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Full Paper

Cytotoxic and Antioxidant Activity of *Mimosa verrucosa* Benth.

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Abstract: The present work describes the antioxidant and cytotoxic properties of the ethanolic extracts of the leaves and stems of *Mimosa verrucosa* Beth. (Fabaceae) found in Northeast Brazil. The antioxidant activity was evaluated using the stable radical sequestration essay of 2,2-diphenyl-1-picrylhydrazyl (DPPH), demonstrating that the leaves had the highest antioxidant activity (IC_{50}= 372.7 ± 0.3 μg.mL^{-1}) and the cytotoxic activity was obtained by evaluating the mortality of *Brine shrimp* larvae, revealing the highest cytotoxic potential of the roots (DL_{50} = 145.5 ± 21.5 μg.mL^{-1}). The analysis of the chromatograms obtained in HPLC-DAD and the thin layer chromatography plates with specific reagents indicated the presence of steroids, flavonoids, saponins and tannins in the different extracts, to which the antioxidant and cytotoxic activities were attributed.

Keywords: *Mimosa verrucosa* Benth; jurema branca; antioxidant activity; *Brine shrimp*

1. INTRODUCTION

Plant´s metabolites have contributed greatly to the development of drugs used in modern medicine [1-4]. There is a growing recognition that natural products are viable source of new bioactive molecules, especially in view of the great Brazilian´s biodiversity and the potentialities of endemic species present in threatened biomes [5].

In this context, the species *Mimosa verrucosa* Benth. (Fabaceae), endemic from the Caatinga biome, one of the most threatened biomes in Brazil due to the forest´s devastation, is used as an anti-inflammatory by the population of the Brazilian northeast region [6]. The *Mimosa verrucosa* Benth. is known popularly as "*jurema*", "*jurema branca*", "*jurema da flor rosa*" or "*jurema de oieras*" and is characterized as an arboreal species, xerophyte, widely used by the local population for making both animals´ fences and for feeding small ruminants during the dry season [7, 8]. Either the stem bark or root of different mimosas species, generically called "*jurema*", have been used by several syncretic Brazilian religious practices, such as "*Catimbó*", "*Umbanda*" and "*União do vegetal*", in the form of a hallucinogen drink called "*vinho de jurema*" or "*ajucá*". These hallucinogenic properties are attributed to the presence of tryptamine derived alkaloids, such as N, N-dimethyltryptamine (DMT), which are found in large quantities in *Mimosa tenuiflora* (syn. *Mimosa hostilis*) [9, 10], however there are no studies which prove the presence of these metabolites in all species of the genus *Mimosa*.

The literature describes phytochemical studies presented dealing with isolation of metabolites of *Mimosa tenuiflora* [11], *Mimosa pudica* [12], *Mimosa caesalpiniifolia* [13, 14] and *Mimosa invisa* [15]. However, to date, there is no phytochemical study of the species *Mimosa verrucosa* Benth. or any correlation with their biological activity so far [16, 17].

Thus, this work reports the qualitative analysis of the main metabolites presented in different parts of *Mimosa verrucosa* Benth. and evaluation of the antioxidant and cytotoxic activity of the ethanolic extracts of the roots, stem and leaves of the species.

2. MATERIAL AND METHODS

2.1 Vegetable material

Leaves, branches and roots of *Mimosa verrucosa* Benth. were collected in the city of Barra

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(BA), Brazil, in May 2015 and a voucher specimen is deposited at the Universidade Federal do Oeste da Bahia - UFOB, Campus Barra.

2.2 Preparation of the extracts

The plant material was dried, milled and then macerated with cold ethanol thoroughly for 24 hours. The extracts were combined and concentrated at room temperature, giving a total of 3 different extracts.

2.3 Qualitative analysis of the present compounds in the extracts by thin layer chromatography (TLC)

The extracts were analyzed using silica gel thin layer chromatographic plates 20 x 20 cm from Sigma and submitted a quantitative analysis of the secondary metabolites, consisted in the spraying of distinct chromatographic plaques containing the extracts as reagent Lieberman-Burchard, $FeCl_3$, $AlCl_3$, the foam test and Dragendorff reagent, to evaluate the presence of steroids, flavonoids, saponins, tannins and alkaloids.

2.4 HPLC analysis conditions

Analysis was performed on the HPLC system (Dionex®, Germany). The HPLC system was equipped with a pump (LPG 3X00), auto sampler (ACC3000), column oven, and diode array UV/VIS detector (DAD3000 (RS)). The output signal of the detector was recorded using a Dionex Chromelon Chromatography Data System. The separation was executed on a Dionex C_{18} column (5 µm, 120 µ, 4.6 mm × 150 mm). The mobile phase was composed of water and methanol with the gradient elution system at a flow rate of 0.3 ml/min, for 20 min.. The injection volume was 3.0 µl. The detection UV wavelength was set at 330 nm. The column temperature was set at 27°C.

2.5 Cytotoxic activity and antioxidant test

The extracts were submitted to a series of biological tests like the cytotoxic one, observed through the lethality of the larvae of *Brine shrimp* in five concentrations (500 $\mu g.mL^{-1}$, 250 $\mu g.mL^{-1}$, 125 $\mu g.mL^{-1}$, 62.5 $\mu g.mL^{-1}$ and 50 $\mu g.mL^{-1}$). The crude extracts were dissolved in dimethyl sulfoxide (DMSO) and solubilized in 25 ml of saline water. To the test execution, the *Brine shrimp* nauplii were incubated in artificial sea water in room temperature, in a 24 hour period. After hatching 10 larvae were distributed in different vials, with distinct concentrations. The test was done triplicated added the original one in neutral environment according to the Meyer method in (1982) [18].

Another biological test done with warty Mimosa extract was radical sequestering qualitative test stable 2,2-diphenyl-1-picrylhydrazyl (DPPH)[15]. The test consisted of spraying the DPPH solution 0.1 $mol.L^{-1}$ on a chromatographic plate containing the eluted extracts the chromatographic system 8/2 $CHCl_3$/MeOH. Then the most active extract was subjected to quantitative evaluation of antioxidant activity [19]. In this test, 2.7 ml of the solubilized extract in ethanol at five different concentrations (50 $ug.mL^{-1}$, 62.5 $ug.mL^{-1}$, 125 $ug.mL^{-1}$, 250 $ug.mL^{-1}$, 500 $ug.mL^{-1}$) was mixed with 0.3 ml of ethanolic solution of DPPH 0.1 $mol.L^{-1}$. After 15 min. of rest in the absence of light, the consumption of DPPH radical was monitored by measuring the decrease in absorbance of the sample was transformed into percentage of kidnapping (% SRL) by means of control absorbance minus the absorbance of the sample divided by the absorbance of the control multiplied by 100.

The UV-VIS Spectrophotometer (Varian Cary®) was operated without a wavelength of 517 nm using gallic acid as positive control.

3. RESULTS AND DISCUSSION

The bioautography in TLC of the extracts of *M. verrucosa* with different revealing reagents, presented results considered positive by the appearance of color, precipitate and / or specific foam of each test described by Matos (1998) [20]. In this evaluation, we searched the chemical classes present in each extract tested by the appearance of a purple, yellow and blue color when the chromatographic plates are sprayed with solutions of Lieberman-Burchard, $AlCl_3$ and $FeCl_3$, indicating, respectively, the presence of steroids, flavonoids and tannins. In addition to foam testing to confirm the presence of saponins and the formation of precipitate in the test for alkaloids in the test with Dragendorff's reagent.

The secondary metabolites detected in plant extracts play different roles in the plant, such as growth, defense against ultraviolet rays, against attacks by pathogens and predators, and attract

pollinators. Table 1 shows the detection of steroids and saponins in the branches and roots of *M. verrucosa*, in addition to flavonoids in the leaves and tannins in the stem (Table 1).

Table 1. Presents the results of quantitative chemical tests of extract of the studied specie.

Chemical Group	Applied tests	Leaves *M. verrucosa*	Stem *M. verrucosa*	Root *M. verrucosa*
Esteroids	Lieberman-Burchard	-	+	+
Flavonoids	$AlCl_3$	+	-	-
Saponins	Test of foam	-	+	+
Tannin	$FeCl_3$	-	+	-
Alkaloids	Dragendorff	-	-	-

(+): detected; (-) not detected

Although the species *M. tenuiflora* (syn. *M. hostilis*) [21], *M. ophthalmocentra* [22], *M. scabrella* [23] and *M. sominians* [24] are well known to contain hallucinogenic alkaloids such as N,N-dimethyltryptamine (DMT), test performed with *M. verrucosa* did not reveal alkaloid presence in detectable amounts. This feature may explain the fact that this species does not present many reports of use in rituals of Brazilian syncretic religions, although the species used in these ritualistic drinks are quite similar, and therefore quite confused.

The chromatographic profile of the crude extract was obtained in HPLC-DAD indicating that the species is rich in phenolic compounds, presenting many peaks in typical wavelengths of phenolic compounds (330nm), especially flavonoids, as confirmed in qualitative tests (figures 1, 2 and 3).

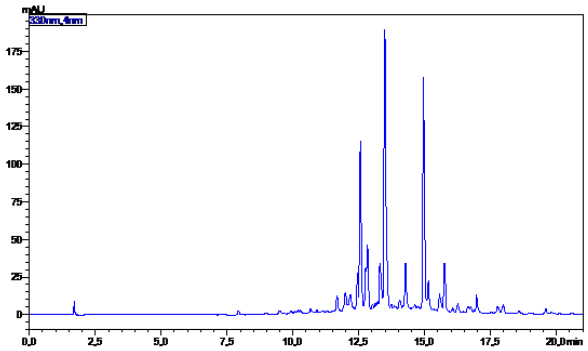

Figure 1. HPLC-DAD chromatogram of crude MeOH extract of *Mimosa verrucosa* leaves.

The test of activity with *Brine shrimp* shows a good correlation with the cytotoxic activity in some human tumors, characterizing an important preliminary test for extracts. According to David (2001) [25], a substance that represents LD_{50} in the intervals between 100 and 900 $\mu g.mL^{-1}$ can be considered active.

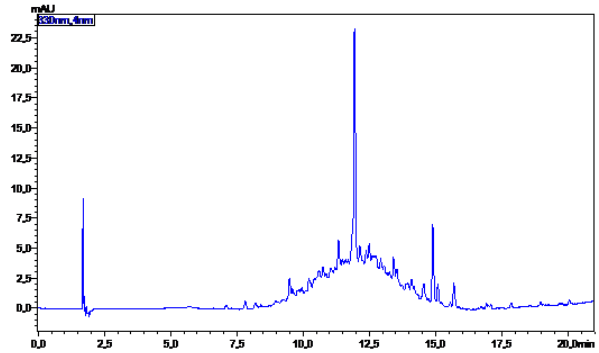

Figure 2. HPLC-DAD chromatogram of crude MeOH extract of *Mimosa verrucosa* stems.

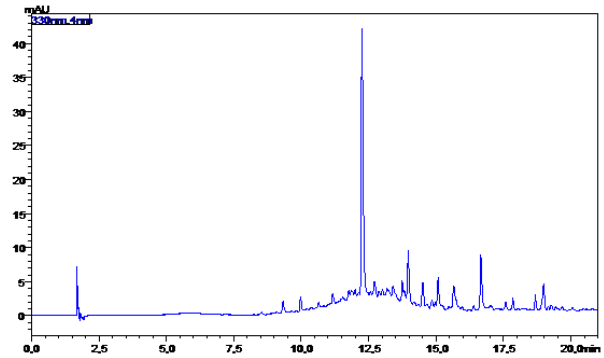

Figure 3. HPLC-DAD chromatogram of crude MeOH extract of *Mimosa verrucosa* roots.

Analyzing the Table 2, we observe that the leaves and roots of the *M. verrucosa* are moderately active. Such results reveal potential to activities cytotoxic of the respective extract, being necessary isolating the metabolites responsible for the activity.

The antioxidant activity of the three extracts tested was positive, although the antioxidant activity of the leaves of *M. verrucosa* was more significant (Table 3). This result indicates that the leaves of the species is a great choice for isolation studies of antioxidant metabolites studies.

These results led to the quantitative test DDPH

radical sequestration to the leaves of this species providing IC_{50} = 372.7 ± 0.3 µg.mL^{-1} (Figure 4).

Table 2. Lethality from different parts of *Mimosa verrucosa* warty front *Brine shrimp.*

Vegetable extract	Lethality front *Brine shrimp*		
	LD_{50} (µg.mL^{-1})	Standard deviation*	Cytotoxic activity
Leaves	513.3	2.5	active
Stem	>1000	-	Not active
Root	145.5	21.5	active

95% of trust in the interval.

Table 3. Qualitative antioxidant activity of extracts of *Mimosa verrucosa*.

Vegetable extracts	DPPH radical quenching
Leaves	++
Stem	+
Roots	+

(+): moderate activity; (++) strong activity.

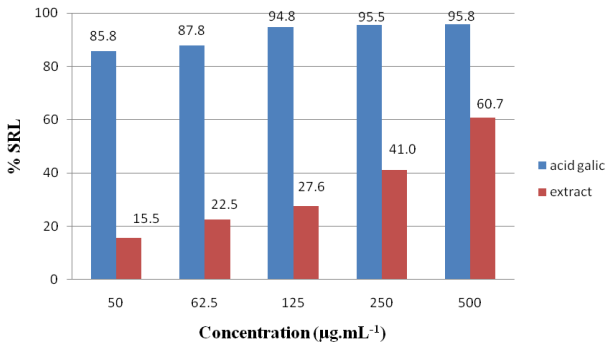

Figure 4: Antioxidant activity of the EtOH extract of the leaves of *Mimosa verrucosa*.

4. CONCLUSION

The phytochemical study of the extracts of *M. verrucosa* confirmed that the genus is rich in bioactive flavonoids, due to the positive tests for flavonoids obtained by the reaction with $AlCl_3$ and the analysis of the chromatographic profile obtained by HPLC-DAD. The detected flavonoids were responsible for the greater antioxidant activity of the leaves of the species and the steroids and saponins were responsible for the greater cytotoxic potential of the root. The present work constitutes one of the few reports of the evaluation of the chemical composition of *M. verrucosa*, and that unlike the most studied species, *M. tenuiflora* (syn. *M. hostilis*), the presence of alkaloids in detectable amounts was not verified.

5. ACKNOWLEDMENTS

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