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NOTE ON THE ACTION OF SOME ESSENTIAL OILS ON FUNGI

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SUMMARY

The essential oils of **Cinnamomun zeylanicum** *(bark and leaf),* **Satureja hortensis, Rosmarinus officinalis** *and* **Cymbopogon citratus** *were tested on 113 fungal strains representative of 18 genera of yeasts and filamentous fungi, among which dermatophytes and moulds. Dermatophytes* (**M. gypseum** *complex,* **M. canis, E. floccosum, T. verrucosum, T. mentagrophytes, T. rubrum**) *resulted the most sensitive fungi to the four essential oils and particularly to cynnamon bark oil.*

RESUMEN

Los aceites esenciales de **Cinnamomum zeylanicum** *(corteza y hoja),* **Satureja hortensis, Rosmarinus officinalis** *y* **Cymbopogon citratus** *fueron probados sobre 113 cepas fúngicas representativas de 18 géneros levaduriformes y filamentosos, entre ellos dermatofitos y mohos. Los dermatofitos (***M. gypseum** *complex,* **M. canis, E. floccosum, T. verrucosum, T. mentagrophytes, T. rubrum***) resultaron ser los más sensibles a los cuatro aceites esenciales estudiados y particularmente para el aceite de corteza del cinamón.*

INTRODUCTION

Studies on the antifungal activity of volatile oils and their active components have been reported by numerous investigators (Ceruti et al., 1982; Deshmukh et al., Garg and Dengre, 1988), Various essential oils are already reported to possess fungitoxic activities on a number of food storage fungi, as species of genera **Aspergillus, Mucor, Rhizopus** (Thompson, 1986; 1989) and on fungi commonly associated with dermatophytosis as species of genera **Trichophyton and Microsporum** (Yousef et al., 1978).

The serum agar-cup-plate serial dilution in solid agar media and agar diffusion are the methods adopted for estimating **in vitro** the antifungal activity of volatile oils.

In this study the antifungal activity of four essential oils of cinnamon (**Cinnamomun zeylanicum** Nees), savory (**Satureja hortensis** L.), rosemary (**Rosmarinus officinalis** L.) and lemongrass (**Cymbopogon citratus** (D.C.) Stapf.) was essayied **in vitro** against 113 fungal strains, representative of yeasts and filamentous fungi among which dermatophytes and moulds.

Among the yeasts of the genus **Candida** the following species were tested: **C. albicans,** a normal inhabitant of the human body, **C. tropicalis, C. parapsilosis, C. pseudotropicalis, C. guilliermondii, C. krusei** and a few others which may occasionally be isolated from skin and the mucous membranes, but not so often to be regarded as a persistent part of the normal microbiota of man.

Among the dermatophytes our attention has applied to some species of the genera **Microsporum, Trichophyton** and **Epidermophyton**; they include geophilic, zoophilic and anthropophilic species isolated by us from soil, animal and man on previous study.

Among the moulds, a very heterogeneous group of microscopic filamentous fungi, species of the genera **Fusarium, Cladosporium, Alternaria,**

Penicillium, Mucor, Rhizopus, Trichoderma and **Helminthosporium** were tested. They include saprobic moulds and some parasites of plants which form localized **leafspots**, generalized **wilts** and **blights.**

They also are a major cause of spoilage in cereal grains and their growth and multiplication in foods are potential health hazards for fungal species implicated in the production of mycotoxins.

MATERIALS AND METHODS

Fungal strains.

The strains of yeast, dermatophytes and moulds used were from the fungal culture collection kept at this Institute: these are listed in Table 1

Medium.

Yeast-morphology agar (YMA) and Sabouraud's dextrose agar (SDA) are the media that we have used for yeasts, moulds and for dermatophytes in our screen.

Essential oils.

Cinnamomum zeylanicum (leaf and bark), **Satureja hortensis** and **Rosmarinus officinalis**, were obtained from Givaudan, Segrate (Milano, Italy); **Cymbopogon citratus** from Ortho Diagnostic Systems, Raritan (New Jersey, USA).

Essential oils were diluted in Polyoxyethylene sorbitan monolaurate (Tween 20) at 1/10, 1/100, 1/1000. Determination of inhibitory concentration was done by dilution technique; each dilution of the essential oil was assessed by applying 20 ul droplets of the dilution series on paper disks of 6 mm in diameter (blank discs OXOID).

Yeast inocula are made up in water at 10^5 colony forming units (cfu)/ml from overnight cultures on YMA, dermatophyte inocula from 2 weeks old cultures on SDA and mould inocula from 1 week old cultures on SDA; 1 ml of each fungal suspension was pipetted into Petri dishes of 10 cm diameter and mixed with 20 ml cooled agar medium YMA and SDA (c. 45º C). The plates were allowed to dry for 30 min. in the incubator. One paper disk per plate of 6 mm in diameter was placed centrally on medium and disks dipped in essential oil dilutions. Plates were incubated in the inverted position for 3-14 days at 28º C and then examined for growth. As controls disks dipped in Tween 20 were made.

Triplicate plates were set up for each test fungus.

The concentration of each essential oil which produced a definitite zone of inhibition has been recorded.

RESULTS

Characteristic results obtained for essential oils are shown in Table 1.

According to their behaviour fungi can be roughly divided into three groups.

Group I - The fungal growth of this group is inhibited completely by all pure essential oils and also partially at low concentrations (1/10, 1/100, 1/100). fungi belonging to this group include geophilic, zoophilic and antrhopophilic species of dermatophytes: **M. gypseum complex, M. canis, T. verrucosum, T. mentagrophytes, T. rubrum** and **E. floccosum.** All were sensitive to various concentrations of essential oils, particularly common bark and leaf. It is interesting to note that the inhibitory concentration of cinnamon essential oil was 1000 ppm against **M. canis.**

Group II - Growth of fungi in this group is inhibited at higher essential oil concentrations and growth inhibition occurs again into a restricted fungal taxa. Among filamentous fungi tested, only the moulds and in particular **F. moniliforme, A. ochraceus, A. clavatus, D. oryzae, P. brevicompactum, P. oryzae and P. thomii** were inhibited by cynnamon and lemongrass diluted at 1/10, 1/100, 1/1000, although even these differed somewhat in their sensitivity.

Group III - The fungal growth of this group is partially inhibited by some pure essential oils. These little or nothing sensitive fungi include imperfect genera of ascomycetous yeasts or basidiomycetous-type yeast and the arthrosporeous **Geotrichum.**

In general this initial test for antifungal activity of four essential oils against different fungal taxa, shows a particular sensitivity of some fungal **taxa** to essential oils, rather than a different antifungal activity.

TABLA 1

Antifungal activity of essential oils. Inhibitory concentration and percentage inhibition.

Fungi	Nº of strains	Cinnamomun zeylanicum								Cymbopogon citratus				Satureja hortensis				Rosmarinus officinalis				Test (tween 20)
		bark				leaf																
		p.o	1/10	1/100	1/1000	p.o	1/10	1/100	1/1000	p.o	1/10	1/100	1/1000	p.o	1/10	1/100	1/1000	p.o	1/10	1/100	1/1000	
Candida albicans	(6)	30	15	0	0	30	20	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0
C. famata	(3)	30	20	0	0	38	0	0	0	30	0	0	0	25	0	0	0	0	0	0	0	0
C. guilliermondii	(3)	50	15	0	0	60	35	0	0	0	0	0	0	25	0	0	0	20	0	0	0	0
C. parapsilosis	(2)	25	0	0	0	30	20	0	0	15	0	0	0	30	0	0	0	10	0	0	0	0
C. pseudotropicalis	(2)	40	15	0	0	45	45	0	0	25	0	0	0	15	0	0	0	25	10	0	0	0
C. stellatoidea	(3)	40	15	0	0	40	30	0	0	30	0	0	0	20	0	0	0	0	0	0	0	0
C. tenuis	(1)	35	10	0	0	40	15	0	0	0	0	0	0	10	0	0	0	15	0	0	0	0
C. tropicalis	(3)	40	0	0	0	20	15	0	0	25	0	0	0	25	0	0	0	0	0	0	0	0
C. zeylanoides	(2)	50	20	0	0	20	0	0	0	30	0	0	0	10	0	0	0	10	0	0	0	0
Cryptococcus albidus	(3)	50	50	20	0	50	50	40	0	50	50	0	0	50	20	0	0	50	0	0	0	0
Geotrichum terrestre	(4)	30	20	0	0	50	20	0	0	50	0	0	0	30	0	0	0	10	0	0	0	0
Hansenula anomala	(1)	25	5	0	0	30	15	0	0	20	0	0	0	20	0	0	0	7	0	0	0	0
Rhodotorula rubra	(3)	36	31	8	0	36	34	10	0	24	10	0	0	24	0	0	0	18	0	0	0	0
Saccharomyces cerevisiae	(3)	25	15	0	0	25	25	10	0	20	10	0	0	20	0	0	0	15	0	0	0	0
Epidermophyton floccosum	(5)	100	100	50	30	100	100	100	50	100	100	20	0	100	100	50	0	100	100	50	0	0
Microsporum audouinii	(1)	100	100	100	10	100	100	100	40	100	100	40	10	100	100	15	0	100	100	20	0	0
M. canis	(10)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	50	100	100	100	0	0
M. gypseum	(5)	100	100	100	50	100	100	100	100	100	100	100	100	100	100	100	50	20	10	0	0	0
Trichophyton mentagrophytes	(5)	100	100	100	50	100	100	100	50	50	30	0	0	100	100	0	0	25	15	0	0	0
T. rubrum	(3)	100	100	30	10	100	100	100	50	100	100	100	100	100	100	0	0	100	30	0	0	0
T. verrucosum	(1)	100	100	10	0	100	100	100	50	100	100	100	100	100	100	0	0	20	15	0	0	0
Asperguillus clavatus	(2)	40	40	0	0	40	30	0	0	40	30	0	0	10	0	0	0	30	0	0	0	0
A. fumigatus	(4)	50	50	0	0	50	50	10	0	50	30	0	0	50	0	0	0	0	0	0	0	0
A. niger	(3)	40	10	0	0	40	10	0	0	0	0	0	0	40	0	0	0	0	0	0	0	0
A. ochraceus	(4)	40	40	0	0	40	30	0	0	40	30	0	0	10	10	0	0	30	0	0	0	0
Cladosporium cladosporioides	(3)	40	30	0	0	20	10	0	0	0	0	0	0	50	30	0	0	20	0	0	0	0
Drechslera oryzae	(1)	40	30	0	0	40	30	0	0	30	20	0	0	50	30	0	0	20	0	0	0	0
Fusarium moniliforme	(3)	50	40	0	0	40	30	0	0	20	0	0	0	40	0	0	0	0	0	0	0	0
F. oxysporum	(4)	35	20	0	0	30	15	0	0	15	0	0	0	35	0	0	0	0	0	0	0	0
F. roseum	(1)	50	20	0	0	50	25	0	0	25	0	0	0	40	30	0	0	0	0	0	0	0
Helminthosporium maydis	(2)	40	30	0	0	15	0	0	0	20	0	0	0	30	0	0	0	0	0	0	0	0
Penicillium brevicompactum	(3)	50	50	20	0	50	50	25	10	50	50	0	0	50	0	0	0	15	0	0	0	0
P. charlesii	(1)	50	30	0	0	50	40	0	0	15	0	0	0	40	0	0	0	20	0	0	0	0
P. chrysogenum	(1)	30	20	0	0	45	25	0	0	35	0	0	0	40	0	0	0	0	0	0	0	0
P. thomii	(1)	40	30	0	0	40	25	0	0	25	0	0	0	50	0	0	0	0	0	0	0	0
Pyricularia oryzae	(2)	40	30	0	0	40	15	0	0	15	0	0	0	20	0	0	0	0	0	0	0	0
Rhizopus stolonifer	(3)	50	50	0	0	50	50	0	0	50	50	0	0	50	15	0	0	50	60	0	0	0
Trichoderma harzianum	(3)	30	0	0	0	45	0	0	0	20	10	0	0	40	0	0	0	0	0	0	0	0
T. viride	(3)	35	0	0	0	30	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0