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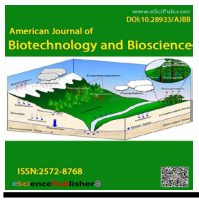

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CHARACTERIZATION OF BIOACTIVITY OF EXTRACTS OF THE PLANT SPECIES *JACARANDA RUGOSA* A.H. GENTRY

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ABSTRACT

The use of plants by man as a way to treat diseases is an ancient practice and widely used within the communities. Plants said to be medicinal present in their composition substances capable of altering the systematic functioning of organs, and may influence the treatment of diseases. The branch of science that studies plants and their ethnofarmacologic characteristics has attracted industries and researchers to elucidate their real biological influences in the organism. The Jacaranda rugosa, native plant of the caatinga biome, has been used in the treatment of infections and skin wounds and in the treatment of diseases. In order to evaluate the bioactivities of this species, the methodology of phytochemical prospecting was used, through thin layer chromatography, the verification of the antioxidant potential of leaf and branch extracts, through techniques such as neutralization of DPPH and ABTS radicals, in order to identify the functioning of the lity and the performance of cellular protection against oxidizing agents. In addition, microbiological techniques were used to evaluate the efficiency of extracts in acting as bactericide against disease-causing pathogens. As results, phytochemical prospection demonstrated the presence of secondary metabolites tannins, flavonoids and coumarins, the analyses of antioxidant activities by jacanda rugosa's DPPH radical showed considerable activities from 62.5 mg/ml. The analysis of the neutralization of ABTS radicals demonstrated efficacy of 46% and 49% of ability to react against antioxidant substances. Against the microorganisms Salmonella spp.and Staphylococcus aureus, there was the activity of the extract for Salmonella spp in 5 mg/mL of the extract, but there was no activity for S. aureus..

Keywords: Caatinga. Antioxidant. Bioprospecting.

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1INTRODUCTION

The medicinal use of plants by men has been reported since prehistoric periods[1]. Medicinal plants have characteristics that help in the treatment of diseases or that improve people's health conditions, and they are producers of substances that are capable of altering the functioning of organs and systems, and some of these plants may be an excellent source for therapeutic innovation, considering that the Brazilian flora is highly diversified in species[2].

The interest in the use of preparations obtained from medicinal plants has increased the interest of ethnopharmacologists for scientific validation of the properties of these plants, seeking to avoid inappropriate and even harmful use of them[2]. This confirmation may direct the formation of new drugs, whether these compounds are obtained by synthesis from prototype molecules or by isolation in sources of origins. These plant materials apparently have less toxicity compared to synthetic drugs, which makes them attractive candidates for drug development[3].

Brazil is the country with the largest biodiversity on the planet, with more than 45,000 cataloged plant species[4]. Among the Brazilian biomes we find the Caatinga, which is an exclusively Brazilian biome containing a vast biodiversity still little studied. Composed of a xerophyte vegetation, the plant species that occur there are adapted to the dry and arid climate and the low availability of water having acquired adaptive mechanisms that allowed their survival in such adverse conditions[5].

The *Jacaranda rugoso*, belonging to the family Bignoniaceae is a tree that can reach up to 15 meters high, are perennial, containing fopposite looks, composed bipinada, 10 to 25 cm long, with small leaflets, glabrous and serrated maple[6]. It is a tree endemic to Brazil, being prevalent in the states of Pernambuco and Bahia[7]. It has two flowering peaks, however, with low flower production at each peak. They are restricted to the Caatinga biome, where it develops in sandy and stony soils. Vegetation is subject to three situations of threat: agriculture with the consequent degradation of soil, cattle ranching and logging[8]. There are constant collections of its components, even if these are not much represented before scientific collections before the research. It is protected by the Serra do Catimbau National Park[9].

The use of folk medicine with rosewood has been widely employed in the treatment of infections and skin wounds through the use of its boiled leaves. In addition, there are reports of the treatment of syphilis, a sexually transmitted disease caused by the bacterium *Treponema pallidum*[10].

Thus, the determination of the biological potential of medicinal plants that will elucidate popular knowledge has been a focus for academic research. Free radicals are unstable molecules released by cellular metabolism[11]. These molecules are capable of causing oxidative stress generating mainly cardiovascular damage[12]. The main methods of oxidative stress analysis are PDDH and ABTS, where both behave as a free radical and measure the potential of the analinete to neutralize these compounds[13,14] .

The potential bactericidal factor offered by the plant according to folk medicine led to a microbiological determination. Salmonella spp. is one of the most common enterogastrite-causing pathogens in the population in view of its broad host spectrum[15]. Thus, the objective of this work is to elucidate the biological characteristics of *Jacaranda rugosa* leaves in view of the popular belief of the therapeutic use of this plant.

2MATERIAL AND METHODS

2.1 Plant material

The branches (EAB) and leaves (EAL) of *Jacaranda rugosa* were collected at the Armadillo Bola Wildlife Refuge located in the pernambucano hinterland. The plant parts were brought to the Biochemistry Department - Campus Recife - UFPE, where they were dried

for 48 h in a greenhouse at 40° and crushed after drying. The powders obtained were used for the production of aqueous extracts.

2.2 Preparation of the extract

The extracts were prepared under reflux, in a water bath at 100 °C for 30 minutes, in the proportion 10 % (w/v). At the end, they were cooled and filtered under vacuum with cotton. They were then kept under refrigeration for 3 days. Finally, they were submitted to the lyophilization process for 48 h to obtain the aqueous crude extracts.

2.3 Phytochemical prospecting

The extracts and patterns were applied manually in chromatographic plates of silica gel 60 - F_{254}. The plates were developed in vats after saturation with the mobile phase (Chart 1). The tank was saturated for approximately 30 minutes at room temperature. The bands were applied with a width of 5 mm and with a distance between them and the edges of the plates of 5 mm. The length and width size of the chromatographic plates was 10 x 20 cm. The samples were applied to 5 mm of the origin and with 5 mm end of the end of the plate. After elution of the plates they were dried at room temperature and observed under ultraviolet light of 254 and 365 nm and visible light, then were scanned. Subsequently, they were revealed with specific reagents for each metabolite (Chart 1). The bands obtained were compared to the bands of the corresponding patterns.

Table 1 - Systems, developers and patterns used.

Metabolite Class	**System**	**Developer**	**Default**
Hidrolisables tannins	90:5:5	NEU + PEG	Galic acid and Elargic acid.
Condensed tannins	90:5:5	Hydrochloric vanillin	Catechin
Flavonoids	90:5:5	NEU + PEG	Quercetin and Rutin
Cinamic derivatives	90:5:5	NEU + PEG	Cafeic acid and. Chlorogenic acid
Cumarins	50:50:50	KOH + Δ	Coumarin

Legend: NEU =ethyl borilaminosester acid; PEG= Polyethylene glycol

2.4 Antioxidant Activity by DPPH radical

The elimination activity of DPPH-free radical from the extracts was performed according to the methodology of BrandWilliams et al (1995)[16], with some modifications. A stock solution of DPPH diluted in methanol (200 ^M) was diluted in methanol to obtain UV-VIS absorbance between 0.6-0.7 nm up to 517 nm, obtaining the DPPH working solution. From 1 mg of the extract diluted in 1 ml of water there were serial dilutions to obtain 6 distinct concentrations, 500; 250; 125; 62.5; 31.25 and 15.6 µg/mL. On a plate of 96 wells pipettes 40 µl of each concentration in triplicate (3) and then added 250 µL of the DPPH solution, we left at rest for 30 minutes in the dark. To validate the test we made a negative control where we pipetteed 40 µL of methanol and added 250 µL of the DPPH solution. After rest, the plate was read in the ELISA® at wavelength at 517 nm. The result was expressed as an inhibition percentage by the formula below:

$$\%\, ATV.\ ANTIOXIDANT = \frac{100 - (ABS.\ SAMPLE - ABS.WHITE}{ABS.CONTROL} X\ 100$$

2.5 Antioxidant activity by radical ABTS

The ABTS+• free radical method was performed according to Sánchez-González et al. (2005)[17]. The ABTS solution was prepared in aqueous

medium, from the reaction between 7mM of the stock solution of ABTS, with 2.45 mM of potassium persulfate, resulting in the formation of an ABTS+• cation. The mixture was stored in amber bottle and at room temperature for 16 hours before use. The ABTS+• solution was diluted with ethanol to absorbance from 0.7 to 730 nm. In addition, 1 mg of the extract was diluted in 1 ml of ethanol, and from this solution 20 µL was removed and placed in triple-shaped test tubes (3) and then adding 2 ml of ABTS+• solution, left at rest in the dark for 6 minutes. The readings were made in spectrophotometer in an absorbance of 730 nm after a reaction period between 6 and 7 minutes. The result was expressed as an inhibition percentage by the formulation below:

$$I\% = \frac{(ABS.\ WHITE - ABS.SAMPLE}{ABS.CONTROL} X\ 100$$

2.6 Biological material and cultivation conditions

As strains of *Staphylococcus aureu*s 02 and Salmonella *spp.* were provided by the collection of microorganisms from the department of antibiotics - UFPE. The isolates were kept in glycerol 15% (-80° C) and reactivated in Brain Heart *Infusion* (BHI) at 37° C / 24h.

2.7 Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined by the microdilutionmethod, according to CLSI (2018)[18]. 50 mg of extract were weighed and solubilized in 1 ml of stereo distilled water. Plates of 96 flat bottom wells were previously filled with 100µl of Müller-Hinton (MHB) medium. After that, 100µL of the solubilized extracts were added and then homogenized, then 100 µL was removed to be placed in the next well in serial dilution of 1/2 (initial concentration: 25 mg/mL). Soon after, 10 µL of bacterial suspension (approximately 1.5 × 10^8 CFU /mL) were added to the wells. The samples were incubated for 24 hours at 37 °C. The optical density (OD) reading was performed to verify the mortality of microorganisms.

3RESULTS AND DISCUSSION

3.1 Phytochemical profile

The qualitative analysis of the phytochemical content of the EAL and EAB were summarized in table 2. The results of this study indicate the presence of hydrolysable tannins, flavonoids and coumarins; as well as absence of condensed tannins and cinnamic derivatives.

Table 2 - Phytochemical content of the aqueous extract of *Amburana cearensis* bark (EAAc).

Metabolite Class	**Branch**	**Leaf**	**Default**
Hidrolisables tannins	_	-	Galic acid and Elargic acid.
Condensed tannins	-	-	Catechin
Flavonoids	-	+	Quercetin and Rutin
Cinamic derivatives	+	+	Cafeic acid and. Chlorogenic acid
Cumarins	+	-	Coumarin

The presence of Cinamic derivatives, coumarin and flavonoid derivatives provided the activities described ahead, these metabolites are widely described in the literature as holders of biological and therapeutic activities.

3.2 Evaluation of eliminating the DPPH radical

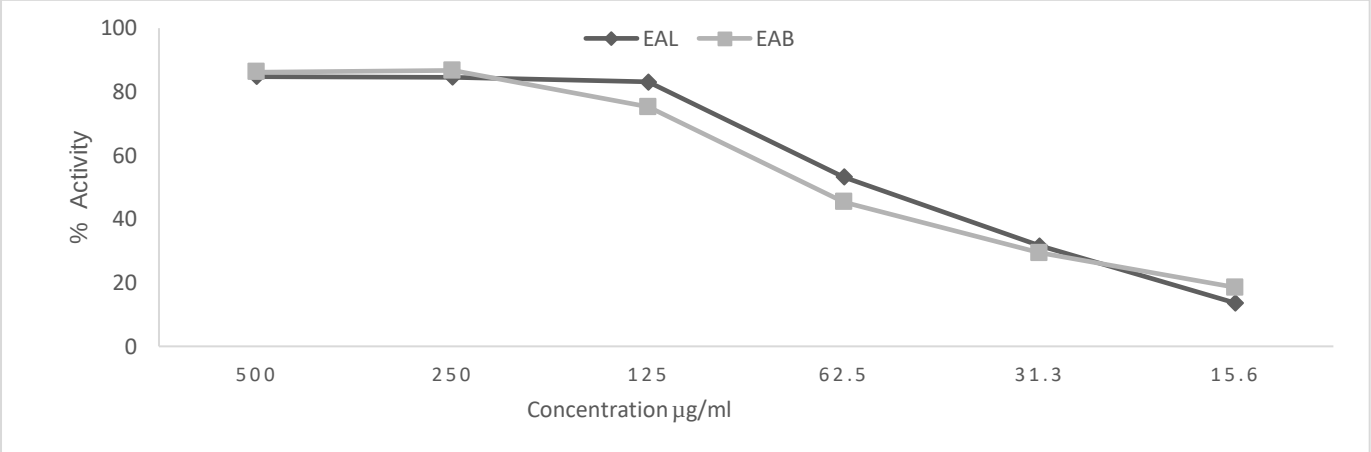
The DPPH radical has the function of being similar to free radicals produced naturally by metabolism, but unlike natural ones, they present good stability in the absence of light bringing advantage to the test[13]. The electron transfer of the antioxidant compound to the

DPPH molecule will reduce this molecule, leaving it neutral[19].

The neutrality of the free radical is visualized by the loss of the purpura color of the solution, transforming into a yellowish color[20] .

According to graph 1 it is possible to notice a decay of the antioxidant response extracts of rosewood as it decreases its concentration. Thus, it is possible to observe that from the concentration of 62.5 mg/mL there were satisfactory antioxidant responses, because they present prevention power to free radical attacks greater than 50 % when compared to the standard. The concentrations of 31.25 and 16.6 mg/mL were not satisfactory in the prevention of oxidative stress, because its antioxidant power does not exceed the neutralization of the minimum of 50 % of the DPPH.

Graph 1: Decay of antioxidant response to DPPH radical. A response greater than 50 % of free radical neutralization occurs between concentrations of 62.5 and 125 µg/mL in both extracts.



3.3 Evaluation of the elimination of the ABTS radical

The antioxidant evaluation was based on the incorporation of an electron to molecule in oxidized state[21]. The neutralization of charge made by antioxidant molecules, and visualized from the bleaching of the solution that is initially blue-green, and may become transparent coloring[22] . The ABTS molecule has been widely adopted as a standard and safe indicator for the measurement of antioxidant capacity, and it can act in various reactions[22]. The ability to neutralize the oxidized molecule is expressed as a percentage (%) and illustrates the potential that the extract has in keeping molecules stable and being highly conserved. In the ABTS assay, the EAF and EAB presented 46 % and 49 % of the ability to react to antioxidant substances, this portrays a protective power to agents that cause cell oxidation.

3.4 Antimicrobial activity assay

The antimicrobial capacity of oils can vary depending on biotic and abiotic factors, such as environmental temperature, soil composition, climate and season, these factors can contribute to increase or decrease in the bactericidal capacity of plants[23]. The minimum inhibitory concentration (IMC) predicted the death of microorganisms from a minimum oil concentration[24]. Both extracts were active against *Salmonella spp*., having an ic50 of 5mg/mL. In versus starting, there were no satisfactory responses to inhibition of *Staphylococcus aureus.*

4CONCLUSION

The present work reports a biological performance of the oil extracted from the leaf of the *Jacaranda rugosa.* Based on these results it is possible to have a guidance on the ability that vegetables have to influence on biological factors from their metabolites, Thus, the leaves of Jacarandá proved effective in reversing

products of cellular metabolism, such as free radicals, as well as had the ability to intervene on infections caused by external pathogens such as *Salmonella spp.*

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