Chapter 192

Histochemical Localization and Antioxidant Potential of Lipids from the Root, Stem and Leaf of Salicornia neei Lag

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ABSTRACT
Investigations were carried out with the Salicornia neei Lag plant, evaluating the biotechnological potential in the synthesis of lipids, starting with the histochemical location in the tissues of the stem and leaf structures, observing the intense coloration of the oil droplets present in the cells, which were stained black or dark blue, in both structures. In this study, the content of total lipids were evaluated, in addition to evaluating their potential to antioxidant activities. The total lipids of the root and leaf showed higher values corresponding to approximately 10,06% and 5.67%, respectively, and the stem 3.07%. The studies carried out also demonstrated high antioxidant activity in the root, stem and leaf, allowing the application of Salicornia neei in the food industry and as a candidate for the pharmaceutical area.

Keywords: Halophyte, Lipids, Active biomolecules, Antioxidant activity.

1 INTRODUCTION

The study of plants for many years has shown great relevance as a source of food, home remedies or medicines and is based on bioactive compounds, which

occur naturally. In addition to many plants being considered important for human nutrition, considering the high content of vitamins and minerals, which confers great economic value to certain species. However, major problems have been occurring due to the continuous degradation of agricultural lands by salinization due to the decrease of freshwater and groundwater (MUNNS and TESTER, 2008; PANTA et al., 2014; QADIR et al., 2014).

The salinization of agricultural soils, caused by natural and anthropogenic factors, has been restricting agricultural productivity worldwide, becoming a challenge for global food security and

environmental sustainability. It is estimated that soil salinization in the world reaches 1.125 billion hectares, affecting 76 million hectares by human activities (MUKHOPADHYAY et al., 2021; ABOELSOUD et al., 2022; SPARKS et al., 2023).

Salicornia species (Chenopodiaceae, Salicornioideae) are halophytic, succulent plants that respond to saline stress with anatomical, physiological and metabolic adaptations. Species of the Chenopodiaceae family have in their composition a high content of minerals, polyphenols, fatty acids and other bioactive compounds, being attractive and with several industrial applications (BOSCAIU et al., 2013; SÁNCHEZ-GAVILÁN et al., 2021). Human consumption of these halophytes provides numerous health benefits, such as stimulation of immune responses, protection against oxidative stress and prevention of obesity. (PATEL, 2016; RAHMAN et al., 2018).

Salicornia species are considered oilseeds with fatty acid composition similar to other common edible vegetable oils (GOUDA and ELSEBAIE, 2016; PENTEADO et al., 2022; COELHO et al., 2021). Seeds of Salicornia spp. produce a percentage of 28% (ANWAR et al., 2002). Fatty acids are bioactive compounds present in the genus Salicornia, mainly in the stem and seeds. Some species are studied as a source of linoleic acid and oleic acid (LOCONSOLE et al., 2019; SÁNCHEZ-GAVILÁN et al., 2021).

The use of natural substances has been adopted as an important resource for health, in addition to becoming more popular, in particular, the recovery of antioxidants, minerals, pigments, polymers, and oils from fresh plant matrices and agro-industrial by-products. Other molecules such as nutraceuticals, obtained mainly from extracts of food plants, are very successful due to their nutritional benefits and functional properties (PATEL, 2016; BAJWA et al., 2023).

Salicornia neei is a species native to South America, originating from naturally saline soils (ALVES et al., 2020), which has been used in food due to its bioactive properties and high nutritional content (RIQUELME et al., 2016; DE SOUZA et al., 2018).

Considering the need to expand knowledge on *Salicornia neei*, investigations were carried out aiming at the histochemical location of lipids in different parts of the plant, in addition to evaluating the antioxidant activity of lipids, as viable and efficient biomolecules. And yet, the expansion of biotechnological studies with Salicornia neei makes possible the knowledge of the potential for future application in the food and/or pharmaceutical industries.

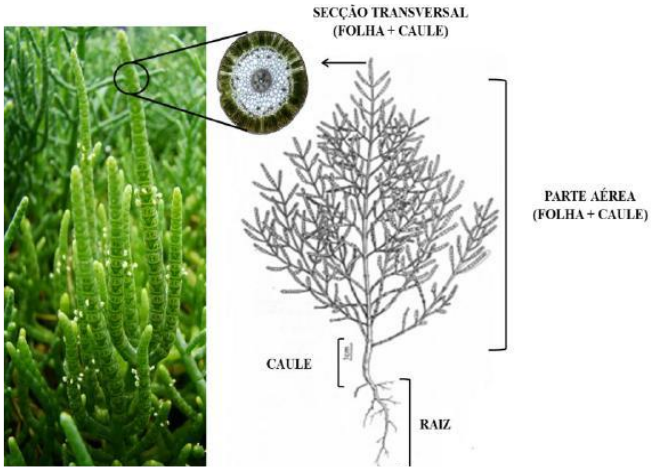
2 MATERIALS AND METHODS

2.1 BOTANICAL MATERIAL: SALICORNIA NEEI LAQ

Salicornia neei with 90 days of growth was collected in a greenhouse at the headquarters of the Agronomic Institute of Pernambuco (IPA), Recife, Pernambuco. The succulent seedlings were washed in running and distilled water, and later, separated into parts of the stem and leaves, cut into pieces of 8 to 10 cm. The genus Salicornia, was previously confused with the genus Sarcocornia, however, Salicornia presents characteristics (Figure 1) such as: spongy stems with scales, and its leaves, flowers and fruits are

discreet. The genus Sarcocornia, on the other hand, is presented with the following characteristics: perennial, branched plants, with prostrate-ascending branches, whose height varies according to the species, but is generally not greater than 70 cm (PATEL, 2016).

Figure 1 – Característica da *Salicornia neei Lag.*



2.2 HISTOCHEMICAL LOCALIZATION OF LIPIDS IN SALICORNIA NEEI

The localization of lipids in *Salicornia neei* was performed according to the method described by Sheehan and Storey (1947), modified. Transversal sections approximately 1 mm thick from the stem and leaf of fresh S. neei were fixed on the slide with buffered glycerin and observed by optical microscopy. The oil droplets present in the cells were stained black or dark blue.

2.3 EXTRACTION OF TOTAL LIPIDS

Fresh samples of stem and leaf of *Salicornia neei* were subjected to total lipid extraction according to the methodology described by Manocha et al., (1980). The plant parts were subjected to successive extractions with a chloroform: methanol solvent system (2:1; 1:1; 1:2 v/v). At the end, the extracts of each part of the plant were gathered and, after total evaporation, the samples were placed in a desiccator until constant weight. The yield of total lipids present in the samples was quantified by gravimetry and expressed as a percentage using Equation 1.

$$\text{Lipids (\%)} = \frac{P_2 - P_1}{M_1} \cdot 100 \qquad \text{(equation 1)}$$

P_2 (Final weight)

P_1 (Starting weight)

M_1(Sample weight)

2.4 EVALUATION OF ANTIOXIDANT ACTIVITY

The DPPH (2,2-diphenyl-1-picryl-hydrazyl) colorimetric method was used to evaluate the antioxidant capacity, according to the methodology described by BRAND-WILLIAMS et al. (1995) modified by SÁNCHEZ-MORENO et al. (1998), in which the fractions of extracts from the stem and leaf of Salicornia neei at different concentrations diluted in methanol (3.83; 1.91; 0.95 mg/mL) were analyzed. In a dark environment, 2.5 mL of each sample concentration was added to 1.0 mL of methanol solution containing the oxidizing radical DPPH, at a concentration of 0.18 mM. And after a reaction period of 30 minutes, the samples were read in a spectrophotometer at 515 nm. The percentage inhibition was calculated using the following equation:

Equation (2)

$$\% \text{ DPPH} = \frac{(Ac - As)}{Ac} \times 100$$

Ac: control absorbance

As: sample absorbance

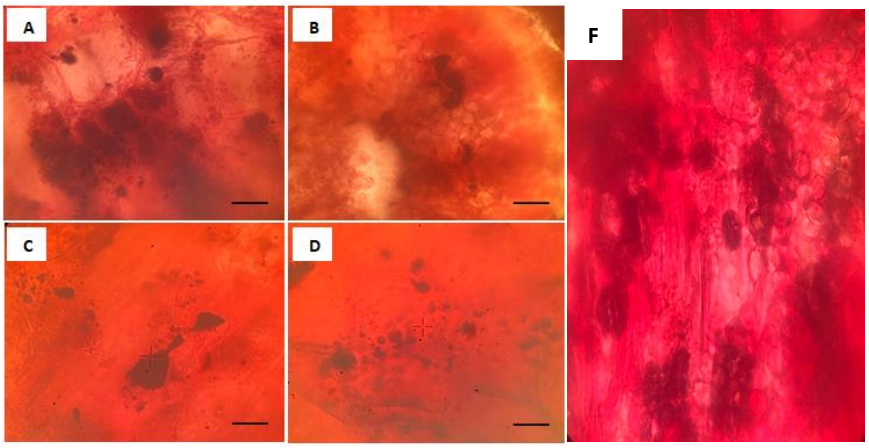
3 RESULTS AND DISCUSSION

The investigations carried out with Salicornia neei demonstrated the potential of lipid synthesis in the root, followed by the stem and leaf of the halophyte plant, being the first time that it is evidenced by histochemistry, considering the information in the literature. The literature refers to lipids in other species of Salicornia mostly directed to lipids in seeds (EL-MALLAH et al., 1994; BASHAN et al., 2000; EGANATHAN et al., 2006; ELSEBAIE et al., 2013).

3.1 DETECTION OF LIPIDS IN SALICORNIA NEEI

The stem (Figures 1A and 1B), leaf (Fig. 1C and 1D) and Fig 1 E (root) showed positive reactions with the Sudan Black dye, proving the presence of lipids in all structures, with emphasis on Figure 1E, of the root with a greater amount. The sensitivity of the histochemical method with Sudan Black was described by Shepherd et al., (2005) using the dye to detect lipids in embryos and seeds of several species of Chenopodiaceae, including other species of the genus Salicornia. However, positive reactions were only observed in Sarcocornia perennis and Allenrolfea occidentalis embryos, with no reactive lipid bodies observed in Salicornia bigelovii, S. europaea and S. bigelovii seeds. The authors suggested that the age of the seed or the form of the lipids are determining factors for the positive reaction with the dye. However. lipids continue to be one of the most enigmatic classes of biological molecules, according to Flores et al. (2020).

Figure 1. Cross-sections showing cell layers of Salicornia neei L. stained with Sudan Black in (A) lipid bodies stained in dark blue in the stem parenchyma; (B) lipid bodies stained in blue close to the vascular bundle of the stem; (C) large blue-stained lipid bodies in leaf parenchymal cells, (D) small blue-stained lipid bodies in leaf cells, and (E) large amounts of blue-stained lipid bodies. Scales: 50µm.



3.2 TOTAL, LIPID CONTENT IN *SALICORNIA NEEI*

The levels of lipids presented in leaves of Salicornia neei Laq were higher, corresponding to 56.40 mg/g of plant, compared to the stem (30.40mg/g) (Table 1).

However, for Min et al. (2002) in S. herbacea L. the lipid content was higher in the stem (0.3%) and in the root (0.3%), while the leaf presented a smaller fraction (0.2%). Lu et al., (2010) when analyzing the tips of the branches of Salicornia bigeloviia obtained a very low lipid content (0.37mg/g).

According to Turki (1999) several species of Salsola, a succulent of the Chenopodiaceae family, have lower lipid levels than the leaves of S. neei. The author also demonstrated lower levels of lipids in S. schnvinjiirrhii (15.3 mg/g) and produced by the species S. longifolia (32.2 mg/g). Similar results were obtained by Ahmed et al. (2022), in which species of the same family, Arthrocnemum macrostachyum Moric and Kochia indica Wight produced 56.42 mg/g and 32.18 mg/g, respectively.

Table 1. Total lipid content of leaves and stems of *Salicornia neei*

Part of the Plant	Mass (mg/g)	Percentage (%)
Sheet	56,40	5,64
Stalk	30,70	3,07
Root	100,06	10,06

Source: Own authorship (2023).

3.3 QUANTITATIVE AND QUALITATIVE ANALYSIS OF ANTIOXIDANT ACTIVITY

Several studies report the antioxidant activity related to phenolic extracts, alkaloids, flavonoids and terpenoids from halophytic plants of the genus Salicornia. In addition to its culinary relevance, medicinal attributes such as immunomodulatory, hypolipidemic, antiproliferative, osteoprotective and hypoglycemic, make this plant less known from the swamp and more significant for phytochemical studies (PATEL, 2016).

Table 2 presents the values of quantitative and qualitative antioxidant activity of lipids in leaves, stems and roots of the Salicornia neei plant. The leaf reached its maximum value (51.65) in the

concentration (0.95mg). In the case of the stem, the antioxidant activity that reached a maximum value of 85.47%) was also at the minimum concentration (0.95mg) of lipids. However, the highest values were 97.01%, observed in the root at a concentration of 1.91mg of lipids, and 88.83% at a concentration of 3.88mg of lipids, respectively. However, in relation to Salicornia neei there are no reports of studies of antioxidant activity with lipid extracts. Studies carried out evaluating the stability of antioxidant activity in oils extracted from Salicornia herbacea seeds demonstrated that the oil contained high levels of α-tocopherol (249.2 mg/kg of oil) (CHOI et al., 2014). The literature demonstrates the efficiency of vitamin E (α-tocopherol, α-Toc) as an antioxidant present in lipid systems (BAROUH et al., 2022). This information suggests that lipids extracted from parts of the Salicornia neei plant may also have high levels of α-tocopherol, allowing future studies.

Table 2. Antioxidant activity in lipid extracts of *Salicornia neei*

LIPID CONCENTRATION (mg)	**ANTIOXIDANT ACTIVITY (%)**		
	Sheet	**Stalk**	**Root**
0,95	51,65	85.47	57,46
1,91	39,21	37.13	97,01
3,88	4,35	7.88	88,83

4 CONCLUSIONS

The histochemical location of lipids in the root, stem and leaf demonstrated the accumulation of lipids, being evidenced for the first time in the halophyte plant Salicornia neei. The total lipid content was consistent with the histochemical location, with the root having the highest content, followed by stem and leaf.

It is noteworthy that the lipids of Salicornia neei also demonstrated for the first time the high antioxidant potential of the root, stem and leaf oils, showing future perspectives for further studies, as well as future applications in the food and/or pharmaceutical industries.

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