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COMUNICACIÓN BREVE

Inhibitory activity of *Conyza bonariensis* (L.) Cronquist tincture against fungi and bacteria causing superficial infections

Actividad inhibidora de la tintura de *Conyza bonariensis* (L.) Cronquist contra hongos y bacterias causantes de infecciones superficiales

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ABSTRACT

Introduction: *Conyza bonariensis* (L.) Cronquist is a herbaceous plant of the underbrush group. It is distributed worldwide and is used to treat a variety of skin conditions.
Objective: Evaluate the *in vitro* inhibitory activity of the tincture from *C. bonariensis* leaves against fungi and bacteria causing superficial infections.
Methods: The tincture was obtained by alcoholic maceration of dry leaves of *C. bonariensis*. Minimum inhibitory concentration (MIC) of the tincture was determined against 53 isolates from patients with superficial lesions and 6 reference strains; 20 *Malassezia* (7 *M. sympodialis*, 7 *M. furfur*, 6 *M. globosa*), 16 *Candida* (8 *C. albicans*, 8 *C. parapsilosis*), 17 dermatophytes (6

Trichophyton rubrum, 6 *Trichophyton mentagrophytes*, 5 *Microsporum canis*) and 6 *Staphylococcus aureus*.
Results: A notable reduction in the viability of most of the microorganisms evaluated was obtained at low concentrations (under 10 % v/v) of the C. bonariensis tincture.
Conclusions: Results allow to conclude that the C. bonariensis tincture displays antibacterial activity against Staphylococcus aureus and antifungal activity against the main fungi causing superficial infections, such as dermatophytes, Candida and Malassezia. MIC variations were found between genera and species, but not within each species. These results may help experts find a scientific explanation for the empirical use of the C. bonariensis extract to treat various skin infections and revalue traditional ethnomedical knowledge.

Key words: *Conyza bonariensis* (L.) Cronquist, antifungal activity, antibacterial activity, *s*kin infections.

RESUMEN

Introducción: *Conyza bonariensis* (L.) Cronquist es una planta herbácea de distribución mundial que forma parte del grupo de las malezas y es utilizada para tratar diversas afecciones de la piel.
Objetivo: Evaluar la actividad inhibitoria *in vitro* de la tintura de las hojas de *C. bonariensis* contra hongos y bacterias causantes de infecciones superficiales.
Métodos: La tintura se obtuvo mediante maceración alcohólica de las hojas secas de *C. bonariensis*. La concentración inhibitoria mínima (MIC) de la tintura se determinó en 53 aislados de pacientes con lesiones superficiales y en 6 cepas de referencia; 20 *Malassezia* (7 *M. sympodialis*, 7 *M. furfur*, 6 *M. globosa*), 16 *Candida* (8 *C. albicans*, 8 *C. parapsilosis*), 17 dermatofitos (6*Trichophyton rubrum*, 6 *Trichophyton mentagrophytes*, 5 *Microsporum canis*) y 6 *Staphylococcus aureus*.
Resultados: Se obtuvo una marcada reducción de la viabilidad de la mayoría de los microorganismos evaluados con bajas concentraciones (menores a 10 % v/v) de la tintura de *C. bonariensis*.
Conclusiones: Los resultados permitieron concluir que la tintura de *C. bonariensis* tiene actividad antibacteriana contra *Staphylococcus aureus* y actividad antifúngica contra los principales hongos que causan infecciones superficiales como dermatofitos, *Candida* y *Malassezia*. Se observaron variaciones de las MIC entre género y entre especies, pero no se observaron variaciones de las MIC dentro de las especies. Estos resultados contribuyen a dar una explicación científica del uso empírico del extracto de *C. bonariensis* en el tratamiento de varias infecciones cutáneas y a revalorizar el conocimiento etnomédico tradicional.

Palabras clave: *Conyza bonariensis* (L.) Cronquist; actividad antifúngica; actividad antibacteriana; infecciones de la piel.

INTRODUCTION

Since the World Health Organization[1] supported the introduction of resources of traditional medicine in health systems everywhere, the use of medicinal plants has shown a marked increase in the world. This decision was reaffirmed in the Ottawa Charter for Health Promotion.[2] At present, the program "Traditional Medicine" have a plan of health monitoring which purpose is to recover natural medical practices.[3] Moreover, application of scientific methods in selection and study of plants to validate the properties from which are traditionally known,[4] is increasingly important in the field of research.[5] The WHO 2014-2023 strategy on Traditional Medicine reevaluate the strategy 2002-2005,[3] and points the course of traditional and complementary medicine for the next decade.

The use of specific plants to cure some skin infections has been the traditional medicine of different ethnic groups. Through ethnomedical information, is known that juice of crushed fresh leaves of *Conyza bonariensis* (L.) Cronquist (*C. bonariensis*) is used to treat various skin lesions in Manabi province (Ecuador). Rural people use it to cure "manchas blancas" (white macules), referring to pityriasis versicolor.[6] *C. bonariensis* is a native Latin American herbaceous plant member of the *Asteraceae* family, spread all over the world forming part of weeds group. Commonly it is known as "canilla de venado", "rama negra", "mata negra" or "hierba carnicera" by indigenous peoples.[7-10]

The aim of this study was to evaluate the *in vitro* inhibitory activity of the tincture of *C. bonariensis* leaves against fungi and bacteria causing superficial infections.

METHODS

Plant material

Leaves of *C. bonariensis* (L.) Cronquist (Asteraceae) were hand harvested in the agricultural farm of the Escuela Superior Politécnica del Litoral (ESPOL), Guayaquil, Ecuador. A voucher (code CIBE002) was deposited in the Herbario Nacional de Ecuador, Quito. The contaminated and diseased parts of plants were discarded.

Tincture preparation

Leaves were washed under running water for 10 minutes and shade dried for 1 hour at room temperature. After, treated with sodium hypochlorite solution 5 % for 3 minutes and washed 3 times with sterile distilled water, according to Bissegger and Sieber, 1994. Next, leaves were dried in a hot air oven (Memmert SFB-400) at 45 °C to constant weight. Once dry, the leaves were ground in a manual grinder and sifted with a mesh of 2 mm in diameter.

The tincture was obtained by maceration of *C. bonariensis* leaves with ethanol 96° for 24 hours in a closed vessel, with periodic stirring, in a final ratio of 1 herb part for 20 alcohol parts (0.05 g/mL). Then, it was filtered and stored in light protected sterile glass bottles.

Microorganisms tested

A total of 53 isolates including yeast, dermatophytes and bacteria obtained from patients with skin infections were studied: 20 *Malassezia* (7*M. sympodialis*, 7 *M. furfur*, 6 *M. globosa*), 16 *Candida* (8 *C. albicans*, 8 *C. parapsilosis*), 17 dermatophytes (6 *Trichophyton rubrum*, 6*Trichophyton mentagrophytes*, 5 *Microsporum canis*) and 6 *Staphylococcus aureus*. All isolates were previously identified by molecular and conventional methods and deposited at Mycology and Bacteriology Culture Collection of Instituto de Medicina Regional (IMR), Universidad Nacional del Nordeste, Resistencia, Argentina.

Malassezia isolates were obtained from pityriasis versicolor, seborrheic dermatitis and atopic dermatitis lesions. *Candida* isolates were obtained from cutaneous candidiasis, mucocutaneous candidiasis and onychomycosis; dermatophytes isolates were obtained from *tinea capitis*, *tinea corporis*, *tinea unguium*, *tinea pedis*, *tinea interdigitalis* and *tinea cruris* lesions and bacteria isolated from skin infections.

As reference strains were used *M. furfur* CBS 7019,*M. sympodialis* CBS 7222, *M. globosa* CBS 7886, *C. albicans* ATCC 64548, *C. parapsilosis* ATCC 22019 and *Staphylococcus aureus* ATCC 25923.

Inhibitory activity assay

The inhibitory activity of the tincture was evaluated using the agar dilution technique by surface dissemination.[12]

The inoculum suspensions used were adjusted to a concentration equivalent to a 0,5 McFarland standard for fungus and bacterial concentration was adjusted to 4×10^6 colony forming units per mL (CFU/mL). After the inhibition test, bacterial viability was quantified in Mueller-Hinton agar after 24 hours of incubation at 37 °C.[13]

Petri dishes of 9 cm with culture medium containing dilutions from 20 % v/v of the tincture were used. Then, according to the obtained growth, intermediate dilutions were performed.

Culture medium, temperature and time of incubation, varied according to the microorganism studied:

Malassezia spp.: Dixon agar, incubation at 32 °C up to 5 days.

Candida spp.: Sabouraud agar, incubation at 37 °C up to 48 hours.

Dermatophytes: potato dextrose agar (PDA), incubation at 28 °C up to 10 days.

Staphylococcus aureus: trypticase soy agar (Mueller-Hinton), incubation at 35 °C up to 48 hours.

Plates were inoculated with 0,5 mL of the inoculum suspensions poured onto the agar surface (flooding) and the excess liquid was immediately removed.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the tincture at which growth was not observed and was expressed as % (v/v).

As growth control, each microorganism was tested: a) only with the extraction solvent, to rule out any possible effect of the solvent and b) without addition neither the tincture nor the extraction solvent, as growth positive control.

RESULTS

Table shows MICs against the main fungi and bacteria causing superficial infections.

Table. Minimum inhibitory concentration % (v / v) of *C. bonariensis* tincture against microorganism species tested

Strain	Source	MIC% (v/v)	Strain	Source	MIC% (v/v)
Candida albicans	Reference strain ATCC 64548	9	*Malassezia globosa*	Reference strain CBS 7886	5
C. albicans (IMR-ML 333)	Onychomycosis	9	*M. globosa* (IMR-MM 175)	Pityriasis versicolor	5
C. albicans (IMR-ML	Cutaneous candidiasis	9	*M. globosa* (IMR-MM 176)	Seborrheic dermatitis	5

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C. albicans (IMR-ML 347)	Onychomycosis	9	*M. globosa* (IMR-MM 187)	Pityriasis versicolor	5
C. albicans (IMR-ML 248)	Mucocutaneous candidiasis	9	*M. globosa* (IMR-MM 370)	Seborrheic dermatitis	5
C. albicans (IMR-ML 249)	Cutaneous candidiasis	9	*M. globosa* (IMR-MM 189)	Pityriasis versicolor	5
C. albicans (IMR-ML 250)	Cutaneous candidiasis	9	*Trichophyton rubrum* (IMR-M 806)	*Tinea cruris*	4.5
C. albicans (IMR-ML 198)	Mucocutaneous candidiasis	9	*T. rubrum* (IMR-M 786)	*Tinea pedis*	4.5
Candida parapsilosis	Reference strain ATCC 22019	4.5	*T. rubrum* (IMR-M 789)	*Tinea interdigitalis*	4.5
C. parapsilosis (IMR-ML 111)	Cutaneous candidiasis	4.5	*T. rubrum* (IMR-M 793)	*Tinea corporis*	4.5
C. parapsilosis (IMR-ML 127)	Cutaneous candidiasis	4.5	*T. rubrum* (IMR-M 794)	Onychomycosis	4.5
C. parapsilosis (IMR-ML 258)	Cutaneous candidiasis	4.5	*T. rubrum* (IMR-M 797)	*Tinea pedis*	4.5
C. parapsilosis (IMR-ML 369)	Onychomycosis	4.5	*Trichophyton mentagrophytes* (IMR-M 784)	*Tinea pedis*	9.5
C. parapsilosis (IMR-ML 741)	Cutaneous candidiasis	4.5	*T. mentagrophytes* (IMR-M 785)	*Tinea interdigitalis*	9.5
C. parapsilosis (IMR-ML 150)	Onychomycosis	4.5	*T. mentagrophytes* (IMR-M 795)	*Tinea corporis*	9.5
C. parapsilosis (IMR-ML 357)	Cutaneous candidiasis	4.5	*T. mentagrophytes* (IMR-M 794)	Onychomycosis	9.5
Malassezia sympodialis	Reference strain	7	*T.*	*Tinea pedis*	9.5

	CBS 7222		*mentagrophytes* (IMR-M 800)		
M. sympodialis (IMR-MM 162)	Pityriasis versicolor	7	*T. mentagrophytes* (IMR-M 805)	*Tinea cruris*	9.5
M. sympodialis (IMR-MM 167)	Pityriasis versicolor	7	*Microsporum canis*(IMR-M 780)	*Tinea capitis*	4
M. sympodialis (IMR-MM 362)	Pityriasis versicolor	7	*M. canis* (IMR-M 781)	*Tinea capitis*	4
M. sympodialis (IMR-MM 170)	Seborrheic dermatitis	7	*M. canis* (IMR-M 782)	*Tinea corporis*	4
M. sympodialis (IMR-MM 171)	Seborrheic dermatitis	7	*M. canis* (IMR-M 801)	*Tinea corporis*	4
M. sympodialis (IMR-MM 172)	Atopic dermatitis	7	*M. canis* (IMR-M 802)	*Tinea capitis*	4
Malassezia furfur	Reference strain CBS 7019	6.5	*Staphylococcus aureus*	Reference strain ATCC 25923	6.5
M. furfur (IMR-MM 157)	Pityriasis versicolor	6.5	*S. aureus* (IMR-B 065)	Piodermitis	6.5
M. furfur (IMR-MM 159)	Pityriasis versicolor	6.5	*S. aureus* (IMR-B 067)	Piodermitis	6.5
M. furfur (IMR-MM 155)	Seborrheic dermatitis	6.5	*S. aureus* (IMR-B 071)	Nail infection	6.5
M. furfur (IMR-MM 156)	Seborrheic dermatitis	6.5	*S. aureus* (IMR-B 075)	Leg ulcer	6.5
M. furfur (IMR-MM 358)	Seborrheic dermatitis	6.5	*S. aureus* (IMR-B 080)	Piodermitis	6.5
M. furfur (IMR-MM 361)	Seborrheic dermatitis	6.5			

DISCUSSION

Ethnopharmacology is defined as an ensemble of knowledge and belief about plants, parts thereof or products deriving from them. It focuses on the scientific study of indigenous medicines in order to contribute, in the long-run, to improved health care in the

regions of study, as well as search for pharmacologically unique principles from existing indigenous remedies.[14-16]

Medicinal plants can be used whole or parts of them (leaves, roots, seeds, flowers), fresh or in different preparations as aqueous extracts, tinctures, essential oils, resins, balsams.[17] As inhabitants of Manabi do, several studies conducted in different parts of the world showed that leaves are used more than others parts of a plant.[18-21]

Tinctures are solutions of alcoholic or hydroalcoholic extraction obtained from parts of medicinal plants[22,23] and it is preferred by the people because they concern a more practical use and better results.[24]

Table shows MICs obtained, proving the antibacterial and antifungal activity of *C. bonariensis* tincture against the main fungi and bacteria causing superficial infections. No MICs variations were observed among isolates of the same species, but MICs variations were observed mainly with the genus.

Controls carried out demonstrated that in no case the microorganism growth was affected by solvent. All microorganisms studied grew in the absence of tincture.

Dermatophytes are the most frequent agents of superficial mycoses. Showing different activity against species of the same group, in this study the lowest and the highest MIC were obtained with dermatophytes fungi (*M. canis* and *T. mentagrophytes*). Moreover, different activity was observed against species of the same genus such us *T. rubrum* and *T. mentagrophytes*.

Similar to that observed with dermatophytes, *Candida* species showed highly variable results. *C. albicans* the most frequently agent of superficial candidiasis showed lower susceptibility than *C. parapsilosis*.

Malassezia yeast species are considered to be the etiological agents of pityriasis versicolor and *Malassezia* folliculitis, and can be related as an associated agent or a contributory factor in other dermatological entities. Considering its recognized use in traditional medicine in Ecuador for the cure of the "white stain" (pityriasis versicolor), the tincture of *C. bonariensis* was highly active against the three *Malassezia* species tested, showing a low variation of MICs interspecies. *M. sympodialis, M. globosa* and *M. furfur* are the most frequent species related to skin lesions as pityriasis versicolor, seborrheic dermatitis and atopic dermatitis.[25-27]

S. aureus was included in this study because is frequently isolated from skin infections. Some studies showed that ethanolic

extract of *C. bonariensis* is active against *S. aureus* and other bacteria.[17,28] In this work, *C. bonariensis* tincture leaves has proved to have activity activity against *S. aureus* too.

Traditional "healers" mainly use water to extract the compounds with antimicrobial activity; however, extracts obtained with organic solvents have a more consistent antimicrobial activity compared to aqueous extracts as they allow greater extraction of active compounds.[29]

El Zalabani et al. evaluated the antimicrobial activity of hydroalcoholic extract of *C. bonariensis* but only against *C. albicans.*[30] Others studies showed that methanol, hexane and ethyl acetate extracts of the aerial parts of *C. bonariensis* did not show any activity against*C. albicans* and *S. aureus.*[31,32] However, *Shah* et al. studied the methanolic extract of *C. bonariensis* and observed its antifungal activity against *C. albicans.*[33] In this work, ethanol was an effective solvent for extraction of bioactive compounds and the ethanolic extract proved to be active against the most microorganisms tested. In addition, we must consider that ethyl alcohol has low toxicity unlike methyl alcohol which is very dangerous, it can lead to blindness and in severe intoxication cases cause death.[34,35]

Nowadays, the phytomedicine and ethnopharmacological knowledge are advancing in great strides. The application of the scientific method allows corroborate the traditional use of plants as medicines and find more properties to those already known since many years. This particular case opens up the possibility to demonstrate the *in vivo* effectiveness of *C. bonariensis* tincture leaves as an antifungal and antibacterial suitable to treat superficial infections, both for use in humans and veterinary medicine. A natural product would help to resolve the problem of adherence to current therapy associated with the use of synthetic drugs, because of its high costs, sometimes with bad results and collateral effects.

The results allowed concluding that *C. bonariensis* tincture has antibacterial activity against *Staphylococcus aureus* and antifungal activity against the main fungi causing superficial infections, such as dermatophytes, *Candida* and *Malassezia*.

MICs variations were observed intergenus and interspecies, but no MICs variations were observed among intraspecies isolates.

These results contribute to the scientific explanation of the empirical use of *C. bonariensis* extract in treating several skin infections and with the revaluation of the traditional ethnomedical knowledge.

Declaration of interest

The authors declare no conflict of interest.

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