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Antimicrobial Activity of *Cariniana legalis* (Lecythidaceae) Seedlings Subjected to Different Light Intensities

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ABSTRACT: *Cariniana legalis*, Lecythidaceae is an emergent species endemic to the Brazilian Atlantic Rainforest. Popularly known as jequitibá-rosa, this sapwood has been used in reforestation, and in folk medicine to treat leucorrhoea, female reproductive disorders and back pain. The aim of this study was to evaluate the *in vitro* antimicrobial activity of the aqueous extract of leaves of *C. legalis* seedlings subjected to different light environments, in shade houses. From the leaves, the aqueous extracts were made by means of decoction and lyophilization. The antifungal and antibacterial activity against the fungi *Candida albicans*, *C. krusei* and *C. parapsilosis* and the bacteria *Escherichia coli* and *Staphylococcus aureus* were evaluated. The minimum inhibitory concentration and minimum fungicidal/bactericidal concentration were determined. The results revealed that the aqueous extracts of leaves of *C. legalis* seedlings have bacteriostatic action for *E. coli* and fungicidal activity against *C. krusei* and *C. parapsilosis*. Our results suggest the antimicrobial action of anthocyanins produced under higher light radiation in the extracts of *C. legalis* leaves. However, further studies are needed to characterize with accuracy the substances responsible for the antimicrobial activity.

Key words: jequitibá-rosa, aqueous extract, bacteriostatic, fungicide

INTRODUCTION

The Lecythidaceae A. Rich. family has a pantropical distribution, concentrated in the Neotropical wet forests, including about 24 genera and 341 species of trees, shrubs and lianas (Smith et al. 2016). *Cariniana legalis* (Mart.) Kuntze, jequitibá-rosa, is an emergent and endemic tree of the Brazilian Atlantic Rainforest (Mori 1995; Smith et al. 2016). The habitat loss has been causing the decline of its populations and *C. legalis* is currently on the Red List of the International Union for Conservation of Nature and Natural Resources (ARW 1998), within the vulnerable category. This species is indicated for reforestation, in mixed or pure plantations, because the high quality of its wood (Rolim et al. 2018) and in the traditional medicine, sapwood is used in the treatment of leukorrhea (Fenner et al. 2006), female reproductive disorders and back pain (Gonçalves and Pasa 2015).

Although antimicrobial usage surveillance efforts made by the World Health Organization (WHO 2018) microorganisms are increasingly resistant to the drugs currently used (WHO 2014). So, the extracts from medicinal plants can be used as a therapeutic alternative in infections caused by these pathogens. Currently, there are an increasing of scientific studies that aim at the acquisition of substances of plant origin that present antimicrobial action or potentiate the activity of the medicines used (Rios and Recio 2005; Silva and Fernandes Júnior 2010; Selvamohan et al. 2012). This would reduce microbial resistance and certain negative effects that are commonly presented by commercial synthetic compounds, such as high toxicity, since natural products depending on their own level of toxicity may be less harmful to humans (Zuzarte 2002; Guldiken et al. 2018). The genus *Candida* comprises yeast fungi that constitute the normal microbiota of the oral cavity, skin, bronchial secretions, digestive tract and genitourinary tract living as commensal organisms. Occasionally, these microorganisms act as opportunists and produce superficial mucosal lesions to generalized dissemination (Papon et al. 2013; Colombo et al. 2017). The number of infections caused by *Candida* spp has increased, which is linked to the growing number of people with some immune system damage - HIV infected, for example - treatment by chemotherapy, organ transplantation, invasive surgery and prolonged use of antibiotics (Sanglard and Odds 2002; Colombo and Guimarães 2003). The Gram-negative bacteria *Escherichia coli* lives naturally in the intestines of humans, as well as in most animals. Certain strains of *E. coli* can cause several diseases, among which

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are diarrhea, neonatal meningitis, urinary tract infections and septicemia (Orskov and Orskov 1992). *Staphylococcus aureus* is a Gram-positive bacterium that also normally inhabits the human body, but can be considered a pathogenic microorganism of importance, considering its capacity to produce biofilms and toxins (Vuong et al. 2016).

There are studies in the literature that confirm the biotechnological potential of some species of the genus *Cariniana* spp., such as the presence of triterpenes and the anti-inflammatory activity for *C. rubra* Gardner ex Miers (Lima et al. 2002; Silva 2014). For *C. brasiliensis* Casar. (Baurin et al. 2002) inhibitory activity of the enzyme tyrosinase has been reported. Carvalho et al. (2011) reported moderate in vitro activity against the fungus *Alternaria alternata* for *C. estrellensis* (Raddi) Kuntze. However, there were no studies evaluating the antimicrobial action of extracts obtained from the leaves of *C. legalis* seedlings subjected to different light availability. Thus, the aim of this study was to evaluate the in vitro antimicrobial activity of *C. legalis* seedlings subjected to contrasting light environments.

MATERIAL AND METHODS

Plant material and extract preparation

Seedlings of *C. legalis* were produced in Instituto Floresta Viva (IFV) nursery, located in the district of Serra Grande, Uruçuca, BA, Brazil (www.florestaviva.org.br). Seedlings plants with visually uniform sizes and approximately 90 days of age were selected. The plants were brought to the nursery of the Universidade Estadual de Santa Cruz (UESC), Ilhéus, Bahia, Brazil (14º47'S and 39º10'W) and transferred to black bags with 1.5 l capacity containing forest soil. The plants were irrigated daily and two fertilizations with mono-ammonium phosphate (MAP), KCl and urea, as sources of phosphorus, potassium and nitrogen, respectively, were carried out during the experiment. Nine shade houses of 1.2x1.2x1.0 m were built. The shade houses were divided into three replicates of three light radiation environments. The shade houses were coated with different layers of black shading screens, thereby establishing an available photosynthetically active radiation (PAR) gradient. To characterize PAR inside shade houses, S-LIA-M003 sensors coupled to Hobo Micro Station Data Loggers (Onset Computer, Massachusetts, USA) were programmed to perform readings at 1-second intervals, with an average of every 10 minutes. Seedlings grown in shade houses for 77 days. The average PAR obtained for the light gradient in ascending order was of 1.1±0.04, 2.3±0.13 and 5.9±0.03 mol photons/m^2/day.

Leaves were dried in a forced air circulation oven at 50 ºC until reaching a constant mass. Dried leaves were submitted to aqueous extraction. For that, 200 ml of distilled water was added to a 5 g of leaves cut in 5 mm slices in a covered 500 ml graduated beaker. After 10 min boiling the liquid part was transferred to a 50 ml polypropylene tube, submitted to -20 ºC and freeze-dried using Freeze drier (Freezone 6 - Labconco, Kansas City, MO) to produce the dry aqueous extract. The solid part was discharged.

Microorganisms

Microorganisms of clinical importance were used in this study: *Candida albicans*, ATCC 14057; *C. parapsilosis*, ATCC 22018; *C. krusei*, ATCC 6258, *Staphylococcus aureus* INCQS 00249 (ATCC 25904) and *Escherichia coli* EPEC INCQS 00182 (CDC 086H35) obtained from Collection of Reference Microorganisms on Health Surveillance (CMRVS, FIOCRUZ-INCQ, Rio de Janeiro-RJ, Brazil). Yeast and bacterial strains were cultivated and maintained in Sabouraud dextrose agar supplement with chloramphenicol (50 µg/ml) and nutrient agar (NA) (Acumedia, Indaiatuba, Brazil), respectively.

Antifungal and antibacterial tests

Microdilution techniques were used for both antibacterial and antifungal evaluation. The susceptibility test was performed on bacteria using the Clinical and Laboratory Standards Institute M100-S22 document (CLSI 2006) method with adaptation. Briefly, for antibacterial tests 96 well plates were filled with 90 µl of serial dilution of plant extract from 1,000 to 7.8 µg/ml in brain heart infusion broth (BHI) (Kasvi, São José do Pinhais, Brazil) and 10 µl of bacterial inoculum giving a final volume of 100 µl by well. Plates were incubated at 37 °C for 24 h. Chloramphenicol (NeoQuímica**,** Anápolis, Brazil) at 50 µg/ml, BHI, DMSO (0.15%), and extracts without bacteria were used as controls. After the incubation period, 20 µl of resazurin (Sigma-Aldrich, Darmstadt, Germany) (0.01%) were added to each well to determine the bacterial growth (pink color) or inhibition (blue color) (Palomino et al., 2002). Also, 10 µl of each well was transferred to a plate containing 20 ml nutrient agar and incubated for 24 h at 37 °C. The MBC was determined by lack of visible bacterial growth. To perform the microdilution evaluation for yeast, an international norm recommended by the CLSI M27-A2 document (CLSI 2002), with adaptations was used. In 96 wells polystyrene plates, 100 µl of Roswell Park Memorial Institute (RPMI) 1640 culture medium (Vitrocell-Embriolife, Campinas, Brazil), containing the inoculum ($2.6x10^3$ cells/ml) and 100 µl of extract at a final concentration ranging from 1.95 to 250 µg/

ml were added in triplicate. The controls consisted of the RPMI medium with the inoculum, saline solution (NaCl 0.9%), and chlorhexidine (25 µg/ml), dimethyl sulfoxide with RPMI medium at the same concentration of the lowest dilution of the extract and extract without inoculum. After 48 h of incubation at 37 °C, plates were evaluated for the presence of precipitation in the bottom of the well. The lowest concentration where a precipitation in the bottom appeared (yeast death) was considered the minimal inhibitory concentration (MIC). To determine the minimal fungicidal concentration (MFC), 10 µl were transferred from a replicate of each treatment to the Sabouraud Dextrose Agar and plates were incubated at 28 °C for 72 h. The MFC was defined as the lowest concentration where no yeast grown was seen. Experiments were done in triplicates and repeated twice.

RESULTS AND DISCUSSION

The effect of light availability on the antimicrobial action of leaves of *C. legalis* seedlings cultivated under different light environments revealed different susceptibility of microorganisms, with the best results obtained at higher light condition tested in this study (5.9 mol photons/m²/day) (Table 1). The antifungal and antibacterial revealed antimicrobial potential of *C. legalis* leaves aqueous extract. From all agents tested, antimicrobial effect was seen for *C. krusei* and *C. parapsilosis* yeast and *E. coli* being *C. krusey* the most sensitive. *Candida albicans* and *S. aureus* were resistant at all concentrations used. Regarding the mechanism of action (death or only inhibition of growth) on microorganisms, fungicide effect was seen for all susceptible agents and bacteriostatic effect was seen for the Gram-negative bacteria *E. coli*.

It is well known that restriction of light availability leads to chlorophyll accumulation in the leaves, in order to optimize light absorption (Valladares and Niinemets, 2008; Valladares et al. 2012). Conversely, in high light availabilities, plants tend to redirect its metabolism to produce photoprotective pigments such as anthocyanins, a flavonoid that protects the photosynthetic apparatus from excessive energy of sun rays (Close and Beadle 2003; Lev-Yadun; Gould 2009). Young leaves of *C. legalis* naturally exhibit high concentrations of anthocyanins, conferring the characteristic reddish coloration of this species (Costa et al. 2019). In addition, the concentration of anthocyanins in leaves of young plants of *C. legalis* increases as a function of the increased availability of light radiation (Costa et al. 2019). So, the antimicrobial effect, in particular the antifungal, may be due to the high content of anthocyanin in the leaves of seedlings that grew at about 5.9 mol photons/m²/s. However, further studies are still needed to identify and quantify the substances responsible for the antimicrobial activity. It is recommended the use of leaves of *C. legalis* seedlings exposed to higher values of PAR, which may have more content of anthocyanin and, consequently, highest antimicrobial action.

The results obtained in this study revealed that the aqueous extracts of the leaves of *C. legalis* seedlings have antifungal activity for *Candida krusei* and *C. parapsilosis* and bacteriostatic for *E. coli*, having probable relation with the phytochemical compounds present in the leaves of the plant, especially the anthocyanins. Also, *C. legalis* is widely recommended for use in forest restoration projects and timber industry (Rolim et al. 2018), and the antimicrobial activity of its leaves adds a new potential value to this tropical tree species.

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TABLE 1. Antimicrobial effect of leaves aqueous extract (µg/ml) obtained from *C. legalis* seedlings subjected to different light treatments.

	5.9 mol photons/m²/day		2.3 mol photons/m²/day		1.1 mol photons/m²/day	
Microorganism	MIC	MFC/MBC	MIC	MFC/MBC	MIC	MFC/MBC
C. albicans ATCC14057	R	R	R	R	R	R
C. krusei ATCC6258	≥250	≥250	≥250	≥250	500	500
C. parapsilosis ATCC22018	≥250	≥250	R	R	R	R
E. coli EPEC-CDC086H35	500	R	500	R	500	R
S. aureus ATCC25904	R	R	R	R	R	R

R: resistant (presence of bacterial/yeast growth). MIC: minimal inhibitory concentration. MFC: minimal fungicidal concentration. MBC: minimal bactericidal concentration.

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AUTHORS' CONTRIBUTION

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by TDV, JOC, MCF, and ACD. The first draft of the manuscript was written by MSM and AOC and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The author declares no competing interests.

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