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Original Article

Antimicrobial Screening of Medicinal Plants Popularly used in Mato Grosso for Treating Infections: Advances on the Evaluation of *Conyza bonariensis* (L.) Cronquist *in vitro* and *in vivo* Antibacterial Activities

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ABSTRACT

Objective The aim of this study was to screen a group of medicinal plants' extracts used in the treatment of ailments related to infections in the Brazilian popular medicine. And to carry out *in vivo* toxicity and antibacterial studies on *Conyza bonariensis* (Asteraceae) leaves and roots methanolic extracts selected based on the screening. **Methods:** Eleven methanolic extracts obtained from nine plants, reportedly used in the treatments of infections from the state of Mato Grosso, Brazil, were initially screened for their *in vitro* antibacterial and antifungal activities employing disc diffusion and broth micro dilution assays. Preliminary phytochemical analysis was carried out. The most promising extract based on our results and previous literature reports was then evaluated in the *in vivo* antibacterial activities using mouse model of bacterial infection induced by *Staphylococcus aureus* and *Escherichia coli*. In addition, *in vivo* acute toxicity was conducted to evaluate the safety profile of the extracts. **Results:** All of the extracts tested were active against at least one of the bacterial and fungal strain tested with activities ranging from moderate to weak. Phytochemical analyses of ME*Cb*l and ME*Cb*r demonstrated the presence of free steroids and coumarins in ME*Cb*l and flavonoids, tanins, free steroids, reduced anthraquinones and coumarins in MECBr. Oral administration of ME*Cb*l and ME*Cb*r up to 5000 mg/kg did not provoked any toxicological events in the mice, thus suggesting that the LD_{50} is higher than 5000 mg/kg. *In vivo* antibacterial assay demonstrated superior prophylactic activity of ME*Cb*l compared to ME*Cb*r. **Conclusion:** ME*Cb*l and MEC*br* are safe when administered acute orally at doses up to 5000 mg/kg. Methanolic extracts of *Conyza bonariensis* possessed *in vitro* antibacterial and antifungal activities. Considerable *in vivo* antibacterial activities were observed in bacterial infection model for both MECbl and MECbr, effects comparable to that of meropenem, in some cases. Both extracts present in common free steroids and coumarins. The current *in vivo* antibacterial activity study further lend supports to the use of *Conyza bonariensis* in the treatment of infections in many traditional medicines.

Key words: Medicinal plants, *Conyza bonariensis*, Antimicrobial, Mato Grosso, Acute toxicity, Preliminary phytochemistry

INTRODUCTION

Human infections are a serious public health problem because many pathogens such as bacteria and yeast are becoming increasingly resistant to antibiotics.[1]

Despite the great advances achieved by science, among the ten main causes of death worldwide, which include ischaemic heart disease, stroke, diarrhea, HIV/AIDS, malaria, tuberculosis, pre-term birth complications and birth asphyxia and birth trauma, the lower respiratory infections top the list.[2] It is estimated that the so called 'super-microorganisms' alone will be responsible for over 10 million deaths by 2050, making it imperative the search for alternative treatments to microorganisms which are unresponsive to most modern antibiotics.[3] Similarly, opportunistic fungal infections and resistance to antifungal agents have increased significantly in immunocompromised patients and these infections are responsible for a high rate of morbidity/mortality in severe cases.[1,4]

The growing need for more effective and safe antimicrobial agents has led to the renewal of multidisciplinary investigation on natural products, where new approaches combined with traditional techniques, are accelerating tracking of substances, which present antimicrobial activities. In addition, it has also allowed identification of the molecular targets responsible for their effects, moreover, many of these substances present new mechanisms of action.[4] Medicinal plants constitute an arsenal of chemicals

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that could be exploited by human to prevent microbial invasion and have been a major source for drug development. All over the world, plant extracts and their products are used in the treatment of bacterial, fungal and viral infections.[5] The use of plants and preparations made from them to treat infections is an ancient practice used by a large portion of the world's population, particularly in developing countries where there is a reliance on traditional medicine for a variety of diseases.[6-7] Many plants are used in Brazil in the form of crude extracts, infusion or poultice to treat common infections, without any scientific evidence. Due to the mega biodiversity of the Brazilian medicinal plants, many studies have been conducted in an attempt to validate the antimicrobial properties of popular use in a given region, of these preparations.[8-9]

Brazil possesses the largest floristic diversity on Earth, containing six continental biomes, the Amazon rainforest, the Cerrado the Caatinga, the Atlantic forest, the Pantanal and the Pampas, with Amazon rainforest the most noteworthy, since it is the largest tropical forest in the world. In addition, the diversity of plant species constitutes an endless source for the research on herbal remedies for the development of new molecules with biological activities.[2] The state of Mato Grosso (MT), the largest farming and livestock producer in Brazil, contains three important bio geographical regions (Amazon rain forest, Brazilian Savannah and Pantanal) and a rich ethnic-cultural diversity, represented by 42 indigenous groups and traditional *Quilombola*, *Cabocla* and Riverine Communities.

In vitro antimicrobial tests allow selection of crude extracts of plants with potential properties through use of chemical and pharmacological studies. In fact, majority of studies on antimicrobial potentials of medicinal plants are restricted to *in vitro* studies.[10-12] However, *in vitro* susceptibility testing is only one-step in the evaluation of the potential efficacy of antimicrobial agents against microbial organisms. Based on the aforementioned, this study aimed at screening selected medicinal plants from Mato Grosso, through *in vitro* antimicrobial activity methods with the view of selecting the most promising for *in vivo* antibacterial study.

As part of our on-going research towards development of new antimicrobial for use in humans, the aim of the present study was to screen medicinal plants used popularly in the state of Mato Grosso for treating infections, with the sole purpose of selecting the most promising plant. *Conyza bonariensis* extracts were selected for further studies based on its *in vitro* antimicrobial activities and the availability of extensive reports of its use in ethnomedicine. The important uses include among others microbial infections; wound healing, constipation, diarrhea, and inflammation, just to mention but few. Numerous species of the genus *Conyza* have been extensively used in popular folk medicine. The plant has traditionally been used to treat rheumatism, gout, cystitis, nephritis, dysmenorrhoea, dental pain, and headache.[8,13-20]

There are also reports of several biological, phytochemical and pharmacological studies of different extracts or derivatives from this plant that have supported its popular use in many cases. Despite several *in vitro* studies, none has ventured to evaluate *Conyza bonariensis in vivo* antibacterial activity in experimental rodents. Thus in the present work, we present its *in vitro* activities using different methods, the acute toxicity and it's *in vivo* prophylactic effect in systemic infection model.

MATERIALS AND METHOD

Experimental Animals

Albino mice *Mus musculus*, Swiss-Webster strain (25-30 g) were used for the *in vivo* anti-bacterial studies. Animals were maintained in polypropylene cages at 26°C in a 12 h light-dark cycle, with free access to standard laboratory chow and water. Groups of six animals were used for each experiment. The experimental protocol followed the International Principles for the Biomedical Research Involving Animal[13] and was approved by the Committee on the Use of Animal for experimentation (CEUA/UFMT) with protocol number 23108,047577/09-1. The number of animals and the intensity of the stimuli used were minimum required to demonstrate in a consistent manner the effect of treatments.

Microorganisms

All bacterial and fungal strains used in *in vitro* experimental models were from American Type Culture Collection (ATCC) strains commercially acquired from Newprov (Paraná, Brazil). These were *Enterococcus faecalis* (ATCC-29212), *Enterobacer aerogene* (ATCC-13048), *Escherichia coli* (ATCC-25922), *Klebsiella pneumonia* (ATCC-13883), *Pseudomonas aeruginosa* (ATCC-27853), *Proteus mirabilis* (ATCC-25933), *Shigella flexneri* (ATCC-12022), *Staphylococcus aureus* (ATCC-25923), *Strepto-*

Table 1: Plants collected, place of collection in Mato Grosso State, Brazil, and voucher number.

Scientific name/ Family	Main vernacular name	Part collected/Medicinal use	Place of collection	Voucher number	Reference
Cariniana rubra Gardner ex Miers/ Lecitidaceae	Jequitibá-vermelho	Leaves/depurative, ulcer, throat inflammation	Cuiabá, latitude: 15º 35' 46" and longitude 56º 05' 48"	18,337	[5]
Lafoensia pacari A. St.-Hil./Lythraceae	Mangava-brava	Stem bark/ infections, diarrhea with blood, venereal disease, chilblain, furuncle, female infection (with discharge), tuberculosis; inflammation, uterine inflammation, uterus and ovary infection, kidney infections, wound healing	Várzea Grande, latitude: 15º 51' 58" S and longitude 52º 15' 37" W	35,577	[5]
Stryphnodendron rotundifolium Mart./ Fabaceae	Barbatimão	Stem bark/ antimicrobial, anti-ulcer and anti-inflammatory properties	Santo Antonio do Leverger, latitude: 15º 51' 56" S and longitude: 56º 04' 36" W	35,584	[5]
Anacardium humile A. St. -Hil./ Anacardiaceae	Cajuzinho-do-campo	Leaves and stem bark/diarrhea, (superficial skin mycoses), general Infection, gastritis, wound healing, throat infection	Cuiabá, latitude: 15º 32' 50" S and longitude 56º 09' 26"W	31,789	[5]

Scientific name/ Family	Main vernacular name	Part collected/Medicinal use	Place of collection	Voucher number	Reference
Handroanthus heptaphyllus (Vell.) Mattos/Bignoniacee	Ipê-roxo	Stem bark/ DE: wound healing; malaria, stomach infection in the ovary , osteoporosis, uterine problems antibiotic, rheumatism , Bladder infection , urinary infection	Cuiabá, latitude: 15º 35' 46"S and longitude 56º 05' 48"W,	39,140	[5]
Gossypium barbadense L./Malvaceae	Algodão	Leaves/ infections, female infection (with discharge) flu , inflammation, ovarian infection, uterine infection, vaginal infection with discharge, uterine inflammation, uterine and ovarian inflammation, wound healing, injury	Poconé, Latitude:16º 02'90'S and longitude 0 56 43'49' 'W.	31,755	[5]
Plantago major L./ Plantaginaceae	Tanchagem	Leaves/ skin diseases, infectious diseases, digestive organs, respiratory organs, reproduction, tumours, pain, fever	Santo Antônio do Leverger, latitude: 15º 51' 56" S and longitude: 56º 04' 36" W	31,790	[14]
*Cecropia pachystachya*Trécul/ Cecropiaceae	Embaúba	Leaves/ anti-inflammatory, antitussive, expectorant, anti-asthmatic and hypoglycaemic effects	Cuiabá, latitude: 14°47'23.200" S and longitude: 56°19'10.008"W	34,119	[5]
Conyza bonariensis (L.) Cronquist/	Margaridinha-do-campo	Leaves and roots/ laxative, diarrhoea, cough, aphrodisiac, gastrointestinal problems including diarrhoea	Campo Verde, latitude: 15º 32' 48" S and longitude: 55º 10' 08" W	21,438	[15]

coccus pyogenes (ATCC-19615), *Candida albicans - fluconazole-resistant* (ATCC® 10231™), *Candida albicans* (ATCC-64550), *Candida grablata* (ATCC-90030), *Candida kruzei* (ATCC-6258) and *Candida parapsilosis* (ATCC-40058). For the experiments, all bacteria were cultured at 37°C in agar Muller- Hinton and yeasts on Sabouraud agar, 24 h prior to testing to become viable and reproducible experiment

Botanical materials

Different parts of the plants used in the screening assays were collected during the period of 2008 – 2010 from various locals situated in the different municipalities indicated in the Table 1. All plants were identified by the taxonomist Dr. Rosilene Rodrigues Silva and were deposited in the Herbarium of Universidad Federal de Mato Grosso (UFMT). The accepted plants names were checked with www.theplantlist.org, on May 21, 2015, while the geographical origin status was based on Rio de Janeiro Botanical Garden database of list of species of the Brazilian flora (available at http: http://floradobrasil.jbrj.gov.br/).

The antimicrobial activities of nine plants (11 extracts) with popular uses related to bacterial or fungal infections were evaluated. These were *Anacardium humile* A. St. -Hil. (leaves and stem bark), *Cecropia pachystachya* Trécul (leaves), *Gossypium barbadense* L. (leaves), *Plantago major* L. (leaves), *Cariniana rubra* Gardner ex Miers (leaves), *Lafoensia pacari* A.St.-Hil. (Stem bark), *Stryphnodendron rotundifolium* Mart. (stem bark), *Handroanthus heptaphyllus* (Vell.) Mattos (stem bark), *Conyza bonariensis* (L.) Cronquist (leaves and root) on Gram-positive and Gram-negative bacteria and fungal strains.

Extract preparations

The extracts of the plants were prepared at the Natural Products Laboratory of Pharmacology, Faculty of Medicine, UFMT. The parts of the plants were collected cleaned and dried in the shade at room temperature for a period of 7 days, were milled and sieved using electric miller, resulting in 100 g of powdered plant material. After which they were obtained by soaking each part of powder in cold absolute methanol solvent (1:10 w/v) for 7 days at 25°C.

The extracts were filtered and concentrated in vacuum at 600 mm Hg rotary evaporator and the residual solvent was removed in an oven at 40°C. At the time of use, extracts were dissolved in Tween 80 (Synth). The extracts were *Anacardium humile* leaves and stem bark (ME*Ah*l and ME*Ahs*), *Cecropia pachystachya* (ME*Cp*), *Gossypium barbadense* (ME*Gb*), *Plantago major* (*MEPm*), *Cariniana rubra* (ME*Cr*), *Lafoensia pacari* (ME*Lp*), *Stryphnodendron rotundifolium* (ME*Sr*), *Handroanthus heptaphyllus* (ME*Hh*), *Conyza bonariensis* leaves and root (ME*Cb*l and ME*Cb*r, respectively).

Preliminary phytochemical analysis

Preliminary phytochemical tests were performed to identify the following principal secondary metabolite groups: tannins, flavonoids, steroids and triterpenoids, saponins, alkaloids, coumarins and quinones, through a process of qualitative prospecting.[16] The preliminary phytochemical analysis was carried out by using the following standard methods. *Test for tannins:* 10 mL of bromine water was added to the 0.5 g crude extracts. Decoloration of bromine water showed the presence of tannins.

Tests for flavonoids shinoda test: Pieces of magnesium ribbon and HCl concentrated were mixed with crude plant extracts after few minutes and pink color showed the presence of flavonoid.

Test for steroids: steroids was sought by the reaction of Liebermann, 10 mL of crude extracts were evaporated. The residue was dissolved in 0.5 mL of hot acetic anhydride; we added 0.5 mL of the filtrate chloroforme. Treated with the reagent of Libermann Burchardt. The appearance, at the interphase, a ring of blue-green, showed a positive reaction.

Test for triterpenoids: Liebermann - Burchard's test 2 mg of dry extracts were dissolved in acetic anhydride, heated to boiling, cooled and then 1

mL of concentrated sulphuric acid was added along the sides of the test tube. Formation of a pink colour indicates the presence of triterpenoids.

Test for saponins: 5.0 mL of distilled water was mixed with crude plant extracts in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Test for anthraquinones: 10 mL of benzene was added in 6 g of the crude plant extracts in a conical flask and soaked for 10 min and then filtered. Further 10 mL of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 s and pink, violet, or red color indicated the presence of anthraquinones in the ammonia phase.

Test for alkaloids: Dragendorff's test To 2 mg of the crude extracts 5 mL of distilled water was added, 2 M HCl was added until an acid reaction occurs. To this 1 mL of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicates the presence of alkaloids.

Test for coumarins: Evaporate 5 mL of ethanolic solution, dissolve the residue in 1-2 mL of hot distilled water and divide the volume into two parts. Take half the volume as a witness and to add another volume of 0.5 mL 10% NH_4OH. Put two spots on filter paper and examined under UV light. Intense fluorescence indicates the presence of coumarins.

Antimicrobial assays

Disc diffusion assay

The disc diffusion method was used for the tests disc. Sterile Filter papers (7 mm in diameter, (Sensibiodisc-Cecon, São Paulo, Brazil) impregnated with extract solution (20 µL) were placed on Muller-Hinton agar (Oxoid, Thermo Fisher Scientific, São Paulo, Brazil) and Saubouraud agar (Oxoid, Thermo Fisher Scientific, São Paulo, Brazil), according to the method of Kirby *et al*.[17] against nine bacteria species, being 6 Gram-negative and 3 Gram-positive, and 5 leveduriforms (*Candida* spp.). The test plates were prepared with Müller-Hintonand Saubouraud agar and were inoculated on the surface with bacterial and fungal suspension respectively, prepared in sterile saline (0.9%).

The concentration of the bacterial suspension was adjusted to 0.5 MacFarland scale ($1x10^5$ CFU/mL) and the fungal suspension was adjusted to 1 MacFarland scale ($1x10^5$ UFC/mL). The extracts were tested at different concentrations (20 - 0.009 µg/disc), using chloramphenicol (30 µg/disc, Sensibiodisc-Cecon, São Paulo, Brazil) and amphotericin B (100 µg/disc, Sensibiodisc-Cecon, São Paulo, Brazil) as the standard drugs. The negative controls for the extracts were as follows: distilled water for ME*Ah*l, ME*AH*c, ME*Cr*, ME*Hh*, DMSO (0.04%) and Tween 80 (2%) in distilled water for ME*Pm*, ME*Gb*, ME*Cp*, ME*Cb*l, ME*Cb*r and ME*Lp*. The plates were placed in a refrigerator for 4 h, so that the test drug will diffuse throughout the medium. After this period, the plates were incubated at 37°C for 24 h and we subsequently proceeded to measure the zones of inhibition of bacterial growth, considering the active zones of inhibition of bacterial growth ≥ 10 mm.[6] Tests were performed in duplicates.

Broth micro dilution

The antibacterial activities of the extracts were evaluated by determining the minimal inhibitory concentration (MIC) according to guidelines established by Clinical and Laboratory Standards Institute (CLSI). MICs were determined using micro plates of 96 wells according to CLSI guidelines.[18] Stock solutions of the extracts in distilled water were diluted to give serial twofold dilutions that were added to each medium, resulting in concentrations ranging from 1000 - 1.9 µg/mL of the extracts. Inoculum of 100 µL (final concentration 10^4 CFU/mL) were added to Mueller-Hinton broth. Chloramphenicol (50 - 3.1 µg/mL) (Sigma, São Paulo, Brazil) was used as positive control. The culture medium 0.04% DMSO served as the negative control. Plates were incubated for 24 h at 35°C.

The same procedure was used to evaluate the antifungal activity, using the Saubouraud medium (Acumedia, São Paulo, Brazil) incubated for 24 h. Amphotericin B (100 - 3.25 µg/mL) (Sigma, São Paulo, Brazil) was used as standard drug. The reading of MIC was performed manually or visually, considering the presence of turbidity in each microplate.[19]

The reading was performed using the microplate reader method. The criteria used to classify the activity of the extracts were: MIC ≤ 100 µg/mL good antimicrobial activity; when the MIC between 100 -500 µg/mL, moderate activity; MIC above 500 - 1000 µg/mL, weak activity and MIC ≥ 1000 µg/mL inactive.[20] The MIC is the lowest concentration of the test drug that was able to inhibit completely the bacterial growth in the medium. All tests were conducted in duplicates.

Acute toxicity screening test

The effect of ME*Cb*l and ME*Cb*r on the general behavior of conscious animals was evaluated in mice, as previously described by Malone and Robichaud.[21] Briefly, male and female mice (n=3/group) received by gavage (p.o.) ME*Cb*l and ME*Cb*r at doses of 500, 1,000, 2,000 and 5,000 mg/kg body weight (b.w.). One control animal per group, received the vehicle (distilled water, 10 mL/kg). Animals were observed individually in open field at 5, 10, 15, 30, 60, 120 and 240 min and once a day, for a period of 14 days, noting any clinical signs or mortality.

Systemic bacterial infection in mice

For the systemic infection experiments,[22] the ME*Cb*l and ME*Cb*r were used against two bacterial clinical isolates of *S. aureus* and *E. coli*. Swiss albino male and female mice, weighing between 25-35 g were allocated into 10 groups of 10 animals each. The negative control group received distilled water (vehicle) orally and the positive control group received meropenem (Biochimico, São Paulo, Brazil) 20 mg/kg subcutaneously as treatment. In the test groups, different doses (0.01; 0.1; 1; 10; 50; 100; 200; 300 and 500 mg/kg) of the tested extracts were given orally. The bacterial strains were plated on nutrient agar, 24 h before the experiment (Biobrás, São Paulo, Brazil).

The bacterial inoculum of *S. aureus* was adjusted to MacFarland 6 scale ($21x10^8$ CFU/mL), for *E. coli* the scale was MacFarland 3 scale ($9x10^8$ CFU/mL). These bacterial concentrations are capable of inducing systemic infection in the animals and causing death in 100% of the animals in less than 14 days. Bacterial infection was induced by the intraperitoneal administration (0.2 mL) of the bacterial suspension in BHI broth (Biobrás®, São Paulo, Brazil). Treatments of the animals were done immediately and 4 h after inoculation of the animals, and they were observed for 14 days to record mortality.

Data analysis

The Bartlett's test was used to test for homogeneity of variance between groups. When no significant heterogeneity was detected, one-way analysis of variance (ANOVA) was applied, followed by Student-Newman-Keuls multiple comparison test. $P < 0.05$ level was considered as significant. Graph Pad Prism© version 5.01 for Windows (Graph Pad Software, USA) was used for statistical analysis.

RESULTS

Preliminary phytochemical analysis

Preliminary phytochemical analysis of the extracts revealed the presence of flavonoids, tanins, alkaloids, free steroids, saponins, reduced anthraquinones, triterpenes and coumarins (Table 2).

ME*Ah*l and ME*Ahs*: methanolic extract of *Anacardium humile* leaves and stem bark, respectively, ME*Cp*: methanolic extract of *Cecropia*

Table 2: Preliminary phytochemical analysis of selected medicinal plant methanolic extracts from the state of Mato Grosso, Brazil.

Extracts	Flavonoids	Tanins	Alkaloids	Free steroids	Saponins	Reduced anthraquinones	anthraquioiones	Triterpenoids	Coumarins
ME*Ahl*	+	+	-	+	+	+	-	-	+
ME*Ahs*	-	+	-	+	-	+	-	-	+
ME*Cp*	+	+	-	+	-	+	-	-	+
MEGb	-	+	-	+	-	+	-	-	+
ME*Pm*	+	-	-	+	-	+	-	-	+
ME*Cr*	+	+	-	-	+	+	-	+	+
ME*Lp*	+	+	+	-	+	+	-	-	+
ME*Sr*	+	+	-	+	+	+	-	-	+
ME*Hh*	+	-	-	-	-	+	-	-	+
ME*Cb*l	-z	-	-	+	-	-	-	-	+
ME*Cb*r	+	+	-	+	-	+	-	-	+

+ Present in the methanolic extract ; -Absent in the methanolic extract

pachystachya, MEGb: methanolic extract of *Gossypium barbadense*; ME*Pm*: methanolic extract of *Plantago major,* ME*Cr:* methanolic extract of *Cariniana rubra*, ME*Lp*: methanolic extract of *Lafoensia pacari*: ME*Sr*: methanolic extract of *Stryphnodendron rotundifolium*, ME*Hh*: methanolic extract of *Handroanthus heptaphyllus*; MECbl and MECbr: methanolic extract of *Conyza bonariensis* leaves and roots, respectively.

Antimicrobial activity

Disc diffusion assay

Antibacterial activities of the plants' methanolic extracts obtained against the Gram-positive and Gram-negative bacteria organisms in the disc diffusion method are shown in Table 3. All the plant extracts tested demonstrated antibacterial activity against one or more bacterial agents. However, they differ in their spectrum of activities against the microorganisms. On one hand, none of the extracts was active against *K. pneumoneae, S. flexneri* and *P. mirabilis,* whereas, *E. feacalis* was the most sensitive bacterial strain. Chloramphenicol, the standard antibiotic used in this assay was active against all the tested strains (Table 3).

ME*Ah*l and ME*Ahs*: methanolic extract of *Anacardium humile* leaves and stem bark, respectively, ME*Cp*: methanolic extract of *Cecropia pachystachya*, MEGb: methanolic extract of *Gossypium barbadense*, ME*Pm*: methanolic extract of *Plantago major,* ME*Cr:* methanolic extract of *Cariniana rubra*, ME*Lp*: methanolic extract of *Lafoensia pacari*, ME*Sr*: methanolic extract of *Stryphnodendron rotundifolium*, ME*Hh*: methanolic extract of *Handroanthus heptaphyllus*; MECbl and MECbr: methanolic extract of *Conyza bonariensis* leaves and root, respectively.

Broth microdilution assay

All the plant extracts showed moderate to weak activities against the Gram-positive and Gram-negative bacteria tested in this assay, with MICs ranging from 250 - 1000 µg/mL as shown in Table 4. Whereas chloramphenicol, the standard drug, demonstrated good activity against all tested bacteria with MIC ranging between 0.5 and 2.0 µg/mL.

MIC = Minimum inhibitory concentration. Good activity: MIC ≤ 100 µg/mL; Moderate activity: 100 < MIC < 500 µg/mL; Weak activity: 500 < MIC < 1000 µg/mL; Inactive: ≥ 1000 µg/mL.[20]

Ef = Enterococcus faecalis; Sa = Staphylococcus aureus; Sp = Streptococcus pyogenes; Ec = Escherichia coli; Kp = Klebsiella pneumoniae; Pa = Pseudomonas aeruginosa; Sf = Shigella flexneri; Pm = *Proteus mirabilis*; Ea = *Enterobacter aerogenes*.

ME*Ah*l and ME*Ahs*: methanolic extract of *Anacardium humile* leaves and stem bark, respectively, ME*Cp*: methanolic extract of *Cecropia pachystachya*, MEGb: methanolic extract of *Gossypium barbadense*; ME*Pm*: methanolic extract of *Plantago major,* ME*Cr:* methanolic extract of *Cariniana rubra*, ME*Lp*: methanolic extract of *Lafoensia pacari*: ME*Sr*: methanolic extract of *Stryphnodendron rotundifolium*, ME*Hh*: methanolic extract of *Handroanthus heptaphyllus*; MECbl and MECbr: methanolic extract of *Conyza bonariensis* leaves and root, respectively.

Antifungal activity

Disc diffusion assay

The antifungal activities of the extracts using disc diffusion method can be seen in Table 5. Only 5 of the extracts demonstrated activity against the yeast strains employed, with *C. bonariensis* displaying higher spectrum of antifungal activity.

ME*Ah*l and ME*Ahs*: methanolic extract of *Anacardium humile* leaves and stem bark, respectively, ME*Cp*: methanolic extract of *Cecropia pachystachya*, MEGb: methanolic extract of *Gossypium barbadense*; ME*Pm*: methanolic extract of *Plantago major,* ME*Cr:* methanolic extract of *Cariniana rubra*, ME*Lp*: methanolic extract of *Lafoensia pacari*: ME*Sr*: methanolic extract of *Stryphnodendron rotundifolium*, ME*Hh*: methanolic extract of *Handroanthus heptaphyllus*; MECbl and MECbr: methanolic extract of *Conyza bonariensis* leaves and root, respectively.

Broth microdilution assay

Similar to the results obtained in the antibacterial micro broth dilution assay, all plants' extracts demonstrated moderate to weak activity against all the fungal strains tested. However, Amphotericin B showed superior activity against all the leveduriform strains with MIC ranging between 0.25 and 1.0 µg/mL (Table 6).

ME*Ahs* and ME*Ahl*: methanolic extract of *Anacardium humile* stem and leaves, respectively, ME*Cr:* methanolic extract of *Cariniana rubra*, ME*Cp*: methanolic extract of *Cecropia pachystachya*, MECbl and MECbr: methanolic extract of *Conyza bonariensis* leaves and root, respectively.MEGb: methanolic extract of *Gossypium barbadense*; ME*Lp*: methanolic extract of *Lafoensia pacari*: ME*Pm*: methanolic extract of *Plantago major,* MESo: methanolic extract of *Stryphnodendron rotundifolim*, ME*Hh*: methanolic extract of *Handroanthus heptaphyllus*.

In the selection of the plants extracts that were included in the *in vivo* toxicological and *in vivo* antibacterial studies, we employed various

Table 3: **Results of antibacterial activity by disc diffusion method of selected medicinal plant methanolic extracts from the state of Mato Grosso, Brazil.**

Extract	Concentration (µg/disc)	Diameter of inhibition zone (mm)								
		Bacteria								
		Ef	*Sa*	*Sp*	*Ec*	*Kp*	*Pa*	*Sf*	*Pm*	*Ea*
MEA*h*l	2.5	--	--	--	--	--	--	--	--	--
	5	--	--	--	--	--	--	--	--	--
	10	--	--	--	--	--	--	--	--	--
	20	--	--	--	--	--	--	--	--	--
MEA*h*s	2.5	--	--	--	--	--	--	--	--	--
	5	--	--	--	--	--	--	--	--	--
	10	--	--	--	--	--	--	--	--	--
	20	--	--	--	--	--	--	--	--	--
MEC*p*	2.5	--	10	--	--	--	--	--	--	--
	5	--	12	--	--	--	--	--	--	--
	10	--	--	--	--	--	--	--	--	--
	20	--	--	--	--	--	--	--	--	--
MEG*b*	2.5	10	10	--	12	--	--	--	--	--
	5	10	10	--	11	--	--	--	--	--
	10	12	10	--	--	--	--	--	--	--
	20	--	--	--	11	--	10	--	--	--
MEP*m*	2.5	11	--	--	--	--	--	--	--	--
	5	13	--	--	--	--	--	--	--	10
	10	15	--	--	--	--	--	--	--	--
	20	16	--	--	--	--	--	--	--	--
MEC*r*	2.5	--	--	--	--	--	--	--	--	--
	5	--	--	--	--	--	--	--	--	--
	10	--	--	--	--	--	--	--	--	--
	20	--	--	--	--	--	--	--	--	--
MEL*p*	2.5	--	10	--	--	--	--	--	--	--
	5	--	10	--	--	--	--	--	--	11
	10	--	10	--	--	--	--	--	--	10
	20	--	--	--	--	--	--	--	--	--
MES*r*	2.5	--	--	--	--	--	--	--	--	--
	5	--	--	--	--	--	--	--	--	--
	10	--	--	--	--	--	--	--	--	--
	20	--	--	--	--	--	--	--	--	--

Extract	Concentration (µg/disc)	Diameter of inhibition zone (mm)									
		Bacteria									
		Ef	*Sa*	*Sp*	*Ec*	*Kp*	*Pa*	*Sf*	*Pm*	*Ea*
ME*Hh*	2.5	--	--	--	12	--	--	--	--	--
	5	10	--	--	--	--	--	--	--	--
	10	10	--	--	--	--	--	--	--	--
	20	10	--	--	--	--	--	--	--	--
MECbl	2.5	--	--	10	--	--	--	--	--	--
	5	--	--	12	--	--	--	--	--	--
	10	13	--	15	--	--	--	--	--	--
	20	14	--	16	--	--	--	--	--	--
MEC*br*	2.5	--	--	--	--	--	--	--	--	--
	5	--	--	--	--	--	--	--	--	--
	10	--	--	--	--	--	--			10
		20	11	--	--	--	--	--	--	13
Chloramphenicol	30	30	30	28	25	25	30		28	28

-- = no bacterial inhibition observed; Antibacterial activity: inhibition zones ≥ 10 mm;[6] *Ef* = *Enterococcus faecalis*; *Sa* = *Staphylococcus aureus*; *Sp* = *Streptococcus pyogenes*; *Ec* = *Escherichia coli*; *Kp* = *Klebsiella pneumoniae*; *Pa* = *Pseudomonas aeruginosa*; *Sf* = *Shigella flexneri*; Pm = *Proteus mirabilis*; Ea = *Enterobacter aerogenes*

criteria. These criteria specifically were: the preponderance of reports that have demonstrated scientific evidence of the ethnomedicinal uses of the plant in question; the potential antibacterial and antifungal activities observed; literature evidence concerning its pharmacological and biological activities, reports of toxicity, if any, and if there is study with human subject. We therefore proceeded only with the methanolic extracts of *Conyza bonariensis* leaves and root for the *in vivo* toxicological and antimicrobial evaluations.

In vivo acute toxicity study

The *in vivo* oral acute toxicity study of the two extracts MEC*b* extracts (root and leaves) demonstrated that both extracts are safe at doses up to 5000 mg/kg, as no behavioural or deaths were recorded after 14 days of observations (Table 7).

Systemic bacterial infection in mice

Table 8, shows the protective effects of MEC*b*l, *MECbr* and meropenem, on a murine systemic infection model induced by a variety of pathogens. The protective effect of MEC*b*l was comparable to that of imipenem and stronger than that of MEC*br* for infections induced by *S. aureus.* For the other gram-negative bacterial infection i.e. *E. coli*, its protective effect was inferior to that meropenem, but superior to MEC*br*, which lack effect on the *E. coli*. In of general, by observing the *in vitro* and *in vivo* results, it is evident that *S. aureus* was more susceptible to the extracts than the *E. coli.* Intriguingly, at the maximum dose of 500 mg/kg there seems to be reductions in the prophylactic activities of the two extracts.

DISCUSSION

As part of our research goals, in identifying medicinal plants with potential for phytotherapeutic ends, we screened selected medicinal plants from the Cerrado of the state of Mato Grosso for potential antimicrobial use. Generally, the antimicrobial activities of natural products are screened using *in vitro* biological assays susceptibility testing.[37] Several plants were selected based on initial ethnobotanical survey using for screening of their antibacterial activity the disc diffusion, agar diffusion and micro dilution methods, that are the most commonly used for screening plant extracts with potential antimicrobial activities.[38]

Initial screening of the 11 extracts showed that all the extracts displayed antibacterial and antifungal activities to more than one pathogen tested, although at varying degrees. Some of these plants have been previously studied, with different solvents, parts and sometimes using different strains and different methodological approachs.[23-36] Although, there are some reports concerning antimicrobial activities of some of the plants tested (Table 9), the main difficulties in comparing previous studies, lies in the fact that the criteria, method and end-points used for reporting the activity are very diverse. As can be seen in the table in the case of *Gossypium barbadense*, the minimum concentration used in the study by Ikobi *et al.*[32] and regarded to represent antibacterial activity, is considered in our study to be too high (10 folds increase compared to the maximum dose we utilized), and regarded as not having activity. The genus *Conyza*(Asteraceae) is comprised of approximately 400 species and several species are known for their use in traditional medicine.[39]Many ethnobotanical studies have documented the use of *C. bonariensis* in the ethnomedicines of different cultures.[40-44] Previous studies have confirmed bioactive properties for specific *Conyza* species.[45-51]

C. bonariensis (leaves (MEC*bl*) and root (MEC*br*) extracts) were selected based on its modest *in vitro* antibacterial activity results and the vast amount of studies done on different parts of the plants from different parts of the world.

In the popular medicines, different parts of *C. bonariensis,* in the form of infusion or decoction of its parts, are used as antiseptic, anti-ulcer-

Table 4: Antibacterial activity in broth microdilution assay of selected medicinal plant methanolic extracts from the state of Mato Grosso, Brazil.

Plants	Bacteria (MIC, µg/mL)								
	Ef	*Sa*	*Sp*	*Ea*	*Ec*	*Kp*	*Pa*	*Pm*	*Sf*
ME*Ah*l	1000	1000	500	500	500	500	500	500	250
ME*Ah*s	1000	500	250	250	250	500	500	1000	500
MEC*p*	1000	1000	500	500	500	500	250	500	1000
MEGb	250	1000	250	250	1000	500	500	1000	1000
ME*Pm*	500	500	500	1000	1000	1000	1000	1000	1000
MEC*r*	500	1000	500	1000	1000	1000	250	1000	1000
ME*Lp*	1000	1000	250	1000	1000	1000	250	1000	1000
MES*r*	500	1000	1000	125	500	500	500	500	500
Me*Hh*	250	500	1000	1000	1000	1000	1000	1000	500
MEC*b*l	250	1000	500	500	1000	1000	500	1000	1000
MEC*b*r	500	1000	500	1000	1000	1000	500	1000	1000
Chloramphenicol	2.0	1.0	1.0	0.5	1.0	2.0	2.0	1.0	1.0

Table 5: Antifungal activity of selected medicinal plant methanolic extracts from the state of Mato Grosso, Brazil, by agar disc diffusion method.

Extract	Diameter of inhibition zone (mm)				
	Leveduriform				
	Candida kruzei	*Candida parapsilosis*	*Candida glabrata*	*Candida albicans*	*Candida albicans*
ME*Ah*l	--	--	--	--	--
ME*Ah*s	--	--	--	--	--
--	--	--	--	--	
--	--	--	--	--	
MEC*p*	--	--	--	--	--
ME*Gb*	10	--	--	--	--
10	--	--	--	--	
10	10	10	--	--	
ME*Pm*	--	15	--	--	--
--	15	--	--	--	
MEC*r*	--	--	--	13	--
ME*Lp*	--	10	--	--	10
--	10	--	--	10	
--	--	--	--	13	
MES*r*	--	--	--	--	--
--	--	--	--	--	
--	--	--	--	--	
ME*Hh*	--	--	--	--	--
--	--	--	--	--	
--	--	--	--	--	
MEC*b*l	10	15	--	--	--
10	15	--	--	--	
15	14	--	10	--	
MEC*b*r	15	10	--	10	10
--	--	--	--	--	
--	--	--	--	--	
Amphotericin B	11	20	18	18	25

-- no inhibition of fungal growth observed ; Antifungal activity: inhibition zone ≥ 10 mm;[6]

Table 6: Antifungal activity in broth microdilution assay of selected medicinal plants from the state of Mato Grosso, Brazil.

	Leveduriform (MIC, µg/mL)				
Extract	***Candida albicans* ATCC 10231**	***Candida albicans* ATCC 64550**	***Candida glabrata* ATCC 90030**	***Candida kruzei* ATCC 6258**	***Candida parapsilosis* ATCC 40058**
ME*Ah*s	500	250	500	1000	1000
ME*Ah*l	500	500	500	500	500
ME*Cp*	500	500	500	1000	500
ME*Gb*	500	500	500	250	250
ME*Pm*	500	500	500	500	250
ME*Cr*	250	1000	1000	1000	1000
ME*Lp*	250	500	1000	250	250
ME*Sr*	500	500	125	500	1000
ME*Hh*	125	1000	500	500	250
MEC*b*l	500	500	1000	500	125
MEC*br*	250	500	500	1000	250
Amphothericin B	1.0	1.0	0.5	1.0	0.25

MIC = Minimum Inhibitory Concentration. Good activity: ≤ 100 µg/mL; Moderate activity: > 100 < 500, 100-500 µg/mL; Weak activity: > 500 < 1000 µg/mL; Inactive: ≥ 1000 µg/mL

Table 7: Acute effects of oral administration of methanolic extracts of *Conyza bonariensis* leaves and root on general behavior activities in mice.

Plant extracts	Dose (mg/kg p.o.)	Behavioral changes	Death
MEC*bl*	500	None	0/3
	1000	None	0/3
	2000	None	0/3
	5000	None	0/3
MEC*br*	500	None	0/3
	1000	None	0/3
	2000	None	0/3
	5000	None	0/3

MEC*bl* and MEC*br*: methanolic extract of *Conyza bonariensis* leaves and root, respectively.

Table 8: *In vivo* antibacterial activity methanolic extracts of *Conyza bonariensis* leaves and root in the systemic infection models in mice by *Staphylococcus aureus* and *Escherichia coli*.

Plant extracts	Doses (mg/kg, p.o.)	Survival (%)	
		Bacterial species	
		Sa	*Ec*
MEC*bl*	10	100	50
	50	100	50
	100	100	60
	500	100	30
MEC*br*	10	85	10
	50	85	00
	100	43	00
	500	29	00
Meropenem	20	100	100

Sa: *Staphylococcus aureus*; Ec: *Escherichia coli;* MEC*bl* and MEC*br*: methanolic extract of *Conyza bonariensis* leaves and root, respectively.

ative and hepatoprotective, in addition to several other ethno medicinal uses.[45-51] In fact, promising results were obtained with the methanolic extract of *C. bonariensis* from Pakistan, as it demonstrated to be active in DMBA-induced skin carcinogenesis *in vivo* studies.[52]

Reasonable comparisons with previous studies could not be made for many reasons. We have summarized these studies in Table 9, with short comments added for clarifications. These include among others, the use of different antimicrobial assay methods from those we employed in this work and/or sometimes the experimental conditions were poorly described. For example, Avancini and Wiest[53] (only reported that 1g of the extract was macerated in 10 mL of hydroethanolic solution of *C. bonariensis* without stating the concentration of ethanol used, nor the yield of the extract so as to ascertain the active concentration. Sometimes different parts of the plants are used and or its essential oils[54] or different solvents in most cases. Moreover, in some occasions, the concentrations used are ten or more folds higher than the maximum concentration we employed (Table 9).

We encountered similar impediments, as in the case of the *in vitro* antibacterial studies of *C. bonariensis*, while trying to compare our results with previously reported *in vitro* antifungal studies. Most reports with previous studies. See Table 9 for more details on these issues..[55] *In vivo* acute toxicity is usually performed on drug candidate for the purposes of: classification and labeling, to provide basic information on the mode of toxic action of a substance if any, to help in the choice of dose of a new compound, as well as to help in dose determination in animal studies.[56] We therefore conducted the Hippocratic screening, to evaluate the potential toxic properties of the extracts. The acute toxicity test of the extracts administered orally demonstrated the high safety margin of MEC*br* and MEC*b*l, suggesting lack of toxicity at the level of dose to be used in the *in vivo* studies. The no adverse effect level (NOAEL) in the oral acute toxicity study of MEC*bl* and MEC*br* was calculated to be above 5000 mg/kg b.w. The human equivalent dose (HED) of 5000 mg/kg in the rats using body surface area was 405.4 mg/kg b.w.[57] Although, there are no reports of the toxicity studies of *Conyza bonariensis* in the literature, toxicity of some other species of *Conyza* have been studied. Biological and pharmacological studies have been carried out to confirm these ethnomedicinal claims.[45-51]

Table 9: Summary of literature search and comparisons of antibacterial studies of plants screened in the present study.

Reference	Part /type of extract tested	Method used	Activity tested		Concentrations tested	Study Conclusion	Observations
			Antibacterial: sensitive species	antifungal			
			Conyza bonariensis				
Shah *et al.* [23]	crude methanolic extract and its subsequent solvent fractions.	Disk diffusion assay and Agar tube dilution Method for antibacterial and antifungal assay, respectively	Sensitive bacterial species: *Escherichia coli, Pseudomonas aureginosa, Klebsiella pneumoniae*	Sensitive fungal species: *Cladosporium cucumerinum*, and *Candida albicans*	6000 - 18000 µg/mL and 24,000 µg/mL – for the antibacterial and antifungal study, respectively	**+WA** – weak/moderate activity	Criteria for activity not stated, concentration of DMSO used in dissolving the extracts was not stated. The identity or source of species used were not stated
Thabit *et al.* [24]	whole plants/ethanol ultrasonic extraction	agar well diffusion	*Shigella dysenteriae* CMCC 51302, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* CMCC 50013, *Streptococcus pyogenes* ATCC 12344,*Staphylococcus aureus* ATCC 25923		5000, 10,000, and 20,000 µg/mL	Activity at (20000 µg/mL)	Minimum concentration (5000 µg/mL) used in the study was 5 times higher than the maximum concentration (1000 µg/mL) we used in our study.
Zalabani *et al.* [25]	Leaves/ethanol	Agar dilution	*Bacillus subtilis,Escherichia coli, Mycobacterium phleiListeria innocua* "LMG 2710"*Enterococcus faecalis, Staphylococcus aureus* "Non-pathogenic LMG 3242",*Staphylococcus aureus* "Pathogenic LMG 3240", *Staphylococcus aureus* " Lab. Strain"		200 – 800 µg/mL	Weak activity	
Yaseen *et al.* [26]	Aerial parts /ethanol and chloroform	Disk diffusion	*Staphylococcus aureus, Escherichia coli and Sarcina lutea*		10,000 – 20,000 µg/mL	760 – 3000 µg/mL activity	Minimum concentration (10,000 µg/mL) used in the study was 10 times higher than the maximum concentration (1000 µg/mL) we used in our study. Activity not classified
Araujo *et al.* [27]	Essential oil of fresh leaves	Broth micro dilution			MIC 25-200 µg/mL		Essential oil used
			Anacardium humile A. St. -Hil./Anacardiaceae				
Pereira *et al.* [28]	Leaves/ ethanol, n-hexane, n-butanol	Agar and microdilution	*S. mutans* (ATCC 70069), *Staphylococcus aureus* (ATCC 12692), and *Actinobacillusactinomy cetemcomitans* (ATCC 33384)	*Candida* albicans (ATCC 18804),	0.512 mL - 0.008 mL	weak/moderate activity	

Reference	Part /type of extract tested	Method used	Activity tested		Concentrations tested	Study Conclusion	Observations
			Antibacterial: sensitive species	antifungal			
			Cariniana rubra Gardner ex Miers/Lecitidaceae				
Silva Junior *et al.* [29]	Stem bark/ hexane, dichloromethane, ethyl acetate and ethanol 75%	micro-broth dilution		*Aspergillus fumigatus*	100-1000 µg/mL	No antibacterial activity reported, but weak activity against *Aspergillus fumigatus*	Moderate activity against *Pseudomonas aeruginosa,* (weak activity against all other strains)
Larissa da Silva *et al.* [30]	stem bark / hydroethanol 70%	micro-broth dilution	*Helicobacter pylori* (100),*Enterococcus faecalis* (400),*Staphylococcus aureus* (200),*Streptococcus pyogenes* (400) µg/ml	*Aspergillus fumigatus* 200 µg/ml	6.25 – 800 µg/ml	weak activity against susceptible strains (100 ≤ MIC ≤ 500)	
			Cecropia pachystachyaa Trécul/Cecropiaceae				
Souza *et al.* [31]	leaves and stems / ethanol and methanol fraction	micro-broth dilution	*Staphylococus aureus* ATCC 25923, *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442) and *Klebsiella pneumoniae* (ATCC 4362)	-	312.5 - 39.06 µg/mL and 312.50 - 78.13 µg/mL	weak/moderate activity against *Staphylococcus aureus* 358, *Escherichia coli* 27	
			Gossypium barbadense L./Malvaceae				
Ikobi *et al.* [32]	Leaves / methanol	Agar well diffusion	*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, and Shigella sonnei*	-	10000 µg/mL - 30,000 µg/mL	dose dependent activity against all the test organisms except *Escherichia coli*	The minimum concentration (10 mg/mL is 10 folds higher than the maximum concentration used by us.
Essien *et al.* [33]	essential oil from leaf	Agar well diffusion	*Staphylococcus aureus (NCTC 6571), Gardinella spp (UCHSTC 2182) , Klebsiella aerogenes (UCHSTC 2134) - Neisseria gonorrhoea (UCH 2303) - Neisseria gonorrhoea (ATCC 19424) - Escherichia coli (UCH 2306) , Escherichia coli (NCTC 9001) , Pseudomonas aeruginosa (UCH 2145) -*	*Candida albicans (UCHSTC 2112)*		Moderate activity against *S. aureus, Gardinella spp, Escherichia coli and Candida albicans*	

Reference	Part /type of extract tested	Method used	Activity tested		Concentrations tested	Study Conclusion	Observations
			Antibacterial: sensitive species	antifungal			
Luciano-Montalvo *et al.* [34]	Fruit/decoction	Disc diffusion	*Staphylococcus saprophyticus (ATCC 15305) and Staphylococcus aureus (ATCC 6341); the Gram-negative bacteria Escherichia coli (ATCC 4157), Haemophilus influenzae (ATCC 8142), Pseudomonas aeruginosa (ATCC 7700), and Proteus vulgaris (ATCC 6896)*	*Candida albicans (ATCC 752).*	110.5 - 27.9 µg/mL	25% growth inhibition against *Staphylococcus aureus* and *S. saprophyticus saprophyticus*	No classification criterion for the antibacterial activity. Reported percentage inhibition relative to the positive control (solvent)
Lafoensia pacari A. St.-Hil./Lythraceae							
Silva Junior *et al.* [29]	Stem bark/ hexane, dichloromethane, ethyl acetate and ethanol 75%	Micro-dilution		*Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019, *Cryptococcus neoformans* ATCC 32264	100-1000 µg/mL	**+WA** – weak/moderate activity	
Pereira *et al.* [28]	leaves, roots, stem/ ethanol, n-hexane, n-butanol	Agar and microdilution	*Streptococcus mutans* (ATCC 70069), *Staphylococcus aureus* (ATCC 12692), and *Actinobacillusactinomy cetemcomitans* (ATCC 33384)	*Candida* albicans (ATCC 18804),	0.512 mL - 0.008 mL	**+WA** – weak/moderate activity	
Plantago major L./Plantaginaceae							
Stanisavljević *et al.* [35]	Leaves/ethanol (70%)	well-diffusion method	*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231)	*Saccharomyces cerevisiae* (ATCC 9763) and *Aspergillus niger* (ATCC 16404)	20 mg/ml	**+WA** – weak/moderate activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae* and *Candida albicans*	
Stryphnodendron rotundifolium Mart./Fabaceae							
Oliveira *et al.* [36]	Stem bark/ hydroethanolic	micro-broth dilution method	*Escherichia coli* (EC27) and *Staphylococcus aureus* (SA358)		512 µg/mL	**+WA** – weak/moderate activity	

With these promising results of both the *in vitro* antimicrobial activities and the lack of *in vivo* acute toxicity, we proceeded to evaluate the *in vivo* antibacterial effects of the plant using *S. aureus* and *E. coli* systemic infection models.

However robust might be the results of *in vitro* studies, *in vivo* testing is without doubt one of the recognized, if not the most important, essential links between *in vitro* sensitivity testing and clinical studies in humans. Based on this fact, several regulatory agencies in many countries have made it as explicit requirements of experimental evaluation of new compounds in animals, destined for human, as part of guidelines for the clinical evaluation of efficacy and toxicity of anti-infective drugs, being prerequisites to clinical trials.[58]

Staphylococcus aureus is an important human pathogen responsible for many infectious diseases, sometimes life-threatening, including skin and soft tissue infections (SSTIs), foreign-body infections, bloodstream infections, just to mention but few, in both hospital and community settings. On the other hand, *E.coli*, is a known pathogen and one of the most frequent and lethal causes of bloodstream infections.[59] We therefore selected these two bacterial strains for the *in vivo* antibacterial activity, based on their clinical relevance.

Our results from *in vivo* murine systemic infection model revealed that treatments with MEC*b*l and MEC*br* demonstrated potential antibacterial activities, particularly, their prophylactic activity in the systemic infections caused by Gram-positive and Gram-negative microorganisms (*S. aureus* and *E. coli*).

The effect of MEC*b*l was similar to that of the standard antibiotic, meropenem, in the case of *S. aureus* systemic infection, but milder in the case of *E. coli*. Thus, demonstrating the potential of this plant as an anti-bacterial agent. Considering the maximum dose (500 mg/kg) used in these studies, the HED is estimated at 40.5 mg/kg. Simple comparison of this value with HED of the NOAEL shows that it is 10 folds less, further testifying to its high safety margin. In general, the *in vivo* antibacterial effect of MEC*b*l and MEC*b*r seem to be more effective on the Gram-positive bacterium (*S. aureus*) than the Gram-negative (*E. coli*) bacterium. The Gram-negative bacteria are implicated in the pathogenesis of severe sepsis and septic shock, although the exact mechanism is uncertain. A number of studies have been conducted to decipher the pathophysiological differences in bacteraemia with different causative bacterial species. In the study of patients admitted to the general intensive care unit (ICU) of a university teaching hospital by Abe *et al.*[60] the authors observed that the incidence of Gram-negative bacteraemia was significantly higher in bacteraemia ICU patients with septic shock than in those with sepsis or severe sepsis. They concluded that the Gram-negative bacteraemia induces greater magnitude of inflammatory response than Gram-positive bacteraemia. In fact, these authors showed that the C-reactive protein and IL-6 levels were significantly higher in Gram-negative bacteraemia than in Gram-positive bacteraemia. These may actually explain the difference in the response to the extracts by these two bacterial strains, representing the Gram-negative and Gram-positive strains.[61]

We also observed that maximal positive response occurred at up to certain dose level, beyond which it declines (Table 8). The exact mechanism responsible for this effect is not known, but is sometimes seen in the effects of plants extracts and phytochemical compounds.[62] However, the disc diffusion method is restrict to evaluate antimicrobial activities of plant extracts because the activity of the substances present in the extracts depends on the solubility of metabolites in the medium to act in the micro-organism. In the case of ME*Sr* the preliminary phytochemical analysis indicated the presence of various classes of secondary metabolites (free steroids, coumarins, reduced anthraquinones, saponins, tannins and flavonoids) many of non-polar categories. So it is probable that these substances have difficulty to diffuse across the agar, but if these are in direct contact with the bacterium like in the broth micro dilution method the solubility of the substances is not an impairment factor.[63] Another possibility is that *Enterobacter aerogenes* is a Gram-negative bacterium and consequently is more resistant to antibiotic, because it has outer membrane that is not present in Gram-positive bacterium like *Streptococcus pyogenes.* The presence of saponins in the ME*Sr* may facilitate the penetration of the compounds across the outer membrane of bacterium. On the other hand, MEC*b1* presented in preliminary photochemical analysis only two classes of secondary metabolites (free steroids and coumarins). It is possible that the substances presents in MEC*bl* have sufficient capacity to diffuse on the agar and exert their action against-*Streptococcus pyogenes,* a Gram-positive bacterium. It is noteworthy that we are talking about two different species of bacteria, one Gram-positive that is usually more sensitive to antibiotics and another Gram-positive in general, more resistant to antibiotics.[64] Moreover, various hypotheses have been postulated to explain this phenomenon. These include the fact that many phytochemical compounds are pleitropic molecules that may act by binding to certain receptor. Desensitization of such receptor(s) may occur at higher drug dose, thereby resulting in little or no effect as compared to the lower dose. Increases in the dose may also triggered an untoward effect on other body systems, thereby provoking a negative response toward the antibacterial activity of observed. The induction or enzymatic systems (phase II), responsible for detoxification of xenobiotic. Taken together, it is probable that a higher dose predisposes the physiological system to excrete more of MEC*b*l and MEC*b*r, thus lowering their effective physiological concentration, and hence, diminished protective effects.

Preliminary phytochemical analysis of MEC*b*l revealed the presence of flavonoids, coumarins and free steroids. There are considerable in formation in the literature detailing the antibacterial activities of the identified phytochemical constituents.

The antibacterial effects of MEC*b*l and MEC*br* may therefore be due in part to the presence of the aforementioned metabolites, and possibly through a synergistic and or combined effects and may be responsible for its antibacterial activity established in this study. To the best of our knowledge, this is the first study dealing with the *in vivo* antibacterial activity of MEC*b*l and MEC*b*r.

CONCLUSION

In conclusion, systemic infection studies demonstrated that *C. bonariensis* had *in vivo* antimicrobial activity comparable to that of meropenem. This *in vivo* antimicrobial activity study confirmed that methanol extracts of *C. bonariensis* has high activity and deserves further investigation. The present results confirm previous *in vitro* studies by many researchers on different extracts of *C. bonariensis* further lending support to its use as anti-infective in traditional medicine. There is need for further studies to identify probable metabolites responsible for the *in vivo* antibacterial activity and possible mechanism of action of the extracts.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interests.

ABBREVIATIONS

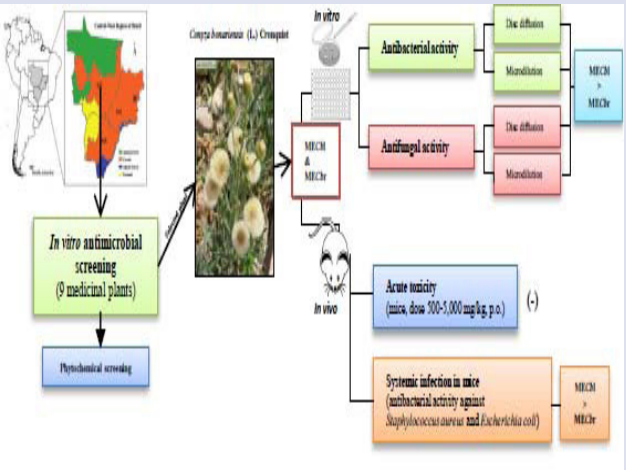
ANOVA: Analysis of variance; **ATCC:** American Type Culture Collection; **CEUA** Committee on the Use of Animal for experimentation; **LD:** Lethal Dose 50; **MEAhl and MEAhs:** Methanolic extract of *Anacardium humile* leaves and stem bark; **MECp:** Methanolic extract of *Cecropia pachystachya*; **MECbl and MECbr:** Methanolic extract of *Conyza bonariensis* leaves and roots; **MECr:** Methanolic extract of *Cariniana rubra;* **MEGb:** Methanolic extract of *Gossypium barbadense;* **MEHh:** Methanolic extract of *Handroanthus heptaphyllus;* **MELp:** Methanolic extract of *Lafoensia pacari;* **MEPm:** Methanolic extract of *Plantago major;* **MESr:** Methanolic extract of *Stryphnodendron rotundifolium;* **MIC:** Minimal inhibitory concentration; **sUFMT:** Universidade Federal de Mato Grosso.

REFERENCES

1. Nolte O. Antimicrobial resistance in the 21st century: A multifaceted challenge. Protein Pept Lett. 2014;21(4):330-5.
2. World Health Organization. The top 10 causes of death. Fact sheets. http://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. Published 2018. Accessed June 24, 2018.
3. Jim O'Neill. Review on Antimicrobial Resistance. Tackling a Global Health Crisis: Initial Steps. 2015. https://amr-review.org/sites/default/files/Report-52.15.pdf (Accessed on: 04.09.2017).
4. Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov. 2015;14(2):111-29.
5. Ribeiro RV, Bieski IG, Balogun SO, De Oliveira, Martins DT. Ethnobotanical study of medicinal plants used by Ribeirinhos in the North Araguaia microregion, Mato Grosso, Brazil. J Ethnopharmacol. 2017;1(1):1-8.
6. Silva IF, Filho VC, Zacchino SA, Lima JC, Martins DT. Antimicrobial screening of some medicinal plants from Mato Grosso Cerrado. Brazilian J Pharmacogn. 2009;19(1):242-8.
7. Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol. 2005;100(1-2):80-4.
8. Bieski IG, Leonti M, Arnason JT, Ferrier J, Rapinski M, Violante IM, *et al*. Ethnobotanical study of medicinal plants by population of Valley of Juruena Region, Legal Amazon, Mato Grosso, Brazil. J Ethnopharmacol. 2015:173;383-423. doi:10.1016/j.jep.2015.07.025.
9. Kremer D, Košir IJ, Končić MZ, Čerenak A, Potočnik T, Srečec S, *et al*. Antimicrobial and Antioxidant Properties of *Satureja montana* L. and S. Subspicata Vis. (Lamiaceae). Curr Drug Targets. 2015;16(14):1623-33.
10. Pereira EM, Gomes RT, Freire NR, Aguiar EG, Brandão MD, Santos VR. *In vitro* antimicrobial activity of Brazilian medicinal plant extracts against pathogenic microorganisms of interest to dentistry. Planta Med. 2011;77(4):401-4.
11. Gangoue-Pieboji J, Eze N. The *in-vitro* antimicrobial activity of some medicinal plants against β-lactam-resistant bacteria. J Infect Dev Ctries. 2009;3(9):671-80.
12. Shah NZ, Muhammad N, Azeem S, Khan AZ, Samie M, Khan H. Antimicrobial and phytotoxic properties of *Conyza bonariensis*. Pharm Pharmacol Res. 2013;1(1):8-11.
13. CIOMS/WHO. International ethical guidelines for biomedical research involving human subjects. Bull Med Ethics. 2002;(182):17-23.
14. Samuelsen AB. The traditional uses, chemical constituents and biological activities of Plantago major L. A review. J Ethnopharmacol. 2000;71(1-2):1-21.
15. Bukhari IA, Shah AJ, Khan RA, Meo SA, Khan A, Gilani AH. Gut modulator effects of *Conyza bonariensis* explain its traditional use in constipation and diarrhea. Eur Rev Med Pharmacol Sci. 2013;17(4):552-8.
16. Matos FJA. Introdução à Fitoquímica Experimental 3, Third. Ed. UFC, Fortaleza. 2009.
17. Kirby W, Bauer A, Sherris J, Turk M. Antibiotic susceptibility testing by standard single disk method. Am J Clin Pathol. 1966;45(4):493-6.
18. CLSI. Clinical and Laboratory Standards Institute, formely NCCLS (National Committee for Clinical Laboratory Standards). Methods for Diluition Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard-NCCLS Document M7-A6, sixth ed. Pennsylvania, USA. 2003.
19. Rex JH, Alexander BD, Andes D, Arthington-Skaggs B, Brown SD, Chaturvedi V, *et al*. Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard - third edition. Clin Lab Stand Inst. 2008:1-25.
20. Holetz FB, Pessini GL, Sanches NR, Cortez DA, Nakamura CV, Dias Filho BP. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz. 2002;97(7):1027-31.
21. Malone MH, Robichaud RC. A Hippocratic screen for pure or crude drug materials. Lloyd. 1962;25(4):320-32.
22. Meyer B, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine Shrimp?: A Convinient General Bioassay for Active Plant Consyituents. Kournal Med Plant Res. 1982;45(5):31-4.
23. Shah NZ, Muhammad N, Azeem S, Khan AZ, Samie M, Khan H. Antimicrobial and phytotoxic properties of *Conyza bonariensis*. Pharm Pharmacol Res. 2013;1(1):8-11.
24. Thabit RAS, Cheng XR, Tang X, Sun J, Shi YH, Le GW. Antioxidant and antibacterial activities of extracts from *Conyza bonariensis* growing in Yemen. Pak J Pharm Sci. 2015;28(1):129-35.
25. Zalabani SM El, Hetta MH, Ismail AS. Genetic profiling, chemical characterization and biological evaluation of two conyza species growing in Egypt. J Appl Pharm Sci. 2012;2(11):54-61.
26. Yaseen M, Irshad N, Qayyum MI, Kamal Y. Evaluation of antibacterial and cholinomimetic activities of different extracts of *Conyza bonariensis*. Indian J Adv Plant Res. 2014;1(4):37-40.
27. Araujo L, Moujir LM, Rojas J, Rojas L, Carmona J, Rondon M. Chemical composition and biological activity of *Conyza bonariensis* essential oil collected in Mérida, Venezuela. Natural Product Communications. 2013:8(8):1175-8.
28. Pereira EM, Gomes RT, Freire NR, Aguiar EG, Brandão MDGL, Santos VR. *In vitro* antimicrobial activity of Brazilian medicinal plant extracts against pathogenic microorganisms of interest to dentistry. Planta Med. 2011;77(4):401-4.
29. Silva Junior IE, Cechinel Filho V, Zacchino SA, Lima JC, Martins DT. Antimicrobial screening of some medicinal plants from Mato Grosso Cerrado. Revista Brasileira de Farmacognosia. 2009:19(1B):242-8.
30. Larissa SI, Karuppusamy A, Fabio M, Ivana MPV, Isanete GCB, Sikiru OB, *et al*. Antimicrobial and antioxidant activities of selected plants used by populations from Juruena Valley, Legal Amazon, Brazil. Int J Pharm Pharm Sci. 2017;9(5).
31. Souza, Daniele O, Saulo RT, Fernando GF, Maria CMB, Maria FBMB, *et al*. "Antibacterial and modulatory activity of Cecropia pachystachya Trécul on the action of aminoglycosides."Revista Cubana de Plantas Medicinales.2014;19(3):121-32.
32. Ikobi EU, Igwilo CI, Awodele O, Azubuike PC. Antibacterial and wound healing properties of methanolic extract of dried fresh *Gossypium barbadense* leaves. Asian Journal of Biomedical and Pharmaceutical Sciences.2012;2(13):32.
33. Essien EE, Aboaba SO, Ogunw IA. Constituents and antimicrobial properties of the leaf essential oil of *Gossypium barbadense* (Linn.).Journal of Medicinal Plants Research.2011;5(5):702-5.
34. Luciano-Montalvo C, Boulogne I, Gavillán-Suárez J. A screening for antimicrobial activities of Caribbean herbal remedies. BMC Complementary and Alternative Medicine.2013;13(1):126.
35. Stanisavljević IT, Stojičević SS, Veličković DT, Lazić ML, Veljković VB. Screening the antioxidant and antimicrobial properties of the extracts from plantain (*Plantago major* L.) leaves.Separation Science and Technology.2008;43(14):3652-62.
36. Oliveira DR, Brito-Junior FE, Bento EB, Matias EF, Sousa ACA, Costa JG, et al. Antibacterial and modulatory effect of *Stryphnodendron rotundifolium*. Pharmaceutical Biology. 2011;49(12):1265-70.
37. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. 2007. doi:1-56238-525-5.
38. Barry A, García F, Thrupp L. An improved single-disk method for testing the antibiotic susceptibility of rapidly growing pathogens. Am J Clin Pathol. 1970;113(1):149-58.
39. Martínez RMG, Richard-Greenblatt M, Juárez ZN, Av-Gay Y, Bach H, Hernández LR. Antimicrobial, Anti-Inflammatory, Antiparasitic, and Cytotoxic Activities of *Laennecia confusa*. Sci World J. 2012;1-8.
40. Bekalo TH, Woodmatas SD, Woldemariam ZA. An ethnobotanical study of medicinal plants used by local people in the lowlands of Konta Special Woreda, southern nations, nationalities and peoples regional state, Ethiopia. J Ethnobiol Ethnomed. 2009;5(1):26. doi:10.1186/1746-4269-5-26
41. Bieski IGC, Leonti M, Arnason JT, *et al*. Ethnobotanical study of medicinal plants by population of Valley of Juruena Region, Legal Amazon, Mato Grosso, Brazil. J Ethnopharmacol. 2015;173:383-423. doi:10.1016/j.jep.2015.07.025
42. Almeida CFCBR, Ramos MA, Silva RRV, *et al*. Intracultural variation in the knowledge of medicinal plants in an urban-rural community in the atlantic forest from northeastern Brazil. Evid Based Complement Alternat Med. 2012;679373. doi:10.1155/2012/679373
43. Liporacci HSN. Plantas medicinais e alimentícias na Mata Atlântica e Caatinga: uma revisão bibliográfica de cunho etnobotânico. 2015.
44. Duke JA. Duke's Handbook of Medicinal Plants of Latin America. (Bogenschutz-Godwin MJ, Ottesen AR, eds.). New York, NY: CRC Press, Taylor and Francis Group. 2009. doi:10.1201/9780849382031
45. Agra MF, Silva KN, Basílio IJLD, Freitas PF, Barbosa-Filho JM. Survey of medicinal plants used in the region Northeast of Brazil. Rev Bras Farmacogn. 2008;18(3):472-508. doi:10.1590/S0102-695X2008000300023
46. Zalabani SM El, Hetta MH, Ismail AS. Genetic profiling, chemical characterization and biological evaluation of two conyza species growing in Egypt. J Appl Pharm Sci. 2012;2(11):54-61. doi:10.7324/JAPS.2012.21110
47. Yaseen M, Irshad N, Qayyum MI, Kamal Y. Evaluation of antibacterial and cho-

linomimetic activities of different extracts of *Conyza bonariensis*. Indian J Adv Plant Res. 2014;1(4):37-40.
48. Calzada F, Cedillo-Rivera R, Mata R. Antiprotozoal activity of the constituents of *Conyza filaginoides*. J Nat Prod. 2001;64(5):671-3.
49. Chaudhry BA, Janbaz KH, Muhammad, Uzair ASE. Biological Studies of Conyza and Euphorbia species. J Res. 2001;12(1):85-88.
50. Harraz FM, Hammoda HM, Ghazouly MG, Farag MA, El-Aswad AF, Bassam SM. Chemical composition, antimicrobial and insecticidal activities of the essential oils of Conyza linifolia and Chenopodium ambrosioides. In: Natural Product Research. 2015:29(9);879-82. doi:10.1080/14786419.2014.988714
51. Calzada F, Cedillo-Rivera R, Mata R. Antiprotozoal activity of the constituents of Conyza filaginoides. J Nat Prod. 2001;64(5):671-3.
52. Saleem M, Naseer F, Hussain K. Cytotoxic effect of methanol extract of on DMBA-induced skin carcinogenesis: An *in vivo* study. Bangladesh Journal of Pharmacology. 2015;10(2):467-74.
53. Avancini CAM, Wiest JM. Etnomedicina veterinária, etnonosotaxia e etnoterapêutica de doenças de pele como referência para seleção e avaliação preliminar da atividade antibacteriana de plantas nativas no sul do Brasil. Rev Bras Plantas Med. 2008;10(1):21-8.
54. Araujo L, Moujir LM, Rojas J, Rojas L, Carmona J, Rondón M. Chemical composition and biological activity of *Conyza bonariensis* essential oil collected in Mérida, Venezuela. Nat Prod Commun. 2013;8(8):1175-8.
55. Silva, Fernandes. Biological properties of medicinal plants?: A review of their antimicrobial activity. 2010;16(3):402-13.
56. Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM. Toxicological Studies of Hydromethanolic Leaves Extract of *Grewia crenata*. Int J Pharm Sci Drug Res. 2012;4(4):245-9.
57. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. The FASEB journal. 2008;22(3):659-61.
58. Arunachalam K, Ascêncio SD, Soares IM, Souza ARW, Da Silva LI, De Oliveira RG, *et al*. Gallesia integrifolia (Spreng.) Harms: *In vitro* and *in vivo* antibacterial activities and mode of action. J Ethnopharmacol. 2016;184:128-37.
59. Wang S, Zhao SY, Xiao SZ, Gu FF, Liu QZ, Tang J, *et al*. Antimicrobial Resistance and Molecular Epidemiology of Escherichia coli Causing Bloodstream Infections in Three Hospitals in Shanghai, China. PLoS One. 2016;11(1):e0147740.
60. Abe R, Oda S, Sadahiro T, Nakamura M, Hirayama Y, Tateishi Y, *et al*. Gram-negative bacteremia induces greater magnitude of inflammatory response than Gram-positive bacteremia. Crit Care. 2010;14(2):R27.
61. Alexandraki I, Palacio C. Gram-negative versus Gram-positive bacteremia: what is more alarmin(g)?. Crit Care. 2010;14(3):161.
62. Beserra AM, Calegari PI, Souza MD, Dos Santos RA, Lima JC, Silva RM, *et al*. Gastroprotective and ulcer-healing mechanisms of ellagic acid in experimental rats. J Agric Food Chem. 2011;59(13):6957-65.
63. De Bona E, da Silva Pinto FG, Fruet TK, Jorge TC, de Moura AC. Comparação de métodos para avaliação da atividade antimicrobiana e determinação da concentração inibitória mínima (cim) de extratos vegetais aquosos e etanólicos. Arquivos do Instituto Biológico. 2014:81(3):218-25.
64. Vasconcelos JAA, Menezes EA, Cunha FA, Cunha MD, Braz BH, Capelo LG, Silva CL, *et al*. Comparação entre microdiluição e disco difusão para o teste de susceptibilidade aos antifúngicos contra Candida spp. Semina Cienc. Biol. Saude. 2012;33(1):135-42.

GRAPHICAL ABSTRACT



SUMMARY

- This study was aimed to advancing on the *in vivo* antibacterial activity of a medicinal plant popularly employed in the Brazilian ethnomedicine, after careful screening using *in vitro* antibacterial and antifungal studies. *Conyza bonariensis* (Asteraceae) was selected and evaluated using *in vitro* and *in vivo* experimental models, in addition to evaluating its *in vivo* safety. Methods: Eleven methanolic extracts obtained from nine plants, reportedly used in the treatments of infections from the state of Mato Grosso, were initially screened for their *in vitro* antibacterial and antifungal activities employing disc diffusion and broth microdilution assays. Results: All of the extracts tested were active against at least one of the bacterial and fungal strain tested with activities ranging from moderate to weak. Phytochemical analyses of ME*Cb*l and ME*Cbr* demonstrated the presence of free steroids and coumarins in ME*Cb*l and flavonoids, tanins, free steroids, reduced anthraquinones and coumarins in MECBr. Oral administration of ME*Cb*l and ME*Cb*r up to 5000 mg/kg did not provoked any toxicological events in the mice, thus suggesting that the LD_{50} is higher than 5000 mg/kg. The *in vivo* antibacterial assay demonstrated superior prophylactic activity of ME*Cb*l compared to ME*Cb*r. Conclusion: The current *in vivo* antimicrobial activity study further lend supports to the use of *C. bonariensis* in the treatment of infections in several popular medicine practices from many countries. *C. bonariensis* may represent a potential antibacterial agent based on its potent *in vivo* antibacterial activity.

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