Research, Society and Development, v. 9, n. 11, e3369119992, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i11.9992

Botanical studies, antimicrobial activity and cytotoxity of *Eleutherine bulbosa* (Mill.) Urb

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Estudios botánicos, actividad antimicrobiana y citotoxicidad de *Eleutherine bulbosa* (Mill.) Urb

Received: 06/11/2020 | Reviewed: 10/11/2020 | Accepted: 11/11/2020 | Published: 15/11/2020

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Abstract

The use bulbs of *Eleutherine bulbosa* for treatament of diseases caused by malaria, amoeba and bacteria. This study accomplished the botanical and phytochemical characterization, antimicrobial activity and cytotoxic of *E. bulbosa*. For the anatomical studies, the studied material was fixed in formaldehyde – acetic acid and ethanol and it was dyed in safranin and

in astra blue. For histochemistry, was fixed in buffered neutral formalin and in ferrous sulphate in formalina. Ethanolic Extract (EE) was submitted to fractionation in a chromatographic column and four (4) fractions were obtained from it. The Dichloromethane Fraction (DF) was submitted to a new fractionation. The biological activity was evalueted by diffusion in agar, microdilution and celular viability MTT. The bulb of *E. bulbosa is characterized by a* reduced caulinar axis and by succulent amiliferous cataphylls, epidermis with the presence of anthocyanins, homogeneous mesophyll with idioblasts of prismatic crystals of calcium oxalate and phenolic compounds. In agar diffusion it was observed that EE, DF and ethyl acetate fraction (ACF) were active for *Staphylococcus aureus.* In microdilution, DF (Inhibitory minimum concentration= 125 µg/ mL) was more active. For all samples the Minimous Bactericidal Concentration was >1000 µg/ mL. The fractionation contributes positively with the citotoxicity, being subfractions S1 and S2 the most citotoxic ones. The Fraction Dichloromethane was the most active one for *S. aureus* and more citotoxicity to VERO cells. Probably the less citotoxicity of EE is related to the presence of anthocyanins that are present on bulbs epidermis.

Keywords: *Eleutherine bulbosa*; Cataphylls; Isoeleutherin; Citotoxicity; Antimicrobial activity.

Resumo

Bulbos de *Eleutherine bulbosa* são usados para tratamento de malária, ameba e bactérias. Este estudo realizou a caracterização botânica e fitoquímica, atividade antimicrobiana e citotóxica de *E. bulbosa*. Para os estudos anatômicos, o material estudado foi fixado em formaldeído - ácido acético e etanol e corado em safranina e azul astra. Para histoquímica, foi fixado em formalina neutra tamponada e em sulfato ferroso em formalina. O Extrato Etanólico (EE) foi submetido ao fracionamento em coluna cromatográfica e a partir dele foram obtidas quatro frações. A Fração Diclorometano (DF) foi submetida a um novo fracionamento. A atividade biológica foi avaliada por difusão em ágar, microdiluição e viabilidade celular MTT. O bulbo de E. bulbosa é caracterizado por eixo caulinar reduzido e por catafilos amilíferos suculentos, epiderme com presença de antocianinas, mesofilo homogêneo com idioblastos de cristais prismáticos de oxalato de cálcio e compostos fenólicos. Na difusão em ágar observou-se que EE, DF e fração acetato de etila (ACF) foram ativos para *Staphylococcus aureus*. Na microdiluição, o DF (concentração mínima inibitória = 125 µg / mL) foi mais ativo. Para todas as amostras, a Concentração Bactericida Mínima foi> 1000 µg / mL. O fracionamento contribui positivamente com a citotoxicidade, sendo as subfrações S1 e S2 as mais

citotóxicas. A Fração Diclorometano foi a mais ativa para *S. aureus* e com maior citotoxicidade para as células VERO. Provavelmente, a menor citotoxicidade do EE está relacionada à presença de antocianinas que estão presentes na epiderme dos bulbos.

Palavras-chave: *Eleutherine bulbosa*; Catafilos; Isoeleuterina; Citotoxicidade; Atividade antimicrobiana.

Resumen

El uso de bulbos de *Eleutherine bulbosa* para el tratamiento de enfermedades causadas por malaria, ameba y bacterias. Este estudio logró la caracterización botánica, fitoquímica, antimicrobiana y citotóxica de *E. bulbosa*. Para los estudios anatómicos, el material estudiado se fijó en formaldehído - ácido acético y etanol y se tiñó con safranina y azul astra. Para histoquímica, se fijó en formalina neutra tamponada y en sulfato ferroso en formalina. El Extracto Etanólico (EE) se sometió a fraccionamiento en columna cromatográfica y de él se obtuvieron cuatro (4) fracciones. La Fracción de Diclorometano (DF) se sometió a un nuevo fraccionamiento. La actividad biológica se evaluó mediante difusión en agar, microdilución y viabilidad celular MTT. El bulbo de *E. bulbosa* se caracteriza por un eje caulinar reducido y por suculentos catafilos amilíferos, epidermis con presencia de antocianinas, mesófilo homogéneo con idioblastos de cristales prismáticos de oxalato cálcico y compuestos fenólicos. En difusión en agar se observó que EE, DF y fracción de acetato de etilo (ACF) fueron activos para Staphylococcus aureus. En microdilución, DF (concentración mínima inhibitoria = 125 µg / mL) fue más activo. Para todas las muestras, la concentración bactericida mínima fue> 1000 µg / ml. El fraccionamiento contribuye positivamente con la citotoxicidad, siendo las subfracciones S1 y S2 las más citotóxicas. La Fracción Diclorometano fue la más activa para S. aureus y más citotoxicidad para las células VERO. Probablemente la menor citotoxicidad de EE esté relacionada con la presencia de antocianinas presentes en la epidermis de los bulbos.

Palabras clave: *Eleutherine bulbosa*; Catafilos; Isoeleuterina; Citotoxicidad; Actividad antimicrobiana.

1. Introdução

Eleutherine bulbosa (Mill.) Urb. Iridaceae popularly known in brazilian amazon region as “marupazinho”, nambu-tutano and anajai (Matos & Lorenzi, 2002; Coelho-Ferreira, 2009). Native species of tropical America, including the dry fields of Amazon region (Matos &

Lorenzi, 2002). It belongs to the family Iridaceae and it has as a botanic synonymous *Eleutherine plicata* Herb. (Bianchi & Ceriotti, 1975).

Ethnobotanical studies of this species related the following use allegations: antidiarrheal action (Lin, et al., 2002; Coelho-Ferreira, 2009), antiparasitic action specially fora malaria, amoeba and giardy (Schultes & Raffauf, 1990; Mors, et al., 2000; Martins, et al., 2005; Pinto & Barbosa, 2009), antifungal action (Alves, et al., 2003) and antibacterial action (Ding & Huang, 1983; Panda, et al., 2016; Santos, et al., 2020). Ifesan, et al. (2009) studied the antibacterial activity of ethanolic extract obtained from bulbs of *Eleutherine americana* in 14 strains of bacteria, 6 strains of fungi and 2 strains of yeasts. This extract presented activity against all Gram positive bacteria, besides that, it was active against fungi *Aspergillus niger, Rhizopus.* spp, *Penicillum.* spp, while all dermatophyte fungi and yeasts tested showed resistance.

Isoeleutherol and isoeleutherin have already been isolated from the ethanolic extract obtained from bulbs of *E. bulbosa* (Malheiros, 2008). Eleutherin, under concentrations of 50 e 25 µg/ mL, inhibited 87% of the production of melanin (Kusuma, et al., 2010). This substance also presented antitumor activity through inhibition of topoisomerase II (Krishnan & Bastow, 2000).

The objective was to accomplish the anatomic characterization of the bulbous cataphylls of *E. bulbosa*, its phytochemical analysis, its antibacterial activity, its cytotoxic potential and the influence of its fractionation on the tested microorganisms.

2. Metodologia

This work used the experimental scientific model of comparison with positive and negative controls of biological activities in vitro(Pereira, et al., 2018).

2.1 Obtainment of vegetal material, of ethanolic extract and of fractionation.

The bulbs of *E. bulbosa* were colected in September of 2012 in a place called Vila Fátima, County of Tracuateua - Pará, Brazil, Road BR 318, Lat. 1.1436°, Longitude. 46.95511°, Altitude 88 feet. The testimony sample obtained the registration MG. 202631 in the Herbarium of Museu Paraense Emílio Goeldi.

2.2 Anatomical Studies

After being washed in running water, the bulbs were fixed in glutaraldehyde 2,5% phosphate buffer 0,1 M - pH 7,2 (Gerlach, 1977), AAF – acetic acid and ethanol 70 °GL (Johansen, 1940), FSF (ferrous sulphate in formalin) and NTF (neutral buffered formalin). After its fixation, the material was gradually dehydrated in tertiary ethyl and butyl alcohols (Johansen, 1940), included in histological paraffin (Johansen, 1940), sectionated in a rotating microtome with thickness of 12 µm, dyed with astra blue and alcoholic safranin (Braga, 1977) and mounted in synthetic resin. The material was analysed in a photonic microscope. The results were registered by fotomicrographs prepared with support of a Canon digital photographic camera connected to a model AXIOLAB Zeiss microscope.

For the achievement of histochemical tests, fresh cuts and pre-fixed ones in NTF and in FSF and freshly detached epidermis were used. They were submitted to the following dyes and reagents: Astra blue (cellulose), basic fuchsin (cutin, suberin), lugol (starch), ferric chloride, FSF (phenolic compounds) and safranin (lignin, cutin, suberin).

2.3 Phytochemical Analysis

The bulbs were washed in running water, kept in forced air circulation oven at 40º C for a week and, then, the dry material was submitted to milling in a knife mill. The ethanolic extract was prepared from its powder by maceration, followed by concentration in a Rotary evaporator up to residue.

The ethanolic extract (EE) obtained from the bulbs of *E. bulbosa* (5g) was fractionated by an open chromatographic column, silica gel was used as a stationary phase (35-70 mesh) as well as solvents of increasing polarity (hexane, dichloromethane, acetate and methanol). The fraction dichloromethane (DF) was submitted to chromatography in a thin preparative layer, with silica gel for chromatography in a thin layer (Borges, 2012) and eluted with dichloromethane, being obtained 3 diferent subfractions. These subfractions were analysed in spectrophotometry of TLC and NMR.

2.4 Antimicrobial Activities

The microbial suspensions used for the test of diffusion in paper disc were obtained as from the cultivation of 24 hours in Müller Hinton broth from the following strains:

Staphylococcus aureus (ATCC 25923), *Escherichia coli* (ATCC 25318); *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 14053), diluted in saline solution, according to 0,5 of Mac Farland scale which corresponds approximately to (1 a 2 $x10^8$ CFU/mL; Bauer, et al., 1996).

The test was accomplished in triplicate, distributing the microbial suspention for all the surface of media of Agar Müller Hinton and Sabouraud with the support of sterile *swab*. The 6 mm diameter paper discs (NM 618) were prepared by impregnation of 10 µL of ethanolic extract and fractions (500 µg/disc). For the negative control, paper discs were impregnated with 10 µL of DMSO and for the positive control of the strains of *C. albicans,* paper discs were impregnated with solution of nystatin (120 µg/disc) from a suspension containing 100.000 UI/mL. For making of strains of *E. coli* and *S. aureus* chloramphenicol in concentration of 30 µg was used and for strains of *P. aeruginosa,* gentamicina in concentration of 10 µg was used, both were commercially acquired.

The discs were distributed for all the surface of the media with the use of tweezers, and the plates were taken to incubation at 35°C/24 hours for bacteria and 30ºC/48 hours for the fungus. After the period of incubation, the halo of inhibition formed around the discs containing the strata was measured with the use of pachymeter (including the diameter of the disc; Kartal, et al., 2003; CLSI, 2012).

For determination of MIC the method of dilution in broth was used, according to recommendation by CLSI (2012) with modification in microplates of 96 wells for extract that presented antimicrobian activity in difusion test in agar. The extracts were solubilized in DMSO 10 % (2 mg/mL) and the concentrations of extracts obtained after serial dilutions resulted in concentrations that varied from 1000 to 31.5 µg/mL (CLSI, 2012).

For the preparation of inoculum from 3-4 colonies of cultivation of 24 hours in saline solution were used, corresponding to the 0,5 Mac Farland scale approximately equivalent 1.2 x 10^8 CFU/mL, being this one adjusted to 10^6 CFU/mL.

The plates were prepared by putting an aliquot of 10 µL of each extract in the concentrations of 1000, 500, 250, 125, 62,5, e 31,25 µg/mL in the wells containing Müller Hinton broth and microbial suspension for a final volume of 200 µL/mL in each well (CLSI, 2012) and incubated at 35ºC/24 h. After incubation, in all wells 10 µL of MTT were added and again incubated for 4 hours at maximum with the objective of verifying, through visual reading, the wells where there was microbial growth.

The acceptance criteria of antimicrobial activity of vegetal extracts were, according to Holetz, et al. (2002), that is, it was considered active MIC $\leq$ 100 µg/mL. For determination of

MBC 10 µL of equal and superior concentrations to MIC was withdrawn, seeded in Petri plates containing Agar Müller Hinton and incubated at 35º C for 24 hours. To calculate the minimum bactericidal concentration (MBC), samples were colected in equal and superior concentrations to MIC. These samples were seeded in plates containing Agar Müller and it was observed if there was bacterial growth or not.

2.5 Cytotoxic Potencial

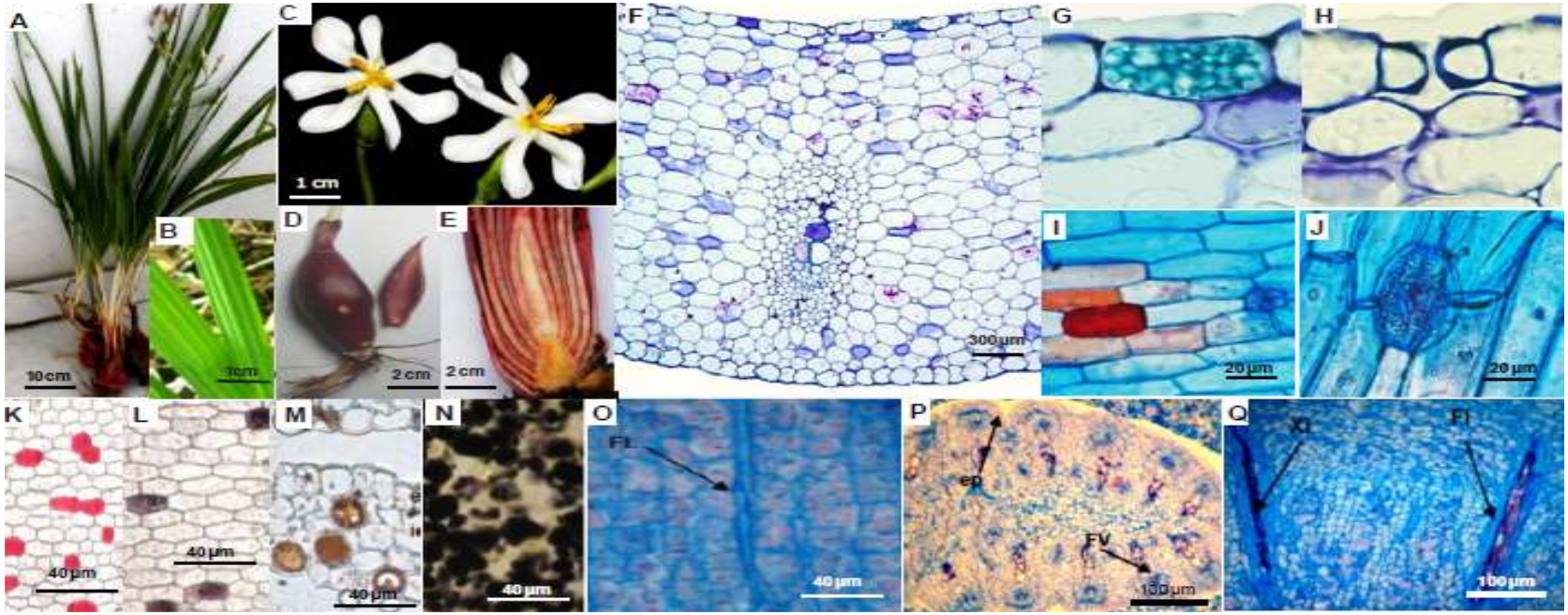
The test of cell viability was developed according to Mosmann (1983). With the use of plates of 96 wells, VERO cells were distributed ($8x10^3$ cells/mL medium DMEM supplemented with 10% of FBS). The plates were incubated at 37°C in humid atmosphere with 5% of CO_2. After 24h of incubation, the treatment with five growing concentrations (12.5, 25, 50, 100 and 200µg/mL) of Ethanolic Extract (EE), Dichloromethane Fraction (DF), and of subfractions S1 and S2 of *E. bulbosa* and of positive control with NMU (N-metil-N-nitrosurea). After 24 hours of exposition, the supernatant part was discarded and the MTT was added (bromide of [3-(4,5-dimethylthiazole-2-il) -2,5-diphenyltetrazolium]); at a concentration of 500µg/mL. The absorbances of the wells were read in a scanning spectrophotometer of multiple wells, using a wave-lenth at a 570 nm reference. The values of CC_{50} (50% cytotoxic concentration) were calculated with the use of dose response curves from three independent experiments (Brandão, et al., 2020).

3. Resultados e Discussão

3.1 Botanical Studies

Eleutherine bulbosa perennial herb, bulbous, has simple, parallel-veined leaves, with elongated limb, about 30 cm long, cylindrical peduncles, white flowers with inferous ovary and three stamens opposite to petals (Fig. 1A-C). During cultivation this species presented teratological stamens and variations in number of petals. According to taxonomist guidance, those changes are frequent in cultivated plants, but they must be considered during the species identification (Fig. 1C). Their bulbs are made by several layers of overlapping modified leaves similar to onions, denominated cataphylls, they are wine-coloured and exude white latex when they are cut (Fig. 1D and 1E).

Figure 1. *Eleutherine bulbosa* (Mill.) Urb. A- Habit; B- Parallel-veined leaves; C- Variable petals and stamens; D– Bulb e cataphyll; E- Longitudinal section of the bulb; F- Longitudinal section of the cataphyll; G e H- Stomata and idioblasts of phenolic compounds in epidermis; I, J, K e L – In frontal view, the epidermis with stomata, idioblasts with vacuoles containing a pink substance, suggestive of anthocyanins, besides reacting positively to the presence of phenolic compounds (brown coloured), when they are treated with ferric chloride; M- Epidermis and mesophyll with idioblasts of phenolic compounds of brown color evidenced by CFF; N- Mesophyll with starch grains evidenced by Lugol's solution; O- mesophyll with libriform fibers; Stem: P – transversal section; Q – longitudinal section. ep: epidermis; Fl: phloem; XI: xylem; FV: vascular bundle.



Source: Authors.

The cataphylls epidermis, in transversal section, is constituted by rectangular cells, covered by a thin cuticula with a discreet difference in their formats: cells of adaxial surface are laterally more elongated, while the cells of abaxial surface are more rounded (Fig. 1F). These modified leaves, the cataphylls, present stomata and idioblasts in adaxial and in abaxial epidermis as well (Fig. 1F-1J). In front view, the cells are polyhedral, heterodimensional, with straight anticlinal walls (Fig. 1I-J and 1K-L). The stomatal cells are involved by four (predominant), five and six subsidiary cells, that don't differ in format and in size from the other epidermal cells, characterizing them as of tetracytic, pentacytic and hexacytic types (Fig. 1F-1J). Among monocotyledon plants, excpt in Poaceaes the stomata are diacytic ones. Anomocytic stomata were found in leaf blade of species of Tigridieae, among them *Eleutherine latifolia* (Rudall, 1990).

It can be seen in adaxial and in abaxial epidermis as well, idioblasts with vacuoles containing a pink substance, suggestive of anthocyanins, besides reacting positively to the presence of phenolic compounds (brown coloured), when they are treated with ferric chloride (Fig. 1I, 1K and 1L). The presence of tannins in its extract can be responsible for antimicrobial activity of the plant (DJIPA, et al., 2000). And in a study with leaves of *Eleutherine plicata* Herb (marupazinho) reducing sugars, phenols and tannins, steroids and terpenoids, azulenes, carotenoids, depsides and depsidones and derivatives of cumarina were detected. Alves et al. (2003) related the presence of naphtoquinones in extracts prepared from bulbs of marupazinho. Anthocyanins are largely used as natural dyes and they have great pharmacological value due to antioxidante action, protective activity against DNA damages (Ojeda, et al., 2010).

The mesophile of the cataphylls is undifferentiated and it's formed by several layers of parenchimatical cells that are predominantly spherical, heterodimensional, similar to idioblasts of phenolic compounds and of amyliferous reservation content, being possible to see the radial hilum of starch grains (Fig. 1F, 1M, 1N and 1Q). Still in mesophile, frequent idioblasts of crystals of calcium oxalate were seen, of a prismatic format (Fig. 1N). In general, crystals are useful as structural support, protection against herbivorous animals and maintenance of ionic balance, by storage of calcium oxalate in idioblasts (Franceschi & Horner-Junior, 1980). The differences between presence or absence of these crystals constitute an important taxonomic feature (Prychid & Rudall, 1999). In Iridaceae specimens, most present crystals of styloid type (Goldblatt, et al., 1984; Wu & Cutler, 1985; Wolter, 1990; Rudall, 1994, Rudall 1995).

Still in mesophile, in longitudinal section, not lignified fiber of libriform type dispersed for all over the amiliferous parenchyma can be seen. (Fig. 1O).

The vascular bundles are of distinct calibres in which the xylem circles the phloem characterizing as if anphyvasal type, whose tracheal elements of xylem have helicoidal type thickening (Fig. 1P). The anphyvasal type is seen in some monocot specimens as *E. bulbosa*, even about in family Iridaceae there had already been related that diferentiated bundles present anphyvasal arrangement (Rodrigues & Estelita, 2009).

A series of histochemical tests demonstrated the heterogeneous nature of the contitution of cataphylls, confirming the presence of starch grains, in parenchymatous cells of mesophyll (Fig. 1N). These ones in contact with FSF evidenced phenolic compounds of brown colour, as well as, in its epidermis, it was detected the presence of pink anthocyanins (Fig. 1I and 1K). The justification for the usage of bulbs in treatment of microbial afections is that it may be associated to the presence of those phenolic compounds that, according to Trigui, et al. (2013) they have antimicrobial efect due to its capacity of braking the integrity of microbial membrane when it links to peptidoglycan. They can also generate hydrogen peroxide during the auto oxidation of tannins and the privation of substrates necessary to microbial growth. In their turn, anthocyanins are known for their powerful antioxidant capacity, their capacity of regulating the abnormal lipid metabolism, of inhibiting the oxidation of lipoproteins of low density and diminishing the lipid levels in blood (Jiang, et al., 2017).

On the stem, in transversal section, the root distal region presents unstratified epidermis with epidermal cells of rectangular format (Fig. 1P). You can see adjacent to epidermal cells, spherical parenchyma cells, heterodimensional ones that acumulate numerous starch grains. In the root proximal region, you can see a radial parechyma constituted by two types of cells: the adjacent and the distal to epidermis ones. The proximal to epidermis cells are spherical, isodiametric and full of starch grains. The distal ones are more tabular presenting a bigger dimension in radial direction, being arranged in parallel to vascular bundle characterizing a parenchyima as being of radial type.

On the stem, the vascular bundles are dispersed in fundamental parenchyma, characterized as being of anficrival type, that means, phloem externaly disposed to xylem. The vascular bundles are radially dispersed cross parenchyima in two ways: phloem e xylem transversely interlaced and phloem going around the tracheids of xylem (Fig.1P and 1Q). In the most central region of the stem, the vascular bundles are randomly distributed for all the region, interleaved by spherical cortical parenchyma cells (Fig.1P).

3.2 Phyitochemical Studies

The TLC analisys of EE and DF showed three bands yellow, suggesting it to be naphthoquinone. From some species of *Eleutherine*, the following 1,4-naphthoquinones have already been isolated: eleuterin, eleuterinone, isoeleuterin. A previous study carried out by the group led to the isolation of the dichloromethane fraction, of the eleuterine and isoleuterine naphthoquinones. It also demonstrated that antimalarial activity is related to these metabolites (Vale, et al., 2020).

The fraction S2, probably is isoeleutherin, a naphthoquinone already isolated from *E. americana* Mer (Hara, et al.,1997); *E. bulbosa* Mill (Alves, et al., 2003) and *E. bulbosa* (Malheiros, 2008). The spectrum of NMR^{1}H of S2 has characteristic signs of aromatic compounds, that corroborates with the data obtained by the spectrum of UV and with data of literature. In the region of aromatic compounds there are the signs in δ 7,75 (dd, J = 7,5 and 1,2 Hz) attributed to H 6; δ 7,65 (dd, J = 8,1 and 7,5 Hz) attributed to H 7 and δ 7,28 (dd, J = 1,2 and 8.1 Hz) attributed to H 8. The data of NMR^{1}H of S2 were compared with the literature (Malheiros, 2008) and they showed total similarity to isoeleutherin.

Studies in NMR^{1}H of subfraction S3 suggest it deals with naphthoquinone, but it was not possible to determine its chemical structure. Due to the small quantity of S3 avaiable, it was not possible to make the NMR^{13}C.

3.3 Antimicrobial Activity

For this preliminary study, the ethanolic extract and its fractions were tested only in the concentration of 500 µg/mL. This extract inhibited the growth of *S. aureus* (inhibition halo =16.19 mm ± 0.13), but it showed up inactive for *E. coli*, *P.aeruginosa* and *C. albicans*. In another study, There was inhibition of *S.aureus* by EE in the same concentration (Ribeiro, 2008).

Table 1. Determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and relation between MBC/MIC of EE of bulbs of *E. bulbosa* and its fractions.

Samples	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC
EE	500	> 1000	> 2
ACF	250	>1000	>4
DF	125	>1000	>8
S2	250	>1000	>4
S3	250	>1000	>4

Legend: EE: ethanolic extract; ACF: ethyl acetate fraction; DF: dichloromethane fraction; S2 and S3: subfractions. Source: Authors.

The fractions DF and ACF inhibited the growth of *S. aureus* (inhibition halo = 18.05mm ± 1.62; 12.66 ± 1.92, respectivelly), but they haven't presented activity for *P. aeruginosa, E. coli and C. albicans*. The MF (methanolic fraction) hasn't inhibited the growth of any microorganism. The productivity of HF (hexane fraction) hasn't permited the achievement of any test.

A previous study demonstrated that DF had, predominantly, naphtoquinones, in particular isoeleutherin (Malheiros, 2008). So, the initial hypothesis was that the antimicrobial activity of this fraction was related to naphtoquinones. That fraction was submitted to fractionation, being isolated three naphtoquinones and spectrophotometric studies permited to confirm that S2 was isoeleutherin.

The EE and its active fractions and naphtoquinones were submitted to avaluation of inhibitory activity on *S. aureus* by the use of microdilution technique. The EE (MIC= 500□g/mL), ACF (MIC= 250□g/mL) and DF (MIC = 125 □g/mL) presented moderate antimicrobial activity (Holetz, et al., 2002). Isoeleuterine and another naphtoquinone presented a MIC of 250µg/mL (Table 1).

The fractionation of EE contributed positively for antimicrobial activity, due to DF present a higher activity. However, the fractionation of DF contributed negatively to the activity, due to the isolated substances have a higher MIC (Table 1). Rios, et al. (1987) suggest that the antimicrobial activity of vegetal extracts occurs by conjunct action of chemical compounds present in plants, not by activity of isolated compounds, what was demonstrated in this work.

With the purpose of investigating the bactericidal potencial of the active samples (EE, DF, S2 and S3) the MBC was determined. For all samples tested it was observed the reversibility of inhibitory efect (MBC >1000 □g/mL). That is, in the absence of the samples,

bacteria restarted to grow significantly (Table 1). This fact suggests that the efect promoted by EE, DF, ACF, S2 and S3 is of bacteriostatic type.

Another interesting fact was the relation MBC/MIC. The fraction with the highest antimicrobial activity (DF), was also the biggest difference between MBC/MIC=8. When the relation MBC/MIC was done, it was observed that in DF, ACF and pure substances it was superior to the one of EE (Table 1), suggesting that probably the activity against the strain of *S. aureus* is a bacteriostatic one.

Several antomicrobial agents, used to treatament of bacterial infections, have bacteriostatic activity, but this kind of response can cause therapeutic failure in immunosuppressed pacients. The association of antimicrobial agents must be done, preferably, with bactericidal drugs.

3.4 Cytotoxicity

Although four fractions have been obtained in the phytochemical study of ethanolic extract, the option made was for the evaluation of cytotoxity only of the fraction of dichloromethane and its fractions. Such fact is due to information obtained in other studies (Borges, 2012; Veiga, 2013) where the biological activity was observed in this fraction and in its subfractions. Also, with the aim of evaluating whether the fractionation contributs in the toxidity, the extract EE was included in the study of cytotoxidity.

The EE was submitted to evaluation of toxidity with the use of VERO cells, where it was observed that the cytotoxic concentration 50% (CC_{50}) was of 28.71 µg/mL $\pm$ 1.054. A former study evaluated the cytotoxity of EE, with the use of the same methodology, in cells of lineage HepG2 A16, where a CC_{50}= 61,55µg/mL was obtained, being considered little cytotoxic, but it was observed that the Reading of MTT was accomplished with a filter of 570 nm, and background in 630 nm (Silva, 2012).

In a similar way the cytotoxity of DF was evaluated where a CC_{50} = 26.19 µg/mL $\pm$ 1.670 was obtained. When the obtained result for EE is compared in relation to DF, the growth of cytotoxity is observed. This growth may be related to the fact of the DF contains more concentration of naphtoquinones. It is known that the cytotoxidity of naphtoquinones may be related to production of superoxid radicals (Dubin, et al., 1990).

The evaluation of cytotoxity of subfractions of DF resulted in CC_{50} = 14.36 µg/mL $\pm$ 1.12 for S1 and in CC_{50} = 18.67 µg/mL $\pm$ 1.25 for S2. The fractionation of EE and of DF contributed positively for cytotoxic activity, due to the CC_{50} DF and its fractions decreased,

therefore, it was able to destroy 50% of cells VERO in lower concentration when it was compared to CC_{50} of EE.

The antimicrobial and antiparasitic activity of extracts and fractions of naphtoquinones-rich plants has been related to the redox potential of these components, in other words, naphtoquinones arre involved in biological oxidative processes. Also, the cytotoxic efects of many quinones are usually associated to the reduction of an eléctron that results in semiquinones. Those semiquinones get in a redox cicle with molecular oxygen producing ROS and producing oxidative stress (Thor, et al., 1982); semiquinones as well as ROS can generate hydroxyl radical that causes a breaking in DNA strand. Another fator of cytotoxity is the inhibition of topoisomerase II (Verma, 2006). In summary, the growth of cytotoxity of subfractions of DF (fraction naphtoquinone-rich) may be related to oxidative stress.

4. Conclusion

It was concluded that the phenolic compounds are located on the epidermis and on the mesophile of bulbs cataphylls. Phytochemistry sutdy identified the naphtoquinone isoleutherin. It was observed that EE, DF and ACF were active against *Staphylococcus aureus*, without having significant antimicrobial activity against the other tested microorganisms. The fractionation of the ethanolic extract *E. bulbosa* led to a fraction (DF) with a higher antimicrobial activity and a higher cytotological potential. A subsequent fractionation doesn't contribut for antimicrobial activity, but it increased the cytotoxity in VERO cells.

In order to clarify the toxic potential of EE, fractions and naphthoquinones, *in vivo* studies of acute and chronic toxicities are necessary, as well as studies of genotoxicity, mutagenicity and carcinogenicity. In addition, studies aimed at identifying possible mechanisms of action in bacteria are important

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