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Antifungal activities of nine traditional Mexican medicinal plants

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Abstract

Eighteen plant extracts from nine traditional Mexican medicinal plants were tested for antifungal activity against two dermatophyte fungal species (*Trichophyton mentagrophytes* and *Trichophyton rubrum*), one non-dermatophyte (*Aspergillus niger*), and one yeast (*Candida albicans*). The strongest effect was manifested by the hexane extracts from *Eupatorium aschenbornianum* and *Sedum oxypetalum*, as well as the methanol extracts from *Lysiloma acapulcensis* and *Annona cherimolia*.

Keywords: Antifungal activity; Medicinal plants; Plant extracts; Folk medicines

1. Introduction

Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide (World Health Organization, 1998). During the past several years, there has been an increasing incidence of fungal infections due to a growth in immunocompromised population such as organ transplant recipients, cancer and HIV/AIDS patients. This fact coupled with the resistance to antibiotics and with the toxicity during prolonged treatment with several antifungal drugs (Giordani et al., 2001) has been the reason for an extended search for newer drugs to treat opportunistic fungal infections (Fostel and Lartey, 2000).

This paper reports the in vitro antifungal screening of 18 extracts from nine medicinal plants against four human pathogenic fungi. The plant species selected are used as traditional medicines in Morelos State, Mexico, for the treatment of illnesses which, according to the symptomatology described, include respiratory, genital, gastrointestinal, urinary and skin infections. The ethnobotanical data on the use of these plants and the selection of the plant part to be tested were complemented with a bibliographic review and interviews with empirical midwives and herbalists of the rural areas linked to the research project (Table 1). Minimum inhibitory concentration (MIC) was established for hexane and methanol extracts from each one of the nine species.

2. Materials and methods

2.1. Plant materials

Asclepia curassavica, *Bixa orellana*, *Eupatorium aschenbornianum*, *Galphimia glauca*, *Lysiloma acapulcensis*, *Malva parviflora*, *Sedum oxypetalum* and *Senecio angulifolius* (Table 1) were collected in November 2000, in their natural habitat from different regions of the Morelos State, Mexico. Voucher specimens were identified at the Herbarium of the Instituto Nacional de Antropología e Historia de Morelos (INHAM) in Cuernavaca City, Morelos State and were deposited at this institution. *Annona cherimolia* was collected in the same month from Arteaga, Michoacan State, and a voucher specimen was identified and deposited at the Herbarium of the Escuela Nacional de Ciencias Biológicas Instituto Politécnico Nacional (HENCB), Mexico City (Table 1).

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Table 1
List of medicinal plants used in the antifungal assay

Species (family) (voucher specimen number)	Local name	Popular uses (route of administration) (references)
Annona cherimolia Miller (Annonaceae) (HENCB 504-1)	Chirimoya	For diarrhea, stomach infections, vomiting, dysentery, flatulence, dandruff, pimples, inflammation (oral and topic) (Argueta et al., 1994; Fuentes and Avilés, 1997; Monroy and Castillo, 2000)
Asclepia curassavica L. (Asclepiadaceae) (INHAM 230-1)	Quiebra muelas	For skin problems, pimples, gonorrhea, warts, headaches, parasitism, cough, angina, colds, vaginal infections, herpes, muscular ache, eye infections, rabies, vomiting, scorpion stings, fever (oral, topic, vaginal, and eye wash) (Argueta et al., 1994; Avilés and Suárez, 1994; Fuentes and Avilés, 1997; Monroy and Castillo, 2000)
Bixa orellana L. (Bixaceae) (INHAM 2001)	Achiote	For measles, allergy, leprosy, wounds, diarrhea, stomach upset, scabies, ulcers, angina, tumors, skin problems, urinary affections (oral and topic) (Argueta et al., 1994; Avilés and Suárez, 1994; Fuentes and Avilés, 1997)
Eupatorium aschenbornianum Schauer (Asteraceae) (INHAM 141-1)	Axihuitl	For skin problems, wounds, tumors, aphtas (oral and topic) (Argueta et al., 1994; Avilés and Suárez, 1994; Fuentes and Avilés, 1997; Monroy and Castillo, 2000)
Galphimia glauca Cav. (Malpighiaceae) (INHAM 360-1)	Corpionchi	For wounds, pimples, womb inflammation, vaginal flow, skin problems, vaginal infections (topic and vaginal) (Argueta et al., 1994; Avilés and Suárez, 1994; Monroy and Castillo, 2000)
Lysiloma acapulcensis (Kunth) Benth. (Fabaceae) (INHAM 2002)	Tepehuaje	For wounds, diarrhea, bladder infections (oral and topic) (Argueta et al., 1994; Avilés and Suárez, 1994; Monroy and Castillo, 2000)
Malva parviflora L. (Malvaceae) (INHAM 2004)	Alacle	For sores, inflammation, abscesses, pimples, angina inflammation, intestinal infections, parasitism, kidney infections, vaginal inflammation, fever, headaches, aphtas, spider stings, bronchitis, cough, colds, pharingitis, tuberculosis, vaginal infections (oral, vaginal, and topic) (Argueta et al., 1994; Avilés, 2001; Avilés and Suárez, 1994; Monroy and Castillo, 2000)
Sedum oxypetalum HBK. (Crassulaceae) (INHAM 1126-1)	Siempreviva	For burns, skin infections, mouth infections, eye affections (topic, oral and eyes wash) (Argueta et al., 1994; Avilés, 2001; Avilés and Suárez, 1994; Fuentes and Avilés, 1997; Monroy and Castillo, 2000)
Senecio angulifolius DC. (Asteraceae) (INHAM 325-1)	Cachane	For vaginal infections (vaginal) (Avilés and Suárez, 1994; Fuentes and Avilés, 1997; Monroy and Castillo, 2000)

2.2. Extractions of plant materials

The powder of the dried plant materials was extracted sequentially with *n*-hexane and methanol (100 g/1500 ml) at room temperature. Each solvent was replaced three times with fresh solvent, remaining in contact with the plant material for 48 h in each occasion. After filtration, the extracts were concentrated under low pressure at 40 °C. Finally, the percentage yield for each extract was determined.

2.3. Preparation of tested extracts

Stock solutions of the extracts and controls were prepared in dimethyl sulphoxide (DMSO, Merck), at concentrations of 60 and 5 mg/ml, respectively, resulting in homogeneous solutions of each one. Further dilutions were performed using sterile distilled water. Positive controls were prepared with Ketoconazole (Sigma) and Nystatin (Merck). Final concentrations of DMSO in the test were less than 2% (v/v).

2.4. Microorganisms

The microorganisms used for the biological evaluation were purchased from the American Type Culture Collection (ATCC, Rockville, MD). They were: *Aspergillus niger* ATCC 10335, *Trychophyton mentagrophytes* ATCC 28185, *Trychophyton rubrum* ATCC 28188, and *Candida albicans* ATCC 10231. The filamentous fungi were maintained on potato dextrose agar (PDA, Merck) at 27 °C. Sabouraud's glucose agar (SGA, Merck) was used to maintain the yeast and as an assay medium.

2.5. Antifungal assay

The antifungal assay was performed by the agar dilution method using 100 mm × 15 mm petri dishes (Falcon). The stock solutions of the extracts and controls were two-fold serial dilution in concentrations in the range of 8–0.125 mg/ml and 128–1 μg/ml, respectively. A final inoculum of 10^5 cell/ml for the *Candida albicans* and 10^6 spore/ml for the filamentous fungi was spouted on top of the solidified agar with a loop calibrated to deliver 0.010 ml. Experiments were triplicated. The plates were incubated at 29 °C. The fungal growth was checked, first in control plates prepared without any test sample, after 24, 48 and 72 h, depending on the period of incubation time required for a visible growth; 24 h for *Candida albicans*, 48 h for *Aspergillus niger* and 72 h for the dermatophytes. MIC was defined as the lowest concentration of extract that inhibited visible growth on agar.

3. Results and discussion

In Table 2, the specific plant parts employed, percentage yield and MIC values of the corresponding extracts are summarized. Considering that in this study only crude extracts were employed, extracts with MIC values of 8 mg/ml or below against any of the fungi tested, were considered active. The results obtained in the antimicrobial evaluation give evidence of pronounced antifungal activity of hexane extracts of *Eupatorium aschenbornianum* and *Sedum oxypetalum*, and methanol extracts of *Lysiloma acapulcensis* and *Annona cherimolia* which demonstrated inhibitory growth of all tested fungi.

The MIC results indicate that *Eupatorium aschenbornianum* hexane extract was found to be the most active against the dermatophytes *Trychophyton mentagrophytes* and *Trychophyton rubrum* with MIC values of 0.03 and 0.3 mg/ml, respectively, while against *Candida albicans* and *Aspergillus niger*, the MIC values were 8.0 and 4.0 mg/ml, respectively. *Sedum oxypetalum* hexane extract showed a MIC value of 8.0 mg/ml against all tested microorganisms, whereas the methanol extract was active against *Aspergillus niger* and *Trychophyton rubrum* with MIC values of 8.0 and 2.0 mg/ml, respectively. *Lysiloma acapulcensis* methanol extract showed activity against all tested fungi, giving MIC values of 1.0 mg/ml against *Trychophyton mentagrophytes* and *Trychophyton rubrum*, 2.0 mg/ml against *Candida albicans* and 4.0 mg/ml against *Aspergillus niger*. *Annona cherimolia* methanol extract demonstrated antifungal activity against all fungi tested, with MIC values of 4.0 mg/ml for *Trychophyton mentagrophytes*, *Aspergillus niger* and *Candida albicans* and 8.0 mg/ml for *Trychophyton rubrum*. The remaining five plants were active against only one or two of the fungal species (Table 2).

Previous reports on the chemical nature or antimicrobial activity have been described for the following species: chromones and benzofurans for *Eupatorium aschenbornianum* (Gómez et al., 1982); flavonoids, pyrrolidines and piperidines for *Sedum* spp. (Stevens et al., 1996; Kim et al., 1996); tannins in *Lysiloma acapulcensis* (Argueta et al., 1994); alkaloids and acetogenins for *Annona cherimolia* seeds (Ríos et al., 1989); terpenes, fernesylacetone, geranyl geranyl octadecanoato, C20-terpene alcohol and minor carotenoids for *Bixa orellana* (Jondiko and Pattenden, 1989; Mercadante et al., 1999). Previous reports have demonstrated that *Bixa orellana* possessed antifungal activity against *Cryptococcus neoformans* and *Microsporum gypseum*, with MIC values at 8 and 2 mg/ml, respectively

Table 2
Antifungal activity of crude extracts of nine traditional Mexican medicinal plants

Species	Plant parts tested[a]	Extract[b]	Extract yield (%)[c]	Antifungal activity MIC (mg/ml)			
				Candida albicans	*Aspergillus niger*	*Trychophyton mentagrophytes*	*Trychophyton rubrum*
Annona cherimolia	S	H	4.3	>8.0	>8.0	>8.0	>8.0
		M	3.2	4.0	4.0	4.0	8.0
Asclepia curassavica	L, St	H	5.9	>8.0	>8.0	>8.0	2.0
		M	5.1	>8.0	>8.0	>8.0	2.0
Bixa orellana	L, St	H	3.5	>8.0	>8.0	>8.0	>8.0
		M	3.7	>8.0	>8.0	2.0	2.0
Eupatorium aschenbornianum	L, St	H	2.8	8.0	4.0	0.03	0.2
		M	4.0	8.0	>8.0	1.0	0.5
Galphimia glauca	Ap	H	2.4	>8.0	>8.0	8.0	4.0
		M	4.5	>8.0	>8.0	8.0	>8.0
Lysiloma acapulcensis	B	H	2.6	>8.0	>8.0	>8.0	>8.0
		M	5.2	2.0	4.0	1.0	1.0
Malva parviflora	Ap	H	2.3	8.0	8.0	>8.0	>8.0
		M	4.9	>8.0	>8.0	>8.0	>8.0
Sedum oxypetalum	L, St	H	2.1	8.0	8.0	8.0	8.0
		M	3.6	>8.0	8.0	>8.0	2.0
Senecio angulifolius	L, St	H	4.6	>8.0	>8.0	8.0	>8.0
		M	4.1	>8.0	>8.0	8.0	>8.0
Myconazol				NT	0.016	0.008	0.008
Nystatin				0.008	NT[d]	NT	NT

[a] Plant parts tested: Ap, aerial parts; B, bark; L, leaves; S, seeds; St, steam.
[b] Extract: H, hexanic; M, methanolic.
[c] Dry residue of the extract in terms of dry starting material.
[d] NT: not tested.

(Cáceres et al., 1998). Studies on latex of *Asclepia curassavica* have also identified terpenes and cardenolides, which are presumed to be responsible for the growth inhibition of *Candida albicans* (Moulin-Traffort et al., 1990). No data on antimicrobial activity of *Eupatorium aschenbornianum*, *Sedum oxypetalum*, *Lysiloma acapulcensis*, *Senecio angulifolius* and *Galphimia glauca* appear to have been published.

The results of the present work indicate that the plant species assayed possess antifungal properties. This explains the use of these plants in folk medicine for the treatment of various diseases whose symptoms might involve fungal infections, and underline the importance of the ethnobotanical approach for the selection of plants in the discovery of new bioactive compounds. Further phytochemical research is needed to identify the active principles responsible for the antifungal effects of some of these medicinal plants.

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