterilizing the affected area with alcohol-soaked cotton to remove bacteria and substances that could obstruct examination. The samples were taken from various areas and were intended for direct microscopic examination. Samples were collected from different areas, which are the following:

- The skin scales were collected from the edge of the affected area, as they contain the fungal hyphae formed by the fungus causing the infection, by scraping the area using a sterile surgical blade.
- Hair and scales samples were collected from the edge of the affected area in the head, using sterile forceps.

The samples were divided into two parts - one for microscopic examination and the other for cultivation on SDA media. Microscopic examination was performed on a clean glass slide, while the rest of the scales were planted on the central region of the SDA media for growth and identification (1).

Diagnosis

Direct microscopic examination

Small amount of skin scrapings, hair, or thin keratinized residue from the skin were taken using inoculation needles, one small part of samples was placed on a clean, sterile glass slide and added (1 or 2) drops of 15% potassium hydroxide solution, then covered with a cover. The slide was heated gently by moving it over the flame of a bunsen lamp two or three times, avoiding boiling. Then the slide was left for several minutes until the keratin dissolved and gently pressed on it to spread the sample, then examined microscopically under 10X and 40X magnification to see the mycelium structures and hyphae conidia (10).

Culturing of samples

The isolates were taken and grown in petri dishes with a diameter of 9 cm containing 20 ml of SDA medium. The dishes were incubated at 28±2^{o}C for a period of 2-4 weeks (11).

Phenotypic examination of fungal colonies

Characteristic features were used to differentiate between dermatophytes, which include growth rate or speed (fast or slow), the surface of fungal colony (flat or containing regular or irregular folds), the texture of the colony (yeasty, smooth, granular, velvety, or cottony), morphology of colony (appearance): (powdery, cottony, fluffy), and color of colony (12).

Colonial microscopy

Microscopic examination was performed to observe the various fungal structures, such as the fungal filaments, shapes, and Macroconidia and Microconidia; (shapes, sizes, number, and wall thickness), chlamydospores and arthrospores, by taking a part of growth fungal colony using a sterile needle and placing it in a drop of Lactophenol stain (LPCB) on a slide and then examined using a light microscope with a lens magnification of 10X, 40X according to Dismukes *et al*., Ellis *et al*., and Jorgensen *et al*., (13-15).

Plant sample collection and extraction

Plant sample collection, identification, and drying were done according to the method described by Esazah (16). Plant samples (*Lawsonia inermis, Frangula alnus* and *Curcuma longa*) were purchased

ready-made from stores that sell dry plant fine powders, crushed locally using an electric grinder, and stored in Sterile plastic bags, and preserved until used to making aqueous plant extracts. Also, plant oils extracts (*Sesamum indicum, Nigella sativa,* local *Olea europaea*) were prepared manually, but *Salvia rosmarinus* and non-local *Olea europaea* were obtained from herbal shops.

Preparing the aqueous extract

Twenty grams of dry powder of plants (*Lawsonia inermis, Frangula alnus* and *Curcuma longa*) were taken and placed in conical flasks and added 200 ml of distilled water. The samples were left to settle for one day in a dark place, when in use were filtered with three layers of medical gauze, then the extracts were placed in plastic boxes and stored in the refrigerator until use, (10% concentration), according to Jannah and Al-Muhaishi (17), using following equation:

Volumetric mass percentage (%) = powder mass (g) / distilled water volume (ml) x 100.

RESULTS

Prevalence rate of dermatophytosis among primary school students

Out of 105 samples collected, 31 samples from the ear, 14 from hair, 13 samples from hand scrapings, and 47 samples from leg scale. The results showed that the percentage of infection was 60.95%, this result was in agreement with a study of Al-Said and Al-Assawi (18). The highest fungi infection rate was leg scale samples 33(70.21%; Table 1 and Fig. 1), the same result was observed by Al Masoudi (19), in which the highest fungal infection rate was of skin scrapings 75.32%. There is a significant relationship between infected and uninfected samples at (*P-value* =0.00), and Chi-square ($\chi 2$) = -63.64.

Identification and distribution of isolated dermatophytes

The dermatophytes identified were *Epidermophyton floccosum, Trichophyton violaceum, Trichophyton mentagrophytes, Trichophyton verrucosm, Microsporum canis, Trichophyton rubrum and Candida albicans* (Table 2, Figs. 2 & 3; 18). The highest percentage of isolated fungi was *Epidermophyton floccosum* 14 (21.5%), where the most infected areas of the body were leg scrapings (8 isolates). The least isolated fungus was *Trichophyton rubrum* (11,18). There is no significant relationship between the species of isolated fungi and their locations in the body (*P-value* = 0.649).

Table 3 shows the distribution of prevalence of isolated dermatophytes according to age groups, the highest rate of dermatophytes infection was 87.7% of age group (11-15 years), followed by age group (≤10) years with an infection rate (10.8%), forever, the group of children over than 15 years of age was the least rate (1.5%), the statistical analysis showed that there is no significant relationship between the isolated fungal species and age groups (*P-value* = 0.084).

Table 1: The percentage of dermatophytes prevalence during the study

Samples	Number	Percentage(%)	Samples infected		Samples non-infected		*p-value*	Chi-square ($\chi 2$)
			No.	%	No.	%		
Ear	31	29.52%	14	45.16%	17	54.83%	0.00	63.64-
Hair	14	13.33%	9	64.28%	5	35.71%		
Hands	13	12.38%	8	61.53%	5	38.46%		
Legs	47	44.76%	33	70.21%	14	29.78%		
Total	105	100 %	64	60.95%	41	39.04%		

Degree of confidence (significant) at *P value* level: ≤ 0.05 Chi-square ($\chi 2$) = 0.2172

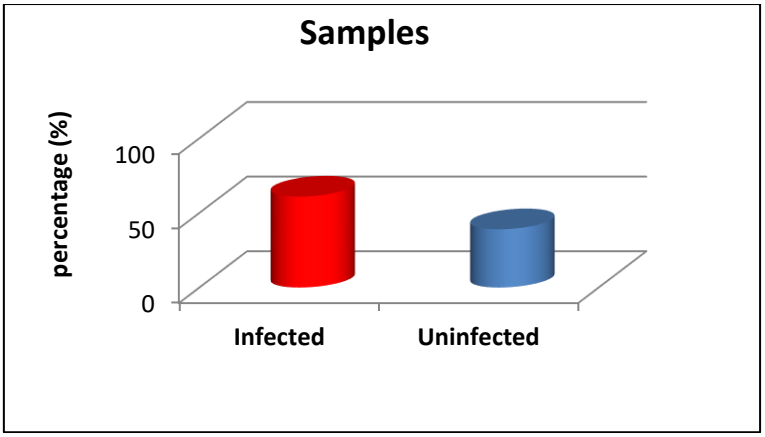


Fig.1: Percentage of samples tested and rate of dermatophyte infection

Table 2: Percentage of dermatophyte species isolated according to their location of body

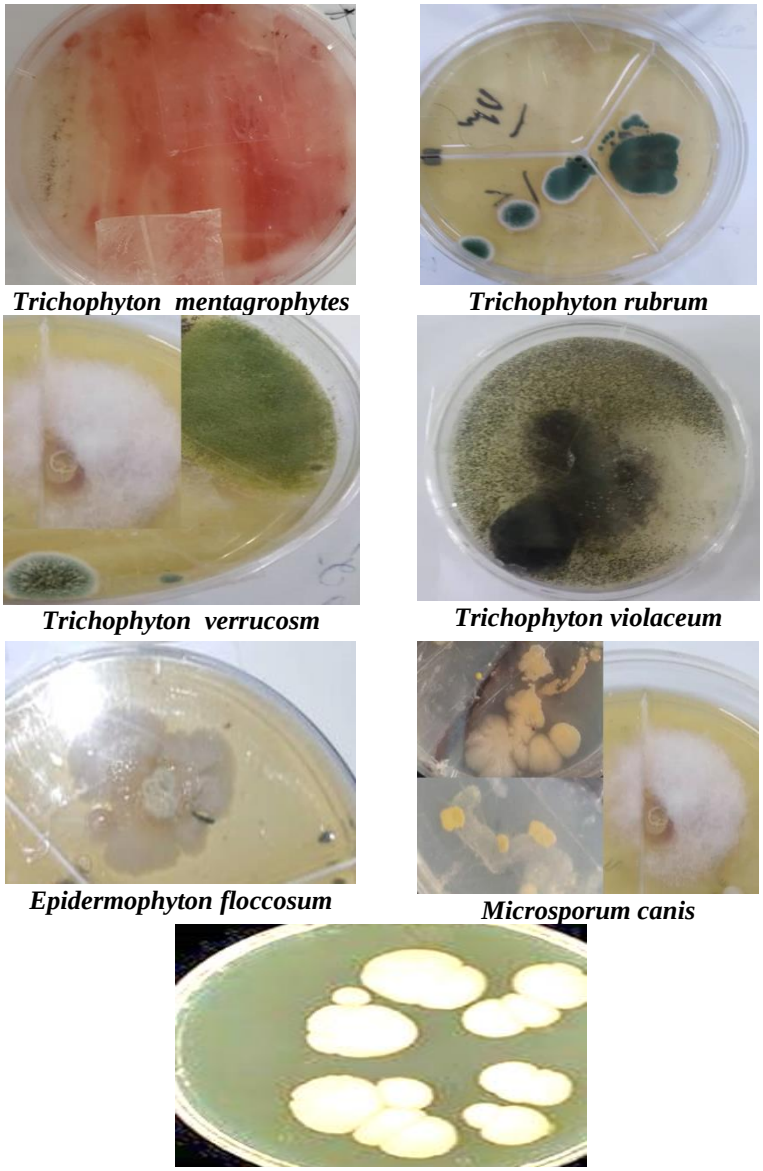
Isolated fungi	Number of isolated fungi from samples				Total (%)	*p-value*
	Ear	Hair	Hands	Legs		
Epidermophyton floccosum	4	0	2	8	14) 21.5%(	0.649
Microsporum canis	2	1	0	2	5) 7.7%(	
Candida albicans	0	2	3	8	)13 20%(	
Trichophyton violaceum	4	1	1	5	11)16.9%(	
Trichophyton rubrum	0	2	0	3	5) 7.7%(	
Trichophyton mentagrophytes	4	1	0	4	9)13.8%(	
Trichophyton verrucosm	1	2	2	3	8)12.3%(	
Total (%)	15 23.1%)(	9)13.8%(	8)12.3%(	33)50.8%(	65	

Degree of confidence (significant) at *P value* level: ≤ 0.05

Table 3: Distribution of the prevalence of isolated dermatophytes according to age groups

Age group (year)	Isolated dermatophytes							Total (%)	*P value*
	E. floccosum	*M.canis*	*C.albicans*	*T. violaceum*	*T. rubrum*	*T. menta-rophytes*	*T. verrucosm*		
≤10	1	2	0	2	0	2	0	7(10.8%)	0.084
15-11	13	3	13	8	5	7	8	57(87.7%)	
15<	0	0	0	1	0	0	0	1(1.5%)	
Total (%)	14(21.5%)	5(7.7%)	13(20%)	11(16.9%)	5(7.7%)	9(13.8%)	8(12.3%)	65(100%)	

Degree of confidence (significant) at *P value* level: ≤ 0.05)



Trichophyton mentagrophytes ***Trichophyton rubrum***

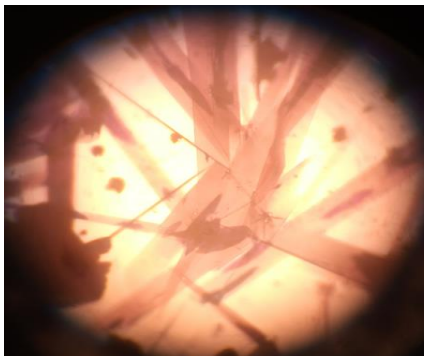
Trichophyton verrucosm ***Trichophyton violaceum***

Epidermophyton floccosum ***Microsporum canis***

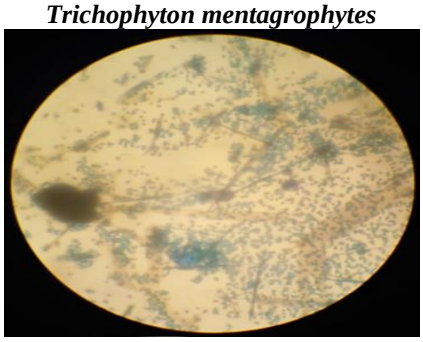
Candida albicans

Fig. 2: Morphology of dermatophyte isolated after cultivation in the culture medium (SDA)

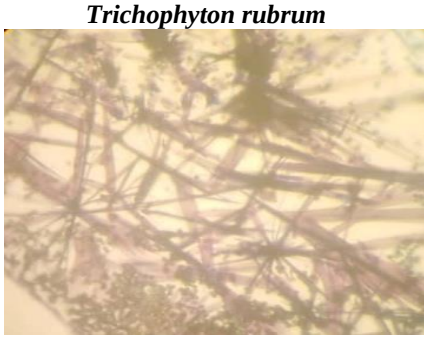
Trichophyton mentagrophytes



Trichophyton rubrum



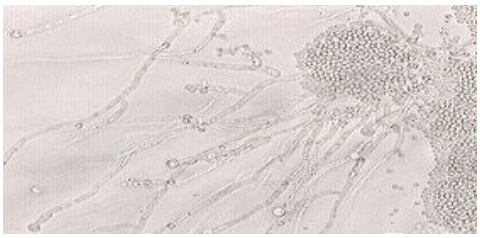
Trichophyton verrucosm



Trichophyton violaceum

Epidermophyton floccosum

Microsporum canis



Candida albicans

Fig. 3: Microscopic examination of isolated dermatophyte stained with methylene blue at 40x magnification, showing mycelium and hyphae

Table 4: The effect of plant aqueous and oil extracts on the growth of isolated dermatophytes, showing inhibition zones

Isolated dermatophytes	**Diameters of inhibition zones (mm) Extracts plant (aquatic and oily)**										
	Lawsonia inermis		*Curcuma longa*		*Frangula alnus*		*Sesamum indicum*	Local *Olea europaea*	*Global Olea europaea*	*Salvia rosmarinus*	*Nigella sativa*
	Concentration										
	10%	15%	10%	15%	10%	15%	natural	natural	Un-natural	Un-natural	natural
E. floccosum	23.6	24	21.6	28	22.3	0	22	0	0	24.3	27
M. canis	18.3	18.3	0	0	0	25.6	0	40	0	19.6	0
C. albicans	18.3	28.6	25	0	23	0	0	26	0	0	23
T. violaceum	24.6	24	0	0	0	0	0	23.3	0	0	26
T. rubrum	24.6	24	5	0	0	0	0	0	0	0	0
T.mentagrophytes	31	28.3	0	0	0	0	0	25	0	0	23.3
T. verrucosm	25	28	0	26.6	0	0	0	0	0	0	44

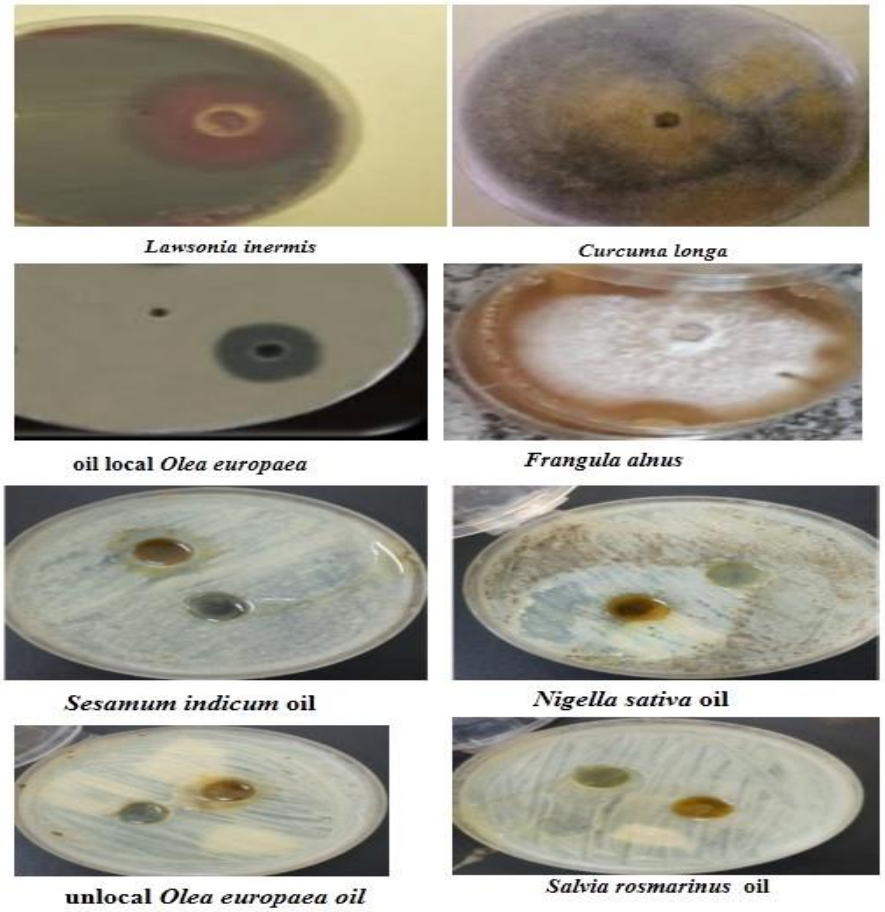


Fig. 4: The effect of plant aqueous and oil extracts on the growth of isolated dermatophytes, showing inhibition zones

DISCUSSION

Dermatophyte infection is a highly contagious condition that can be transmitted through person-to-person contact or via inanimate objects like clothing and combs, posing a significant public health problem worldwide (20). Sixty-five dermatophyte fungi, belonging to seven different species, were isolated and classified based on references by Ellis *et al* (14) and Jorgensen *et al.* (15). The playing habit of children may make them in contact with contaminated soils and animals like gout, sheep, cats, and local dogs which are a known source of infections (20). A study conducted by Kafi and Al-Harir (21) found that the highest infection of dermatophyte fungi was observed in the age group of 11-20 years. Exposure to contaminated soils and animals such as gout, sheep, cats, and local dogs can lead to the transmission of infections (20) This study agrees with Kafi and Al-Harir (21), who indicated that the highest infection of dermatophyte fungi was in the age group of 11-20 years.

Effect of some plant aqueous and oil extracts on the growth of isolated dermatophytes

The results showed that the inhibitory effectiveness of aqueous and oily plant extracts used against the isolated fungi depended on type of extract, its concentration, and the species of fungus. In this study, inhibitory effectiveness of *L. inermis* extract was highest against growth of *T. mentagrophytes* at a concentration of 10%, where the average diameter of the inhibition zone was about (31 mm). As for the *Curcuma longa* extract, it was more effective inhibiting against *E. floccosum* at a concentration of 15%, as the inhibition zone was (28 mm). *Frangula alnus* extract showed inhibitory activity only against *M. canis*, as the inhibition zone reached (25.6 mm) at a concentration of 15% (Table 4).

L. inermis leaves contain the main active compounds, which possess antibiotic properties against microbes and have a toxic effect on fungi (22). Also, it contains phenolic compounds (which are found in high concentrations and are among the most prominent natural antioxidants, including flavonoids, tannins, carotenoids, and phenolic acids), flavonoids, carbohydrates, proteins, tannins, alkaloids, and fatty acids (23). *Frangula alnus* leaves contain flavonoids, tannins, and alkaloids (24). In addition, it contains saponins, sterols and triterpenes as active compounds (25).

In this study, *Nigella sativa* oil extract was the highest inhibitory effective compared to the other oils, as it had an inhibitory effect against five species of isolated dermatophytes, with inhibition zone reaching about (44 mm) against the growth of *T. verrucosm* isolates, because *Nigella sativa* oil contain active compounds (alkaloids, resins, saponins, tannins, coumarins, and

flavones). It also contains alkaloids of its own that are not found in other medicinal plants. They are called Nigellicine, oxide, Nigellimine-N-, and Nigellidine (26). Followed by (local) *Olea europaea* oil, as it had an inhibitory effect against four species of isolated dermatophytes, the highest inhibitory effect was against *M. canis* isolates about (40 mm), followed by *Salvia rosmarinus* oil. had an inhibitory effect against only two species of isolated fungi, as it's the highest inhibition zone reached about (24.3 mm) against *E. floccosum*, followed by *Sesamum indicum* oil was inhibitory effect against *E. floccosum* only (22 mm) in diameter. However, (global) *Olea europaea* oil did not show any inhibitory activity against all dermatophyte isolates (Fig. 4). This result is confirmed by Hbeel, who pointed out that natural *Olea europaea* (olive) oil is antifungal because it contains oleic and palmitic acid along with antioxidants such as (flavonoids, vitamin E, and carotene; 27). In addition, *Sesamum indicum* seeds contain active compounds (flavonoids, fucoumarins and tannins; 28). The *Salvia rosmarinus* plant contains aromatic oils, their oil is composed of terpenes, borneol, and cineol, which are formed in its leaves and used as antioxidants. Its seeds also contain substances like antibiotics(29).

CONCLUSION

Herbal therapies have been used for thousands of years to treat dermatological disorders and dermatophytosis. They are gaining popularity among patients and physicians as they have shown significant therapeutic potential. However, more *in vivo* studies are needed to determine their clinical effectiveness.

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CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

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