

Full Length Research Paper

**Antiviral, antimicrobial and anti-inflammatory activities
of *Ureia baccifera* (L.) Gaudich**

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Ureia baccifera (Urticaceae) is a shrub used in folk medicine for rheumatic diseases and mycosis. This study aimed to test the antiviral, antinociceptive, anti-inflammatory and antimicrobial activities of *U. baccifera* leaves and roots. The cytotoxic and antiviral activity was evaluated against Herpes virus type 1, *in vitro*. The antimicrobial activity was determined by the broth microdilution method based on Clinical and Laboratory Standards Institute (CLSI) M27-A3, M38-A2 and M7-A6 standardized reference method. *In vivo* anti-inflammatory and antinociceptive activity were evaluated in ear edema measurement, complete Freund's adjuvant-induced nociception, measurement of cold allodynia and spontaneous nociception in male adult Swiss mice. *U. baccifera* roots and leaves presented anti-herpetic activity. The plant extract, predominantly the ethyl acetate and butanol fractions, also inhibit *Klebsiella pneumoniae*, *Prototheca zopfii* and *Saccharomyces cerevisiae* and did not show antinociceptive and anti-inflammatory effect on the tested experiments. The presence of biologically active products and the low cytotoxicity demonstrated by the extract and fractions of *U. baccifera* makes these extracts promising antiviral candidates. This nettle can also be considered a moderated antimicrobial agent against *K. pneumoniae*, *P. zopfii* and *S. cerevisiae*. However, antinociceptive and anti-inflammatory effects to the samples in this study were not observed.

Key words: Stinging nettle, herpes simplex type 1, Urticaceae, *Klebsiella pneumonia*, *Prototheca zopfii*, *Saccharomyces cerevisiae*.

INTRODUCTION

Ureia baccifera (L.) Gaudich Ex. Wedd is a shrub with one meter and a half of height, having many stinging trichomes on the stem and leaves. The stinging hairs of

the plant gave it its folk names as nettles in English and ortigas in Spanish. This shrub is popularly used for inflammatory diseases, where leaves or roots are prepared

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by infusion to oral or topic use (Badilla et al., 1999a), and the rubefacient effect is also used for rheumatic pains and arthritis (Badilla et al., 1999a; Valadeau et al., 2009). By topic use, this plant is also used for dermal diseases. There are no references concerning the compounds of *U. baccifera* in the literature.

Herpes simplex type 1 is a pathogen of children and adults responsible for several disorders, including gingivostomatitis, pharyngitis, keratoconjunctivitis and encephalitis (Whitley, 2001). Despite the symptoms caused by herpes infections are self-limiting in healthy individuals, these can be extensive and prolonged in immunocompromised patients (Chen et al., 2000). Nucleoside analogues such as acyclovir and penciclovir are some drugs licensed for the treatment of Herpes simplex virus (Whitley, 2001). However, these agents may cause a variety of toxic side effects, and the emergence of viral strains resistant to these compounds have become a growing problem, especially in immunocompromised patients (Van De Perre et al., 2008). Thus, there is an increasing need for the discovery of more specific antiviral agents effective against the herpes simplex virus.

Natural products like plant extracts are very promising sources of compounds with antiviral activity, due to the very low toxicity that they show for cells, and also due to the great variety of chemical constituents they have (Jassin and Najj, 2003). Fractions obtained from aerial parts of *U. baccifera* showed a significant activity against HSV-1-ACVr (Martins et al., 2009). Moreover, studies of other species of Urticaceae also have demonstrated anti-HSV activity (Silva et al., 2010; Uncini et al., 2005).

The anti-inflammatory (Badilla et al., 1999a, b) tests already been performed with the aerial parts of *U. baccifera* obtained good results in the dosage of 25 to 100 and 500 mg/kg intraperitoneally administrated. Besides, *U. baccifera* aqueous extract in 250 and 500 mg/kg was capable of reducing the edema caused in mice by a snake venom intraperitoneally (Badilla et al., 2006). The roots and leaves of this nettle showed antimicrobial effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Onofre and Herkert, 2012), although the metanolic extract of leaves did not show effect against *E. coli* and *S. aureus* in other studies (Melendez and Capriles, 2006). Therefore, the aim of this study was a screening of cytotoxicity and antiviral activity against HSV-1, *in vitro*, besides that to test the antiviral, anti-inflammatory and antimicrobial activities of crude extract and chloroformic, ethyl acetate and butanol fractions from the roots and leaves of *U. baccifera*.

METHODS AND MATERIALS

Preparation of the sample extracts and fractions

The leaves and the roots of *U. baccifera* were collected in São

Francisco de Assis, RS, Brazil (S 29° 37.115' W 054° 53. 970'; precision: 2.5 m; altitude 150 m) in May, 2010. Prof. Dr. Renato Aquino Záchia identified the plant and a voucher deposited on the University Herbarium under number 13.070. The material (1,000 g of roots and 400 g of leaves) was dried, milled and taken to maceration with ethanol 70% (30 g of plant in 100 ml solvent), for 28 days, with daily agitation. Once every week, each extract were filtrated, giving the respective hydro-ethanolic extract and fresh extraction solvents were added. The extracts were pooled and the ethanol was eliminated on rotary evaporator, giving the aqueous extracts (roots and leaves separately). Part of the aqueous extract was totally dried, becoming the crude extract (CE), and part was fractionated with solvents of crescent polarity: chloroform (CHCl₃), ethyl acetate (EtOAc) and butanol (BuOH). In the fractionation process, to each 400 ml of aqueous extract, 200 ml of solvent were added, which was renovated many times until the exhaustion of the extract, when the next solvent is added in crescent order of polarity. The fractions were equally dried. The income of the leaves and roots were, respectively: CE: 31.5 and 22.3%; CHCl₃: 2.2 and 0.54%; EtOAc: 1.1 and 0.3%; BuOH: 2.1 and 1.8%.

Animals

Male adult Swiss mice (25 to 35 g), 56 animals, maintained in home cages under a 12:12 h light-dark cycle (lights on 06:00 h) and constant room temperature (22 ± 2°C) were used. The animals were acclimatized in the laboratory for at least 1 h before testing. The experiments were performed with the agreement of Ethics Committee of the Federal University of Santa Maria (Protocol number 67/2010) and were carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experiments in conscious animals (Zimmermann, 1983). The number of animals and intensity of noxious stimuli were the minimum necessary to demonstrate consistent effects of drug treatments.

Cells and viruses

HEp-2 cells were maintained in minimum essential medium (MEM – GIBCO Invitrogen Corporation, Grand Island, NY, USA) containing 10% fetal bovine serum (SFB – GIBCO Invitrogen Corporation, Grand Island, NY, USA), penicillin (100 U/ml), streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml). For the cytotoxicity and antiviral activity tests, cell cultures were prepared in 96 well plates and incubated at 37°C and 5% CO₂. The strain KOS of HSV-1 was kindly provided by Dr. Paulo Roehe from the Federal University of Rio Grande do Sul (UFRGS). The virus stocks were prepared and titrated as previously described (Simões et al., 1999), and the aliquots were kept at -70°C.

Cytotoxicity assay

The cytotoxic and antiviral tests were performed through the MTT colorimetric assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] according to Freitas et al. (2009), with minor modifications. HEp-2 cells were seeded into 96-well plates (2 × 10⁴ cells/well) and incubated for 24 h at 37°C. After that, the minimum essential medium with 10% of fetal bovine serum and 200 µl/well of different concentrations of the crude extracts and fractions (1.95 to 250 µg/ml) was added onto subconfluent cells in six replicates for each concentration of the samples. Control wells without the extracts, containing only the culture medium, and the culture medium plus 1% ethanol were included. After 3 days of incubation

at 37°C and 5% CO₂, the growth medium was removed and 50 µl of MTT solution (1 mg/ml) was added. The plates were re-incubated for 4 h and after removal of the MTT solution, dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals (100 µl/well). The supernatant was transferred to a new plate and the readings performed in the enzyme linked immunosorbent assay (ELISA) reader at a wavelength of 540 nm. The viable cells percentage for each compound was calculated according to the formula: absorbance of the compound/absorbance of the cell control × 100%. The cytotoxic concentration of the sample that reduced the viable cell number by 50% (CC₅₀) was determined from dose-response curves. Additionally the maximum noncytotoxic concentration (MNCC) of each compound was determined for subsequent use in antiviral tests (Freitas et al., 2009).

Antiviral evaluation

Briefly, 100 µl/well of MNCC of the compounds were added to preformed monolayers of HEp-2 cells and diluted 1:2 in 96 well plates. After that, a suspension of 100 µl/well containing 10⁴ TCID₅₀/ml of virus was added to all the wells with the exception of the cell control. Control virus wells were kept without addition of the compound. After 72 h of incubation at 37°C and 5%, MTT was added following the procedure described. Acyclovir (10 µg/ml) was used as positive control for HSV-1 inhibition. The inhibition for each compound was calculated according to the formula: (compound absorbance – viral control absorbance) / (cell control absorbance – viral control absorbance) × 100%. The 50% inhibitory concentration (IC₅₀) was defined as the concentration that inhibited 50% of viral replication when compared to virus controls and was determined from dose-response curves. The selectivity index (SI) was calculated as CC₅₀/IC₅₀ (Freitas et al., 2009).

Antimicrobial activity

The crude extract and the chloroform, ethyl acetate and butanol fractions were individually evaluated against *Aspergillus flavus* (clinical isolate), *Candida parapsilosis* (American Type Culture Collection - ATCC 90018), *Candida tropicalis* (ATCC 750), *Candida glabrata* (ATCC 2301), *Candida dubliniensis* (clinical isolate), *Candida albicans* (ATCC 28967), *Saccharomyces cerevisiae* (ATCC 2601), *Cryptococcus neoformans* (ATCC 2857), *Cryptococcus gattii* (ATCC 56990), *Malassezia pachy* (clinical isolate), *Prototheca zopfii* (clinical isolate), *Micrococcus* sp. (ATCC 7468), *Proteus mirabilis* (ATCC 7002), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Aeromonas* sp. (clinical isolate), *Enterococcus faecalis* (ATCC 51299), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus agalactiae* (clinical isolate) and *Escherichia coli* (clinical isolate). The minimal inhibitory concentration (MIC) of each fraction against the tested microorganisms was determined by the broth microdilution method based on CLSI M27-A3, M38-A2 and M7-A6 standardized reference method. The experiments were performed in duplicate. Each extract and fraction was serially diluted in DMSO to generate stock solutions. After that, it was serially diluted in RPMI 1640 broth for fungi and Brain Heart Infusion (BHI) broth for bacteria, to obtain final concentrations ranging from 2000 to 15.625 µg/ml. The inoculums of bacteria were prepared from 24 h-culture in BHI agar; filamentous fungi from 7-day-culture in potato dextrose agar; yeasts from 48-h-culture in Sabouraud dextrose agar. After 24 h of incubation at 37°C for bacteria and 48 h at 30°C for fungi, MIC endpoint was considered the lowest concentration of extract and fraction that inhibited 100% growth. The results of the samples

were compared to the standard antibiotic amphotericin B.

In vivo anti-inflammatory and antinociceptive activity

Only the crude extracts of leaves and roots of *U. baccifera* were used in the *in vivo* anti-inflammatory and antinociceptive assays.

Ear edema measurement

Edema was expressed as an increase in ear thickness, which was measured before and after induction of the inflammatory response, using a digital micrometer (Starret Series 734) in animals anesthetized with isoflurane. The micrometer was applied near the tip of the ear just distal to the cartilaginous ridges. The thickness was recorded in µm. To minimize variation, a single investigator performed the measurements throughout each experiment. The root (10 to 10000 µg/ear) or leaf (1000 to 10000 µg/ear) of *U. baccifera* or vehicle (acetone) was applied to the right ear by topical administration according to the method described previously, with some modifications (Silva et al., 2011).

Nociceptive parameters

CFA- induced nociception

To produce a nociceptive response, mice were lightly anaesthetized with 2% halothane via a nose cone and received 20 µl of complete Freund's adjuvant [CFA-1 mg/ml of heat killed *Mycobacterium tuberculosis* in paraffin oil (85%) and mannide monooleate (15%)] subcutaneously on the plantar surface (intraplantar, i.pl.) of the right hind paw (Ferreira et al., 2001). To evaluate the nociceptive response, 48 h after CFA administration, the animals received oral administration of leaf and root extract of *U. baccifera* (100 mg/kg) or saline (10 ml/kg) used as vehicle.

Measurement of cold allodynia

Cold allodynia was used as a parameter of nociception, which was characterized by a nocifensive reaction of animals after evaporative cooling of topically applied acetone (Caspani and Heppenstall, 2009). For this, 20 µl of acetone was applied on the dorsal hind paw, ipsilateral to the injury, and the behavior was assigned as an arbitrary score. The nociceptive score was assigned as follows: 0 = no behavioral response, 0.5 = licking response, 1 = flinching and brushing of the paw, 2 = strong flinching, 3 = strong flinching and licking. Behavior was observed during the first 30 s after acetone application and evaluated before (basal) and several times after treatments or CFA injection.

Spontaneous nociception

Briefly, the animals that received previous injection of CFA were placed individually in chambers (transparent glass cylinders of 20 cm in diameter) for an adaptation period before treatment, and spontaneous nociception induced by only CFA was observed as described. The behavior of spontaneous nociception was observed during 30 s before (basal) and at 0.5, 1 and 2 h after treatment with leaf and root extract of *U. baccifera* (100 mg/kg) or saline solution (10 ml/kg).

Table 1. Results of cytotoxicity and antiviral activity of the roots and leaves.

Parameter	CC ₅₀ µg/ml (±SD)	IC ₅₀ µg/ml (±SD)	SI (CC ₅₀ /IC ₅₀)	
Roots	CE	>1083.32±102.34	27.39±4.52	39.55
	EtOAc	283.54±10.93	8.08±2.37	35.09
	CHCl ₃	131.36±8.43	9.23±3.58	14.23
	BuOH	>385.55±9.85	25.97±3.26	14.85
Leaves	CE	162.04±6.79	17.97±2.54	9.02
	EtOAc	176.04±8.54	25.30±4.69	6.96
	CHCl ₃	98.72±6.48	10.59±3.22	9.32
	BuOH	181.63±6.81	6.21±2.41	29.25
ACV (10 µg/ml)	>138.23	1.25±0.32	>110.58	

CE: Crude extract; CHCl₃: Chloroform fraction; EtOAc: Ethyl acetate fraction; BuOH: butanol fraction; ACV: acyclovir.

Statistical analysis

Statistical analysis was carried out by Student's t-test or one-way or two-way analysis of variance (ANOVA) followed by Student-Newman-Keuls' or Bonferroni post tests when appropriate, using GraphPad Software 4.0 (GraphPad, USA).

RESULTS

Antiviral activity

The results of the cytotoxicity and viral inhibition tests are shown in Table 1. *U. baccifera* best results was obtained with the CE and EtOAc fraction of the roots, and the BuOH fractions of the leaves. The BuOH fraction of the roots showed very low toxicity to the cells, however its IC₅₀ was high, implying a lower SI as compared to the fractions already cited. The cytotoxicity of this fraction was even lower than the cytotoxicity of the ACV. Although the remaining fractions of both the roots and the leaves showed higher cytotoxicity, they also demonstrated antiviral activity (Table 1). The cytotoxicity in this method can be evaluated by the CC₅₀ (cytotoxic effects on 50% of cultured cells) value, such that the lower it gets, the more toxicity the extract brings to the cells. Thus, the CE of roots can be considered a promising candidate of an antiviral phytochemical, due its high CC₅₀ (>1083.32 ± 102.34) and moderate IC₅₀ (27.39 ± 4.52) which resulted in the higher SI of the samples (39.55).

Antimicrobial activity

The large amount of microorganisms tested in this study have the finality of making a screening analysis, to verify the possible action of *U. baccifera* extracts against a specific microbe. The serial dilution has the objective of evaluation, which is the smaller concentration that

was effective against the microorganisms. Usually, quantitative MIC determination is well accepted for proper evaluation of the antimicrobial activity of a plant extract (being the MIC of a plant extract below 1000 µg/ml) considered as significant (Rios and Recio, 2005). Therefore, only the samples that showed minimal inhibitory concentration (MIC) lower or equal to 1000 µg/ml for most of the microorganisms were listed on Table 2. The fractions obtained from the leaves were the only samples that have action against *K. pneumoniae*. EtOAc of the roots was the only sample that inhibited *S. cerevisiae*. However, to the alga *P. zopfii*, the fractions BuOH and EtOAc from both parts of the plant presented a MIC value considered significant, similarly to the roots CE.

Anti-inflammatory activity

On the ear edema evaluation, a graphic with time-course and dose-response curves of mouse ear edema induced by topical application of the plant was obtained (Figure 1). The data show that there is no occurrence of edematogenic effect, suggesting that *U. baccifera* leaves and roots does not have irritating effect. In the CFA-induced nociception test, it was observed that the roots and the leaves of the plant was not capable to reduce the paw withdrawal threshold induced by CFA at all time tested (data not shown). Therefore, *U. baccifera* did not present antinociceptive effect in this model of inflammatory pain. The antinociceptive pain against allodynia induced by acetone is shown in the Figure 2. The plant extract was not capable to revert the nociception induced by acetone on the animals who received CFA. In the spontaneous nociception experiment, the animals did not present spontaneous pain (Figure 3). The test shown that the crude extracts of the plant did not decrease the spontaneous pain of the mice in comparison

Table 2. Results of antimicrobial activity.

Parameter	CE (µg/ml)	CHCl ₃ (µg/ml)	EtOAc (µg/ml)	BuOH (µg/ml)
Leaf				
<i>S. cerevisiae</i>	>1000	>1000	>1000	>1000
<i>P. zopfii</i>	>1000	>1000	250	250
<i>K. pneumoniae</i>	>1000	312	312	156
Root				
<i>S. cerevisiae</i>	>1000	>1000	250	>1000
<i>P. zopfii</i>	500	>1000	500	250
<i>K. pneumoniae</i>	>1000	>1000	>1000	>1000

CE: Crude extract; CHCl₃: Chloroform fraction; EtOAc: Ethyl acetate fraction; BuOH: butanol fraction.

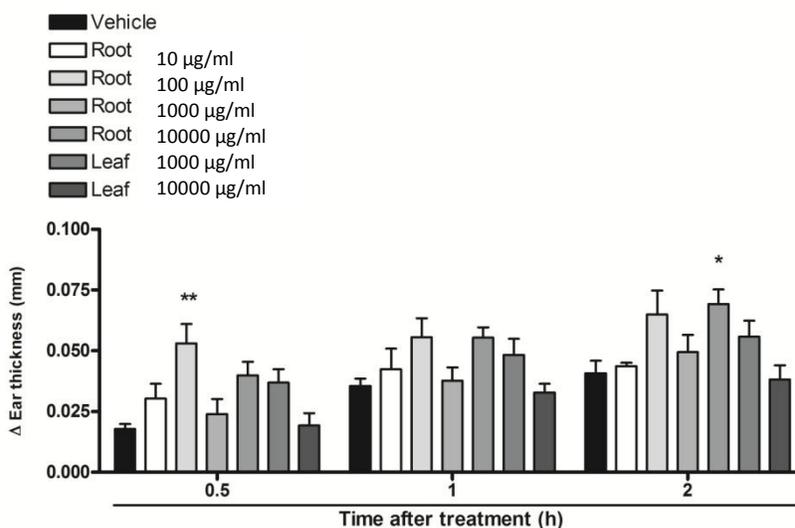


Figure 1. Time-course and dose-response curves of mouse ear edema induced by topical application of root and leaf of the *Uretra baccifera*. The effects of these extracts are expressed as a change in ear thickness. Each column represents the mean ± S.E.M. for 6 animals. *P < 0.05 and **P < 0.01 vs. vehicle.

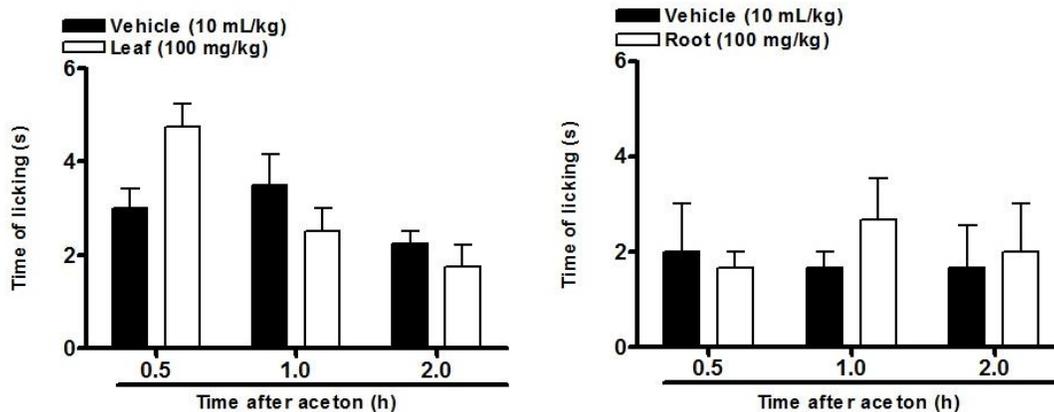


Figure 2. Evaluation of antinociceptive pain against cold allodynia induced by acetone.

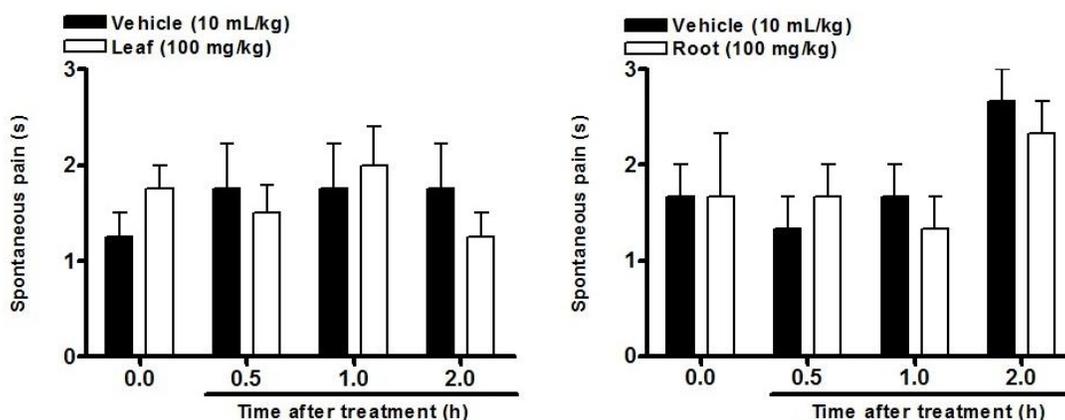


Figure 3. Evaluation of spontaneous pain.

with the vehicle.

DISCUSSION

Antiviral activity

The MTT method allows the evaluation of cytotoxicity of the samples in parallel with their antiviral activity, what is particularly important, since cytotoxic compounds sometimes behave as if they were active (Takeuchi et al., 1991). All samples tested showed anti-herpetic activity according to the parameters proposed by Amoros et al. (1992) and Cos et al. (2006) in order to consider a product as a potential antiviral candidate. *U. baccifera* is a plant classified in the Urticaceae family. The antiviral activity of other Urticaceae species has been confirmed in several studies (Silva et al., 2010; Uncini et al., 2005). The crude extract and the fractions methanol and butanol of *Cecropia glaziovii* Sneth leaves showed high activity against HSV-1 and HSV-2 (Silva et al., 2010). In addition, another study has shown that the species *Urtica dioica* was very effective to inhibit the syncytium formation by the feline immunodeficiency virus (FIV), which is regarded as a model for HIV (Uncini et al., 2005).

The plants have a wide variety and complexity of chemicals that are responsible for various biological effects, such as flavonoids, terpenoids, lignans, polyphenols, coumarins, saponins, alkaloids, thiophenes, proteins, peptides, and others (Jassin and Naji, 2003). The presence of phenolic compounds and flavonoids was previously confirmed in qualitative and quantitative tests for the roots and leaves of *U. baccifera* (Mannion and Menezes, 2010). The samples tested in this study have their content analyzed in a previous work (Gindri et al., 2014). The results showed 29.98 ± 1.27 , 35.54 ± 0.45 and 49.05 ± 0.88 mg/g of poly-phenols and 16.13 ± 0.22 , 15.38 ± 0.75 and 26.61 ± 0.25 of flavonoids, to the CE

CE and EtOAc of the roots, and the BuOH of the leaves, respectively. The phenolic compounds (Hudson, 1990; Sakagami et al., 1995; Van Den Berghe et al., 1986), particularly the flavones and flavonoids (Amoros et al., 1992), have been reported as having antiviral activity.

The proposed mechanisms by which the plant extracts and its compounds act on the viruses are variable, however several studies has shown that polyphenols act before virus entry, directly on the virus particle or avoiding the virus binding to the cells (Hudson, 1990; Sakagami et al., 1995; Van Den Berghe et al., 1986).

According to Hudson (1990), the viral inactivation is due to the *in vitro* binding of the polyphenols to the virus coat proteins. There are also studies showing that the antiviral activities of many natural products may occur by polyphenols binding either to the virus or to the proteins of the host cell membrane, thus preventing viral adsorption (Van Den Berghe et al., 1986). This view is reinforced by studies performed by Sakagami et al. (1995) which suggests that polyphenols act through direct inactivation of the virus and/or inhibition of the binding of the virus to the cells. Thus, the polyphenols may have an important role in the antiviral activity of the extracts of *U. baccifera*, although further studies are necessary to identify the mechanisms.

Furthermore, in our conditions, the BuOH fraction of the leaves of *U. baccifera* showed high antiviral activity against HSV-1, differently from the other fractions of the leaves (Table 1). Similar results have been observed by Martins et al. (2009) who reported that the butanol fractions of the aerial parts of *U. baccifera* showed high antiviral activity against HSV-1- ACVr (99.4%) *in vitro*, although in the same study the extracts did not show expressive percentage of viral inhibition against HSV-2- ACVr. It is important to highlight that in the *Urera* genus, there is the unique study available concerning the antiviral activity. Afterward, the probable presence of biologically

active compounds and the low cytotoxicity demonstrated by the extract and fractions of *U. baccifera* make this plant a promising antiviral candidate. Even though the indication for treatment is not yet possible, the observed activity justify further studies on the elucidation of the mechanisms of virus inhibition. Moreover, the relatively high toxicity of the drugs approved for the treatment of herpetic infections and the continuous propagation of resistant viral strains emphasize the need for new antiviral agents (Van De Perre et al., 2008).

Antimicrobial activity

CHCl₃, EtOAc and BuOH fractions of the leaves showed a good activity against *Klebsiella pneumoniae*, gram-negative bacteria with a prominent polysaccharide capsule, which can cause important hospital infections among adult and pediatric populations, showing that the fractionation of the crude extract can evidence very important antimicrobial activities, not accessible when only crude extracts are tested. The best value was obtained for BuOH (MIC: 156 µg/ml), and based on the criteria mentioned earlier, the fractions of the extracts of *U. baccifera* leaves could be considered active against this important pathogen. Being a well-known raw material in the baking and brewing industry, *Saccharomyces cerevisiae* is also used as a probiotic in humans. However, this fungus can be a singular cause of infections in humans by different forms of invasive infection after ingestion (Sidrim et al., 2003). Taking this to account, EtOAc and BuOH fractions of the roots can be a promising inhibitor of this microorganism, reaffirming the importance of fractionation of the crude extract.

In the same way, the EtOAc and BuOH fractions of leaves and roots and the crude extract of roots presented activity against *Prototheca zopfii*, algae, particularly difficult to eradicate with drugs and without protocol to treatment (Sidrim et al., 2003). Many plant constituents are related to antimicrobial activities as phenolics and polyphenols, terpenoids and essential oils, alkaloids, lectins and polypeptides (Cowan, 1999). The flavonoids are described to possess antifungal, antiviral and antibacterial activity in several studies (Cowan, 1999; Cushnie and Lamb, 2005; Hernandez, Tereschuk and Abdala, 2000; Oh et al., 2011) and can be responsible of the *U. baccifera* activity against *K. pneumonia*, *S. cerevisiae* and *P. zopfii*.

Anti-inflammatory activity

In this study were tested the anti-nociceptive and anti-inflammatory activity of *U. baccifera* in rats, using the concentrations of 100 and 10 to 1000 mg/kg of animal

weight administered orally, to the roots and the leaves, respectively, and these extracts did not show antinociceptive and anti-inflammatory effect in these experiments. Conversely, the plant showed anti-inflammatory and antinociceptive effects (Badilla et al., 1999a, b, 2006) only when the extracts were administered intraperitoneally, and in the last two studies, the dosages used were higher than those used in the present work, indicating that the plant can display anti-inflammatory and antinociceptive activities, but only when administrated intraperitoneally and in high concentrations. Alkaloids (Barbosa-Filho et al., 2006; Matu and Staden, 2003) and diterpenes (Matu and Staden, 2003), as well as flavonoids (Ferrándiz and Alcaraz, 1991; Guardia et al., 2001), were cited as anti-inflammatory constituents of plants. Alkaloids and flavonoids were quantified in this plant, even though *U. baccifera* did not show anti-inflammatory and antinociceptive activities.

This study tested the antiviral, antinociceptive, anti-inflammatory and antimicrobial activities of *U. baccifera* leaves and roots. The antinociceptive and anti-inflammatory effects were not evidenced when orally administrated at the tested doses. On the other hand, this work provides new information about the antimicrobial activity of *U. baccifera*, showing that the fractions of this plant could be considered active against *K. pneumoniae*, *P. zopfii* and *S. cerevisiae*. Additionally, the plant showed a very good anti-herpetic activity, and the best results were obtained with the crude extract and ethyl acetate fractions of the roots and the butanol fractions of the leaves. The probable presence of biologically active compounds and the low cytotoxicity displayed by the extract and fractions of *U. baccifera* make these compounds promising antiviral candidates. This study became important due to the fact that there are few studies concerning the pharmacological activities of *U. baccifera*, and no study regarding activities of other species of *Urera* genus, which makes the genus a promising source of study.

Conflict of interest

No competing interests were disclosed.

ABBREVIATIONS

ACV, Acyclovir; **ATCC**, american type culture collection; **BuOH**, butanol fraction; **CC₅₀**, cytotoxic concentration 50% (µg/ml); **CE**, crude extract; **CFA**, complete freund's adjuvant; **CHCl₃**, chloroform fraction; **DMSO**, dimethyl sulfoxide; **EtOAc**, ethyl acetate fraction; **FBS**, fetal bovine serum; **HSV**, herpes simplex virus; **HSV-1**, Herpes simplex type 1; **HSV-1-ACVR**, acyclovir-resistant Herpes simplex virus type 1; **IC₅₀**, inhibitory concentration; (µg/ml); **i.pl.**, intraplantar (plantar surface); **MNCC**, maximum noncytotoxic concentration; **SD**, standard deviation; **SI**, selectivity index; **TCID₅₀**, 50% tissue culture infectious

doses.

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