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Antidermatophytic Activities of Nine (9) Essential Oils

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The antidermatophytic activity of nine essential oils from 7 plants against 6 fungi was investigated. Fungistatic effect was observed for the oil of *Coreopsis bonariensis* (2 mg/ml), *Laggera alata* var *alata* (1 mg/ml) against *Microsporum audouinii* and for *Cupressus lusitanica* oil against *Trichophyton mentagrophytes* (2 mg/ml). Fungicidal effect was observed for the *C. lusitanica* (leaves) oil (2 mg/ml) against *Trichophyton mentagrophytes*, *Erigeron floribundus* (leaves) oil (1 mg/ml) against *Candida albicans* and for oil of the flowering ends of *C. lusitanica* against *Trichophyton mentagrophytes* at 1 mg/ml. Finally, the essential oils of *C. lusitanica* were found to be the most active while *M. audouinii* was the least resistant fungus.

Keywords: Antidermatophytic , essential oils.

INTRODUCTION

The treatment of skin diseases and especially dermatophytosis is mainly by synthetic drugs (polyenes, azoles and allylamines) [1]. These drugs are few in number and have a narrow safety margin. Some functional disorders like nephrotoxicity may result due to their administration [2]. This explains why researchers have recently been concentrating on the search for new plants derived substances with more activity and less toxicity. This interest for medicinal plants is well understood, taking into account the large population of the developing countries, which exclusively use these plants for treatment. In fact according to the World Health Organization (WHO), this fraction of population is about 80 % [3]. This orientation towards plants shows that the plants are potential reservoirs of efficient antimicrobial substances, which are biodegradable [4]. The essential oils that are more often, complex mixtures of components derived from aromatic plants could then be one of the possible sources. In this investigation, we describe the *in vitro* activity of nine essential oils against six (6) dermatophyte isolates, which are considered as a major cause of morbidity and mortality [5].

EXPERIMENTAL

Plant material and isolation of essential oils

The essential oils used in this study were obtained from *Cupressus lusitanica* Mill. (flowering ends), *Erigeron floribundus* (H.B. & K.) Sch. Bip (leaves and flowers) and leaves of *Coreopsis bonariensis*, *Coreopsis asperta, Laggera pterodonta* Sch. Bip*, L. alata* (Sch. Bip ex Oliver var *alata, L. alata var montana* (table 1). These plants were harvested in and around the campus of the University of Dschang. All voucher specimens have been deposited at the Laboratory of plant Biology, Faculty of Science, University of Dschang. Fresh plant material (1kg) was subjected to hydrodistillation for 8 hours. The oil was dried over anhydrous sodium sulfate and stored in refrigerator till usage.

Screening for antifungal activity

Six (6) fungal isolates, *Trichophyton mentagrophytes* 10a, *Trichophyton mentagrophytes* DL92, *Trichophyton mentagrophytes* var *interdigitale* F120b, *Trichophyton rubrum* Ma2, *Microsporum audouinii* and *Candida albicans* isolated clinically, were used to establish the antifungal activity of essential oils.

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Table 1: Essential oils and tested fungi

Essential oils		Fungi
Cupressus lusitanica	Flowering ends	*Trichophyton mentagrophytes* 10a
		Trichophyton mentagrophytes DL92
		Trichophyton mentagrophytes var *interdigitale* F120b
		Trichophyton rubrum Ma2
Cupressus lusitanica	Leaves	*Trichophyton mentagrophytes* 10a
		Candida albicans
Coreopsis bonariensis	Leaves	*Microsporum audouinii*
		Trichophyton mentagrophytes 10a
Coreopsis asperta	Leaves	*Trichophyton mentagrophytes* 10a
		Trichophyton mentagrophytes DL92
Laggera alata var *alata*	Leaves	*Microsporum audouinii*
Laggera alata var *montana*	Leaves	*Microsporum audouinii*
		Trichophyton mentagrophytes DL92
Laggera pterodonta	Leaves	*Trichophyton mentagrophytes* DL92
Erigeron floribundus	Leaves	*Trichophyton mentagrophytes* 10a
		Candida albicans
Erigeron floribundus	Flowers	*Trichophyton mentagrophytes* 10a
		Candida albicans

All the fungi were maintained on Sabouraud Dextrose Agar. The basic culture medium used for the assays was Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol. Final oil doses of 0.5, 1 and 2 mg/ml in the medium were obtained after dispersion of the essential oil in water containing Tween 20. The concentration of Tween 20 in the medium did not exceed 3 % and it was used as positive control. Thus, a precise volume of a stock solution (0,1 g/ml) of essential oil was pipetted and added to sufficient volume of culture medium for 20 ml so as to reach the desired concentration of the essential oil in the medium.

Determination of the Effects of the essential oils on mycelial growth

The technique used was similar to that described by Ngane [6]. The fungus was allowed to grow first on SDA for 7 days at 25 ± 2 °C after which time a circular piece of fresh mycelium was transferred into petri dishes containing SDA with different essential oil doses. The petri dishes were incubated for 10 days at 25 ± 2 °C, during which the length of mycelium was measured every 24 h. All culture media were done in triplicate. If no growth were observed after 10 days, the piece of mycelium was transferred on to oil free SDA in a petri dish and incubated at 25 ± 2 °C in order to determine whether it was still viable. Minimum Inhibitory Concentration (MIC) was considered as the smallest oil concentration to inhibit mycelial growth. The mycelial growth (Mg) and the percentage of inhibition (I %) were respectively calculated from these relations:

$$Mg = (\theta_1-\theta_2)/2 - \theta_0 \quad (I)$$
$$I\% = ((\theta_t-\theta_e)/\theta_t) \times 100 \quad (II)$$

θ_1 and θ_2 are perpendicular diameter of mycelial growth, θ_0 the diameter of the initial circular piece, θ_t the diameter of the mycelial growth of the negative control (petri dish without essential oil) and θ_e the diameter of the mycelial growth in a test petri dish.

Statistical Analyses

The means of the percentages of inhibition and the efficacy of essential oils were compared using analyses of variance taking into account both isolates and essential oil factors. At this effect, the SPSS 0.1 computer program was used. The differences at $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The results obtained in this work are shown in table 2. From the results, it is evident that the tested essential oils possessed different antifungal activities. The difference in the activity varies with the type of fungi species. The difference in activity observed between the essential oil could be due to their chemical dissimilarities both quantitatively and qualitatively.

Table 2: Effects of essential oils on various fungi isolates

Fungi	Essential Oils		1(not)	MIC (mg/ml)	Observations
T. mentaqggrophytes 10a	*C. lusitanica*	Leaves	$88.55^{b,c}$	2	Fungicidal
	C. lusitanica	Flowering ends	90.32^{b}	2	Fungistatic
	C. bonariensis	Leaves	59.76^{g}	>2	-
	C. asperta	Leaves	75.65^{d}	>2	-
	E. floribundus	Leaves	52.13^{h}	>2	-
	E. floribundus	Leaves	50.71^{h}	>2	-
T. mentagrophytes DL92	*C. lusitanica*	Flowering ends	82.12^{c}	>2	-
	C. bonariensis	Leaves	78.45^{d}	>2	-
	C. asperta	Leaves	81.25^{c}	>2	-
	L. pterodonta	Leaves	72.25^{e}	>2	-
	L. alata var montana	Leaves	77.18^{d}	>2	-
C. albicans	*C. lusitanica*	Leaves	6.60^{f}	>2	-
	E. floribundus	Leaves	$95.08^{a,b}$	2	Fungicidal
	E. floribundus	Flowers	83.43^{c}	>2	-
M. audouinii	*C. bonariensis*	Leaves	56.59^{g}	2	-
	L. alata var alata	Leaves	92.16^{b}	1	Fugistatic
	L. alata var montana	Leaves	100^{a}	0.5	-
T. rubrum	*C. bonariensis*	Flowerings ends	98.99^{a}	1	-
T .metagraphytes var *interdigitale* F 120b	*C. lusitanica*	Flowering ends	71.48^{e}	>2	-

I: Percentages of inhibition followed by the same letter are not significantly different at p=0.05Mic: Minimum inhibitory concentration.

Indeed, the essential oil of *L. alata* var *montana* is mainly constituted of dimethoxy-p-cymene (34%) and epi-γ-endesmol (21.4%) [7]. Those of leaves and flowers of *E. floribundus* are rich in limonene (9.5 % for leaves and 3.7 % for the flowers) and in polyacetylated compounds like (E)-2-lachnophyllum ester (23.7 % for leaves and 3.4 % for the flowers) [8]. The observed activities could be attributed to these major components while recognizing that this activity is often the result of the combined action (antagonism, synergy and potentiation) of all the constituents of an essential oil taking into consideration even those present in trace [9]. This is why, essential oil *L. alata* var *montana* inhibits *M. audouinii* at 100 % compared 56.59 % for *C. bonariensis* on the same fungus.

On the other hand, the differences in susceptibility, which characterize the isolates of this study, could be due to their belonging either to different species or to different varieties for the same species (e.g *T. mentagrophytes*). Thus, they could have divergent genetic constitution. The highest susceptibility of *T. rubrum* (98.99 %) to the EO of flowering ends of *C. lusitanica* against 71.48 % for *T. mentagrophytes* is a good illustration of this variation.

Also, the decrease in activity of the essential oils with time could be attributed to their volatility. On the other hand, the increase of the activity with the dose could be explained through their cumulative effect and abundant proportion of the active substance (s), which lead to the death of the fungus.

CONCLUSION

Essential oils used in this work possess antifungal activity against dermatophytes. The overall results show that *M. audouinii* is the most susceptible fungus whereas the essential oil of flowering ends of *C. lusitanica* is the most active.

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