Salicornia ramosissima: secondary metabolites and testicular protection induced by ethanol extract

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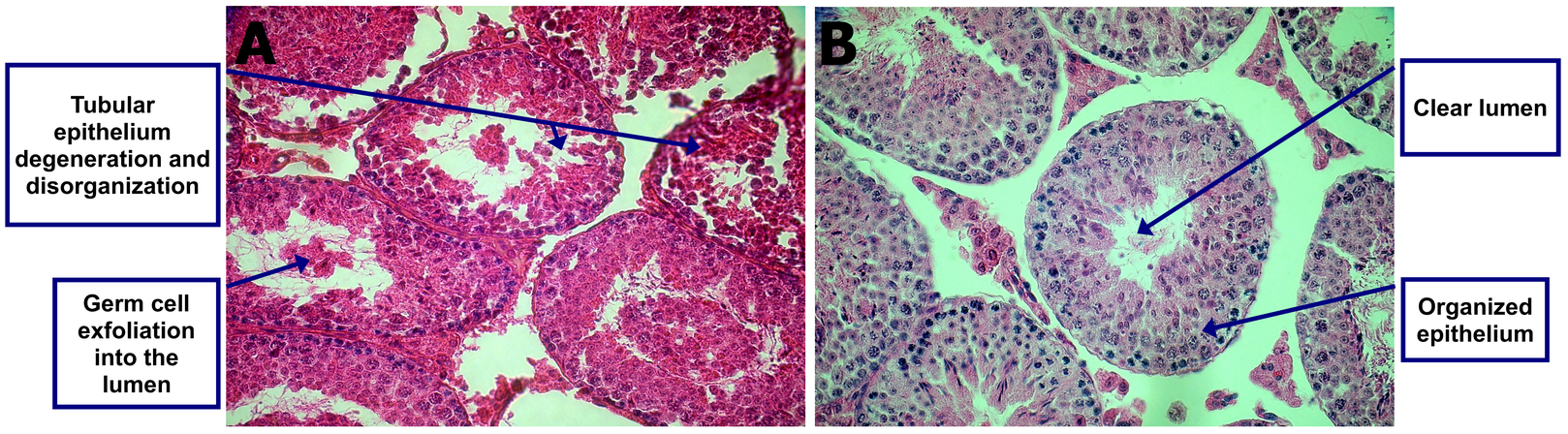
Introduction

Several *Salicornia* species are used in folk medicine for several disorders, such as constipation, obesity and diabetes [1]. Furthermore, extracts of the aerial parts of *Salicornia* spp. exhibit promising biological activities and their phytochemical studies revealed the presence of flavonoids, chromones and alkaloids, also known for their biological activities [1]. Given these bookmarks, we initiate a phytochemical and toxicological study of *S. ramosissima* J. Woods (Chenopodiaceae), an annual salt tolerant plant, broadly distributed in the salt marshes and salt pans of Ria de Aveiro [2]. Previous studies have shown the presence of scopoletin [3] and a new aromatic compound with *t*-butyl substituents (saliramofenol) [4], from the dichloromethane extract of *S. ramosissima* aerial parts, while the *S. ramosissima* ethanolic extract (SREE) showed hepatotoxicity and renal impairment of mice [5].

Results and Discussion

One aim of this study was to assess the effects of SREE on mice testis under carbon tetrachloride (CCl_4) exposure. CCl_4-induced testicular injury is due to free radical action, which results in lipid peroxidation of cell membranes and altered antioxidant enzyme status [6].



CCl_4-treated group SREE+CCl_4-treated group

The images above clearly showed a marked reduction of histopathological findings and demonstrated that SREE had a protective effect on CCl_4-induced testicular injury. In conclusion, SREE preserved testicular structure and subsequently the reproductive function. The histological findings herein described may reflect the single or combined action of the isolated compounds (**1**, **2** and **3**) or others, since derivatives from cinnamic acids and PUFAs are known to have antioxidant activity.

Material and Methods

Fresh aerial part of *S. ramosissima* (1.2 Kg) were collected from a salt pan, Ria de Aveiro. Small pieces were extracted with ethanol at room temperature for 24h. The mixture was filtered and concentrated *in vacuo* at 35°C. The resulting extract (SREE) was then freeze-dried. Dried aerial parts of *S. ramosissima* (1.1 Kg) were extracted with dichloromethane at room temperature (2x 72 h). The extract (SRDE) was evaporated to dryness.

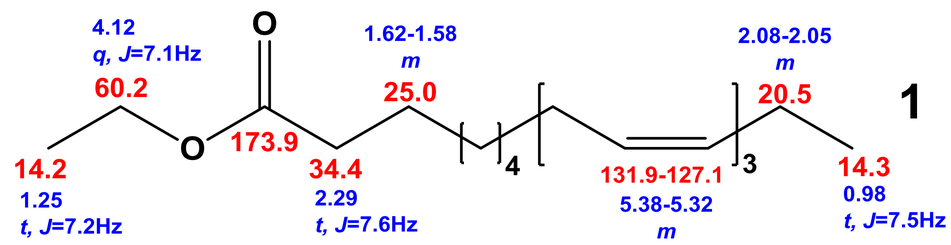
Lyophilized SREE was orally administered during 3 weeks to male ICR-CD1 mice (50 mg/kg/b.w.), and followed by a single injection of CCl_4 solution (0.2 mL/kg/b.w.). Control and CCl_4-treated groups were also considered. Testes were collected 24h after the treatment and prepared for histology. Animal procedures were followed according to guidelines for ethics and animal care.

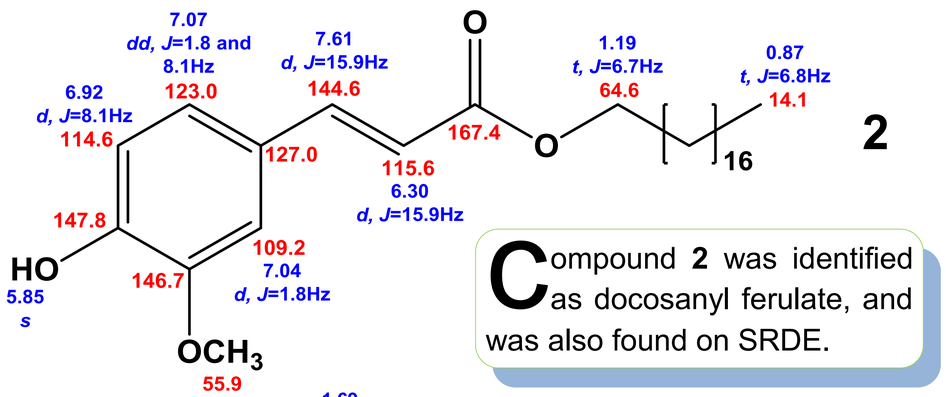
SREE (33 g) was re-suspended in 500 mL of H_2O and successively partitioned with n-hexane, dichloromethane and ethyl acetate. The *n*-hexane layer (2 g) was fractionated by silica CC eluted with *n*-hexane/EtOAc (100:0 - 80:20 v/v). The obtained fractions were purified by silica GF_{254} tlc, eluted with different polarity eluent mixtures. SRDE was fractioned by column and preparative tlc on silica gel, eluting with solvent mixtures of different polarity.

The structure of the isolated compounds **1** and **2** from SREE, and **3** from SRDE were elucidated by NMR and MS spectroscopic data.

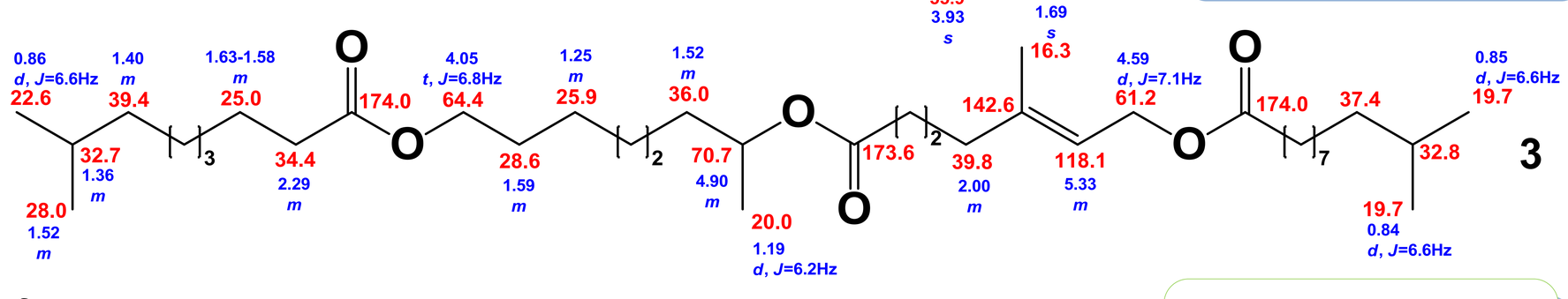
Concerning phytochemical screening of SREE, a total of 2 compounds were isolated and characterized spectroscopically.

The ESI-MS of compound **1** showed a signal at *m/z* 329 corresponding to the $[M+Na]^+$ ion. The compound **1** was identified as ethylated linolenic acid (PUFA ω3). It is not an artifact since the extract was obtained at room temperature and other ethylated derivative was identified on SRDE [3].





Compound **2** was identified as docosanyl ferulate, and was also found on SRDE.



From SRDE was identified a wax with a rare structure **3**, saliramoester.

All these compounds were isolated and identified for the first time on *Salicornia* spp.

References

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Acknowledgements

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