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ANTIBACTERIAL, ANTIFUNGAL AND CYTOTOXIC ACTIVITIES OF EIGHT ASTERACEAE AND TWO RUBIACEAE PLANTS FROM COLOMBIAN BIODIVERSITY

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ABSTRACT

Thirty crude extracts of eight plants belonging to Asteraceae and two to Rubiaceae families collected at different places from the Regional Natural Park Ucumarí (RNPU), Colombia, were tested for their antibacterial activity against two Gram-positive and two Gram-negative bacteria and three fungi. Both the antibacterial and the antimycotic activities were tested by the agar well diffusion method. The cytotoxic activity on the same plant extracts was determined through the brine shrimp lethality bioassay. The extracts from the Asteraceae family were more bioactive against *Bacillus subtilis* and *Staphylococcus aureus*. The extracts of both families studied were bioactive against the fungi *Candida albicans* and *Fusarium solani*. In addition, the extracts of Asteraceae species displayed the greatest cytotoxic activities. However, the most important specie in this research was *Gonzalagunia rosea* Standl (Rubiaceae) because of the strong and moderate activities of the methanol and dichloromethane extracts against *C. albicans* and *F. solani*, respectively; as well as the strong cytotoxic activity of the methanol extract. None of these ten plants has previously been reported for their biological activities.

Key words: agar well diffusion method, antimicrobial screening, *Artemia salina*, bioprospection, Colombian flora

INTRODUCTION

Although approximately 20% of the world plants have been submitted to pharmacological or biological test, it could be concluded that natural products from plant origin are an important source to discover new leads with economical and pharmaceutical importance and great possibilities to be developed as drugs, dyes, fragrances and pesticides, among others (7). To obtain novel and promissory substances many plant extracts have to be assayed. For example, Suffredini et al. (26) assayed 705 plant extracts and found only three extracts with strong antibacterial activity. Furthermore, the screening of plant extracts as antimicrobial agents is necessary to go insight into medicinal flora and get the molecules responsible for this activity and add value to natural resources from tropical areas (21).

Even though in the last three decades pharmacological industries had produced a considerable number of currently available commercial antibiotics, resistance to these drugs by microorganism has increased. In general, bacteria have the genetic ability to in heritage and acquire resistance to drugs, which have been used as therapeutic agents (2,15). Therefore, actions must be taken to reduce this problem, for example, to develop new drugs, either from synthetic or natural sources (16).

The Asteraceae family is well distributed in Colombian flora, it is constituted by herbs, while shrubs or trees are rare (4); by its floral structure and chemical composition, it is considered one of the most evolutionated family from all the Dicotyledonous (6). The Regional Natural Park Ucumarí (RNPU) holds 33 Asteraceae species (3). The phytochemical screening on this plant family has revealed sesquiterpene lactones as the principal secondary metabolites responsible for their antimicrobial activities (5).

On the other hand, the Rubiaceae family is one of the biggest in Dicotyledonous, it is abundant in the tropical regions around the earth; the species reported for Colombia constituted 45% of the total world distribution, which is conformed by trees, shrubs or herbs (9). The RNPU holds 27 Rubiaceae species most of them belonging to the genus *Palicourea* (3). From the known genera

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39 biosynthesized alkaloids and in a high number of cases, these secondary metabolites are responsible for their biological activities.

Colombia is a country with a high number of jungles and natural reserves. The high and accelerated jungle destruction implies not only the extinction of plants, but also of other important natural ecosystem constituents. Because of that, it is a priority to perform bioprospection studies to analyze the greatest number of species, prior to the irrational extinctions of plants from those natural sanctuaries.

Our research group conscious of the accelerated natural reserves destruction had been performing some biological screenings with plant extracts from the RNPU (13,17). In this paper are described the results of the antibacterial, antifungal and cytotoxic evaluation of eight plant species of the Asteraceae and two of Rubiaceae families, that have never been examined for these biological activities.

MATERIALS AND METHODS

Plant material

The plant aerial parts used for this study were collected in October 2001 at different zones of Regional Natural Park Ucumarí (RNPU). They were classified taxonomically by Dr. F. J. Roldán. Voucher specimen were made for all accessions and conserved at Herbarium of Universidad de Antioquia (HUA, Medellín, Colombia) (Table 1).

The plant aerial samples were oven dried at 50ºC, ground and extracted successively by lixiviation with *n*-hexane, dichloromethane and methanol. The different extracts were concentrated at reduced pressure to dryness and stored at -10ºC until assayed.

Microorganism in vitro assays

The following bacteria strains were employed in the screening: Gram-positive *Bacillus subtilis* (ATCC 21556), *Staphylococcus aureus* (ATCC 6538) and the Gram negative *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 9637). In the antifungal screening the following fungi were tested: *Candida albicans* (ATCC 18804), *Aspergillus fumigatus* (ATCC 1022) and Fusarium solani (ATCC 11712). The nutritive media used during the investigation included nutrient Mueller-Hinton II agar for bacteria and Sabouraud dextrose agar for fungi, both from Becton - Dickinson (France).

Antibacterial and antimycotic activities of crude plant extracts were examined by the well diffusion method according to Ríos *et al*. (22). Each plant extract was dissolved in 95% ethanol and tested at five different concentrations (5.00, 2.50, 1.25, 0.62 and 0.31 mg/mL), they were evaluated in triplicate for each bacterium or fungus. As positive control for the antibacterial and antimycotic tests cefotaxime and ketoconazole at different concentrations were used, respectively. In all assays the negative control was the same solvent used to dissolve the respective extract.

Cytotoxic activity assays

Cytotoxic *in vivo* brine shrimp lethality tests were carried out using brine shrimp *Artemia salina* (Leach) larvae (Brine shrimp Eggs, San Francisco, USA) following the procedure described by McLaughlin *et al*. (12).

The *n*-hexane and dichloromethane extracts were dissolved in the same solvent while the methanol ones were dissolved in water. Each plant extract was tested at concentrations of 1.00, 0.10 and 0.01 mg/mL. The concentrations were obtained by transferring the corresponding volume from stock solutions to different vials for evaporation during 16 h. After evaporation, 10 mL of artificial seawater were added to each vial with gentle shaking. Then, 10 shrimps were transferred to each vial. All tests were performed in a temperature controlled chamber at 28ºC, under a continuous light regime. Three replicates were used for each treatment and control. In this test as positive control gallic acid was used. The negative control was obtained by adding the solvent used to dissolve the extracts in the assays, and it was allowed to evaporate during 16 h.

Phytochemical screening

For each plant extract a phytochemical screening was performed testing the presence of secondary metabolites such as phytosterols, polyphenols (tannins, flavonoids), saponins, alkaloids and lactones by using wet reactions and thin layer chromatography (TLC), following the procedures described by Harborne (8) and Stahl (24).

Data analysis

Antibacterial and antimycotic activities. In this procedure, the degree of microorganism inhibition by each plant extract was essayed by measuring the diameter of the inhibition zone (mm). Then, the minimum inhibitory concentration (MIC) was obtained.

Cytotoxic activity

Mortality percentage (% M) for each concentration was calculated by applying the Abbot formula (18). Lethal concentration fifty (LC_{50}) in mg/mL for each plant extract was obtained by interpolation in the graph of mortality percentage versus the concentration (mg/mL) through a linear regression analysis with the software Microsoft Excel (Office XP).

The cytotoxic activity was designated as weak, moderate and strong when the LC_{50} was between 0.50 to 1.0, 0.10 to 0.50 and 0.00 to 0.10 mg/mL, respectively.

RESULTS AND DISCUSSION

Results of the antimicrobial and cytotoxic activities of the crude plant extracts of the Asteraceae and Rubiaceae families collected in the RNPU are shown in Table 1. Among the 10 plants examinated they showed variable degree of antibacterial

and antifungal activities against one or more than one microorganism.

The n-hexane plant extracts from *Aspilla quinquenervis* and *Chromolaena tequendamensis* (Asteraceae) gave moderate and weak activities respectively against *B. subtilis* while the dichloromethane and methanol extracts from *C. tequendamensis* exhibited weak activities against *S. aureus*. These results are in concordance with those found by Mothana and Lindequist

Table 1. Minimum inhibitory concentration (MIC, mg/mL) and lethal concentration fifty (LC_{50}, mg/mL) of crude plant extracts from Asteraceae and Rubiaceae families collected at Regional Natural Park Ucumarí (RNPU).

SPECIES (Voucher number)	EXTRACTS[1]	Bacteria (MIC, mg/mL)				Fungi (MIC, mg/mL)			*Artemia salina* (LC_{50}, mg/mL)
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. fumigatus*	*F. solani*	
		ASTERACEAE							
Aspilla quinquenervis Blake (FJR 3750)	H	-	1.25	-	-	-	-	-	0.96
	DC	-	-	-	-	-	-	-	1.00
	Mt	-	-	-	-	-	-	-	0.40
Chromolaena tequendamensis (Hieron.) (FJR 3730)	H	-	2.50	-	-	-	-	-	1.00
	DC	5.0	2.50	-	-	-	-	-	0.62
	Mt	2.5	-	-	-	-	-	-	0.32
Liabum asclepiadeum Sch. Bip. (FJR 3720)	H	-	-	-	-	-	-	-	0.62
	DC	-	-	-	-	0.62		-	1.00
	Mt	-	-	-	-	-		-	0.01
Montanoa sp (FJR 3749)	H	-	-	-	-	-	-	-	1.00
	DC	-	-	-	-	1.25	-	-	0.90
	Mt	-	-	-	-	0.62	-	-	0.50
Munnozia polymonioides (DC.) Rob & Bret. (FJR 3716)	H	-	-	-	-	-	-	-	1.00
	DC	-	-	-	-	-	-	-	1.00
	Mt	-	-	-	-	-		-	0.04
Munnozia senecionidis Benth. (FJR 3721)	H	-	-	-	-	-	-	-	1.00
	DC	-	-	-	-	-	-	-	1.00
	Mt	-	-	-	-	2.50	-	-	0.92
Schistocarpha sinforosi Cuatrec. (FJR 3725)	H	-	-	-	-	-	-	-	0.60
	DC	-	-	-	-	-	-	-	0.80
	Mt	-	-	-	-	2.50	-	1.25	0.36
Verbesina nudipes Blake (FJR 3746)	H	-	-	-	-	-	-	-	1.00
	DC	-	-	-	-	-	-	-	1.00
	Mt	-	-	-	-	2.50	-	-	0.56
		RUBIACEAE							
Dioicidendron diocum Steyerm (FJR 3748)	H	-	-	-	-	-	-	-	1.00
	DC	-	-	-	-	-	-	-	1.00
	Mt	-	-	-	-	-	-	-	1.00
Gonzalagunia rosea Standl. (FJR 3731)	H	-	-	-	-	-	-	-	0.54
	DC	-	-	-	-	2.50	-	1.25	0.58
	Mt	-	-	-	-	1.25	-	0.31	0.01
Positive control	Cefotaxime	0.25	5.00	0.50	0.50	NE	NE	NE	NE
	Ketoconazole	NE	NE	NE	NE	0.25	0.25	0.25	NE
	Tannic acid	NE	NE	NE	NE	NE	NE	NE	0.01

[1]Extracts: H= n-hexane; DC= dichloromethane, Mt= methanol; - = No growth inhibition at the concentration tested; NE = No evaluated.

(14), in the sense that Gram-positive displayed some degree of antibacterial activities. In addition, the above results correlate very well with those from the ethanol leaf extract of *Pulicaria orientalis* (Asteraceae), that exhibited weak activity against *S. aureus* (1). Besides, the crude ethanol extract from *Bidens pilosa* and *Sigesbekia orientalis* L. (Asteraceae) displayed weak activities against the Gram positives *B. subtilis* and *S. aureus* (10).

None of the plant extracts evaluated in this research inhibited the growth of the Gram-negative *E. coli* and *P. aeruginosa* neither of the fungus *A. fumigatus*. These results are in concordance with those obtained with the methanol extracts from *Artemisia diffusa* and *Artemisia scopana* and the ethanol leaf extract of *Pulicaria orientalis* (1) which did not display any antibacterial activity against the Gram negatives *E. coli* and *P. aeuriginosa*; in addition, the methanol extracts from the Asteraceae *Artemisia oliveriana* and *Artemisia turanica* did not show any antibacterial activity against *E. coli* (20). Moreover, the crude ethanol extract from *B. pilosa* displayed weak activity against *E. coli*, but fail to be active against *P. aeruginosa* (10).

The dichloromethane and methanol extracts of *Gonzalagunia rosea* (Rubiaceae) displayed moderate and strong activities simultaneously against *C. albicans* and *F. solani*, respectively; in addition, the methanol extracts of the Asteraceae *Montanoa* sp. and *Schistocarpha sinforosi* Cuatrec. displayed moderate activity against *C. albicans* (Table 1). Results in this research correlates very well with those from the methanol and chloroform extracts from *Carphalea obovata* (Rubiaceae) that were inactive against *P. aeruginosa* and *E. coli* (14). Furthermore, this correlates with the information on crude leaves methanol extract of *Psychotria microlabastra* (Rubiaceae) that displayed moderate activity against *E. coli* but was inactive against *P. aeruginosa*. No crude extract or fraction exhibited any activity against the fungi tested, including *C. albicans* (11).

The strongest cytotoxic activities were exhibited by the methanol extracts of *Liabum asclepiadeum*, *Munnozia polymonioides* (Asteraceae) and *Gonzalagunia rosea* (Rubiaceae). The methanol extracts of the Asteraceae *A. quinquenervis*, *C. tequendamensis* and *S. sinforsi* showed moderate cytotoxic activity. These results correlate with the brine shrimp lethality assay activities obtained with plant extracts of the Asteraceae *Achillea biebersteinni* (25), *Chromolaena christieana*, *Achyrocline saturoides* (23) and *Ladenbergia undata* (Rubiaceae) (19).

The phytochemical screening performed in this research (data not shown) revealed the presence of sterols, triterpenes and saponins as the main components of the methanol extracts of *A. quinquenervis*, *C. tequendamensis*, *M. polymonioides* and *S. sinforsi*. In addition, the methanol extracts of A. quinquenervis and *G. rosea* presented high contents of tannins and flavonoids, respectively. It is possible that these secondary metabolites might be the responsible for the bioactivity found on these plant extracts. None of the methanolic plant extracts showed presence of alkaloids neither of lactones. The dichloromethane extracts showed presence of sterol and the *n*-hexane extracts did not show the presence of the secondary metabolites tested on this phytochemical analysis.

The dichloromethane and methanol extracts of *Gonzalagunia rosea* and *Montanoa* sp. displayed important biological activities against fungi, followed by *Schistocarpha sinforosi* Cuatrec. These plants are candidates in the search for new bioactive phytocompounds, suggesting that a more extensive biological and chemical bioassay-guided fractionation is required in order to isolate and identify such bioactive compounds.

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RESUMO

Atividades antibactariana, antifúngica e citotóxica de oito plantas Asteraceae e duas Rubiaceae da biodiversidade colombiana

Trinta extratos crus de oito plantas da família Asteraceae e a duas plantas da família Rubiaceae, coletadas em diferentes lugares do Parque Natural Regional Ucumarí (PNRU), Colombia, foram avaliadas quanto à atividade contra duas bactérias Gram-positivas, duas Gram-negativas e três fungos. As atividades antibacteriana e antimicótica foram determinadas pelo método de difusão em agar. A atividade citotóxica dos mesmos extratos foi determinada através do bioensaio de *Artemia salina*. Os extratos da família Asteraceae foram os mais bioativos contra *Bacillus subtilis* e *Staphylococcus aureus*. Os extratos de ambas famílias estudadas foram bioativos contra os fungos *Candida albicans* e *Fusarium solani*. Os extratos da família Asteraceae exibiram maior atividade citotóxica. A espécie mais importante desta pesquisa foi *Gonzalagunia rosea* Standl (Rubiaceae) devido à atividade intensa e moderada dos extratos de metanol e diclorometano contra *F. solani* e *C. albicans*, respectivamente, bem como a intensa atividade citotóxica do extrato metanólico. Não se encontraram estudos prévios acerca das atividades biológicas das dez plantas avaliadas nesse estudo.

Palavras-chave: atividade antimicrobiana, *Artemia salina*, bioprospeção, flora Colombiana, método de difusão em ágar

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