

## Antifungal activity of some medicinal plants used in Jeddah, Saudi Arabia

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### Abstract

Development of more effective and less toxic antifungal agents is required for the treatment of dermatophytosis. Plants and their extraction preparations have been used as medicines against infectious diseases. In this research, the lemon grass [*Cymbopogon citrates* DC.] Stapf., lantana (*Lantana camara* L.), nerium (*Nerium oleander* L.), basil (*Ocimum basilicum* L.) and olive leaves (*Olea europaea* L.) were extracted with either water or different organic solvent to investigate their antifungal activities *in vitro*. The methanol extract of lemon grass, lanta and nerium followed by their ethyl acetate extracts showed the highest activities against *Trichophyton rubrum*. These inhibited the growth of *T. rubrum* by 85-90 and 80-85%, respectively at a concentration of 100 µg ml<sup>-1</sup>, while aqueous extracts inhibited the growth of this fungus at the same concentration by 32-77%. The activity of the methanolic extracts of the 5 selected plants was determined against different pathogenic fungi including *Microsporium canis*, *M. gypseum*, and *T. mentagrophytes*. Extracts of lemon grass were the most effective followed by lantana. Nerium and basil showed moderate activities. The lowest activity was recorded for olive extract. The five dermatophytes differed with regard to their susceptibility to plant extracts. *Trichophyton rubrum* was the most susceptible dermatophyte, followed by *Microsporium canis*, *M. gypseum*, and *T. mentagrophytes*, respectively. The MICs of these most active plants ranged from 25 to 125 µg ml<sup>-1</sup>. In conclusion, ethanolic extracts of some medicinal plants can be used to treat infections with pathogenic fungi.

**Key words:** Antifungal activities, dermatophytes, MIC, *Microsporium*, *Trichophyton*.

### Introduction

Skin, hair, nail, and subcutaneous tissues in human and animal are subjected to infection by several organisms, mainly fungi named dermatophytes and cause dermatophytoses (Valeria *et al.*, 1996; Amer *et al.*, 2006). Dermatophytoses are one of the most frequent skin diseases of human, pets and livestock (Tsang *et al.*, 1996). The disease is widely distributed all over the world with various degrees and more common in men than in women. There are three genera of mould that cause dermatophytosis. These are *Epidermophyton*, *Trichophyton* and *Microsporium*. Contagiousness among animal communities, high cost of treatment, difficulty of control and the public health consequences explain their great importance (Chermette *et al.*, 2008). A wide variety of dermatophytes have been isolated from animals, but a few zoophilic species are responsible for the majority of the cases, viz. *Microsporium canis*, *Trichophyton mentagrophytes*, *Trichophyton equinum* and *Trichophyton verrucosum*, as also the geophilic species *Microsporium gypseum* (Hasegawa, 2000; Mahmoudabadi and Zarrin, 2008). According to the host and the fungal species involved, the

typical aspect of dermatophytic lesions may be modified. A few antifungal agents are available and licensed for use in veterinary practice or human being treatment. The use of systemic drugs is limited to treat man or animal due to their high toxicity and problems of residues in products intended for human consumption (Araujo *et al.*, 2009). Different treatments have been recommended to control dermatophytes. In general, pharmacological treatment option include antifungal agents [Aly, 1997; Agwa *et al.*, 2000], but recently the use of some natural plant products has been emerged to inhibit the causative organisms. The antimicrobial and antitoxin properties of some plants, herbs, and their components have been documented since the late 19th century (Saadabi, 2006). These natural plants involve garlic, lemon grass, datura, acacia, a triplex, ginger, black seed, neem, basil, eucalyptus, alfalfa and basil (Omarand Abd-El-Halim, 1992; Aly *et al.*, 2000; Aly and Bafiel, 2008). They are safe to human and the ecosystem than the chemical antifungal compounds, and can easily be used by the public who used them for thousands of years to enhance flavor and aroma of foods as well as its economic value (Shelef *et al.*, 1980; Shelef, 1983).

Early cultures also recognized the value of these plant materials in medicine. Plant extract has been used traditionally to treat a number of infectious diseases including those caused by bacteria, fungi, protozoa and viruses (Soylu et al., 2005; Yoshida et al., 2005; Nejad and Deokule, 2009). A number of reports are available in vitro and in vivo efficacy of plant extract against plant and human pathogens causing fungal infections (Natarajan et al., 2003). The activity of plant extract against dermatophytosis i.e. the superficial infections of skin or keratinised tissue of man and animals can be very well visualized from the reports of Venugopal and Venugopal (1995). They reported the activity of plant extracts against 88 clinical isolates of dermatophytes which includes *Microsporium cannis*, *M. audouinii*, *Trichophyton rubrum*, *T. mentagrophytes*, *T. violaceum*, *T. imbricatum*, *T. verrucosum*, *T. erinacei* and *Epidermophyton floccosum* by agar dilution technique. While Vlietinck et al. (1995) reported clinical findings of Rwandese medicinal plants (267 plant extracts) used by traditional healers to treat microbial infections and found 60% of these extracts were active against dermatophytes. All the above reports and many others have utilized plant extract, juice or oil for the in vitro or in vivo evaluation of the infections caused by various species of dermatophytes viz. *Trichophyton*, *Microsporium*, *Epidermophyton* and yeast like fungi of genera *Candida*, *Cryptococcus*, *Rhodotorula* and *Torulopsis trichosporon*. Up to now more than 200 different biologically active substances have been isolated from plant extract, among them organosulphur compounds such as allicin, azoenes and diallyltrisulfide. Eugenol, phenolic compound, the most important biologically active compound found in many plant extract (Kähkönen et al., 1999; Aly and Bafiel, 2008).

The present study was designed to evaluate the in vitro antidermatophyte activity of some plant extracts. The antifungal activities of water and organic plant extract are compared. The percentage of inhibition and MIC are also recorded.

## Materials and Methods

### Pathogenic fungi

The fungi used were obtained from the culture collection of Dr. R. Bonally, Laboratoire de Biochimie Microbienne, Fac. De Pharmacie, Nancy, France. *Microsporium ferruginum*, *Trichophyton mentagrophytes* and

*Epidermophyton sp.* were isolated and identified from contaminated dust samples collected from different hospitals in Garbia, Egypt (Amer et al., 2006). All fungi were stored on sabouraud dextrose agar (Oxoid) slants in the refrigerator at 4 °C prior to use.

### Medicinal plant materials

Samples of five medicinal plants, i.e. basil leaves (*Ocimum bacilicum*), lantana leaves and flowers (*Lantana camara*), lemon grass stalk and leaves (*Cymbopogon citratus*), nerium leaves (*Nerium oleander*) and olive leaves (*Olea europaea*) were collected during October 2007 from different districts of Jeddah city, Saudi Arabia and identified by Botany department, Faculty of Sciences, Tanta Uni., Egypt. The plants were brought to the laboratory and thoroughly washed in running tap water to remove debris and dust particles and then rinsed in distilled water.

### Preparation of aqueous and organic medicinal plant extracts

For aqueous and organic extraction, 10 grams of each sun-dried medicinal plant material, were cut into small pieces and then macerated by blender 1–2 mm separately and the powder produced was blended with 100 ml of either distilled water (cold or hot) or organic solvent (ethyl alcohol, methanol, *n*-butanol, ethyl acetate or chloroform), (1:10 w/v). Then, they were extracted under cold conditions for 24 h. The resultant extract was filtered through a glass wool filter and then rinsed with a small quantity (about 30 ml) of 96% ethyl alcohol. The extracts solutions were evaporated under reduced pressure at 40 °C. Subsequently, the extracts were diluted by distilled water and stored in the deep freezer at -10 °C and later lyophilized in a freeze dryer.

### Antimicrobial activity

Antimicrobial activity of the above mentioned extracts was determined, using the agar well diffusion assay method as described by Holder and Boyce (1994). Dimethyl sulfoxide DMSO was used as a negative control and Griseofulvin was used as a positive control. The plates were done in triplicates and were incubated at 37 °C. The antimicrobial activity was taken on the basis of diameter of zone of inhibition, which was measured after 7 days of incubation and the mean of three readings is presented. The presence of inhibition of the treated fungus was calculated using Griseofulvin as standard (100% inhibition). The plant extract and the standard antifungal

agents were dissolved in DMSO, 100% biologically inert substances.

#### Determination of minimal inhibitory concentration of plant extract on fungal growth

The MIC was determined by the methods described by Chand *et al.* (1994) and modified by Aly (1997). Each well of a 96 well ELISA tray was filled with 175 µl of an exponentially growing culture ( $10^6$ – $10^7$  CFU ml<sup>-1</sup>). To each well, 20 µl solution of each concentration of the test substance, or the appropriate solvent as control, was added. The ELISA trays were incubated for 40 minutes before 5 µl of a 0.2% w/v solution of Fluorescein diacetate (FDA) in acetone was added. Incubation was continued for 90 minutes more and the resulting green color from the hydrolysis of FDA was measured at 490 nm (referenced to 630 nm) and blanked against control wells containing microbial cultures only, using an MR7000 automatic ELISA tray reader. The agar plates were incubated overnight (37 °C) and CFU was counted using a colony counter. The MIC corresponded to the minimum concentration of the compound that caused 99% cell inhibition with respect to the CFU's in a control which contained microbial cultures and sterile distilled water or solvent replacing the test compound.

#### Statistical analysis

Each experiment has three replicates and three determinations were conducted. Means of variable and standard deviation were recorded.

### Results and Discussion

Many investigations were carried out to discover plant products that inhibit the fungi like *Trichophyton rubrum* and *Microsporum canis*. These two species cause common infections in humans which are difficult to control effectively, and the pharmaceutical arsenal currently available against them is rather limited (Evans and White, 1967; Levine, 1982; Gupta *et al.*, 1991; Jansen *et al.*, 1991). Hence, plant products that inhibit their growth without harming the host represent potential therapeutic agent. As stated earlier, five different plants belonging to different families (Table 1) used rationally by Saudi Arabia people were collected from Jeddah, and extracted with water or organic solvents and their antifungal activities were detected against *T. rubrum* which is considered one of the fungi usually causes disease in keratinized epithelial structures such as hairs and nails and can invade the dermis, particularly in immunocompromised patients (Maoz and

Neeman, 1998). The antifungal activities of the plant extracts obtained using different organic solvents were compared with that of Griseofulvin and the % of inhibition was calculated (Table 2). Extracts were obtained through the extracting action of the appropriate solvent on a dry plant and the active compounds are thus contained in the solvent used. Each type of extract is defined by the way it is prepared and the nature of the solvent. The extraction process is always studied to respect the integrity of the active molecules. In this experiment, Methanol extract of lemon grass was the best to suppress the growth of *T. rubrum* (90% inhibition), followed by lantana and nerium methanolic extract (85-88% inhibition). The methanol extract of basil as well as olive inhibited *T. rubrum* growth by 73-75%. Extraction of basil and olive with chloroform was found to be the best (77-80% inhibition) compared to the other extracts due to the presence of some essential oil which could be extracted with chloroform. Extraction of lemon grass, lantana or nerium with either ethyl acetate extract, n-butanol or diethyl ether was less active against *T. Rubrum* compared to their ethanol extract. Aqueous extract of cold or hot water of all examined plants showed the lowest activity against *T. Rubrum* compared to different organic solvents used.

The activity of methanol extract of the five selected plants against different dermatophytes were summarized in Table 3. It was found that lemon grass extract showed maximum antifungal activity against *T. mentagrophytes*, followed by *T. verrucosum*, *M. canis* and *E. floccosum* (Table 3). Moderate activity was recorded against different dermatophytes by using lantana. Less activities were recorded for nerium, basil and olive. Plant derived compounds are of interest in this context because they comprise safer or more effective substitutes for synthetically produced antimicrobial agents (Dupuis *et al.*, 1972). The plant extracts used in folkloric medicine in Palestine, Saudi Arabia (Abdulmoniem, M. A. and Saadab, 2006; Aly and Bafiel, 2008), Egypt (El-Fadaly *et al.*, 1999), Mexico (Navarro *et al.*, 1996) and India (Jain *et al.*, 2004) were investigated for their antifungal activity and their use to treat pathogenic fungi. Lemon and lantana extracts showed excellent antidermatophytic properties compared to other plant extract which may be due to free and bound flavonoid fractions, showed the greatest fungicidal properties. The maximum zone of inhibition was recorded in the presence of free flavonoid fraction of the plant extract against *T. rubrum* and *T. terrestre*, which were the most susceptible fungus for all the extracts tested (Jain

*et al.*, 2004). Lemon grass extract has shown itself to be among the most significant of these newly uncovered natural, nontoxic therapies, and has proven itself to be one of the most important antimicrobial agent successfully used for treatments of all kinds of infections arising from fungi, virus, bacteria, parasites, and other microscopic invaders (Mohamed *et al.*, 2006).

The methanol extracts of lantana (leaves and flowers) showed antifungal activity (20 mm) against *M. gypseum*, *T. mentagrophytes*, *M. canis*, and *T. gypseum*. On the contrary, olive extract showed the lowest activity against all tested dermatophytes (8-10 mm). More activities by olive leaves were recorded against plant pathogenic fungi including, *Alternaria solani*, *Botrytis cinerea* and *Fusarium culmorum* (Winkelhausen *et al.*, 2005). They added that the activity was attributed to the presence of phenolic compounds which can be hold a good promise as a natural fungicide against common pathogens of crops. Nwachukwu and Umechuruba (2006) found that Leaf extracts of neem, basil, bitter leaf and paw-paw, which are cheap and environmentally safe, are promising for protecting African yam bean seeds against major seed-borne fungi. Many of the herbs properties can be traced back to the flavonoids that plant contains. Similarly, Olive leaves contain oleuropein, eleonic Acid and other qualities that can be of benefit to treat humans dermatophytes. In this research, the percentage of inhibition (Table 4) was calculated after comparing with Griseovulvin (100% inhibition). The maximum activity was obtained from lemon grass which was ranged from 75-95%, followed by lantana extract which inhibited the fungal growth by 50-80% and nerium and basil extract decreased growth by 30-50%. The lowest activity was recorded for olive leaves which inhibited the growth by 20-33.3 %. The activity index was calculated. It was ranged from 65-69% for both lemon grass and lantana, 38-39% for basil and nerium and 27% for olive leaves.

MICs of the six plant extracts were calculated by using flurocin diacetate method (Table 5). It was ranged from 1.0-1.5 µg/ml for Griseofulvin. The MIC for the different plant extracts were ranged from 25-75 for both lemon grass, lantana and basil and from 100-175 µg/ml for nerium and olive extract. The antifungal activities of griseofulvin were determined by Araújo *et al.* (2009) using broth microdilution technique, against dermatophytes and the minimal

inhibitory concentrations (MICs) for *Trichophyton mentagrophytes*, *T. rubrum* and *Microsporum canis* were ranged from 0.03-1 µg/ml. It can be concluded that, MICs calculated were greater than that obtained for Griseofulvin. Further studies are needed to determine the antifungal compound(s) in such plant extract (isolation, separation and identification) as well as its formulation to be applicable as alternative methods to be used in treatment of skin and skin structures diseases in human and animal. Therefore, such results are of a significant value that confirms the therapeutic potency of some plants used in traditional medicine. It should form a good basis for further phytochemical and pharmacological investigation (Prasad *et al.*, 2009). Useful antimicrobial phytochemicals are: phenolics and polyphenols (such as simple phenols and phenolic acids, quinones, flavones, flavonoids, and flavonols, tannins, coumarins); terpenoids and essential oils; alkaloids; lectins and polypeptides; plus other compounds. The mechanisms thought to be responsible for these phytochemicals against microorganisms vary and depend on these compounds (Aly and Bafiel, 2008). Their mechanism of actions may include enzyme inhibition by the oxidized compounds, and act as a source of stable free radical and often leading to inactivation of the protein and loss of function. They have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls and disrupt microbial membranes (Ali, 1999), some have ability to intercalate with DNA, formation of ion channels in the microbial membrane, competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (Cowan, 1999).

## Conclusion

The ultimate conclusion of this study supports the traditional medicine use of different plant extracts in treating different infections caused by pathogenic fungi in Saudi Arabia either by using a single or combined extracts. It also suggests that a great attention should be paid to medicinal plants which are found to have plenty of pharmacological properties that could be sufficiently better when considering a natural food and feed additives to improve human and animal health.

**Table 1:** Common and scientific names of some plants used to detect their antifungal activities in vitro.

Common name	Scientific name	Family	Used part
Lemon grass	<i>Oymbopogon citrates</i>	Gramineae	Stalk and leaves
Lantana	<i>Lantana camara</i>	Verbenaceae	Leaves and flower
Nerium	<i>Nerium oleander</i>	Apocynaceae	leaves
Basil	<i>Ocimum basilicum</i>	Labiatae	Stem and leaves
Olive	<i>Olea europaea</i>	Oleaceae	leaves

**Table 2:** The % of fungal inhibition of aqueous and organic extract of different plants at concentration 100 µg/ml compared to Griseofulvin (100% inhibition) against *Trichophyton rubrum*.

Used plant	Type of the extract						
	Methanol Extract (control)	Water extract (cold)	Aqueous extract (hot)	Diethyl ether	Ethyl acetate	n-butanol	chloroform
Lemon grass	90	66	77	80	85	84	80
Lantana	88	44	32	80	85	80	60
Nerium	85	32	42	80	80	67	30
Basil	73	24	30	44	73	57	80
Olive	75	26	30	67	75	55	77

The results were compared with that obtained for Griseofulvin which considered 100% inhibition.

**Table 3:** The antifungal activity of methanolic extract (diameter of the inhibition zone, mm) of different plant extracts against different pathogenic fungi.

Pathogenic fungi	Diameter of the inhibition zone (mm)					
	GSF control	Lemon grass	Lantana	Nerium	Basil	olive
<i>Microsporum canis</i>	40 ± 2.5	30 ± 1.5	20 ± 0.9	15 ± 0.7	12 ± 0.7	10 ± 0.6
<i>Microsporum gypseum</i> ,	40 ± 1.5	22 ± 0.5	20 ± 0.7	14 ± 0.8	15 ± 0.6	10 ± 0.5
<i>Trichophyton mentagrophytes</i>	40 ± 0.9	38 ± 1.4	20 ± 0.6	16 ± 0.6	16 ± 0.5	10 ± 0.4
<i>Trichophyton verrucosum</i>	40 ± 0.9	30 ± 1.6	20 ± 1.5	20 ± 0.9	20 ± 0.3	8 ± 0.5
<i>Epidermophyton floccosum</i>	38 ± 0.5	30 ± 1.5	18 ± 0.9	14 ± 0.4	12 ± 0.5	10 ± 0.4
Activity Index*	39.5	30	19.5	16	15	9.5

GSF: Griseofulvin, \*Activity index was calculated as the mean value of net zones of inhibition (mm) against the five fungal test strains

**Table 4:** The % inhibition of the of different plant extracts compared to Griseofulvin (100% inhibition) against different dermatophytes.

Pathogenic fungi	Inhibition compared to Griseofulvin%					
	Griseofulvin	Lemon grass	Lantana	Nerium	Basil	olive
<i>Microsporum canis</i>	100	75.0	50.0	37.5	30.0	25.0
<i>Microsporum gypseum</i> ,	100	55.0	80.0	35.0	37.5	25.0
<i>Trichophyton rubrum</i>	100	95.0	66.6	33.3	42.4	33.3
<i>T. verrucosum</i>	100	75.0	75.0	50.0	50.0	20.0
<i>Epidermophyton floccosum</i>	100	79.0	78.9	36.0	31.5	26.3
Activity Index	100	65.0	69.0	38.0	39	27.0

\*Activity index was calculated as the mean value of net zones of inhibition (mm) against the five fungal test strains.

**Table 5:** Minimal inhibitory concentration (MIC) µg/ml of different plant extract using Fluorescein diacetate method and compared with Griseofulvin.

Dermatophytes	Minimal inhibitory concentration (MIC)					
	(µg/ml)					
	GSF	Lemon grass	lantana	Nerium	Basil	Olive
<i>Microsporum canis</i>	1.5 ± 0.3	25 ± 3.6	50 ± 6.1	100 ± 11.5	50 ± 7.0	175 ± 3.0
<i>Microsporum gypseum</i>	1.0 ± 0.1	25 ± 4.4	50 ± 4.1	100 ± 14.0	50 ± 6.0	175 ± 7.8
<i>Trichophyton rubrum</i>	1.5 ± 0.5	25 ± 2.9	75 ± 7.0	100 ± 8.0	125 ± 11.0	150 ± 12.0
<i>T. mentagrophytes</i>	1.5 ± 0.7	25 ± 5.2	50 ± 5.0	125 ± 6.3	50 ± 3.0	100 ± 5.9
<i>T. verrucosum</i>	1.0 ± 0.4	50 ± 3.9	50 ± 4.4	125 ± 11.1	75 ± 4.2	125 ± 13.0

GSF: Griseofulvin, All the values given, are the mean value of three reading.

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