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Brugmansia suaveolens Bercht. & J. Presl: phytochemistry, cytotoxicity and its larvicidal activity against *Aedes aegypti* L. (Diptera: Culicidae)

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Abstract
The mosquito family (Diptera: Culicidae) contains several species of great public health relevance due to their role as vectors of human diseases. *Aedes aegypti*, is responsible for transmitting some of the most important vector-borne viruses that affect humanity, including Dengue, Chikungunya and Zika. The widespread and intensive use of chemical insecticides has caused significant adverse environmental effects and contributed to the emergence of populations, reducing their efficiency. Therefore, it becomes urgent to develop new alternative tools for vector control. In this context, our study aimed to trace the profile of the main secondary metabolites, followed by determining the larvicidal action against the *A. aegypti* mosquito lineage; and in vitro safety, through cytotoxicity in eukaryotic cells. The extracts were obtained by maceration with better yields for leaves, in relation to the phytochemical profile, the presence of different classes of secondary metabolites was identified, such as triterpenes, flavonoids, tannins, saponins and alkaloids. Low larvicidal lethality was observed at the concentrations tested (250-1000 ppm). The analysis of the cytotoxic potential showed a low toxic activity after direct exposure of the extracts to *Saccharomyces cerevisiae* cells, even though the species was classified as toxic. The study presented results not determined in previous research of this species, providing an innovative, relevant and significant character for future research.
Keywords: *Brugmansia suaveolens* Bercht. & J. Presl; Phytochemistry; Larvicidal.

Resumo

A família dos mosquitos (Diptera: Culicidae) contém várias espécies de grande relevância para a saúde pública devido ao seu papel como vetores de doenças humanas. *Aedes aegypti*, é responsável pela transmissão de alguns dos mais importantes vírus transmitidos por vetores que afetam a humanidade, incluindo Dengue, Chikungunya e Zika. O uso generalizado e intensivo de inseticidas químicos tem causado efeitos ambientais adversos significativos e contribuído para o aparecimento de populações, reduzindo sua eficiência. Portanto, torna-se urgente desenvolver novas ferramentas alternativas para o controle de vetores. Nesse contexto, nosso estudo teve como objetivo traçar o perfil dos principais metabólitos secundários, seguido de determinar a ação larvicida frente à linhagem do mosquito *A. aegypti*; e segurança *in vitro*, através da citotoxicidade em células eucarióticas. Os extratos foram obtidos por maceração com melhores rendimentos para folhas, em relação ao perfil fitoquimico foi identificada a presença de diferentes classes de metabolitos secundários, tais como os triterpenos, flavonoides, taninos, saponinas e alcaloides. Baixa letalidade larvicida foi observada nas concentrações testadas (250-1000 ppm). A análise do potencial citotóxico mostrou uma baixa atividade tóxica após a exposição direta dos extratos frente células de *Saccharomyces cerevisiae* mesmo a espécie sendo classificada como tóxica. O estudo apresentou resultados não determinados em pesquisas anteriores desta espécie, proporcionando um caráter inovador, relevante e significativo para futuras pesquisas.

Palavras-chave: *Brugmansia suaveolens* Bercht. & J. Presl; Fitoquímica; Larvicida.

Resumen

La familia de mosquitos (Diptera: Culicidae) contiene varias especies de gran relevancia en salud pública por su papel como vectores de enfermedades humanas. *Aedes aegypti*, es responsable de transmitir algunos de los virus más importantes transmitidos por vectores que afectan a la humanidad, incluidos el Dengue, el Chikungunya y el Zika. El uso generalizado e intensivo de insecticidas químicos ha causado importantes efectos ambientales adversos y ha contribuido al surgimiento de poblaciones, reduciendo su eficiencia. Por lo tanto, se vuelve urgente desarrollar nuevas herramientas alternativas para el control de vectores. En este contexto, nuestro estudio tuvo como objetivo trazar el perfil de los principales metabolitos secundarios, seguido de la determinación de la acción larvicida contra el linaje de mosquitos *A. aegypti*; y la seguridad in vitro, a través de la citotoxicidad en células eucariotas. Los extractos se obtuvieron por maceración con mejores rendimientos por hojas, en relación al perfil fitoquímico se identificó la presencia de diferentes clases de metabolitos secundarios, como triterpenos, flavonoides, taninos, saponinas y alcaloides. Se observó una letalidad larvicida baja a las concentraciones probadas (250-1000 ppm). El análisis del potencial citotóxico mostró una actividad tóxica baja después de la exposición directa de los extractos a las células de *Saccharomyces cerevisiae*, a pesar de que la especie fue clasificada como tóxica. El estudio presentó resultados no determinados en investigaciones previas de esta especie, otorgando un carácter innovador, relevante y significativo para futuras investigaciones.

Palabras clave: *Brugmansia suaveolens* Bercht. & J. Presl; Fitoquímica; Larvicida.

1. Introduction

Aedes aegypti remains one of the most important vectors of human arboviruses worldwide, including yellow fever, Dengue, Chikungunya and Zika (Muktar et al., 2016). Mosquito control remains the main component of all prevention and control campaigns, which usually rely on strategies such as pesticide spraying, use of biological control agents, environmental management, and others (CDC, 2017).

Dengue is considered one of the main public health problems in the world, affecting 390 million people a year, of which 96 million have clinical manifestations of the disease (WHO, 2016). The ability of the dengue virus to spread is associated with the cosmopolitan and hematophagous behavior of the females of its main vector, the culicid *Aedes aegypti* (Shamsi et al., 2018), which also transmits other viral diseases, caused by arboviruses that have been gaining prominence in the world. Brazil in recent years (Fujiwara et al., 2017; Pavela et al., 2019).

Therefore, the development of innovative, ecologically correct and efficient tools for mosquito control is of paramount importance to ensure the future ability to prevent and control diseases transmitted by these insects. Given the epidemiological importance of the arboviruses transmitted by *A. aegypti* due to the difficulty in controlling the vectors, it is necessary to identify natural products with larvicidal activity as alternatives for the control of dengue and other arboviruses. In this sense, experiments showed that plant extracts are promising and economically viable candidates for use against *Aedes* spp. (Benelli, 2020; Falkowski et al., 2020; Rodrigues et al., 2019; Rodrigues et al., 2018; Rodrigues et al., 2020; Silvério et al., 2020, Simas, 2013, Tavares et al., 2018).

Due to their vast biological diversity, plants represent an important source of potential new insecticides. Furthermore, the natural complexity of plant-derived insecticidal extracts can provide an additional advantage in combating pesticide resistance: as these extracts often contain mixtures of various chemical compounds that can act synergistically on different molecular targets within the insect, the likelihood of survival of individuals exhibiting resistance mechanisms against any of these chemicals is greatly reduced (Thiyagarajan et al., 2014; Siegwart et al., 2015).

Solanaceae are a group of plants comprising approximately 2,700 species, grouped into 98 different genera. This botanical family has a cosmopolitan distribution and includes several species of great economic importance (Jorgensen & León-Yánez, 1999; Costa et al.; 2021) and several species belonging to this family are reported to be used as insecticides by local communities (De La Torre 2008).

Brugmansia suaveolens Bercht. & J. Presl represents a promising source of new active molecules. This lack of comprehensive reports enables unprecedented studies using substances of natural origin, stimulating the discovery of new products with different applications (Aguiar et al., 2014; Schley et al., 2018). Brugmansia suaveolens, is a species native to South America, popularly known as trumpeter, used in folk medicine. The main medicinally valuable alkaloids found in *B. suaveolens* Bercht. & J. Presl are tropanes, such as scopolamine and atropine, in addition to flavonoids and terpernes (Costa et al., 2021).

Thus, our study aims to trace the profile of the main secondary metabolites, followed by determining the larvicidal action against the *Aedes aegypti* mosquito lineage; and *in vitro* safety, through the cytotoxicity of crude extracts in an experimental model with eukaryotic cells of the plant species *Brugmansia suaveolens* Bercht. & J. Presl.

2. Methodology

2.1 Plant material

Aerial parts (leaves and flowers) were obtained. Two collections of subspontaneous populations of white and pink biotypes were carried out, with 37 individuals of leaves and 43 flowers between 19:00 and 22:00, time in which the preservation of phytochemical constituents is sought. The samples were transported, sanitized with running water in order to remove dirt, followed by manually fractioning the samples, the extraction process started 2 hours after collection. The samples were collected in the buffer area of the PARNASO conservation unit, protecting an important remnant of Atlantic Forest, in Serra do Mar, in the city of Teresópolis, RJ, Brazil (geographical coordinates: altitude of 895 m, -22º26'09.38" and; -42º58'33.22" O), in April and September 2019, period in which the species blooms. The mild and humid climate of this region is favorable for the cultivation of this species (Costa et al., 2021). The study of plant material was carried out under the registration of the Brazilian System of Genetic Management of Heritage and Associated Traditional Knowledge (SISGEN) (Process number A76B905).

2.2 Extraction method

The extracts were obtained through the dynamic maceration technique for 7 days, in an amber bottle (in order to preserve photosensitive substances), at room temperature, homogenizing every 24 hours, in order to optimize the extract obtaining process. The leaves and flowers were used *in natura*, sanitized, crushed to help in the extraction process. To standardize the extraction, 1 kg of leaf sample infused in 4 L of 70% ethyl alcohol and 1.17 kg of flowers in 6 L of 70% ethyl alcohol were used. The cold maceration method was chosen to minimize and preserve the substances present in the extract. The extracts were filtered through simple vunil and concentrated in a Tecnal® rota-evaporator at 50º C and the extraction yield was calculated. The extracts were stored at 4º C (Costa et al., 2021).

2.3 Liquid-liquid extraction process

After concentrating the ethanol extracts, they were resuspended in methanol/water (9:1 v/v) starting the liquid-liquid extraction process in a separatory funnel, using solvents with different polarities: *n*-hexane, dichloromethane, ethyl acetate and butanol (Costa et al., 2021).

Subsequently the fractions were concentrated under reduced pressure. The crude extracts of leaves and flowers were analyzed by chromatography and subjected to tests to detect the secondary metabolites saponins, tannins, polysaccharides, terpenes, flavonoids and alkaloids.

2.3.1 Secondary metabolite detection tests, according to Radi & Terrones (2007)

2.3.1.1 Research of saponins

The extractive solution for searching saponins was obtained by adding 10 mL of distilled water to 2 g of plant material and then heating to boiling for 3 min. The product obtained was filtered through cotton into a test tube. Then, the test tube containing the extractive solution was shaken vertically and vigorously for 20 s. The positive result was observed by the presence of persistent foam for 20 min resistant to the addition of 1 mL of 2N HCl.

2.3.1.2 Research of polysaccharides

The hydroethanolic extract was prepared with 40 g of the vegetable and 200 mL of water in a water bath for 1 h at 70° C. Then the material was filtered and the volume was adjusted with water to 200 mL. 2 ml of aqueous extract and 2 ml of Lugol were added. The appearance of a blue color indicates a positive result.

2.3.1.3 Research of alkaloids

a) Tube 1: 2 mL of alcoholic extract, 1 mL of hydrochloric acid (HCl) and 4 drops of Bouchardt reagent.
b) Tube 2: 2 mL of extract, 1 mL of HCl and 4 drops of Dragendorff reagent
c) Tube 3: 2 mL of extract, 1 mL of HCl and 4 drops of Mayer's Reagent in a 3rd tube.

The formation of insoluble and flocculent precipitates confirm the presence of alkaloids.

2.3.1.4 Research of tannins

In a test tube containing 2 mL of the extract, three drops of an alcoholic solution of $FeCl_3$ were added, and after vigorous stirring, any color variation was observed. Blue color precipitate indicates the presence of hydrolysable tannins, and green, the presence of condensed tannins.

2.3.1.5 Research of flavonoids

2.3.1.5.1 Shinoda's reaction

From the final extract, an aliquot of 1 mL was transferred to a test tube and then 2 cm of metallic magnesium tape and concentrated hydrochloric acid were added dropwise. The positive result was observed by the appearance of pink, brown or red coloration after hydrogen evolution.

2.3.1.5.2 Reaction with ferric chloride

A few drops of 2% ferric chloride were added to 1 mL of the final extract. Depending on the flavonoid present, the color varies between green, yellow and violet.

2.4 Thin layer chromatography

Plates (3 X 10 cm) were used, coated with silica gel 60 with flowering indicator (Model Alugram Sil G / UV254 – Merck Brand), activated in an oven at 100ºC for 10 min. The samples were applied to chromatoplates with glass capillaries. Retention factors (RF) were determined through the expression: RF = DR/DM. Where: DR = distance (cm, mm) traveled by the substance. DM = distance (cm, mm) traveled by the front of the mobile phase.

After elution, the plates were dried and visualized under UV light 254nm and 365nm. The colorations of the chromatography were compared with the figures present in the Atlas by Wagner and Bladt (1996).

2.5 Evaluation of larvicidal activity on *Aedes aegypti*

The crude extracts and their respective fractions of the species under study were evaluated for activity on *A. aegypti* larvae in the 3rd stage of development. The eggs were hatched in a tray with 3 L of water in about 24 h, giving rise to mosquito larvae, which were preserved in temperature (T= 27 ± 2ºC), controlled humidity (RH = 70 ± 5%) and light exposure time of 12 h (Carvalho et al., 2014; WHO 2005; Simas et al., 2013).

The tests were performed following the methodology recommended by the World Health Organization (WHO) with adaptations. In a beaker containing 15 mL of filtered water, 100 µL of the sample were placed at concentrations of 250 – 1000 ppm. After 30 minutes of homogenization of the test sample, 4.9 mL of filtered water and five third-instar A. aegypti larvae were added. The solubilization of the samples was performed using the solvent ethanol, acetone or dimethyl sulfoxide (WHO 2005; Simas et al., 2013).

In all tests, controls for solvents and water used were maintained. The tests were performed in triplicate. The reading was performed after 24 h, verifying the number of dead larvae. The mortality percentages for each treatment were calculated by the following formula: % mortality = LM/LT x 100. Where, LM = number of dead larvae after treatment; LT = total number of larvae used in the test for each treatment (Carvalho et al., 2014; WHO 2005; Simas et al., 2013).

***2.6 Saccharomyces cerevisiae* cellular cytotoxicity assay**

To determine the sensitivity of eukaryotic organisms to the crude extracts, a suspension of the yeast *Saccharomyces cerevisiae* in sterile distilled water at a concentration of 1.0x107 cel/mL was prepared. An aliquot of 500µL of this suspension was added to different concentrations of extracts (100µL - 1000µL) and after homogenization the cells were inoculated in Mueller Hilton medium and incubated for 96 hours at 28 ºC. In parallel, control experiments were carried out. At the end of incubation, the number of colonies was quantified. These analyzes were compared to the results obtained with the control (Prasad et al., 2019; Reers et al., 1991).

2.7 Statistical analysis

Statistical analysis of experimental data was performed using the Prism 5.01 GraphPad program (GraphPad Software, Los Angeles, CA), with p values < 0.05 being considered statistically significant. The experiments were performed in triplicate (n = 3). Values were expressed as mean ± standard deviation (SD).

3. Results and Discussion

3.1 Extractive process

Extraction is a fundamental step for the separation of bioactive compounds from plant materials and their use for the development of natural products (Yasir et al., 2016). The yields of crude extracts of flowers and leaves obtained the following yields: 5.3% for the leaf extract and 5.1% for the flower extract.

Thus, among the hydroalcoholic extracts prepared, the one that presented the best yield was that of flowers, reaching a yield of 6.10%, while for the leaves, the yield of the crude extract reached 5.23%. The extracts are defined as concentrated preparations that can be presented in different consistencies, obtained from vegetable raw materials, which have or have not undergone previous treatment (crushing, grinding, drying, among others) and prepared by processes involving a solvent, the process extractive will depend on the final objective for using the product or on the results to be investigated. Extraction consists of a mass transfer operation, where soluble and volatile solids can migrate to the solvent due to continuous contact and auxiliary techniques, such as maceration (Dutra, 2012).

The grinding process used in the study is considered a factor that allows the increase of diffusivity and solubility in the extractive process of secondary metabolites. The properties of the extraction solvent, the particle size of the raw materials, the solvent to solid ratio, temperature and duration of extraction are other factors that must be considered, as they are associated with extraction efficiency, factors in which they are linked to the income generated.

In this way, a process such as crushing, reducing the size of the raw material, can improve the extraction result. Extraction efficiency will be increased by small particle size due to greater penetration of solvents and diffusion of solutes.

Wei et al. (2013) obtained ursolic acid from *Cynomorium* with a yield of 38.21 mg/g by the Soxhlet extraction method. However, he observed the degradation of catechins due to the high extraction temperature applied. The concentrations of total polyphenols and total alkaloids of the Soxhlet extraction method at 70°C decreased compared to the maceration method applied at 40°C, similar parameters used in our study. In another study by Vongsak et al. (2013), various extraction methods, such as compression, decoction, maceration, percolation, were used for *Moringa oleifera* extract. The results show that maceration was more advantageous than other methods of extracting phenolics and flavonoids with greater antioxidant activity, which demonstrates that the extractive method should be evaluated for screening in biological assays, a similar result observed by Chanda et al. (2012) in which they report a comparison between three different methods to extract antioxidants from leaves of *Syzygium cumini* L. These studies corroborate the data obtained in the present research, since the maceration and hydroethanolic solvent present low bust, ecologically and economically viable in scales enlarged for reproducibility.

It is important to point out that the seasonality of the plant is a parameter that must be considered, since the leaves are produced abundantly throughout the year, while the flowers have a specific period for flowering, as well as the place of production, a fact in which the leaves become attractive in an expanded productive scale of the extracts, being considered due to its ease of obtaining, however, other empirical factors must be associated and are discussed in the course of the work as the evaluated biological activities.

With regard to standardization and quality control, the identification and quantification of active principles/markers, reach greater relevance for the development of a desired phytotherapic, since the vegetable raw material is subject to important variations in its chemical composition. A given species, whether domesticated or not, may present variations in its secondary metabolism (intrapopulational, interpopulational, seasonal, circadian or ontogenetic) related to genetic and environmental factors (Gobbo-Neto; Lopes, 2007; Hartman, 1996; Wisdom; Rodriguez, 1982). The drying, stabilization, fragmentation and storage processes can also significantly influence the quality of the vegetable raw material.

The active substances are in the same complex matrix (extract) along with other non-active substances (most of the time, being unknown): in many cases plants have several compounds in small concentration that act synergistically (with the same or different mechanisms of action). or different mechanisms); other times, there is an association of mechanisms by substances acting on different molecular targets, responsible for the total therapeutic effect; action antagonism may also occur; some metabolites may exhibit unacceptable toxicity, circumstances in which they are addressed in subsequent chapters.

3.2 Phytochemical study

The main classes of substances present in the ethanolic extracts of leaves and flowers of *Brugmansia suaveolens* Bercht. & J. Presl are represented in Table 1.

Table 1: Secondary metabolites found in extracts of *Brugmansia suaveolens* Bercht. & J. Presl. (+) present; (-) absent.

Metabolites	Method	Results	
		flower	leaves
Saponins	Persistent foam	+	+
Tannins	Reactions with Iron Salts	+	+
Flavonoids	2,2-diphenyl-1-picrylhydrazyl (DPPH)	+	+
	Shinoda	+	+
	Ferric chloride	+	+
Polysaccharides	Lugol	-	-
Triterpenes	Sulfuric Vanillin	-	+
Alkaloids	Dragendorff	+	+
	Bouchardat	+	+

Source: Authors.

The thin layer chromatography (TLC) technique (Table 2) was used in the different fractions, with revelation by the natural solution products-polyethylene glycol reagent (NP/PEG) (flavonoid developer), showing relatively large spots in the ethyl acetate fraction, followed by another blue spot in both extracts (all of them with a yellowish color, indicative of flavonoids). The analysis showed, after revelation with sulfuric vanillin, the presence of two yellow bands in all fractions (Rf 0.12 to 0.46), which also indicate the presence of flavonoids and, therefore, the ethyl acetate fraction also showed a pinkish band suggestive of terpenes, tannins or steroids.

After revelation with the Dragendorff and Bouchart reagent, orange and brown bands were observed, indicating the presence of alkaloids or nitrogenous compounds that are predominant in the species under study. However, it should be noted that the extract was not prepared by the classic method of acid-base extraction for alkaloids, however this class presented a high concentration in all fractions evaluated. In Table 2, the retention factors, coloration obtained after development and possible metabolites are tabulated.

Table 2: Retention factors, staining obtained after development and possible secondary metabolites found in the thin chromatography technique. RF: Retention Factor A: Hexane Fraction B: Ethyl Acetate Fraction C: Dichloromethane Fraction SQ: Quercitin Standard PE: Standard Scopolamine SA: Standard Atropine SK: Standard Kanpherol VS: Sulfuric Vanillin DR: Dragendrof NP: natural products-polyethylene glycol reagent BR: Bouchardat LV: Leaves FW: Flowers.

Band	Retention Factor			Coloring	Location	Metabolite	Reagent	Origin
	FL	FR	PR					
1	0,49	-	0,46	Yellow	B	Flavonoide	VS, NP	LV, SQ
2	0,26	0,29	0,28	Blue	B	Flavonoide	NP	LV,NP, SK
3	0,12	0,14	-	Yellow	B	Flavonoide	VS	LV, FW,
1	0,12	-	-	Purple	B	Triterpeno	VS	LV
1	0,32	0,41	0,3 a 0,45	Yellow	A,B,C	Alcaloide	DR,BR	LV, FW, SA
2	0,57	0,59	0,55 a 0,65	Yellow	A,B,C	Alcaloide	DR, BR	LV, FW, PE

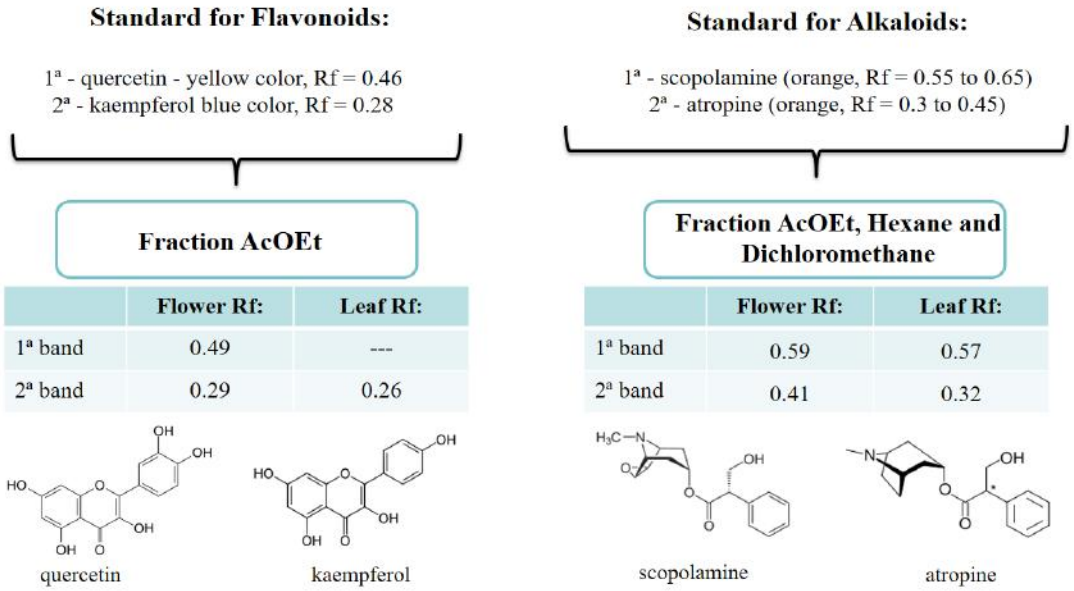
Source: Authors.

According to the chromatographic profile observed in the fractions, the standards of quercetin and kaempferol (for flavonoids), atropine and scopolamine (for alkaloids) were selected for comparative analysis in terms of color and Rf value in the fractions of the extracts. Quercetin and kaempferol were selected because they are flavonoids that occur in the species as

previously reported by Geller et al., (2014).

According to the analysis of both extracts by TLC, the presence of flavonoids with coloring and Rf similar to quercetin (yellow coloring, Rf = 0.46) and to kaempferol (blue coloring, Rf = 0.28) in the acetate fraction was observed. ethyl and the alkaloids scopolamine (orange, Rf = 0.55 to 0.65) and atropine (orange, Rf = 0.3 to 0.45) in all fractions. Therefore, CCD visualization suggests the presence of the flavonoids quercetin, kaempferol and the alkaloids scopolamine and atropine. There are reports in the literature of the isolation of flavonoids and tropane alkaloids from hydroethanolic extracts of flowers and leaves, as shown in Figure 1 (Geller, et al., 2014; Schenkel et al., 2001; Oliveira et al., 2003).

Figure 1: Representation of secondary metabolites observed by the layer chromatography technique and their respective retention factors.



Source: Authors.

3.3 Larvicide assay

In each sample obtained from the liquid-liquid partition, solubility tests of the samples were carried out, to carry out the larvicidal tests in which 0.03 mg of each sample were used. The samples were solubilized in about 300 µL of solvent, and the following solvents were tested: acetone, ethanol, water, dimethyl sulfoxide (DMSO). The samples showed higher solubility in ethanol.

The results of the assay for larvicidal activity of the species *B. suaveolens* Bercht. & J. Presl against A aegypti mosquito larvae are shown in Table 3 described below.

Table 3: Values of lethal concentrations in percentage of larvicidal activity against *A. aegypti* (Rockefeller strain) of fractions from *B. suaveolens Bercht*. & J. Presl.

Samples	250 ppm		500 ppm		1000 ppm	
	Flowers	Leaves	Flowers	Leaves	Flowers	Leaves
Gross Extracts	0%	0%	2,2%	0%	2,2%	6,6%
Ethyl Acetate Fraction	0%	0%	0%	0%	0%	0%
Hexane Fraction	6,6%	0%	6,6%	0%	6,6%	0%
Dichloromethane Fraction	0%	0%	2,2%	0%	6,6%	6,6%

Source: Authors.

The ethanolic extract of the species and its fractions did not show significant larvicidal activity even at its highest concentration (1000 ppm), the larvae remained alive, which did not make it possible to calculate the values of LC_{50}, LC_{95} and LC_{99}. Based on the results, samples from *B. suaveolens* Bercht. & J. Presl were considered inactive regarding their larvicidal activity.

The results presented by Santos et al. (2013) in larvicidal tests against *Ancylostoma* ssp of the species *B. suaveolens* Bercht. & J. Presl has been shown to be one of four toxic species to be active against this helminth species. Coelho et al. (2009) in a larvicidal study against *A. aegypti* larvae, with the extract of leaves of the species *Solanum lycocarpum* belonging to the Solanaceae family, the same family as the species *B. suaveolens*, a low larvicidal activity was also observed (13.3%). Therefore, this species does not have constituents capable of killing mosquito larvae, but may have activity against other larvae.

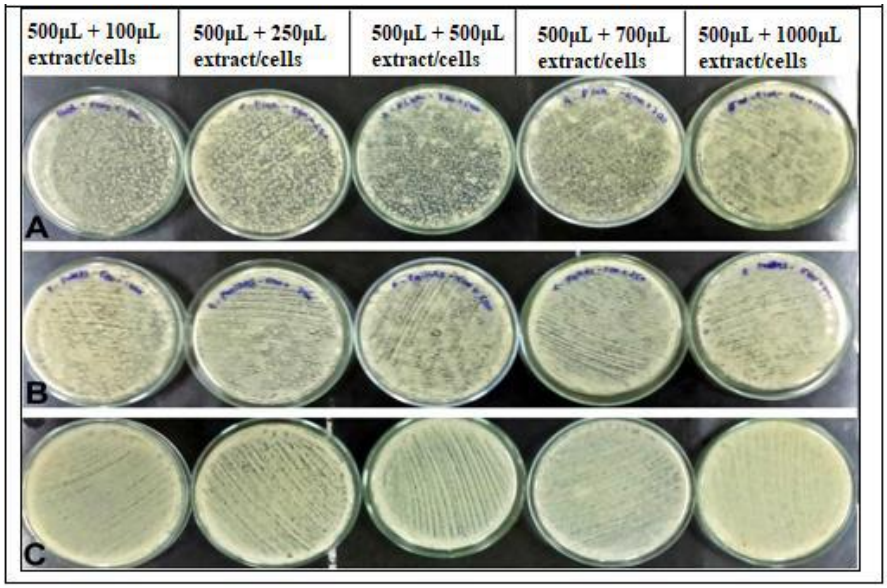
3.4 S. cerevisiae cellular cytotoxicity assay

The yeast S. cerevisiae was used for this experiment because it is a eukaryotic organism and presents similar cellular composition to mammalian cells in terms of the presence of macromolecules, organelles and proteins. This characteristic makes this yeast an important model for studies of metagenesis and DNA repair for eukaryotic models (Costa & Ferreira, 2001).

The yeast was treated with different concentrations of the extract. This assay makes it possible to observe the cytotoxicity of the intrinsic ability of a compound to cause cell death, as a consequence of damage to cell functions (Einsenbrand et al., 2002).

Saccharomyces cerevisiae and was exposed to different concentrations of extracts (100 µL - 1000 µL), but no significant inhibition of cell proliferation was observed (Figure 2), that is, the extracts were not cytotoxic for this eukaryote.

Figure 2: Cytotoxic assay in *S. cerevisiae* cells. Caption: (A) eukaryotic cells + flower extract. (B) eukaryotic cells + leaf extract. (C) control eukaryotic cells.



Source: Authors.

4. Conclusion

Our results suggest that the extract that were detected classes of secondary metabolites such as phenolic acids, flavonoids and tannins. Regarding larvicidal activity, samples derived from the species *B. suaveolens* Bercht. & J. Presl were considered inactive because they are not toxic at concentrations up to 1000 ppm. The sensitivity of eukaryotic cells (*Saccharomyces cerevisiae*) indicated that the extracts did not induce changes in the genetic material, not being considered cytotoxic to these yeasts.

Based on these results, further studies should be conducted through the Brazilian diversity, leaving through this research subsidy for further investigations related to the Solanaceae family, commonly related as a new source of insecticides against insect species of relevance to public health. However, more research is needed to (a) identify and characterize the specific chemicals responsible for insecticidal activity (b) understand the exact biological mechanisms for the low cytotoxicity under the eukaryotic cell model, as the species is related to toxic activity and (c) assess the potential effects of these chemicals on the environment and on non-target organisms. Once this information is available, it will be possible to establish new compounds through natural products that should be considered for future development as insecticides.

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