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Structural and cytotoxic characterization of the marine red algae *Sarconema filiforme* and *Laurencia obtusa*

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ABSTRACT

Rhodophyta (red algae) has been recognized as a rich source of natural compounds with a wide range of medicinal and biological properties, including anti-oxidants, anti-proliferative, anti-tumor, anti-viral, and anti-coagulant properties. The main goal of this study is to structurally characterize and assess cytotoxicity of both ethanolic and chloroform extracts of *Sarconema filiforme and Laurencia obtusa* red seaweed collected from the Suez Canal coasts, Egypt. Extracts of *S. filiforme and L. obtusa* were prepared and characterized using GC/MS analysis and MTT assay was used to examine their toxicity against human breast cancer (MCF-7 and MDA-MB-231 cells) and lung adenocarcinoma (A549 cells) cell lines. Using GC/MS analysis, a total of 34 and 29 different compounds were identified in the extracts of *S. filiforme* and *L. obtusa*, respectively. The identified components include alkanes, phenol derivative, benzoic acid esters, fatty acids, fatty acid esters, terpenes, fatty acyls, steroids, and prostaglandin. The most abundant constituents in both algae species are cholesterol and diisooctyl phthalate. The in vitro assays revealed a mild to moderate cytotoxicity of the two crude extracts of both species on MCF-7, MDA-MB-231, and A549 cells. Based on GC/MS analysis, it is obvious that *S. filiforme* and *L. obtusa* extracts contain various biologically active compounds with potential antimicrobial, antioxidant, antiviral, anti-inflammatory and antitumor activities. Further bioguided fractionations are recommended to identify and isolate antitumor active compounds of both extracts.

INTRODUCTION

Marine environment have always been a valuable source of natural therapeutic agents throughout human history. The ocean covers almost 70% of the earth's surface and is home to a diverse range of ecosystems. It has around 97% of the world's water and 50% of all biodiversity **(Yue *et al.*, 2017)**. However, only approximately 5% of the ocean has been examined **(www. water.usgs.gov)**; in fact, there is a tremendous amount of important aquatic biological and chemical resources that have yet to be resolved **(Fan *et***

***al.*, 2018)**. Due to the uniqueness of the marine environment, marine species have evolved a variety of biochemical and physiological systems to help them thrive in this dynamic and competitive environment. As a result, maritime flora and animals include a wide range of chemicals with various biological activity and possible health advantages **(Yue *et al.*, 2017)**.

Green algae (Chlorophyta), red algae (Rhodophyta), and brown algae (Phaeophyta) are macroscopic, multicellular creatures that are popularly known as seaweeds **(Mickymaray and Alturaiki, 2018)**. Global seaweed production has recently increased significantly which over 291 species utilized for food, paper, fertilizer, medicinal, and industrial product purposes **(Susanto *et al.*, 2019)**. This could be due to the growing interest in seaweeds as nutraceuticals, functional foods, cosmetics, and medications due to their unique nutrients and bioactive components **(Susanto *et al.*, 2019)**.

Due to extreme climatic and environmental stress, such as salinity, light, temperature, and marine chemical diversions, seaweeds produce many novel secondary metabolites (polysaccharides, phycobilins, sterols, tocopherols, terpenes, polyphenols, and phycocyanins) in addition to the primary metabolites (protein, fiber, vitamins, minerals, and polyunsaturated fatty acids) required for normal growth **(Murphy *et al.*, 2014)**. Anti-obesity, anti-diabetes, anti-cancer, antimicrobial, antiviral, antioxidant, anti-inflammatory, and cardioprotective actions are among the possible benefits of these bioactive substances for human health **(Robertson *et al.*, 2015; Cardoso *et al.*, 2015; Chater *et al.*, 2015; Sharifuddin *et al.*, 2015; Sharifuddin *et al.*, 2015; Shannon and Abu-Ghannam, 2016; Gheda *et al.*, 2016; de Alencar *et al.*, 2016; Gutiérrez-Rodríguez *et al.*, 2018; Admassu *et al.*, 2018; Alves *et al.*, 2018; Circuncisão *et al.*, 2018; Gómez-Guzmán *et al.*, 2018; Seca and Pinto, 2018; Viera *et al.*, 2018)**. It's more important to mention that seaweed composition varies greatly (even within the same species), with many chemicals being unique to a particular group. For example, brown seaweeds are the only ones that contain fucoxanthin, and only green seaweeds contain ulvan (a sulfated polysaccharide) **(Murphy *et al.*, 2014)**.

Seaweeds such as red algae (Rhodophyta) are rich in polysaccharides (floridean starch and sulfated galactans such as carrageenans, proteins and derived peptides (phycobiliproteins, phycolectins, and mycosporine-like amino acids), minerals, and other valuable compounds such as polyphenols and lipids **(Cian *et al.*, 2015; Dhanalakshmi and Jayakumari, 2018)**. Traditional uses of red seaweed include eating the whole algae, while agars and carrageenans are extracted for culinary, medicinal, and biotechnological reasons **(Torres *et al.*, 2019)**.

The shore of the Red Sea has a long coastline with a large variety of marine organisms. Of the Rhodophyta, the species *S. filiforme* and *L. obtusa* are both marine red algae in the class Florideophyceae under the phylum Rhodophyta. In rats, *S. filiforme* reduced symptoms of metabolic disorders and inflammation caused by high-carbohydrate and fat diet **(Du Preez *et al.*, 2020)**.

Laurencia is found in tropical water across the world and is responsible for almost half of all red algal chemicals identified to date. It is one of the most researched red algae. *Laurencia* has a total of 421 species that are currently known. In addition to sesquiterpenes and diterpenes, this species produces acetogenins **(Zaleta-Pinet *et al.*, 2014; Guiry and Guiry, 2015)**. *Laurencia* is the world's chemically most complex seaweed genus because it produces the most secondary metabolites and the greatest variety of secondary metabolites. There are a wide variety of biological activities associated with the *Laurencia* family. These activities vary from antibacterial, insecticidal, antifungal, antiviral activity, anti-inflammatory, antiproliferative, antifouling, antifeedant, cytotoxic, ichthyotoxic, to insecticidal properties **(Shaaban *et al.*, 2021)**.

Despite the availability of seaweeds on the Egyptian coasts, few systematic studies investigated both the structural analysis and biological activity of the red seaweed *S. filiforme* and *L. obtusa*. Therefore, the purpose of this study was to assess GC/MS analysis of these species and study their cytotoxic effect on human breast and lung cancer cell lines MDA-MB-231, MCF-7, and A549.

MATERIALS AND METHODS

1. Chemicals and reagents

Dulbecco's modified Eagle's medium (DMEM), RPMI-1640 medium L-Glutamine, phosphate buffered saline (PBS) and antibiotic (penicillin/streptomycin) were purchased from Biowest (Maine et Loire, France); while fetal bovine serum (FBS) was purchased from Seralab (UK, cat# EU-000-H). Sigma (St. Louis, MO, USA) provided the trypsin, while Amresco provided the dimethyl sulfoxide (DMSO) (Solon, OH, USA). MTT is obtained from Lonza Verviers SPRL Belgium. Ethanol and chloroform were purchased from Fisher Scientific, United Kingdom.

2. Sampling and identification of algal species

Two algal species were sampled from the subtidal and intertidal zones of Fayed coasts, Suez Canal, during September (Egypt; 2018). Fresh samples were washed in seawater to remove encrusting material and then thoroughly cleaned with running water to get rid of excess salt. The samples were identified as *Laurencia obtusa* (Hudson) Lamouroux and *Sarconema filiforme* (Sonder) Kylin by Dr. Nehal Osman using the marine herbarium of Botany Department, Faculty of Science, Suez Canal University, (Ismailia, Egypt) **(BØrgesen, 1935; Jaasund, 1976; Aleem, 1978)**. The samples were air-dried in shade, grinded, packaged in airtight containers and kept for further use.

3. Preparation of algal extracts

For extraction, 100gm of each grinded sample was separately percolated in 95% ethanol and chloroform, putted in a shaking incubator at 25^{o}C overnight and then the

extract was harvested. The process was repeated 3 times or till the solvent extracts became clear and then it was combined. The extracts of each alga were then filtered via Whatman No: 4 filter paper and evaporated till dryness under reduced pressure vacuum at a maximum temperature of 35 °C (**Awaad *et al.*, 2017**). The crude extracts were weighed and a stock solution of them was prepared in phosphate buffered saline (PBS) at a concentration of 50 mg/ml and kept at -20 °C until further use. The algal extracts were diluted in PBS to make working solution of 2000 µg/ml, and then various concentrations were made by further diluting in complete medium to make final concentrations of 125, 250, 500, and 1000 µg/ml for each algal extract.

4. Gas Chromatography-Mass Spectroscopy (GC-MS) analytical conditions of algal extracts

Using Ultra Gas Chromatographs (Thermal Scientific Corp., USA) and a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer), GC-MS analysis of ethanolic and chloroform extracts from *S. filiforme* and *L. obtusa* was carried out. In addition, a TR-5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness) was used in the GC-MS system. The following temperature program was used to conduct analyses using helium as the carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10: 60°C for 1 minute; then 240°C for 1 minute at a rate of 4.0°C/min. At 210°C, the injector and detector were maintained. Always, diluted samples (1:10 hexane, v/v) of 1 µl of the mixtures were injected. Electron ionization (EI) at 70 eV01 was used to generate mass spectra with a spectral range of m/z range of 40-450. GC had a total running duration of 25 minutes.

5. Chromatographic and mass spectroscopic data for identification of components

The GC retention durations, percentage composition (peak area %), and retention indices of the two algal extracts impacted the identification of their chemical components. The chemical components of the algal extracts were identified by their retention indices (relative to n-alkanes C_8-C_{22}), mass spectrum matching to authentic standards (where available), Wiley spectral library collection, and NSIT library database using AMDIS software (www.amdis.net). To determine the known pharmacological characteristics associated with these compounds, empirical searches were done using the PubChem Project (https://pubchem.ncbi.nlm.nih.gov/) and previously published data on marine algae.

6. Cell lines and cell culture

The human cell lines MDA-MB-231, MCF-7, were obtained from VACSERA, Egypt, while A549, and MDCK were gift from Centre of Experimental and Clinical Infection research, Germany. A human triple negative and luminal A breast cancer cell lines MDA-MB-231 and MCF-7 respectively, were propagated in RPMI-1640 medium L-Glutamine, while human lung cancer cell line (A549) and normal Madin-Darby Canine

Kidney (MDCK) cell line were propagated in DMEM medium High Glucose, both media were supplemented with 10% FBS and 1% antibiotic. The cells were incubated in 5% CO_2 humidified at 37°C for growth. Every 2 to 3 days, the culture was sub-cultured and any contamination was carefully examined under an inverted microscope.

7. Evaluation of cell proliferation by MTT assay

The anti-proliferative effects of the different algal extracts on the viability of human cell lines (MCF-7, MDA-MB-231, and A549) were evaluated using MTT assays. This cytotoxicity test is based on the reduction of the substrate (MTT) by cellular dehydrogenase to produce water insoluble formazan so the treated sets had lower color intensity than the untreated ones **(Paul and Kundu, 2013)**. The colorimetric MTT (3-[4, 5-methylthiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide) test was slightly modified to assess the cell growth inhibitory efficacy on cancer cell lines **(Safi *et al.*, 2016)**. The cancer cell lines under investigation were treated with range of concentrations (125, 250, 500, and 1000 µg/ml) of algae extracts dissolved in PBS for 48 hrs. Briefly, following cell count and viability were determined using trypan blue dye; cancer cells ($1x10^4$ cells/well) were seeded in a 96-well plate and left to adhere for 24 hrs. The media were changed in the next day with new media containing the specified concentrations of the tested algal extracts, and the cells were incubated for another 48 hrs. After the incubation period, adding 10 µl of MTT (5 mg/mL PBS) in each well and the plates were incubated for 4 hrs. After completing the incubation, 100 µl of DMSO was introduced to each well, gently mixed using the pipette and then the plates were incubated for 10 minutes at room temperature in order to precipitate formazan crystals. A Bio-Tek microplate reader was used to estimate the number of live cells by measuring the optical density at 490 nm. The tests were carried out in triplicate.

The following formula was used to compute data as a percentage of cell viability: % cell viability = (Mean absorbance in tested wells / Mean absorbance in control wells) x 100.

For each tumor cell line, the relationship between surviving cells and concentration of extract is plotted to produce the survival curve after treatment **(Kameyama *et al.*, 2005)**. As a result of graphic plots of dose response curves, Graphpad Prism software was used to determine the 50 % inhibitory concentration (IC_{50}), the quantity necessary to induce harmful effects in 50 % of intact cells.

Statistical analysis

All results were presented as mean ± SEM. Graphpad Prism software, version 7, was used to compare means using a Student t-test followed by a one-way analysis of variance. Differences at $p < 0.05$ were considered statistically significant.

RESULTS

1. GC-MS analysis

Quality control analysis in the pharmaceutical and food product sectors is one of the many analytical applications for GC-MS analysis. This approach may be used to detect drugs, analyze the environment and identify unknown materials **(Bajwa *et al.*, 2016)**. The present data found some important compounds in the extracts of *S. filiforme* and *L. obtusa* by GC-MS analysis. The complete lists of compounds with their retention times (RT), molecular formula, molecular weight (MW), peak area percentage (PA%) and matching factor (MF) are presented in **Tables 1- 4**.

1.1. GC-MS analysis of S. filiforme extracts

The GC-MS analysis of *S. filiforme* ethanolic extract **(Fig.1 and Table 1)** showed that the major constituents were cholesterol, which appear at peak 16.76 (PA 19.11%), diisooctyl phthalate at Peak 13.46 (PA 15.67%), oleic acid at peak 9.25 (PA 10.91%), ethyl oleate at peak 11.10 (PA 8.61%), 2,4-ditert-butylphenol at peak 5.71 (PA 5.02%), phthalic acid, butyl tridecyl ester at peak 9.56 (PA 4.09%) and prostaglandin A1-biotin at peak 21.35 (PA 4.02%). On the other hand, the predominant compounds in chloroform extract of *S. filiforme* are diisooctyl phthalate (PA 41.62%), cholesterol (PA 11.01%), hexadecyl phenyl carbonate (PA 9.94%), octadecyl phenyl carbonate (PA 5.48%), 3-hydroxypyridine, erucylamide and heptadecane (PA 4.70%, 4.47 and 4.33% respectively) as shown in **Fig. 1 and Table 2**. While the rest compounds had less than 4% composition by peak area in both extracts.

1.2. GC-MS analysis of *L. obtusa* extracts

In addition, the highest PA% for the detected compounds in the ethanolic extract of *L. obtusa* **(Fig. 2 and Table 3)** was 22.03%, and 16.12% for cholesterol and diisooctyl phthalate respectively followed by oleic acid (PA 8.37), phthalic acid, hex-3-yl isobutyl ester (PA 8.37%), 2,4-ditert-butylphenol (PA 6.56), ethyl hexadecanoate, dotriacontane, butyl 8-chlorooctyl phthalate and 1H-purin-6-amine, [(2-fluorophenyl) methyl]-, 9,18-diphenyl tetra benz [A,C,H,J] anthracine (PA 5.42%, 5.40%, 5.32%, 4.80% and 4.38% respectively). On the other hand, the major constituents in the GC-MS analysis of chloroform extract of *L. obtusa* **(Fig. 2 and Table 4)** were diisooctyl phthalate (PA 54.25%), cholesterol (PA 9.55%), didecan-2-yl phthalate (PA 5.61%), octadecyl phenyl carbonate (PA 5.44%), heptacosane (PA 5.32%), phthalic acid, hex-3-yl isobutyl ester (PA 4.89%) and methyl 2-(acetyloxy)-10-(2-hexylcyclopropyl) decanoate (PA 4.0%). While the rest compounds had less than 4% composition by peak area in both extracts. Among the 50 compounds obtained through GC-MS study from all extracts, 18 compounds have been reported to show significant biological activities as listed in **Table**

5, and their chemical structures in **Fig. 3**. These compounds are responsible for various pharmacological actions of both algae extracts.

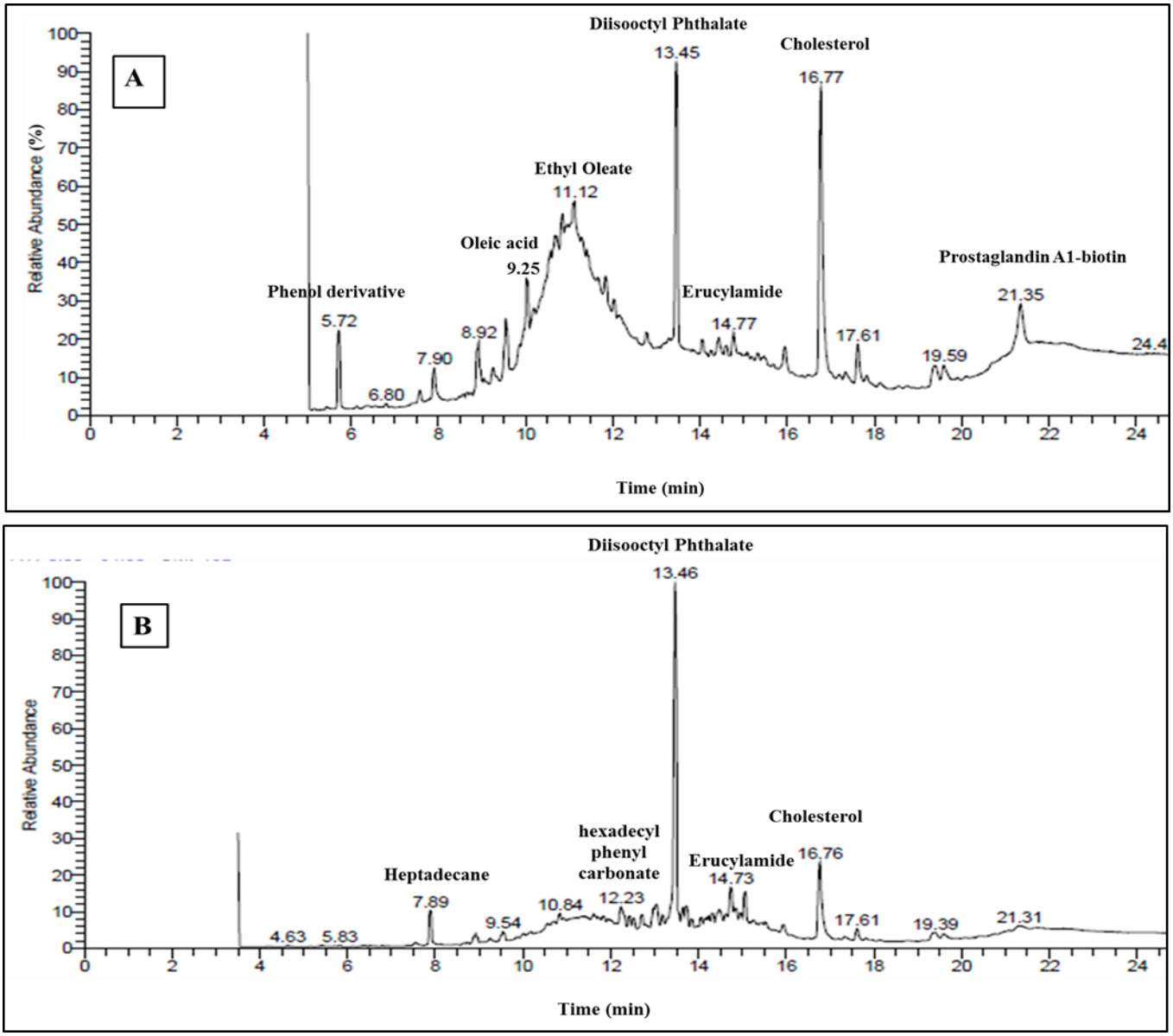

Fig. 1 GC-MS Chromatograms of both crude extracts of *S. filiforme* (Ethanolic (A) and Chloroform (B)) showing relative abundance and retention time of active compounds (using ultra gas chromatographs coupled with a single quadrupole mass spectrometer). The Wiley spectral library collection and NSIT library database were used for the identification of the chemical components.

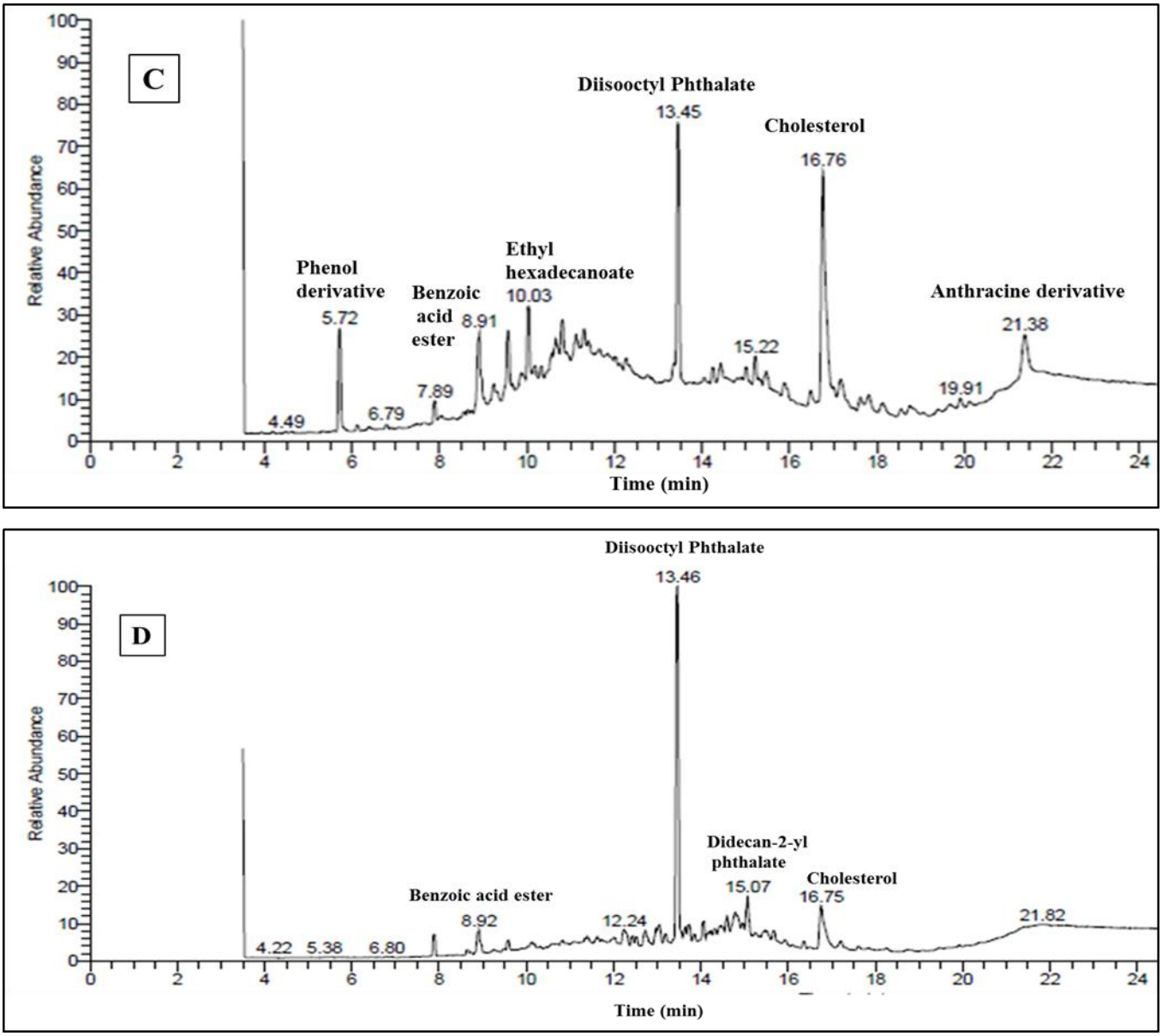

Fig. 2 GC-MS Chromatograms of both crude extracts of *L. obtusa* (Ethanolic (C) and Chloroform (D)) showing relative abundance and retention time of active compounds (using ultra gas chromatographs coupled with a single quadrupole mass spectrometer). The Wiley spectral library collection and NSIT library database were used for the identification of the chemical components.

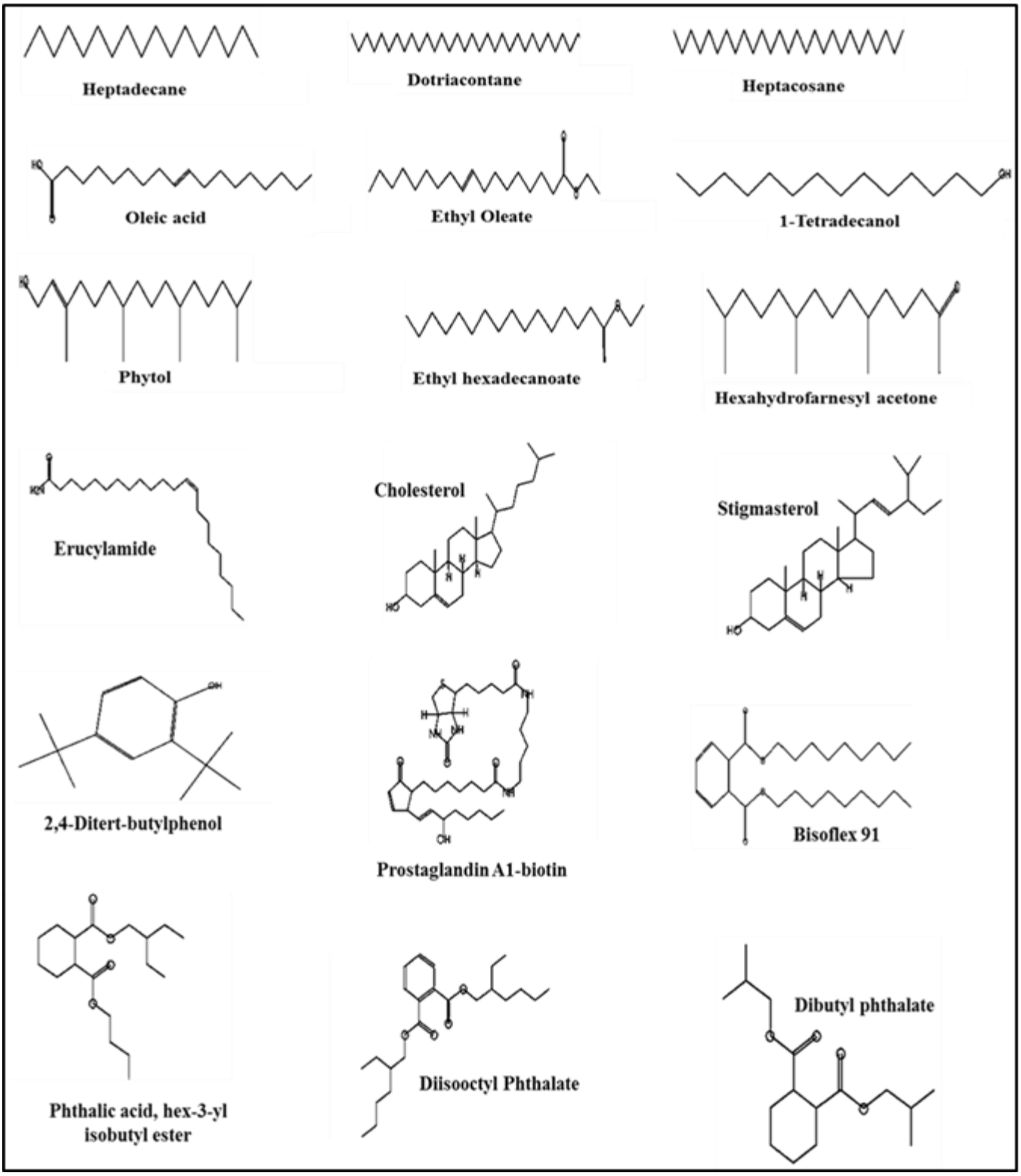

Fig. 3 Chemical structure of bioactive compounds detected in the ethanolic and chloroform crude extracts of *S. filiforme* and *L. obtusa*.

Table 1. GC-MS identified components in the ethanolic crude extract of *S. filiforme* (Compounds are listed in ascending order of retention time)

S.no	RT (min)	Compound Name	Chemical Formula	MW	PA (%)	MF
1	5.71	2,4-Ditert-butylphenol	$C_{14}H_{22}O$	206	5.02*	954
2	7.56	1-Tetradecanol	$C_{14}H_{30}O$	214	0.86	895
3	7.89	2-Dodecoxyethanol	$C_{14}H_{30}O_2$	230	2.49	891
4	8.89	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	3.67	883
5	9.25	9-Octadecenoic acid (Z)- **(Oleic acid)**	$C_{18}H_{34}O_2$	282	10.91*	707
6	9.56	Phthalic acid, butyl tridecyl ester	$C_{25}H_{40}O_4$	404	4.09*	914
7	10.03	Palmitic acid, ethyl ester **(Ethyl hexadecanoate)**	$C_{18}H_{36}O_2$	284	3.93	854
8	10.18	Tricosane	$C_{23}H_{48}$	310	0.79	660
9	10.83	Phytol	$C_{20}H_{40}O$	296	1.95	841
10	11.10	9-Octadecenoic acid (Z)-, ethyl ester **(Ethyl Oleate)**	$C_{20}H_{38}O_2$	310	8.61*	855
11	11.42	Dotriacontane	$C_{32}H_{66}$	450	1.25	726
12	11.84	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	1.57	664
13	13.46	Diisooctyl Phthalate	$C_{24}H_{38}O_4$	390	15.67*	907
14	14.06	Docosane	$C_{22}H_{46}$	310	1.56	679
15	14.41	1,2-Benzenedicarboxylic acid, dinonyl ester **(Bisoflex 91)**	$C_{26}H_{42}O_4$	418	1.73	786
16	14.76	13-Docosenamide, (Z)- **(Erucylamide)**	$C_{22}H_{43}NO$	337	1.93	785
17	15.32	Dimethoxycurcumin	$C_{23}H_{24}O_6$	396	0.95	688
18	15.44	6,8-Di-C-á-Glucosylluteolin	$C_{27}H_{30}O_{16}$	610	0.96	729
19	15.94	2-Dodecen-1-yl(-) succinic anhydride	$C_{16}H_{26}O_3$	266	1.81	723
20	16.76	Cholesterol	$C_{27}H_{46}O$	386	19.11*	858
21	17.33	Cholest-5-en-3-ol (3á)-	$C_{27}H_{46}O$	368	0.97	606
22	17.61	9-Octadecenylsuccinic acid	$C_{22}H_{40}O_4$	368	2.75	677
23	19.39	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	3.40	701
24	21.35	Prostaglandin A1-biotin	$C_{35}H_{58}N_4O_5S$	646	4.02*	891
		Total Percentage Composition			**100**	

RT: Retention time of the identified compound; **PA:** Peak area; **MW:** Molecular weight of the identified compound; **MF:** Matching factor, which indicates the structural similarity. The accepted values of MF range > 600. (*Denotes the crude extract with highest composition of that compound).

Table 2. GC-MS identified components in the chloroform crude extract of *S. filiforme* (Compounds are listed in ascending order of retention time)

S.no	RT (min)	Compound Name	Chemical Formula	MW	PA (%)	MF
1	7.89	Heptadecane	$C_{17}H_{36}$	240	4.33*	923
2	8.89	1,2 Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	1.22	913
3	8.94	2-Pentadecanone, 6,10,14-trimethyl **(Hexahydrofarnesyl acetone)**	$C_{18}H_{36}O$	268	1.20	872
4	9.57	(3R*,4S*)-3-(2-nitro-4 methoxyphenyl)-4-(4 hydroxyphenyl)hexane	$C_{19}H_{23}NO_4$	329	1.93	962
5	10.83	Phytol	$C_{20}H_{40}O$	296	1.37	841
6	12.23	Hexadecyl phenyl carbonate	$C_{23}H_{38}O_3$	362	9.94*	861
7	12.52	Hexanedioic acid, mono (2-ethylhexyl) ester	$C_{14}H_{26}O_4$	258	1.70	804
8	12.71	3-Hydroxypyridine	C_5H_5NO	95	4.70*	957
9	13.47	Diisooctyl Phthalate	$C_{24}H_{38}O_4$	390	41.62*	907
10	13.84	Octadecyl phenyl carbonate	$C_{25}H_{42}O_3$	390	5.48*	860
11	14.49	Spiro[anthracene-1(4h),1'-cyclopropane]-9,10-dione, 4-ethoxy-4a,9a-dihydro-4a-methyl-, (4à,4Aá,9Aá)-(.+-.)-	$C_{19}H_{20}O_3$	296	2.68	987
12	14.73	13-Docosenamide, (Z)- **(Erucylamide)**	$C_{22}H_{43}NO$	337	4.47*	765
13	14.85	Phthalic acid, bis(7-methyloctyl) ester	$C_{26}H_{42}O_4$	418	2.64	831
14	15.06	Didecan-2-yl phthalate	$C_{28}H_{46}O_4$	446	3.78	866
15	16.75	Cholesterol	$C_{27}H_{46}O$	386	11.01*	861
16	17.61	9-Octadecenylsuccinic acid	$C_{22}H_{40}O_4$	368	1.93	690
		Total Percentage Composition			**100**	

RT: Retention time of the identified compound; **PA**: Peak area; **MW**: Molecular weight of the identified compound; **MF**: Matching factor, which indicates the structural similarity. The accepted values of MF range > 600. (*Denotes the crude extract with highest composition of that compound).

Table 3. GC-MS identified components in the ethanolic crude extract of *L. obtusa* (Compounds are listed in ascending order of retention time).

S.no	RT (min)	Compound Name	Chemical Formula	MW	PA (%)	MF
1	5.71	2,4-Ditert-butylphenol	$C_{14}H_{22}O$	206	6.56*	933
2	7.89	Heptacosane	$C_{27}H_{56}$	380	1.03	740
3	8.89	Phthalic acid, hex-3-yl isobutyl ester	$C_{18}H_{26}O_4$	306	7.27*	937
4	9.23	26,27-Dinorergost-7-en-6-one, 3-(formyloxy)-14,24-dihydroxy-, (3á,5á)-	$C_{27}H_{42}O_5$	446	1.13	730
5	9.31	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312	0.45	737
6	9.57	Butyl 8-chlorooctyl phthalate	$C_{20}H_{29}ClO_4$	368	5.32*	911
7	9.86	9-Octadecenoic acid (Z)- (Oleic Acid)	$C_{18}H_{34}O_2$	282	8.37*	752
8	10.03	Ethyl hexadecanoate (Palmitic acid, ethyl ester)	$C_{18}H_{36}O_2$	284	5.42*	875
9	10.17	Dotriacontane	$C_{32}H_{66}$	450	5.40*	768
10	10.33	1H-purin-6-amine, [(2-fluorophenyl) methyl]-	$C_{12}H_{10}FN_5$	243	4.80*	709
11	11.10	Ethyl oleate	$C_{20}H_{38}O_2$	310	1.60	775
12	11.29	3',4',7-Trimethylquercetin	$C_{18}H_{16}O_7$	344	1.09	732
13	12.25	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	$C_{27}H_{42}O_4$	430	0.49	660
14	13.45	Diisooctyl Phthalate	$C_{24}H_{38}O_4$	390	16.12*	906
15	14.44	6,8-Di-C-á-Glucosylluteolin	$C_{27}H_{30}O_{16}$	610	2.31	731
16	15.21	2-Phenyl-4,5-di (methoxy carbonyl)-3,6-heptanopyridine	$C_{22}H_{25}NO_4$	367	1.51	897
17	15.49	11-Octadecenal	$C_{18}H_{34}O$	266	1.05	738
18	15.94	cis-9-Tetradecenoic acid, heptyl ester	$C_{21}H_{40}O_2$	324	0.60	736
19	16.48	Stigmasterol	$C_{29}H_{48}O$	412	1.05	729
20	16.75	Cholesterol	$C_{27}H_{46}O$	386	22.03*	879
21	17.16	Ergosta-7,22-Dien-3-ol, (3á,22E)-	$C_{28}H_{46}O$	398	2.02	754
22	21.37	9,18-diphenyl tetra benz [A,C,H,J] anthracine	$C_{42}H_{26}$	530	4.38*	898
		Total Percentage Composition			**100**	

RT: Retention time of the identified compound; **PA**: Peak area; **MW**: Molecular weight of the identified compound; **MF**: Matching factor, which indicates the structural similarity. The accepted values of MF range > 600. (*Denotes the crude extract with highest composition of that compound).

Table 4. GC-MS identified components in the chloroform crude extract of *L. obtusa* (Compounds are listed in ascending order of retention time).

S.no.	RT (min)	Compound Name	Chemical Formula	MW	PA (%)	MF
1	7.89	Heptadecane	$C_{17}H_{36}$	240	3.31	912
2	8.89	Phthalic acid, hex-3-yl isobutyl ester	$C_{18}H_{26}O_4$	306	4.89*	937
3	12.23	Octadecyl phenyl carbonate	$C_{25}H_{42}O_3$	390	5.44*	858
4	12.97	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	2.32	650
5	13.05	Hexadecyl phenyl carbonate	$C_{23}H_{38}O_3$	362	2.86	872
6	13.46	Diisooctyl Phthalate	$C_{24}H_{38}O_4$	390	54.25*	899
7	13.72	3-Hydroxypyridine	C_5H_5NO	95	2.45	999
8	14.06	Heptacosane	$C_{27}H_{56}$	380	5.32*	748
9	14.78	Methyl 2-(acetyloxy)-10-(2-hexylcyclopropyl) decanoate	$C_{22}H_{40}O_4$	368	4.0	719
10	15.06	Didecan-2-yl phthalate	$C_{28}H_{46}O_4$	446	5.61*	884
11	16.76	Cholesterol (Cholest-5-en-3-ol)	$C_{27}H_{46}O$	386	9.55*	858
		Total Percentage Composition		**100**		

RT: Retention time of the identified compound; **PA**: Peak area; **MW**: Molecular weight of the identified compound; **MF**: Matching factor, which indicates the structural similarity. The accepted values of MF range > 600. (*Denotes the crude extract with highest composition of that compound).

Table 5. List of bioactive compounds identified by GC-MS analysis from the ethanolic and chloroform crude extracts of *S. filiforme* and *L. obtusa* with their reported biological activities.

S.no	Compound Name	Compound Class	Reported biological Activities	Reference
1*	2,4-Ditert butyl phenol	phenol derivative	Antioxidant, antifungal, anti-inflammatory, antiviral, antibacterial, insecticidal, nematicidal, antimalarial, **anticarcinogenic,** allelopathy.	(Kusch et al. 2011; Sang &Kim 2012; Rajamani 2018; Zhao et al. 2020)
2	1-Tetradecanol	Fatty acyls (long-chain fatty alcohol)	Antifungal, antibacterial	(Rajamani 2018; Subbaiyan et al. 2014)
3	Dibutyl phthalate	Benzoic acid esters	Antimicrobial, antifungal, antimalarial.	(Khatiwora et al. 2012; Akpuaka et al. 2013)
4*	Oleic acid	Fatty acids	Hypercholesterolemic, dermatitigenic, anti-inflammatory, **anti-tumor**, antiandrogenic, flavor, 5-alpha reductase inhibitor, anemiagenic insectifuge.	(Beulah et al. 2018; Gideon 2015)
5	Ethyl hexadecanoate	Fatty acyls (fatty acid esters)	Antioxidant, lubricant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavor, hemolytic 5-Alpha reductase inhibitor.	(Soosairaj et al. 2016)
6*	Phytol	Diterpene	Lipid metabolism regulator, antiparasitic, antihelmintic, antiprotozoal (Leishmania), histamine release inhibitor, spasmolytic, antimicrobial anti-inflammatory, diuretic, **anticancer,** antioxidant, antifungal active against Salmonella typhi, resistant Gonorrhoea, joint dislocation, headache, hernia, antibacterial, a stimulant and antimalarial.	(Akpuaka et al. 2013; Beulah et al. 2018; Adnan et al. 2019; Dulara et al. 2019)
7	Ethyl Oleate	Fatty acid esters	Perfumery	(Harborne et al. 1994; Setia and Gupta 2004)
8*	Diisooctyl Phthalate	Benzoic acid esters derivative	Antimicrobial, antioxidant, **anticancer,** antifouling.	(Okoro et al. 2019; Beulah et al. 2018)
9*	Bisoflex 91	Benzoic acid esters derivative	Antimicrobial, antifouling	(Beulah et al. 2018)
10	Erucylamide	Fatty acyls (fatty amides)	Antimicrobial, sugar-phosphatase inhibitor, anti-infective, prostaglandin E1 antagonist, antitoxic, anti-inflammatory, intestinal, albendazole monooxygenase inhibitor.	(Okoro et al. 2019; Adnan et al. 2019)
11*	Cholesterol	Cholestane steroid	Antioxidant, antimicrobial, **anticancer.**	(Thilaga et al. 2014; Jamebozorgi et al. 2019; Motallebi 2020)
12	Prostaglandin A1-biotin	cyclopentenone prostaglandins	**Anti-proliferative**	(Dave et al. 2018)

Table (5) to be continued

13	Heptadecane	Alkanes	Antibacterial activity	(Uma and Parvathavarthini 2010)
14	Hexahydrofarnesyl acetone	Organic oxides (Sesquiterpenoid)	Allelopathic, antimicrobial antibacterial activity against Gram +ve and Gram-ve bacteria.	(Yayli et al. 2006; Govindappa et al. 2014)
15	Phthalic acid, hex-3-yl isobutyl ester	Benzoic acid esters	Antimicrobial, antifungal.	(Dulara et al. 2019)
16	Dotriacontane	Alkane	Antimicrobial, antioxidant, antispasmodic.	(Soosairaj et al. 2016)
17	Heptacosane	Alkane	Antibacterial, antioxidant	(Akpuaka et al. 2013; Khatua et al. 2016)
18	Stigmasterol	Stigmastanes (Triterpenoids)	Dermatologic، antiacne, antiinflammatory, anti-protozoal (Leishmania), antisecretoric، bone formation stimulant.	(Adnan et al. 2019)

The activity of the identified compounds was based on Dr. Duke's Phytochemical & Ethnobotanical Databases and previous literature on GC-MS analysis of marine algae. * means anticancer component.

2. Cytotoxicity of *S. filiforme* and *L. obtusa* extracts

MTT assay on MCF-7, MDA-MB-231, and A549 cells was conducted to test the different concentrations of algal extracts (125, 250, 500 and 1000 µg/ml) at 48 hrs incubation period. With a few exceptions, the obtained data showed that the examined algal extracts had a direct cytotoxic impact on the tested cell lines in a concentration-dependent manner, where the number of nonviable cells increased with elevating concentration of all extracts. As a consequence of the study, *S. filiforme* extracts had the lowest cell viability percentage and showed significant cytotoxic activity, followed by *L. obtusa* extracts. The IC_{50} values of ethanolic extract of *S. filiforme* were 750.21, 553.49, and 742.09 µg /ml against MCF-7, MDA-MB-231, A549 cells respectively, while the IC_{50} values of chloroform extract of *S. filiforme* were 589.50, and 782.0 µg /ml against MDA-MB-231, A549 cells respectively **(Table 6)**. More importantly to mention that ethanolic extracts of both algae showed a higher inhibition of cancer cell growth as compared to chloroform extracts. Ethanolic extracts of both algae showed higher content of anticancer compounds from GC-MS analysis compared with that of chloroform extracts.

Table 6. IC_{50} (µg/ml) values of algal crude extracts on MCF-7, MDA-MB-231, A549, and MDCK cell lines.

Algal crude extract	IC_{50} (µg/ml)		
	MCF-7	**MDA-MB-231**	**A549**
EthOH *S. filiforme*	750.21	553.49	742.09
Chf *S. filiforme*	NA	589.50	782.00
EthOH *L. obtusa*	NA	NA	NA
Chf *L. obtusa*	**NA**	**NA**	**NA**

NA: indicates not active.

DISCUSSION

However marine algae have recently attracted a lot of interest for its incredible medicinal potential, few investigations have been conducted on red marine algae. In this communication, we have used a combination of structural (GC-MS analysis) and physiological approaches to characterize two species (*S. filiforme and L. obtusa*) of marine red algae dominant in the Suez Canal.

The extraction of bioactive components from *S. filiforme* and *L. obtusa* was done using ethanol and chloroform solvents. The GC–MS analyses revealed that various bioactive compounds (fifty compounds) were identified in both extracts. According to published database, eighteen of which have various biological activities, while the others have not known biological activities reported so far. The identified components included a wide range of chemical classes, namely alkanes, phenol derivative, benzoic acid esters, fatty acids, fatty acid esters, terpenes (diterpenoids, triterpenoids, sesquiterpenoids), fatty acyls, steroids, and prostaglandin.

GC-MS analysis of both extracts of *S. filiforme* indicated the existence of thirty-four different compounds (6 of which found in both extracts) while GC-MS analysis of both extracts of *L. obtusa* revealed the presence of twenty-nine different compounds (4 of which found in both extracts). Interestingly, the components found in the ethanolic extracts don't differ so much from the components found in the chloroform extracts of both algae species, in which most of their components were alkanes, benzoic acid esters, fatty acids, fatty acid esters, fatty acyls, and steroids.

More compounds were identified in the ethanolic extracts than chloroform extracts of *S. filiforme* (24 vs 16 compounds) and *L. obtusa* (22 vs 11 compounds). Compared to chloroform, ethanol was the most effective solvent for extracting bioactive chemicals. Two common compounds, namely diisooctyl phthalate and cholesterol were detected in all extracts. Four common compounds, namely phytol, erucylamide, bisoflex 91, and 9-octadecenylsuccinic acid were specific to the extracts of S. filiforme only while two common compounds, namely phthalic acid, hex-3-yl isobutyl ester, and heptacosane were specific to L. obtusa extracts only.

It is reported that all tested extracts of both algae species contain many biologically active compounds, such as antimicrobial, antifungal, antioxidant, anti-inflammatory, antiviral and anticancer activity. GC-MS data showed that cholesterol and diisooctyl phthalate were the major components of *S. filiforme* and *L. obtusa* in both extracts which were reported in the previous literature for their antioxidant, antimicrobial, and anticancer activities **(Thilaga *et al.*, 2014; Jamebozorgi *et al.*, 2019; Okoro *et al.*, 2019; Motallebi, 2020)**. This conclusion is similar to that of **Patterson (1971)** and **Rahelivao (2015)** that cholesterol is the major sterol in most of the red algae (Rhodophyta) **(Patterson, 1971; Rahelivao *et al.*, 2015)**.

In addition, GC-MS data showed the presence of phytol, erucylamide, bisoflex 91, and 9-octadecenylsuccinic acid in the extracts of *S. filiforme* only. Phytol, a key acyclic diterpene alcohol, was found to have antibacterial, antiparasitic, antihelmintic, antiprotozoal (Leishmania), anti-inflammatory, anticancer, antifungal and antimalarial activities **(Akpuaka *et al.*, 2013; Beulah *et al.*, 2018; Adnan *et al.*, 2019; Dulara *et al.*, 2019)**. Erucylamide is used as antistatic agent. Erucylamide is utilized in the production of food packaging materials and personal daily care items such as fragrances and detergents as well as lotions, moisturizer, soaps, toothpaste, and talcum powder. It also has biological activities such as antimicrobial, sugar-phosphatase inhibitor, anti-infective, prostaglandin E1 antagonist, antitoxic, and anti-inflammatory activity **(Adnan *et al.*, 2019; Okoro *et al.*, 2019)**. Bisoflex 91 was observed to have antifouling and antimicrobial **(Beulah *et al.*, 2018).**

Laurencia is a chemically diverse genus, containing mostly sesquiterpenoids, diterpenoids, and terpenoids. Sesquiterpenes were isolated from *Laurencia species*, including *L. tristicha*, *L. mariannensis*, *L. similis*, *L. saitoi*, and *L. okamurai* **(Mickymaray and Alturaiki, 2018)**. In the present work, phthalic acid, hex-3-yl isobutyl ester; heptacosane; and stigmasterol (triterpenoid) were only detected in the extracts of L. obtusa. These compounds were known to have various activities like antifungal, antimicrobial, antioxidant, antiacne, and anti-inflammatory activities **(Akpuaka *et al.*, 2013; Khatua *et al.*, 2016, Adnan *et al.*, 2019; Dulara *et al.*, 2019)**. These findings are consistent with previous studies that stated that certain terpenes biosynthesized by *L. obtusaecies* have pharmacologically significant potential due to their antiviral **(Sakemi *et al.*, 1986)**, antibacterial **(Vairappan *et al.*, 2001; Vairappan *et al.*, 2004)**, antimalarial **(Topcu *et al.*, 2003)**, antileishmanial **(Santos *et al.*, 2010)**, antitrypanosomal **(Veiga-Santos *et al.*, 2010)**, anti-inflammatory **(Chatter *et al.*, 2011)** and anti-cancer **(Kladi *et al.*, 2006; Kim *et al.*, 2008; Lhullier *et al.*, 2010)** actions. Moreover, *L. obtusaecies* contains terpenoids with significant anti-epibiosis action, which might be utilised in the production of antifouling paints **(Da Gama *et al.*, 2002; Da Gama *et al.*, 2003; Pereira *et al.*, 2003)**. The sesquiterpene (−)-elatol, for example, was the subject of a Brazilian patent application for its use as an antifouling agent **(White *et al.*, 2008)**.

The ethanolic extracts of *S. filiforme* and *L. obtusa* showed the presence of common compounds such as 2,4-ditert butylphenol (2,4-DTBP), oleic acid, ethyl hexadecanoate, ethyl oleate, dotriacontane, and 6,8-di-C-á-Glucosylluteolin. Many investigations have highlighted 2,4-DTBP's antioxidant and anti-inflammatory properties. More crucially, phenol was hazardous to all tested creatures, including the producers; for example, cytotoxicity in human cells and animal cells were both seen, in addition to its anticancer, antifungal, antimalarial, antimicrobial, phytotoxicities, insecticidal and nematicidal activities **(Kusch *et al.*, 2011; Sang and Kim, 2012; Rajamani, 2018; Zhao *et al.*, 2020)**. Ethyl oleate is used as perfumery agent **(Harborne *et al.*, 1994; Setia and Gupta, 2004)**. The compounds oleic acid, ethyl hexadecanoate, and dotriacontane, have different activities like antitumor, antiandrogenic, antimicrobial, antioxidant, antiacne, and anti-inflammatory activities **(Kumar *et al.*, 2010; Gideon, 2015; Nithya *et al.*, 2015; Beulah *et al.*, 2018)**.

The bioactive compounds 1-tetradecanol, dibutyl phthalate, and prostaglandin A1-biotin are present in the ethanolic extract of *S. filiforme* by GC-MS analysis. The previous studies suggested that 1-tetradecanol has antifungal **(Rajamani, 2018)** and antibacterial **(Subbaiyan *et al.*, 2014)** activities. Prostaglandin A1-biotin has anti-proliferative activity **(Dave *et al.*, 2018)**, while dibutyl phthalate has antimicrobial, antifouling, antifungal, and antimalarial activities **(Khatiwora *et al.*, 2012; Akpuaka *et al.*, 2013)**. However, heptadecane that has antibacterial activity found only in chloroform extracts of both *S. filiforme* and *L. obtusa* **(Uma and Parvathavarthini, 2010)**. Hexahydrofarnesyl acetone, found in chloroform extract of *S. filiforme*, has some activities such as allelopathic, antimicrobial and antibacterial activity against Gram +ve and Gram-ve bacteria **(Yayli *et al.*, 2006; Govindappa *et al.*, 2014)**. The biological activities of these compounds give a strong indication about the pharmacological potential of these algae.

The antiproliferative effect of algal extracts under investigation was evaluated using MTT test on human breast cancer cell lines (MCF-7, and MDA-MB231), and human lung cancer cell line (A549). In general, from the MTT assay it was cleared that cell death of MCF-7, MDA-MB-231, and A549 was concentration dependent, where the number of nonviable cells increased with elevating concentration of all extracts. More importantly to mention that ethanolic extracts of both algae showed a higher inhibition of cancer cell growth as compared to chloroform extracts. Ethanolic extracts of both algae showed higher content of anticancer compounds from GC-MS analysis compared with that of chloroform extracts.

CONCLUSION

Data of the present article showed that the highest number of compounds (twenty four) was deteced in the ethanolic extract of *S. filiforme* followed by ethanolic extract of *L. obtusa* (twenty two), chloroform of *S. filiforme* (sixteen) and chloroform of *L. obtusa* (eleven) which indicated that hydrophilic solvent provided more compounds than

hydrophobic solvent. It can be concluded from this study that the extracts of *S. filiforme* and *L. obtusa* contain many bioactive constituents which have with potential pharmacological properties and should experimentally tolerated. It would be worthwhile for the isolation, identification, characterization and understanding the physiological and pharmacological effect of compounds from these two algae species which might have some importance and societal benefit.

Authors' contributions

AAH, and MAR conceived the idea and design the present study. WKT, MAT, NAH conduct the experiments. WKT wrote the initial draft of this article and analyzed the data. AAH, MAR, MAT reviewed the article and aided in interpreting the results in its coordination. All the authors (WKT, MAT, NAH, MAR, and AAH) contributed to approved the final version of this manuscript.

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