

ANTIMICROBIAL ACTIVITY OF DICHLOROMETHANE EXTRACTS OF ELEVEN PLANTS FROM THE FLORA OF ENTRE RÍOS (ARGENTINA)

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Summary

Ethnobotanical knowledge of many plants with appreciated antimicrobial actions requires scientific evidence about their activities. Many species from the vascular flora of Entre Ríos (Argentina), are used in the popular culture for the treatment of dermatitis, infections, pimples and other affections. Eleven of them were selected to be studied: *Acacia bonariensis*, *Baccharis articulata*, *Blepharocalyx salicifolius*, *Castela tweedii*, *Eichhornia crassipes*, *Erythrina crista – galli*, *Gaillardia megapotamica*, *Hydrocotyle bonariensis*, *Phytolacca dioica*, *Senna scabriuscula* and *Typha latifolia*. Dichloromethane extracts were assayed against a group of standard and clinical strains of Gram + and Gram - bacteria using the diffusion method in solid media. Results show that extracts of aerial parts of *E. crassipes* and bark of *E. crista–galli* were active against *Bacillus subtilis* and *Staphylococcus aureus*, while the extracts of aerial parts of *H. bonariensis*, *B. articulata*, *B. salicifolius* and roots of *T. latifolia*, were active against *B. subtilis*. The assays of *B. salicifolia* and *E. crassipes* show activity against a clinical methicillin resistant strain of *S. aureus* (MRSA).

Keywords: Medicinal plants, Antibacterial activity, Flora Argentina.

Resistance to traditional antibiotics has appeared since the past few years in several infectious agents, especially in bacteria. Ethnobotanical data indicate that many plants reputed to be antimicrobial require further studies to show scientific evidence of their bioactivity.

The flora of the province of Entre Ríos (Argentina) has about five hundred medicinal plants for the treatment of many illnesses. Eleven of them were selected among the species reported to be useful in the treatment of wounds, infections, rashes, pimples and other microbial affections.

The infusion of fruits and flowers of *Acacia bonariensis* Gillies ex Hook. & Arn. are used to heal wounds⁽¹⁾, and the leaves against diarrhea and ophthalmic diseases⁽²⁾.

The decoction of the stems of *Baccharis articulata* (Lam.) Pers. is employed for skin treatments, rheumatism and wounds, and the powder to treat syphilitic ulcers.

The decoction of bark and leaves of *Blepharocalyx salicifolius* (Kunth) O. Berg is popularly believed to have antitusive, expectorant, pectoral and astringent properties, as well as against diarrhea⁽³⁾; the powder of ground leaves of this plant is applied with navel bandages⁽⁴⁾.

A historically well reported plant is *Castela tweedii* Planch.; decoctions of bark and the leaves are said to have cured dysentery, fever and gastroenteritis of entire legions of soldiers ⁽⁵⁾.

Head aches are said to lessen applying smashed leaves of *Eichhornia crassipes* (Mart.) Solms on the forehead, as well as pains in other parts of the body ⁽⁶⁾; leaves and roots of this plant macerated in wine are reported to be useful in the treatment of dysentery, gonorrhoea and vaginal infections; the infusion of flowers is considered stimulating ⁽⁷⁾ and aphrodisiac ⁽⁸⁾; acid and alkaline extracts of leaves, stems and roots are effective against Gram + and Gram – bacteria ⁽⁹⁾.

Smashed seeds of *Erythrina crista – galli* L. in alcohol are applied in wounds to kill worms; bark, flowers and leaves of this species are used in poultices to heal wounds; the infusion is applied externally for bruises, and in gargles and enemas for recto-vaginal infectious diseases ^{(3), (7), (10)}; the decoction of leaves is hypnotic and sedative; flowers are used to prepare cough syrups.

Ground dried parts of aerial parts of *Gaillardia megapota mica* (Spreng.) Baker var. *scabiosoides* (Arn. Ex DC.) Baker, are used as a sneeze-inducing and in decoctions against headaches and colds ⁽⁷⁾. Aerial parts macerated in alcohol are employed in massages to control dandruff and seborrhea ⁽²⁾.

Smashed juice or poultices of *Hydrocotyle bonariensis* Lam. are applied externally to heal wounds or complexion problems, including freckles. Internally, its decoction is diuretic, antiphlogistic, hepatic, against dropsy and pectoral; the juice is emetic, diuretic, and useful against illnesses of lungs, liver, spleen and bladder ^{(7), (10)}.

Leaves or smashed fruits juice of *Phytolacca dioica* L. are drastic purgatives; poultices made of leaves or their infusion are used externally to heal wounds ⁽⁷⁾; the decoction of the roots is taken internally against rheumatism; externally, the ashes of the root bark are supposed to be useful as antiseptic, astringent and healing agents ⁽³⁾.

Decoctions of smashed leaves of *Senna scabriuscula* (Vog-Zuber) H. S. Irwin & Barneby, are externally employed as disinfectant, resolvent and to heal pimples ^{(2), (11)}, flowers and leaves in decoctions are used in affections of the rectum and in dysentery ⁽¹¹⁾; flowers are also febrifugal and expectorant ⁽¹¹⁾; roots in decoction have digestive, diuretic and pectoral properties taken internally, as well as against menorrhagias; externally they are used to eliminate lice; seed decoctions are supposed to be tonic, diuretic, stimulant, pectoral and in strong amounts abortive ⁽¹¹⁾.

Infusions made of leaves of *Typha latifolia* L. are laxative and useful to soothe kidney pains and blenorhoea. The decoctions of rhizomes are used to cure sores ⁽³⁾: Internally, pollen is used against hemorrhages, painful menstruations, cancer of the lymphatic system and postpartum periods ⁽¹²⁾.

Material and methods

Plant material

Voucher specimens are deposited at the Herbarium of the Faculty of Agricultural Sciences, Entre Ríos National University (ERA): *Acacia bonariensis* (Muñoz 4730); *Baccharis articulata* (Lam.) Pers. (Muñoz 4805); *Blepharocalyx salicifolius* (Kunth) O. Berg, (Muñoz 603); *Castela tweedii* Planch., (Muñoz 3857); *Eichhornia crassipes* (Mart.) Solms (Muñoz 3482); *Erythrina crista-galli*

L. (Muñoz 4796); *Gaillardia megapotamica* (Spreng.) Baker var. *scabiosoides* (Arn. ex DC.) Baker (Muñoz 4772); *Hydrocotyle bonariensis* Lam. (Muñoz 4761); *Phytolacca dioica* L. (Muñoz 4493); *Senna scabriuscula* (Vog.-Zuber) H.S. Irwin & Barneby (Muñoz 3252); *Typha latifolia* L. (Muñoz 2502).

The parts of the species popularly considered to have antimicrobial properties were investigated as mentioned in the introduction. .

Preparation of extracts

Twenty-five grams of dried and ground plant material were extracted twice overnight with 100 ml of dichloromethane and filtered. Both extracts were mixed and dried by vacuum solvent evaporation.

Antibacterial disc diffusion assays

Extracts were confronted with a set of selected microorganisms and were trialed by means of the diffusion test in solid medium by impregnating onto filter paper discs (6 mm) of 1,000 µg of dry extract dissolved in 10 µL of the corresponding extraction solvent and dry at room temperature.

The inoculum was prepared like was described by Kirby-Bauer's method⁽¹³⁾. Essentially, 1×10^6 microorganisms/mL suspensions were swabbed on solidified growth medium of 25 mL, [agar for antibiotics N 1, (Britania, Argentina) pH 6.6 and agar for antibiotics N 11, (Britania, Argentina) pH 7.9], placed into Petri dishes (90mm).

The saturated paper discs were placed on the agar swabbed surface and then the Petri dishes were incubated for 24 hours at 37°C. We adopted the presence or absence of inhibition zone criterion to determinate the activity or inactivity of de crude extracts.

To avoid false positive results originated by the action of the paper discs components on the tested bacteria, a blank was used consisting on a disc without extracts on each studied dish. All the tests were performed in duplicate, and one Ampiciline disc (Britania) was added to each of them as an positive control of the inhibition.

Test microorganisms

The microorganisms used were a set of Gram positive and negative bacteria : *Bacillus subtilis* (ATCC 6633); *Escherichia coli* (ATCC 25422); *Staphylococcus aureus* (ATCC 25923); *Pseudomonas aeruginosa* (ATCC 27853), and a clinical methicillin resistant strain of *Staphylococcus aureus* *Meti R* (MRSA) donated by the Microbiología Clínica chair of Universidad Nacional del Litoral (Argentina).

Results and discusion

The results of the antibacterial screening of dichloromethane extracts of eleven selected Argentine medicinal species are shown in Table1.

Table 1: Antibacterial activity of dichloromethane extracts:

Bacteria	Parts	<i>B. subtilis</i>		<i>P. aeruginosa</i>		<i>S.a. (MRSA)</i>		<i>S. aureus</i>		<i>E. coli</i>	
		ATCC 6633		ATCC 27853		Clinical train		ATCC 25923		ATCC 25422	
		AM 1	AM 11	AM 1	AM 11	AM 1	AM 11	AM 1	AM 11	AM 1	AM 11
<i>Erythrina crista galli</i>	stem/lv	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>Erythrina. crista galli</i>	bark	(+)	(+)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(-)
<i>Phytolacca dioica</i>	aerial	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>Phytolacca dioica</i>	bark	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>Hydrocotyle bonariensis</i>	aerial	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>Typha latifolia</i>	root	(+)	(*)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>Baccharis articulata</i>	aerial	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>Acacia. bonaerensis</i>	aerial	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>Gaillardia megapotá mica</i>	aerial	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>Eichhornia crassipes</i>	aerial	(+)	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(-)	(-)
<i>Castella tweedii</i>	aerial	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>Blepharocalyx salicifolius</i>	aerial	(-)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)
<i>Senna scabriuscula</i>	aerial	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>Typha latifolia</i>	aerial	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

References: **AM1**: Agar for antibiotics núm.1 (pH 6.6); **AM 11**: Agar for antibiótics núm.11 (pH 7.9); (+): presence of halo inhibition ; (-): absence of halo inhibition y (*): very little activity.

Antibacterial activity was found in the extracts of aerial parts of *E. crassipes* and bark of *E. crista-galli* against *B. subtilis* and *S. aureus*. The extracts of aerial parts of *H. bonariensis*, *B. articulata*, *Blepharocalyx salicifolius* and *Typha latifolia* (bark), also show activity against *B. subtilis*

The extracts from aerial parts of *B. salicifolius* and *E. crassipes* evidence activity against the clinical methicillin resistant strain of *S. aureus* (MRSA) mention in recent studies because this bacterium is becoming a serious world wide problem for public health⁽¹⁴⁾.

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References

- 1.- Kelner, M. *Plantas Mágicas en la Medicina*. Editorial Albatros. 1979. Buenos Aires
- 2.- Burgstaller Chiriani, C. H. *La Vuelta a los Vegetales*. Editorial Hachette. 1974. Buenos Aires.
- 3.- Lahitte, H., Hurrell, J. A., Belgrano, M.J. et al. *Plantas Medicinales Rioplatenses*. Editorial L.O.L.A. 1998. Buenos Aires.
- 4.- Berro, M. B. *La Vegetación Uruguaya*. En: Arechavaleta, J. Anales del Museo Nacional de Montevideo (dir.). Tomo II, fasc. 11. 1899. Montevideo.

- 5.- Domínguez, J.A. *Urquiza y Bonpland. Antecedentes Históricos*. Trabajos del Instituto de Botánica y Farmacología N° 59. 1939. (Facultad de Ciencias Médicas de Buenos Aires).
- 6.- Villafuerte, C. *Diccionario de Árboles, Arbustos y Yuyos en el Folklore Argentino*. Editorial Plus Ultra. 1984. Buenos Aires.
- 7.- Hieronymus, J.. *Plantae Diaphoricae Florae Argentinae*. Bol. Acad. Ciencias. Córdoba. 1882. 4:199-589.
- 8.- Marzocca, A. *Vademécum de Malezas Medicinales de la Argentina Indígenas y Exóticas*. Editorial Hemisferio Sur. 1997. Buenos Aires.
- 9.- Nickell, L. G. *Antimicrobial Activity of Vascular Plants. Economic Botany* . 1959. 13 (4): 281-318.
- 10.- Manfred, L.. *Siete Mil Recetas Botánicas a Base de Mil Trescientas Plantas Medicinales*. Editorial KIER. 1973. Buenos Aires.
- 11.- González Torres, D. M.. *Catálogo de Plantas Medicinales Usadas en Paraguay*. Edic. del autor. 1992. Asunción.
- 12.- Bown, D.. *Enciclopedia de las Hierbas y sus Usos*. The Royal Horticultural Society. GRIJALBO. 1996. Barcelona.
- 13.- Bauer AW, Kirby WMM, Sherris JC, Turk M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966;45:493-6
- 14.- Moran, G. J.; Krishnadasan, A.; Gorwitz, R.J. et al.. *Methicillin – Resistant Infections among Patients in the Emergency Department (Emergency ID Net Study Group)*. *The New England Journal of Medicine*, 2006; 355 666-674.