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DISCOVERY OF ANTIFUNGAL PLANTS IN ARGENTINEAN SAN LUIS PROVINCE: ETHNOMEDICAL INFORMATION OR RANDOM SELECTION?

DESCOBERTA DAS PLANTAS ANTIFÚNGICAS EM SAN LUIS PROVÍNCIA DE ARGENTINA ETNOMEDICINA INFORMAÇÕES OU SELEÇÃO ALEATÓRIA?

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ABSTRACT: This study reports the antifungal evaluation of eighty-two crude methanolic extracts of plants from San Luis province, Argentina, which were selected on the basis of their reported ethnomedical uses and compared them with plants selected at random.
The extracts were screened for antifungal properties against yeasts and filamentous fungi and the concentration that completely inhibited the fungal growth (MIC) was determined. For the antifungal evaluation, the microbroth dilution assay recommended by the Clinical and Laboratory Standards Institute was used. For the statistical analysis the Pearson's Chi Square test and the Score's test were used.
The results showed that 25 out of the 40 PE plants (62.5%) were active (MIC ≤ 1000 µg/mL) unless in one group of fungi; 6 (15%) were active in yeasts; 4 (10%) were active in *Aspergillus* spp. and 25 (62.5%) were active in dermatophytes. In turn, among the 42 PW species, 4 (10%) were active (MIC ≤ 1000 ug/mL) unless in one group of fungi, 1 (2.5%) were active in yeasts, 1 (2.5%) were active in *Aspergillus* spp. and 4 (10%) were active in dermatophytes. In addition, it is observed a clear trend of extracts to display

lower MICs within PE group, against dermatophytes ($p < 0.01$) fungi responsible of superficial infections. These findings suggest that the ethnopharmacological approach is useful in guiding the discovery of antifungal plants.
KEYWORDS: Ethnomedical information, San Luis province, Argentinean plants, antifungal activity

RESUMO: Este estudo mostra a avaliação da atividade antifúngica de oitenta e dois extratos metanólicos de plantas na província de San Luis, Argentina, que foram selecionados com base em seus usos ethnomedical relatados e comparados com as plantas selecionadas aleatoriamente.

Os extratos foram selecionados para as propriedades antifúngicas contra leveduras e fungos filamentosos, ea concentração que inibiu completamente o crescimento de fungos (MIC) foi determinado. Para a avaliação da atividade antifúngica, o teste de microdiluição recomendados pelo Clinical Laboratory Standards Institute. Para a análise estatística de Pearson Chi quadrado e teste do Score foram utilizados.

Os resultados mostraram que 25 das 40 plantas de PE (62,5%) eram ativos (MIC $\leq$ 1000 1,000 µg/mL) pelo menos em um grupo de fungos, 6 (15%) estavam ativos em leveduras, 4 (10%) estavam ativos em *Aspergillus* spp. e 25 (62,5%) estavam ativos em dermatófitos. Além disso, observa-se uma clara tendência de extratos para mostrar MICs menores no grupo PE, contra dermatófitos ($p <0,01$) fungos responsáveis de infecções superficiais. Estes resultados sugerem que a abordagem etnofarmacológica é útil para orientar a descoberta de plantas antifúngicos.
PALAVRAS-CHAVES: Informação ethnomedical, província de San Luis, Argentina plantas, atividade antifúngica

INTRODUCTION

Invasive fungal infections have increased in frequency in the past two decades, having an enormous impact on morbidity and mortality in immunocompromised patients[1-2]. Fungi with low virulence for immunocompetent persons, can be life-threatening[3] for neonates, cancer patients receiving chemotherapy, organ transplant and burn patients, in addition to those with acquired immunodeficiency syndrome (AIDS). Other risk factors include corticosteroid and antibiotic treatments, diabetes, epidermic and dermis injuries, malnutrition, neutropenia, and

surgery[4]. In addition, an increasing number of normal individuals, including children in third-world nations[5-6] that receive deficient sanitary attention and education, suffers superficial fungal infections (those involving the skin and mucosal surfaces) which diminish the quality of their lives.

Although it appears to be a big armamentarium of antifungal drugs in clinical use, in fact a modest number of drugs, derived from five antifungal classes, are available[7].

All of the available antifungals possess some of the following inconveniences: they have a limited spectrum of action, are fungistatic rather than fungicide thus producing recurrence, develop resistance or are toxic[8].

Plants provide countless opportunities for the isolation of new antifungal compounds because of the peerless availability of chemical diversity[9]; in fact, numerous antifungal compounds have been isolated from them[10-13] among many others.

But the first important concern within a program of discovery of new plants with antifungal properties is the selection of species to be submitted to biological evaluation.

According to a recent review of Ríos and Recio[14] a wide range of criteria have been followed to select plants to be submitted to antimicrobial studies. Some studies focused on the ethnopharmacological uses; others, investigated plants growing in a specific region or country; a third group focused on the finding of inhibitors of one or more clinically important fungal species with an *at random* selection of plants.

Among the different concluding recommendations, Ríos and Recio[14] suggested to avoid the at random criterion and to select plants following an ethnopharmacological perspective since this approach appeared to enhance the probability of success in new drug-finding efforts. These authors based their recommendation on some works[15-19] that, unfortunately, all were performed with the agar diffusion method. In this qualitative method, the diffusion plays an important role in the diameter of the inhibition halo, and therefore it could not reflect the true antifungal activity of the extract[20].

With the aim of determining the importance of following the ethnopharmacology knowledge to detect antifungal plants growing in San Luis province of Argentina in comparison to the at random criterion, we made a bibliographic survey of the ethnomedical information on plants of this region used for ailments related to antifungal infections.

Among the flora of different regions of Argentina, San Luis province represents one important source of material with pharmacological activity due to its biodiversity.

A five-stage process of documentation, evaluation and analysis of results was conducted: (1) selection of words that could describe the ethnopharmacology use related to fungal infections; (2) a survey of specialized literature on ethnopharmacological uses of plants from San Luis; (3) collection and preparation of extracts of each plant. (4) antifungal evaluation of the selected plants; (5) statistical analysis of the results.

A group of 40 plants used medicinally for ailments related to fungal infections in San Luis province of Argentina (PE) were selected. The following conditions of traditional therapeutic indications were chosen: anti-alopecia, antibiotic, antifungal, antiseptic, dandruff, dental and general infections, diarrhœa, itching, leucorrhœa, respiratory diseases, skin ailments, skin infections, skin ulcerations, urinary infections, vaginitis, venereal diseases, vulnerary, women diseases and wound healing.

Another group of 42 plants without reported traditional use, or with traditional use not related to fungal infections (PW group) was chosen *at random* and collected in the San Luis province of Argentina.

The taxonomic identity of the plant of both groups was established by the authors, Prof. Elisa Petenatti and Luis Del Vitto.

A crude methanolic extract of aerial parts of each plant was evaluated for antifungal properties against the same panel of fungi with the microbroth dilution assay recommended by the Clinical and Laboratory Standards Institute[22].

MATERIAL AND METHODS

Plants

Aerial parts of the plants detailed in Table 1 and 2 were collected in the different environments of San Luis province.

Voucher specimens were deposited at the Herbarium of the National University of San Luis (UNSL) and classified by Professors Elisa Petenatti and Luis Del Vitto.

Extracts preparation

Aerial parts of each plant species were air dried and then ground in a mill. Powders were submitted to maceration with MeOH (3x) 24 h each. Plant extracts were filtered, pooled, vacuum evaporated at < 40 ºC in a rotary

evaporator and stored at -80 ºC until tested.

Antifungal evaluation

Microorganisms and media

For the antifungal evaluation, standardized strains from the American Type Culture Collection (ATCC), Rockville, MD, USA, and Reference Center in Mycology (CEREMIC, C, Rosario, Argentina) were used. *Candida albicans* ATCC 10231, *Candida tropicalis* C 131, *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264, *Aspergillus flavus* ATCC 9170, *A. fumigatus* ATTC 26934, *A. niger* ATCC 9029, *Trichophyton rubrum* C 110, *T. mentagrophytes* ATCC 9972, *M. gypseum* C 115 and *Epidermophyton floccosum* C 114. Strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30 °C, maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid) and sub-cultured every 15 days to prevent pleomorphic transformations. Inocula were obtained according to reported procedures and adjusted to 1-5 $x10^3$ cells/spores with colony forming units (CFU) /mL[21].

Antifungal susceptibility testing

Minimal Inhibitory Concentration (MIC) of each extract or compound was determined by using broth microdilution techniques according to the guidelines of the National Committee for Clinical Laboratory Standards for yeasts (M27-A2) and for filamentous fungi (M 38 A)[21], MIC values were determined in RPMI-1640 (Sigma, St Louis, Mo, USA) buffered to pH 7.0 with MOPS. Microtiter trays were incubated at 35 °C for yeasts and hialohyphomycetes and at 28-30 °C for dermatophyte strains in a moist, dark chamber. MICs were visually recorded at 48 h for yeasts, and at a time according to the control fungus growth, for the rest of fungi.

For the assay, stock solutions of extracts were two-fold diluted with RPMI-1640 from 1000 - 0.98 µg/mL (final volume = 100 µl) and a final DMSO concentration ≤ 1%. A volume of 100 µl of inoculum suspension was added to each well with the exception of the sterility control where sterile water was added to the well instead. Ketoconazole and Amphotericin B were used as positive controls for yeasts and *Aspergillus* spp. Terbinafine was used as a positive control for dermatophytes. Endpoints were defined as the lowest concentration of drug resulting in total inhibition (MIC) of visual growth compared to the growth in the control wells containing no antifungal. Tests were made by duplicate.

Statistical analysis

The comparison of the number of positive plants in PE and PW groups was analyzed with the Chi Square test. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Eighty-two crude methanolic extracts of plants from San Luis province were screened for antifungal properties with the microbroth dilution assay against yeasts and filamentous fungi and the concentration that completely inhibited the fungal growth (MIC) was determined for each extract. A plant with MICs ≤ 1000 µg/mL was considered active.

The fungal panel comprised 11 fungi included yeasts, molds of *Aspergillus* genus and dermatophytes. Among yeasts, *Candida albicans* was included because is the main causal agent of fungal infections in immunocompromised hosts, followed by other non-*albicans Candida* species such as *C. tropicalis*[1]. On the other hand, *Cryptococcus neoformans* is one of the fungus of the panel because it is the main cause of meningoencephalitis in AIDS patients[22]. Among molds, *Aspergillus fumigatus* was included because it is one of the pathogens responsible for invasive, and many times fatal, mycoses in immunocompromised patients. Some other *Aspergillus* species such as *A. flavus* and *A. niger* are the cause of severe fungal infections too[23,24].

Among dermatophytes, the most common skin mycoses are those known as tineas. Tinea unguium, tinea pedis and tinea manuum are mainly produced by *Trichophyton rubrum* and *T. mentagrophytes*. Instead, tinea cruris is due to *T. rubrum* and *Epidermophyton floccosum,* while tineas corporis, capitis and barbae are produced by *Microsporum canis* and *T. mentagrophytes*[25].

Plants were divided in two groups: group PE included plant species that possess ethnopharmacological uses related to fungal infections (total species in this group = 40). Group PW included plant species that possess either ethnopharmacological uses not related to fungal infections or do not possess any reported traditional use (= 40). PE and PW are listed alphabetically by family and within family, by genus, in Tables 1 and 2 along with the antifungal activities against the different groups of fungi: yeasts (that comprise *Candida* and *Cryptococcus* spp.; *Aspergillus* spp. which include *A. flavus, A. fumigatus* and *A. niger* and dermatophytes that comprise species of the *Microsporum* and *Trichophyton* genus.

Results in Table 1 showed that 25 out of the 40 PE plants (62.5%) were

active (MIC ≤ 1000 µg/mL) unless in one group of fungi; 6 (15%) were active in yeasts; 4 (10%) were active in *Aspergillus* spp.; and 25 (62.5%) were active in dermatophytes.

In turn, among the 40 PW species, 4 (10%) were active (MIC ≤ 1000 ug/mL) unless in one group of fungi, 1 (2.5%) were active in yeasts, 1 (2.5%) were active in *Aspergillus* spp. and 4 (10%) were active in dermatophytes (Table 2). Data of tables 1 and 2 are schematically showed in Figure 1.

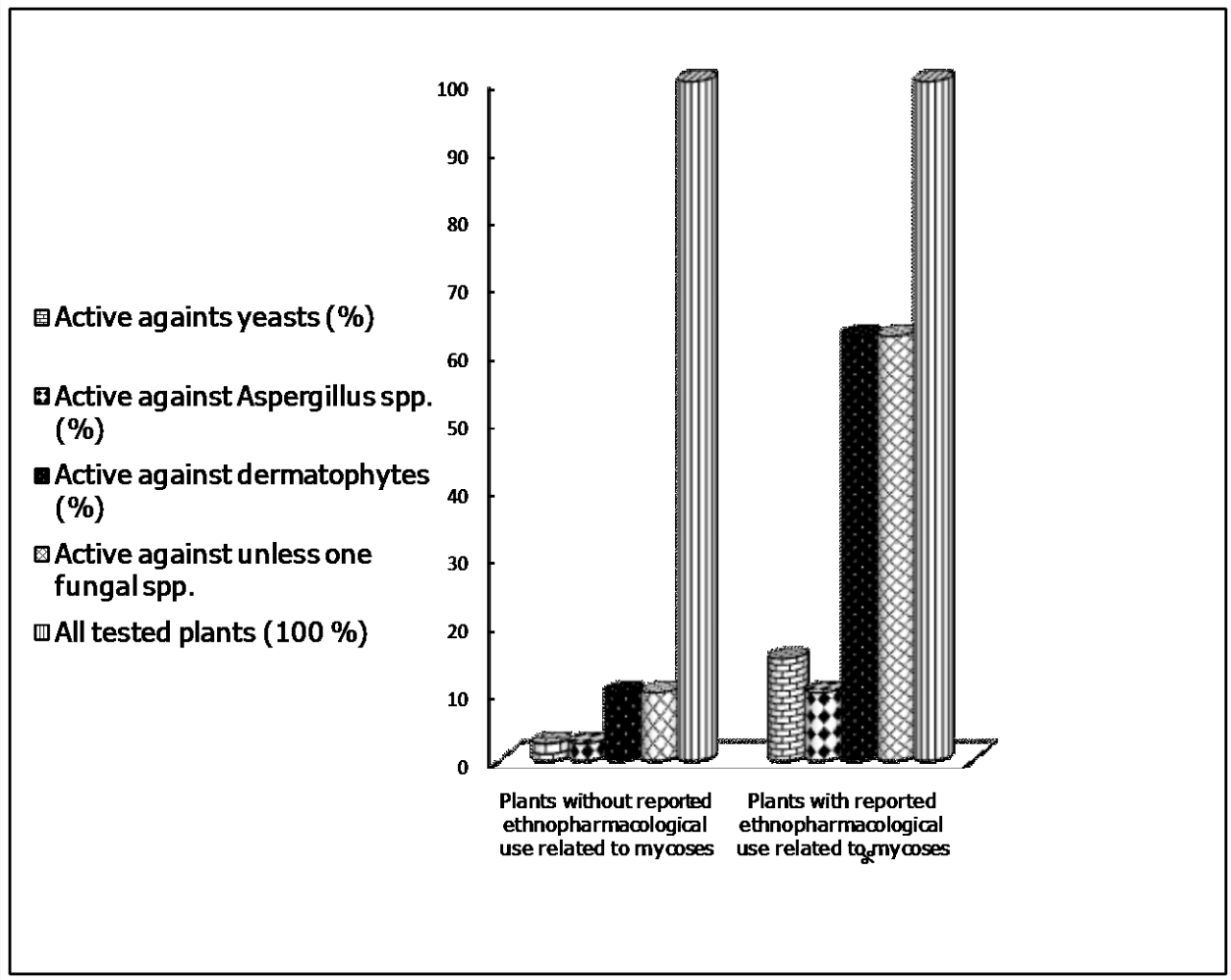

Figure 1: Percentage of active extracts against: unless one fungal species; yeasts; Aspergillus spp.; dermatophytes, within each of both groups of plants: PW (plants without ethnopharmacological uses related to mycoses, bars at the left) and PE (plants with ethnopharmacological uses related to mycoses (bars at right).

Table 1: Antifungal activity (MICs in μg/mL) of plant species with reported ethnopharmacological uses to treat conditions related to fungal infections (I: MIC > 1000 μg/mL).

	Family	Plant name Voucher specimen	Ethnopharmacological uses	Yeasts	*Aspergillus* spp.	Dermatophytes
1	Amaranthaceae	*Gomphrena pulchella* Mart. subsp. *rosea* (Griseb.) Pedersen Del Vitto & Petenatti 9271-UNSL	diarrhoea[27-29]	I	I	125-250
2	Anacardiaceae	*Schinus fasciculata* (Griseb.) I.M. Johnst. Del Vitto & Petenatti 9287-UNSL	vulnerary[27,30]	I	I	1000
3		*Schinus molle* L. Del Vitto & Petenatti 3844-UNSL	vulnerary[28]	I	I	500-1000
4	Aristolochiaceae	*Aristolochia argentina* Griseb. Del Vitto & Petenatti 9284-UNSL	antifungal[28]	250-1000	250-1000	125-250
5	Asteraceae	*Baccharis grisebachii* Hieron. Del Vitto & Petenatti 234-UNSL	vulnerary[31-34]	I	I	250-500
6		*Conyza bonariensis* (L.) Cronquist Del Vitto & Petenatti 5546-UNSL	vulnerary, cicatrizant[28-29, 33, 35-39]	I	I	I
7		*Flaveria bidentis* (L.) Kuntze Del Vitto &	antifungal, antiseptic[35, 40-41]	I	I	I

	Petenatti 9238-UNSL				
8	*Gaillardia megapotamica* (Spreng.) Bak. var. *megapotamica* Del Vitto & Petenatti 5825-UNSL	antialopecic, antidandruff and seborrhea[28, 42]	I	I	250-1000
9	*Gaillardia megapotamica* (Spreng.) Bak. var. *radiata* (Griseb.) Baker. Del Vitto & Petenatti 7143-UNSL	antialopecic, antidandruff and seborrhea[28, 42]	I	I	250-1000
10	*Gaillardia megapotamica* (Spreng.) Bak. var. *scabiosoides* (Arn. ex DC.) Baker. Del Vitto & Petenatti 5811-UNSL	antialopecic, dandruff and seborrhea[28, 42]	I	I	250-1000
11	*Gochnatia glutinosa* (D. Don) Hook. & Arn. Del Vitto & Petenatti 7461-UNSL	Antifungal[28]	500-1000	I	50-62.5
12	*Mikania periplocifolia* Hook. & Arn. Del Vitto & Petenatti 8521-UNSL	vulnerary for washing wounds, for venereous diseases[33, 43-44]	I	I	50-1000
13	*Pterocaulon virgatum* (L.) DC. Del Vitto & Petenatti 2217-UNSL	antifungal[29, 45]	I	I	1000
14	*Schkuhria pinnata* (Lam.) Kuntze ex	antifungal[29, 46]	I	I	I

		Thell. Del Vitto & Petenatti 9240-UNSL				
15		*Tagetes filifolia* Lag. Del Vitto & Petenatti 9242-UNSL	for itch, and healing infected wounds[39, 47]	I	I	I
16		*Xanthium spinosum* L. Del Vitto & Petenatti 5064-UNSL	vulnerary, venereous and skin diseases[28, 31, 48]	I	I	80-1000
17	Bignoniaceae	*Jacaranda mimosifolia* D. Don Del Vitto & Petenatti UNSL 499	Wound's cicatrizant venereous diseases[31- 32]	I	I	I
18	Bromeliaceae	*Tillandsia bryoides* Griseb. ex Baker Del Vitto & Petenatti 9306-UNSL	Vulnerary[28-29, 33]	1000	I	250
19	Buddlejaceae	*Buddleja cordobensis* Griseb. Del Vitto & Petenatti 9316-UNSL	Vulnerary[28-29, 43]	I	I	I
20	Celtidaceae	*Celtis tala* Gillies ex Planch. Del Vitto & Petenatti 3490-UNSL	desinfectant[49]	I	I	I
21	Convolvulaceae	*Ipomoea cairica* (L.) Sweet Del Vitto & Petenatti 6259, UNSL	vulnerary[43, 50]	I	I	I
22	Euphorbiaceae	*Acalypha communis*	For skin disorders[51-52]	I	I	250-500

		Müll. Arg. Del Vitto & Petenatti 9262-UNSL				
23	Fabaceae	*Acacia caven* (Molina) Molina Del Vitto & Petenatti 6256-UNSL	antifungal; oral antiseptic, cicatrizant[27, 29, 30, 32, 51-52]	I	I	I
24		*Prosopis nigra* (Griseb.) Hieron. var. *nigra* Del Vitto & Petenatti 4214-UNSL	for venereous diseases[30, 35, 43, 53]	I	I	I
25		*Senna alata* (L.) Roxb. Del Vitto & Petenatti 9278-UNSL	antiseptic, cicatrizant[29]	I	I	I
26		*Zuccagnia punctata* Cav. Del Vitto & al. 9325- UNSL	antifungal[28, 46]	500-1000	500-1000	250-500
27	Lythraceae	*Heimia salicifolia* (Kunth) Link Del Vitto, Petenatti & Petenatti 6653 UNSL	vulnerary[31, 54]	I	I	250-1000
28	Oxalidaceae	*Oxalis erythrorhiza* Gillies ex Hook. & Arn. MERL 27319-UNSL	genito-urinary tract[33, 55]	I	I	500-1000
29	Papaveraceae	*Argemone subfusiformis* G.B. Ownbey Del Vitto & Petenatti 5553-UNSL	oral antiseptic aphta, cicatrizant, alopecia[27-28, 33, 43]	I	I	250-500
30	Polygonaceae	*Polygonum convolvulus* L. UNSL-503	vulnerary, for skin diseases [29]	I	I	250-500

31		*Polygonum hydropiperoides* Michx. UNSL-504	antifungal[29]	I	I	125-250
32		*Polygonum punctatum* Elliott UNSL 705	antiseptic, for skin diseases[28, 56]	I	I	62.5-500
33	Rosaceae	*Acaena magellanica* (Lam.) Vahl Kiesling 1361-UNSL	genito-urinary tract[33, 57]	I	I	I
34	Rutaceae	*Fagara coco* (Gillies) Engl. Del Vitto & Petenatti & 5763-UNSL	antifungal[28]	250-1000	500-1000	250-500
35	Solanaceae	*Cestrum parqui* L'Hér. Del Vitto & Petenatti 8980-UNSL	vulnerary, skin disorders antiseptic[29, 31, 33, 35, 43]	I	I	125-250
36		*Nicotiana glauca* Graham Del Vitto & Petenatti 7473-UNSL	antifungal, anti-itch, vulnerary, for healing wounds[27-28, 58]	I	I	I
37		*Physalis viscosa* L. Del Vitto 8579-UNSL	vulnerary[27-28, 31]	I	I	I
38	Urticaceae	*Parietaria debilis* G. Forst. Del Vitto & Petenatti 7563-UNSL	vulnerary[28, 33]	I	I	I
39	Zygophyllaceae	*Larrea cuneifolia* Cav. Del Vitto & Petenatti 2818-UNSL	dermal, cicatrizant[27, 51, 59]	500-1000	I	25-100

40	*Porlieria microphylla* (Baill.) Descole, O'Donell & Lourteig Del Vitto & Petenatti 7159-UNSL	vulnerary, for venereous diseases[27-29, 31, 43, 51]	I	I	500-1000
	Anfotericina B		0.25-1.0	0.5	-
	Ketoconazol		0.25-0.5	0.25-0.5	-
	Terbinafina		-	-	0.01-0.04

Table 2: Antifungal activities (MIC in μg/mL) of plants selected at random

	Family	Plant name and Voucher specimen	Yeasts	*Aspergillus* spp.	Dermatophytes
1	Acanthaceae	*Justicia gilliesii* (Nees) Benth. Del Vitto & Petenatti 4281- UNSL	I	I	I
2		*Justicia squarrosa* Griseb. Del Vitto & Petenatti 5070-UNSL	I	I	I
3		*Justicia tweediana* (Nees) Griseb. Del Vitto & Petenatti 7231-UNSL	I	I	I
4	Amaranthaceae	*Iresine diffusa* Humb. & Bonpl. ex Willd. Del Vitto & Petenatti 9263-UNSL	I	I	I
5	Asteraceae	*Baccharis medullosa* DC. Del Vitto & Petenatti 9236- UNSL	I	I	I
6		*Baccharis stenophylla* Ariza Del Vitto & Petenatti 5284- UNSL	I	I	I
7		*Bidens subalternans* DC. Del Vitto & Petenatti 6580- UNSL	I	I	I
8		*Eupatorium argentinum* Ariza	I	I	I

		Del Vitto & Petenatti 9281- UNSL			
9		*Gnaphalium gaudichaudianum* DC. Del Vitto & Petenatti 9239- UNSL	I	I	I
10		*Grindelia discoidea* Hook. & Arn. Del Vitto & Petenatti 9264- UNSL	I	I	I
11		*Grindelia pulchella* Dunal *var. discoidea* Bartoli & Tortosa Del Vitto & Petenatti 9264- UNSL	I	I	I
12		*Nassauvia axillaris* (Lag. ex Lindl.) D. Don UNSL 507	I	I	I
13		*Thymophylla pentachaeta* (DC.) Small var. *belenidium* (DC.) Strotter Del Vitto *et al*. 8999-UNSL	I	I	I
14	Calyceraceae	*Boopis anthemoides* Juss. Del Vitto & Petenatti 9253 UNSL	I	I	I
15	Cucurbitaceae	*Abobra tenuifolia* (Gillies ex Hook. & Arn.) Cogn. Del Vitto & Petenatti 9263- UNSL	I	I	I
16	Dipsacaceae	*Dipsacus fullorum* L. Del Vitto & Petenatti 9424- UNSL	I	I	I
17	Ehretiaceae	*Ehretia cuneifolia* Gottschling & Hilger (= *Cortesia cuneifolia* Cav.) Del Vitto & Petenatti 9237- UNSL	I	I	I
18	Escalloniaceae	*Escallonia cordobensis* (Kuntze) Hosseus Del Vitto & Petenatti 9309- UNSL	I	I	I
19	Euphorbiaceae	*Croton argentinus* Müll. Arg. Del Vitto & Petenatti 4615- UNSL	I	I	I
20		*Croton hieronymi* Griseb. Del Vitto & Petenatti 6625- UNSL	I	I	I
21		*Croton parvifolius* Müll. Arg. Del Vitto & Petenatti 9425- UNSL	I	I	1000
22		*Croton sarcopetalus* Müll. Arg.	I	I	I

		Del Vitto & Petenatti 9266- UNSL			
23	Fabaceae	*Adesmia macrostachya* Benth. Del Vitto & Petenatti 7407- UNSL	I	I	1000
24		*Astragalus pehuenches* Niederl. Del Vitto & Petenatti 8636- UNSL	I	I	I
25		*Collaea argentina* Griseb. Del Vitto & Petenatti 9310- UNSL	I	I	I
26		*Coursetia hassleri* Chodat Del Vitto & Petenatti 9288- UNSL	I	I	I
27		*Prosopis nigra* (Griseb.) Hieron. Del Vitto & Petenatti 3367- UNSL	I	I	I
28	Lamiaceae	*Satureja parvifolia* Phil. Del Vitto & Petenatti 8536 UNSL	I	I	I
29		*Teucrium cubense* subsp. *cordobense* Epling Del Vitto & Petenatti 9243- UNSL	I	I	I
30	Loranthaceae	*Tripodanthus flagellaris* Tiegh. Del Vitto & Petenatti 9244- UNSL	I	I	I
31	Malvaceae	*Pseudabutilon virgatum* (Cav.) Fryxell Del Vitto & Petenatti 5782- UNSL	I	I	I
32	Nictaginaceae	*Boungainvillea stipitata* Griseb. Del Vitto & Petenatti 9286- UNSL	I	I	I
33	Plumbaginaceae	*Plumbago caerulea* Kunth Del Vitto & Petenatti 9425- UNSL	1000	1000	1000
34	Polygalaceae	*Polygala stenophylla* Ar. Gray Del Vitto & Petenatti 8512- UNSL	I	I	I
35	Rubiaceae	*Galium latoramosum* (Hook et. Arn.) Clos Del Vitto & Petenatti 5635- UNSL	I	I	I
36	Salviniaceae	*Salvinia adnata* Desv. UNSL 506	I	I	I
37	Scrophulariaceae	*Maurandya antirrhiniflora* Humb & Bonpl. ex	I	I	I

		Willd.			
		UNSL 500			
38		*Agalinis communis* (Cham. & Schltdl.) D´Arcy	I	I	I
		Del Vitto & Petenatti 7865- UNSL			
39	Simaroubaceae	*Ailanthus altissima* (Mill.) Swingle	I	I	I
		Del Vitto & Petenatti 3400- UNSL			
40	Sterculiaceae	*Ayenia lingulata* Griseb.	I	I	I
		Del Vitto & Petenatti 9251- UNSL.			
41	Verbenaceae	*Lippia integrifolia* (Griseb.) Hieron.	I	I	250-1000
		Del Vitto & Petenatti 6089- UNSL.			
42		*Xeroaloysia ovatifolia* (Moldenke) Tronc.	I	I	I
		Del Vitto & Petenatti 9252- UNSL			
		Anfotericina B	0.25-1.0	0.5	-
		Ketoconazol	0.25-0.5	0.25-0.5	-
		Terbinafina	-	-	0.01-0.04

To analyze whether there is a significant difference in the percentage of the detected antifungal plants between PE and PW groups, the Pearson chi square test was applied. Results showed that there is a significant higher chance (62.5% *vs*. 10%, $p < 0.01$) of detected antifungal plants in unless one group of fungi, when the plant has a history of medicinal use related to antifungal properties (PE group) than when plants are chosen *at random* (PW group).

In addition, it is observed a clear trend of extracts to display lower MICs within PE group, against dermatophytes ($p < 0.01$) fungi responsible of superficial infections. These findings suggest that the ethnopharmacological approach is useful in guiding the discovery of antifungal plants in plants of San Luis province, mainly for infections in which the pathological expression can be clearly observed by the population.

This result is in concordance with our previous work regarding antifungal

activity of plants of Latin America[26] but here, within plants of San Luis province, it could be observed a more pronounced correlation between ethopharmacological data and antifungal behavior, both works clearly suggesting that the ethnopharmacological approach is useful in guiding the discovery of antifungal plants.

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