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Evaluation of the Chemical and Biological Potential of the Flower Extract of *Handroanthus Impetiginosus* Mattos

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Abstract: *Handroanthus impetiginosus Mattos species belongs to the Bignoniaceae family and is popularly known as purple ipê. The barks and leaves of this species are used in folk medicine. The chemical composition of H. impetiginosus has been widely studied due to its pharmacological interest however, there are not many studies on the biological properties of its flowers. With this, the present work had as objective to evaluate the biological potential of acetonic and methanolic extracts and chemical composition of the hexanic extract, of the flower of the purple ipê. For this, the flowers went through extraction, partition and chromatography processes. The biological potential was analyzed using a toxicological test with Artemia salina, where the acetone extract had a cytotoxic activity better than the methanolic phase. The agrochemical potential was tested against the Cattleya nobilior species where a stimulus in the number of shoots was observed. The composition of the hexane extract was analyzed in gas chromatography coupled to the mass spectrometer, where it was possible to identify 16 compounds, of which hentriacontane is the identified compound with a major percentage of area. The results showed the potential of H. impetiginosus flower extracts and the importance of continuing studies on them.*

Keywords: *toxicity; ipê; orchid; hentriacontane; lapacho*

1. INTRODUCTION

The Bignoniaceae family, inserted in the order of the Lamiales, showed about 100 genera and 840 species, ranging from large trees to vines [1]. Regarding the taxonomic classification, the *Handroanthus* genus is inserted in the Tecomeae tribe and belongs to the *Tabebuia* Alliance.

In Brazil, there are 15 *Handroanthus* species widely distributed, these species also known as “ipê” [2]. *Handroanthus impetiginosus* (Mart. Ex DC.) Mattos is traditionally used as a remedy for inflammation, cancer, syphilis, malaria, fevers, trypanosomiasis, fungal infections, bacterial infections, stomach ulcers and inflammatory process [3,4].

Phytochemical studies of the bark and leaves of *H. impetiginosus* are widely found in the literature. The chemical composition includes cyclopentene dialdehydes [5], flavonoids [6], benzoic acid, benzaldehyde derivates and, principally quinones, just like lapachol and its derivative, β-lapachone [7]. Its flowers are lush and its use in gastronomy has been increasing in recent years however there are few studies related to the chemical composition and biological potential of these flowers.

For this purpose, a toxicity test is performed to assess the safety or hazards presented by substances such as pharmaceuticals, natural products, and industrial chemicals and is considered a useful tool for the preliminary assessment of toxicity plants [8]. Agrochemical research was also carried out to investigate the ability to induce growth of the *Cattleya nobilior*. Called the Queen of the Cerrado,

Cattleya nobilior is an orchid native to Brazilian savanna. It is a species that has flowers up to 15 cm in diameter with colors ranging from white to pinkish purple [9] (WATANABE, 2002). Due to its beautiful ornamental characteristics, it has great economic importance, which generates the need to find new alternatives for its cultivation. Regarding the chemical composition, the hexane extract was evaluated by GC-MS for the analysis of the major volatile compounds.

2. MATERIAL & METHODS

2.1. Plant Material

Fresh flowers of *H. impetiginosus* Mattos were collected in Gurupi (Tocantins, Brazil, 11° 44' 27" S e 49° 3' 49" W) in August 2019 and, 2020. The plants were authenticated at the Herbário do Tocantins (HTO-UFT) and a voucher specimen (HTO 12115) was deposited. The materials were separated according to the plant parts and dried (ca. 45 °C).

2.2. Extraction

The flowers (191.1 g) were ground and extracted three times at room temperature with *n*-hexane, acetone and methanol (ca. 200 mL, 24h and shaken manually every 12 h for 2 min for each extraction), successively. The process resulted in hexane (HE; 2.7 g), acetonic (AE; 4.5 g) and, methanolic extract (ME).

The methanolic extract was viscous and underwent fractionation with Amberlite XAD-16 column (40 g, 30.0 × 2.5 cm) eluting with H_2O (300 mL) and MeOH (150 mL), successively, to give aqueous fraction (AF 1.0 g) and methanol fraction (MF 10.3 g).

2.3. GC-MS Analyze

The composition of the hexane extract was established by GC-MS analyses. Portions (0.5 mg) of hexane extract were dissolved with the GC-graden-hexane (1 mL), filtered through a PVDF 0.45-µm membrane, and analyzed by GC-MS Shimadzu QP2010 coupled to an AOC-5000 mass spectrometer (GC-MS) equipped with a Phenomenex ZB-5MS capillary column (30 m x 0,25 x 0,25 mm). The injector temperature was kept at 260°C and the oven temperature program was from 140° to 320°C at a rate of 3 $^{o}C.min^{-1}$. The GC-MS operated in electronic ionization mode at 70eV, with the transfer line maintained at 250 ^{o}C. Helium (1.3 $mL.min^{-1}$) was used as carrier gas. Retention indices for all compounds were determined according to the equation proposed by van Den Dool and Kratz using n-alkanes as standards. Components were identified based on a comparison of their mass spectra and retention time with those at the NIST/EPA/NIH Mass Spectral Database.

2.4. Toxicity Testing Using Artemia Salina

The brine shrimp lethality assay was performed by the method of McLaughlin [10]. Brine shrimp eggs (Artemia salina) were hatched in a saline solution of NaCl (25 $g.L^{-1}$) with pH 8 and were incubated for 24 h. The saltwater solution was aerated continuously during incubation with an aquarium air pump. The nauplii (10 units) were added to each set of tubes containing acetonic extract (AE) and a methanolic fraction (MF) (solubilized in saline solution containing 1% DMSO). The samples were tested in triplicate at concentrations of 150, 200, 250, 300, 350, 400 e 500 $mg.L^{-1}$ for MF and 10, 100, 500 e 1000 $mg.L^{-1}$ for AE. For each concentration, three test tubes containing the same volume of DMSO plus seawater and brine shrimp nauplii were used as a control group. Survival was measured after 24 h incubation. The collected data were computerized and LC_{50} values determined by Probit analysis.

2.5. Effects of Different Methanolic Extract Concentrations of in Vitro Growth of Cattleya Nobilior

The experiment was carried out at the Laboratory of Plant Ecophysiology and Laboratory of Tissue Culture Molecular Analysis of Plants of the Federal University of Tocantins, Campus of Gurupi-TO using *Cattleya nobilior* plants cultivated in vitro. The experiment consisted of four treatments: 0, 4, 6 and 8% of a methanolic compound from *Handroanthus impetiginosus* flowers diluted in Murashige & Skoog (1962) [11] culture medium plus 6 $g.L^{-1}$ of agar and supplemented with 5 $g.L^{-1}$ of sucrose in media with the methanolic extract and 30 $g.L^{-1}$ of sucrose in the medium without it (control). All media had their pH adjusted to 5.8. After preparation, the media were poured into test tubes, making a total of 10 tubes (replications) per treatment. Subsequently, they were sterilized in an autoclave for 25 minutes

at 120º C. Then, the tubes were taken to a laminar flow cabinet where, by tube, a microplant of *Cattleya nobilior* with about 5 mm in height was inoculated. After 190 days, the evaluations were: the number of shoots in vitro culture pots, shoot height (cm) and main root length (cm) by using a ruler. The data were tabulated in Excel to be analyzed in the R software (R Core Team, 2021) with the help of the platform R Studio, (2021), where the statistical analysis was performed. For root length, the Tukey test was performed with 5% significance, while the variables, numerous shoots and Aerial Part Length, were analyzed using the Kruskal-Wallis test with Dunn's post-hoc test with alpha 5%.

3. RESULTS & DISCUSSION

The composition of the hexane extract from flowers of *H. impetiginosus* Mattos from biome Cerrado was established by GC-MS analyses by comparison of the linear retention index (I) of the compounds with those of standard samples and data in the literature, as well as by an analysis of their mass spectra (Table 1). A total of 34 compounds were detected and, among these, 10 were identified. These compounds belong to classes like fatty acids, steroids, terpenoids and homologous series of alkanes. The major component (with a relative % peak area above 0.1) was hentriacontane (24.9%), composed of approximately 31 carbons and found in beeswax [12].

Table1. *Composition of hexane extract determined by GC-MS*

Peaks	Time (min)	Area %	Identification Proposal			
			IR_{cal}	IR_{lit}	Similarity %	Compound
1	15.911	0.06	1792	1792	93	Octadecene
2	16.137	0.02	1800	1800	91	Octadecane
3	17.534	0.07	1844	1844	91	Hexahydrofarnesyl acetone
4	21.147	0.84	1959	1958	97	*n*-Hexadecanoic acid
5	22.210	0.08	1993	1992	95	Eicosene
6	24.937	0.20	2081	2090	95	Heneicosene
7	26.376	0.18	2129	2130	94	Linoleic acid
8	26.574	0.66	2136	2141	95	Oleic Acid
9	27.292	0.39	2160	2156	94	1-Nonadecanol
10	28.299	0.06	2193	2195	94	1-Docosene
11	45.039	0.15	2829	2831	92	Supraene
12	46.740	24.01	2902	2900	97	Nonacosane
13	48.954	1.83	3000	3000	91	Triacontano
14	51.191	24.94	3102	3100	97	Hentriacontane
15	53.266	1.31	3200	3200	94	Dotriacontane
16	53.779	0.61	3224	3203	95	Sitosterol

The brine shrimp (*Artemia salina*) lethality bioassay is widely used as a preliminary toxicity screen of several samples. Several studies have demonstrated that there is a correlation between LC_{50} and the results of oral acute toxicity assay in mice [8]. The toxicity testing using brine shrimp of methanolic fraction and acetonic extract showed median lethal concentration (LC_{50}) values of 812.8 and 475.7 $mg.L^{-1}$. The results showed a direct relationship with the mortality rate of *Artemia salina*, as the concentration of the extracts increases. It has been found that crude extract with LC_{50} less than 1000 ppm is considered active while a value higher than 1000 ppm is considered non-active [13,14]. The samples are considered actives, suggest the presence of bioactive constituents that exhibited cytotoxic properties.

Considering the study with *Cattleya nobilior* a significant reduction was detected in root length when 6% from the extract was used (Fig 1a). The aerial part of *C. nobilior* showed a greater growth per seedlings with a dose of 4% in relation to the control seedlings (Fig 1b). The shoots number per seedling increased when using 4 and 6% of the extract in relation to the control (Fig 1c). However, the presence

of the extract did not change the root formation per seedling, because no significant difference was observed between the number of formed roots during the experiment.

In *in vitro* cultivation of orchids, the well-known MS medium is used (Murashige and Skoog, 1962). In that is recommended to use 30 $g.L^{-1}$ of sucrose, it was observed that even using half of this amount (15 $g.L^{-1}$) with the plant extract an effect was observed: increasing the growth of orchid seedlings or increasing the number of shoots per seedling. Based on previous works by the research group, it was observed that the extracts and methanolic fractions are rich in cyclitols, information consistent with the part of the plant studied. It was probably not the amount of sugar available in the extract, but the type of sugar. So we suggested that these have had an effect, because experiments carried out with cyclitols, for example myo-inositol. SEPAHVAND et al. (2012) [15] tested myo-inositol concentrations on micropropagation of GF677 (peach× almond hybrid), they observed that lower concentration of myo-inositol produced normal plants, and higher concentrations produced dwarf and rosette shots because of the higher concentrations of carbohydrates in the medium MS with myo -inositol.

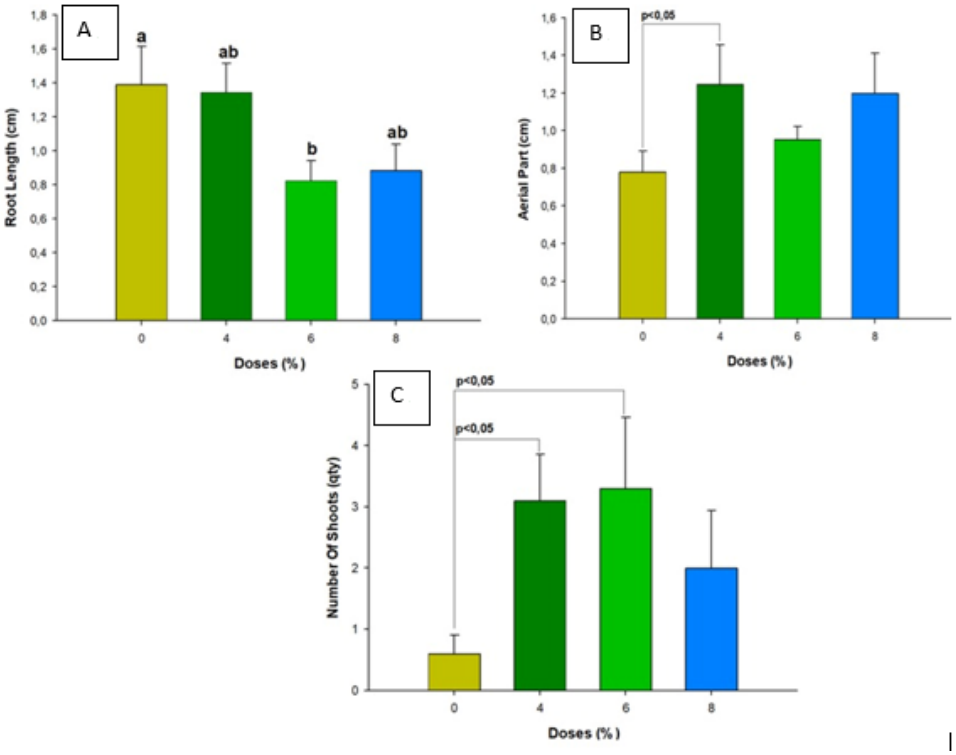

Figure1. *Root length (a), shoot height (b) and number of shoots (c) in Cattleya nobilior seedlings submitted to methanolic extract of Handroanthus impetiginosus flowers in vitro cultivation*

4. CONCLUSION

Phytochemical studies involving flowers of *Handroanthus* species are rarely reported in the literature. From the GC-MS analysis, it was possible to determine 16 compounds. The toxicological test showed that the acetone extract presents moderate toxicity and the methanolic fraction low toxicity. The methanolic extract was able to stimulate an increase in the number of shoots of species of *Cattleya nobilior*.

The results obtained in this study show the potential that flowers of the species *Handroanthus impetiginosus* Mattos have and demonstrate the importance of continuing phytochemical studies to isolate compounds from extracts that can enhance biological activities.

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