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ANTIBACTERIAL AND ANTIDERMATOPHYTE ACTIVITIES OF SOME ESSENTIAL OILS FROM SPICES

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كفاءة الزيوت الطيارة المستخلصة من بعض التوابل في مقاومة أنواع من البكتريا الممرضة والفطريات التي تصيب الجلد

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دراسة عن التأثير المثبط للزيوت الطيارة المستخلصة من بعض التوابل على نمو إحدى عشر سلالة من أنواع البكتريا الممرضة . أوضحت الدراسة أن زيوت كل من الزعتر ، القرفة والحبهان (هال) ذات كفاءة عالية ضد جميع أنواع البكتريا المختبرة ، بينما كانت الزيوت الطيارة لكل من النعناع، البردقوش حصا لبان (الأكليل) ذات كفاءة متوسطة ضد الأنواع المختلفة من البكتريا . أظهرت الدراسة أيضاً أن الزيوت الطيارة لكل من القرفة الصيني (الدار الصيني) ، القرنفل ، الكمون وأوراق الكافور ذات كفاءة محدودة أو منعدمة الكفاءة في مقاومة أنواع البكتريا المختبرة .

اشتمل البحث أيضاً على دراسة التأثير المثبط لهذه الزيوت الطيارة ضد ثمان سلالات لأنواع مختلفة من الفطريات التي تصيب الجلد . وجد أن الزيوت الطيارة لكل من الزعتر والقرفة يليهما زيت النعناع ثم زيت الحبهان ذات كفاءة عالية ضد نمو جميع أنواع فطريات الجلد المختبرة .

Key Words: Antibacterial, antidermatophyte activities, essential oils

ABSTRACT

The inhibitory effects of ten essential oil from different spices against the growth of various isolates of bacteria representing Gram-positive (seven isolates) and Gram-negative (four isolates) were studied. Eight antibacterial agents were included for comparative purposes. Results show that essential oils of thyme (*Thymus vulgaris* L.), cinnamon (*Cinnamomum verum* Presl (Syn. *C. zylanicum* Blume) and cardamom (*Elettaria cardamum* White and Maton) were highly active against both Gram-negative and Gram-positive bacteria. The essential oils of peppermint (*Mentha piperita* var, *officinalis*), marjoram (*Majorana hortensis* L.) and rosemary (*Rosmarinus officinalis* L.) showed moderate effects against the test bacteria. The essential oils of Chinese cassia (*Cinnamomum cassia* Presl), clove (*Syzygium aromaticum* L.) Merr and Perry (Syn. *Eugenia caryophyllata* Thunb), cumin *(Cuminum cyminum* L.) and eucalyptus *(Eucalyptus globulus* L.) had no or little effect against Gram-negative and Gram-positive bacteria.

The inhibitory effect of the essential oils against eight different species of dermatophytic fungi were also studied. Thyme and cinnamon oils were highly effective inhibitory agents against all the isolates tested when added at 200 and 500 ppm. Peppermint oil completely inhibited the growth of all the fungi tested at 500 ppm and three isolates when added as 200 ppm. Essential oil of cardamom only inhibited the same three isolates when added at 500 ppm. The other essential oils tested, showed partial inhibition against the growth of the test fungi.

INTRODUCTION

It has been known for some time that herbal drugs and spices contain substances with antimicrobial activity in their essential oils (Okazaki and Oshima, 1952a-d; Maruzzella and Henry, 1958a, b; Frazier, 1967; Dupuis *et al.*, 1972; ASTA, 1976; Bullerman *et al.*, 1977; FDA, 1978; Hitokoto *et al.*, 1978,1980; Dikshit *et al.*, 1981; Sharma *et al.*, 1984; Shukla and Tripathi, 1987). The utilization of essential oils for the fungus-free storage of food has also been successfully

established (Dubey *et al.*, 1982; Dikshit *et al.*, 1983).

Although the antifungal effects of spices are known, very few reports are available on the effect of spices or spice oils against dermatophytic fungi which are capable of invading the skin in man and animals causing skin changes. Disease produced by dermatophytes, collectively known as dermatophytoses (e.g. ringworm). The object of the present study was directed to examine inhibitory effects of ten essential oils of different spices on the growth of eleven isolates of bacteria and eight isolates of dermatophytic fungi.

MATERIALS AND METHODS

Extraction of essential oils from different kinds of spice. The plant materials of peppermint, cardamom, chinese cassia, cinnamon, clove, cumin, eucalyptus, marjoram, rosemary and thyme, cut into small pieces (Ca 100 gm) were placed in a flask (2 1) together with double distilled water (1.5 1). A continuous steam distillation extraction head was attached to the flask, after steam distillation (Ca 5 hours), the oil was collected and dried over anhydrous sodium sulfate (Farag *et al.*, 1989).

Antibacterial activity. The disc-diffusion method was used to measure the antimicrobial activity (Sletgh and Timburg, 1981). Test bacteria were each preincubated in 10 ml of Difco Nutrient Broth for 20 h at 37°C. The agar medium used in this study composed of: beef extract, 3 gm; peptone, 5 gm; agar 15 gm/l. The medium was adjusted to pH 7 before sterilization at 121°C for 15 min and maintained in a 50°C water bath. Twenty ml of the medium were poured in each Petri dish (10 ml diameter). Bacterial solutions prepared by Difco Nutrient Broth were diluted 5 times with sterilized physiological saline and streaked on the agar plate with a sterilized loop. After 6 h of inoculation (at room temperature), discs containing the different oils were placed upon the surface of the medium. The essential oils were added at a concentration of 0.5 mg/disc (Whatman No. 3 filter paper, 0.5 cm diameter). The plates were incubated at 37°C for 24 h and inhibition zones of microbial growth produced by the different essential oils were measured (mm). All tests were carried out in triplicate.

Antifungal activity: The antifungal activity of each essential oil was evaluated by an agar plate method. Sabouraud dextrose agar medium with a pH of 5.5 was used as the plating medium for all of the dermatophytic fungi. The medium comprised (gm/1), peptone from meat, 10; glucose; 40; agar, 15 (Moss and McQuown, 1969).

Solid medium was prepared in one-liter amounts and autoclaved at 121°C for 20 min. After cooling the oils were transferred aseptically to the sterilized medium to eliminate the possibility of any loss of oil due to volatilization during sterilization. The doses of the different oils used were 200 and 500 ppm. Petri dishes were poured with media containing the essential oils. After solidification of the agar, one drop of spore suspension (about 10^6 spores/ml) of the test organism was placed in the center of the plate with a loop of 3.0 mm inside diameter. The plate was incubated in an upright position for 24 hours and then inverted to prevent the seeding of new colonies. An incubation temperature of 28°C±2°C was used throughout the inhibition studies. After ten days of incubation the diameter of the colony was measured.

Microorganisms. The antimicrobial spectrum of the different essential oils was tested with eleven strains of bacteria and eight isolates of dermatophytes.

A - Bacteria: *Escherichia coli* (No. 502), *Klebsiella pneumoniea* (No. 903), *Proteus vulgaris* (No. 131), *Pseudomonas pyocyanea* (No. 141), *P. fluorescens* (No. 142), *Salmonella paratyphi* (No. 150), *Serratia rhadnii* (No. 152), *Bacillus anthracis* (No. 201), *B. cereus* (No. 203), *Micrococcus luteus (Sarcina)* (No. 111) and *Staphylococcus aureus* (No. 153).

B - Dermatophytic fungi: Pure cultures of local isolates of these fungi were obtained from the Culture Collection of Botany Department, Faculty of Science, Assiut University, Assiut, Egypt. These isolates were: *Chrysosporium carmichaelli* Van Oorschot (No. 1886), *C. indicum* (Randhawa and Sandhu) Gray (No. 1887), *C. keratinophilum* (D. Frey) Carmichael (No. 1888), *C. queenslandicum* Apinis and Rees (No. 1889), *C. tropicum* Carmichael (No. 1890), *Microsporum canis* Bodin (No. 4650), *Trichophyton mentagrophytes* (Robin) Blanohard (No. 7001), *T. simii* (Pinoy) Stockdale, Machenzie and Austwick (No. 7003).

Antibiotic and antifungal standard references: A multidisc standard (manufactured by Oxoid Ltd, England) contained eight separate antimicrobial agents as reference antibiotics were used for comparative purposes. Each multidisc contained: Chloramphenicol (C_{50} µg); Colistin sulphates (CT_{10} µg); Nitrofurantion (F_{200} µg); Sulphafurazole (SF_{500} µg); Nalidixic Acid (NA_{30} µg); Ampilicillin (PN_{25} µg); Streptomycin (S_{25} µg) and Tetracycline (TE_{50} µg).

Three antimycotic agents, Griseofulvin, produced by Kahira Pharm. and Chem. Ind. Co., Cairo, A. R. E.; Mycostan, produced by Memphis Chem. Co., Cairo, A. R. E. and Batrafen, produced by Hoechst Orient S. A. A. Cairo, under licence of Hoechst AG, Frankfurt (Main) (Germany), were also tested simultaneously with the essential oils against dermatophytic fungi for comparison.

RESULTS AND DISCUSSION

Antibacterial activity

The data (Table 1) for inhibition zones of various microorganisms indicated that the essential oils of Chinese cassia, clove, cumin and eucalyptus had no or little effect against Gram-negative and Gram-positive bacteria. The essential oils of peppermint, marjoram and rosemary showed moderate effects against the test bacteria. On the other hand, essential oils of thyme, cinnamon and cardamom were highly active against both Gram-negative and Gram-positive bacteria. Generally the Gram-positive bacteria were more sensitive to the different essential oils than Gram-negative bacteria. These results are in full agreement with that previous recorded by several authors. Garg and Dengre (1984) showed that essential oil from *Capillipendrum factidum* displayed high antibacterial activity against Gram-positive bacteria and moderate to Gram-negative strains. Farag *et al.* (1989) recorded also that Gram-negative bacteria were more resistant to essential oils of sage, rosemary, caraway, cumin, clove and thyme than Gram-positive bacteria.

The results obtained during this study also showed that four kinds of essential oils tested (eucalyptus, cumin, clove and Chinese cassia) had no or low inhibitory effects against all the isolates of bacteria examined. Three essential oils (peppermint, marjoram and rosemary showed moderate inhibitory effects. However, essential oils of thyme, cinnamon and cardamom proved to be highly active against the eleven strains of bacteria examined. These three oils were previously recorded as potent antibacterial agents. Similar findings have been reported by other investigators (Patakova and Chladek,

Table 1
Effect of different essential oils on the growth of various isolates of bacteria tested, diameter of inhibition zone in mm.

Essential oil	Gram - negative bacteria							Gram-positive bacteria			
	Escherichia coli	*Klebsiella pneumonia*	*Proteus vulgaris*	*Pseudomonas pyocyanea*	*Pseudomonas fluorescens*	*Salmonella paratyphi*	*Serratia rhadnii*	*Bacillus anthracis*	*Bacillus cereus*	*Micrococcus (Sarcina)*	*Staph aureus*
Peppermint	11	NI*	8	NI	NI	9	7	14	11	14	15
Cardamom	20	20	18	11	30	20	10	25	27	16	28
Chinese cassia	10	NI	NI	6	10	NI	NI	11	16	6	15
Cinnamon	18	20	35	10	30	16	14	26	30	30	29
Clove	10	NI	9	NI	NI	NI	NI	10	7	NI	NI
Cumin	NI	NI	7	NI	7	8	NI	7	8	7	11
Eucalyptus	11	8	NI	NI	NI	NI	NI	11	NI	7	NI
Marjoram	11	12	15	7	12	NI	NI	25	16	10	20
Rosemary	12	12	10	6	12	11	7	12	15	10	15
Thyme	25	18	20	NI	20	20	8	22	20	20	30
Antibiotics standards**											
TE50	12	NI	NI	NI	NI	NI	NI	15	20	16	20
C50	20	NI	20	18	20	NI	NI	22	26	18	25
CT10	10	11	NI	12	15	12	12	NI	NI	NI	12
F200	NI	15	15	NI	30	16	11	21	25	NI	24
SF500	NI	NI	NI	NI	NI	NI	NI	NI	20	NI	NI
NA30	20	20	20	NI	25	20	20	20	24	11	20
PN25	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
S25	20	NI	20	18	NI	NI	18	23	30	12	14

*NI = No Inhibition
**TE_{50} = Tetracycline 50 µg/disc
C_{50} = Chloramphenicol 50 µg/disc
CT_{10} = Colistin sulphates 10 µg/disc
F_{200} = Nitrofurantion 200 µg/disc
SF_{500} = Sulphafurazole 500 µg/disc
NA_{30} = Nalidixi Acid 30 µg/disc
PN_{25} = Ampicillin 25 µg/disc
S_{25} = Streptomycin 25 µg/disc

Table 2
Effect of different essential oils of some spices at two concentrations (200 ppm and 500 ppm) on the growth of different isolates of dermatophytic fungi (Number = diameter of colony, cm).

Essential oil	Dermatophytes fungi tested															
	Chrysosporium carmichaelii		*C. indicum*		*C. keratinophilum*		*C. queenslandicum*		*C. tropicum*		*Microsporum canis*		*Trichophyton mentagrophytes*		*T. simii*	
	200	500	200	500	200	500	200	500	200	500	200	500	200	500	200	500
Control	3.4		3.2		3.6		2.8		3.7		3.9		2.9		3.5	
Peppermint	3.4	---	1.8	---	2.4	---	2.2	---	2.4	---	---	---	---	---	---	---
Cardamom	2.0	1.8	0.6	---	2.5	1.3	2.6	2.0	1.4	1.1	1.6	---	3.0	---	1.3	---
Chinese cassia	3.0	2.2	3.3	2.8	3.0	2.1	3.3	3.3	2.7	2.7	3.9	2.6	3.1	2.6	4.9	4.1
Cinnamon	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Clove	2.5	1.1	2.8	1.9	2.8	1.5	2.5	1.3	3.0	3.0	2.0	2.0	3.0	3.0	2.5	1.8
Cumin	3.4	2.1	2.8	2.5	2.8	2.3	3.1	2.0	2.8	2.5	---	---	3.0	---	3.1	---
Eucalyptus	3.0	2.5	2.6	2.2	3.0	3.0	2.5	3.0	3.0	3.0	---	---	3.0	1.8	3.0	2.5
Marjoram	3.6	3.1	2.8	1.6	3.7	2.6	2.8	2.3	2.4	2.1	3.8	0.8	2.7	---	4.6	2.3
Rosemary	3.2	2.1	2.4	2.6	3.7	1.8	3.0	3.0	2.8	2.6	3.1	2.3	3.0	2.5	3.6	2.4
Thyme	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Antimycotics standards																
Batrafen	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Griseofulvin F.P.	0.5	---	2.7	2.5	0.5	0.5	3.1	2.9	2.1	2.0	---	---	---	---	1.1	0.5
Mycostan	---	0.6	0.6	---	1.3	0.6	2.3	1.6	0.5	---	2.5	---	2.5	0.6	3.9	1.8

1974; Bouchberg and Allegrini, 1976; Farag *et al.*, 1989).

It appears that there is a relationship between the chemical structure of the most abundant compounds in the essential oils and the antimicrobial activity. Farag et al., (1989) reported that thyme oil proved to contain thymol (42.7%) and P-cymen (36.0%) as the most prevalent compounds. The extent of the inhibitory effect of essential oils could be attributed to the presence of aromatic nucleus containing a polar function group, Farag et al. (1989) concluded that the higher inhibitory action of thymol might be due to the presence of phenolic OH group. It is well known that OH group is quite reactive and easily forms hydrogen bonds with active sites of target enzymes. Thyme at a concentration as low as 0.5% in culture media proved to be highly toxic to *Vibrio parahaemolyticus* (Beuchat, 1976), while very low concentrations (0.125-1.0 mg/ml) of the essential oil of thyme were quite sufficient to prevent the growth of eight different isolates of bacteria (Farag et al., 1989). Farag et al., (1988) used thyme oil in an attempt to prevent butter deterioration during storage at room temperature. Their results showed that this oil decreased both total bacterial and lipolytic bacteria counts.

Similar to the results obtained in this study, Frazier (1967) classified cinnamon as one of the more effective bacteriostatic agents. Cinnamon contains 0.5 to 1.0% volatile oil composed mainly of cinnamaldehyde (65-75%), eugenol (4-10%), cinnamic acid, and 0-methoxycinnamaldehyde (Shoda, 1958; Karig, 1975; Ross, 1976). Morozumi (1978) studied the inhibition of bacterial growth by 0-methoxycinnamaldehyde and concluded that this compound was effective only against *Staphylococcus aureus* and *Clostridium botulinum* out of eight different isolates of pathogenic bacteria tested. Cinnamylphenol compounds have been recognized as a new group of natural products occurring in species of *Dalbergia* and *Macharium* (Gregson *et al.*, 1968; Ollis, 1968). Dupuis *et al.* (1972) tested the antibacterial activity of cinnamylpyrogallol and found that strong antibacterial activity was shown in tests with *Streptomyces griseus* and *Bacillus mycoides*, but inhibition of *Serratia marcescens* and *Escherichia coli* was absent.

Antidermatophytic activity

The data presented in Table (2) clearly show that thyme and cinnamon oils were highly effective against all the isolates of fungi tested. They completely inhibited mycelial growth of all fungi when added to solid medium at the low concentration (200 ppm). The inhibitory effect of essential oils of thyme and cinnamon compared well with the antimycotic batrafen (used simultaneously for comparison), which also completely inhibited all the dermatophytic fungi tested at the lower concentration used (200 ppm).

Thyme was previously recorded as inhibitory to growth of different toxigenic fungi, and thymol which constitute 42-54% of the essential oil of thyme, was the inhibitory agent (Hitokoto et al., 1980; Llewellyn et al., 1981). Cinnamon which showed pronounced growth inhibiting effect against dermatophytic fungi has been previously recorded as being strongly inhibitory against several pathogenic fungi. As mentioned above, cinnamon contains 0.5-1.0% volatile oil which is composed of approximately 65-75% cinnamicaldehyde, 4-10% eugenol, some cinnamic acid, 0-methoxycinnamaldehyde and various other compounds (Merory, 1960). Bullerman *et al.* (1977) studied the inhibition of fungal growth and aflatoxin production by cinnamaldehyde, eugenol and 0-methoxycinnamaldehyde and concluded that these compounds are the major antifungal substances of cinnamon oil. Out of 1300 samples of different higher plants in Japan including spices and herbal drugs, cinnamaldehyde exhibited the highest antifungal properties (Okazaki and Kogawara, 1951; Okazaki and Oshima, 1951, 1952a-d, 1953a,b; Okazaki et al. 1953). Morozumi (1978) also carried out a chemical study on the inhibitory factor of cinnamon and identified it as 0-methoxycinnamaldehyde. He also pointed out that this substances had a strong inhibitory effect on the growth of dermatophytes such as *Microsporum, Trichophyton* and *Epidermatophyton*. The minimum inhibitory concentration (MIC) for five species of dermatophytes was 3.12 to 6.25 µg/ml. Dupuis *et al.* (1972) reported that cinnamylpyrogallol was found to be an inhibitor for fungal growth. In general it displayed some antibiotic activity against 26 species of fungi. They also recorded that cinnamyl alcohol was the most active against four isolates of dermatophytic fungi. Azzouz and Bullerman (1982) tested 16 kinds of herb and spice against seven mycotoxin producing moulds and reported that clove followed by cinnamon were the strongest antifungal spices.

The essential oil of peppermint came the third potent oils tested. It completely inhibited all the fungi tested when added as 500 ppm and also inhibited three isolates (*Microsporum canis, Trichophyton mentagrophytes* and *T. simii*), when added as 200 ppm (Table 2). Fungicidal action of peppermint was previously recorded by Sarbhoy *et al.*, (1978), Hitokoto *et al.* (1980) and Mabrouk and El-Shayeb (1980). Mint oil contains 50-78% free methanol, 5-20% combined in various esters, L-limonene, menthone, cineol and phellandrene (Claus, 1962). These constituents, which are terpenes and their oxidized derivatives (Campbell, 1967) may be responsible for the inhibition of fungal growth by mint. Furthermore, the presence of valeric acid in mint (Claus, 1962) may also add to its inhibitory action.

Cardamom also shows some inhibitory effect to the different eight isolates of dermatophytes. Volatile oil of cardamom containing B-Orneol and L-Limonene. The inhibitory effect of L-limonene which is also the major constituent of citrus oils was also recorded (Braverman, 1949; Kirchner, 1961).

In the testing procedures used in this investigation, the moulds were grown under near optimum laboratory conditions with controlled temperature and adequate nutrients. Hence, the concentration of essential oils necessary to suppress growth of dermatophytic fungi in this study would be expected to be greater than that necessary under conditions less favourable for fungal growth.

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