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Original Article

Chemical and biological profile of the fractionated extract of the species *Campylopus savannarum* (Müll. Hal.) Mitt.

Perfil químico e biológico do extrato fracionado da espécie *Campylopus savannarum* (Müll. Hal.) Mitt.

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Abstract

The indiscriminate use of allopathic drugs has selected resistant bacterial and fungal populations which represents a severe public health problem worldwide. On the other hand, plants are in a prominent position due to the capability to synthesize structurally complex bioactive metabolites that can be an alternative to resistant microorganisms' control. In this work, we evaluated the chemical composition and the antimicrobial, antioxidant, and cytotoxic potential of the fractionated extract of *C. savannarum* in ethyl acetate. The extract of *C. savannarum* was divided into 12 fractions that were submitted to phytochemical screening, minimum inhibitory concentration (MIC), reduction of 1,1-diphenyl-2-picrylhydrazine (DPPH), and hemolytic activity of sheep erythrocytes assays. During the investigation, all extract fractions presented alkaloids, triterpenoids, steroids, and phenolic compounds in qualitative analyses, while in the quantitative evaluation, we observed the presence of both phenols and flavonoids in these fractions. Among the fraction, the highest phenolic content was observed in the Cs23-24 fraction (2.480 mg EAG/g), while the Cs31-34 fractions presented the highest amount of flavonoid (182.25 µg EQ/100 mg). Nine of the 12 fractions of the moss species' extract showed antimicrobial action Against Gram-positive bacteria: *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis;* Gram-negative bacteria: *Pseudomonas aeruginosa*; and also antifungal activity against *Candida albicans* and *Candida glabata*. The cytotoxic assay demonstrated that the tested fractions did not induce hemolysis at concentrations 10 and 100(µG/ML). In the antioxidant evaluation, the Cs55-69 fractions were the ones that presented the highest scavenging activity (57, 0%) followed by the Cs45-54 fraction (42.7%). Overall, the evaluation of the biological potential of the fractionated extracts of *Campylopus savannarum* showed promising data, in the search for natural antimicrobial compounds.

Keywords: antimicrobial activity, toxicity, antioxidant, fractionated extract, *Campylopus savannarum*.

Resumo

O uso indiscriminado de drogas alopáticas tem selecionado populações bacterianas e fúngicas resistentes, o que representa um grave problema de saúde pública mundial. Por outro lado, as plantas ocupam posição de destaque devido à capacidade de sintetizar metabólitos bioativos estruturalmente complexos que podem ser uma alternativa ao controle de microrganismos resistentes. Neste trabalho, avaliamos a composição química e o potencial antimicrobiano, antioxidante e citotóxico do extrato fracionado de *C. savannarum* em acetato de etila. O extrato de *C. savannarum* foi dividido em 12 frações que foram submetidas aos ensaios de triagem fitoquímica, concentração inibitória mínima (CIM), redução de 1,1-difenil-2-picrilhidrazina (DPPH) e atividade hemolítica de eritrócitos de ovinos. Durante a investigação, todas as frações do extrato apresentaram alcalóides, triterpenóides, esteróides e compostos fenólicos nas análises qualitativas, enquanto na avaliação quantitativa, observou-se a presença de fenóis e flavonoides nessas frações. Dentre as frações, o maior teor de fenólicos foi observado na fração Cs23-24 (2,480 mg EAG/g), enquanto as frações Cs31-34 apresentaram a maior quantidade de flavonoides (182,25 µg EQ/100 mg). Nove das 12 frações do extrato da espécie de musgo apresentaram ação antimicrobiana contra bactérias Gram-positivas: *Bacillus cereus, Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis;* Bactérias Gram-negativas: *Pseudomonas aeruginosa*; e também atividade antifúngica contra *Candida albicans e Candida glabata*. O ensaio citotóxico demonstrou que as frações testadas não induziram hemólise nas concentrações 10 e 100(µG/ML). Na avaliação antioxidante, as frações Cs55-69 foram as que apresentaram maior atividade seqüestradora (57,0%) seguida da fração Cs45-54 (42,7%). No geral, a avaliação do potencial biológico dos extratos fracionados de Campylopus savannarum mostrou dados promissores, na busca de compostos antimicrobianos naturais.

Palavras-chave: atividade antimicrobiana, toxicidade, antioxidante, extrato fracionado, *Campylopus savannarum*.

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1. Introduction

The use of medicinal plants to treat diseases is an ancient strategy used worldwide. Extracts with biological activity derived from natural resources are largely used to fulfill human needs, promote better living conditions, and increase the chances of survival (Moraes and Santana, 2001; Andrade et al., 2007). In terms of variety, Brazil displays the most abundant vegetal diversity on the planet. From Brazilian flora, there is a large number of unique medicinal plants, among which some have not yet been studied (Bosso, 2010). It is estimated that only 6% of the existing plant species have been pharmacologically investigated, and about only 20% were submitted to phytochemical tests (Cragg and David, 2013).

Research on the numerous biological activities of different types of plants has reached great scientific interest. However, Zhu et al. (2006) highlighted that among existing plants, bryophytes are highlighted as one of the most relevant and favorable sources of antibiotics and biologically active compounds in nature.

Bryophytes are the second largest group of plant species and can be divided into three groups: Marchantiophyta, Anthocerotophyta, and Bryophyta (Goffinet et al., 2009). The use of bryophytes as a source of bioactive compounds was neglected for a long time due to the generally small size of its structures, which implies working with low biomass (Asakawa, 1981). On the other hand, this group of plants considered basal is eminently easy to withstand biotic and abiotic stresses, indicating the ability to produce several secondary metabolites (Asakawa, 2007).

Research on the medicinal potential of bryophyte species is still scarce in Brazil. There are reference studies such as Pinheiro et al. (1989), where the authors studied bryophytes from the Amazon as possible producers of substances with antibiotic properties, as well as a recent study in which the diversity of secondary metabolites of *Syzygiella uvaulis* (Nees) Steph was investigated (Costa et al., 2018).

In this perspective, bryophytes become promising organisms in the production of biologically active compounds, some studies with the Marchantiophyta division show that the chemical composition of these bryophytes has several biological activities, such as antifungal, anticancer, antibacterial, anti-inflammatory, and antioxidant agents, among others (Asakawa, 1981). Liverworts have been studied for their wealth of oil bodies, capable of accumulating lipophilic terpenoids and aromatic compounds, thus being the most studied group (Marko et al., 2001).

The *Campylopus savannarum*, the specie studied in this work, lacks chemical and biological information in the scientific literature. They are characterized by belonging to the group of mosses (Bryophyta) and have a pantropical distribution, with reports on the occurrence in mountainous regions, restinga, cerrado, and caatinga (Santos, 2011). Thus, the objective of this work was to evaluate the chemical composition, as well the antimicrobial, antioxidant, cytotoxic, and hemolytic activity of *Campylopus savannarum*.

2. Material and Methods

2.1. Botanical material and extracts preparation

Samples of the species *C. savannarum* were collected in the city of Alagoinhas, Bahia state, northwest of Brazil, in a remnant of Atlantic Forest on the edges of the forest (12º10'42.62"S/38º24'39.52'W). Botanical identification was carried out in the laboratory of plant taxonomy at the Universidade Estadual de Feira de Santana (UEFS). Reference specimens were deposited in the Herbarium of the University (HUEFS) with the registration number (14710). The extracts were prepared using the whole plant (phyllid, stem, and rhizoid). The botanical material was dried at 40°C and ground using ethyl acetate solvent. Three successive extractions were performed with intervals of 72 h in closed containers, protected from light. The filtrate remained exposed to room temperature for complete evaporation of the solvent and concentration of the extracts, then it was stored at 4ºC until use.

2.2. Fractionation of the extracts

The crude extract was subjected to column chromatography. Kiesel silica gel 60 (0.063-0.200 mm) was the adsorbent material used as the stationary phase. Which was coupled to a valve at its lower end to control the volume of the collected fractions and ensure the depletion of the solvent used as eluent. The crude extract sample used for the experiment was 2.5141 grams. The solvents used for the elution of the compounds followed the increasing order of polarity, were Hexane, Ethyl acetate, and Ethanol. So that the increase in polarity of the system was gradual, mixtures of solvents in different proportions were used. The volume for each eluent and/or mixture was 100 ml. Fractions were collected at the end of the passage of 100 ml of each mobile phase. At the end of the passage of the mobile phase (solvents), the fractions were collected in properly identified and labeled glass beakers and conducted to evaporate the solvents at room temperature.

2.3. Chromatographic analysis of the fractionated extracts

After complete evaporation of the solvents, the samples were again monitored by thin-layer chromatographic (TLC) on analytical plates prepared with Kiesel silica gel (Merck) using the system (9:1) as eluents with hexane and ethyl acetate solvent and read in a UV chamber (254 and 365 nm). The samples were pooled according to the chemical profile of the visible fractions, stored in glass flasks, and kept at 4 ºC until further analyses.

2.4. Phytochemical characterization

The extracts were submitted to a qualitative chemical screening to determine classes of chemical constituents. The developers used were specific for each metabolite class investigated. For the determination of flavonoids and terpenoid compounds, a solution of ceric sulfate was used, while for tannins a ferric chloride solution was used. The detection of alkaloids was performed using Drangendorffi's reagent while for steroids/triterpenoids, Lieberman-Burchard's reagent was used.

2.5. Dosage of phenols and flavonoids

The characterization of the total phenol content present in the fractions was performed through spectrometry with absorbance measuring at 620nm, using the Folin-Ciocalteu reaction (Slinkard and Singleton, 1977). The obtaining of the gallic acid calibration curve was similar to the preparation of extracts (y= 0.64x + 0.1868. R^2= 0.9511). As for the contents, they were exposed to similar milligrams of gallic acid per gram of extract (mg EAG/g of extract). All analyzes were performed in triplicate. The flavonoid content was characterized using the methodology described by Arvouet Grand et al. (1994). Absorbances were measured in a spectrophotometer at 620 nm, and represented in milligrams of quercetin per gram of crude extract (µg EQ/100 mg of extract) in triplicate. The quercetin calibration curve equation was acquired under the same preparation conditions (y= 0.0038x + 0.0745. R^2= 0.9882).

2.6. Evaluation of antimicrobial activity - minimum inhibitory concentration (MIC)

The determination of the MIC was based on the M7-A6 document from the CLSI – Clinical & Laboratory Standards Institute (CLSI, 2012). The MIC was performed using 100 µl of Broth medium distributed in 96-well plates, followed by 100 µl of the extracts to the first wells, after homogenization and serial dilution, 100 µl of microbial suspension were added to each well and different concentrations were obtained (2000 to 3.9 µg/ml). Kanamycin and DMSO were used as positive and negative controls, respectively. Plates were stored at 37°C for 24h for tests with bacteria and 48h for tests with fungi. The test was considered highly active when MIC was within 50-500µg/ml, moderately active within 600-1500µg/ml, and weak active when above 1500µg/ml (Sartoratto et al., 2004). The tests were performed against the following strains of bacteria and fungi: *Bacillus subtilis* ATCC® 6633™; *Micrococcus luteus* ATCC® 10240™; *Pseudomonas aeruginosa* ATCC® 15442™; *Escherichia coli* ATCC® 94863™, *Staphylococcus aureus*; ATCC® 06538™; *Candida albicans* ATCC® 18804™; *Candida glabrata* ATCC® 728™.

2.7. Toxicity test

Extract fractions that were bioactive in antimicrobial trials were selected for hemolytic activity evaluation in suspensions of 1 ml of 2% defibrillated sheep blood were prepared with extracts at concentrations of 1000, 100, and 10 µg/ml, incubated for 3 hours with homogenization in the first 30 minutes. Then, the samples were centrifuged for 5 minutes at 3000 rpm, and the appearance of hemolysis was analyzed by the reddish coloration of the plasmatic fraction. Distilled water was used as the positive control while DMSO and saline solution were used as the negative controls.

2.8. Antioxidant activity

The same previously selected extract fractions from the antimicrobial trials were also evaluated on their antioxidant capacity through photo colorimetric determination of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), in 26 and 96 wells-plates (Brand-Williams et al., 1995). A spectrophotometer at 620 nm was used to read the maximum absorption of the radical under study. Tests were performed in triplicates and at the previously mentioned concentrations. Ascorbic acid was used as a standard. From the absorbances obtained, the antioxidant capacity of the extracts was expressed by the percentage of DPPH free radical scavenging (% SRL) through the Equation 1:

$$\%SRL = \left\{ \left[\left(Sample\ Abs - Control\ Abs \right) \times 100 \right] / Control\ Abs \right\} \quad (1)$$

where: Abs control matches the absorbance of DPPH and ethanol. Abs sample corresponds to the absorbance of the extract after reaction with DPPH (Ashraf et al., 2016).

3. Results

3.1. Raw and fractionation of extracts

The yield of the raw extract obtained from maceration in ethyl acetate was 2.5141 grams. From the raw extract, 77 fractions were obtained with a final mass of 1.6427 g. The chromatographic column retained 0.8714 g of biomass.

3.2. Chromatographic analysis of the fractionated extracts

The fractions obtained through the extractive technique were analyzed by thin-layer chromatography. The plates were viewed in a UV chamber (254 and 365 nm). The samples were pooled according to the chemical profile of the visible fractions (according to Table 1) and stored in glass vials, being weighed to check the yield.

3.3. Phytochemical evaluation

During the phytochemical screening, it was detected the presence of alkaloids, triterpenoids, steroids, and phenol (according to Table 2). The most frequently present compound was triterpenes and alkaloids, found in all fractions evaluated (Cs16-20, Cs21-22, Cs23-24, Cs25-26, Cs27-30, Cs31-34, Cs35-36, Cs37-38, Cs39-44, Cs45-54, Cs55-69, and Cs70-77) and in the raw extract.

Table 1. *C. savannarum* plant extract fractions (Cs) pooled after chromatographic analysis.

Separated and merged fractions	Extraction performance (g)
Cs16-Cs20	0.5826
Cs21- Cs22	0.0818
Cs23-Cs24	0.0469
Cs25-Cs26	0.073
Cs27-Cs30	0.114
Cs31-Cs34	0.0565
Cs35-Cs36	0.0403
Cs37-Cs38	0.0375
Cs39- Cs44	0.0725
Cs45-Cs54	0.1012
Cs55-Cs69	0.2076
Cs70-Cs77	0.2288

It is also worth mentioning the presence of phenolic compounds, absent only in the Cs55-69 and Cs70-77 fractions.

Regarding the phenol content present in the extract, the Cs23-24 fraction expressed the highest phenolic content (2.480 mg EAG/g), successively from the Cs25-26, and Cs27-30 fractions, with values of 1.791 mg EAG/g and 1.530 mg EAG/ g, respectively. On the other hand, the total flavonoid content was higher in the Cs31-34, Cs23-24, and Cs70-77 fractions with values of 182.25 µg EQ/10mg; 37.76 µg EQ/100 mg; and 27,316 µg EQ/100 mg, respectively (according to Table 3).

3.4. Antimicrobial activity evaluation

The antimicrobial evaluation by MIC analysis revealed that the six extract fractions of *C. savannarum* exhibited activity against the bacteria *B. cereus* (Cs23-24, Cs25-26, Cs27-30, Cs31-34, Cs35 -36, and Cs37-38), one against *S. aureus* (Cs45-54), one against *M. luteus* (Cs31-34), three against *B. subtilis* (Cs27-30, Cs31-34, and Cs35-36), and seven against *P. aeruginosa* (Cs27-30, Cs31-34, Cs35-36, Cs37-38, Cs45-54, Ch55-69, and Cs70-77) with concentration varying between 500-62.5 µg/ml, showing bactericidal activity and bacteriostatic activity was observed only against *P. aeruginosa* with concentration 62.5 µg/ml (according to Table 4). Fractions Cs35-36, Cs 45-54, and Cs55-69 showed an inhibitory effect on yeasts *C. albicans* and *C. glabata* (according to Table 5). Only three fractions did not present antimicrobial activity against the mentioned microorganisms (Cs16-20, Cs21-22, and Cs39-44) and did not follow to antioxidative and toxicity evaluations.

3.5. Toxicity assay

The hemolytic activity assay using sheep erythrocytes detected that the fractions Cs23-24, Cs25-26, Cs27-30, Cs31-34, Cs 35-36, Cs37-38, Cs45-54, Cs55-69, Cs70-77, and Cs B (according to Table 6), the presence of hemolytic activity at 1000 µg/mL.while was not observed at concentrations of 10 and 100 µg/mL.

During the antioxidant activity analysis, it was verified that both the raw extract and its fractions in ethyl acetate were able to reduce free radicals in different ratios (according to Table 7). The maximum free radical reducing capacity was observed using Cs55-69 fraction with a ratio up to 57.0% (2.0 mg/mL).

Table 2. Phytochemical screening of *C.savannarum* extract fractions.

Extract	Alkaloid	Triterpenoid	Tannin	Steroids	Phenol	Flavonoid
TRE	+	+	_	_	+	_
Cs21-22	+	+	_	+	+	_
Cs23-24	+	+	_	+	+	_
Cs25-26	+	+	_	+	+	_
Cs27-30	+	+	_	_	+	_
Cs31-34	+	+	_	_	+	_
Cs35-36	+	+	_	_	+	_
Cs37-38	+	+	_	_	+	_
Cs39-44	+	+	_	+	+	_
Cs45-54	+	+	_	+	+	_
Cs55-69	+	+	_	_	_	_
Cs70-77	+	+	_	_	_	_

Cs: *Campylopus savannarum* ; TRE: total raw extract; CS16 – 77: TRE fractions; (+) presence; (-) absence.

Table 3. Content of phenols (mg EAG/g) and total flavonoids (µg EQ/100 mg) in the fractionated extract of *C. savannarum* (mean ± standard deviation).

Fractions	Phenols	Flavonoid
TRE	1.246±0.188	27.31±0.074
Cs23-24	2.480 ± 0.0385	37.76 ± 0.109
Cs25-26	1.791 ± 0.379	20.836 ± 0.005
Cs27-30	1.530 ± 0.379	11.48 ± 0.004
Cs31-34	1.143 ± 0.042	182.25 ± 0.001
Cs35-36	0.686 ± 0.002	0.0257± 0.005
Cs37-38	0.513 ± 0.113	3.223 ± 0.062
Cs45-54	0.449 ± 0.041	6.376 ± 0.019
Cs55-69	0.439 ± 0.064	5.391 ± 0.018
Cs70-77	1.4552 ± 0.210	27.316 ± 0.050

TRE: total raw extract ; Cs: *Campylopus savannarum*

Table 4. Minimum Inhibitory Concentration (µg/ml) of the fractions and raw extract of *C. savannarum* in bacterial models.

Extract	*Gram-positive*						*Gram-negative*					
	B. cereus		***S. aureus***		***M. luteus***		***B.subtilis***		***E. coli***		***P. aeruginosa***	
TRE	**250**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Cs16-20	-	-	-	-	-	-	-	-	-	-	-	
Cs21-22	-	-	-	-	-	-	-	-	-	-	-	-
Cs23-24	125	BT	-	-	-	-	-	-	-	-	-	-
Cs25-26	125	BT	-	-	-	-	-	-	-	-	-	-
Cs27-30	250	BT	-	-	-	-	500	BT	-	-	125	BT
Cs31-34	250	BT	-	-	500	BT	500	BT	-	-	125	BT
Cs35-36	125	BT	-	-	-	-	500	BT	-	-	62,5	BC
Cs37-38	125	BT	-	-	-	-	-	-	-	-	62,5	BC
Cs39-44	-	-	-	-	-	-	-	-	-	-	-	-
Cs45-54	-	-	125	BT	-	-	-	-	-	-	500	BT
Ch55-69	-	-	-	-	-	-	-	-	-	-	62,5	BC
Cs70-77	-	-	-	-	-	-	-	-	-	-	125	BT
Chlora	62,5	-	62,5	-	63,5	62,5	62,5	-	62,5	-	62,5	-

Cs: *Campylopus savannarum;* TRE: total raw extract; CS16 – 77: TRE fractions; (-) absence of growth inhibition; BC: bactericidal; BT: Bacteriostatic; Chlora: chloramphenicol.

Table 5. Minimum Inhibitory Concentration (µg/ml) of the fractions and raw extract of *C.savannarum* in fungal models.

Extract	Yeast			
	Candida albicans		***Candida glabata***	
Cs35-36	-	-	500	FC
Cs45-54	250	FC	-	-
Cs55-69	250	FC	-	-
Ciclop.	62.5	-	62.5	-

Cs: *Campylopus savannarum;* TRE: total raw extract; CS16 – 77: TRE fractions; (-) absence of growth inhibition; FC: Fungicide; Ciclop: Ciclopirox.

Table 6. Toxicity evaluation of *C. savannarum* extract fractions by the hemolysis test.

Extract	**Concentration (µG/ML)**		
Fraction	**10**	**100**	**1000**
TRE	**-**	**-**	**+**
Cs 23-24	**-**	**-**	**+**
Cs 25-26	**-**	**-**	**+**
Cs 27-30	**-**	**-**	**+**
Cs31-34	**-**	**-**	**+**
Cs 35-36	**-**	**-**	**+**
Cs 37-38	**-**	**-**	**+**
Cs 45-54	**-**	**-**	**+**
Cs 55-69	**-**	**-**	**+**
Cs 70-77	**-**	**-**	**+**

Cs: *Campylopus savannarum;* TRE: total raw extract; CS16 – 77: TRE fractions; (-) hemolysis absence; (+) hemolysis presence.

Table 7. Percentage of DPPH reduction using fractionated extract of *C. savannarum* (mean).

Fraction (2,0 mg/mL)	%FRS
TER	49.6
Cs23-24	19.0
Cs25-26	4.96
Cs27-30	5.79
Cs31-34	12.9
Cs35-36	37.0
Cs37-38	33.1
Cs45-54	42.7
Cs55-69	57.0
Cs70-77	35.3

Cs: *Campylopus savannarum* ; TRE: total raw extract; CS16 – 77: TRE fractions; %FRS= Percentage of free radical scavenging by the Mann-Whitney method.

4. Discussion

The search for biological activities of different types of plants has reached great scientific interest. In this scenario, *C. savannarum* extracts and their composition exhibit important properties against microorganisms.

Research on different bryophytes from Europe demonstrated antimicrobial activity against *E. coli*, *B. cereus*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *M. luteus*, *B. subtilis*. Besides the antimicrobial potential reported on the European bryophytes, our findings on C. savannarum only presented inhibition on E. coli's growth. However, it is important to consider that the chemical composition of bryophytes can differ between species, growth environment, and geographic location (Klavina et al., 2013).

The evaluated fractions were able to inhibit the growth of both gram-negative and gram-positive bacteria, demonstrating a broad antimicrobial spectrum. According to Bodade et al. (2008), the antibiotics that are most used in therapeutic treatments are active against gram-positive bacteria, which highlights the importance of this study, once the bacteria gram negatives are more difficult to inhibit their growth in the function of their cellular structure.

The gram-negative bacterium *P. aeruginosa* was more susceptible with MIC up to 62.5 µg/ml and with bactericidal and bacteriostatic action. Studies reveal that the activity can be considered strong for MIC values between 50-500 µg/mL, moderate activity values for MIC between 600–1500 µg/mL, and weak activity above 1500 µg/mL (Sartoratto et al., 2004).

Thus, in agreement with the literature, the obtained data from this research may consider that *C.savannarum* extracts present strong activity against the tested bacterial strains with the exception of *E. coli*. Considering this potential, these findings may encourage future research on *C. savannarum* proprieties.

The antifungal effect observed was also relevant, as they showed an inhibitory effect on the yeast *C. albicans* and *C. glabata*. These microorganisms have demonstrated a problematic antifungal resistence profile. According to Sanglard and Odds (2002), this resistance is mainly associated with changes in the lipid composition of their cell membrane. Based on our findings, the tested fungi's sensitivity to the extract of *C. savannarum* demonstrates the antifungal potential existing in mosses, which is not usually reported once the studies of the antifungal activity are better seen in liverwort species (Marko et al., 2001

Previous studies have investigated that the genus *Campylopus* has biological properties such as antioxidants, and antibacterials, among other medicinal properties (Veljić et al., 2008; Mukhopadhyay, 2013; Shin et al., 2016). Despite this, the biologically active compounds of *C. savannarum* are unknown, as well as the nature of their activity.

Although the accentuated use of plants as a therapeutic resource in recent decades has been constant, there are still few studies that provide information about the toxicity of the fractionated extract of the species *C. savannarum*. Toxicological research involves, above all, biological processes and has become an alternative for tracking plants that have toxic effects and/or adverse effects caused by the interaction between chemical substances and living organisms (Bednarczuk et al., 2010; Markowicz-Piasecka et al., 2018).

Our findings suggest that the antimicrobial potential of C. savannarum may be associated with the presence of different classes of bioactive constituents such as alkaloids, triterpenoids, steroids, phenolic compounds, and flavonoids. According to Commisso et al. (2021), the phytochemistry of bryophytes shows a formidable variety of biologically active compounds. In addition, such plants have substances that are widely used as antitumors, antipyretics, insecticides, and antimicrobials.

Our results on the chemical profile of *C. savannarum* are similar to the data from Vidal et al. (2012), analyzing the species of bryophytes *Octoblephum albidum hedw*, where they obtained similar results, also demonstrating the presence of these compounds.

Furthermore, the phytochemical compounds found in the studied fractions are usualy related to different pharmacological properties. Among all the compounds found in plants, all extract fractions presented alkaloids. Generally, alkaloids may exhibit analgesic, spasmolytic, anti-inflammatory, and/or anticancer effects. On the other hand, steroids are highly linked to the application of cholesterol control, nutritional and cosmetics applications, and others as the formulation of contraceptive molecules according to Queiroz (2009). In a similar way, triterpenoids may present analgesic, hepatoprotective, anti-inflammatory, antimycotic, immunomodulatory, antibiotic, virostatic, and tonic effects (Dzubak et al., 2006).

Regarding the levels of phenols present in the extract's fractions, the Cs23-24 expressed the highest phenolic content, while the greatest flavonoid content was observed in the Cs31-34 fraction.

Hexane extract of the species of *Leucobryum aduncum* and *Campylopus schmidii*, also present high levels of phenolic content (119.87 ± 11.51 mg GAE/g of extract), what may be considered a strong antioxidant property (Makajanma et al., 2020) For Crozier et al. (2006), the association and accumulation of phenolic compounds must be driven in response to environmental conditions, often related to specific stages of plant development.

Although the constated absence of classes of secondary metabolites such as flavonoids and tannins during the quantitative phytochemical screening, the presence of flavonoids was even lower. This observation could be related to the difference in the sensitivity of the tests, once the quantitative assessment is more sensitive to the presence of these compounds.

C. savannarum extracts also showed high antioxidant capacity. Maximum free radical-reducing activity up to 57.0% (2.0 mg/mL) was found. The result found corroborates to the report by Makajanma and collaborators (2020), on species of mosses of the genera *Leucobryum* and *Campylopus* also presented antioxidant activity. The antioxidant practice was analyzed, showing the concentration of the extract that resulted in a 50% reduction of 1,1-diphenyl-2-picrylhydrazine DPPH. These concentrations were described >2000 mg/L for *L. aduncum* extracts and 1329.02 ± 7.8 mg/L for *C. schmidii,* respectively.

Most of the existing plants are abundant in antioxidant biomolecules that have the effect of delaying, preventing, or removing oxidative damage from a target molecule. These effects establish endogenous or exogenous defense mechanisms against free radicals (Halliwell and Gutteridge, 2007). Thus, this antioxidant function is attributed to the presence of phenolic compounds in its composition, produced by the secondary metabolism of plants, such as flavonoids (Hirata et al., 2004; Nascimento et al., 2011).

Considering the antioxidant activity, the DPPH has been a commonly used method and is based on evaluating the antioxidant capacity through the reducing activity of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Henrigues et al., 2016). The inhibition of DPPH radicals basically consists of the transfer of hydrogen atoms from an antioxidant compound establishing the stable compound diphenylpicrylhydrazine DPPH-H (hydrazine) (Silva et al., 2006).

In this study, the results of free radical-reducing activity demonstrate the significant antioxidant property of the species, suggesting that the compounds present in the extracts were great electron or hydrogen donors. The data corroborate in part with previous studies on bryophytes species where Montenegro et al. (2009) also portrayed by means of the photo colorimetric assay of free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH), demonstrating the antioxidant properties of *Sphagnum magellanicum.*

As for the evaluation of erythrocyte toxicity, the fractions tested did not show hemolytic activity at concentrations 10 and 100 µG/ML, being considered non-toxic. The hemolytic screening is standardized through compatibility and interaction with the biological system. The erythrocyte fragmentation can be constated by the by the detection of hemolysis, reducing the ratio of red cells in the blood (Neto et al.2022).

The nine fractions that presented hemolytic activity at a concentration of 1000 µG/ML (Cs23-24, Cs25-26, Cs27-30, Cs31-34, Cs 35-36, Cs37-38, Cs45-54, Cs55-69, Cs70-77) also exhibited the presence of compounds of triterpenic, steroidal, phenolic, and/or alkaloidal nature. In addition, these same nine fractions presented antimicrobial action. The low toxicity demonstrated here encourages the development of new studies on the therapeutic traits using this species. According to Brandão et al. (2005) the determination of the cytotoxic activity of a plant extract is of great importance considering the capacity of several chemical compounds to generate toxic effects. The widespread cytotoxicity is often caused not exclusively by only one compound, but by the coexistence of several components present in the extract.

5. Conclusion

Overall, our investigation explored for the first time aspects of the bioactive properties of Campylopus savannarum. We demonstrated through phytochemical analysis the present of important biomolecules classes such as: alkaloids, triterpenoids, steroids and phenolic compounds. Also, here we provided consistent findings that indicate the antimicrobial, antioxidant, and non-toxic potential of Campylopus savannarum extract and derived, which could support the future studies on the field.

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