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IDENTIFICATION OF *BAMBUSA VULGARIS* (BAMBOO) EXTRACT SECONDARY COMPOUNDS AND ITS BIOLOGICAL EFFECTS

IDENTIFICAÇÃO DE COMPOSTOS SECUNDÁRIOS DE EXTRATO DE *BAMBUSA VULGARIS* (BAMBU) E SEUS EFEITOS BIOLÓGICOS

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ABSTRACT

Among the various species of medicinal plants used as treatment, *Bambusa vulgaris* is indicated due to its medicinal properties for a range of pathological signs and symptoms. The present study had the objective to conduct the phytochemical and biological evaluation of the aqueous extract of *B. vulgaris* dried leaves. Extracts were prepared in the different concentrations and which were subjected to the following tests: identification of secondary metabolites, toxicity when exposed to *Artemia salina*, evaluation of the antioxidant potential, in vivo test with *Allium cepa* and antimicrobial activity. The results showed that the aqueous extract of *B. vulgaris* has alkaloids, coumarins, flavonoids, saponins and triterpenes. It did not present toxicity when exposed to *A. salina* (DL50= 1.016 ug/mL) and has antioxidant activity (EC50=130.5 mg/mL). Regarding the test with *A. cepa*, it was possible to observe that the extract in the highest concentrations inhibited root growth as well as the cellular division. The micronucleus formation was detected in all tested concentrations, but the extract was not mutagenic. The results suggest that aqueous extract of *B. vulgaris* has some biological effects.
Keywords: Medicinal Plants, Therapeutic Use, Ethnobotany, Popular Usage.

RESUMO

Dentre as diversas espécies de plantas medicinais utilizadas como tratamento, a *Bambusa vulgaris* é indicada devido as suas propriedades medicinais para uma variedade de sinais e sintomas patológicos. O presente estudo teve como objetivo realizar a avaliação fitoquímica e biológica do extrato aquoso das folhas secas de *B. vulgaris*. Foram preparados extratos em diferentes concentrações e submetidos aos seguintes testes: identificação de metabólitos secundários, toxicidade frente a *Artemia salina*, avaliação do potencial antioxidante, teste in vivo com *Allium cepa* e atividade antimicrobiana. Os resultados demonstraram que o extrato aquoso de *B. vulgaris* possui alcaloides, cumarinas, flavonoides, saponinas e triterpenos. Não apresentou toxicidade frente a *A. salina* (DL50= 1.016 ug/mL) e possui atividade antioxidante (EC50=130.5 mg/mL). Com relação ao teste com *A. cepa*, verificou-se que o extrato nas maiores concentrações inibiu o crescimento radicular bem como a divisão celular. Constatou a formação de micronúcleo em todas as concentrações testadas, porém o extrato não foi mutagênico. Os resultados encontrados sugerem que extrato aquoso de *B. vulgaris* possui alguns efeitos biológicos
Palavras-chave: Plantas Medicinais, Uso Terapêutico, Etnobotânica, Uso Popular.

1. INTRODUCTION

The use of plants for curing diseases and symptoms relief has occurred in many cultures since the early days of mankind and, despite advances in technology, this therapeutic procedure is still widely used. In Brazil, the influence of cultural interactions among indigenous, black and Portuguese peoples was the responsible for the dissemination of the knowledge inherited in relation to the cultivation and the use of plant species [1,2].

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Studies carried out in several parts of Brazil show that a large part of the population has already consumed medicinal plants as a form of treatment, which proves the great diffusion of this practice among Brazilian people [3,4].

Bambusa vulgaris (Bamboo) species, that belongs to the family *Poaceae*, subfamily *Bambusoideae*, is among the plants used. It is found in many parts of the world, but it is uncommon in Europe [5]. Bamboo stands out because it is used for various purposes, such as: construction industry; paper producti on; furniture and toy manufacturing, in addition to be used as phytotherapic product [6].

In some cultures, bamboo is used as a treatment for complaints of paralysis, inflammation, diabetes, fever, as preparation of astringent and ophthalmological solutions, stomach problems, as well as being used as an abortifacient in Nigeria [7,8]. The usage of Bamboo in Brazil stands out for its use in ornamentation, in furniture and handicraft manufacturing, however its phytotherapic use had not been found in the literature until reports from Northern Brazil identified it [9].Moreover, it has a high nutritional value, thus offering vitamins, proteins, amino acids, among other compounds [10,11]. It is possible to verify in literature that in other works using *B. vulgaris* were found several secondary metabolites such as alkaloids, flavonoids, triterpenes, saponins, coumarins, among others, that are possibly responsible for conferring the previously described properties, since these metabolites are directly related to the medicinal properties of plants [12,13].

Considering the usage of *B. vulgaris* as a form of treatment, the present study aims to present a phytochemical and biological evaluation of the aqueous extract of *B. vulgaris* dry leaves.

2. MATERIALS AND METHODS

2.1 Plant material collection and extract preparation

B. vulgaris leaves used in this study were collected in August, 2016 in the municipality of Ouro Preto do Oeste, Rondônia, Brazil, submitted to botanical identification and had their exsicata deposited in Antônio Dalla Marta herbarium of Lutheran University Center of Ji-Paraná (Centro Universitário Luterano de Ji-Paraná) – CEULJI / ULBRA, under number 270.

The collected leaves were sent to Pharmacognosy and Phytochemistry Laboratory of CEULJI / ULBRA, where they were dried in a kiln at 44° C for 48 hours and ground to powder.

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The aqueous extracts were prepared by the infusion technique [14], in the following concentrations: 250 mg/mL; 125 mg/mL; 62,5 mg/mL; 31,25 mg/mL; 15,62 mg/mL; 7,81 mg/mL and 3,9 mg/mL. Hydroethanolic extract (80%) was also prepared using the maceration technique for 96 hours for the antimicrobial activity.

2.2 Phytochemical Prospection

For phytochemical prospecting, the compounds Alkaloids, Flavonoids, Triterpenes [15], Tannins, Anthraquinones [16], Saponins, volatile Coumarins [17] and Aurones and Chalcones [18] were investigated, following the recommended methodology.

2.3 Cytotoxic Activity

The cytotoxic activity of *B. vulgaris* aqueous extract was determined by the lethality test when exposed to *Artemia salina* microcrustacean [19], making dilutions from the initial concentration (250 mg/mL) in Sodium Chloride solution (NaCl 3,5%)to obtain the pre-established concentrations for the study and after,5mL were transferred to a tube, and then adding 10 Naupils of *A. salina*. After 24hours dead and living microcrustaceans were counted to determine the median lethal dose (DL_{50}) [20]. Only NaCl solution (3,5%) was used as negative control.

Data interpretation was made as described by Mayer et al. [19], who classifies the samples that present DL50 < 1000 µg/mL as toxic or active, and the ones that present DL50 >1000 µg/mL as non-toxic or inactive.

2.4 Antioxidant activity

The antioxidant potential of *B. vulgaris* aqueous extract was determined by the free radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity, which in the presence of an antioxidant properties agent undergoes discoloration, going from violet to yellow [21].

The technique consisted in adding 3,9 mL of DPPH (0,06 mMol) and 100 µL of the extract to be tested in a test tube, then the analysis of the absorbance in 515 nm and the monitoring were done, to verify the test stabilization. Based on the results, the value of EC_{50}, which is the sample capacity to reduce DPPH concentration by 50%, was calculated. For the

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negative control, 100 µl of water was added in 3,9mL of DPPH (0,06 mMol), while the methyl alcohol was used as white.

The percentage of free radical sequestration (%SRL) is due to the decrease in absorbance of the sample (As) related to the absorbance of the control (Ac): %SRL = (Ac – Aam/Ac) x 100.

The standard curve of DPPH was carried out based on the absorbance values of test extracts (10 µM, 20 µM, 30 µM, 40µM, 50 µM and 60 µM) of DPPH methanolic solution, using methyl alcohol as white.

2.5 Antimicrobian activity

B. vulgaris aqueous and hydroethanolic extracts were used for antimicrobial activity. *Staphylococcus aureus* (ATCC 2494), *Shigella* sp., *Salmonella* sp and *Escherichia coli* (ATCC 25922) bacteria were tested, all of them from the bacteria collection of CEULJI/ULBRA.

According to *Clinical and Laboratory Standards Institute* [22] (adapted), the determination of antimicrobial activity was performed in two tests (Disk diffusion and Agar diffusion), for which bacterial suspensions in physiological solution equivalent to 0,5 on Macfarland scale were prepared. The culture medium used was Mueller-Hinton Agar.

2.6 - Disk Diffusion Test

Sterile filter paper disks (6 mm) were inoculated in the same 20 µl of the extracts to be tested, remaining for 48 hours in the dissector. Afterwards, the discs were put in Mueller-Hinton Agar plates with the bacterial inoculum, sending these to the kiln for 24 hours.

2.7 - Agar Diffusion Test

With the support of a mold, 6 mm wells were prepared in Mueller-Hinton Agar plates, which received 20 µl of the extracts. It took 30 minutes for complete absorption of the extract by the culture medium, and then the plates were incubated in a kiln for 24 hours.

After the incubation period the inhibition halos were measured with a pachymeter. Penicillin (30ug) was used as a positive control to *S. aureus*, ceftriaxone (30ug) to *Shigella* sp. and *E. coli* and ciprofloxacin (30ug) to *Salmonella* sp. All tests were performed in triplicates.

***2.8 Allium cepa* Test Submission**

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The onion bulbs were submerged in distilled water for 24 hours before the test begins, and then the lower germinative part of the bulbs were submerged in each solution used in the test for a period of five days, under an average temperature of 24ºC (±2).In total, 90 bulbs were used in the experiment, 10 of them for each series (concentration and control).

After 48 hours, the meristems put in methanol/acetic acid (3:1) for 12 hours were cut and then washed with distilled water, after their hydrolysis with Hydrochloric Acid (HCl) were done at 60º C for 6 minutes. After the process described previously, the meristems were placed on slides (duplicate) and stained using a stain set (Panótico Rápido LB), then the squash was prepared with a coverslip to provide a better visualization of the meristematic cells [23].

Based on the analysis of the slides made with 40x and 100x lenses, the formation of micronuclei in 1,000 cells could be observed. In addition to the mitotic index, which is based on dividing the number of cells in mitosis (prophase, metaphase, anaphase and telophase) by the total number of cells (interphase and mitosis), the result obtained is multiplied by 100 [24,25]. On the fifth day of the experiment, the largest root was selected and a pachymeter was used to analyze its length [26].

2.9 Statistical Treatment

The statistical treatment of this study was determined by one-way analysis of variance (ANOVA) and for comparison of means of treatments, Dunnett's and Tukey's methods were used at a significance level of 1 and 5%.

3. RESULTS

As shown in Table 1, the results of *B. Vulgaris* aqueous extract phytochemical prospection indicated the presence of five secondary metabolites.

Table 1. Secondary metabolites identified in *Bambusa vulgaris* aqueous extract.

Compounds	Results
Alkaloids	Positive
Antraquinone	Negative
Chalcones and Aurones	Negative
Cumarins	Positive

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Flavonoids	Positive
Saponins	Positive
Tannins	Negative
Triterpenes	Positive

The percentage of mortality observed on the test with *A. salina* is shown in Figure 1, with the logarithm (log) value of the tested concentrations. The linearity of the test (R^2= 0,8229) resulted in LD50 of 1.016ug/mL. Considering the studies of Meyer et al. (1982), the species was classified as non-toxic or inactive, however it could be observed that it is extremely close to the limit, since values below 1000 µg/ml are considered toxic.

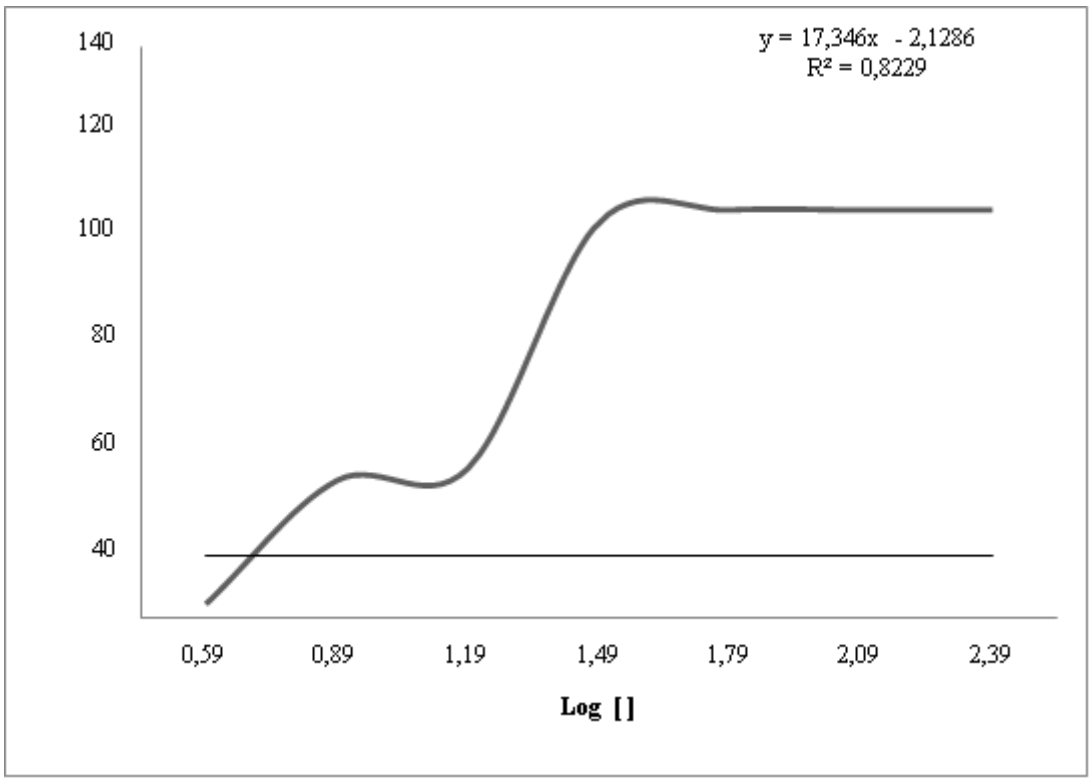

Figure 1. Distribution of mortality percentage of Artemia salina by the log of the different tested concentrations (mg/mL).

Regarding the antioxidant activity, the first indicative of positivity for the extract concerned was the change in color from violet to yellow; however, this change of color was more evident in the 4 higher concentrations, 2 of them became slightly yellow and the last one, unchanged. Such preliminary result was confirmed by the absorbance (Figure 2), showing that the higher the concentration, the lower the absorbance.

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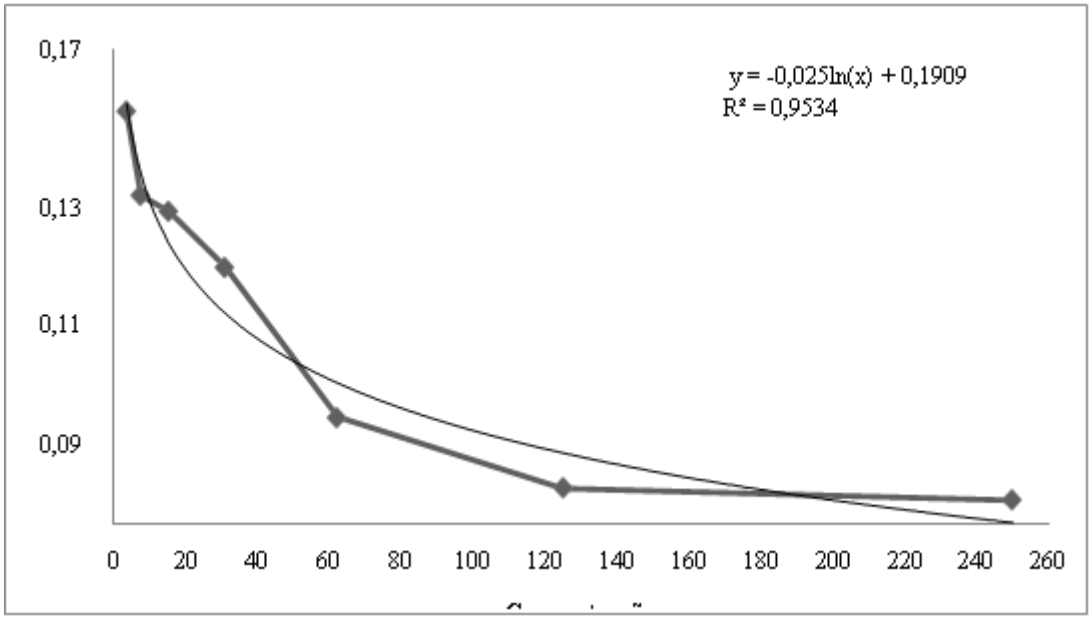

Figure 2. DPPH Curve of Bambusa vulgaris aqueous extract

The lower tested concentration got an absorbance of 0,154 nm and, when compared to the absorbance of the negative control employed (0,155 nm), confirmed that it did not develop antioxidant activity.

The value of EC_{50} was 130,5 mg/mL, so it was necessary 130,5mg of *B. vulgaris* to reduce by half the DPPH solution.

Table 2 shows the results obtained for *A. salina* lethality test, and it can be observed that in the highest concentrations the extracts were toxic, leading to 100% of *A. salinas* lethality.

Table 2. Comparison of test concentrations of *Bambusa vulgaris* when exposed to biological toxicity testing.

Concentrations (mg/mL)	% of *A. Salina* mortality
3,9	3,4
7,81	33,4
15,625	36,7
31,25	97
62,5	100
125	100
250	100

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Regarding the antimicrobial activity, no activity was observed in any of the extracts and methods used.

On the root growth, a dose-dependent relationship was observed inversely proportional, that is, the lower the concentrations, the higher the radicles, since the concentration of 250mg / mL obtained a mean of 0.03 ± 0.06cm, while the (3mg / mL) presented a mean of 4.1 ± 1.96cm.

Statistical analysis showed that the size of the roots of the positive control and of the concentrations used showed a significant difference when related to the negative control (Figure 3).

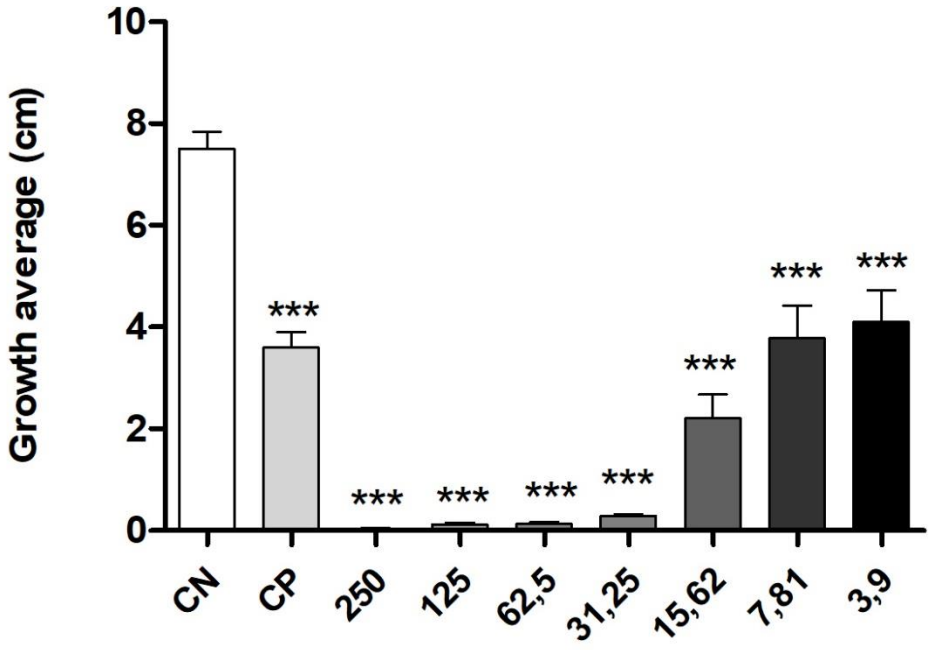

Figure 3. Distribution of root growth averages by concentration of Bambusa vulgaris extract. *** Analysis of variance ANOVA by Tukey test at 5% significance (P <0.05)

The inhibition of the mitotic index was observed at all concentrations when compared to the positive control, but the concentrations of 250mg/mL and 125mg/mL showed a statistically significant inhibition in relation to the other treatments (Table 3).

Table 3. Number of cells in Mitosis analyzed and Mitotic Index obtained in *Allium cepa*.

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Treatment	Interphase	Mitotic Phase				MI
		Prophase	Metaphase	Anaphase	Telophase	
CN	19048	916	0	0	0	4.54bc
CP	17083	2588	44	46	54	13.11a
250	1884	98	0	1	6	0.49d
125	1852	137	0	3	5	0.69d
62,5	7416	553	0	8	8	2.77cd
31,25	12816	1140	0	12	21	5.71bc
15,625	14570	1382	1	15	23	6.93bc
7,81	12709	1251	0	14	22	6.27bc
3,9	14452	1494	0	11	36	7.49b

NC = Negative Control. PC = Positive Control. Analysis of variance ANOVA –Tukey's at a significance level of 1% ($p<0.01$)

Figure 4 shows the micronucleus index, where it is possible to verify that the positive control and the concentration of 250mg/mL presented a significant difference in relation to the NC and other concentrations.

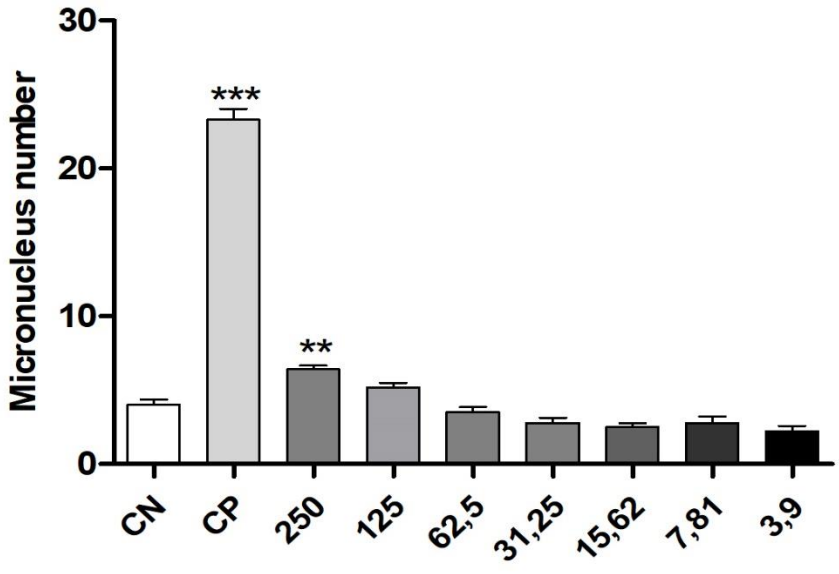

Figure 4. Average of micronuclei per concentration of *Bambusa vulgaris* aqueous extract. *** Analysis of variance ANOVA by Tukey's test at a significance level of 5% ($P < 0.05$).

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4. DISCUSSION

Faced to the relentless search for new substances, plants have become one of the main targets, considering their secondary metabolites production, which are components related to plant protection and defense mechanisms against potential hazards such as predators and pathogens. The substances of diverse species have already proved to be efficient against human diseases, in addition to being useful for the development of fragrances, pesticides, among others [27,28].

Therefore, after analyzing the results found in the present study, it was verified that the *B. vulgaris* used in the research has secondary compounds (alkaloids, flavonoids, coumarins, saponins and triterpenes), thus indicating that it may have some medicinal properties.

The alkaloids are a broad group of compounds in which there are numerous subclasses due to great structural variation, the main ones are the indole alkaloids, imidazolic, isoquinolic, piperidinic, pyruvic, quinolytic, and tropic alkaloids [29]. Therefore, several biological and pharmacological activities are related to this group, such as: cytotoxic, platelet antiaggregant, antibacterial, antifungal and antiplasmic [30], emetic, amebicidal [31], contraceptive, anti-inflammatory, antimalarial, anti-HIV, leishmanicidal, antitumor, anti-hypertensive, anticholinesterase, central nervous system stimulant and many others [32].

Flavonoids are associated with antimicrobial, antineoplastic, antioxidant, anti-inflammatory and several other activities [33]. Coumarins have a variety of pharmacological properties, such as antidepressant, antitumor, antimicrobial, antioxidant, anti-inflammatory and antiasthmatic activities [34,35]. Saponins are also known for their antioxidant potential, however they also have cytotoxic effects that have been exploited to act against tumor cells [36]. Among triterpenes biological activities are cytotoxic, antitumoral [37], antiparasitic [38,39] and antimicrobial activities [40].

In a similar study, GOYAL et al. [13] verified the presence of all the compounds studied (Saponins, steroids, alkaloids, tannins, carbohydrates, flavonoid, anthraquinones, glycosides and reducing sugars) when evaluating the methanolic extract of *Bambusa vulgaris* "Vittata". However, Yakubu and Bukoye [12] found positive results for saponins, alkaloids, tannins, phenolics, flavonoids, anthraquinones and glycosides; and negative results for steroids, terpenes and chalcones when evaluating the constituents of *B. vulgaris* aqueous extract.

The lethality test with *A. salina* provides a preliminary result of the general toxicity level of the extract to be studied [41]. In this study it was verified that the extract analyzed is non-toxic (LD 50 of 1.016 µg / mL), being near to the tolerable limit [19].

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According to the National Poisoning Information System (Brasil - SINITOX) [42], in Brazil, between 2012 and 2015, 3637 cases of poisoning by plants were registered and resulted in 4 deaths. Sinitox does not present isolated cases of intoxication caused by ingestion of plants, however the data presented lead us to believe in the risk plants may represent despite the number of intoxications being low. Oliveira et al. [43] and Monseny et al. [44] hypothesize that the number of cases of intoxication by plants is greater, since many cases are not reported or reported as exposure to an unknown toxic agent.

Yakubu et al. [45] observed different reactions at the end of the experiment of *B. vulgaris* leaves aqueous extracts in pregnant rabbits. Alade et al. [46], testing *B. vulgaris* extract in male rats, concluded that the extract administration may have low toxicity, resembling the present study, that the result may suggest a relative toxicity due to the proximity of the limit.

The data for the antioxidant activity found in this study showed that *B. vulgaris* aqueous extract presented antioxidant capacity (EC_{50}=130,5 mg/mL). Palioto et al. [47] point out that the lower the value of EC50, the greater the antioxidant activity. The antioxidant activity of the present study may be related to the presence of secondary compounds such as flavonoids, saponins and coumarins, since they have the capacity to stop the unpaired electrons without damaging the cellular structure, due to the stability these compounds present [25,36].

In recent years, the research of agents with antioxidant properties has been intensified, since several pathological processes such as cancer, Alzheimer's disease, among others, may be related to free radicals, as well as the aging process [48,49].

According to Gasparri [48], free radicals are constituted of molecules that have one or more unpaired electrons in the last orbital, provoking the same instability, besides becoming very reactive, promoting great affinity with several molecules present in the cellular structure. The free radicals in our organism are combated by the antioxidant agents produced by our body or by those ingested in our diet, but when an imbalance occurs between the production of free radicals and the antioxidants present in the body, oxidative stress occurs, triggering several pathological problems, requiring the ingestion of food/antioxidant drugs [49,50].

Goyal et al. [13] found a result of EC50 superior (EC50 = 269.53 mg / mL) to the one found here when analyzing the methanolic *B. vulgaris* "Vittata" extract. Tripathi et al. [51] verified greater results than in the present study when investigated the antioxidant capacity of *B. vulgaris* using chloroform, acetone and methanol as solvent. These authors also analyzed the *Bambusa mutans* species with the same solvents and, when comparing with the results obtained by *B. vulgaris* extracts, they observed that *B. mutans* had better antioxidant activity, but when

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compared with the results of this work, only the methanolic extract presented lower EC50 (EC_{50}=123.45 mg/mL).

The aqueous and hydroethanolic extracts of *B. vulgaris* did not present antimicrobial activity for the tested microorganisms, but it cannot be said it does not have the potential to be a new antibiotic, since there are other microorganisms, solvents and extraction methods.

Compared to the results found, Owolabi and Lajide [52] found the antibacterial activity of *B. vulgaris* against *S. aureus*, *Bacillus cereus*, *E. coli* and *Klebsiella pneumoniae* using 3 different solvents (chloroform, n-hexane and ethyl acetate). Macherla et al. [53] found antimicrobial activity of *B. vulgaris* in the three concentrations used (10, 50 and 100 mg / mL) for the *S. aureus*, *Bacillus subtilis*, *E. coli* and *K. pneumoniae* microorganisms, using the methanolic extract.

The obtained data for antimicrobial activity were negative, though the extract analyzed indicated the presence of flavonoids, coumarins and triterpenes, classes known by several compounds with antimicrobial properties, however they are large families of compounds, which not all present such activities [33,35,40]. Therefore, the difference among the results found here and those available in the literature can be explained since *B. vulgaris* aqueous extract analyzed probably does not have the compounds that condition the antimicrobial activity or have them in extremely low amounts. In addition, the presence of secondary metabolites in plants is influenced by natural and artificial factors to which the plant is exposed to, since solar incidence, soil, season, pollutants, among others, interfere directly in production of metabolites, which explains the results found [54].

It was observed that the extract analyzed influenced the root growth in the test with *A. cepa*: in the highest concentrations tested the growth was almost null, and it increased when the concentration decreased. This result indicates a probable allelopathic activity of the *B. vulgaris* extract when exposed to the roots of *A. cepa*, suggesting its toxicity. The fact observed in this study may be related to the presence of alkaloids, flavonoids, saponins and triterpenes, as they perform allelopathic activities along with other secondary metabolites [55,56].

It was observed that the mitotic index in the first concentrations of *A. cepa* tested presented low values, when compared with the values obtained in the lower concentrations. Fachinetto et al. [56] point out that high concentrations of certain compounds may exert an inhibition or stimulation of the cell cycle, therefore, when relating it to the results found, the extract of *B. vulgaris*, in its highest concentrations, inhibited root growth and, consequently, cell division. According to Turkoglu [57] cell cycle blockade in G_2 phase, or even an

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interruption on DNA synthesis results in a decrease of the mitotic index, suggesting the concentration is cytotoxic.

The frequency of Micronuclei observed in the present study may have a directly proportional relation, as the lower the concentrations the smaller the number of them. As noted here, Poletto et al. [58] and Fão et al. [59] also found a proportional relationship between concentrations and micronucleus formation.

The higher concentration tested stimulated a significant growth of micronuclei, which shows that at the concentration of 250 mg / mL *B. vulgaris* aqueous extract interfered more intensely, triggering the appearance of micronuclei, which are DNA found in cytoplasm, originating from small pieces of acentric chromosomes [60]. However, even in contact with micronuclei, *B. vulgaris* extract was not mutagenic in any of the concentrations, since the micronucleus amount was low.

It should be emphasized that the observed results come from a plant system, which does not mean it will occur in a human body, but it is an indication that *B. vulgaris* aqueous extract can interfere in the animal system. However, Barros e Davino [61] reports it is necessary that the toxic agent reaches specific locations, in concentrations and sufficient time, to harm or interfere in an animal organism.

CONCLUSION

B. vulgaris aqueous extracts showed the presence of some secondary metabolites and their various therapeutic properties and biological activities that have been described in the literature, also showing high antioxidant potential. However, it could be observed that the toxicity to *A. salina* can almost reach the toxic limit. For antimicrobial activity, the two extracts tested presented no *S. aureus*, *Shigella sp*, *E.coli* and *Salmonella* sp. In addition, *B. vulgaris* aqueous extracts showed inhibition of root growth and mitotic index, presenting certain toxicity and allelopathic activities, not demonstrating it was mutagenic. Micronuclei were present in *Allium cepa* meristematic cells, establishing a directly proportional relationship in larger concentrations. However, such results elucidate possible activities and properties of the plant for better guidance, regarding empirical medicinal use and future research.

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