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ORIGINAL ARTICLE

Activity of Some Natural Oils on Dermatophytes Isolated from Assuit University Hospitals

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ABSTRACT

Background: *Dermatophytosis constitutes a major public health problem in different countries. The most common factors affecting the distribution and transmission of dermatophyte infections are climatic conditions, general hygiene, and animal contact. In Egypt, the prevalence of dermatophytosis has not been well clarified. Several antifungal agents are available to manage these infections but drug resistance, severe side effects and its very poor penetration can result in treatment failure.* ***Objectives:*** *This study aimed to detect the prevalence of dermatophyte infections in Assuit University Hospital and to screen for anti-dermatophytic activity of some plant essential oils to discover their medicinal potential for future applications as antifungal agents.* ***Methodology:*** *Samples were collected from 50 patients attending dermatology clinic at Assuit University Hospital and clinically diagnosed as tinea infections. Isolation and laboratory identification of dermatophytes were performed by direct microscopic examination, Sabouraud's dextrose agar and rice grains medium culture. Extracellular enzymatic activities (urease, lipase and protease) were tested. Some natural oils (dill seed, garlic, ginger, nigella seed, olive, peppermint and riccinus) were screened for their anti-dermatophytic activities by disc diffusion methods.* ***Result:*** *Dermatophytosis was diagnosed in 40 (80%) of all patients examined, the prevalence of dermatophytosis in male (57.5%) was higher than in female (42.5%), the most common dermatophyte infection diagnosed was tinea capitis (65%), followed by tinea pedis (17.5%). Four species belonging to two genera were identified, 20 (50%) T. violaceum, 14 (35%) M. canis, 3 (7.5%) T. mentagrophytes and 3 (7.5%) T. rubrum. All tested oils have dermatophytic activities except dill and olive oils. Nigella oil was the most active against growth of T. mentagrophytes and T. rubrum. Ginger oil was the most active against T. violaceum and M. canis. Garlic and ginger oils had active effect against T. rubrum.* ***Conclusion:*** *Nigella, ginger and garlic oils had proved a promising effectiveness against dermatophytes and can be used in the development of new anti-dermatophytic preparations.*

INTRODUCTION

Human mycoses are caused by primary pathogenic fungi that invade the tissues of a normal host, or by opportunistic fungi that invade the tissues of individuals with severe alterations in the immune system. Dermatomycosis is one of the most frequent dermal lesions that affect human and animals, and is caused by dermatophytes of the genera *Epidermophyton*, *Microsporum* and *Trichophyton*. These fungi infect keratinized tissue such as skin, nails and scalp and their lesions are characterized by irritation, scaling, local redness, swelling and inflammation[1].

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Dermatophytic fungi prevalence is much higher in developing than in industrialized countries. They are believed to affect 20% to 25% of the world's population and the incidence continues to increase. The most common factors affecting the distribution and transmission of dermatophytes infections are climatic conditions, general hygiene, and animal contact [2, 3].

Infection is generally cutaneous and restricted to the nonliving cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of immunocompetent hosts [4]. Reactions to a dermatophyte infection may range from mild to severe as a consequence of the host's reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection[5]. Exoenzymes found to be produced by dermatophyte are keratinase, lipase and phospholipase, protease, elastase, and collagenase[6].

Despite of different techniques which have been used for detection of fungal exo-enzymes, the use of solid media permits a rapid screening of large numbers of fungal isolates for enzyme production [7].

Several antifungal agents can be used to manage these infections. Unfortunately, drug resistant can result in treatment failure[8]. The available antifungal agents include allylamine, azoles and others have severe side effects and are inefficient. Dermatologists often attribute treatment failures to short therapies and lacking the drug specificity. Actually the penetration of antifungal drugs is very poor in respect to nails [9].

It is worth noting that natural oils have proved promising effectiveness against dermatophytes, and may be incorporated into pharmaceutical preparations[10,11,12]. The present study was undertaken to establish the anti-dermatophytic activity of some natural oils. Also to detect the prevalence of dermatophyte infections in Assuit University Hospital

METHODOLOGY

1. Clinical examination and specimen collection:

Skin scrapings, hair fragments, toe nail cuttings and finger nail cuttings were collected aseptically from 50 patients with suspected dermatophytic infections were referred to Dermatology Clinic at Assiut University Hospital. They were presented with tinea pedis, tinea capitis, tinea corporis, tinea cruris and onchomycosis.

2. Direct Microscopic examination and culture of collected specimens:

a. Direct Microscopic Examination:

Wet mounts were prepared from each sample by treating the sample first with few drops of 10–20% potassium hydroxide (KOH) with or without dimethyl sulfoxide (DMSO) followed by gentle heating to allow digestion of the keratin and then examined microscopically to detect fungal elements; hyphae or spores[13].

b. Culture:

The samples were inoculated on the surface of the Sabouraud' s Dextrose Agar (SDA) plates (Himedia, India) supplemented with Chloramphenicol (250 mg/L) and Cyclohexamid (0.5 g/L). Cultures were incubated at 25 °C for to 4 weeks [14].

3. Identification of dermatophyte isolates:

a- Macroscopic and microscopic features on SDA:

Dermatophytes were identified according to their macroscopic features on SDA plates and microscopic features in Lacto phenol cotton blue (LPCB) (Himedia, India) stained wet mounts [15, 16].

b- Growth on rice grains medium:

Autoclaved rice grains were used as a selective medium to induce sporulation and differentiation of *M. canis.* After inoculation with the test fungi, plates were incubated at 25 °C for 7-14 days [17].

4. Detection of extracellular enzymatic activities of isolated dermatophytes:

a. Lipase production:

It was tested on the medium that has the composition of (gm/L): peptone 10, $MgSO_4$.$7H_2O$ 0.2, $CaCI_2.2H_2O$ 0.2, Tween 20 10 ml and agar agar 15. The medium was sterilized by autoclaving at 121°C for 15 minutes. The Tween 20 was autoclaved separately and added to the sterile and cooled basal medium. The medium was dispensed aseptically in 15 cm test tubes (10 ml/tube). Test tubes were inoculated on the surface of the medium and incubated at 25 ºC for 10 days. The lipolytic ability by a fungus was observed as a visible precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme. The depth of each visible precipitate (in mm) was measured [18].

b. Protease production:

It was tested using casein hydrolysis medium which has the composition of (g/L): KH_2PO_4 1.0, KCL 0.5, $MgSO_47H_2O$ 0.2, $CaCI_2.2H_2O$ 0.1, 15 % skimmed milk 25 ml, glucose 10 and agar agar 15. The medium was sterilized by autoclaving at 121°C for 15 minutes. The skimmed milk was sterilized separately by autoclaving at 115°C for 10 minutes. Both parts are left to cool, mixed together and poured in sterile 15 cm test tubes (10 ml/tube).

The test tubes containing medium were inoculated with the tested fungal isolates and incubated at 25 °C for 7 days. After incubation, lipolytic activity of the fungus is detected as complete degradation of milk proteins that appears as clear depth in the tube. The clear depth below the colony was measured (in mm) [19].

c. Urease production:

Christensen's urea medium was employed with some modifications. The medium was prepared as 2 separate parts, the first part composed of (g/L): peptone 1, KH_2po_4 1, KCl 0.05, yeast extract 1and phenol red 0.012. These components were dissolved in 800 ml distilled water. The second part composed of (g/L): glucose 5, $MgSO_4.7H_2O$ 0.5 and distilled water 200 ml.

The two parts were mixed after autoclaving and cooled to 50°C. Aliquots of 5 ml of 40 % solution of sterilized urea were added to each 100 ml of the medium. The medium was poured into 15 ml test tubes (5 ml for each) and the tubes were inoculated with the desired fungi and incubated at 25 °C for 3 days. After incubation the results were recorded as positive after appearance of a deep pink color in the broth medium [20].

5. Anti-dermatophytic activities of some natural oils by disc diffusion method [21]:

Seven pure extracted natural oils (dill seed, garlic, ginger, nigella seed, olive, peppermint and riccinus) were collected from the local market to evaluate their anti-dermatophytic activity. Major components and functional groups of these oils are shown in table 1. Sterilized filter paper discs 0.5 cm diameter (Wattman No.1) was saturated with each oil with concentration of

99%. Similarly, filter- paper discs were impregnated in solution of standard antifungal (Fluconazole, Sigma) (10 mcg/disc) for comparison of antifungal activity of tested natural oils.

Pure cultures of the 40 tested fungal isolates were used; spore suspension of each isolate was prepared in a test tube by transferring of approximately 1cm^2 from a seven days-old culture on SDA and suspending it in 5ml distilled water supplemented with two drops of Tween 80 followed by vigorous shaking. Aliquots 1ml of each fungal spore suspension were transferred under sterilized conditions into sterile Petri dishes before the SDA medium was poured, swirled and allowed to solidify. After agar solidification, three discs of each oil sample and fluconazole standard were transferred to each plate inoculated by one of the tested organisms. Three replicates were done for each tested oils, diameters of the inhibition zones were measured in mm and average values of inhibition zones were calculated. The activity index of each tested oils was calculated. Activity Index = Average of inhibition zones of sample / Inhibition zone of standard.

Table 1: Major components and functional groups of tested essential oils:

Common Name	***Plant Botanical Name and family***	***Phytochemical screening***	***References***
Dill seed oil	*Nethum graveolens* (Apiaceae)	Tannins, Glycosides, Saponins, Steroids, Terpenoids	**22**
Garlic oil	*Allium sativum*	tannin, flavonoid, saponin, alkaloid and glycoside	**23**
Ginger oil	*Zingiber officinale* (Zingiberaceae)	alkaloids,saponins, flavonoids, polyphenols,cardiac glycosides	**24**
Nigella seed oil	*Nigella sativa* (Ranunculaceae)	Flavonoid, Tannin, Steroid and Triterpine, Saponin, Alkaloid, Cardiac glycoside	**25**
Olive oil	*Olea europaea* (Oleaceae)	palmetic acid, Z-nerolidol, octacosane, caryophyllene oxide and tetracosane.	**26**
Peppermint oil	*Mentha piperita* Lamiaceae (Mint Family)	alkaloids, flavonoids, steroids, tannins, and phenols	**27**
Castor Oil	*Ricinus communis* (Euphorbiaceae)	Alkaloids, Resins and flavonoids	**28**

RESULTS

Among 50 patients examined, 40 (80%) patients (23 males and 17 females) showed positive clinical presentations of dermatophytes infections (tables 2). 21 (52.5%) of these positive cases had history for contact with domestic animals (i.e. cats, dogs and cattle) and 12 (30%) of them had previous dermatophytes infections (tables 3).

Table 2: Clinical presentations of patients included in the study

Clinical presentation	***NO.***	***%***
Tinea capitis	26	65
Tinea pedis	7	17.5
Tinea corporis	5	12.5
Onychomycoses	2	5
Total	40	

Table 3: History of animal contact and previous dermatophytes infections among patients included in the study

Cases	**Positive**		**Negative**	
	NO.	**%**	**NO.**	**%**
Animal contact	21	52.5	19	47.5
Previous dermatophytes infection	12	30	28	70

For these patients, 14 samples (35%) were positive with direct microscopic examination compared to 26 (65%) positive samples when inoculated on SDA (tables 4).

Table 4: Results of Direct Microscopic Examination versus culture on SDA

Method	***Positive***		***Negative***	
	NO.	**%**	**NO.**	**%**
Direct Microscopic Examination	14	35	26	65
Culture on SDA	26	65	14	35

Four species belonging to two genera were identified, 20 (50%) *T. violaceum* isolates, 14 (35%) *M. canis* isolates, 3(7.5%) *T. mentagrophytes* isolates and 3(7.5%) *T. rubrum* isolates (Fig. 1, 2, 3 and 4 and Table 5). These isolates showed high lipolytic, proteolytic and urealytic activities (Table 6).

Table 5: Dermatophytes isolates as identified on SDA and rice grains medium

Species	***NO.***	***%***
T. violaceum	20	50
M. canis	14	35
T. mentagrophytes	3	7.5
T. rubrum	3	7.5
Total	40	

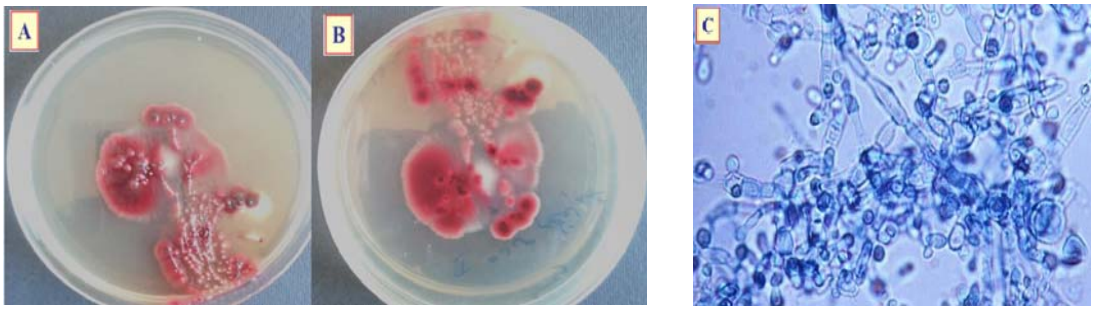

Fig. 1: *T. violaceum*; A. surface of the colony on SDA, B. dorsum of the colony on SDA and C. microscopic appearance in LCB stained wet mount.

Fig. 2: *M. canis*; A. surface of the colony on SDA, B. dorsum of the colony on SDA and C. microscopic appearance in LCB stained wet mount.

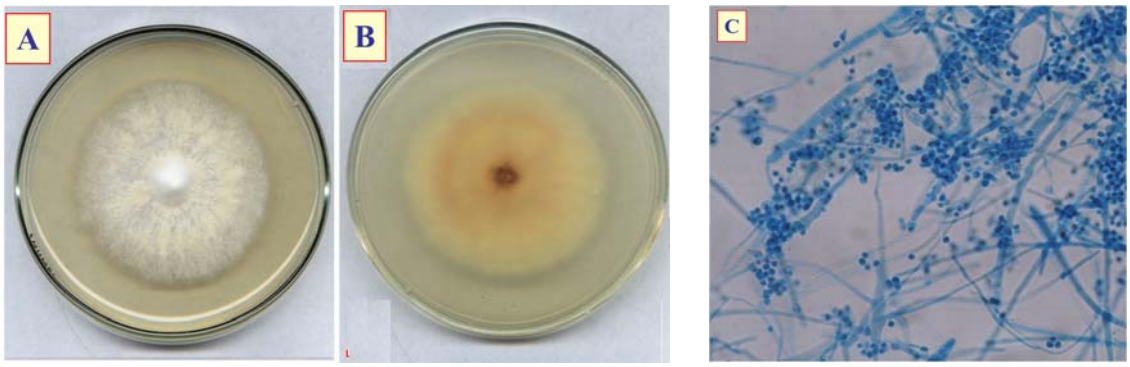

Fig. 3: *T. mentagrophytes*; A. surface of the colony on SDA, B. dorsum of the colony on SDA and C. microscopic appearance in LCB stained wet mount.

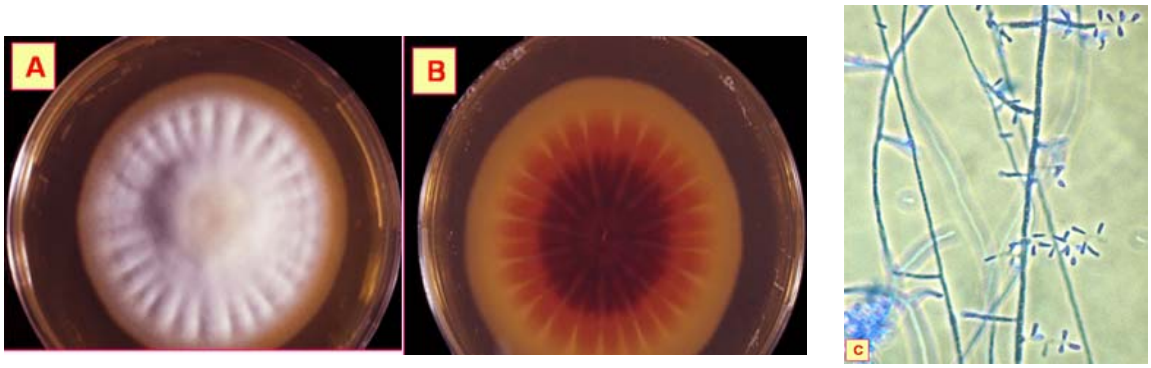

Fig. 4: *T. rubrum*; A. surface of the colony on SDA, B. dorsum of the colony on SDA and C. microscopic appearance in LCB stained wet mount.

Table 6: Screening of isolated dermatophytes for some extracellular enzymes production:

Species	*Lipase*		*Protease*		*Urease*	
	+ve	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**
T. violaceum (20 isolates)	14 (70%)	6 (30%)	15 (75%)	5 (25%)	18 (90%)	2 (10%)
M. canis (14 isolates)	12 (85.7%)	2 (14.3%)	12(85.7%)	2 (14.3%)	14 (100%)	0 (0%)
T. mentarophytes (3 isolates)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0(0%)
T. rubrum (3 isolates)	1(33.3%)	2 (66.7%)	1 (33.3%)	2 (66.7%)	3 (100%)	0 (0%)
Total (40)	30(75%)	10(25%)	31(77.5%)	9(22.5%)	38(95%)	2 (5%)

Regarding the inhibitory effects of the tested natural oils against dermatophytic isolates; dill seed and olive oil had no effect against all tested dermatophytic fungal isolates while garlic, ginger, nigella, peppermint and castor oils displayed variable anti-dermatophytic activities (table7).

Table 7: Sensitivity of dermatophyte isolates to tested natural oils.

Natural oil	*No. and percent of dermatophyte isolates sensitive to tested oils*				*Total*
	***T. violaceum* (20)**	***M. canis* (14)**	***T. mentarophytes* (3)**	***T. rubrum* (3)**	*(40)*
Dill seed oil	Zero	Zero	Zero	Zero	Zero
Garlic oil	2 (10%)	2(14.3%)	Zero	One (33.3%)	5(12.5%)
Ginger oil	5 (25%)	10 (71.4%)	Zero	2(66.6%)	17 (42.5%)
Nigella seed oil	5 (25%)	11 (78.6%)	3(100%)	2(66.6%)	21 (52.5%)
Olive oil	Zero	Zero	Zero	Zero	Zero
Peppermint oil	6 (30%)	8(57.1%)	2(66.6%)	2(66.6%)	18(45%)
Castor Oil	2(10%)	4(28.57%)	2 (66.6%)	2(66.6%)	10(25%)

DISCUSSION

The spreading of dermatophytosis in most developed countries of the world represents a considerable public health and economic problem. In poor developing countries, mycoses appear endemic and their treatment often fails because of the lack of efficient antifungals and also duo to drug resistance.

In Egypt, the prevalence of dermatophytosis has not been well clarified. In this study, dermatophytosis was diagnosed in 40 (80%) of the 50 patients examined in Dermatology Clinic at Assiut University Hospital. Our study showed that the prevalence of dermatophytosis in male (57.5%) was higher than in female (42.5%), Similar finding were obtained by Aghamirian and Ghiasian [29].

We found that Tinea capitis accounts for (65%) of manifestation of dermatophytosis followed by Tinea pedis (17.5%) and Tinea corporis (12.5%). Similarly, some studies revealed that tinea capitis is dominant in Africa [29]. In Europe, tinea corporis and tinea pedis were the most common clinical forms of dermatophytosis [30].

Four species belonging to two genera were identified; *T. violaceum* was the most frequently isolated dermatophyte (20 isolates, 50%), *M. canis* (14 isolates, 35%), *T. mentagrophytes* (3 isolates, 7.5%) and *T. rubrum* (3 isolates, 7.5%). In fact, *T. violaceum* has been detected as the most prevalent species in Libya (50**%**) [30], Ethiopia (81.6%) [32] and Pakistan (65%) [33].

Microsporum canis is the most important dermatophyte of domestic animals. The frequency of *M. canis* infection in dogs and cats varies from 40% to 90%[34]. High prevalence of *M. canis* isolates in our study reflects the degree of contact between the human and animal populations, as twenty one (52.5%) of the positive cases had history for contact with domestic animals.

Extracellular enzymatic activities of isolates (urease, lipase and protease exoenzymes) were done to determine their degree of virulence. These isolates showed high lipolytic, proteolytic and urealytic activities. The role of these enzymes is suggested to maintain the function of the fungal cell membrane and to aid in the invasion of host tissue [35].

The production of lipase enzymes were detected by most of isolates, 14 (70%), 12 (85.7%), 3(100%) and 1 (33.3%) by *T. violaceum, M. canis, T. mentarophytes and T. rubrum* respectively. Similar results reported that over 75% of *M. gypseum, M. canis, E. feoccosum* and *T. mentagrophytes* are lipase producers, while only one strain of T. rubrum secreted this enzyme [35].

Dermatophytes showed protease activity which may be needed by these pathogens merely for penetration of the skin or to utilize protein for their nutrition [36].

The plant oils have been reported to have antibacterial, antifungal, antiviral, anti-parasitic and anti-dermatophytic properties. It is now considered as a valuable source of natural products for development of medicines against various diseases and also for development of industrial products[37]. For this purpose we investigated the anti-dermatophytic activities of seven natural oils from medicinal plants against our dermatophytic isolates.

All selected oils had dermatophytic activities except dill and olive oils. Regarding number of

inhibited tested isolates; nigella oil (*N. sativa*) was the most active one followed by peppermint oil then ginger oil. The pharmacological properties of *N. sativa* and its ingredients had been investigated *in vitro* and *in vivo* studies conducted on human and laboratory animals. These studies showed that *N. sativa* and its ingredients have a wide range of pharmacological effects; immune-stimulatory, anti-inflammatory, hypoglycemic, antihypertensive, antiasthmatic, antimicrobial, antiparasitic, antioxidant and anticancer effects [38, 39].

The second and third oils ranking were peppermint oil (*Mentha piperita*) and ginger (*Zingiber officinale*), respectively. They had inhibitory action against 18 and 17 isolates contributing 25% and 24% of total studied dermatophytic fungal isolates with range of inhibition zone from 0.8±0.1 to 1.5±0.4 and 0.8±0.05 to 1.5±0.2, respectively.

Moghaddam *et al*.[40] support these results because they analyzed the essential oil of *Mentha piperita* and evaluated it *in vitro* for antifungal activity against *Dreschlera spicifera*, *Fusarium oxysporum* and *Macrophomina phaseolina* and found that major compounds identified in peppermint oil were menthone, menthol, menthofuran, β-phellandrene, isomenthone, menthol acetate, pulegone, β-caryophyllene, neomenthol, 1,8-cineole and the oil was found to be effective against these fungal pathogens under laboratory screening [39].

Also, Sa-Nguanpuag *et al*.[41] confirm our result where they investigated capacity of ginger oils to inhibit microorganisms was studied. They found that the major constituents of ginger oil inhibited *Bacillus subtilis*, *Bacillus nutto*, *Pseudomonas aerugenosa*, *Rhodoturola* sp., and *Fusarium* sp.**.**

The least active oil was garlic (*Allium sativum*) which had inhibitory effect against only five tested isolates (range: 0.5±0.1 to 1.5±0.3) followed by castor oil (*Ricinus communis*) which had inhibition activity against ten studied dermatophytic fungal isolates (range: 0.5±0.1 to 0.9±0.2). Verma *et al*.[42] agree with our obtained results, they investigated the antifungal activities of essential oils of nine medicinal plants among them *R. communis*, *Olea europaea* and *Mentha piperita* against two genus, *Aspergillus niger* and *Geotrichum candidum* .Their results revealed that the essential oils of *M. piperita* completely inhibited the mycelial growth of two tested micro-organisms and displayed the highest inhibition but the oils of *R. communis* and *O. europaea* exhibited least antifungal activities.

CONCLUSION

It deserves to be mentioned that dill and olive seed had no activity against any one of the tested isolates. In our knowledge, no previous literatures about antifungal activity of these two oils. The present study will be helpful in the realistic approach for the development of antifungal drugs.

REFERENCES

1. Kyle AA, Dahl MV. Topical therapy for fungal infections. Am J Clin Dermatol 2004; (5):443–451.
2. Popoola TOS, Ojo DA and Alabi RO. Prevalence of dermatophytosis in junior secondary schoolchildren in Ogun State, Nigeria. Mycoses 2006; (49):499–503.
3. Borman AM, Campbell CK, Fraser M. and Johnson EM. Analysis of the dermatophyte species isolated in the British Isles between 1980 and 2005 and review of worldwide dermatophyte trends over the last three decades. Med Mycol. 2007, (45):131-141.
4. Dei Cas E and Vernes A. Parasitic adaptation of pathogenic fungi to mammalian hosts. Crit. Rev. Microbiol. 1986, (13):173–218.
5. King RD, Khan HA, Foye JC, Greenberg JH and Jones HE. Transferrin, iron, and dermatophytes. I. Serum dermatophyte inhibitorycomponent definitively identified as unsaturated transferrin. J. Clin. Med. 1975; 86:204–212.
6. Muhsin TM, Aubaid AH and Al-Duboon AH. Extracellular enzyme activities of dermatophytes and yeast isolates on solid media. MYCOSES. 1997, (40): 465-469.
7. Hankin L and Anagnostakis SL. The use of solid media for detection of enzyme production by fungi. Mycologia., 1975; (67): 597-607.
8. Keyvan P, Leila B, Zahra R, Manuchehr S and Kamia Z. *In vitro* activity of six antifungal drugs against clinically important Dermatophytes. Jundishapur Journal of Microbiology 2009; 2(4): 158-163.
9. Yehia AG, Mahmoud. *In vitro* Evaluation of Antidermatophytic Activity of Egyptian Bee Propolis in Combination with Plant Essential Oils in Sheep Hoof Plate: An Experimental Model. Mycobiology, 2003; 31(2): 99-104.
10. Prasad C. S., Shukla R., Kumar A. and Dubey N. K., 2010. *In vitro* and *in vivo* antifungal activity of essential oils of *Cymbopogon martini*and *Chenopodiumambrosioides* and their synergism against dermatophytes. Mycoses (53): 123-129.
11. Saddiq AA and Khayyat SA. Chemical and antimicrobial studies of monoterpene: Citral. Pestic. Biochem. Phys. 2010; (98): 89-93.
12. Zore GB, Thakre AD, Rathod V and Karuppayil SM. Evaluation of anti-*Candida* potential of geranium oilconstituents against clinical isolates of *Candida albicans*differentially sensitive to fluconazole: inhibition of growth, dimorphism and sensitization. Mycoses, 2011; (54): e99-109.
13. Lilly KK, Koshnick RL, Grill JP, Khalil ZM, Nelson DB and Warshaw EM. Cost-effectiveness of diagnostic tests for toenail onychomycosis: a repeated-measure, single-blinded, cross-sectional evaluation of 7 diagnostic tests. J Am Acad Dermatol. 2006; (55): 620–626

14. Brun S, Bouchara JP, Bocque A, Basile AM, Contet-Audonneau N. and Chabass D. Evaluation of five commercial Sabouraud gentamicin-chloramphenicol agar media. Eur J ClinMicrobiol Infect Dis. 2001; (20):718–723.
15. Larone DH. Medically important fungi. A guide to identification. 4th ed. ASM press Washington, D.C.
16. Domsch K.H., Gams W. and Anderson T., 2007. Compendium of soil fungi. 2nded. IHW-Verlag. Eching, 2002.
17. Ellis D, Davis S, Alexion H and Handke R. Description of medical fungi 2nd ed. Mycology unit. North Adelaide, Australia, 2007.
18. Ullman U and Blasins C. A simple medium for the detection of different lipolytie activity of microorganisms. Zbl. Bakt. Hyg., II Abt. Orig. A, 1974; (229): 264-267.
19. Paterson RRM and Bridge PD. Biochemical Methods for Filamentous Fungi. IMI Technical Handbooks No.1.Wallingford UK: CAB International, 1994.
20. Seeliger HPR. Use of a urease test for the screening of dermatophytic infections in Alberta, Canada. Mykosen 1956; 29 (6): 255-262.
21. Sharma R. and Malik A. Activity of natural products derived essential oils against dermatophytes. World journal of pharmacy and pharmaceutical sciences 2005; 4(7):1203-1209.
22. Dahiya P and Purkayastha S. Phytochemical Analysis and Antibacterial Efficacy of Dill Seed Oil against Multi-Drug Resistant Clinical Isolates. Asian Journal of Pharmaceutical and Clinical Research, 2012; (5): 2.
23. Garba I, Umar AI, Abdulrahman AB, Tijjani MB, Aliyu MS, Zango UU and Muhammad A. Phytochemical and Antibacterial Properties of Garlic Extracts .Bayero Journal of Pure and Applied Sciences 2013; 6 (2): 45 – 48.
24. Osabor VN, Bassey FI and Umoh UU. Phytochemical Screening and Quantitative Evaluation of Nutritional Values of *Zingiberofficinale* (Ginger). American Chemical Science Journal 2015; 8 (4): 1-6.
25. Elshiekh YH and Mona AM. Phytochemical screening and antimicrobial activity of *Strigahermonthica* and *Nigella sativa* seeds. American Journal of Research Communication 2015; 3(3): 24-33} www.usa-journals.com
26. Boukhebti H, Adel NC, Takia L and Messaoud R. Chemical and antimicrobial properties of essential oils of *Olea europea*L. Inernational Journal of Pharmacology & Toxicology 2015; 5(1): 42-46.
27. Sujana P, Thulasi MS, Penchalaneni J and Challagundla VN. Antibacterial Activity and Phytochemical Analysis of *Menthapiperita*L. (Peppermint)—An Important Multipurpose Medicinal Plant. American Journal of Plant Sciences 2013; (4): 77-83.
28. Kensa VM and Yasmin S. Pytochemical screening and antibacterial activity on *Ricinus communis*. Plant Sciences Feed 2011; 1(9): 167-173.
29. Aghamirian MR and Ghiasian SA. Dermatophytoses in out patients attending the Dermatology Center of Avicenna Hospital in Qazvin, Iran. Mycoses. 2008; 51(2):155-160.
30. Gargoom AM, Elyazachi MB, Al-Ani SM, Duvb GA. Tinea capitis in Benghazi, Libya. Int J Dermatol 2000; 39:263–5.
31. Panasiti V, Devirgiliis V, Borroni RG, Mancini M, Curzio M, Rossi M, et al.,2007. Epidemiology of dermatophytic-infections in Rome, Italy: a retrospective study from 2002 to 2004. Med Mycol. 2007; 45(1):57–60.
32. Woldeamanuel Y, Mengistu Y, Chryssanthou E, Petrint B.. Dermatophytosis in Tulugudu Island, Ethiopia. Med Mycol 2005; 43:79–82.
33. Hussaini I, Aman S, Haaron TS, Jahangir M, Nagi AH. Tinea capitis in Lahore, Pakistan. Int J Dermatol. 1994; (33):255–257
34. Viani FC, Dos Santos JI, Paula CR, Larsony CE and Gambale W. Production of extracellular enzymes by *Microsporum canis* and their role in its virulence. ISHAM, *Medical Mycology,* 2001; (39): 463-468.
35. Das SK and Banerjee AB. Phospholipids of *Trichophyton rubrum. Sabouraudia,* 1974; (12): 281-286.
36. North M. Comparative biochemistry of the proteinases of eucaryotic microorganisms. *Microbiol. Rev.* 1982; (46): 483-486.
37. Sharma R and Choudhary N. Study on role of keratinophilic fungi in nature: AREVIEW. Biolife journal 2014; 2(2):690-701.
38. Randhawa M.A., 2008. An update on antimicrobial effects of *Nigellasativa* and experience at King Faisal University, Dammam, SaudiArabia. JSSDDS 12 (1): 36–43.
39. Randhawa MA and Alghamdi M.S. Anticancer activity of *Nigellasativa* (Black Seed)—a review. Am. J. Chin. Med. 2011; 39 (6): 1075–1091.
40. Moghaddam M, Pourbaige M, Tabar HK, Farhadi N and Hosseini SMA. Composition and Antifungal Activity of Peppermint (*Mentha piperita*) Essential Oil from Iran. TEOP. 2013; 16 (4): 506–512.
41. Sa-Nguanpuag K. Kanlayanarat S, Srilaong V, Tanpraser K and Techavuthiporn Ch. Ginger (*Zingiber officinale*) Oil as an Antimicrobial Agent for Minimally Processed Produce: A Case Study in Shredded Green Papaya.International Journal of Agriculture & Biology.http://www.fspublishers.org, 2011 895–901.
42. Verma RK, Chaurasia, L. and Kumar M. Antifungal activity of essential oils against selected building fungi. Indian Journal of Natural Products and Resources, 2011; Vol. 2(4): 448-451.