

Antimicrobial Activity of Extracts Obtained from *Urera baccifera* (L.) Gaudich

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Abstract The purpose of this work was to assess the antimicrobial activity of extracts from *Urera baccifera*. Aqueous, ethanol and methanol extracts made from the leaves, bark and roots of *U. baccifera* were tested, at different concentrations using the disk diffusion method, against the bacteria *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Inoculated plates were incubated at 35° C ± 1 C for 24 hours and the inhibition halos were assessed and interpreted. The methanol extracts from the leaves (ML) and roots (MR) had greatest antimicrobial activity against the three bacteria tested. The MICs of the ML and MR extracts against *E. coli* were 6.25 and 0.19 mg/L, respectively, and against *P. aeruginosa* and *S. aureus* they were 3.12 and 0.19 mg/L (for both species). The results show that the methanol extracts of the leaves and roots of *U. baccifera* are antimicrobially active against *E. coli*, *P. aeruginosa* and *S. aureus*.

Keywords Antimicrobial, Natural Products, Medicinal Plants, Secondary Metabolites

1. Introduction

Since antiquity medicinal plants have been used in the treatment of several illnesses that afflict humans. This type of medicine corresponds to any plant-derived matter that is administered to a living being and presents a pharmacological property for the treatment of a pathological condition[1].

Within the pharmaceutical industry, medicinal plants represent a primary source of substances that are used to create new medicines. Plants are considered natural biochemical laboratories that synthesize several active principles[2], such as phenols, quinones, flavonoids, tannins, coumarins, terpenes, alkaloids and lectins, which have antimicrobial activity *in vitro* and can be extracted by organic solvents or water[3].

The use of plants in medicine is currently of great interest due to how difficult it is to treat some diseases, the resistance of microorganisms[4] and the increased cost of synthetic medications[5] (Fuck et al., 2005). Although there are several types of antimicrobials in clinical use, many are ineffective against some types of microorganisms because of the ability of these organisms to quickly develop a resistance to the medicine[6].

For these reasons, research is focused on finding new active principles that can be used in the production of new

medications. Considering the high biodiversity in Brazil, the popular know-how about the properties of medicinal plants and the unknown chemical characteristics of most species, the scientific assessment of the therapeutic value of plants with still-unknown properties is becoming increasingly promising[5].

Urera baccifera (Urticaceae)[7] (Figure 1) is a perennial, erect shrub, ranging in height from 1.5 to 2.5 m, with simple, alternate leaves, which have stinging hairs that cause skin burns. The flowers are small, unisexual, and grow in axillary inflorescences. The fruits are small, achene- or drupe-shaped[8], spongy, hydrated and rich in carbohydrates and proteins[9]. This species is popularly known as nettle and can be found along forest edges in Tropical America, where there is a humid, shady environment[10].

Among many components, the leaves of *U. baccifera* contain proteins (23% of the total), calcium (5%), potassium (3.1%), magnesium (0.54%), phosphorus (0.27%), sulfur (0.27%), iron (0.0209 mg.kg⁻¹), sodium (0.0108 mg.kg⁻¹), manganese (0.0072 mg.kg⁻¹), boron (0.0053 mg.kg⁻¹), zinc (0.0039 mg.kg⁻¹) and copper (0.0008 mg.kg⁻¹)[11].

Martins et al.[12] tested the activity of extracts of the aerial parts of *U. baccifera* against the herpes simplex virus (HSV) and observed that these extracts only had activity against HSV-1, and not HSV-2. They also reported that butanol extracts showed high antiviral and viricide activity (85.9%), and that extracts of ethyl acetate inhibited the cellular receptors by 90% and viral penetration by 85.9%, but did not show viricide activity. In addition, the ethanol extracts in their study showed antiviral activity because they inhibited the virus from penetrating the cells (94.4%).

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Figure 1. *Ureia baccifera* (L.) Gaudich. ex Wedd. – Urticaceae

Popularly, *Ureia baccifera* is used in the treatment of hemorrhoids, hemorrhages, rheumatism, hair loss, skin diseases and chilblain[13]. Despite being of natural origin, it should not be used indiscriminately in infusions, because it may have several metabolites that can cause damage or toxicity to the organism[14].

In addition, other species of Urticaceae show hypoglycemic[15], hypotensive[16], antibacterial and antiviral activity [17, 18].

Based on this, the goal of the present study was to assess the antimicrobial activity of extracts made from *Ureia baccifera* (L.) Gaudich against pathogenic bacteria.

2. Materials and Methods

2.1. Collection Site

The plant material was collected in the countryside in the town of Dionísio Cerqueira, Santa Catarina, Brazil (26°24'17.19"S, 153°38'49.27"W; elevation 511m). The specimens were identified and stored in the collection at the Botanical Laboratory at Paranaense University, Campus of Francisco Beltrão, PR. When the collections arrived at the laboratory, material was selected by separating the leaves, trunk and roots, which were dried in a stove for seven days at 60° C ±4. The material was then ground and stored in paper bags in a dry environment. All of the collections were authorized by IBAMA under the permit number 13.234-2 (1 August, 2006).

2.2. Obtainment of the Ethanol and Methanol Extracts

The extracts were obtained by macerating 30 g of each dried part of the plant and then immersing the material in 300 mL of 99.8% methanol or 96% ethanol, for 24 hours, after the solution was quickly boiled. The material was then filtered and concentrated in an exhaustion chamber at room temperature until about 20% of the initial volume was left. The finished extracts were kept in amber flasks at room temperature until use[18].

2.3. Obtainment of the Aqueous Extract

To obtain the aqueous extracts, 50 g of each part of the plant were separately added to 500 mL of sterile distilled water. The material was kept at rest at room temperature, for 24 hours, after the solution was quickly boiled. Next, the extracts were filtered and concentrated in an exhaustion chamber at room temperature until the concentrated solution was 20% of the initial volume. The extracts were kept in amber flasks at room temperature until use[19].

2.4. Preparation of the Samples

Flasks were numbered from 1 to 10 according to the different concentrations prepared. dimethylsulphoxide (DMSO) was employed as a solvent for the ethanol and methanol extracts, and water was employed for the aqueous extracts. The concentrations prepared were 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19 mg/L. Next, discs of filter paper, 6 mm in diameter, were soaked with the extracts and placed on the surface of the culture medium.

2.5. Preparation of the Inoculate

For the antimicrobial assessments, strains from the American Type Culture Collection (ATCC) that belong to the Microbiology Laboratory at UNIPAR were used: *Staphylococcus aureus* (ATCC 25923), a microorganism that dwells in skin, mucosa and upper respiratory tract and can cause cutaneous and even systemic infections; *Escherichia coli* (ATCC 25922), a member of the intestinal microbiota, is involved in enteritis, urinary infections and nosocomial bacteremias; and *Pseudomonas aeruginosa* (ATCC 27853), an important opportunistic bacterium, is involved in hospital and urinary infections and in sepsis[20]. The microbial cultures were standardized to tube 0.5 of the McFarland scale.

2.6. Test of Sensitivity

The method used was that of Kirby Bauer (disk diffusion), which involves the inoculation of a standard solution of a specific microorganism on an agar surface. Paper disks, previously saturated with the samples whose antimicrobial activity is to be investigated, are placed on the agar. The substances impregnated on the paper disks diffuse through the culture medium and, if the sample has inhibitory activity against the tested microorganism, an inhibition halo forms around the disk. After the incubation period, defined specifically for the microorganism, the zones of inhibition are measured[21-22].

The antibiotics Amoxicillin 10 mg and Chloramphenicol 30 mg (Newprov®¹) were used as positive controls. The negative controls were Dimethyl Sulphoxide, Methanol 99.8% and Ethanol 96%.

2.7. Essay of Disk Diffusion

The suspensions of microorganisms were inoculated on

¹ Newprov – Laboratory Products Ltd - Primeiro de Maio St., 590 - Pinhais-Paraná – Brazil.

plates containing Mueller-Hinton (MH) agar. The disks containing the extracts were then transferred to the media containing each inoculate. The plates were incubated at $35^{\circ}\text{C} \pm 1$ for 24 hours. After this period, they were inspected for the presence of inhibition halos and these, when present, were measured (in mm).

2.8. Broth Dilution Method

This method was used to determine the MIC (Minimum Inhibitory Concentration) of bioactive samples was carried out using serial dilutions in the ratio 1:2, using 2 ml of BHI broth. Two groups were controls, positive and negative, respectively, formed by the culture medium (BHI) plus $2\ \mu\text{L}$ of microbial suspensions, and this culture medium without the addition of the inoculum[23-25].

The samples were solubilized in a solution of dimethylsulfoxide (DMSO) 25%. Because the intensity of color of the material analyzed, which alter the color in the medium, making the visual reading of the results was added 0.2 mL of triphenyl tetrazolium chloride (TTC) 2%.

The Minimal Inhibitory Concentration (MIC) was considered as the lowest concentration of the extract capable of inhibiting microbial development.

3. Results and Discussion

From the nine extracts assessed in this work, only four showed antimicrobial activity. These were the ethanol extract from the bark (EB), the ethanol extract from the roots (ER), the methanol extract from the leaves (ML) and the methanol extract from the roots (MR). These data are shown in Table and Figure 2.



Figure 2. Activity on the root extract against *S. aureus* - (ATCC 25923)

An analysis of this data revealed that most of the antimicrobial activity of the four extracts was against *Escherichia coli*, because all four extracts inhibited its growth. The MIC was 100 mg/L for the EB, exhibiting a 7.00 ± 1.25 mm inhibition halo. This behavior was also observed with the ER at the concentration of 100%, which

had a 6.75 ± 0.54 mm inhibition halo. The methanol extracts of the leaves and roots showed higher inhibitions, with halos of 11.85 ± 0.75 mm[MIC 6.25] and 7.00 ± 1.00 mm[MIC 0.19], respectively. The EB and ER extracts did not exhibit antimicrobial activity against *P. aeruginosa* and *S. aureus*. On the other hand, the ML and MR extracts inhibited their growth, with MICs of 3.12 and 0.19 mg/L (for both). The inhibition halos for *P. aeruginosa* were 10.00 ± 0.25 mm and 9.00 ± 1.00 mm, and for *S. aureus* they were 7.32 ± 0.32 mm and 8.25 ± 0.45 mm, respectively.

The MR reached 38 mm against *S. aureus*, 37 mm against *P. aeruginosa* and 29 mm against *E. coli* at 100 mg/L concentration. The inhibitory activity against the first two bacteria was higher than that of the control antibiotics.

The positive controls – disks soaked in Amoxicillin and Chloramphenicol – had inhibition halos that averaged 25 mm and 30 mm, respectively. The negative controls – disks impregnated with DMSO, ethanol 96% or methanol 99.6% – did not inhibit the growth of the microorganisms.

In an investigation by Meléndres and Capriles[26], 172 species of plants were tested for their antimicrobial activity from methanol extracts of *Ureca baccifera* (L.) Wedd leaves, and the extracts did not show activity against *E. coli* and *S. aureus*. The discordance between this and the present results are probably because the production of secondary metabolites varies in plants.

Active principles generally come from the secondary metabolism of plants, and play a role in the interaction with the environment, especially biotic (herbivores, pathogens, pollinators, seed dispersers, symbionts) and abiotic factors (light, temperature, water, nutrients)[27]. Secondary products can vary according to the conditions a plant is exposed to during development, its stage of development and its needs.

Secondary metabolites can be divided into three chemically distinct groups: terpenes, phenolic compounds and nitrogenous compounds. Terpenes are insoluble in water, volatile or not, and toxic to plague insects and herbivorous mammals, but attractive to pollinators. Major examples of this class are saponins, cardenolides, limonoids, limonene, menthol and phytoecdisones. Phenolic compounds can be either soluble in organic solvents or water, or insoluble; many act against herbivores and pathogens, and others attract pollinators, protect the plant against ultraviolet light, sustain the plant or reduce the growth of neighboring plants.

This class includes lignine, coumarine, anthocyanin, isoflavone, tannin and isoflavonoids; the latter two are known to exhibit antimicrobial activity. Nitrogenous compounds, also known as alkaloids and cyanogenic glycosides, are soluble in aqueous solvents, act against predators and some are used by the pharmaceutical industry for many purposes. The most well-known alkaloids are morphine, nicotine, caffeine and vinblastine[28].

The fact that the aqueous extracts did not show antimicrobial activity can be explained by the absence, or low concentration, of hydrophilic substances that have antimicrobial potential. The opposite was observed for the

extracts obtained from the organic solvents, ethanol and methanol, which had antimicrobial activity.

Table 1. Inhibitions halos followed by Minimal Inhibitory Concentration (Halos[MIC]) observed for the four extracts obtained from *Urera baccifera* against the bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Extract	<i>E. coli</i> (Halos/MIC)	<i>P. aeruginosa</i>	<i>S. aureus</i>
Ethanol (EB)	7.00±1.25[100]	R	R
Ethanol (ER)	6.75±0.54[100]	R	R
Methanol (ML)	11.85±0.75[6.25]	10.00±0.25[3.12]	7.32±0.32[3.12]
Methanol (MR)	7.00±1.00[0.19]	9.00±1.00[0.19]	8.25±0.45[0.19]

*Means and standard deviations (Mean±Standard Deviation) and concentration of the extract. R – Resistant. EB – Ethanol of the bark. ER – Ethanol of the roots. ML – Methanol of the leaves. MR – Methanol of the roots.

4. Conclusions

Based on data collected in this study, it can be concluded that, among the extracts assessed, the ethanol extracts from the bark and the root of *Urera baccifera* (L.) Gaudich only had antimicrobial activity against *Escherichia coli* (ATCC 25922) and that the methanol extracts obtained from the leaves and roots had antimicrobial activity against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923).

Conflict of Interest

The authors declare no conflict of interest.

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