



ANTIMICROBIAL ACTIVITY OF LATIN AMERICAN MEDICINAL PLANT EXTRACTS

Haag G^{1,3*}, Marín GH², Brignoles P³, ME Del Valle ME¹, S Magarinos MC² and Debenedetti SL¹

¹Cátedra de Farmacognosia, Facultad de Ciencias Exactas, La Plata, Argentina.

²Cátedra de Farmacología Básica, Facultad de Ciencias Médicas, La Plata, Argentina.

³Cátedra de Higiene y Salud Pública Facultad de Ciencias Exactas, UNLP. La Plata, Argentina.

ABSTRACT

Resistance of pathogenic microorganisms to several drugs has increased due to the widespread use of antibiotics to treat infectious diseases. Hence, development of new antimicrobial drugs from plants is an area of active research in the search for medicinal, veterinary or agricultural industry use. For this work 19 plants species was collected, dried in the shade and oven. 20 g powder plant were macerated in cold either with dichloromethane (DCM) or methanol (MeOH) filtered, evaporated to yield determination expressed in grams per 100g of dry plant. Methods: Antibacterial activity assays of the DCM and MeOH extracts were performed by agar diffusion. Paper disks were then impregnated with 10 ul of extract (range: 340-614mg/disc for MeOH; 300-500mg/disc for DCM). Solvents of DCM or MeOH extracts were used as negative controls (NC) and Gentamicin and Cephazoline were used as positive control (PC). The microorganisms used for testing were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853. The inoculum was incorporated to Petri plates and paper disks with extracts were added, and incubated for 24 hours at 37°C. Results: All DCM extracts tested except *Gentianella parviflora*, *Baccharis crispa*, *Bauhinia candicans*, *Terminalia langiflora* and *Picrosia australis* showed activity against *Staphylococcus aureus*. The DCM extracts of the three species of Gaillardia showed to be as well active against *Escherichia coli*. Only the MeOH extracts of *Baccharis crispa*, *Gentianella achalensis*, *Lippia turbinata*, *Lippia germinata*, *Terminalia australis* and *bicolor adesmia* were active against *Staphylococcus aureus*. Conclusion: dichloromethane extracts of Gaillardia and Protousnea poepiggii species might be important sources for the isolation of compounds with antimicrobial activity with a potential use in the pharmaceutical industry.

Keywords: Plants, Herbal, Antimicrobial Activity, Extracts.

INTRODUCTION

Despite major advances in the chemotherapy of infectious diseases, these affections are far from being controlled or eradicated and they remain as one of the leading causes of death worldwide. In recent years, the resistance of pathogenic microorganisms to several drugs has increased due to the widespread use of antibiotics to treat infectious diseases. The research and development of new antimicrobial drugs from plants is an area of active research in the search for new molecules with different spectrum of activity or novel chemical structures that serve as seed for the development of drugs or extracts from medicinal veterinary or agricultural industry use [1].

In this study, 19 plant species of plants with traditional uses associated with several health properties were selected for analysis of their antimicrobial activity (table 1).

Achyrocline satureioides, *Bicolor adesmia*, *Baccharis crispa*, *Bauhinia candicans*, *Equisetum giganteum*, *Gaillardia megapotamica* var. *radiata*, *cabrerae* *Gaillardia*, *Gaillardia megapotamica* var. *scabiosioides*, *Gentianella achalensis*, *Gentianella parviflora*, *germinata* *Lippia* *Lippia turbinata*, *Macfadyna ungui - cati*, *Pellaea flavens*, *Picrosia longiflora*, *Protousnea poepiggii*. *Pterocaulon polystachyum*, *Terminalia australis*, *Terminalia triflora*

were evaluated for antimicrobial activity against two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and a Gram positive germ (*Staphylococcus aureus*) with the aim of providing foundations for the development of new products for the treatment of infectious diseases.

MATERIALS AND METHODS

Plant material

The plant material was classified by Dr. Etilo Spegazzini and a herbarium specimen of each is deposited in the Museum of Botany and Pharmacognosy "Carlos Spegazzini". The used portion of each of the 19 species was collected in the wild. The plant material was dried in the shade, then in an oven at 30°C and ground in mechanical grinder.

Preparation of extracts

20 g powder plants were macerated in cold with dichloromethane (DCM) for 48 hours with frequent and regular stirring. The extract was filtered and again macerated for 48 hours. After another filtered, extracts obtained were evaporated in a rotary evaporator to yield determination expressed in grams per 100g of dry plant drug.

The extracted plant was finally dried and again extracted with methanol (MeOH) using the same methodology for obtaining DCM extract.

Antimicrobial activity

Antibacterial activity assays of the DCM and MeOH extracts was performed by the method of agar diffusion Kirby- Bauer [12]. Paper disks were then impregnated with 10 ul of extract, dried on plate heated at 40°C and then kept at room temperature. MeOH

impregnated extracts were in the range of 340-614 mg / disc and the DCM extracts in the range of 300-500 mg / disc. In addition, paper discs were impregnated with 10ul of the appropriate solvents according to DCM or MeOH extracts in order to use them as negative controls (NC). Gentamicin was used as positive control (PC) for gram negative activity and cephazoline for gram positive testing activity (both at 10 mg / disc). Assays were always performed in duplicate.

The microorganisms used for testing were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853. The inoculum was conducted from an overnight culture; it was incorporated into Mueller Hinton Agar (Merck) at a concentration of 10^8 by swabbing the surface using Petri plates of 9 mm in diameter. Once absorbed into the inoculum medium, the paper disks impregnated with the respective extracts to be tested were added, and the plates were incubated for 24 hours at 37°C. It was registered as positive antibacterial activity of the extracts the presence of inhibition zone.

RESULTS

All the DCM extracts tested except *Gentianella parviflora*, *Baccharis crispa*, *Bauhinia candicans*, *Terminalia langiflora* and *Picrosia australis* showed activity against *Staphylococcus aureus*. The DCM extracts of the three species of *Gaillardia* (*Gaillardia cabreræ*, *G. megapotamica* var. *Radiata* and *G. megapotamica* var. *Scabiosoides*) showed to be as well active against *Escherichia coli*. Only the MeOH extracts of *Baccharis crispa*, *Gentianella achalensis*, *Lippia turbinata*, *Lippia germinata*, *Terminalia australis* and *bicolor Adesmia* were active against *Staphylococcus aureus*.

Table 1. Scientific Name and traditional uses

Scientific Name / Common Name	Family	Known Popular Use
<i>Achyrocline satureioides</i> "Marcela female" "Vira vira"	Asteraceae	Choleretic, hepatoprotective, digestive, antimicrobial [2].
<i>Baccharis crispa</i> "Carqueja"		Liver, kidney, digestive diseases, male impotence, antiulcer, antiseptic skin [3,4]
<i>Gaillardia megapotamica</i> var. <i>radiata</i> "Topasaire"		Analgesic, antiseptic, antineuralgic in treating ulcers, influenza, decongestant and acne [5,6]
<i>Gaillardia megapotamica</i> var. <i>Scabiosoides</i> "Buttercup "		
<i>Gaillardia cabreræ</i> "Pampa's Margarita"		
<i>Picrosia longifolia</i> "Achicoria"		Tonic, laxative, diuretic, sudorific [7]
<i>Pterocaulon polystachyum</i> "Bull's Shadow "		Digestive disorders [8] Insect repellent [9]
<i>Macfadyena unguicatis</i> "Nail Cat"	Bignoniaceae	Syphilis, malarial fever, poisonous snake bite
<i>Terminalia australis</i> "Yellow launch"	Combretaceae	Digestive, liver disease, hemorrhoids astringent [7]

	“Yellow Stick”		
<i>Equisetum giganteum</i>	“Horse Tail”	Equisetaceae	Diuretic, astringent, antirheumatic, scabies [7]
<i>Adesmia bicolor</i>	“Babosita”	Fabaceae	Antidiuretic
<i>Bauhinia candicans</i>	Cow hoof		Antidiuretic, antidiabetic.
<i>Gentianella achalensis</i>	sour Grass “nencia”	Gentianaceae	Bitter, digestive, antioxidant, antipyretic tonic [10]
<i>Gentianella parviflora</i>			Bitter, digestive, tonic hepatoprotective [2,11]
<i>Pellaea flavens</i>	“Doradilla”	Pteridaceae	Menstrual regulator, colds, constipation
<i>Lippia turbinata</i>	“Poleo”	Verbenaceae	Digestive tonic, tachycardia, hemenagogo diuretic, abortifacient, nerves
<i>Lippia germinata</i>	“Purple Salvia”		Menstrual regulator, Cold
<i>Protousnea poeppigii</i>	Payun Monguén "Lichen Old Beard"	Polytrichaceae	Antibiotic and healing

Table 2. Antimicrobial activity of different plants extracts

Plants	<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>		<i>Pseudomonas aeruginosa</i>	
	DCM	MeOH	DCM	MeOH	DCM	MeOH
<i>Achyrocline satureoides</i>	+	-	-	-	-	-
<i>Adesmia bicolor</i>	+	+	-	-	-	-
<i>Baccharis crispa</i>	-	+	-	-	-	-
<i>Bauhinia candicans</i>	-	-	-	-	-	-
<i>Equisetum giganteum</i>	+	-	-	-	-	-
<i>Gaillardia megapotamica var. Radiata</i>	+	-	+	-	-	-
<i>Gaillardia megapotamica var. Sacbiosoides</i>	+	-	+	-	-	-
<i>Gaillardia cabreræ</i>	+	-	+	-	-	-
<i>Gentianella achalensis</i>	+	+	-	-	-	-
<i>Gentianella parviflora</i>	-	-	-	-	-	-
<i>Lippia turbinata</i>	+	+	-	-	-	-
<i>Lippia germinata</i>	+	+	-	-	-	-
<i>Macfadyena unguis-cati</i>	+	-	-	-	-	-
<i>Pellaea flavens</i>	+	-	+	-	-	-
<i>Picrosia langiflora</i>	-	-	-	-	-	-
<i>Protousnea poeppigii</i>	+	-	-	-	-	-
<i>Pterocaulon polystachyum</i>	+	-	-	-	-	-
<i>Terminalia australis</i>	-	+	-	-	-	-
<i>Terminalia triflora</i>	+	-	-	-	-	-
CN	-	-	-	-	-	-
CP (gentamicine 10 µl)	+	+	+	+	+	+
CP (cephazoline 10 µl)	+	+	+	+	+	+

CONCLUSION

The discovery of new antibiotic agents, whether from natural or synthetic sources has become a pressing need for medical and scientific community, given the increasingly frequent occurrence of bacterial resistance [13-15]. In recent years, the interest shown in the knowledge in folk medicine was markedly enhanced. Noteworthy, that interest in herbal medicine is certainly less onerous for public health, especially in developing countries [16]. The World Health Organization recognizes

the important dimension that medicinal plants have as therapeutic resource, especially in Primary Health Care [17].

Among the results obtained in our work, it is suggest that the dichloromethane extracts of the three species of *Gaillardia* and *Protousnea poeppigii* are important sources for the isolation of compounds with antimicrobial activity with a potential use in the pharmaceutical and / or agrochemical industry.

REFERENCES

1. Voigt Mota F, Damé Schuch LF, Lambrecht Gonçalves C, Faccin L, Actividad antibacteriana de los extractos de *Syzygium cumini* (L.) Skeels (jambolán). *Rev Cubana Plant Med*, 18(3), 2013.
2. Toursarkissian M. Plantas Medicinales de la Argentina. Hemisferio Sur, Buenos Aires, Argentina, 1980.
3. Del Vitto L, Petenatti E, Petenatti ME. Recursos herbolarios de San Luis (República Argentina) Primera parte, plantas nativas. *Multequina (Latin American Journal of Natural Resources)*, 6, 1997, 49-66.
4. Barboza GE, Cantero JJ, Nuñez COY Ariza L. Espinar Flora Medicinal de la Provincia de Córdoba (Argentina) Pteridófitas y Antófitas silvestres y naturalizadas ED. Museo Botánico Córdoba, 2006, 329.
5. Rosella MA, Matias RLY, Mandrile EL. *Gaillardia* spp. (Asteráceas), Geobotánica y etnofarmacognosia. *Revista Farmacéutica*, 142, 2000, 1-48.
6. Martínez, G. y Planchuela, A. La medicina tradicional de los criollos campesinos de Paravachasca y Calamuchita, Córdoba (Argentina). *Scripta Ethnológica*, XXV (25), 93, 115
7. Alonso JRY, Desmarchelier C. Plantas Medicinales Autóctonas de la Argentina—Bases Científicas para su Aplicación en Atención Primaria de la Salud.- Edic. *Fitociencia*, 2006
8. Lopes AMV. Plantas Usadas na Medicina Popular do Rio Grande do Sul. Caderno Técnico. *Imprensa Universitária, Santa Maria*, 1995, 74.
9. Zardini EM. Etnobotánica de Compuestas argentinas con especial referencia a seu uso farmacológico. *Acta Farmacéutica Bonaerense*, 3, 1983, 77-79.
10. Ratera EL & MO Ratera. Plantas de la Flora Argentina empleadas en Medicina Popular, Ed. Hemisferio Sur. *Buenos Aires*, 1980, 134.
11. Kirby-Bauer. National Committee for Clinical Laboratory Standards Performance Standards for Antimicrobial Susceptibility Testing, Ninth Informatinal Supplement. 1999, 100-S8. NCCLS, Wayne, PA.USA.
12. Gérvas J. La resistencia a los antibióticos, un problema de salud pública. *Aten Primaria*, 25, 2000, 589 - 596.
13. Cabrera Y, Fadragas A, Guerrero L. Antibióticos naturales, Mito o realidad. *Rev Cubana Med Gen Integr*, 21, 2005, 6.
14. Available in,
15. Kunin CM. Why did it take the Infectious Diseases Society of America so long to address the problem of antibiotic resistance. *Clin Infect Dis*, 46, 2008, 1791-1792.
16. Linares EY, Bye R. Plantas medicinales de México, Usos y remedios tradicionales. Segunda edición. Centro de Tecnología Electrónica e Informática (CETEI) e Instituto de Biología de la Universidad Nacional Autónoma de México (IB, UNAM), Eds. México, DF, 1995.
17. OMS. The Promotion and Development of Traditional Medicine, Ed. WHO, Technical Report Series No. 622, 1978, Ginebra.