genera such as *Halodule*, *Halophila,* and *Thalassia*.[12,27,39,40] Minor sterols such as sitosterol were found in our study of *S. filiforme* species, which is in accordance with those reported in *S. isoetifolium* and other marine angiosperms of *Cymodocea*, *Halodule*, *Thalassia,* and *Enhalus* genera from tropical zones after GC-MS analysis.[12,41,42]

On the other hand, phenolic acids derived from benzoic acid were detected. They include ρ-hydroxybenzoic as a major component (2.57%), ρ-hydroxy-3-methoxybenzoic acid (vanillic acid); 3,5-dihydroxy benzoic acid methyl ester; 3,4-dihydroxybenzoic acid, and the benzoic acid itself. It is well known the pharmacologic potential of these compounds, which are also present in the abovementioned marine angiosperms.

Plants are important sources of naturally occurring antimicrobial and anticancer agents. Antimicrobial and cytotoxic activities of plant extracts have been extensively reported in literature.[43-46] Some of these studies have led to the identification of the active components responsible for such activities, contributing to the development of novel drugs for therapeutic use in humans. Because of the emergence of multiple drug resistance in human pathogenic micro-organisms and the adverse effects of cancer chemotherapeutic drugs, the search for new antimicrobial and anticancer molecules from alternative sources, including plants, is receiving attention by the scientific community.[47-50]

Seagrasses are continuously defending from the infection of marine micro-organisms.[51] In this way, many seagrass species are able to produce secondary metabolites with antibacterial and antifungal properties.[51] For example, Engel *et al.* showed the antimicrobial activity of a lipophilic fraction from *S. filiforme* against *S. aggregatum* and *P. bacteriolytica* strains that can affect marine plants.[20] By a similar approach, Yuvaraj *et al.* highlighted the presence of the same activity in *H. ovalis* extracts, which proved effective against several bacteria and *Vibrio*.[32] *T. hemprichii* flavonoids also possessing *in vitro* capabilities of inhibiting different bacteria.[52] Similarly, Aswathi *et al.* demonstratedsignificant antibacterial activity of acetone and methanol extracts from *S. isoetifolium* against Gram stains of human and fish pathogens, proving a mayor antimicrobial effect for the acetone extract.[19] In addition, Iyapparaj *et al.* have also showed the antibacterial and antifouling capacity of acetone, dichloromethane, and methanol extracts from *S. isoetifolium* and other marine plants such as *C. serrulata*.[23]

In the present study, $SfCHCl_3$ exhibited antimicrobial activity against five human pathogens, particularly against *S. aureus*, *P. aeruginosa,* and *S. typhi*; whereas *S. filiforme* crude extract displayed lower effects and was no active against two of the five tested strains. This difference could be related to the high content of FAs in the fraction, since they have been reported to mediate the antibacterial effects of many organic fractions and extracts from marine plants and seaweeds.[20,53,54] Furthermore, azelaic acid has been described as an antimicrobial agent that inhibits the proliferation of food pathogenic bacteria;[55] it is indicated for the treatment of *acne rosacea* and cutaneous infections and has been proven to be well tolerated in numerous clinical trials.[35] This substance has also shown profound anti-inflammatory, antioxidant, and cytotoxic effects,[56] thus being another compound that could synergistically contribute to the observed effects of $SfCHCl_3$.

Cytotoxicity is an interesting pharmacological property that has also been studied for different extracts and pure compounds isolated from seagrasses. For instance, a significant *in vitro* cytotoxic effects against two human lung cancer cell lines were demonstrated by cymodienol from *Cymodocea nodosa*.[57] In contrast, ketosteroids from *C. nodosa* showed no *in vitro* cytotoxicity against these cell lines.[58] Other compounds, such as the syphonoside from *Halophila stipulacea,* showed no cytotoxicity, however, inhibited apoptosis in some of the studied cell lines.[59] On the other hand, El Baz *et al.* demonstrated antitumor activity of sulfolipid fractions, mainly composed for FA derivatives, from various algal species against HepG2 and MCF-7 cell lines, as well as antibacterial activity against *B. subtilis* and *E. coli*.[60] Both activities found are attributable to those kinds of compounds. Similarly, crude extracts of the marine plant *Thalassodendron ciliatum* exhibited cytotoxic effects against selected human cancer cell lines, and some activity against hepatitis A and herpes simplex viruses *in vitro*.[11,61]

According to these previous evidence and based on the preferential antimicrobial activity that $SfCHCl_3$ displays compared with the crude extract, we decided to evaluate the cytotoxicity of the lipophilic fraction in human lung cancer cells, in comparison to *S. filiforme* crude extract and a reference chemotherapeutic agent used for lung cancer treatment, such as cisplatin. This study revealed important cytotoxic effects of $SfCHCl_3$ in the cell line A549, which were similar to the cytotoxicity of cisplatin in the same cell line. As it occurred with the antimicrobial activity, the crude extract was no cytotoxic in comparison with the chloroform fraction and was not able to decrease the viability of A549 cells below an 80%, not even at the highest concentration tested.

The main compound present in $SfCHCl_3$ was palmitic acid. This FA showed cytotoxic effects in A549 human lung cancer cell line through a mechanism that involves endoplasmic reticulum stress, hypercalcemia, and generation of reactive oxygen species in the study carried out by Wong *et al.*[62] Furthermore, other lipophilic mixtures, containing short and long-chain FA exhibit cytotoxic effects in different human cancer cell lines and enhance the activity of cytostatic drugs such as paclitaxel and cisplatin.[63,64] Because of that, some of these acids and their methyl esters have also drawn the attention and have been commercialized as nutritional supplements showing antioxidant, anticancer, and antihistaminic properties.[14,65]

Recently, our group carried out a similar procedure to study *T. testudinum leaves* from Cuban coastal zones and it was found that the same fraction was mainly composed of FAs (80%), where palmitic acid was also the major compound. Similarly, such studied fraction showed a potent cytotoxicity and antiproliferative effect in the same cell lines,[12] which is in agreement with the results obtained in the present study.

As previously mentioned, azelaic acid could be contributing to the cytotoxicity of $SfCHCl_3$. Besides its antimicrobial properties, this compound has demonstrated cytotoxic and antiproliferative effects.[34,66,67] At molecular level, azelaic acid acts as a competitive inhibitor of tyrosinase, a key enzyme for melanin synthesis; consequently, it has been used to treat hyperpigmented disorders including melasma and *lentigo maligna,*[34,35,66] but it has also shown antitumor activity on melanoma, leukemia, and squamous carcinoma cells *in vitro* and *in vivo* in mice with xenotransplanted melanoma tumors.[66-68] This dicarboxilic acid has been tested in humans as well, demonstrating effectiveness against melanoma *in situ* and malignant melanoma.[67]

The present work reveals the importance of non-polar and mid-polar constituents of *Syringodium filiforme* for its pharmacological properties. Since we demonstrate that the crude extract of *S. filiforme*, which is predominantly polar, is not active compared with the chloroform fraction isolated from it, our results indicate that the non-polar components present in *S. filiforme* are critical for the biological activity of products derived from this marine plant. Based on the presented evidence, it can be inferred that the antibacterial and cytotoxic effects demonstrated for $SfCHCl_3$ may be due to the presence of FAs and azelaic acid, in synergy with other minor components of the fraction such as glycerides and flavonoids. This work contributes to the chemo-taxonomical characterization of *S. filiforme* and validates the chloroform fraction derived from the crude extract of this organism as a potential source of natural antimicrobial and cytotoxic molecules.

CONCLUSION

The lipidic extract of the phanerogam *Syringodium filiforme* was characterized by GC-MS. The assays allowed the identification of the main non-polar compounds of this species and, for the first time, demonstrated its antimicrobial effects against human pathogens and cytotoxicity in A549 human lung carcinoma. On the basis of these facts, this seagrass is an interesting potential biotechnological resource.

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Conflicts of interest

There are no conflicts of interest.

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