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EVALUATION OF THE BIOLOGICAL EFFECTS OF AQUEOUS EXTRACTS FROM THE LEAF OF *BAUHINIA BLAKEANA DUNN*

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ABSTRACT

The use of plants as an object of study in the area of concentration in environmental toxicology rekindled the importance of the use of bioassays that allow the evaluation of the real actions attributed to these plants, as well as the exposure of the population to the active principles that constitute such specimens. Bauhiniassp, a plant belonging to the family Leguminosae, commonly known as the "pata-de-vaca" (cow leg), is one of the 71 species of plant related in Renisus, since it is attributed to this genus actions hypoglycemic, diuretic, hypocholesterolemic and other disorders Still in research and discussion. As most of these disorders are related to dysfunctions associated with oxidative stress and cellular damage, the aim of the present study was to evaluate the antioxidant, antibacterial activities from aqueous extracts of leaves of Bauhinia blakeana. Leaves were collected, processed and extracted by infusion, which was used in three different strains of Escherichia coli by the disc diffusion method. A 0.9 % NaCl solution was used as the negative control. For antibacterial evaluation the positive control was attributed to antibiotics and in the antioxidant activity to stannous chloride and hydrogen peroxide. Assuming that B. blakeana is a hybrid of two other species of the genus, the results obtained did not corroborate all the results found in the literature, but such results contribute to the identification and qualification process.

KEYWORDS: Bauhinia blakeana; Escherichia coli; Aqueous Extract; Phytotherapy; Toxicology.

INTRODUCTION

The use of substances of the nature to assist man is quite old. Since the earliest times, plants, animals and chemical elements are part of the medicines produced and used by man.[1] The World Health Organization says during the International Conference on Traditional Medicine for the countries of Southeast Asia, that, for millions of people, a Traditional and Complementary Medicine is the main or only source of health care.[2] In 2005, the Secretariat of Science and Technology and Strategic Inputs (SCTIE), through the Department of Pharmaceutical Assistance and Strategic Inputs (DAF/SCTIE/MS), developed, in partnership with other ministries and with consultant collaborations and researchers, a list of plant species considering those already used in state and municipal health services, compiling traditional and popular knowledge and available chemical and pharmacological studies. Second Nunes et al. (2009)[3] this document subsidized in 2008 the elaboration of the National Relation of Medicinal Plants of Interest to the Unified Health System (Renisus), with the purpose of Renisus to subsidize the development of the entire production chain, including actions that will be developed by others Ministries participating in the National Program of Medicinal and Phytotherapeutic Plants, related to the regulation, cultivation, management, production, commercialization and dispensation of herbal and phytotherapeutic plants. It will also have the function of guiding studies and research that may support the development of Renafito (National Relation of Medicinal Plants and Phytotherapics), the development and innovation in the area of medicinal and phytotherapeutic plants.[4] The Leguminosae family, which belongs to the genus *Bauhinia ssp* is one of the largest botanical families of interest due to reports of their genera being used in traditional medicine. Composed of about 18.000 species in 619 genera, occupying the place of third largest

family, it is divided into four major subfamilies: *Caesalpinioideae*, *Cercideae*, *Faboideae*, *Mimosoideae*.[5] Natural Asia, *Bauhinia ssp*, had its development on lands with typical tropical climate well consolidated.[6] The genus Bauhinia comprises about 300 species distributed on the planet,[7] But only in Brazil have been described more than 200 names, according to Vaz & Tozzi (2005).[8] With several popular names and due to the format of the leaves it is known as: "pata-de-vaca", "casco-de-vaca", "unha-de-boi", "pata-de-boi".[9] Found in Renisus because of the publications regarding medicinal applications, the genus Bauhinia presents diuretic, hypoglycemic, tonic, depurative and in the fight against elephantiasis,[10] analgesic, antidiabetic, anti-inflammatory, antimicrobial, astringent[11,12,13] anti-infective,[14] for the treatment of stomach swellings and tumors, diarrhea and ulcer,[15] The antioxidant activity attributed to the aqueous, ethanolic and butanolic extracts of leaves of *Bauhinia forficate*,[16,17] In addition to the various uses mentioned above, there are decorative and food applications. And although it is among the exotic species of ornamental use and there are few studies on the use for medicinal purposes, *B. blakeana* Dunn is used by the population mainly for this purpose.[18] *Bauhinia blakeana Dunn* is a much sought-after tree as ornamental because of the size and beauty of its flowers. It was described as a new species in 1908 after being discovered off the coast of Hong Kong Island. When brought to Brazil it was fully adapted to local climatic conditions and it is now possible to find it grown in the southeast region, where it is known by the name of "pata de vaca" (cow leg) and considered to have, like other nearby species, Name, hypoglycemic properties. The Brazilian population usually uses the species as a therapeutic alternative in the treatment of diabetes, when consuming their leaves in the form of "tea".[19] Also known as tree-orchid, it is sterile and has been considered a hybrid, possibly from two related species, *B. purpurea* and *B. variegate*.[20] Due to the easy recognition of the plant by the leaf shape and abundance of the genus in different places, the propagation of medicinal use among the communities became indistinct, leading to a disordered use of many species without previous chemical knowledge of the substances present within the material Consumed,[21] whether in the form of tea, infusion or individual parts of the plant applied to the place of infirmity. And despite the recognized ornamental potential of the *Bauhinia genus*, due to the attractive nature of its flowers, it also excels in medicinal, food and timber applications.[22,23] Species of this genus are used in folk medicine in various regions of the world, such as Africa, Asia and Central and South America, in the treatment of various diseases.[14] Among the various reports of popular use of the species of *Bauhinia*, we highlight the use as astringent, analgesic, antimicrobial, antioxidant, antiparasitic, antirheumatic, hypocholesterolemic, diuretic, antidote for venoms and scorpion bites, as well as use against asthma, gastrointestinal disorders, ulcers and wounds, pain and inflammation, and especially diabetes.[24,14,22,25,26,27] The antidiabetic action of different fractions of the leaf extract of *Bauhinia candicans Benth* was evaluated by Fuentes et al. (2004).[28] Treatment significantly reduced blood glucose levels, indicating a more pronounced activity. These results suggest that extracts of *B. candicans* increase glucose metabolism and may contain active principles with hypoglycemic properties.[29] However, in folk medicine, the use of the leaf tea of *Bauhinia forficata* plants for the treatment of this disease is quite common, being this custom passed from generation to generation.[30,31,32] In the literature the hypoglycemic action is among all the therapeutic actions presented by the genus *Bauhinia*, the most reported and investigated. However, divergent results were also found,[33] in which ethanolic extracts of *Bauhinia forficata* did not demonstrate anti-diabetic activity in rats, this species being considered as the "true cow's leg" because it has more studies proving this effect.[14] The aqueous extract of the species Bauhinia divaricata showed a significant effect in rats referring to the hypoglycemic activity test.[34] Thus, since the initial studies, and over time have been studying other species from different locations and used for the same hypoglycemic purpose. Other species investigated, *Bauhinia monandra*, *Bauhinia megalandra* and *Bauhinia cheilantha* also presented hypoglycemic action in rats induced to diabetes, where in some cases the reduction of the hepatic production of glucose and its intestinal absorption.[25] Some studies further explore the rationale for such activities and relate them to the presence of flavonoids isolated from *Bauhinia megalandra* that did not present inhibitory activity on glucose-6-phosphatase, the enzyme responsible for glucose hydrolysis, but on the transporter of this enzyme, proposing a new medium to the mode of hypoglycemic activity.[35] Studies of the pharmacological action of the ethanolic and aqueous extracts of *B. variegata* presented positive results in the decrease of the glycemic rate, as well as of the cholesterol,[36] insulinotropic activity (insulin secretion) in cell lines tested,[37] ethanolic extracts showed chemopreventive activity and cytotoxic effects on mouse liver tumors and cancer cell lines of human origin.[38] As for isolated compounds, the flavonoid rhamnocitrin, has strong antioxidant effects, which can be used in the regulation of cataract;[39] has also been reported with the same antimicrobial action.[40,41] We have reported in the literature the antibacterial activity against S. aureus and E. faecalis from the methanolic extract of B. purpurea leaves,[15,42,43] and in the aqueous and ethanolic extracts of the leaves of *Bauhinia tomentosa*.[44] This statement is also described by Negi et al, (2012),[45] which found activity of the methanolic extract obtained from leaves of *Bauhinia purpurea* only against *S. aureus* and *Bacillus subtilis*, Gram-positive, and negative result against *E. coli*, *Salmonella typhi* and *P. aeruginosa*, Gram-negative. The literature also attributes to *B. purpurea* inhibitory activities against the growth of tumor cells.[46,47,48] Chen *et al*. (2010)[49] affirms the popular use in China of *Bauhinia blakeana* in the treatment against rheumatic pains, rickets and thrombocytopenic purpura.

These data show the medicinal values of this species classified as exotic in Brazil (LORENZI & MATOS, 2002).[18] The most varied species of the genus are organic compounds of different classes and medicinal interest, such as lactones, flavonoids, terpenoids, tannins, quinones[14] and alkaloids.[7] Other compounds, of the class of metabolites found in B. purpurea, were the amino acids and oxepines, a very rare class found in nature, to which they were linked to excellent inhibitory activities against the growth of tumor cells, little biological activity reported in compounds isolated from the genus.[46,48] Below Table 1 with compounds isolated from the species *B. purpurea* and B. Variegata, the putative parents of *B. blakeana*.[5,14]

Table 1: Isolated compounds of the species B. purpurea and B. variegata.

Class / Species	*B. purpurea*	*B. variegata*
Amino acids	Aspartic acid; Threonine; Serine; Glutamic acid; Proline; Glycine; Alanine; Valine; Methionine; Isoleucine; Leucine; Tyrosine; Phenylalanine; Histidine; Cysteine; Lysine; Tryptophan	-
Dihydrodibenzoxepine	Bauhinoxepin J	-
Steroids	-	Sitosterol
Flavonoids and derivatives	-	Ombuina
Glycosylated flavonoids	Astragalin; Routine; Quercetin; Isoquercitrin; Kaempferol-3-galactoside	Kaempferol-3 galactoside; Kaempferol 3,7-ramnoglucoside; Hesperidin; 5,6-dihydroxy-7-methoxyflavone-6-O-β-D-pylopyanoside; Narigenin-5,7-dimethoxy-4-rhamnoglucoside
Lactone	-	Grifonilide
Oxepines	Bauhinias - tatinas1-4	5,6b dihydro-1,7-dihydro-1,1-dihydroxy-3,4-dimethoxy-2-methyldibenzene
Triterpenoids	-	Lupeol

MATERIALS AND METHODS

Collection and extraction

The botanical material of *Bauhinia blakeana* was collected according to the SISBIO guidelines, at the Roberto Burle Marx site in Barra de Guaratiba - RJ (23º01'-23.7 "S and 43º32'-50.0" W), during the month of January / 2016, In the publication of Harri Lorenzi and Luiz Emygdio Mello Filho, entitled Tropic Plants of R. Burle Marx (2001)[50] (Figure 1). During the collection, branches were removed from the tree in different regions of the crown, which were taken to the laboratory for selection. The care was focused on keeping the leaves as unblemished as possible, and then proceeded to the hygiene. After the leaf collection, which was guided by site managers, the material was taken to the Laboratory of chemical and biological analysis/ LAQB, in the Foundation State University Center of the West Zone/ UEZO. Where the selection, hygiene, grinding and subsequent extraction were carried out. At this stage, the sheets were washed in running water and kept in a clean vessel to drain, thereby eliminating excess water. The extracts were obtained by infusion, that is, by the addition of the crushed to the distilled water at 100ºC, in the proportion of 50 g of extract to 500 mL of water. The weighing was performed on a Bel Engineering semi-analytical scale, model 5202. The mixture was kept in closed flasks for 60 minutes to extract the water-soluble compounds and after that period were vacuum filtered. The aqueous extract obtained then had the concentration of 0.1 $g.mL^{-1}$. The extract was then divided into aliquots which were kept in a freezer until the time of use.

Figure 1: Bauhinia blakeana flowers. Source: Tropical Plants R. Burle Marx (2001).

Bacterial strains evaluated

The tested bacterial line of the Escherichia coli species was: AB 1157, wild, proficient in all DNA repair genes, BW 9091, mutant of the xthA gene, whose product, exonuclease III acts in the repair of oxidative lesions of the DNA in phase Exponential growth,[51] and ATCC 25922, β-lactamase negative.[52]

Diffusion in disk

The evaluation assay was performed using bacteria of the species *Escherichia coli*, strains AB 1157, ATCC 25922 and BW 9091. According to the scale of 0.5 McFarland the bacteria were diluted in 5 mL of sterile physiological solution at a concentration of 0.9 % Sown with the aid of a swab. In a biological safety booth (Pachane - Model Pa 400 - ECO), inoculation was performed using a sterile swab embedded in the bacterial solution and passing smoothly and uniformly on the surface of the agar in three directions. Prior to the application of the discs, the seeded plates were left on the stand for approximately five minutes, to allow excess moisture from the surface of the agar to be absorbed. The discs were applied using a sterile forceps to avoid contamination. All discs were gently pressed into full contact with the surface of the agar. The distances of 30 mm between one disc and another and 15 mm of the edge of the plate were maintained, preventing the overlapping of inhibition halos,[52] see representation in Figure 2.

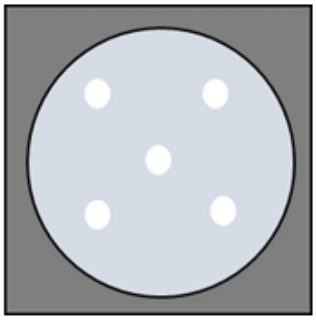


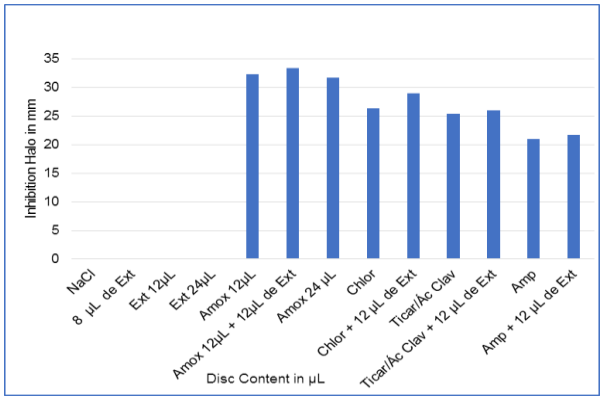
Figure 2: Representative image of agar diffusion technique.

In the plating we used 150 mm petri dishes containing 50 mL of agar medium, 15 plates were prepared, separated into 5 groups, all in triplicate. Following the representation of Figure 2, each disc was added with five discs, except those of the last group, in which only 3 discs were positioned, making a total of 23 disks.[53] In the first plate, five disks were prepared: 24 µL of 0.9% saline solution, 24 µL aqueous extract at 100 $mg.mL^{-1}$, 12 µL aqueous extract at the same previous concentration, 12 µL amoxicillin at 50 $mg.mL^{-1}$ and 12 µL Aqueous extract combined with 12 µL amoxicillin. In a second plaque we only used antibiotics with and without associations: chloramphenicol at 30 $\mu g.mL^{-1}$ (C 30 - OXOID LIMITED), chloramphenicol plus 12 µL aqueous extract, 24 µL amoxicillin, clavulanic acid ticarcillin at 85 $\mu g.mL^{-1}$ (TIM 85-OXOID LIMITED) and ticarcillin/clavulanic acid and 12 µL aqueous extract. We inserted into a third plate five other disks containing the antibiotic ampicillin at 20 $\mu g.mL^{-1}$ (SAM 20 - OXOID LIMITED), ampicillin adding 12 µL of aqueous extract to the plate, 24 µL of stannous chloride ($SnCl_2$), 12 µL of stannous chloride and 8 µL of Stannous chloride. Stannous chloride was prepared at the time of use at a concentration of 5 $mg.mL^{-1}$. The preparation of the fourth plate followed the sequence of disks with 12 µL of stannous chloride plus 12 µL of aqueous extract, 8 µL of aqueous extract, 12 µL of aqueous extract plus 12 µL of 3 % hydrogen peroxide (H_2O_2), 12 µL of hydrogen peroxide plus 12 µL of stannous chloride and a last disc with 8 µL of hydrogen peroxide plus 8 µL of stannous chloride plus 8 µL of aqueous extract. The fifth and last plates were superimposed three discs with 8 µL of hydrogen peroxide, 12 µL of hydrogen peroxide and 24 µL of hydrogen peroxide. At the end of this step the plates were stored in a bacteriological oven (Solab, SL-101) for 24 h at 35°C. In reading the results we used a ruler to measure halos in antibiogram of the brand Laborclin. We finish by treating the results obtained with the program Graph Pad InStat. The means were compared, and the data analyzed from the Variance Analysis (ANOVA) tests and the Tukey-Kramer Multiple Comparison test.

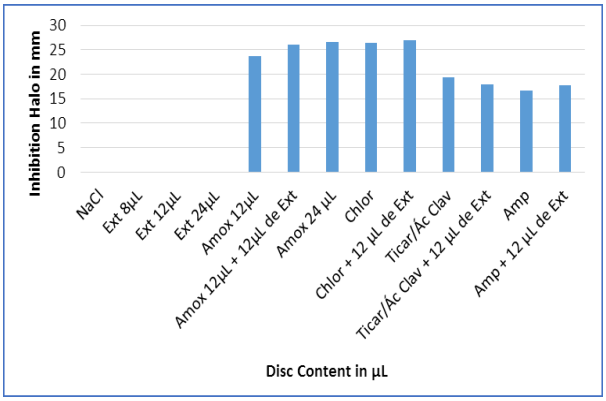
RESULTS AND DISCUSSIONS

Antibacterial Activity

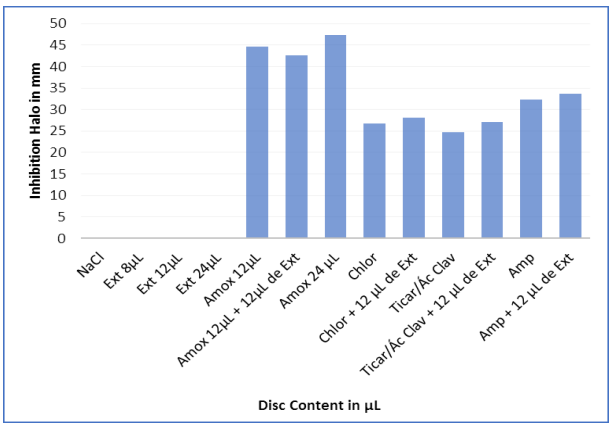
The aqueous extract from Bauhinia blakeana leaves was tested against 3 bacterial cultures by the agar diffusion method. In the literature we find efficient results for antimicrobial activities from organic extracts of the leaf of *Bauhinia splendens* against *Salmonella sp.*, *Streptococcus sp*, *S. aureus* and *S. typhimurium*,[54] methanolic extract of *B. racemosa* stem bark, *Escherichia coli* and other bacteria and fungi,[55] and also *B. forficata* with aqueous and ethanolic extracts from different organs.[56,57] However, *Bauhinia blakeana Dunn* extract showed no efficacy against the bacterium tested, diverging from some of the reports in the literature. However, comparing the results obtained with those described on putative parent species, we can note the similarity with the inefficiency of the *B. variegata* extract,[58] although B. purpura has shown efficacy,[59] both using ethanolic extract of the leaves on cultures of *E. coli*, the target specimen of the present study.



Graph 1: Disk diffusion statistics of strain AB 1157.



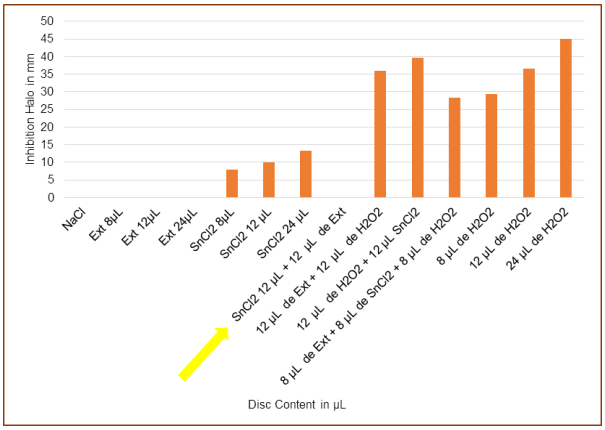
Graph 2: Disk diffusion statistics of strain ATCC 25922.



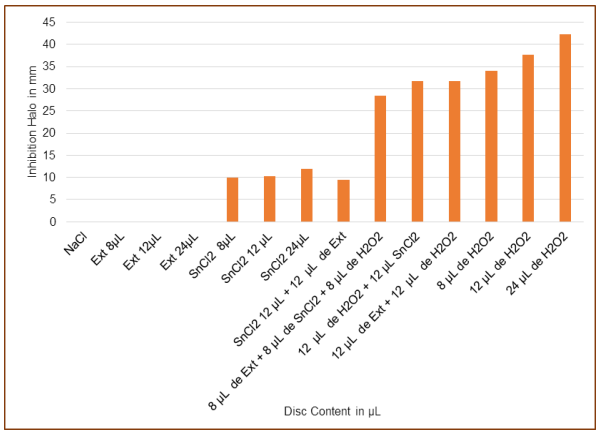
Graph 3: Disk diffusion statistics of strain BW 9091.

Antioxidant Action

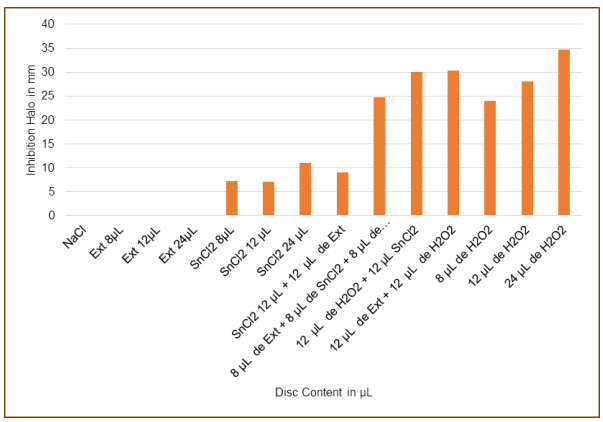
In the present study, the antioxidant action of the aqueous extract of B. blakeana leaves was determined by the agar diffusion method, where the positive controls for the action were stannous chloride ($SnCl_2$) and hydrogen peroxide (H_2O_2). There are in the literature reports of antioxidant activity in experiments performed with methanolic extracts obtained from the stem of *B. racemose*.[55] Another species of this genus was investigated by Argolo et al. (2004)[60] that studying the antioxidant action of the leaves of *Bauhinia monandra Kurz*., Showed that the chloroform and ethyl acetate extracts showed excellent activity. Such positive results were predicted, since studies have been reported on phenolic groups characteristic of the *Bauhinia genus* that have antioxidant activity, and can be affirmed due to the presence of flavonoids.[61,62,63] In the experiments carried out with aqueous extract of the leaves of B. blakeana it was possible to visualize the antioxidant activity when the use inhibited the action of the stannous chloride on the strain BW 9091, whose characteristic is the sensitivity to this effect (Graph 4). It was also observed with respect to the three strains that the concomitant use of the extract plus stannous chloride and hydrogen peroxide did not significantly influence the action of the latter component when compared to the unitary exposure, but potentiated the stannous chloride activity.



Graph 4: Disk diffusion statistics of strain BW 9091.



Graph 5: Disk diffusion statistics of strain AB 1157.



Graph 6: Disk diffusion statistics of strain ATCC 25922.

CONCLUSION

Regarding the evaluation of antibacterial activity, the extract when tested against the three *E. coli* strains showed no inhibition. Simultaneous application of the aqueous extract with the antibiotics tested did not alter the results obtained alone. Such a result suggests that there is no presence of substances potentiating the action of these antibiotics in the extract, such behavior may favor concomitant administration. However, considering the various popular uses of plants of the genus, whose popular identification is only due to the leaf shape, and the widespread dissemination of phytotherapeutic use, it

is necessary to study the effectiveness of the action, as well as the safety in Applicability. However, few studies on *B. blakeana* have been published and none of them have investigated the activity of the plant or its components isolated as to the antibacterial action. This statement highlights the need for more specific studies related to the species.

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