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Antimycotic Activity of 20 Plants from Colombian Flora

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Abstract

The antifungal activities of 65 extracts were evaluated through the agar well diffusion method in 22 plant samples from the Ucumari Natural Regional Park (UNRP). These samples belong to 20 plant species related to the following botanic families: Asteraceae, Euphorbiaceae, Melastomataceae, Podocarpaceae, Rubiaceae and Solanaceae. The plant extracts were obtained in hexanes, dichloromethane and methanol. The 65 extract samples were tested against pathogenic fungi *Aspergillus fumigatus* (ATCC 1022), *Candida albicans* (ATCC 18804) and *Fusarium solani* (ATCC 11712). Ketoconazole was used as positive control. The methanol extracts from *Solanum* spp. (FJR 3155) and *Tibouchina grossa* exhibited the greatest inhibitory activity against the three fungi tested, while the methanol extracts from *Hyeronima macrocarpa, Miconia lehmannii,* and *Sapium stylare* inhibited two of the fungi assayed. The dichloromethane extracts from *Miconia lehmannii* Cong, *Lycinathes acutifolia* and *Solanum* spp. (FJR 3155) inhibited two of the three fungi tested, while the dichloromethane extracts from *Cinchona pubescens* Vahl and *Palicourea* spp. (FJR 3182) inhibited one of the three microorganism tested. Only one of the hexane extracts produced activity against the three fungi tested.

Keywords: Bioactivity, bioassay, Asteraceae, Euphorbiaceae, Melastomataceae, Podocarpaceae, Rubiaceae, screening, Solanaceae, well diffusion method.

Introduction

The Ucumari Natural Regional Park (UNRP) is located on the western side of the Central Colombian Andean mountain chain and has a latitudinal range from 1800 to 2650 m above sea level. UNRP has a total area of 4,240 hectares and has an annual pluviosity range from 2000 to 4000 mm/year (Londoño, 1994). The UNRP is an example of a tropical rainforest and presents a remarkable diversity in fauna and flora (Rangel, 1994). For this reason we considered the UNRP to be a potential source of new compounds with novel and important biological activities.

The search for new antimycotic agents is of great importance since there are currently few therapeutic substances that have shown effectiveness against mycoses. Filamentous fungi can produce systemic and opportunistic mycoses mainly in patients with general defects in their host defense mechanisms, such as AIDS, neoplastic diseases, organ transplants and diabetes (Hamburger & Hostettmann, 1991; Hadacek & Greger, 2000; Eto et al., 2000). For this reason, the search for new, safer and powerful antifungal agents with novel mechanisms of action to overcome these problems has increased dramatically over the last years.

Based on the premise that plant extracts are a source of antimycotic agents and other bioactive substances, many researchers have performed bioprospection studies by using extracts from plant origin growing in different regions around the world. Among the literature review that contributes to the development of this field of research, the following references proved to be of special interest to our investigation: Rojas et al. (1992); McCutcheon et al. (1994); Nick et al. (1995); Alkofahi et al. (1996); Sanabria et al. (1998); Suzuki et al. (2000) among others.

As far as we know, there is no existing bioprospection research with regard to this natural reserve. Hence, the main goal of this work was to perform a preliminary evaluation of the antimycotic activity from plant extracts that belong to some of the more predominant families in the park. In addition, this research seeks to add knowledge to the field of bioprospection.

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Materials and methods

Plant material

Aerial plant samples were collected in February 2000 at different zones of Ucumari Natural Regional Park. A voucher specimen was made for each collection. They were classified and kept at the Herbarium of the Universidad de Antioquia as is shown in Table 1.

The plant materials were oven-dried at 40 °C, grounded and extracted successively with the organic solvents: hexanes, dichloromethane and methanol in a Soxhlet. The different extracts were concentrated at reduced pressure to dryness and refrigerated at 4 °C until tested.

Antifungal in vitro assays

Antimycotic activity was studied by the agar well diffusion following the procedure of Rios et al. (1988) against the following microorganisms: *Candida albicans* (ATCC 18804), *Aspergillus fumigatus* (ATCC 1022), and *Fusarium solani* (ATCC 11712). Each plant extract was evaluated in triplicate against each microorganism (Tables 2 and 3). In this procedure, the degree of fungi inhibition by each plant extract is evaluated by measuring the diameter of each inhibition halo.

Each crude plant extract sample was assayed using 5 different concentrations (10,000, 5000, 2500, 1250 and

Table 1. List of plant species collected in the Ucumari Natural Regional Park.

FAMILY	SCIENTIFIC NAME	HERBARIUM SPECIMEN VOUCHER NUMBER
Asteraceae	*Ageratina popayanensis* (Hieron.) K. & R.	FJR 3174
Asteraceae	*Mikania leiostachya* Benth.[a]	FJR 3175
Asteraceae	*Mikania leiostachya* Benth.[a]	FJR 3176
Asteraceae	*Jungia coarctata* Hieron.	FJR 3195
Euphorbiaceae	*Sapium stylare* Müll.-Arg.	FJR 3160
Euphorbiaceae	*Hyeronima macrocarpa* Müll.-Arg.[b]	FJR 3196
Euphorbiaceae	*Hyeronima macrocarpa* Müll.-Arg.[b]	FJR 3200
Melastomataceae	*Tibouchina grossa* (L.f.) Cogn.	FJR 3157
Melastomataceae	*Miconia lehmannii* Cogn.	FJR 3172
Podocarpaceae	*Nageia rospigliosii* (Pilg.) de Laub.	FJR 3168
Rubiaceae	*Palicourea angustifolia* Kunth	FJR 3158
Rubiaceae	*Cinchona pubescens* Valh	FJR 3161
Rubiaceae	*Hoffmannia asperula* Standl.	FJR 3169
Rubiaceae	*Palicourea petiolaris* Wemh	FJR 3182
Rubiaceae	*Palicourea andaluciana* Standl.	FJR 3183
Rubiaceae	*Palicourea* thyrsiflora (Ruiz & Pav.) DC.	FJR 3184
Solanaceae	*Lycianthes radiata* (Sendtn.) Bitter	FJR 3154
Solanaceae	*Witheringia coccoloboides* (Damn.) Hunz	FJR 3155
Solanaceae	*Lycianthes acutifolia* (Ruiz & Pav.) Bitter	FJR 3156
Solanaceae	*Cestrum olivaceum* Francey	FJR 3159
Solanaceae	*Cestrum ochraceum* Francey	FJR 3166
Solanaceae	*Solanum deflexiflorum* Bitter	FJR 3173

[a] Plant collected at different localities.
[b] Plant at different physiological stages.

Table 2. Percentages of antimycotic activity from the three plant extracts and fungi assayed.

Extract		*Candida albincans*		*Aspergillus fumigatus*		*Fusarium solani*	
Type	No	Positive	%	Positive	%	Positive	%
Hexanes	22	1	4.5	2	9.1	1	4.5
Dichloromethane	21	4	19.1	4	19.1	0	0.0
Methanol	22	7	31.8	8	36.4	6	27.3

625 ppm), which were prepared by weighing 10 mg of each plant extract and adding 1 mL of ethanol. Appropriate dilutions were made to create the variation in concentration of solutions to be tested from each stock solution.

C. albicans, *A. fumigatus* and *F. solani*, were cultured in solid Saboreaud-glucose (1.5%) agar medium (Difco) for a period of 4–5 days. For each of these cultures, 5 portions of 10 mm were transferred to 100 mL of sterile saline solution to give a suspension of about 10^7–10^8 spores/mL for an overnight culture. Next, Saboreaud-glucose (1.5%) agar was prepared and sterilized by autoclaving at 121 °C for 20 min. After sterilization and cooling (40–45 °C), 20 mL of the agar medium was poured into Petri dishes (10 cm diameter), mixed with a 1.0 mL sample of each fungus saline suspension, and allowed to solidify.

Once the agar solidified, seven equidistant 7 mm each diameter wells were made under sterile conditions in each Petri dish. Five wells were filled with 20 µL of the 5 different concentrations to be evaluated for each plant extract. The other two wells were filled with 20 µL of ketoconazole (250 ppm) and 20 µL of ethanol to be used as positive and negative control, respectively.

In the case of *Candida albicans*, the plates were incubated at 25 °C for 48 h while for *Fusarium solani* or *Aspergillus fumigatus* the plates were incubated at 25 °C for 72 h. After the incubation time, the diameters (mm) of the resultant zones of inhibition around each well were measured and recorded. Each experiment was performed in triplicate.

For each plant extract a phytochemical screening was performed following the procedures proposed by Harborne (1973).

Data analysis

The total percentage of the fungi inhibition by each extract was calculated (Table 2). The average for the zones of inhibition was obtained for the three replicates. The degree of inhibition for each extract was evaluated as weak, if the extract was capable to inhibit the growth of a fungus assayed at 1 or 2 of the concentrations tested and as moderate or strong if the extract was able to inhibit the growth of a fungus at 3 or 4 out of 5 of the concentrations tested, respectively. The bigger the inhibition halo the stronger the potency of the respective extract.

Results and discussion

Sixty-five extracts were assayed for their antifungal activity against three fungi. Since none of the extracts assayed were bioactive at 625 ppm, these results were excluded and will not be presented in the tables.

Table 2 shows the organic solvents and fungi used with their respective bioactive percentage including the number of positive extracts. From the 65 extracts assayed in this study, it can be deduced that the highest antimycotic activity was found in the methanol extracts that are related to the Solanaceae and Melastomataceae families, where *A. fumigatus* was the most susceptible (36.4%) and the least sensitive one was *F. solani* (27.3%). The bioactivity of the methanol extracts (which showed the strongest activity) was followed by the dichloromethane extracts where the more active were those from *Miconia lehmannii* Cong and *Solanum spp.* (FJR 3155). The hexanes extracts showed the weakest antifungal activity and only two extracts inhibited the fungi tested in a weak manner. In general, Table 2 illustrates that *A. fumigatus* showed the strongest susceptibility and *F. solani* the weakest bioactivity against the extracts analyzed.

Table 3 points out that the methanol extract from *Solanum* spp. (FJR 3155) showed a strong antifungal activity against *C. albicans*, which is comparable to the inhibitory zone given by ketoconazole at 250 ppm (25 mm). The other methanol extracts with strong inhibition were those from *Miconia lehmannii* and *Palicourea* spp. (FJR 3182) against *C. albicans*. In addition, *Miconia lehmannii* and *Tibouchina grossa* showed strong activity against *A. fumigatus* and *F. solani*

The hexane extract with moderate antifungal activity was the one from *Solanum* spp. (FJR 3155) against *C. albicans* and *F. solani*.

From Table 3 it can be deduced:

- The methanol extracts from *Tibouchina grossa* (Melastomataceae) showed strong activity against the three fungi assayed.
- The methanol extract from *Miconia lehmannii* showed strong bioactivities against *C. albicans* and *A. fumigatus* and the methanol extract from *Palicourea* spp. (FJR 3182) showed strong activity against *C. albicans*.
- The dichloromethane extracts from *Miconia lehmannii* showed strong activities against *C. albicans* and *A. Fumigatus*.

According to the preliminary phytochemical screening (Table 4) on the 65 extracts, it can be deduced that the phytocompounds related to the antifungal activity in methanol crude extracts are mainly alkaloids, lactones or steroids.

It is interesting to note that the Euphorbiaceae species in this study did not show significant antifungal activity against the fungi tested. This finding correlates satisfactorily with those reported by Nick et al. (1995).

In this study, none of the 12 extracts from the Asteraceae family were active against the fungi tested at any concentrations assayed. This is contrary to the results found by Alkofahi et al. (1996) who, working with *C. albicans*, showed that 5 out of 14 Asteraceae species showed inhibition. In addition, the studies performed by Recio et al. (1989) found that 6 out of 17 Asteraceae species were bioactive against *C. albicans*.

Out of two Solanaceae assayed, only one showed activity against *C. albicans* (Alkofahi et al., 1996). The Solanaceae

Table 3. Plant species, extract type and fungi assayed with the well diffusion method.

Plant species	Extract[1]	Antimycotic activity								
		C. albicans			*A. fumigatus*			*F. solani*		
		Strong	Moderate	Weak	Strong	Moderate	Weak	Strong	Moderate	Weak
Lycianthes radiata (Sendt) Bitter.	H	–[5]	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–
Lycianthes acutifolia (Ruiz & Pav.) Bitter.	H	–	–	–	–	–	–	–	–	–
	DC	–	X	–	–	–	X	–	–	–
	M	–	–	X	–	X	–	–	–	X
Solanum spp.[2]	H	–	X	–	–	–	X	–	X	
	DC	X	–	–	–	X	–	–	–	–
	M	X	–	–	X	–	–	X	–	–
Solanum spp.[2]	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–
Cestrum olivaceum Francey.	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	X	–	X	–	–	–	–
Cestrum ochraceum Francey.	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–
Miconia lehmannii Cogn.	H	–	–	–	–	–	–	–	–	–
	DC	X	–	–	X	–	–	–	–	–
	M	X			X	–	–	–	–	–
Tibouchina grossa (L.f.) Cogn.	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	X	–	–	X	–	–	X		
Hyeronima macrocarpa Müell.-Arg[3].	H	–	–	–	–	–	X	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	X	–	–	X	–	–
Hyeronima macrocarpa Müell.-Arg[3].	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	X	–	–	–
Sapium stylare Müell.-Arg.	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	X	–	–	–	X	–
Hoffmania asperula Standl.	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	X	–	–	–	–	–	–	–	–
Palicourea angustifolia Kunth.	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	X
Palicourea andaluciana Standl.	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–
Palicourea spp.[2]	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	X	–	–	–	–
	M	X	–	–	–	–	–	–	–	–
Palicourea spp.[2]	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–
Cinchona pubescens Vahl.	H	–	–	–	–	–	–	–	–	–
	DC	–	X	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–
Nageia rospigliossii (Pilger) Laubenf.	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–

Table 3. Continued

Plant species	Extract[1]	Antimycotic activity								
		C. albicans			*A. fumigatus*			*F. solani*		
		Strong	Moderate	Weak	Strong	Moderate	Weak	Strong	Moderate	Weak
Ageratina popayanensis (Hieron.) K&R.	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–
Mikania leiostachya Benth.	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–
Mikania leiostachya Benth.	H	–	–	–	–	–	–	–	–	–
	DC	NE[4]	NE	NE	NE	NE	NE	NE	NE	NE
	M	–	–	–	–	–	–	–	–	–
Jungia coarctata Hieron.	H	–	–	–	–	–	–	–	–	–
	DC	–	–	–	–	–	–	–	–	–
	M	–	–	–	–	–	–	–	–	–
Ketoconazole (250 ppm)			25			23			20	

[1] H = Hexanes; DC = Dichloromethane; M = Methanol.
[2] Different species and unclear taxonomic classification.
[3] In different development stages.
[4] NE = Not evaluated.
[5] (–) = Did not present activity; (X) = Present activity.

Table 4. Preliminary phytochemical screening from the different plant extracts assayed.

Scientific Name	Phytocompounds																							
	1[a]			2		3			4			5			6			7			8		9	
	H[b]	C	M	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	C	M	C	M
Lycianthes radiata	–[c]	–	+	–	–	–	+	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–
Lycianttties acutifolia	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–
Solanum spp.	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
Solanum spp.	+	–	+	–	+	–	+	–	–	–	–	–	+	–	–	–	–		+	+	–	–	–	–
Cestrum olivaceum	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+	–	–
Cestrum ochraceum	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–
Miconia lehmannii	+	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	+	–	–	–	–
Tibouchina grossa	–	–	+	–	–	–	–	–	+	–	+	–	–	+	–	–	+	–	–	–	–	+	–	–
Hyeronima macrocarpa	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+	+	+	+	–	–	–
Hyeronima macrocarpa	+	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	+	–	+	+	–	+	–	–
Sapium stylare	–	–	+	–	–	–	–	–	–	–	–	–	+	+	–	–	+	–	–	+	–	–	–	–
Hoffmania asperula	+	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	+	–	–	–	–
Palicourea angustifolia	–	+	+	–	–	–	+	–	+	–	+	–	+	+	–	–	+	–	–	+	–	–	–	–
Palicourea andaluciana	–	–	+	–	–	–	+	–	+	–	+	–	–	–	–	–	+	+	–	+	–	–	–	–
Palicourea spp.	+	–	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–
Palicourea spp.	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+	–	–
Cinchona pubescens	+	–	+	–	+	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–
Nageia rospigliosii	–	–	–	–	–	–	–	–	+	–	+	–	–	+	–	–	+	+	+	+	–	–	–	–
Ageratina popayanensis	–	–	+	–	–	–	–	+	+	–	+	–	+	+	+	–	+	+	+	+	–	–	–	–
Mikania leiostachya	–	–	+	–	–	–	–	–	+	–	+	–	+	+	–	–	+	–	–	–	–	+	–	–
Mikania leiostachya	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–
Jungia coarctata	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	+	+	+	–	–

[a] 1 = Alkaloids; 2 = Anthraquinones/quinones; 3 = Coumarins; 4 = Flavonoids; 5 = Lactones; 6 = Phenols; 7 = Saponins; 8 = Steroids/Sterols; 9 = Triperpenoids.
[b] H = Hexanes; C = Dichloromethane; M = methanol.
[c] + = Present; – = Absent.

studied for their antimicrobial activity by Recio et al. (1989) did not show activity against *C. albicans*. These findings are not consistent with our results where the methanol extracts from *Solanum* spp. (FJR 3155) were bioactive against the three fungi evaluated in the concentration range between 10,000–25,000 ppm.

Conclusions

The plant extracts that showed stronger bioactivity were the methanol extracts. This result corresponds very accurately with the point-of-view of Rios et al. (1988) in stating that the agar well diffusion method works adequately for polar extracts.

Based on the measurements of the inhibition zones, it can be concluded that the species with the strongest antimycotic activity were *Solanum* spp., *Miconia lehmannii* and *Tibouchina grossa*.

From the 20 different plant samples studied during this research, a group constituted by 12 (54.5%) of the species showed antimycotic activities against the three fungi tested in a wide range of concentrations. The remaining species (45.4%) did not show any detectable activity. Based on these results, it can be concluded that the flora from the UNRP has great potential to yield new natural compounds with antifungal activity.

It is important to point out that the remarkable inhibition exhibited by the methanol extracts from *Tibouchina grossa*, *Solanum* spp. and *Miconia lehmannii* deserve further screening for antifungal activity. The isolation of their bioactive compounds should be achieved in the near future by using bioassay-guided fractionation.

There is a need for more in depth studies and research associated with the UNRP flora in the continuing search for new and effective antifungal as well as other bioactive phytocompounds.

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