Phytochemical and biological activities evaluation of latex from *Himatanthus obovatus* (Muell. Arg.) Woodson (Apocynaceae)

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**Abstract.** *Himatanthus obovatus* is a tree species that has laticifers in its aerial organs, where it is used in folk medicine. The aim of this work was to evaluate the latex of *H. obovatus* regarding its phytochemical composition and biological activities. The latex was collected and lyophilized, yield (%) was carried out, phytochemical prospection for several groups, determination of the content of flavonoids, phenolics and total tannins, antioxidant activity in reducing DPPH, antifungal activity on the *Candida* genus, antibacterial for *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* serovar *Enteritidis* and *Typhymurium* and *Enterococcus faecalis*, hemolytic activity on human erythrocytes and cytotoxicity bioassay on *Artemia salina*. The latex yield was 19.05%, positive presence of several groups of phytocompounds was observed, total flavonoid content of 78.80 mg QE 100 g⁻¹, total phenolic compounds of 166.51 mg AGE g⁻¹, total tannins of 14.81 mg TAE 100 g⁻¹, antioxidant activity IC₅₀ = 178.87 µg mL⁻¹, antifungal activity for all *Candida* strains and antibacterial only for *E. coli* and *E. faecalis*, hemolytic activity between 68-18% and cytotoxicity with LC₅₀ = 433.97 µg mL⁻¹ on *A. salina*. The latex of *Himatanthus obovatus* demonstrated in this study, important phytochemical data and biological activities.

**Keywords:** Alkaloids, Tannins, Hemolytic activity, *Staphylococcus aureus*, *Candida albicans*.

**Introduction**  
Apocynaceae family has about 550 genera and between 3,700-5,100 species, where it presents
a diversified size from arboreal, shrubby, herbs, vines to subshrubs (Soares et al., 2016). Among the genera that make up the family is Himatanthus, which has high herbal potential (Spina, Bittrich, Kinoshita, 2013; Santos et al., 2021). This genus has thirteen species that are exclusively found inhabiting regions from Panama to countries that make up South America, with tropical and subtropical climate (Linhares; Pinheiros, 2013). In Brazil, the genus Himatanthus is well diversified in almost all biomes and Cerrado domain, with the Amazon region being the largest distribution center, with Himatanthus obovatus (Fig. 1) with wide territorial distribution (Menezes Filho et al., 2021).

H. obovatus is a laticiferous species, popularly known as “janaguba, tiborna or pau-de-leite”, has arboreal size between 4 and 5 m in height, and is found mainly in Cerrado domain environments, Amazon biome and Caatinga where it is well established inhabiting natural or anthropized areas (Linhares et al., 2013). Its distribution has already been presented in numerous studies covering the Brazilian states of Minas Gerais, Bahia, Sergipe, Alagoas, Pernambuco, Rio Grande do Norte, Ceará, Paraíba, Roraima, Maranhão, Pará, Piauí and Goiás (Santos et al., 2021; Menezes; Filho et al., 2021).

The genus Himatanthus has a large number of pharmacological activities such as antineoplastic, anti-inflammatory, antimicrobial and analgesic. Species such as Himatanthus drasticus, Himatanthus bracteatus, Himatanthus sucula and Himatanthus articulatus from leaf extracts, stem bark and latex have potential healing activity (Soares et al., 2015; Pugas et al., 2018). Although the genus Himatanthus has a considerable number of scientific studies on numerous previously described biological activities, the species H. obovatus still has a fine line with a low number of pharmacobotanical studies, thus presenting a need to know this species and its biological activities of phytomedical interest.

The few data on popular pharmacology refer mainly to the leaves of H. obovatus that are used in the treatment of high blood pressure, skin spots, pimples, inflammatory processes and as an antitumor agent. The roots are also used in the treatment of the amastigote form of Trypanosoma cruzi, as an antifungal and leishmanicidal agent, latex has a potential anticancer action (Lucetti et al., 2010; Vale, 2014).

As noted the pharmacological properties described for H. obovatus are due to the large number of complex phytomolecules that make up the special metabolism of the various organs of this plant, especially aerial ones. Phytochemical compounds such as lupeol acetate and triterpenes found in H. obovatus, among other species of the genus Himatanthus, play important roles in the healing action and with anti-inflammatory activity in wounds (Santos et al., 2021). Another special group of phytomolecules found in H. drasticus and especially in H. obovatus are the iridoids, where their angiogenic, anti-inflammatory and antioxidant activities are already known, and which are also involved in cell increase and differentiation in healing processes (Soares et al., 2016; Almeida et al., 2017).

It is known that the phytomedicinal use by the population that seeks through plants means to cure their illnesses, brings important ethno pharmacological data to researchers capable of directing studies to a certain plant species, with this there is a reduction in the time of exhaustive laboratory analysis where it is done. It is necessary to use modern and expensive equipment, which often lead to negative results, requiring the use of reagents, equipment time and research funds (Menezes Filho et al., 2021; Siqueira et al., 2021).

Thus, considering its medicinal importance, this study aimed to evaluate the phytochemical and biological activities of latex obtained from aerial organs of Himatanthus obovatus.

Figure 1. Individual of Himatanthus obovatus in an area of Cerrado stricto sensu. In (A) individual in the fruiting period, in (B) exposed leaf cuts and laticifers (latex). Source: Authors, 2021.
Materials and Methods

Plant material

The latex was obtained from the aerial parts of 15 individuals of *H. obovatus* in September 2021, in a permanent preservation area with Cerrado stricto sensu phytophysigion located in the municipality of Rio Verde, Goiás, Brazil, georeferenced location (17°47'17.9”S and 50°57'57.6”W). The species was identified by biologist Antonio Carlos Pereira de Menezes Filho and an exsiccate was herborized and deposited in the Herbarium of Instituto Federal Goiano, Campus Rio Verde, with Voucher HRV: 12374.

Sample preparation

The latex was collected with the aid of a disposable syringe, and the supernatant placed in a conical tube cooled to -8 °C in an airtight box. The material was then quickly frozen in nitrogen and transferred to a lyophilizer. After lyophilization, the powder obtained from the latex was stored in a refrigerator at -12 °C until analysis.

Determination of mass percentage (%)

The percentage yield of latex was determined using the dry mass (a) and the fresh mass (b) of the obtained latex, according to equation 1.

\[
\%\text{Yield} = \frac{a}{b} \times 100 \text{Eq. 1}
\]

Phytochemical prospecting

Qualitative phytochemical analysis was performed for the following groups: alkaloids, anthraquinones, coumarins, flavonoids, phenolics, quinones, foamy saponin, hemolytic saponin, tannins, reducing sugars, non-reducing sugars and cardiac glycosides as described by Ajuru, Williams and Ajuru (2017), resins, organic acids, azulenes, polysaccharides, purines, catechins, depsides and depsidones, steroids and triterpenoids and double olefins as described by Barbosa et al. (2001), proteins and amino acids, fatty acids, aldehydes and ketones and diterpenes as described by Pandey and Tripathi (2014).

Determination of total flavonoid content

The determination of the total flavonoid content was performed in a 96-well microplate as described by Sembiring, Elya and Sauriasari (2018). As standard, an ethanolic solution 99% of quercetin at a concentration of 10-100 µg mL⁻¹ was used. 1 mg mL⁻¹ of the standard solution in 10 µL mL⁻¹ of 10% aluminum chloride solution (w/v) was added, followed by 150 µL of 99% ethanol. A 10 µL aliquot of sodium acetate concentration 1 Mol L⁻¹ was added to the solution. As an instrumental blank, 99% ethanol was used. The plate was then incubated at room temperature for 40 min in the dark. Absorbance was measured at 415 nm with a microplate spectrophotometer. The total flavonoid content was expressed in mg equivalents in Quercetin (QE) per 100 g⁻¹ of latex.

Determination of total phenolic content

The total content of phenolic compounds was determined in a 96-well microplate by the colorimetric method using *Folin-Ciocalteu* reagent described according to Sembiring, Elya and Sauriasari (2018). A 25 µL aliquot of the latex was diluted in 100 µL of 99% methanol, and then 100 µL of *Folin-Ciocalteu* reagent concentration (1:4) was added and shaken for 60 seconds on a 96-well microplate shaker table.

The solution was kept for 15 min under stirring in a dark place. Soon after, 75 µL of a sodium carbonate solution concentration 100 g L⁻¹ were added, and again stirred for 1 min. After 2 h at room temperature, absorbance was measured at 765 nm using a microplate UV-Vis spectrophotometer. As an instrumental blank, 99% methanol was used. A standard curve was performed using Gallic acid concentration (5-350 mg L⁻¹). The result was expressed in mg equivalents of Gallic acid (AGE) per 100 g⁻¹ of latex.

Determination of total tannin content

The content of total tannins was determined as described by Ojha et al. (2018) and proposed by Price and Butler (1977). A methanolic solution of the latex of *H. obovatus* 500 µL from a 1 mg mL⁻¹ stock solution was added in 8 mL of distilled water, 0.5 mL of a ferric chloride solution concentration 0.1 Mol mL⁻¹ and 0.5 mL of a solution of potassium ferrocyanide concentration 8 mMol mL⁻¹ sequentially, and then incubated at 27 °C for 10 min.

Then, the reading was performed in a UV-Vis spectrophotometer at 720 nm. As an instrumental blank, each reagent was used without the addition of a latex sample. The total content of tannins in the sample was quantified from a standard curve of tannic acid between concentrations (1.5 to 20 µg mL⁻¹) with R² = 0.9998 and expressed in mg equivalent of tannic acid (TAE) per 100 g⁻¹ of latex.

Antioxidant activity

The antioxidant activity in DPPH free radical reduction was performed as described by Sembiring, Elya and Sauriasari (2017) in 96-well microplates. An aliquot of 20 µL of methanolic latex stock solution was prepared at the following concentrations (50-2500 ppm), and then 180 µL of a 0.147 mMol L⁻¹ DPPH solution was added to each well with the aid of a pipette automatic multichannel. After 30 min of incubation at room temperature and in a place free from light, the reading was performed in a microplate UV-Vis spectrophotometer at 517 nm. As an instrumental blank, 99% methanol was used. Ascorbic acid, Quercetin and BHT were used as a positive standard. The 50% inhibition
concentration of DPPH (IC50) was calculated and expressed in µg mL⁻¹.

**Antifungal and antibacterial activity**

For antifungal activity, ATCC standard strains of Candida albicans (ATCC 10231), Candida krusei (ATCC 34135), Candida guilliermondii (ATCC 90877) and Candida tropicalis (ATCC 4563) were used. For antibacterial activity, ATCC standard strains of Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Salmonella serovar Enteritidis (ATCC 13076), Salmonella serovar Typhymurium (ATCC 14028) and Enterococcus faecalis (ATCC 29212) were used. The trial followed as described by Santos et al. (2007) with adaptations.

Inoculums of different microorganisms were prepared from cell transfers with 26 h of cultures in sterile Sabouraud Dextrose Agar (SDA) (fungi) and Mueller-Hinton Agar (AMH) medium. Using the MacFarland 0.5 scale, the cell suspension was adjusted to a turbidity equivalent to 1.5x10⁶ (CFU mL⁻¹) in a UV-Vis spectrophotometer.

The tests were carried out by the diffusion technique on sterile paper discs. In 10 cm diameter Petri dishes, the respective culture media were added, after solidification, 1 mL of the inoculum suspension was homogenized on the surface and laminar flow chamber. Then, 7 mm diameter discs were prepared containing different concentrations of H. obovatus latex (100, 50, 25, 5 and 2.5 mg mL⁻¹) diluted in a 10% (v/v) methanolic solution of dimethylsulfoxide (DMSO). As a negative control, a 10% DMSO solution was used, and as a positive control, Azithromycin 15 µg, Cephalexin 30 µg, Tigecycline 15 µg and Ketoconazole 50 µg. The plates were incubated at 36 °C for 36 h, and after this period, the antibiosis halo was measured using a digital caliper. The minimum defined antibiosis halo was 5 mm.

**Hemolytic activity**

The determination of hemolytic activity followed as described by Ramos et al. (2020) and proposed by Dacie et al. (1975). In 50 mL conical tubes, 5 mL of saline solution at 0.9% concentration were added, and in three conical tubes as a positive control, containing distilled water and 5% human erythrocyte solution in 0.85% saline. The cell suspension was adjusted to a turbidity equivalent to 1.5x10⁶ (CFU mL⁻¹) and used as a positive control in the bioassay, and as a negative control, 100 µL of DMSO and 5 mL of sea water. The assay was carried out varying concentrations between 50-1000 µg mL⁻¹ of a methanolic solution of H. obovatus latex. The samples were incubated at room temperature in an ultra thermostatic bath for 15 min, and then centrifuged at 1000 rpm for 15 min. The supernatant was collected using an automatic pipette and transferred to a 5 mL optical glass cuvette. The reading was carried out in a UV-Vis spectrophotometer by absorption at a wavelength of 540 nm. The percentage of erythrocyte hemolysis was calculated according to equation 2.

\[
\text{%Erythrocytes} = \frac{(\text{AbsS}) \times 100}{(\text{AbsC})} \text{ Eq. 2}
\]

Where: AbsA = sample and AbsC = control.

**Leach lethality bioassay with Leach brine shrimp**

The lethality test on A. salina was performed as described by Pereira et al. (2018) adapted. Eggs of A. salina were incubated in a 1 L beaker, filled with sterile sea water concentration of 23 g L⁻¹ of sea salt and 0.7 g L⁻¹ of sodium bicarbonate, and pH adjusted to 8.5 using aqueous solution, 1 N NaOH concentration at a temperature of 27 °C under constant aeration for 48 h. After hatching, free active nauplii were collected and used for testing.

Ten nauplii were removed using a Pasteur pipette and placed in each vial containing 5 mL of brine solution. In each experiment, 500 µL of the latex was added to 5 mL of saline solution and kept at room temperature for 24 h under light and the surviving larvae were counted. The experiments were carried out together with the control (sea water), different concentrations in methanolic latex solution (35%) at the concentrations (1000, 500, 100, 50, 25 and 1 µg mL⁻¹). Percentage lethality was determined by comparing the mean of surviving larvae from the test and control tubes.

The lethal concentration values (LC50) were obtained from the best fit line plotted from the concentration versus percentage lethality. Potassium dichromate and sea water were used as a positive control in the bioassay, and as a negative control, 100 µL of DMSO and 5 mL of sea water.

**Statistical analysis**

All assays were performed in quadruplicate followed by ± SD. For the cytotoxic assay with A. salina, a linear regression was performed considering the equation of the straight line obtained by the logarithm relation of the concentrations with their respective mortalities. For statistical difference, Tukey test was used (P < 0.05) and Duncan multiple range test significance (P < 0.05). The statistical program used was PAST 3.

**Results and discussion**

The latex of H. obovatus showed a white, liquid, homogeneous, non-aromatic color and yield 19.05 ± 0.95%. Nascimento et al. (2018) found a yield of 0.4% for latex extracted from the stem of H. dracicus.

Phytochemical screening indicated the presence of alkaloids, flavonoids, phenolics, hemolytic saponins, condensed tannins, reducing sugars, organic acids, steroids and triterpenoids, proteins and amino acids, aliphatic compounds and diterpenes for the latex of aerial parts of H. obovatus (Tab. 1).

Several phytochemical studies with Himatanthus have presented important results regarding the prospection of numerous groups of phytomolecules from latex and extracts, as in the study by Herrera-Calderón et al. (2021) where they found 24 compounds in the latex of H. sucuuba as flavonoids (chrysin and apigenin), amino acids,
alkaloids and fatty acid amides. In the study by Nascimento et al. (2018) the researchers found a positive presence for condensed tannins, catechins, flavones, flavanols, xanthones, flavanols and flavanones from latex extracted from the stem of *H. drasticus*. In the study by Santos et al. (2021), although they evaluated the leaf ethanol extract, the researchers verified the positive presence for reducing sugars, tannins, phenols, flavonoids and alkaloids for *H. obovatus*.

According to Ajuru, Williams and Ajuru (2017) and Kittakoop, Mahidol and Ruchirawat (2014), alkaloids have important pharmacological properties such as antimarial, antiasthmatic and anticancer. Cholinomimetic, vasodilator, antiarrhythmic, antihyperglycemic, analgesic, and antibacterial activities are also reported (Russo et al., 2013; Qiu et al., 2014; Cuschnie, Cuschnie, Lamb, 2014). Although alkaloids have a multitude of activities, Guerra and Peters (1991), cited by Elisabetsky and Shanley (1994) describe toxic properties for *Himatanthus succuuba* (Spr. ex Muell. Arg.) Woodson.

The group consisting of flavonoids also has important pharmacological activities against free radicals, with important anti-inflammatory, anti-allergic, anti-carcinogenic, antimicrobial, hepatoprotective, antiviral and platelet protection action (Amri, Hossain, 2018; Veiga et al., 2021). Phenolic compounds also have activities against pathogens, prevent cardiovascular and neurodegenerative diseases and diabetes, are also important for the immune system, immune system stimulators and hormonal modulators (Ajuru, Williams, Ajuru, 2017).

Saponins are used in the treatment of respiratory problems, with anticarcinogenic and anti-diabetic properties, in immune modulation, in the regulation of proliferative and antifungal cells (Urbina et al., 2018; Miao et al., 2020). In the study by Santos et al. (2021) evaluating the leaf ethanol extract of *H. obovatus*, the researchers had not observed this phytochemical group. Tannins (condensed or catechetal) have physiological astrignent and hemostatic properties, accelerating wound healing and are effective in the inflamed mucous membrane, in addition to having antioxidant activity (Top et al., 2017; Dieng et al., 2020).

In the study by Santos et al. (2021), the researchers evaluated the leaf ethanol extract of *H. obovatus* in order to verify the healing activity on wounds in guinea pigs. It can be suggested that the positive presence of the phytochemical groups of tannins and alkaloids showed circumstantial improvement in the regression of the wound area after 14 days, thus, the action of this group of phytomolecules with beneficial effects in medicine is observed.

Organic acids have special antimicrobial activity, the main acids being: formic, malic, lactic, tartaric, benzoic and citric (Dian et al., 2020). Steroids and triterpenoids according to Ilhan et al. (2020) and Shady et al. (2021), have anti-inflammatory, antibacterial and analgesic action. Steroids are a class of special metabolites that exhibit decarboxylations of precursors that originate from triterpenes. Triterpenes are a group of terpenes with high diversity (Shady et al., 2021; Pham et al., 2021).

In plants, triterpenes have a protective function against herbivorous animals, fungi and insects, however, their role is still not well known, with about 40 thousand phytochemical structures, where many of them have some pharmacological activities such as antiproliferative, proapoptotic, anti-inflammatory, analgesic and antipyretic (Shady et al., 2021; Pham et al., 2021). Aldehydes and ketones stand out as antibacterial and antifungal agents (Paludo et al., 2019; Orak, Bahrisefit, Sabudak, 2019). And diterpenes have biological properties in regulating plant growth, with antimicrobial, antiparasitic, cytotoxic and anti-inflammatory activities (Etssasala et al., 2019).

### Table 1. Phytochemical prospection of latex from aerial parts of *Himatanthus obovatus*.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
</tr>
<tr>
<td>Foamy saponins</td>
<td>-</td>
</tr>
<tr>
<td>Hemolytic saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+*</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>No-reducing sugars</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
</tr>
<tr>
<td>Organic acids</td>
<td>+</td>
</tr>
<tr>
<td>Azulenes</td>
<td>-</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>-</td>
</tr>
<tr>
<td>Purines</td>
<td>-</td>
</tr>
<tr>
<td>Catechins</td>
<td>-</td>
</tr>
<tr>
<td>Depsides and deipsoides</td>
<td>-</td>
</tr>
<tr>
<td>Steroids and triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Double olefins</td>
<td>-</td>
</tr>
<tr>
<td>Proteins and amino acids</td>
<td>+</td>
</tr>
<tr>
<td>Aldehydes and ketones</td>
<td>+**</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: (-) absent; (+) present. *Green (condensed). **Yellow (aliphatic compound).

The content of total flavonoids was expressive in the latex of *H. obovatus* as observed in (Table 2), this when compared to the study by Nascimento et al. (2018) evaluating the ethyl acetate extract and in natura latex of *H. drasticus* where they found levels of 16.4 and 22.05 mg QE 100 g⁻¹ of flavonoids. Phenolic compounds play an important role for plants against external and internal factors, as well as for humans, as they are important phytomolecules capable of reducing free radicals such as singlet oxygen and nitric oxide, (Nascimento et al., 2018; Araújo et al., 2020).

Again, the total phenolic content in this study for the latex of *H. obovatus* (Table 2) was higher than that observed by Nascimento et al.
(2018) for ethyl acetate and in natura latex extract of 
H. drasticus with 59.78 and 74.46 mg GAE 100 g⁻¹. As for the antioxidant activity in DPPH free radical reduction, the latex IC₅₀ showed moderate activity in DPPH reduction, however, inferior to standard antioxidants that presented high radical reduction activity, showing a significant difference according to statistical analysis by Tukey’s test.

Free reactive species cause oxidative stress in various biomolecules and living tissues and are thus involved in a wide variety of human diseases such as rheumatoid arthritis, Alzheimer’s, Parkinson’s, amyotrophic lateral sclerosis, heart disease, various types of allergies, diabetes and numerous types of cancers (Ahmadinejad et al., 2017). Thus, it is possible to observe that the phytochemical composition of H. obovatus latex has pharmacological potential, containing significant levels of phenolic compounds, flavonoids and tannins, which are important phytochemical groups with antioxidant activity, as is also observed for other Himatanthus species, H. articulatus, H. drasticus and H. sucuuba (Soares et al., 2016; Almeida et al., 2017).

<table>
<thead>
<tr>
<th>Table 2. Biological parameters of aerial parts latex of Himatanthus obovatus.</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total flavonoid content (mg QE 100 g⁻¹)</td>
<td>78.80 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>Total phenolic content (mg EAG 100 g⁻¹)</td>
<td>166.51 ± 1.68</td>
<td></td>
</tr>
<tr>
<td>Total tannin content (mg EAT 100 g⁻¹)</td>
<td>14.81 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Latex (IC₅₀) µg mL⁻¹</td>
<td>178.87 ± 0.17d</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid (IC₅₀) µg mL⁻¹</td>
<td>2.77 ± 0.33a</td>
<td></td>
</tr>
<tr>
<td>Quercetin (IC₅₀) µg mL⁻¹</td>
<td>3.81 ± 0.60b</td>
<td></td>
</tr>
<tr>
<td>BHT (IC₅₀) µg mL⁻¹</td>
<td>4.11 ± 0.15c</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Statistical analysis by Tukey’s test (P < 0.05) was performed only for antioxidant activity.

Latex at different concentrations showed potential antifungal activity at the highest concentrations between 100-25 mg mL⁻¹ for all Candida strains, except for C. guilliermondii, which showed low fungal inhibition activity only at the highest concentration of 100 mg mL⁻¹ (Table 3). In particular, C. guilliermondii proves to be more resistant when compared to other Candida strains that have been shown to be sensitive to H. obovatus latex. When compared to the reference antifungal, latex showed moderate fungal inhibition activity, however, statistically inferior as observed by the Tukey’s test compared to the reference antifungal Ketoconazole conc. 50 μg.

As for the antibacterial activity, the latex of H. obovatus showed low sensitization activity on most bacterial strains (Table 3). High growth inhibition activity was observed in E. coli at all concentrations, demonstrating that it is a bacterial strain highly sensitive to latex phytocompounds. Discrete inhibition activity for E. faecalis was also observed only at the highest concentrations between 100-25 mg mL⁻¹. It is suggested that some groups of phytochemical compounds observed in phytochemical prospecting may be involved in both antifungal and antibacterial activities such as alkaloids, flavonoids, saponins, organic acids, steroids and diterpenes (Valiatti et al., 2018).

In the study by Sequeira et al. (2009) the researchers evaluated the latex of H. articulatus where they observed antifungal activity on C. albicans, however, they did not observe antimicrobial activity on E. coli, Bacillus subtilis and S. aureus. Silva et al. (2010) found antibacterial activity in H. sucuuba latex, hexane, chloroform and aqueous fractions, although only the aqueous fraction showed antibacterial activity against Proteus mirabilis, E. coli, S. aureus, Staphylococcus epidermidis and Staphylococcus haemolyticus. Almeida et al. (2017) in a systematic review of H. drasticus attributes the species to antifungal activity. In other reviews of the genus Himatanthus, Sequeira et al. (2009) and Soares et al. (2016) attribute in the scientific survey antifungal and antibacterial activity for the latex of H. articulatus and H. lancifolius.

<table>
<thead>
<tr>
<th>Table 3. Antifungal and antibacterial activities of aerial parts latex of Himatanthus obovatus.</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans⁷</td>
<td>16.07 ± 0.19b</td>
<td>13.55 ± 0.21b</td>
</tr>
<tr>
<td>C. krusei⁷</td>
<td>14.32 ± 0.52b</td>
<td>9.03 ± 0.14c</td>
</tr>
<tr>
<td>C. guilliermondii⁷</td>
<td>8.41 ± 0.42b</td>
<td>0.0 ± 0.0c</td>
</tr>
<tr>
<td>C. tropicalis⁷</td>
<td>13.60 ± 0.15b</td>
<td>8.89 ± 0.17c</td>
</tr>
<tr>
<td>Antifungal</td>
<td>25.07±0.32a</td>
<td>28.10±0.63a</td>
</tr>
</tbody>
</table>

**Note:** Statistical analysis by Tukey’s test (P < 0.05) was performed only for antioxidant activity.

The latex of H. obovatus demonstrated in vitro important hemolytic activity on human erythrocytes. A variation of 18-68% is observed between concentrations of 5-1000 μg mL⁻¹ of

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hemolysis activity as observed in (Figure 2). According to Siqueira et al. (2020) the hemolytic activity is considered to be at a moderate level at 40%, thus, in this study, the activity was high, as it presented higher values between 1000-50 µg mL\(^{-1}\). Results below 10% are considered low, although it was not observed in the latex of *H. obovatus* within the concentration range analyzed.

There is no record in the literature about the hemolytic activity of the studied species. Thus, comparisons can be made with synthetic drugs such as Amphotericin B, as discussed by Pereira et al. (2018), where different compounds of plant origin have the ability to inhibit hemolysis induced by this drug. We also emphasize that Amphotericin at a concentration of 40 µg mL\(^{-1}\) has, in studies, a cytotoxic effect on leukocyte concentrates and Vero cells, causing a reduction in cell viability.

It is complemented that, this biological assay is used to know the cytotoxic potential of natural or synthetic products, being able to measure the concentration and the percentage rate on the damage in the plasmatic membrane of erythrocytes. Still, among the main phytochemical groups addressed in this study, saponins stand out, which in studies are linked to disturbances in the cell membrane of erythrocytes, causing the formation of pores and rupture, extravasating the hemoglobin content (Siqueira et al., 2020). It is known that saponins have surface-active activity capable of binding to cell membrane elements of erythrocytes (Ramos et al., 2020).

![Figure 2. Hemolytic activity on human erythrocytes 5% on different concentrations of aerial part latex of *Himatanthus obovatus*. Equal letters between columns do not differ significantly according to Tukey’s test (P < 0.05).](image)

In the toxicity bioassay, the latex of *H. obovatus* at different concentrations has a moderate toxicity effect on *A. salina* with LC\(_{50}\) = 433.97 ± 2.47 µg mL\(^{-1}\). LC\(_{50}\) values equal to and less than 1000 µg mL\(^{-1}\) indicate considerable biological activity, and above this value are considered non-toxic (Meyer et al., 1982). It is suggested that the qualitative isolated substance capable of presenting biological activity as antileishmanicidal and antitumor (hepatoma (HepG2)), on human fibroblasts (HDFa) and keratinocytes (HaCat) (Almeida-Cincotto et al., 2016; Calazans et al., 2019).

**Conclusion**

The latex of *Himatanthus obovatus* presented several phytochemical groups and important biological activities, especially in the reduction of the DPPH free radical due to the contents of phenolics, flavonoids and total tannins; in addition, it was shown to be an excellent antifungal agent against *Candida* and an antibacterial agent against *Escherichia coli* and *Enterococcus faecalis*. Important cytotoxic activity was also observed for presence of groups of saponins and triterpenes in the latex of *H. obovatus* has a recognized cytotoxic effect (Viliatti et al., 2018). The bioassay with *A. salina* presents a quick result which is related to potential biological effects, being, therefore, an important test to know the toxicity levels of a compound or latex causing a strong hemolytic reaction on human erythrocytes and cytotoxicity on *Artemia salina*.

Future work should be carried out quantitatively evaluating the molecules within the main phytochemical groups, as well as evaluating the cytotoxic activity on different cancer cell culture models.

**References**


Ajuru, M. G.; Williams, L. F.; Ajuru, G. Qualitative and quantitative phytochemical screening of some plants.


