ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *Brunfelsia uniflora* LEAF EXTRACT

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ABSTRACT: Finding bioactive compounds with antimicrobial and antioxidant capacity from natural sources has been a challenge., mainly due to the increase in microbial resistance. This study aimed at prospecting the main classes of secondary metabolites and the antioxidant and antimicrobial activity of *Brunfelsia uniflora* leaf extract. The ethanolic extract was obtained by dynamic maceration with solvent, and the antioxidant activity was analyzed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The antimicrobial activity was evaluated against *Escherichia coli*, *Staphylococcus aureus,* and *Candida albicans* by broth microdilution method. The phytochemical analysis of *B. uniflora* leaf extract showed the presence of saponins, tannins and alkaloids. The extract presented minimum inhibitory concentration of 62.5 mg/mL for *E. coli*. The 1.49 mg/mL extract concentration inhibited 50% of free radicals in the DPPH solution at 60 µM. The antimicrobial and antioxidant activities of this plant extract were the basis of studies to develop applications in the pharmaceutical, cosmetics and food industries.
KEY WORDS: Biological activity. *Brunfelsia uniflora.* Free radicals. Manacá.

ATIVIDADE ANTIMICROBIANA E ANTIOXIDANTE DO EXTRATO DE FOLHAS DE *Brunfelsia uniflora*

RESUMO: Encontrar compostos bioativos com capacidade antimicrobiana e antioxidante a partir de fontes naturais tem sido um grande desafio. Esta procura está pautada no aumento da resistência microbiana e na necessidade de conservantes alternativos, não sintéticos, na indústria alimentícia e farmacêutica. Desta forma, este estudo teve como objetivo prospectar as principais classes de metabólitos secundários e a atividade antioxidante e antimicrobiana do extrato de folhas de *Brunfelsia uniflora*. O extrato etanólico de folhas de *B. uniflora* foi obtido por maceração dinâmica com esgotamento do solvente, concentrado em evaporador rotativo e a atividade antioxidante analisada pelo método de DPPH (2,2-diphenyl-1-picrylhydrazyl). A atividade antimicrobiana foi avaliada contra *Escherichia coli*, *Staphylococcus aureus* e *Candida albicans* pelo método de microdiluição em caldo. A análise fitoquímica do extrato das folhas do manacá evidenciou saponinas, taninos e alcaloides. O extrato bruto apresentou concentração mínima inibitória de 125mg/mL para *C. albican*s e *S. aureus*, e 62,5 mg/mL para *E. coli*. A concentração do extrato que inibe 50% dos radiais livres da solução de DPPH a 60 µM foi de 1,49 mg/mL. A atividade antimicrobiana e antioxidante do extrato das folhas desta planta embasa estudos para o desenvolvimento de aplicações nas indústrias farmacêutica, cosmética e de alimentos.
PALAVRAS-CHAVE: Atividade biológica. *Brunfelsia uniflora*. Manacá. Radicais livres.

ACTIVIDAD ANTIMICROBIANA Y ANTIOXIDANTE DEL EXTRACTO DE HOJAS DE *Brunfelsia uniflora*

RESUMEN: Encontrar compuestos bioactivos con capacidad antimicrobiana y antioxidante a partir de fuentes naturales ha sido un gran desafío. Esta demanda está pautada en el aumento de la resistencia microbiana y en la necesidad de conservantes alternativos, no sintéticos, en la industria alimenticia y farmacéutica. De esta forma, este estudio tuvo como objetivo prospectar las principales clases de metabolitos secundarios y la actividad antioxidante y antimicrobiana del extracto de hojas de *Brunfelsia uniflora*. El extracto etanólico de las hojas de *B. uniflora* ha sido obtenido por maceración dinámica con agotamiento del solvente, concentrado en evaporador rotativo y la actividad antioxidante analizada por el método de DPPH (2,2-diphenyl-1-picrylhydrazyl). La actividad antimicrobiana ha sido evaluada contra *Escherichia coli*, *Staphylococcus aureus* y *Candida albicans* por el método de micro dilución en caldo. El análisis fitoquímico del extracto de las hojas del "manacá" se evidenció saponinas, taninos y alcaloides. El extracto bruto presentó una concentración mínima inhibitoria de 125 mg/ml para *C. albicans* y *S. aureus*, y 62,5 mg/ml para *E. coli*. La concentración del extracto que inhibe 50% de los radicales libres

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de la solución de DPPH a 60 µM fue de 1,49 mg/ml. La actividad antimicrobiana y antioxidante del extracto de las hojas de esta planta son la base de estudios para el desarrollo de aplicaciones en las industrias farmacéutica, cosmética y de alimentos.
PALABRAS CLAVE: Actividad biológica. *Brunfelsia uniflora*. "Manacá". Radicales libres.

Introduction

In the last decade, the pharmaceutical, cosmetic and food industries have increased the utilization of plant extracts in detriment of conventional preservatives, mainly those with antioxidant and antimicrobial activity (MARTINS et al., 2009; PEREIRA; CARDOSO, 2012). Antioxidants act on the oxidative protection of cells and can decrease risks to health such as toxic and/or carcinogenic effect (PEREIRA; CARDOSO, 2012). The association of substitution for natural antioxidants and/or antimicrobials such as plant extracts can be an alternative for industrial processes.

The antimicrobial activity of the ethanolic extract from *Brunfelsia uniflora* (Pohl.) D. Don leaves, known as manacá, has been partially studied but did not present antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, important contaminant organisms of food; however, this plant has potential antioxidant activity because its chemical composition contains caffeic acid and phenolic acid (MARTINS et al., 2009). Manaca is a bushy plant that is utilized due to its medicinal properties like other plants from Solanaceae family, especially in the Amazon region and the Atlantic Forest (MARTINS et al., 2009; SCHNEIDER et al., 2015), indicating potential antimicrobial effect. Moreover, preliminary tests done by our research group showed the antimicrobial activity of the extract from *B. uniflora* leaves contradicting the information available in the literature.

Despite the broad variety of the national flora, few phytochemical and biological activity studies have been carried out, mainly for *B. uniflora*. Thus, this study aimed to prospect the major classes of secondary metabolites and the antioxidant and antimicrobial activities of *B. uniflora* leaf extract.

Material and Methods

Harvest and preparation of vegetal material

Brunfelsia uniflora leaves were harvested in the morning at the coordinates 25º 44' 01'' S and 53º 03' 26'' W at 509 m of altitude. The botanical identification and the exsiccate deposit occurred in the Botanical Garden Museum of Curitiba, under number 396292. The leaves were dried outdoors, displayed in 3-mm layers in the shade, and they were turned over three times a day, every 4 h. After drying, the leaves were ground in a blade mill to obtain particles smaller or equal to 350 µm.

Obtaining crude extract from leaves

To obtain the crude extract from *B. uniflora* leaves, 225 g of leaves in powder in hydrated cereal alcohol (96 ºGL) was utilized by dynamic maceration process with solvent depletion, followed by concentration in a rotary evaporator (BRASIL, 2010).

Phytochemical analysis

The phytochemical prospection of leaf extracts was developed by qualitative evaluations for alkaloids, anthraquinones, flavonoids, saponin and tannins. Alkaloids were detected through alkaloid general reagents with which they form turbidity or precipitation in acid medium. The utilized reagents were Dragendorff evidencing the formation of an orange precipitate (DOCTOR; MANUEL, 2014). Tannins were evaluated utilizing tannin-gelatin reaction. The confirmation of tannins was done by the reaction of heavy metals with copper and lead solutions that were precipitated or caused turbidity which are positive result for tannins (DOCTOR; MANUEL, 2014; VAIYAPURI; RAJU; KARUPPUSAMY, 2015). Anthraquinones were identified by modified Borntträger reaction, indicating their presence with reddish-pinkish coloration (DOCTOR; MANUEL, 2014). Flavonoids were detected by Shinoda reaction with concentrated HCl and metallic magnesium, indicating their presence by pink or red coloration (DOCTOR; MANUEL, 2014; VAIYAPURI; RAJU; KARUPPUSAMY, 2015). Foamy saponins were verified by foam index test using agitation for 15 s. The results were considered positive with persistent foam formation for longer than 15 min (VAIYAPURI; RAJU; KARUPPUSAMY, 2015).

Antimicrobial activity

The utilized microorganisms were *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *Candida albicans* (ATCC 27853). The microbial inoculum was prepared by comparison 0.5 McFarland scale and diluted until the final concentration of 5×10^5 CFU/mL. The minimum inhibitory concentration (MIC) of crude extract from *B. uniflora* leaves was obtained by microdilution method in microplates containing 96 wells per serial dilution in Mueller Hinton broth (MHB; Kasvi®) according to CLSI (2015), adapted for plant extract. Each well had 100 µL of MHB, 10 µL of inoculum and 100 µL or control extract or solution. The extracts were dissolved in a 5% dimethyl sulfoxide (DMSO) solution and the final concentrations of the extracts were 250, 125, 62.5 and 31.25 mg/mL in MHB with inoculum. The final concentration of the controls was 0.12 µg/mL of benzylpenicillin, 0.8 µg/mL of nystatin and 50 µL/mL of DMSO in MHB with inoculum. The assays were done in triplicate. MIC was determined after 24 h of incubation at 35 ± 2 ºC for bacteria and at 25 ± 2 ºC for the fungus. The smallest extract concentration without visible microbial growth in the optical microscope was defined as MIC. The confirmation of the absence of microbial growth was determined by subcultivation for 24 h in a series of 100 µL in Petri dishes with Mueller Hinton agar (Kasvi®) for bacteria, and potato dextrose agar (Kasvi®) for fungus.

Antioxidant activity

The antioxidant capacity was evaluated utilizing free radical sequestration method of DPPH (2,2-diphenyl-1--picrylhydrazyl) (BLOIS, 1958). The extract was prepared in methanol at the concentrations of 0.25, 0.75, 1.00, 1.50 and 2.00 mg/mL. From each dilution, 0.1 mL was separated and mixed with 3.9 mL methanolic solution of DPPH prepared at 60 µM. The mixtures were kept at rest for 30 min and the

absorbance of the samples was measured at 515 nm in an UV/VIS spectrophotometer. From the correlation between the absorbance *versus* concentration of the antioxidant sample, and previously knowing the relative absorbance at 50% of DPPH concentrations, the concentration of the antioxidant sample to reduce 50% of the free radicals (IC_{50}) of the sample was determined (ATHMOUNI et al., 2015; RAJ; RADHAMANY, 2010). For the negative control, 0.1 mL of methanol was utilized, and for the positive control 0.1 mL of serial dilutions of an aqueous solution of quercetin was prepared initially at 60 µM.

Results and Discussion

The phytochemical analysis of crude extract from *B. uniflora* leaves evidenced the presence of secondary metabolites such as saponins, tannins, and alkaloids, but anthraquinones and flavonoids were not found (Table 1). Martins et al. (2009) reported the presence of caffeic acid and coumarins in the ethanolic extract from *B. uniflora* leaves, characteristic compounds of flavonoids, differed from our results in which the chemical composition presented saponins, tannins, and alkaloids. Phenolic compounds, also characteristic of flavonoids (rutin and ferulic acid), were found in the aqueous extract of *Brunfelsia cuneifolia* (SCHNEIDER et al., 2015).

Table 1: Phytochemical prospection of crude extract *from Brunfelsia uniflora* leaves.

Reactive compound	Reagents	Result
Saponins	Foamy Saponin	+
Hydrolyzable Tannins	Heavy metal reaction (copper solution)	+
	Heavy metal reaction (lead acetate)	+
Condensed Tannins	Gelatin reaction	+
Anthraquinones	Borntränger reaction	-
Flavonoids	Shinoda eraction	-
Alkaloids	Dragendorf reaction	+

(+) presence or (-) absence of the compound.

Lima and Gomes (2014) found positive result for alkaloids and tannins in ethanolic extract of *Solanum acanthodes*; Anselmo and Lima (2014) analyzing ethanolic extract of *Solanum jamaicense* obtained positive result for alkaloids, flavonoids, tannins, and saponins; Wollenweber and Dörr (1995) verified the presence of flavonoids in *Browallia grandiflora*, *Chamaesaracha sordida*, *Nicotiana tabacum*, *Petunia surfina*, and *Salpiglossis sinuata*, all specimens from Solanaceae family. These differences in the chemical composition found in the members of Solanaceae family can be related to biotic and abiotic effects (LINDE et al., 2016).

MIC of crude extract that inhibited the microbial growth of *C. albicans* and *S. aureus* was 125 mg/mL, and of *E. coli* was 62.5 mg/mL (Table 2). The positive controls for bacteria (benzylpenicillin) and for fungus (nistatin) inhibited the growth of all microorganisms. Klouceket al. (2005) obtained MIC value of 4 mg/mL against *S. aureus* for ethanolic extract from *Brunfelsia grandiflora* root, but did not show action against *E. coli*. Martins et al. (2009) studied concentrations from 1 to 32% (volume/volume) of ethanolic extract from *B. uniflora* leaves and did not find antibacterial activity against *E. coli* and *S. aureus*, a conflicting result from the one found in our study. On the other hand, antibacterial tests with *Brunfelsia latifolia* were effective against *Bacillus cereus, Shigella boydii*, *E. coli*, *Shigella dysenteriae*, *Saccharomyces cerevisiae* and *C. albicans* (BEGUM et al., 2007). Ethanolic extract from *Solanum jamaicense* leaves with the chemical composition of alkaloids, coumarins, tannins, saponins, triterpenes, anthracene derivatives, presented growth inhibition of *C. albicans* (ANSELMO; LIMA, 2014).

Table 2: Antifungal and antibacterial activity of *Brunfelsia uniflora* leaf extract.

Microorganism	Leaf extract (mg/mL)				DMSO (µL/mL)	Benzylpenicillin (µg/mL)	Nistatin (µg/mL)
	250	125	62.5	31.25	50	0.12	0.8
C. albicans	-	-	+	+	+	*	-
E. coli	-	-	-	+	+	-	*
S. aureus	-	-	+	+	+	-	*

(+) presence or (-) absence of microbial growth; (*) not tested. DMSO - Dimethyl sulfoxide. *Candida albicans* (ATCC 27853), *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923)

Alkaloids, tannins, and saponins found in the extract from *B. uniflora* leaves can be involved with the antimicrobial activity of this plant. Alkaloids are described due to their bactericidal and fungicidal action (SCHNEIDER et al., 2015). This class of compounds can inhibit enzymes, responsible for cell division, inhibit the synthesis of nucleic acids and depolarize the cytoplasmic membrane (CUSHNIE; CUSHNIE; LAMB, 2014).

Tannins are characterized by antiseptic, antimicrobial and antifungal activity (MONTEIRO; ALBUQUERQUE; ARAÚJO, 2005). These compounds can complex bacterial and fungal enzymes and modify the metabolism of cell

membranes. Also, tannins can complex essential minerals to the microbial metabolism (LOGUERCIO et al., 2005). Thus, natural products with the capacity to form phytocomplexes have been broadly studied for several uses and the main ones are as antimicrobials and antioxidants (ATHMOUNI et al., 2015).

For the antioxidant activity, the concentration of extract from *B. uniflora* leaves that inhibited 50% of free radicals of a solution of 60 µM of DPPH (IC_{50}) was 1.49 mg/mL. Regarding the positive control quercetin, IC_{50} was 0.016 mg/mL. A greater antioxidant activity of quercetin was expected because it is a pure antioxidant. For crude extracts of plants, the antioxidant activity found for *B. uniflora* is greater than the one described for *Brunfelsia americana* which presented IC_{50} of 0.310 mg/mL (RAJ; RADHAMANY, 2010). These authors associated the antioxidant activity to the presence of flavonoids, tannins, and saponins in the crude extract.

The antioxidant characteristic in plants is a result of a group of substances in their metabolism, but in the case of *B. uniflora* we speculated that these antioxidant properties are a result of saponins (PEREIRA; CARDOSO, 2012) and of phenolic compounds such as tannins found in plants from the Solanaceae family (SCHNEIDER et al., 2015). The phenolic compounds have the capacity to inhibit the lipid peroxidation and lipoxygenase. The antioxidant properties of phenolic compounds are due to the chemical structure and the oxidoreduction properties with performance in the sequestration or neutralization of free radicals without causing damages to cell structures (SANTOS et al., 2011; SCHNEIDER et al., 2015).

In conclusion, the ethanolic extract from *B. uniflora* leaves has antimicrobial and antioxidant activity. The antimicrobial and antioxidant activities of this plant are the basis for other studies in order to develop applications in the pharmaceutical, cosmetic and food industries.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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