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Biological activities and chemical profile from *Batis maritima* (Bataceae), a halophyte species with bioprospecting potential

Atividades biológicas e perfil químico de *Batis maritima* (Bataceae), uma espécie halófita com potencial para bioprospecção

Actividades biológicas y perfil químico de *Batis maritima* (Bataceae), especie con gran potencial para la bioprospeccion

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Abstract

Halophytes are salt-tolerant plants, enriched in metabolic compounds with several industrial applications. *Batis maritima* is a halophyte with occurrence in tropical and subtropical zones and the present work aimed to investigate *B. maritima* leaves extract for biotechnology and bioprospecting approaches. The present study evaluated the phytochemical profile, the role of acetylcholinesterase, and protective activities from *B. maritima*. The phytochemical screening highlighted the presence of phenolic compounds, anthracenes, and saponins. The metal content analysis revealed sodium (Na) as the most abundant salt (1 g/kg), followed by Al (0.35 g/kg), and several other elements in

lower concentrations. Further, DPPH and TAC methodologies showed an significant antioxidant activity (IC_{50} 5.17 ± 0.05 μg/mL and 0.45 ± 0.08 mg.AAEm, respectively). A metal chelating activity was also observed, with IC_{50} 25.76 ± 0.3 μg/mL. Additionally, *B. maritima* aqueous extract disclosed an anticholinesterase inhibitory effect in a dose-dependent manner (IC_{50} = 0.0023 mg/mL). Haemolysis assay did not reveal any toxic effects. On the other hand, the cytotoxicity assay, using murine fibroblasts cells (NIH-3T3), showed a decrease in cell viability at high concentrations.
Keywords: Antioxidant activity; *Batis maritima*; Cytotoxic activity; Metal content; Phytochemical characterization.

Resumo

Halófitas são plantas tolerantes a salinidade, ricas em metabolitos com aplicação em diversas indústrias. *Batis maritima* é uma halófita com ocorrência em zonas tropicais e subtropicais, o presente trabalho objetivou investigar o extrato das folhas de *B. maritima* em uma abordagem de bioprospecção e biotecnológica. O presente estudo avaliou o perfil fitoquímico, o potencial efeito sobre a enzima acetilcolinesterase, além de propriedades protetivas de *B. maritima*. A análise fitoquímica evidenciou a presença de compostos fenólicos, antracenos e saponinas. O conteúdo de metal revelou o Sódio (Na) como o sal mais abundante (1g/Kg), seguido pelo alumínio (Al;0,35 g/Kg), e diversos outros elementos em concentrações menores. As metodologias de DPPH e Capacidade Antioxidante Total indicou efeito antioxidante significativo (IC_{50} 5,17 ± 0,05 μg/mL e 0,45 ± 0,08 mg.AAEm, respectivamente). A atividade quelante de metal também foi observada, com um IC_{50} de 25,76 ± 0,3 μg/mL. Além disso, o extrato aquoso de *B. maritima* apresentou efeito inibitório sobre a acetilcolinesterase de forma dose-dependente com IC_{50} de 0,0023 mg/mL. O ensaio de hemólise não revelou efeitos tóxicos, por outro lado, o ensaio de citotoxicidade, utilizando células da linhagem NIH-3T3, evidenciou uma diminuição da viabilidade celular em concentrações elevadas do extrato.
Palavras-chave: Atividade Antioxidante; *Batis maritima*; Caracterização fitoquímica; Citotoxicidade; Concentração de metais.

Resumen

Los halófitos son plantas tolerantes a la sal, enriquecidas en compuestos metabólicos con varias aplicaciones industriales. Batis maritima es un halófito con presencia en zonas tropicales y subtropicales y el presente trabajo tuvo como objetivo investigar el extracto de hojas de B. maritima para enfoques de biotecnología y bioprospección. El presente estudio evaluó el perfil fitoquímico, el papel frente a la acetilcolinesterasa y las actividades protectoras de B. maritima. El cribado fitoquímico destacó la presencia de compuestos fenólicos, antracenos y saponinas. El análisis del contenido de metales reveló que el sodio (Na) es la sal más abundante (1 g/kg), seguida del Al (0,35 g/kg) y varios otros elementos en menor concentración. Además, las metodologías DPPH y TAC mostraron una actividad antioxidante significativa (IC50 5.17 ± 0.05 μg/mL y 0.45 ± 0.08 mg.AAEm, respectivamente). También se observó una actividad quelante de metales, con IC50 = 25,760 ± 0,3 μg / mL. Además, el extracto acuoso de B. maritima reveló un efecto anticolinesterasa de una manera dependiente de la dosis (IC50 = 0,0023 mg / ml). El ensayo de hemólisis no reveló ningún efecto tóxico. Por otro lado, el ensayo de citotoxicidad, utilizando células de fibroblastos murinos (NIH-3T3), mostró una disminución de la viabilidad celular.
Palabras clave: Actividad antioxidante; *Batis maritima*; Caracterización fitoquímica; Citotoxicidad; Concentración de metales.

1. Introduction

Soil salinity has been associated with incorrect management and unsustainable practices in the environment, which leads to a decrease in crops productivity and enhanced the risk of desertification. In the last decade, several salt-tolerant plant species (also known as halophytes) have been explored as crop plants to control salt and metal concentrations in the environment (Hasanuzzaman et al., 2014). The biomass produced by those halophytes enlists a variety of biological, industrial, and medical applications. Although other major properties of halophytes remain to be investigated, they have been contributing to the advancement of nutraceuticals, industrial and pharmacology approaches (Kong et al., 2008, Jeong et al., 2004; Ventura et al., 2011; Hameed & Khan, 2011; Buhmann & Papenbrock 2013, Munns & Gilliham 2015).

B. maritima (Bataceae) is an herbaceous halophyte that have been found in tropical and subtropical zones in Brazil, its display vastly uses in *folk* medicine for inflammatory and antioxidant purposes (Lonard et al., 2013; Marcone, 2003). As well other several halophytes species, *B. maritima* has been investigated in cultivation systems with high salt content, such as an aquicultural system, to produce biomass and treatment of waste water (Schardong et al. 2018), in mangrove forests regeneration projects as early-colonizer, which in turn improved mangrove seedlings success (Milbrandt & Tinsley, 2006) and

even suggest to be implemented in unproductive agricultural land due to high salinity content (Marcone et al., 2003).

Since there is a tendency of industries to seek feedstock associated with sustainable practices. The investigation of species that can be used to restore, or even attenuate stress factors, in the environment are essential to sustainable development, and to aim this, is important to evaluate the biotechnological potential of candidate species. With that in mind, the present study aimed to elucidate the phytochemical profile and the biochemical activity of the aqueous extract of *B. maritima*. These results might extend the benefit of *Batis maritima* for the bioprospecting endeavours whilst underscoring its biotechnological application.

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2. Methodology

2.1 Extract preparation and phytochemical screening

Batis marítima (Bataceae) leaves were collected in Rio Grande do Norte, Brazil, (°19'58"S 35°02'58"W), An exsiccate was deposited at the Herbário Professor Vasconcelos Sobrinho from Universidade Federal Rural de Pernambuco, under the voucher 55833. To obtain the aqueous extract, the leaves were dried in a stove at 40 ºC for 48h, later the botanical material was grounded to a homogenous powder. Then, the powder was mixed with distilled water (1:10) were placed in a water bath at 100 °C for 30 minutes. Subsequently, the mixture was filtered, and the extract was lyophilized.

A qualitative phytochemical screening was performed to access the secondary metabolites class present on the plant extract, following Matos (1998) modified by Barbosa *et al.* (2021), this protocol is based in the addition of reagent to aliquots of extract, which in turn will reveal the presence or absence of alkaloids, phenolic compounds, coumarins, anthracenes, tannins, terpenoids, steroids, saponins and quinones.

2.2 Total phenolic compound and total flavonoids quantification

Total phenolic quantification was performed using the Folin-Ciocalteu reagent (Sigma-Aldrich) according to Li et al. (2007) using a UV-Vis spectrophotometer, in this methodology, gallic acid (GA) (Sigma-Aldrich) is used as standard. The results were expressed in GA (mg)/dry extract (g) (mg.GAE/g). The total flavonoids quantification was performed according to Woisky and Salatino (1998) methodology, also using UV-Vis spectrophotometer and Quercetin (Q) (Sigma-Aldrich) as standard. The total flavonoid content was expressed as Quercetin equivalents (mg)/ dry extract (g) (mg.QE /g).

2.3 Metal estimations

To derteminate Al, As, Br, Ca, Cu, Fe, K, Mn, Mo, Na, Ni, P, Pb, Se and Zn concentration, 0.5 g of leaves tissue were grounded and digested in 5 mL of nitric acid. Sample digestion was carried out in a microwave digester (Berghof speed wave) at 180 ºC for 20 minutes. The metal content was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), using model 5110 by Agilent, the metal content is expressed in mg/kg.

2.4 Antioxidant Activity

The antioxidant activity was evaluated according to three different methods. DPPH assay, according to Melo-Silveira et al. (2014) protocol, and Hydroxyl radical scavenging activity (Costa et al., 2011) to assess the scavenging activity, while the total antioxidant capacity (TAC) to evaluate the extract reducing potential (Costa et al., 2011).

2.5 Metal Chelating Activity

The Fe^{2+} ion chelating activity was performed according to the methodology described by Melo-Silveira et al. (2014) with modifications. In this methodology, 1 mL of the sample was transferred to 25 mL amber test tubes. 3.7 mL of deionized

water was added to this aliquot: 0.1 mL of 2 mM $FeSO_4$ and 0.2 mL of 5 mM [3-(2-pyridyl)-5,6-bis-(4-phenylsulfonic acid)-1,2,4-triazine] ferrozine. The mixture stirred, and after 20 min, the reading was made at 562 nm. The reduction in absorbance indicates metal chelating activity. All assays were carried out in triplicate. EC_{50} of Metal chelating was calculated based on the linear regression of the percentage of remaining Metal chelating against the sample concentration.

2.6 Acetylcholinesterase inhibition assay

Ellman et al. (1961) protocol was applied for the acetylcholinesterase inhibition assay using Acetylcholinesterase from Sigma-Aldrich (VI-S type). The reaction solutions consisted of 200 µL of DTNB (0.25 mM), Tris-HCl [0.5 M, pH 7,4), 10µL from AChE (1 µg/mL) and 10 µL of extract concentration ranging from 0.0001 to 1.0 mg/mL. The solution was incubated for 60 minutes. The reaction was started by adding 20 µL of acetylcholine (62 mM), and the absorbance was read at 405 nm from time 0 to 180s. The enzymatic activity was expressed in mean ± standard deviation.

2.7 Haemolytic assay

The haemolytic activity was measured by the determination of human erythrocyte lysis (hRBCs) donated from the Hospital of the Federal University of Pernambuco (UFPE). A sample of human blood type O^- was mixed with 0.9 % PBS at a ratio of 1:30 and centrifuged at 2500 rpm for 5 min to isolate the erythrocytes. The erythrocytes were resuspended in 0.9 % PBS to obtain a 0.5% suspension. The activity was evaluated with extract concentration between 125 and 1000 µg/mL. The cell suspension with extract was mixed with saline solution (NaCl 0.9 %) and used as negative control whereas the cell suspension mixed with Triton X-100 (1%) was used as a positive control. The samples were incubated for 4, 8 and 12h at 37 °C, and then centrifuged at 2500 rpm for 5 min. Hemolysis was measured by spectrophotometry at a wavelength of 540 nm in 96-well using 200 µL of samples. All experiments were performed in triplicate and expressed as percentage (Ahmad et al., 2010).

2.8 Cytotoxic activity

The cell lines used in this study were (NIH-3T3) murine fibroblast cells cultured in DMEM (Gibco BRL) containing 10% fetal bovine serum (FBS), 1% penicillin (100 µg/mL) and streptomycin (100 µg/mL) at 37 °C in a humidified incubator with 5% CO2. The cells (5×10^5 cells/mL, 100 µL/well) were seeded in 96-well with different *B. maritima* extract concentration mixed in fresh medium and incubated for 24h. Then, the supernatant was removed, and cells washed with PBS three times. 200 µL of fresh media containing 0.5 mg/mL MTT was added to each well-plate and incubated for 4h at 37 °C. After this period, the supernatant was removed, and 150 µL of DMSO was added to dissolve formazan crystals. The absorbance was measured on a microplate reader at the wavelength 560 nm (Melo-Silveira et al., 2014). The results were expressed as a percentage reduction of the MTT, considering the absorbance of the negative control as a 100% reduction.

2.9 Statistical analysis

All assays were performed in triplicate. Values were considered significantly different when $p < 0.05$. The data were analysed using the GraphPad Prism® version 5.0 and expressed as mean ± SD. Statistically significant differences were calculated by the application of one-way analysis of variance (ANOVA) followed by Turkey post-hoc test.

3. Results and Discussion

3.1 Phytochemical screening

The phytochemical screening revealed the presence of phenolic compounds, anthracenes, and saponins (Table 1). These elements have been extensively required for the food, cosmetic and pharmaceutics industries (Olszowy, 2019). For example, phenolic compounds are famous anti-inflammatory and antioxidant molecules and lately have been highlighted due to their antitumor activity, which modulates body function and induce a brain-protective effect (Abifarin *et al*., 2019; Lim *et al*., 2019; Russo *et al*., 2019; Sudhakaran *et al*., 2019). As for the anthracenes, the literature points to a potent anti-cancer activity (Zagotto *et al*., 2000; De *et al*., 2013), antimicrobial (Kim *et al*., 2009;), and biotechnology application as chemosensory devices (Damme and Prez, 2018). Saponins not only complement a variety of nutraceuticals and functional foods, as also anti-inflammatory, antimicrobial, immunostimulant, hypocholesterolemic, anticarcinogenic, and antioxidant activity (Podolak *et al*., 2010; Kimura *et al*., 2006; Raju and Mehta 2009).

Table 1. Phytochemical constituents from *Batis maritima* aqueous extract.

Phytochemical constituents	***B. maritima***
Phenolic compound	+
Tannins	-
Anthracenes	+
Terpenoid and Steroid	-
Coumarins	-
Saponin	+
Quinones	-
Alkaloids	-

(+): Presence of chemical compound. (-): Absence of chemical compound. Source: Authors

As for the phenolic compounds and flavonoids content, the results are summarized in Table 2. *B. maritima* aqueous extracts displayed significant content of phenolic compounds (29.90 ± 0.05 mg GAE/g extract), and flavonoids (0.99 ± 0.19 mg QE/g extract), since flavonoids are a subclass of phenolic compounds, it is expected that compounds show higher concentrations. Comparing with the literature, our findings are similar to other halophyte species described in the literature, such as *Crithmum maritimum* (Apiaceae) extract, with a range of 33 mg.GAE/g (Meot-Duros and Magné, 2009), as well in higher levels, like Houta *et al*. (2011) in the same specie 14 mg.GAE/g extract. But also, some species presents higher concentrations, like the 5 species investigated by Qasim *et al*. (2017). The production of secondary metabolites varies according to abiotic and biotic factors, even plants from the same species can vary in composition. However, highlight the secondary metabolites that a plant can produce is important to show its potential, especially those plants that can be used in the decontamination process, once they are harvested, knowing the type of molecule that can be found in their composition will help to give an adequate destination to the plant residues. The case of phenolic compounds and flavonoids reveals a potential antioxidant activity, which outlines a variety of medical uses and purposes for the pharmaceutical industries, or if its appropriate to use in other system, like an aquiculture system (Schardong *et al.* 2018).

Table 2. Total phenolic compounds and flavonoids content of aqueous extracts of *Batis maritima*.

	Total phenolic compounds (mg GAE/g extract)	**Flavonoids (mg QE/g extract)**
B. maritima	29.90 ± 0.05	0.99 ± 0.19

GAE: Gallic acid equivalents; QE: Quercetin equivalent. Values described in mean ± standard deviation (n = 3).
Source: Authors

3.2 Metal content

To further explore the bioprospecting properties of *B. maritima* extract, the metal content was quantified by ICP-OES, and the results are shown in table 3. The results revealed the presence of Sodium (Na) (1002,70 mg/kg) and Aluminium (Al) (353.70 mg/kg) as major elements, and lower concentration of several other metals. The metal content in plants varies according to several factors: metal available in the environment, root capacity absorption, water uptake, competition between metallic ions, soil physic-chemical properties, among others. The expressive concentration of heavy metals in halophytes disclosure their tolerance for high salinity-soils (Millic *et al*., 2012; Vahedi, 2013).

The metals not only potentiate oxygen transport, energy production, enzyme activation but also reactive oxygen species production and cell death (Affone and Ifediba, 2020; Zoroddu *et al*., 2019; Leung *et al*., 2010). At low concentration most of those metals sustain organism homeostasis and function (Marini *et al*., 2021). Of note, the levels of metals quantified in *B. maritima* extract are in accordance with the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2009), which may represent possibilities to produce nutraceutical products, even organic salt that benefits health with secondary metabolites and provides essential elements for the body, further studies with different soils and cultivation of *B. maritima* are needed to determine the dynamics of absorption of the metal by the plant.

Table 3. Metal content estimation in *Batis maritima* leaves.

Element	**Concentration (mg/kg)**
Al	353.70
As	0.03
Ba	0.26
Ca	8.05
Cu	0.14
Fe	6.41
K	87.81
Mn	0.51
Mo	0.06
Na	1002,70
Ni	0.24
P	21.28
Pb	0.35
Se	0.16
Zn	0.33

Source: Authors.

3.3 Protective effects

Antioxidant compounds induce a protective effect in the body through the ability of free radical scavengers (Quideau *et al*., 2011). Since various pathologies can be avoided due to antioxidant effects, natural dietary antioxidants can promote the improvement of the human body, as well better performance in physical activities. The antioxidant property of *B. maritima* extract was evaluated by DPPH scavenging, hydroxyl radical scavenging activity, as well total antioxidant capacity, the IC_{50} evaluations are disclosed in Table 4.

Our results shows that *B. maritina* extract have an expressive concentration-dependent antioxidant activity in all proposed assays. Plants extracts are strongly associated with antioxidant effects due to its metabolite's secondaries, that may act as non-enzymatic free radical scavengers, and protecting biomolecules from damage, as demonstrated by the metal chelating activity, since heavy metals may induce a toxic effect on biological system, the metal chelating capacity from metabolites secondaries prevents the free radical formation. Comparing with the literature, our findings suggests that *B. maritima* extract is a potent antioxidant natural source, since other halophytes like *Sesuvium portulacastrum* presents a C_{50} of 871.67 ± 18.42 μg/mL (Chintalapani *et al*., 2018); also, *Beta vulgaris* IC_{50} value of 254.76 ± 2.07 μg/mL (Edziri *et al*., 2019), and *Trigonella foenum graecum* IC_{50} of 172.6 ± 3.1 μg/mL (Akbari *et al*., 2018). In this view, these results support that *B. maritima* might potentiate health surveillance to medical/industrial purposes, specially because it work in different manners, by scavenging free radicals, reducing potential, as well by chelating metals avoiding cell damage.

Table 4. Antioxidant and metal chelating activities of *Batis maritina* aqueous extract.

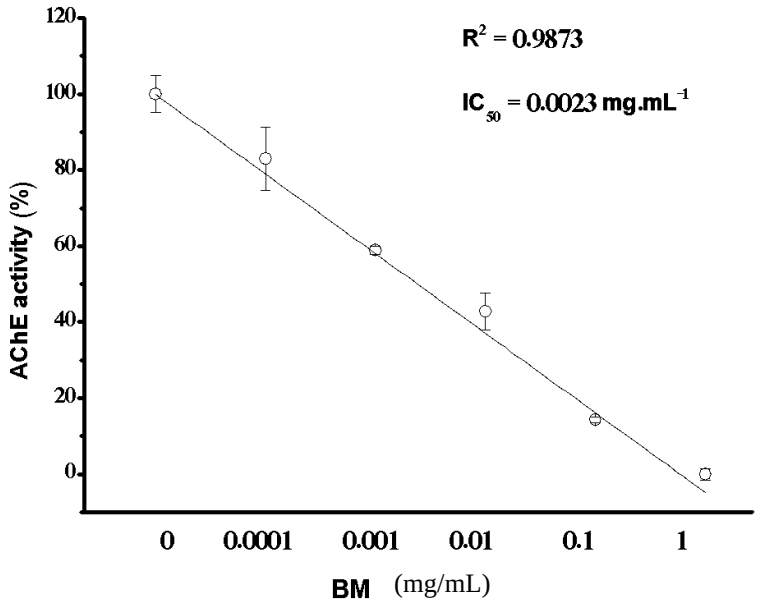
Method	IC_{50}
DPPH	5.17 ± 0.05 μg/mL
Total antioxidant capacity	0.45 ± 0.08 mg.AAE/g
Metal chelating activity	25.76 ± 0.3 μg/mL
Hydroxyl radical scavenging	3.78 ± 0.21 μg/mL

Values expressed in mean ± standard deviation (n = 3). Source: Authors

3.4 Acetylcholinesterase (AChE) activity

Further, the aqueous extract of *B. Maritima* displayed an inhibitory activity against AChE in a dose-dependent manner (IC_{50} of 0.0023 mg/mL) (Figure 1). AChE inhibitors have been emerging as a prevalent choice for neurological disorders, especially Alzheimer's disease treatment (Dvir *et al*., 2010; Singh *et al*., 2013; Li *et al*., 2015; Ahmed *et al*., 2018). Concordantly, the literature demonstrates the AChE inhibitory function sustained by phenolic compounds and terpenes (Roseiro *et al*., 2012; Murray *et al*., 2013; Monteiro *et al*., 2018; Khan *et al*., 2018; Santos *et al*., 2018). The molecular mechanism of AChE inhibition, and its outcome, is beyond the scope of this manuscript, therefore further studies are needed to decipher the therapeutic effect of *B. maritima* extract.

Figure 1. AChE inhibition activity of *B. maritima* aqueous extract in different concentrations.



Source: Authors.

3.5 Haemolytic assay

The Haemolytic assay showed that *B. maritima* did not promote haemolysis of erythrocyte cellular membranes in all tested concentrations (Table 5). As haemolysis can be triggered by the interaction of substances with the membranes, the negative results obtained point to absence of erythrocyte membrane toxicity. Usually, plant extracts described as potent antioxidants present haemolysis, making it impossible to use them, as verified in the extract of *Pandanus tectorius*, which presented a haemolysis rate higher than 5%, at a concentration of 600 µg/mL (Prakash et al., 2016), fact this was not verified in this study, indicating safety, so the absence of haemolysis means that the extract can be safe, as it can also exert a protective effect on the body.

Table 5. Haemolytic index (HI) of *Batis maritima* aqueous extract.

Concentration (µg/mL)	*B. maritima*	
	HI (%)	Remarks
125	1.41 ± 0.02	NH
250	1.41 ± 0.04	NH
500	1.45 ± 0.01	NH
1000	1.36 ± 0.07	NH
Negative Control	1.34 ± 0.19	NH
Positive Control	100.00 ±0.01	H

Values described in mean ± standard deviation (n = 3). HI: Hemolytic index; NH: No Hemolysis; MH: Moderate Hemolysis; H: Hemolysis. Source: Authors.

3.6 Cytotoxic activity

The cytotoxic activity of *B. maritima* aqueous extract effect on murine fibroblast cells proliferation (NIH-3T3) was analysed by MTT formation assay (Table 6), the results shows that the aqueous extracts disclosed cytotoxicity activity as the

concentrations increase. Is worth to mention that plant extracts often shows more than metabolites secondaries on its composition, as we had shown on the metal content topic, several salts are arrested among plant molecules when the vegetal tissue is exposed to the solvent, and those salts may alter the cell homeostasis, leading to cell damage. Still, by showing a decrease in cell viability, it is possible that *B.* maritima extract may be investigated under the light of anti-cancer activity, since halophytes species used in food consumption, such as *Juncus acutus* and *Nitraria retusa*, disclosed cytotoxic activity against tumour cells with absence of toxic effect in normal cells (Zar *et al*., 2013; Rodrigues *et al*., 2014).

Table 6. Effect of extracts *B. maritima* under proliferation of murine fibroblast NIH-3T3 cells.

Concentration (µg/mL)	Cell viability (%)
2000	65.22 ± 3.6 [c]
1000	70.91 ± 5.5 [a]
500	77.09 ± 1.3 [b]
250	78.35 ± 5.4 [b]
125	79.22 ± 3.3 [b]
62.5	85.04 ± 1.8 [b]
31.25	85.35 ± 3.1 [b]
15.62	87.35 ± 3.3 [b]
7.81	96.17 ± 2.3 [a]
3.90	100.00 ± 2.7 [a]
1.95	100.00 ± 3.0 [a]

Values described in mean ± standard deviation (n = 3). Source: Authors.

4. Final Considerations

The present study evaluated several parameters from *Batis maritima*, such as chemical profile, metal content, protective effect, *in vitro* toxicity, as well a potential approach for future studies in human health. Taken together, our discovery supports potential projects that may involve *B. maritima* as a halophyte that can be used in cropping systems with high salinity concentrations, and subsequently apply the crop to industries that could exploit plants in biotech approaches, especially industries that have been seeking to implement a sustainable production cycle, which involves raw materials that do not cause various damages to the environment. Nevertheless, more studies with *B. maritima* are needed for a better understanding of the potential of this species, as well as methodologies and logistics for implementing its cultivation and harvesting.

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