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Article

Promising organic compounds in invasive aquatic plants identified in freshwater lagoons in Cuba

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ABSTRACT

Pistia stratiotes L. and *Eichhornia crassipes* (Mart.) Solms have promising organic compounds. These species are invasive aquatic plants present in some freshwater lagoons in Cuba. Using its phytochemical properties can be a way out of its negative impact. Water quality from two lagoons was evaluated. The content of phenols, anthraquinones and flavonoids were evaluated for both species. The extraction was made with ethanol (90 %), and the High-Performance Thin-Layer Chromatography analytical technique was used for the phytochemical test. Both lagoons were highly polluted. *E. crassipes* were observed in two forms: dense and not dense stands. The content of phenolic compounds ranged from 3.07 to 9.2 mg g^{-1} DW, the anthraquinones ranged from 1.06 to 3.74 mg g^{-1} DW and flavonoids ranged from 33.9 to 18.2 mg g^{-1} DW. The highest content of organic compounds was recorded in the dense stands of *E. crassipes*. Some coincidences for morindin and rubiadin standards were found in *P. stratiotes*. Coincidences for kaempferol and quercetin standards were also found in the samples. The results of this study suggest that both species of plants could be used as a source of organic compounds by the pharmaceutical industry of Cuba.

Keywords: freshwater lagoon; invasive plants; phenols; anthraquinones; flavonoids

INTRODUCTION

In Cuba, programs are being developed to prevent and control invasive species at the national and local levels since their harmful effects on native species, ecosystems, and ecosystem services are widely recognized[1]. Specifically, the invasive plants have great potential to disrupt global biodiversity, forestry, livelihood, and human health[2]. Nevertheless, ecological and socio-economic investigations of invasive plants to facilitate restoration strategies are insufficient in many species [2].

Pistia stratiotes L. and *Eichhornia crassipes* (Mart.) Solms are invasive, noxious, and transformative freshwater aquatic plants[1]. Despite the negative impacts on aquatic ecosystems, these invasive plants can be beneficial. Some authors have found secondary metabolites such as phenols, anthraquinones, and flavonoids, among others, with antibacterial[3,4], antifungal[5,6,7], antiviral[8], anticancer[9,10], antioxidant[11,12,13,14] and bioacaricide[15] properties.

In Cuba, studies on aquatic flora are limited[16]. There are few studies on *P. stratiotes* and *E. crassipes*[17,18,19]. No references have been found on the content of secondary metabolites present in these species. Given the negative impact caused by *P. stratiotes* and *E. crassipes* in the lagoons where they predominate and the need to find solutions to it, this study aimed to identify promising organic compounds present in leaves and roots of *P. stratiotes* and *E. crassipes*. These results could benefit the pharmaceutical industry in Cuba.

MATERIAL AND METHODS

Study area

The province of Ciego de Avila is located in Central Cuba. *P. stratiotes* is present at Vista Alegre lagoon (L.1) (0.013 km^2). It is located in the western part of the city of Ciego de Avila (21°51'9''N-78°46'39''W) and *E. crassipes* is present at La Turbina lagoon (L.2) (0.086 km^2), located in the outskirts of the city (21°50'51''N-78°45'43''W).

Analysis of physicochemical and microbiological of the water

Some physicochemical and microbiological parameters were analyzed to evaluate the water quality of both lagoons. For water analyses, samples were taken at one site in L.1 and three in L.2. The number of samples corresponded with the area occupied by each la-goon. Water samples were taken in the morning (from 08:00 to 09:00 am). To evaluate physicochemical parameters, the water samples were collected in polyethylene bottles, previously washed with hydrochloric acid, and sterilized glass bottles to evaluate microbiological parameters. Some of these parameters (pH, electric conductivity, oxygen saturation, chemical demand of oxygen, and fecal coliforms) were used to calculate the Water Quality Index (WQI)[20]

$$\mathrm{ICAsp} = \sum_1^5 Wi^{*\,qi} \quad (1)$$

Where:
Wi: relative weight of each indicator or parameter.
qi: value (in percentage) obtained from correlation functions.

Analysis of organic compounds

According to Coetzee et al.[21], vast amounts of *E. crassipes* grow near the margins of the lagoons with elongated petioles (up to 1 m tall) (dense stand); however, in sparse infected sites and at the edge of such sites, the petioles are bulbous and short (less than 30 cm) (not dense stand).

The samples of leaves and roots were washed with fresh water, and subsequently with distilled water before drying (constant weight) in a certified oven at 70 °C for 48 hours. The dry samples were powdered to approximately 2 µm in size. The method of solid-liquid maceration was used to obtain the extracts of the studied plants. For the preparation of ethanolic extract, 5 g of the powdered dry mass of plant was placed in 150 mL of ethanol (90 %) (v:v), used as an organic solvent, with a solid-liquid ratio of 1:30 (m:v).

Total Phenolic Content (TPC) was determined using the colorimetric method Folin–Ciocalteu, proposed by Gurr, McPherson and Bowles (1992) in Rivero[22], in Total Anthraquinone Content (TAC) was determined according to the colorimetric method described by Han et al.[23]. For the calculated result, the molar extinction coefficient of 5500 M^{-1}cm^{-1} about alizarin and proposed by Schulte[24] was used. Total Flavonoid Content (TFC) was determined

using the colorimetric method proposed by Kim et al.[25]. The result was expressed as mg per g of dry mass (mg g^{-1} DW).

High Performance Thin-Layer Chromatography (HPTLC)

The identification of phenolic acids, anthraquinones, and flavonoids using High-Performance Thin-Layer Chromatography (HPTLC) was made for both plants (leaves and roots) and in the case of *E. crassipes* in both stands (dense and not dense). HPTLC Plates ALUGRAM® Nano Silica Gel, 8.0 x 10.0 cm, was used to identify the said substances. The plates were activated with oxalic acid (1 %) (m:v) at 100 °C for 2 minutes. Applying samples (10 µL of extract) was performed over each lane at 0.5 cm between each sample and 1.0 cm at the edges. The solvents used for these analyses were toluene, ethyl acetate, methanol, and formic acid in the ratio 32: 14: 12: 5.

Some anthraquinones were used as reference standards: lucidine, alizarin, rubiadin, 1 methyl ether, damnacanthal, emodin, rubiadin, chrysophanic acid and morindine. Some flavonoids were also used, as well as phenolic acids such as quercetin, kaempferol, rutin and gallic acid and the iridoids asperolosidic acid, diacetyl asperolosidic acid. The visualization was carried out at 254 and 366 nm and the retention factor (Rf) was calculated as the quotient between the migration distance by compound from the application point and the migration distance of the solvent front.

Statistical analysis

The TAC, TPC, and TFC data in all the samples were subject to variance analysis using the Kruskal – Wallis non-parametric test. When significant differences from the Kruskal -Wallis test were found, the Wilcoxon test was used to determine the samples with such differences. Statistical analyses were made using the R software version 3.1.2 (R Core Team 2014), package Vegan[26].

RESULTS

Analysis of physicochemical and microbiological of the water

The pH of water was lower at L.1 than at L.2, so dissolved oxygen and total hardness. Other physicochemical parameters were higher at L.2 (Table 1).

Physicochemical and microbiologic parameters	L.1	L.2
pH	7.60 (at 22.0 °C)	7.70 (at 22.0 °C) - 7.86 (at 22.4 °C)
Saturation of dissolved oxygen (%)	12	36
Chemical oxygen demand (mg O_2 L^{-1})	44	15 - 42
Nitrogen from nitrite (N-NO_2^{1-}) (mg L^{-1})	1.7 x 10^{-2}	0.047 - 0.283
Reactive soluble phosphorus (RSP) (mg L^{-1})	0.671	0.386 -0.497
Total alcalinity (mg L^{-1})	405	250 - 255
Total hardness (mg L^{-1})	244	259 -269
Fecal coliforms (MPN/100 mL) (CT)	1.6 x 10^6 NMP/100 ml	1.6 – 5.4x10^6 MPN/100 ml
Water quality index (WQI)	42	45 - 56

Table 1. Physicochemical and microbiological conditions of freshwater lagoons L.1 and L.2

Analysis of organic compounds

The TPC ranged from 3.07 to 9.2 mg g^{-1}DW. The highest mean TPC was found in the roots of the dense stand of *E. crassipes* (9.19 mg g^{-1}DW) without significant differences with the leaves of the dense stand and the leaves of

the not dense stand of *E. crassipes* (Fig. 1a). The TAC ranged from 1.06 to 3.74 mg g^{-1} DW. The highest mean of TAC was recorded for the leaves of *E. crassipes* (dense stand) (3.73 mg g^{-1}DW), followed by the leaves of the not dense stand of *E. crassipes* sample. The lowest TAC was found in the leaves of *P. stratiotes* (Fig. 1b). The TFC ranged from 18.2 mg g^{-1} to 33.9 mg g^{-1}DW. The highest mean of TFC was found in the leaves of the dense stand of *E. crassipes* (33.92 mg g^{-1}DW), without significant differences with the leaves of the not dense stand (Fig. 1c).

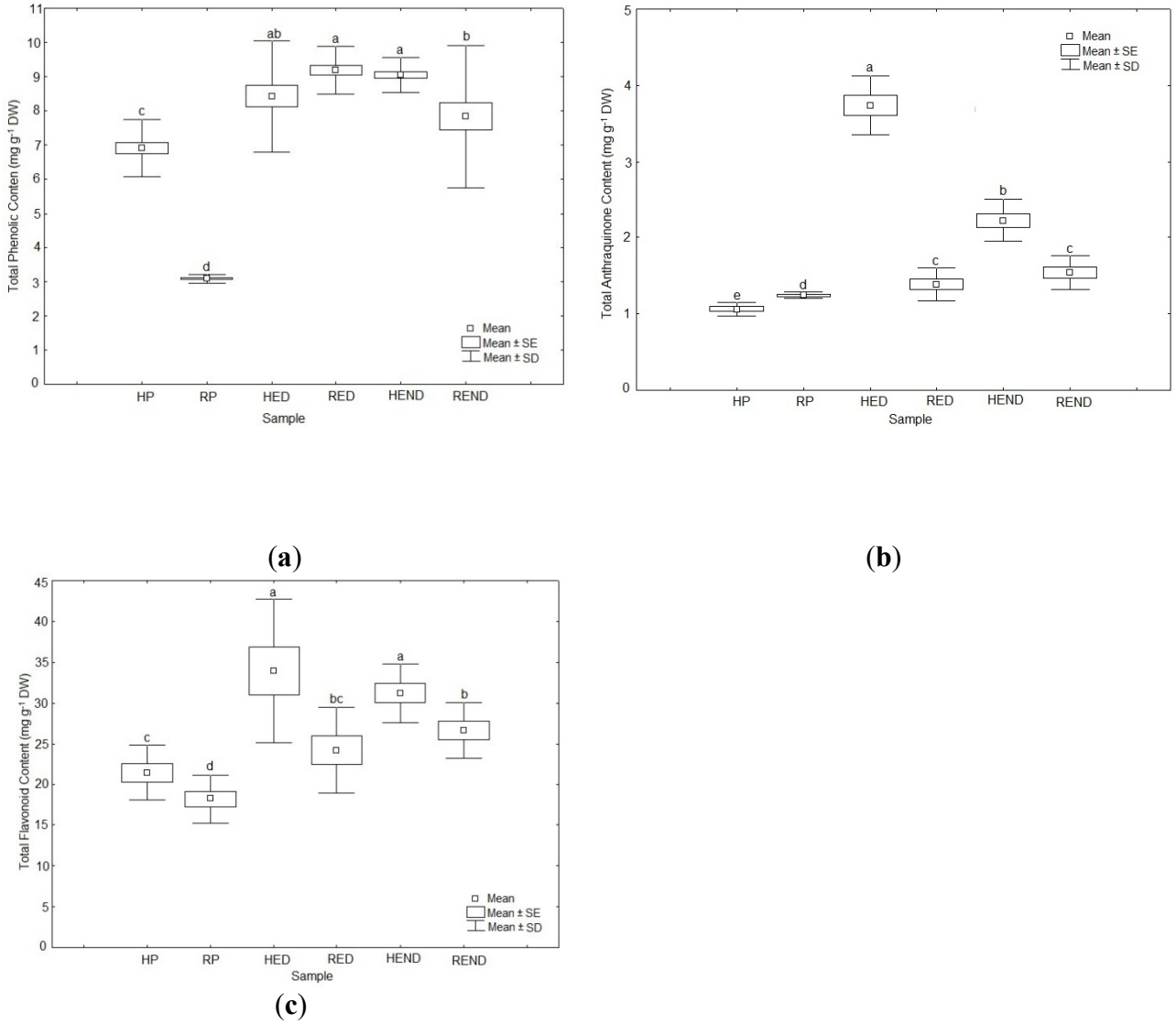

Figure 1. Total Phenolic Content (A) (Kruskal-Wallis chi-squared = 102.36, df = 5, p-value < $2.2e^{-16}$). Total Anthraquinone Content (B) (Kruskal-Wallis chi-squared = 47.046, df = 5, p-value = $5.559e^{-09}$). Total Flavonoid Content (C) (Kruskal-Wallis chi-squared = 34.244, df = 5, p-value = $2.129e^{-06}$) (mg g^{-1} DW) in ethanolic extracts of the leaves (HP) and roots (RP) of *P. stratiotes*, leaves (HED) (HEND) and roots (RED) (REND) of the dense and not dense stand of *E. crassipes*. Letters in the above quartile indicate significant differences among extracts.

High Performance Thin-Layer Chromatography (HPTLC)

Using unrevealed UV365nm (Fig. 2a), the phytochemical composition of extracts from leaves (HP) and roots (RP) of *P. stratiotes* showed similarities in two bands of both parts of the plant (light blue) and (blue-violet) (Fig. 2a). The blue bands were only visible in the leaves of *P. stratiotes*. In ethanolic extracts of *E. crassipes* (Fig. 2a),

the behavior was similar in the leaves of the dense and not-dense stands, with different shades of pink. However, a blue band was observed in HEND that was not present in HED, which could be related to the plant's development stage. For the root samples of *E. crassipes*, results were similar in both stands, with four bands of different shades of blue and a green band.

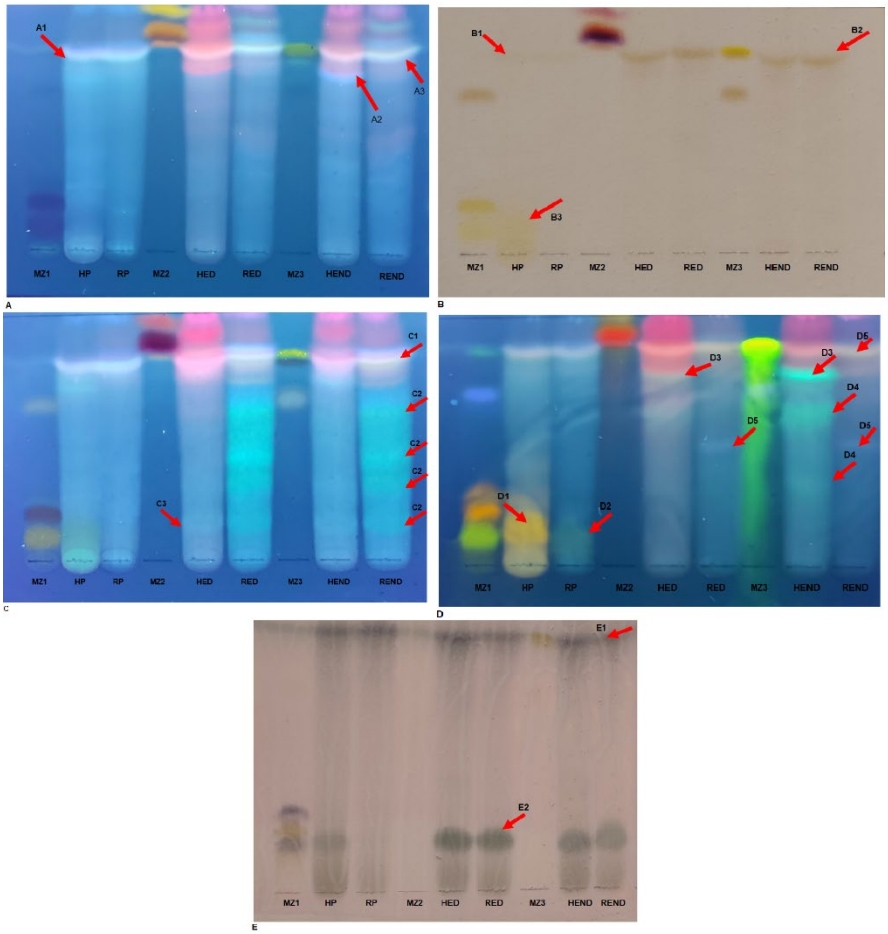

Figure 2. Phytochemical screening using HPTLC in ethanolic extract of leaves (HP) and roots (RP) of P. stratiotes, leaves (HED) and roots (RED) of dense stands of E. crassipes and leaves (HEND) and roots (REND) of not dense stands of E. crassipes. Chromatographic plates run in the solvent IV system. (A) Unrevealed Ultraviolet Light (UV365 nm), (B) Revealed white light with KOH, (C) Revealed ultraviolet light (UV365 nm) with KOH, (D) Revealed ultraviolet light (UV365 nm) with NP and (E) Revealed white light with V/S). Mixtures of standards morindine, diacetyl asperolosidic acid, rutin and Gallic acid (MZ1). Mixtures of standards lucidin, rubiadin-1 methyl ether, emodin, alizarin, damnacanthal and chrysophanic acid (MZ2). Mixtures of standards gallic acid, quercetin and kaempferol (MZ3).

In the chromatogram revealed with KOH and in white light (Fig. 2b), a brown band was observed in the ethanolic extracts, which coincides with standards for quercetin and kaempferol compounds. In HP and RP extracts, this brown band was also present but barely noticeable, which could indicate less content of these compounds. A yellow band coincident with moridina standards was observed in the HP extract.

The chromatogram revealed with KOH (Fig. 2c) at UV365nm showed the bands observed when white light was used. Some other bands (linked to *E. crassipes*) were observed: four green–blue bands in the roots of plants from both stands (dense and not dense), and of these four bands, only one was observed in the leaves (at HED and HEND).

The screening using UV365nm revealed NP (Fig. 2d) showed a barely noticeable yellow-orange band that coincided with rutin and morindine standards. A similar band was present in the ethanolic extract RP but was scarcely perceptible.

Three green-blue bands were observed in the ethanolic extracts of HED and HEND, one more intense in HEND and the other two bands of the same color. These green-blue bands were not present at the roots of *E. crassipes*. In the roots of *E. crassipes* (in both stands), a blue band that was not in the leaves of this plant was present.

The plates revealed in white light and with V/S showed a brown band (Fig. 2e) in all studied extracts that coincided with quercetin and kaempferol standards. It was unclear whether the color coincided when both standards were run at the same Rf, showing a mixture between yellow and brown due to the overlap between both compounds, which did not allow bands to be defined. One olive green band was observed in all extracts (more intense in HED and RED) except for the RP sample. This band was observed at the same Rf as the diacetyl asperolosidic acid standard.

The phytochemical screening of ethanolic extracts of both studied plants, the use of solvent system IV, and three revealers (KOH, NP, and V/S) showed coincidences in Rf and color with morindine or rubiadin in the leaves and roots of *P. stratiotes*. Some coincidences in Rf and color with quercetin, kaempferol, and diacetyl asperolosidic acid standards were found, except for the roots of *P. stratiotes*.

DISCUSSION

This is the first study on the water quality of freshwater lagoons where both invasive plants live. The pH of these lagoons was near the pH of previous reports for Cuban freshwater lagoons[20]. In both lagoons, its oxygen saturation was low, with a clear tendency to hypoxic conditions. This low-dissolved oxygen content was highly related to the high content of organic matter expressed as chemical oxygen demand (COD) that was above the limits of the Cuban standard for water quality (NC.25 in Pelegrín-Morales et al.)[27]. Considering these limits (COD), the water quality of these lagoons could be classified as “poor”. The causes of the high content of organic matter at both lagoons could be related to sewage discharges from neighboring dwellings and to the death of plants that end at the bottom of the lagoons. *P. stratiotes* and *E. crassipes* completely cover the water surface of L.1 lagoon covers almost 30 % of L.2. The decomposition of dead plants at the bottom of these lagoons triggers a significant oxygen uptake by bacteria, which results in the low content of dissolved oxygen in the lagoon waters[28]. Another evidence of sewage disposal into these lagoons is the high contents of phosphorus and nitrite, mainly in L.2. Due to the alkalinity of both lagoons, their waters could be classified as waters with temporal hardness and not sulfated, like the groundwaters, rivers, and dams of this region and Cuba[29].

In Latin America, studies of organic compounds in *E. crassipes* and *P. stratiotes* are scarce[30,] and in the case of Cuba, no reference to this topic has been found. Some studies around the world reported phenolic compounds in both plants, which vary when comparing them with the results of this study[9,6]. López et al.[4] obtained lower TPC in the leaves (2.36 mg g^{-1} DW) and the roots (2.7 mg g^{-1} DW) of *E. crassipes* than the TPC found in this study.

Like TPC, TAC was also determined in both plants. However, Tulika and Mala[8] detected TAC only in *E. crassipes*, not *P. stratiotes*. Tyagi and Agarwal[29] did not detect TAC in the roots of *E. crassipes*. Tyagi[32] found no TAC in *E. crassipe*s leaves with methane extracts. This author did not find TAC with ethanolic extracts. The same author[32] studied TAC in *P. stratiotes* and detected it in the leaves and roots with methanolic and ethanolic extracts. López et al.[4] detected (qualitatively) TAC in the leaves and roots of ethanolic extracts of *E. crassipes*.

In our samples, flavonoids were present. However, Rodríguez et al.[5] did not find these compounds in the leaves of *E. crassipes* and Vargas and Zambrano[3] neither in *E. crassipes*. López et al.[4] recorded lower TFC than the TFC recorded in this study in the leaves (0.75 mg $g^{-}1$ DW) and roots (0.29 mg $g^{-}1$ DW) of *E. crassipes*. The TFC in *P. stratiotes* and E. *crassipes* was higher than TAC and TPC, which coincides with the results obtained by Sudirman et al.[33].

Although this study did not focus on the relationship between the environmental conditions of freshwater lagoons and the content of organic compounds in plants, some authors have suggested the influence of these conditions and other factors such as solvent extraction, season of the year, the age of the plant[32,34,4,10], in the content of organic compounds in *P. stratiotes* and *E. crassipes*. This study demonstrated that the growth state of *E. crassipes* (dense and non-dense) also influences the content of organic compounds. For *E. crassipes*, TAC was higher in the dense stand than in the not dense stand (leaves and roots) and similar values of TAC and TFC were found in both stands of the same lagoon (L.2). Another phase of this research could be an evaluation of how the environmental conditions of each studied lagoon affect the content of organic compounds in both plants.

Identifying some organic compounds using the HPTLC technique showed results that differed from other studies of the same species. For instance, our results showed the presence of quercetin and kaempferol in diacetyl asperolosidic acid. However, Shanab et al.[35] did not find these compounds in the Nile's methanolic extracts of E. crassipes. Using the same extraction solvent (ethanol), Souza[36] identified only anthocyanins in the leaves of *E. crassipes*. López et al.[4] identified gallic acid (a phenolic compound) and catechin (flavonoid) in *E. crassipes*. Khalid et al.[6] and Gebrehiwot et al.[10] found terpenoids and flavonoids, including quercetin and kaempferol and evaluated their antifungal and antibacterial properties. Gebrehiwot et al.[10], stated that as antimicrobial activity, the concentrations of *E. crassipes* extracts can vary from 20 µg mL^{-1} to 500.0 µg mL^{-1}, depending on the plant organ used. Rufchaei et al.[37] determined gallic acid in *E. crassipes* (258.3 ± 10.8 mg g^{-1}) and evaluated their antibacterial activities against *Escherichia coli* and *Pseudomonas aeruginosa*.

Other non-aquatic invasive plants identified in Cuba have potential for use in agriculture, the food industry and pharmaceuticals, such as *Tithonia diversifolia* (Hemsl.) A. Gray[1,2]. This species was studied in Cuba by Herrera et al.[38] who demonstrated the positive effect of climatic factors on the concentration of secondary metabolites, such as phenols. Also, the species *Senna spectabilis* (Lam.) Irwin & Barneby is an invasive plants, identified in Cuba[1], in which Prajitha and Bai[39] identified fifteen important allelochemicals, including phenolic compounds, flavonoids, anthraquinone. However, no studies carried out in Cuba on this subject were found. Therefore, an explicit assessment of *P. stratiotes* and *E. crassipes*, in Ciego de Ávila province, can enable a cost-benefit analysis to help determine its ecological economics while pursuing the targets of the sustainable development and your ecosystems restoration[2]. In this regard, these results can be extended to the rest of the country. To do this, the biological activity of the compounds identified in *P. stratiotes* and *E. crassipes* must be evaluated, and the extraction and purification methods must be optimized.

CONCLUSIONS

Today, the high cost of cleaning these freshwater lagoons and the potential pollution of groundwater is the main reason why the management and use of *P. stratiotes* and *E. crassipes* must be an important issue to be solved by government authorities. The results of this study show that in *P. stratiotes* and *E. crassipes*, the content of phenolic compounds varied from 3.07 to 9.2 mg g^{-1}, anthraquinones from 1.06 to 3.74 mg g^{-1} and flavonoids from 33.9 to 18.2 mg g^{-1} of dry weight, so the use of their extracts in the pharmaceutical industry may be an alternative to manage and control their invasive effects. However, research is required on their antioxidant effects and their antifungal and antibacterial properties.

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