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ORIGINAL ARTICLE

Chemical Composition and Biological Potential of a Chloroform Fraction from the Leaves of Marine Plant *Syringodium filiforme* Kützing

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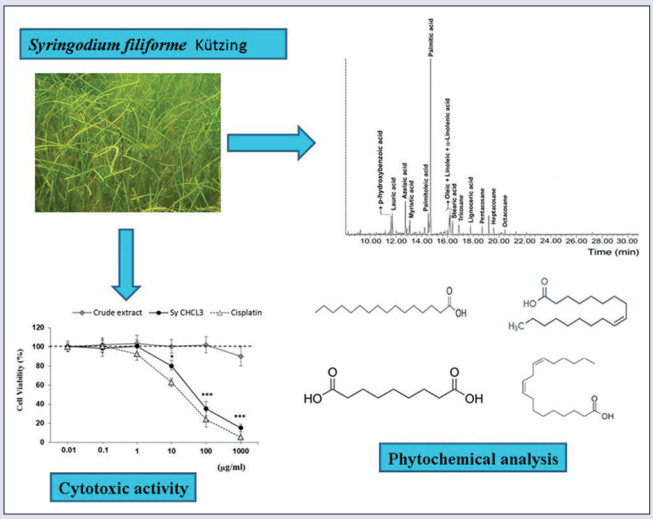
ABSTRACT

Background: Seagrasses are an important component of Nearshore marine ecosystems and a rich source of secondary metabolites with important pharmacological properties. **Materials and Methods:** In this work, crude hydroethanolic extract and chloroform fraction ($SfCHCl_3$) from marine plant *Syringodium filiforme* were evaluated for antimicrobial and cytotoxic potentials. In addition, the chemical composition of chloroform fraction was determined by gas chromatography-mass spectrometry (GC-MS) analysis. **Results:** GC-MS analysis allowed the identification of 68 compounds in the $SfCHCl_3$, where palmitic acid (39.18%) was the main component. The evaluation of antibacterial activity of $SfCHCl_3$ showed good activity against strains of *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), and *Salmonella typhi* (ATCC 9992v), and it was less active against *Escherichia coli* (ATCC 10576) and *Candida albicans* (ATCC 10231), while the crude extract showed low antibacterial activity. Furthermore, an important cytotoxic effect of $SfCHCl_3$ in the A549 human lung carcinoma cell line was evidenced, which were similar to the cytotoxicity of cisplatin in the same cell line. **Conclusion:** The results suggest that compounds may play an important role in the antimicrobial and cytotoxic effects observed for this species. In addition, this research contributes to the chemotaxonomic characterization of *S. filiforme* and validates this species as a potential source of natural antimicrobial and cytotoxic molecules.

Key words: *Syringodium filiforme*, Chemical composition, Chloroform fraction, Antimicrobial, Cytotoxicity

SUMMARY

- Crude hydroethanolic extract and chloroform fraction ($SfCHCl_3$) from marine plant *Syringodium filiforme* were evaluated for antimicrobial and cytotoxic potentials.
- GC-MS analysis allowed the identification of 68 compounds in the $SfCHCl_3$, being palmitic acid (39.18 the predominant substance.
- This study allowed demonstrating antimicrobial and cytotoxic effects of the species *S. filiforme*.



Abbreviations used: $SfCHCl_3$: Chloroform fraction of *Syringodium filiforme*; GC-MS: Gas chromatography-mass spectrometry.

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INTRODUCTION

Marine species are constantly competing for extent their habitats. To survive to this intense competition and such extreme conditions, seagrasses and algae have biosynthetically developed a wide variety of secondary metabolites. These wide range of unique chemical entities have lately drawn the attention of investors from worldwide pharmaceutical companies because of their broad panel of pharmacological activities and their health benefits.[1-8]

In this sense, the presence of diverse potent secondary metabolites in seagrasses is well documented,[9-12] while their antibacterial, anticancer, antiproliferative, antitumor, and antioxidant effects make them being recognized as new potential drugs.[11,13-15]

Previous investigations, only a few studies, have been conducted about the chemical composition and pharmacological activities of *Syringodium* genus. Nussier *et al*. identified in the hydroethanolic extract

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of *Syringodium filiforme* (manatee grass), phenolic compounds such as *p*-hydroxybenzoic, vanillic, caffeic, protocatechuic, *p*-coumaric, ferulic, syringic, chicoric, and caftaric acids.[16] From *Syringodium* flotsam, *L*-chiro-inositol was isolated and characterized, with demonstrated hypoglycemic action.[17] The qualitative analysis of extracts from this species showed high content of flavonoids, phenols, terpenes, anthocyanins, reduced sugars, and alkaloids. From all evaluated extracts, only the total extract and methanol fraction revealed asignificant antioxidant properties against free radicals.[18]

Similarly, a qualitative test of phytochemicals from methanol extracts of *S. isoetifolium* showed the occurrence of phytoconstituents such as saponins, resins, proteins, carbohydrates, glycosides, acidic compounds, reduced sugars, cardiac glycosides, phenols, and alkaloids. The antibacterial activity of methanol and acetone extracts from this species against 17 human pathogens and five fish pathogens were demonstrated,[19] while the lipophilic fraction from *S. filiforme* was active against *Schizochytrium aggregatum* and *Pseudoalteromonas bacteriolytica*.[20]

On the other hand, the cytotoxic activity has been less explored for the *Syringodium* genus. There are reports of the cytotoxicity of hydroethanolic extracts from other marine plants such as *Thalassodendron ciliatum* (Egyptian Red Sea seagrass) and *Thalassia testudinum* (turtle grass).[21,22] In addition, the cytotoxicity of a chloroform fraction recently obtained from *T. testudinum* was assessed in A549 human lung carcinoma.[12] *S. isoetifolium*, together with other marine angiosperms such as *Cymodocea serrulata* have demonstrated potent antifouling and antimicrobial activity against microalgae and pathogenic bacteria.[23] However, the antibacterial and antiproliferative capacity of *S. filiforme*-derived products/extracts has not been characterized. Other research groups have demonstrated the nutritional value of *S. filiforme* to humans and animals;[24-26] nevertheless, its cytotoxic or antimicrobial properties were not evaluated.

In this context, based on the progress recent of the *Syringodium* genus, this research aimed to characterize, a lipophilic fraction of *S. filiforme* seagrass to determine its antibacterial activity against human pathogenic micro-organisms, as well as its cytotoxic effects.

MATERIALS AND METHODS

Plant material

S. filiforme was collected on March 16, 2016, in Guanabo Beach (23°10'44"N - 82°07'01" W) Havana, Cuba. It was authenticated by Dr. A. J. Areces (Institute of Oceanology, Havana) and deposited in the collection of the National Aquarium of Cuba, with number IDO 165. The seagrass was washed with distilled water to remove sand and salts and then dried in an oven at a temperature of 50°C to constant weight.

Extraction and fractionation

The dried and pulverized *S. filiforme* leaves (200 g) were continuously macerated with 2000 mL of ethanol: H_2O (1:1 v/v) over a period of 7 days at room temperature. The extract was filtered and concentrated to dryness under reduced pressure and low temperature (45°C), resulting in 2.5% of the crude hydroethanolic extract. The dry extract was transferred to a vial and placed in desiccators to remove moisture. Four grams of this extract were fractionated using mechanic agitation for 2 h with chloroform (300 mL) to obtain the non-polar fraction after filtration and drying processes, yielding 1.6%.

Chemicals

Analytical-grade reagents and reference patterns for gas chromatography-mass spectrometry (GC-MS) were obtained from Sigma, USA. Culture media and supplements were purchased from GIBCO (Gibco BRL, Paisley, UK).

Gas chromatography-mass spectrometry analysis

Samples of chloroform fraction (5.0 mg) from *S. filiforme* (SfCHCl$_3$) were accurately weighed into a 2 mL vial, then 0.15 mL of *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide were added. Subsequently, the vial was tightly capped, heated at 80°C for 1 h and 0.5 µL were analyzed by GC-MS. The analyses were performed using an Agilent GC 6890N equipped with a mass selective detector 5975 B inert, and a split-splitless injector, in splitless mode, was used (Agilent, Palo Alto, CA, USA). Separations were made on a HP-5Ms fused-silica capillary column (30 m x 0.25 mm), with a film thickness of 0.25 µm D_f (Agilent, Avondale, PA). The GC oven temperature was kept at 60°C for 2 min and programmed to 200°C at a rate of 20°C/min, then from 200°C to 300°C at a rate of 8°C/min and kept constant at 300°C for 5 min. The temperature of the injector was fixed at 320°C and that of the source at 280°C, while MS interface temperature was 250°C. Helium (purity, 99.9995%) was the carrier gas; its flow rate was fixed at 1 mL/min. Ionization of the sample components was performed in electron impact mode (EI, 70 eV). The mass range from 40 to 1000 *m/z* was scanned at a rate of 3.0 scans/s. One microliter of the organic extract was manually injected into the GC-MS system using a Hamilton syringe, for total ion chromatographic analysis. The total running time of the GC-MS system was 70 min. The relative percentage of each extract constituent was expressed as a percentage with respect to peak area normalization. Peak identification was achieved by computer matching mass spectra against commercial libraries (National Institute of Standards and Technology (NIST 2011 GC-MS), as well as MS literature data.[12,27-29]

Determination of antimicrobial activity

Antibacterial activity was determined by the microdilution method according to the Guidelines of National Committee of Clinical Laboratory Standards.[30] The crude extract of *S. filiforme* and SfCHCl$_3$ was assayed in concentrations between 1 and 2000 µg/mL. For this assay reference strains of *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 10576), *Candida albicans* (ATCC 10231), and *Salmonella typhi* (ATCC 9992 vaccine) were used. All micro-organisms were adjusted to 0.5 Mc Farland scale. Double dilutions were made from 2000 µg/mL to 1 µg/mL in 96-well plates. In each well, 25 µL of *Syringodium* extract or fraction plus 25 µL of each micro-organism was added. Furthermore, a positive control was performed by adding 25 µL of each micro-organism plus 25 µL of saline. Streptomycin was used as a reference antimicrobial agent. The plates were incubated at 37°C for 24 h, and then 5 µL of each dilution was plated in triplicate in Tryptone Soya Agar medium and incubated at 37°C for 24 h to determine the minimum inhibitory concentration (MIC). Reading was performed thereafter. MIC was taken as the lowest concentration of the product able to completely inhibit bacterial growth in the well. The experiment was performed in triplicate.

Cytotoxic evaluation by 3-(4,5-dimethylthiazol -2-yl)-2,5-diphenyltetrazolium bromide reduc tion assay

Cell viability of A549 cells (ATCC CCL-185) in the presence of different concentrations of *S. filiforme* crude extract, SfCHCl$_3$, and cisplatin (0.01–1000 µg/mL) were evaluated through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as it has been previously described.[31] The crude extract and cisplatin were dissolved in culture media, whereas the organic fraction was solubilized in culture media with *dimethyl sulfoxide* (DMSO) 1%.

Untreated cells and cells treated with DMSO 1% were used as controls. Cell viability was determined from three independent experiments with three replicas each.

Statistical analysis

The statistical analysis was carried out using the GraphPad Prism software. Values are expressed as the mean ± standard deviation of at least three independent experiments. One way ANOVA with a Tukey posttest ($P < 0.05$ and $P < 0.01$) was used for multiple mean comparisons.

RESULTS

The GC-MS analysis of the obtained $SfCHCl_3$ after chemical derivatization allowed the identification of saturated and unsaturated fatty acids (FAs) (ω-3 and ω-6), phenolic acids, sterols, glycerides, fatty alcohols, and flavonoids [Figure 1 and Table 1]. In the chromatogram, 68 components of the seventy detected were identified, being palmitic acid (C16:0) the main component (39.18%), followed by azelaic acid (5.06%), α-linolenic (C18:3) (3.63%), oleic acid (3.54%), linoleic acid (C18:2) (3.33%), and two isomers of palmitoleic acid (3.21 and 3.04%) [Table 1]. Other acidic, aromatic, and aliphatic compounds were detected in lower proportions.

Table 2 shows the antibacterial activity of *S. filiforme* crude extract, $SfCHCl_3$, and Streptomycin. The crude extract showed low antibacterial activity against the tested strains and in some cases, it was close to zero relative to the fraction obtained from it. Moreover, the organic fraction revealed antibacterial activity in concentrations below 1.5 mg/mL. $SfCHCl_3$ showed good activity against strains of *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), and *Salmonella typhi* (ATCC 9992v), and it was less active against *E. coli* (ATCC 10576) and *Candida albicans* (ATCC 10231). The strains tested are not resistant to multiple drugs; however, it is very important to evaluate the inhibitory effect of natural extracts and fractions obtained from these, since they represent clinically relevant species involved in major human infections and good models to search for new antibacterial candidates. The antibacterial activity shown by this fraction led us to evaluate its cytotoxic potential in human lung cancer cells.

The presence of long-chain FAs, as well glycerides and phenolic acids, suggests possible cytotoxic and antitumor properties for $SfCHCl_3$.

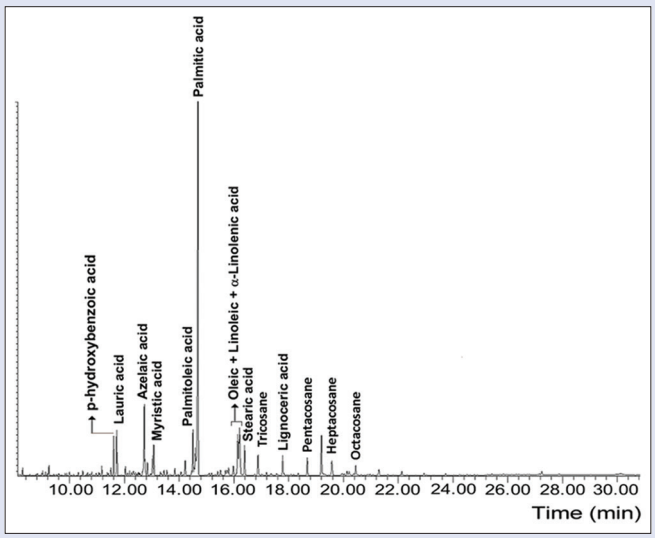

Figure 1: Gas chromatography-mass spectrometry profile of the derivatized chloroform fraction obtained from hydroethanolic extract of *S. filiforme*

In this way, the cytotoxic potential of $SfCHCl_3$ and *S. filiforme* crude extract in comparison to a clinically used anticancer drug such as cisplatin was evaluated in A549 human lung carcinoma. The organic fraction significantly reduced the viability of treated cells in comparison to control cells (no treatment or treatment with DMSO 1%), whereas the crude extract did not affect cell viability at the same concentrations [Figure 2]. $SfCHCl_3$ exhibited concentration-dependent cytotoxicity in A549 cell line. The effects of this fraction in this cell line were comparable to the cytotoxic activity of cisplatin, which also shows a concentration-dependent effect [Figure 2].

DISCUSSION

Sixty-eight compounds were identified in the GC-MS analysis of $SfCHCl_3$. All of them were identified for the first time in *S. filiforme* that grows in Cuban coastal zones. Among the total components detected, FA resulted in the most abundant reaching a value of 70%. Accordingly, palmitic acid was the main metabolite found in *S. isoetifolum*.[10]

Non-polar fractions of some species of seagrasses have been studied by GC–MS. In this sense, the analysis of chemical fractions purified from *Halophila ovalis* R. Br. Hooke (Hydrocharitaceae) revealed a high content of 9-octadecenoic (oleic) acid (27.01%), hexadecanoic (palmitic) acid (21.63%), and octadecanoic (stearic) acid (10.42%) in one of these fractions; while, benzoic acid (11.11%), tetradecanoic acid (6.12%), and hexadecane (3.47%) were found the prevalent components in other non-polar fractions.[32]

Audra *et al.* studied the composition of non-polar fractions of three seagrasses, *Halodule wrightii* (shoal grass), *S. filiforme*, and *T. testudinum*, all plants analyzed contained less than 1% lipid (wet weight) and more than 75% moisture.[33] Freshwater plants lipids were composed of monoacylglycerols, phosphatidylserine, and/or phosphatidylethanolamine. These were the major lipid classes for the seagrasses, along with phosphatidylcholine. The main FA in all plants analyzed included palmitic, linoleic, and linolenic acid, with lesser amounts of palmitoleic and oleic acid. This research demonstrated that

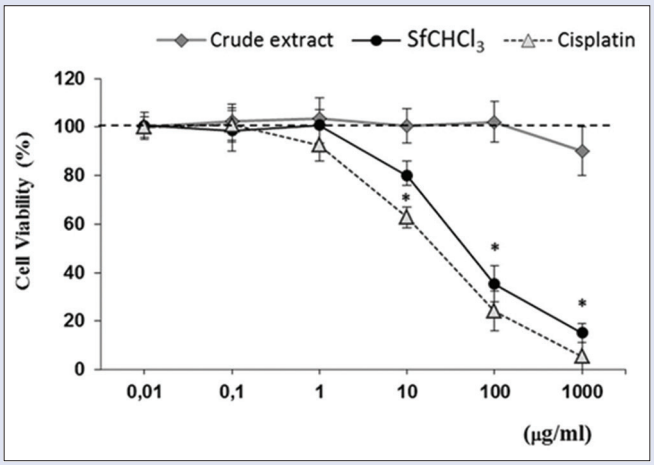

Figure 2: Cytotoxic activity of the chloroform fraction and the crude hydroethanolic extract of *S. filiforme* in A549 cells. Cell viability of A549 cells after 48 h of exposure with different concentrations of Cisplatin, the crude extract and the chloroform fraction of *S. filiforme* was evaluated by MTT reduction assays. The Figure shows the concentration-response curve for the cytotoxicity of the three products from 0.01 to 1000 µg/ml in comparison to untreated control cells and cells treated with *dimethyl sulfoxide* 1% (vehicle control). The values are shown as mean percentages of control from three independent experiments. bars, standard deviation. *$P < 0.05$ and ***$P < 0.001$ versus control (untreated cells)

Table 1: Chemical composition of chloroform fraction from crude extract of *Syringodium filiforme*

Tr (min)	Compound	Percentage	Tr (min)	Compound	Percentage
8.281	3-Hydroxybutyric acid	0.31	14.462	Palmitoleic acid (C16:1)	0.22
8.551	Dihydro-4,4-3-hydroxy-2 (3H) furanone	0.07	14.504	Palmitoleic acid (C16:1)	3.04
8.824	4-Methylvaleric acid	0.14	14.596	Palmitoleic acid (C16:1)	3.21
9.017	Benzoic acid	0.30	14.690	Palmitic acid (C16:0)	39.18
9.117	Octanoic acid (C8:0)	0.20	15.101	2,4,6-Trimethyldecane	0.17
9.183	2,3-Butanediol	0.17	15.184	Methyldecanoate	0.22
9.245	Glycerol	0.63	15.407	Heptadecenoic acid (C17:1)	0.31
9.420	Phenylacetic acid	0.09	15.510	Margaric acid (C17:0)	0.33
9.594	2-Methyl-thiocyclohexene	0.16	15.553	Caffeic acid	0.05
9.897	2-Ethoxyphenol	0.13	15.687	Ethyllinoleate (C18:2et)	0.32
9.995	4-Hydroxybenzaldehyde	0.31	15.752	Ethyllinolenate (C18:3et)	0.35
10.483	Decanoic acid (C10:0)	0.24	15.807	3,7,11,15-Tetramethyl-2-hexadecenol	0.61
10.808	Unsaturated fatty acid	0.23	15.979	Nonadecane (C19)	0.64
11.060	Nonenoic acid (C19:1)	0.14	16.139	Linoleic acid (C18:2)	3.33
11.177	1-Dodecanol ($C_{12}OH$)	0.43	16.185	Oleic acid (C18:1)	3.54
11.415	3-Methylnonadecanoic acid	0.10	16.207	α-Linolenic acid (C18:3)	3.63
11.505	NI	0.43	16.397	Stearic acid (C18:0)	2.19
11.611	*p*-Hydroxybenzoic acid	2.57	16.881	Tricosane (C23)	1.93
11.716	Lauric acid (C12:0)	3.16	17.194	12-Methyloctadec-11-enoic acid	0.23
11.741	3-Hydroxycapric acid	0.90	17.784	Tetracosane (C24)	1.81
12.034	Suberic acid	0.44	18.177	Eicosanoic acid (C20:0)	0.07
12.111	3-Hydroxybenceneacetic acid (D)	0.19	18.346	1-Heneicosanol ($C_{21}OH$)	0.26
12.402	Gluconic acid (D)	0.13	18.684	Pentacosane (C25)	1.45
12.457	1-Tetradecanol ($C_{14}OH$)	0.12	19.201	*bis*-(2-Ethylhexyl) phtalate	3.41
12.543	*p*-Hydroxy-3-methoxybenzoic acid	0.14	19.571	Heptacosane (C27)	1.53
12.725	Azelaic acid	5.06	20.445	Octacosane (C28)	0.72
12.749	Benzoic acid (D)	1.12	21.296	2,6-Dimethylheptacosane	0.55
12.839	NI	0.89	22.130	2-Methyloctacosane	0.25
12.955	3,4-Dihydroxybenzoic acid	0.09	22.940	Nonacosane (C29)	0.27
13.033	4-Hydroxybutanoic acid (D)	1.58	23.732	Hentriacontane (C31)	0.19
13.082	Myristic acid (C14:0)	2.14	25.420	Stigmasterol	0.13
13.445	Sebasic acid	0.30	25.847	β-Sitosterol	0.14
13.552	Methyltetradecanoate (C14:Omet)	0.29	27.242	Triterpenoid	0.38
13.843	Pentadecanoic acid (C15:0)	0.47	27.867	Quercetin (D)	0.13
14.218	Ethylhexadecanoate (C16:0et)	1.12	31.426	Flavonoid	0.18

NI: Nonidentified; Tr: Retention time

Table 2: Antibacterial activity of the crude extract and chloroform fraction of *Syringodium filiforme*

Product	MIC (mg/mL)				
	***Staphylococcus aureus* (ATCC 6538)**	***Pseudomonas aeruginosa* (ATCC 9027)**	***Escherichia coli* (ATCC 10576)**	***Candida albicans* (ATCC 10231)**	***Salmonella typhi* (ATCC 9992v)**
Crude extract	47.7±0.58	NE	38.1±0.75	190.5±0.49	NE
SfCHCl$_3$	0.7±0.35	0.7±0.29	1.5±0.67	1.5±0.87	0.7±0.74
Streptomycin			0.005±0.25		

MIC: Minimum inhibitory concentration; NE: No effect

lipid and FA distributions for seagrasses and freshwater plants, collected in local Florida waters, are similar to those found in other parts of the world. Similarly, the chemical composition of Indian seagrasses (*Enhalus acoroides; Thalassia hemprichii; Halodule pinifolia; S. isoetifolium; C. serrulata* and *C. rotundata*) were determined by GC–MS.[10] In *C. rotundata,* FA was the main phytoconstituents, being palmitic acid the main metabolite. Particularly, *S. isoetifolium* yielded seven compounds: palmitic acid (42.88%), 9-octadecanoic acid (*Z*)-methyl ester (24.04%), 3,7,11,15-tetramethyl-2-hexadecan-1-ol (24.04%), oleic acid (1.51%), tridecanoic acid methyl ester (1.61%), cyclopentaneundecanoic acid methyl ester (3.29%), and 13-octadecenal (3.29%).

Particularly, *S. isoetifolium* yielded seven compounds: palmitic acid (42.88%), Methyl octadecenoate (24.04%), 3,7,11,15-tetramethyl-2-hexadecan-1-ol (24.04%), oleic acid (1.51%), methyl tridecanoate (1.61%), methyl cyclopentaneundecanoate (3.29%), and 13-octadecenal (3.29%).

It is interesting to point out the presence of a high concentration (5%) of azelaic acid in *S. filiforme*. This saturated dicarboxylic acid is reported to be produced by the symbiotic fungus *Pityrosporum ovale* present in human skin and commonly found in terrestrial plants such as *Hordeum vulgare* (barley), *Secale cereale* (rye), some species of *Triticum* genus (wheat) and in some well-characterized angiosperms such as *Arabidopsis thaliana*,[34-37] but it is not abundant in marine organisms. Azelaic acid has been detected in marine aerosols, as a result of photooxidation of FAs produced by phytoplankton,[38] and there is one reference of a 3% content of this acid in the same studied fractions of the macroalgae *Ulva lactuca*,[28] and in the marine species *T. testudinum* but in lower concentration (1%).[12]

Other compounds with around 7% of contribution in this chloroform fraction were hydrocarbons, being tricosane (1.93%), tetracosane (1.81%), pentacosane (1.45%), and heptacosane (1.53%) the most abundant. Some of them have also been detected in other seagrass

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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