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Chemical Profiling and Biological Activities of *Sargassum angustifolium* Volatile Oil: Antioxidant, Antibacterial and Selective Cytotoxic Properties.

Layla Safar Jebur Khaledi[1] and Haider A. Alghanmi[2]
[1] Department of Biology, College of Education for Pure Science, University of Al-Qadisiyah, Iraq
[2] Department of Biology, College of Education for Pure Science, University of Al-Qadisiyah, Iraq
Corresponding Author email: haider.alghanmi@qu.edu.iq, 0096407831319132

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Abstract
Marine brown algae are known to be the sources of biologically active secondary metabolites that have Promising pharmaceutical potential. In the current research paper, the objective was to describe chemical composition of volatile oil found in *Sargassum angustifolium* and to test the antioxidant, antibacterial and cytotoxic properties of the oil using in vitro assays. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the volatile oil and the profile obtained consisted of long-chain aliphatic hydrocarbons, fatty acids, ketones, and terpenoid-related compounds. The antioxidant activity was determined by DPPH radical scavenging test; the activity was concentration-dependent with moderate anti-oxidant power at a higher concentration. The assessment of antibacterial activity was performed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests with the selected Gram-positive and Gram-negative bacteria and showed relatively weak inhibitory and bactericidal action. The MTT assay method was used to determine cytotoxic activity in human dermal fibroblast cells and two types of cancer cells (HepG2 and MCF-7). It was found that the volatile oil had high concentration dependent cytotoxicity on cancer cells with a cell viability exceeding 50% in normal fibroblast cells representing a level of selective cytotoxicity. These results indicate that volatile oil of *Sargassum angustifolium* has bioactive lipophilic compounds that may be effective anticancer agents, despite relatively low antioxidant activity and antibacterial activity.
Keywords: Sargassum angustifolium; volatile oil; GC-MS; DPPH assay; MTT assay

1. Introduction
The marine macroalgae are a rich and chemically diverse assemblage of organisms which has gained growing scientific attention as a source of biologically active natural products [1,2]. Among the marine algae, brown seaweeds of the genus Sargassum are particularly remarkable because of the possibility to biosynthesise a very broad range of secondary metabolites, such as fatty acids, sterols, terpenoids, hydrocarbons, and complex polysaccharides, many of which have antioxidant, antimicrobial, and anticancer effects [3-5]. These are compounds that are vital ecologically in marine ecosystems and which have shown good pharmacological prospects in vitro and in vivo [6,7].
Sargassum angustifolium is a brown alga, which is abundantly found on the Persian Gulf coastline and has predominantly been researched on polar and semi-polar fractions, particularly fucoidan, alginate and crude hydroalcoholic extracts [8-11]. Earlier investigations have shared the presence of antioxidant activity of

polysaccharide rich fractions, moderate cytotoxicity of crude extract against cancer cell lines, and the protection of normal cells to oxidative stress conditions [12-15]. Conversely, the volatile oil fraction of S. angustifolium has also been highly understudied despite the fact that volatile and lipophilic constituents in other Sargassum species may be very important to antimicrobial and cytotoxic effects [4,16].

Gas chromatography-mass spectrometry (GC-MS) is a powerful method of analytical technique used in the characterization of volatile and semi-volatile compounds in marine algae that enables the identification of aliphatic hydrocarbons, fatty acids, ketone, and terpenoid-related compounds that define bioactivity in most cases [5,17]. Moreover, the in vitro trials like the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging tests, minimum inhibitory and bactericidal concentration tests (MIC/MBC), and MTT (Methyl Thiazolyl Tetrazolium) cytotoxicity tests are commonly used to measure the antioxidant capacity, antibacterial potential, and selective cytotoxicity of the substance on cancer and normal cells [18-20]. Nevertheless, a combined analysis of the GC-MS chemical profiling of antioxidant, antibacterial, and cytotoxic activity of S. angustifolium volatile oil has not been reported in depth.

In this regard, the purpose of the current research was to identify the chemical composition of the volatile oil that was extracted during *Sargassum angustifolium* using GC-MS and to determine its antioxidant, antibacterial, and cytotoxic properties in the forms of DPPH, MIC/MBC, and MTT, respectively, to understand the biological significance of the understudied fraction.

2. Materials and Methods

Algal Species

The biological material or brown alga *Sargassum angustifolium* was used in the study. They were collected in the Persian Gulf, Islamic Republic of Iran, and upon the demand of the University of Tehran laboratory. The gathering and pre-preliminary processing were done by University of Tehran, Qeshm, International Campus, Persian Gulf, University of Tehran, Qeshm.

After the collection, algal thalli were washed thrice in distilled water to eliminate sticking salts, sand particles, and epiphytic contents. The samples were cleaned, and shade dried at ambient temperature till a constant weight was obtained. The dry algal material was then ground to a fine powder using a laboratory grinder and kept in airtight containers until it was extracted.

Volatile Oil Extraction

Volatile and lipophilic compounds of *Sargassum angustifolium* were extracted using an organic solvent-based method of extraction based on the procedures of brown marine algae with minor modification [21]. Briefly, 10 g of the dried and powdered algal matter was put into 100 mL of dichloromethane and stirred with this solvent continuously over a 24-hour period at room temperature to allow the extraction of volatile constituents.

The mixture was filtered using a 0.45 mm membrane filter to eliminate insoluble residues after extraction. The filtrate obtained was heated at half the concentrated under reduced pressure at room temperature to about half its initial volume. The concentrated extract was further incubated and transfer to dark and gas-tight vials and stored at 4 degC pending further GC-MS and biological analysis.

Gas Chromatography-Mass Spectrometry (GCMS) Analysis.

Separating and identifying volatile substances were performed with the help of a gas chromatography system coupled with a mass spectrometer (Agilent Technologies) in combination with a 5% phenyl 95% dimethylpolysiloxane (30 m x 0.25 mm internal diameter, 0.25 mm film thickness) capillary column.

The carrier gas was helium, and the injector temperature was maintained at 250 o C. The constant flow rate of the carrier gas was 1.0 mL/min. and samples were injected in split mode. The program on the oven

temperature was set at 50 °C and kept 2 min and then the temperature was gradually increased at a rate of 5 °C/min to 280 o C whereby it was maintained 10 min to allow total elution of high-boiling compounds.
Mass spectrometer was used in electron ionization (EI) mode at 70 eV and mass spectra were collected at m/z between 40 to 550. The identification of compounds was done using reference spectra of standard mass spectral libraries compared with the obtained mass spectra.

Antioxidant Activity Assay (DPPH).
As a standard spectrophotometric technique, the antioxidant power of the volatile extract was determined by use of the DPPH free radical scavenging assay [23]. The volatile extract was mixed with various concentrations with a DPPH solution of 0.2 mM in ethanol. The mixtures of the reactions were incubated at room temperature and in the dark over 1 h and the absorbance was measured at 517 nm with a spectrophotometer [22].
The equation below was used to obtain the percentage of DPPH radical scavenging activity:

$$\text{DPPH scavenging activity (\%)} = [1 - (\text{Absorbance of sample} / \text{Absorbance of control})] \times 100$$

All the measurements were tripled, and the results were represented as mean and standard deviation.

Bacterial Strains
Certified reference bacterial strains which were supplied by the Iranian Genetic Resources Center (IGRC) of the University of Tehran. The strains received their international identification numbers (ATCC and PTCC) as authenticated pure cultures. *Escherichia coli* (PTCC 1330 / ATCC 8739), *Salmonella enterica* (PTCC 1709 / ATCC 14028), *Staphylococcus aureus* (PTCC 1764 / ATCC 33591), and *Bacillus cereus* (PTCC 1015 / ATCC 11778) were used as bacterial strains in this study.
The strains used are habitual in antimicrobial screening researches, and they are Gram-negative and Gram-positive bacteria that have well characterized resistance mechanisms.

Antibacterial Activity Assay (MIC and MBC)
The effectiveness of the volatile extract as an antibacterial agent was assessed by identifying the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the bacterium through a microdilution technique of resazurin as a survival marker. The volatile extract was used as a serial dilution in appropriate growth media and inoculated using standardized bacterial suspension. The color changes were noted after incubation to establish the bacterial growth inhibition and bactericidal effects [23].

Cytotoxicity Assay (MTT)
The MTT assay was used to determine the cytotoxic effects of the volatile extract as earlier mentioned [4]. Cells were planted in 96-well plates and left to incubate a period of 24 h to enable attachment. The cells were then exposed to various dilution of the volatile extract and left to incubate in 72 h. MTT solution was added to all the wells after treatment and left to incubate further to 4 h to form formazan crystals.
Dimethyl sulfoxide (DMSO) was used to dissolve the crystals and absorbance at 570 nm was measured using a microplate reader. The viability of the cells was expressed as a percentage compared with control cells that were not treated and the values of IC 50 were obtained by calculation of the dose-response relationship [24].

Statistical Analysis
Statistical analysis was performed using GraphPad Prism software. Data were expressed as mean ± standard deviation (SD). Differences between groups were analyzed using one-way ANOVA followed by Tukey's post

hoc test. A value of $p < 0.05$ was considered statistically significant. IC_{50} values were calculated from dose–response curves.

3. Results

Identification of Sargassum angustifolium

The brown alga *Sargassum angustifolium* was used as the biological material in this study. The algal thallus employed in all experimental analyses is illustrated in Figure 1, confirming the identity of the species and its suitability for chemical and biological evaluations.

Figure 1. *Sargassum angustifolium* used in the present study.

Chemical Composition of the Volatile Oil Extracted from Sargassum angustifolium

Gas Chromatography–Mass Spectrometry analysis of the volatile oil extracted from *Sargassum angustifolium* revealed a chemically diverse profile, as presented in Table 1 and illustrated by the chromatogram in Figure 2. The volatile oil was characterized by a predominance of long-chain aliphatic hydrocarbons, including tetradecane, pentadecane, hexadecane, octadecane, and eicosane, which are commonly detected in marine algae.

In addition, terpenoid hydrocarbons such as 2-hexadecene, 2,6,10,14-tetramethyl- were identified, along with ketones including 2-heptadecanone. Semi-volatile fatty acids, notably oleic acid and n-hexadecanoic acid (palmitic acid), were also detected. Furthermore, the analysis revealed the presence of a naturally occurring nitrogen-containing compound related to pyridine. These results demonstrate the complex chemical composition of the volatile oil extracted from Sargassum angustifolium.

Table 1. Chemical composition of the volatile oil extracted from *Sargassum angustifolium* as identified by GC–MS analysis.

RT (min)	Identified compound	Compound class	Molecular formula
26.61	Tetradecane	Aliphatic hydrocarbon	$C_{14}H_{30}$
29.17	Pentadecane	Aliphatic hydrocarbon	$C_{15}H_{32}$
29.53	3-(But-3-enyl)-cyclohexanone	Ketone	$C_{10}H_{16}O$
31.61	Hexadecane	Aliphatic hydrocarbon	$C_{16}H_{34}$
36.11	Octadecane	Aliphatic hydrocarbon	$C_{18}H_{38}$
37.00	Bicyclic terpene-like hydrocarbon	Terpenoid	~$C_{10}H_{16}$
37.14	2-Hexadecene, 2,6,10,14-tetramethyl-	Terpenoid hydrocarbon	$C_{20}H_{40}$
38.34	2-Heptadecanone	Ketone	$C_{17}H_{34}O$
40.19	Eicosane	Aliphatic hydrocarbon	$C_{20}H_{42}$
40.27	5-Octadecene (E)	Unsaturated hydrocarbon	$C_{18}H_{36}$
40.40	Oleic acid	Fatty acid (semi-volatile)	$C_{18}H_{34}O_2$
41.10	n-Hexadecanoic acid (Palmitic acid)	Fatty acid (semi-volatile)	$C_{16}H_{32}O_2$
45.98	Pyridine-related natural alkaloid*	Nitrogenous natural compound	~C_6H_7NO

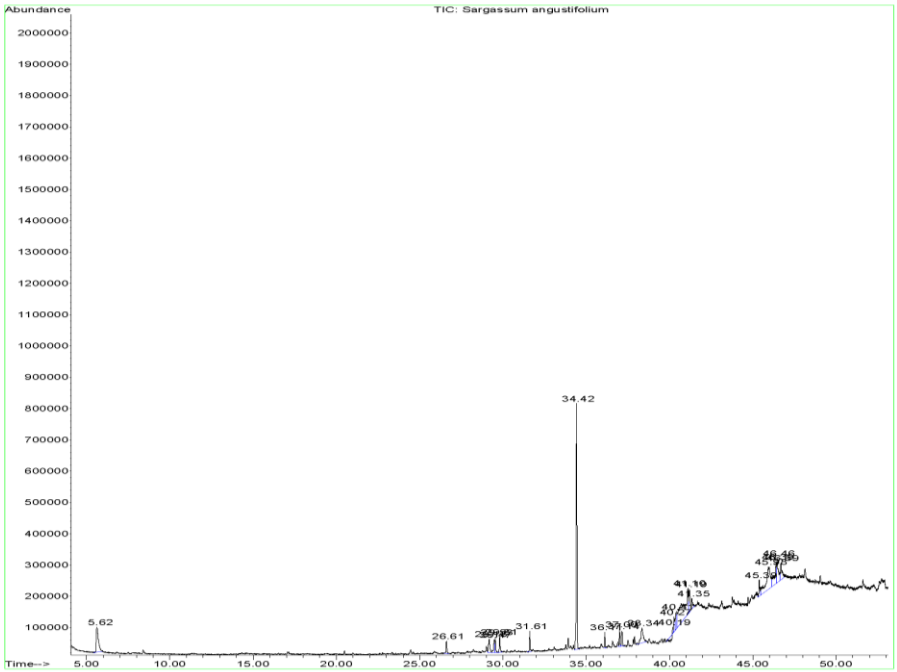

Figure 2. GC–MS chromatogram of the volatile oil extracted from Sargassum angustifolium.

Antioxidant Effect of *Sargassum angustifolium* Volatile Oil through DPPH Assay.

Using the DPPH free radical scavenging assay the antioxidant of the volatile oil of *Sargassum angustifolium* was assessed. The obtained results showed a definite concentration-dependent antioxidant activity, expressed in a slow decrease in the values of absorbance with the increase in the concentration of oil.

The maximum free radical scavenging capacity of 30.86 was observed at the concentration of 25%. The activity of scavenging was dropped to 21.45 when concentration was lowered to 20%. Further dilutions led

to 14.58, 9.44 and 6.36 percent scavenging at 12.5, 6.2 and 3.1 percent, respectively. The control sample did not show any antioxidant activity.

Such findings show that *Sargassum angustifolium* volatile oil possesses moderate antioxidant properties which strongly depend on concentration.

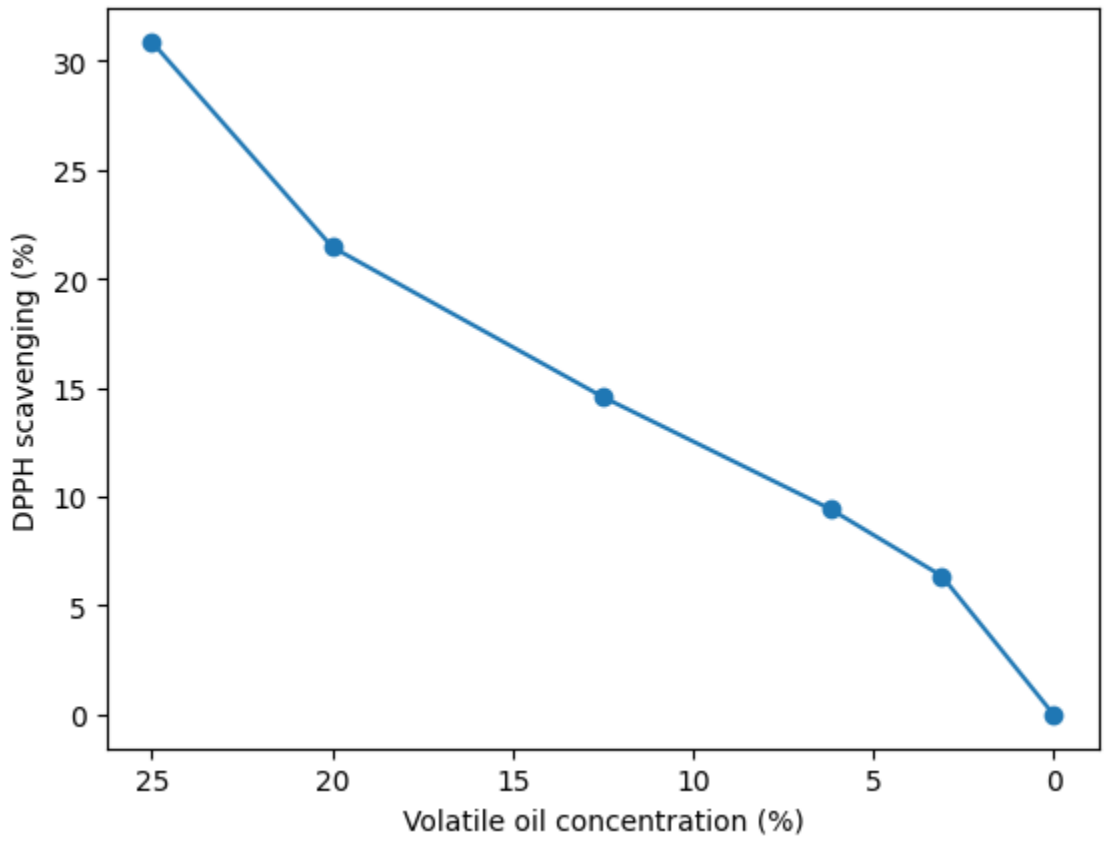

Figure 3. Effect of different concentrations of volatile oil extracted from *Sargassum angustifolium* on antioxidant activity determined by the DPPH assay.

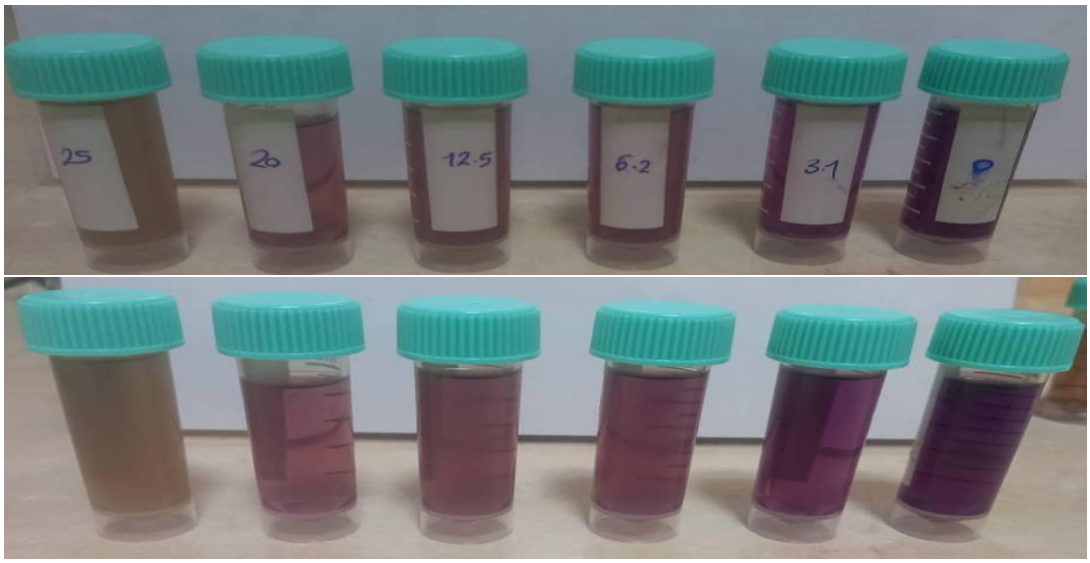

Figure 4. Colorimetric changes observed in the DPPH assay for volatile oil extracted from *Sargassum angustifolium* at different concentrations.

Antibacterial Property of *Sargassum angustifolium* Volatile Oil.

The antibacterial activity of the volatile oil was evaluated by determining the minimum inhibitory concentration (MIC) using the resazurin-based microdilution method. Serial dilutions of the volatile oil were prepared in suitable growth media and inoculated with standardized bacterial suspensions. After incubation, resazurin dye was added to detect bacterial metabolic activity. A color change from blue to pink indicated bacterial growth, whereas the absence of color change indicated inhibition of bacterial growth.

Minimum Inhibitory Concentration Assay

The minimum inhibitory concentration assay was done to measure the antibacterial activity of the volatile oil extracted in the *Sargassum angustifolium* in terms of resazurin dye. The majority of bacterial strains showed changes in colorimetric observation of the colorimetric changes of blue to pink at comparatively high concentrations of oil, as indicated by the colorimetric changes of the microplate assay (Figure 5).

The wells with high and intermediate concentrations were persistently pink or red in color, which corresponded to the presence of bacterial metabolism and low levels of inhibition. In the dilute concentrations, all the bacterial strains were found to convert the entire color to indicate the lack of antibacterial activity. The bacterial control exhibited normal metabolism whereas the medium and oil controls maintained the color blue hence validity of the assay. these observations demonstrate is that the volatile oil of *Sargassum angustifolium* has a relatively low antibacterial activity.

Figure 5. Inhibitory effect of volatile oil extracted from *Sargassum angustifolium* against Escherichia coli, Salmonella enterica, Staphylococcus aureus, and Bacillus cereus determined by the minimum inhibitory concentration microplate assay.

Minimum Bactericidal Concentration

The lowest bactericidal concentration values reflected that the volatile oil obtained in *Sargassum angustifolium* exhibited the lowest bactericidal properties in the studied algae. The bactericidal effects were only observed during the highest level of concentration, whereas none of the bactericidal activities was detected in intermediate and low levels of concentration in most of the bacterial strains (Table 2).

Figure 6 distinctly shows the presence of the bacteria over the longest period of time at all their concentrations, which validates the low bactericidal capacities of the volatile oil that was obtained after the extraction of Sargassum angustifolium.

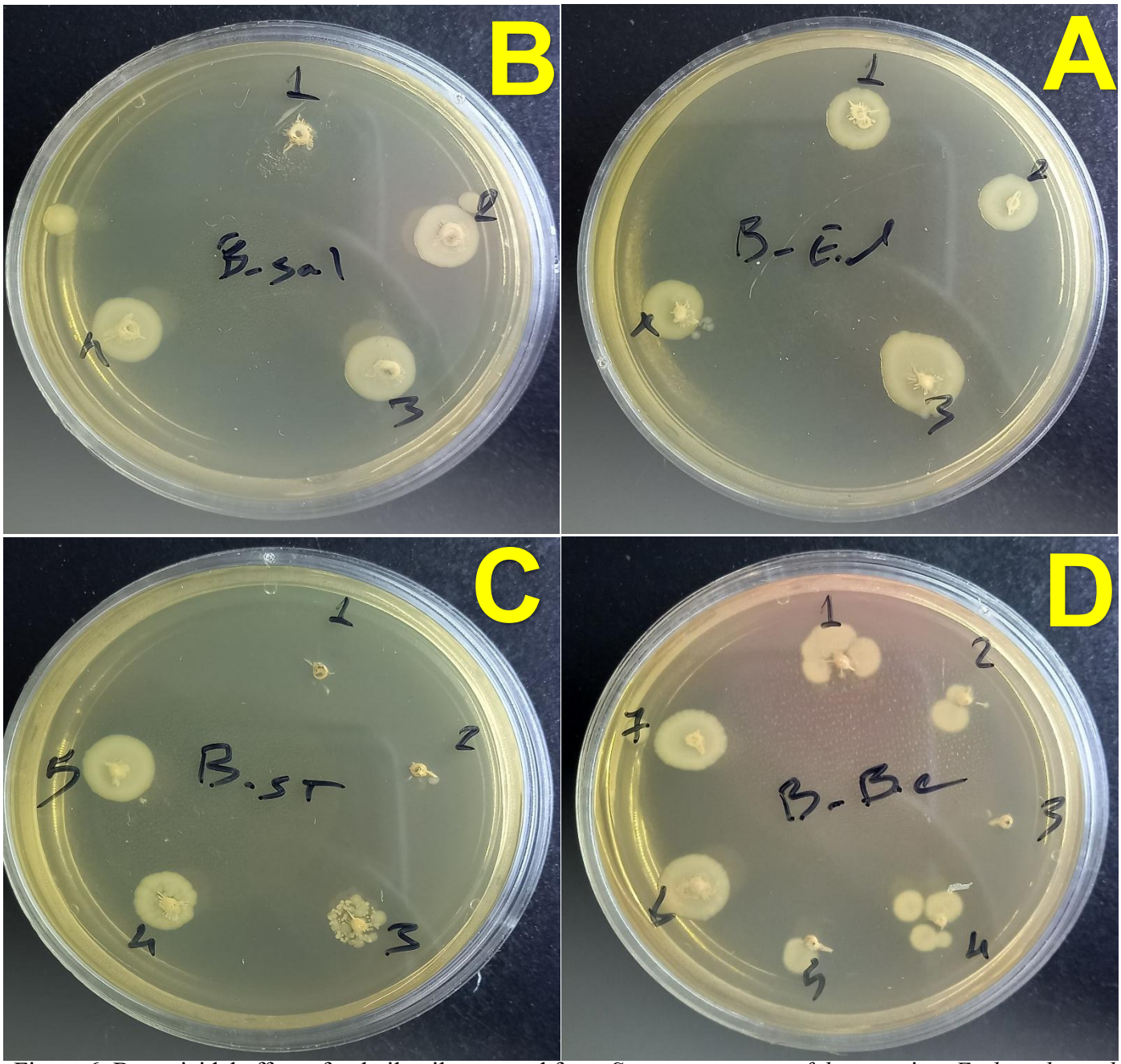

Figure 6. Bactericidal effect of volatile oil extracted from *Sargassum angustifolium* against *Escherichia coli* (A), *Salmonella enterica* (B), *Staphylococcus aureus* (C), and *Bacillus cereus* (D) determined by the minimum bactericidal concentration assay.

Table 2. Minimum inhibitory concentration and minimum bactericidal concentration values of volatile oil extracted from *Sargassum angustifolium* against selected bacterial strains.

No.	Bacterial Species	Abbreviation	Test	Volatile Oil concentration									Control treatment		
				1	2	3	4	5	6	7	8	9	10	11	12
				100 %	50 %	25 %	12.5 %	6.2 %	3.1 %	1.5 %	7.8 %	3.9 %	Bacteria Control	Culture medium control	Algae control
1	*E. coli* PTCC-1330 ATCC-8739	E.C/B	MIC	-	+	+	+	+	+	+	+	+	+	-	-
			MBC	-	+	+	+	+	+	+	+	+			
2	*S. enteric* PTCC-1709 ATCC-14028	Sal-B	MIC	+	+	+	+	+	+	+	+	+	+	-	-
			MBC	+	+	+	+	+	+	+	+	+			
3	*S. aureus* PTCC-1764 ATCC-33591	St/B	MIC	-	-	+	+	+	+	+	+	+	+	-	-
			MBC	-	-	+	+	+	+	+	+	+			
4	*B. cereus* PTCC-1015 ATCC-11778	B.C/B	MIC	+	+	+	+	+	+	+	+	+	+	-	-
			MBC	+	+	+	+	+	+	+	+	+			

(+) Presence of bacterial growth, (−) Absence of bacterial growth.

Toxicity of *Sargassum angustifolium* Volatile Oil on Human Dermal Fibroblast (HDF).

The effects of the compound on human dermal fibroblast cells were studied as follows: 5.1.1 Effect on the Cellular Protective Factor The testing sample included healthy human skin cells or dermal fibroblast cells. The effects of the compound on the cells were examined by analyzing the cellular protective factor before and after the cellular exposure to the compound.

The MTT assay was used to determine the cytotoxic effect of volatile oil extracted *Sargassum angustifolium* on human dermal fibroblast cells (HDF). The viability of the cell as indicated by Table 3 was found to gradually decrease with the increase in the concentration of the oil, but viability was still better than 50% at all levels.

The largest cell viability (99.80) was at the lowest concentration (3.1%), and the smallest viability (79.24) was at the highest concentration (25%). Figure 7 shows the trend of cell viability as a function of time, whereas Figure 8 shows the dose-response behavior. The half-maximal inhibitory concentration was not attained in the case of HDF cells.

Table 3. The MTT assay of cytotoxic effects of volatile oil extracted on *Sargassum angustifolium* regarding human dermal fibroblast cells.

Concentration (%)	OD1	OD2	OD3	Avg OD	Cell Viability (%)
0	0.341	0.348	0.337	0.342	100
3.1	0.348	0.341	0.335	0.3413	99.80507
6.2	0.331	0.327	0.332	0.33	96.49123
12.5	0.299	0.309	0.319	0.309	90.35088
20	0.288	0.291	0.271	0.2833	82.846
25	0.263	0.271	0.279	0.271	79.23977

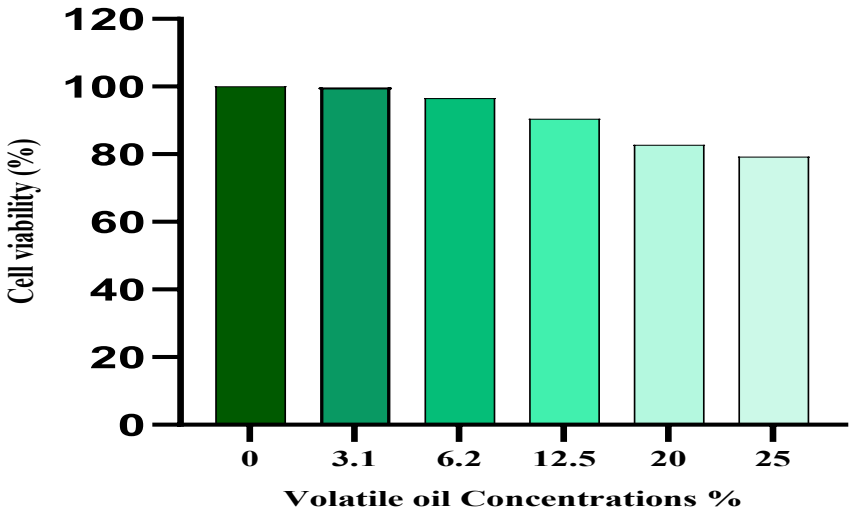

Figure 7. Cell viability response of human dermal fibroblast cells to different concentrations of volatile oil extracted from Sargassum angustifolium.

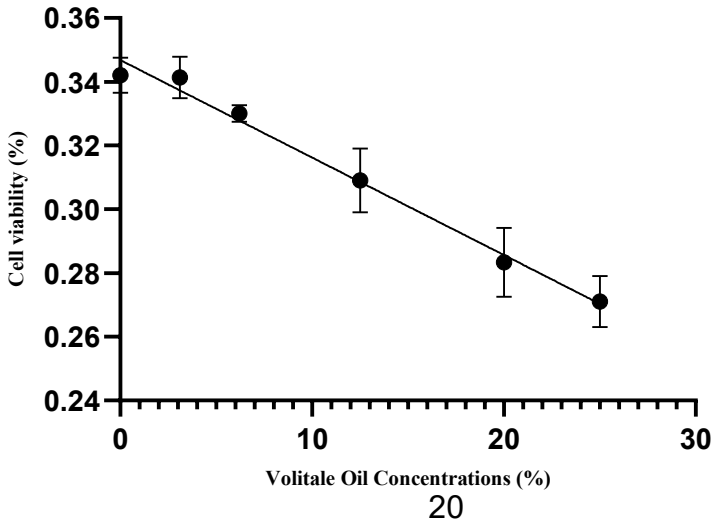

Figure 8. Cytotoxicity evaluation of volatile oil extracted from *Sargassum angustifolium* on human dermal fibroblast cells using the MTT assay.

Toxicity of *Sargassum angustifolium* Volatile Oil on Human Hepatocellular Carcinoma Cells (HepG2).
The volatile oil of *Sargassum angustifolium* had a strong concentration-dependent cytotoxic effect on human hepatocellular carcinoma cells (HepG2). The cell viability peak was reached at the lowest concentration (47.52%, Table 4) and the viability declined drastically with an increase in concentration up to the maximum one (7.74%).
Figure 9 shows the loss of cell viability with concentration and Figure 10 shows the dose response curve that was used in the determination of cytotoxic behavior. The value of half-maximal inhibitory concentration was determined to be 3.621 percent which is a strong cytotoxic one against the HepG2 cells.

Table 4. The MTT assay was done on cytotoxic effects of volatile oil isolated in *Sargassum angustifolium* on human hepatocellular carcinoma cells.

Concentration (%)	OD1	OD2	OD3	Avg OD	Cell Viability (%)
0	0.623	0.676	0.639	0.646	100
3.1	0.305	0.309	0.307	0.307	47.52
6.2	0.263	0.273	0.279	0.2717	42.05
12.5	0.191	0.185	0.188	0.188	29.1
20	0.11	0.103	0.104	0.1057	16.36
25	0.06	0.04	0.05	0.05	7.74

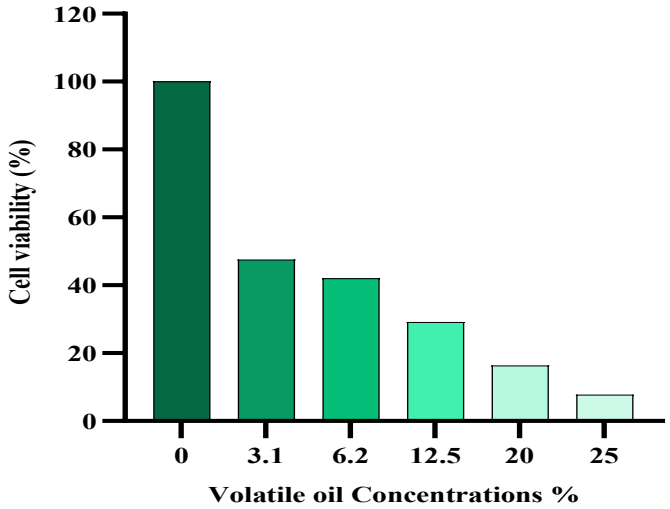

Figure 9. Cell viability response of human hepatocellular carcinoma cells to different concentrations of volatile oil extracted from Sargassum angustifolium.

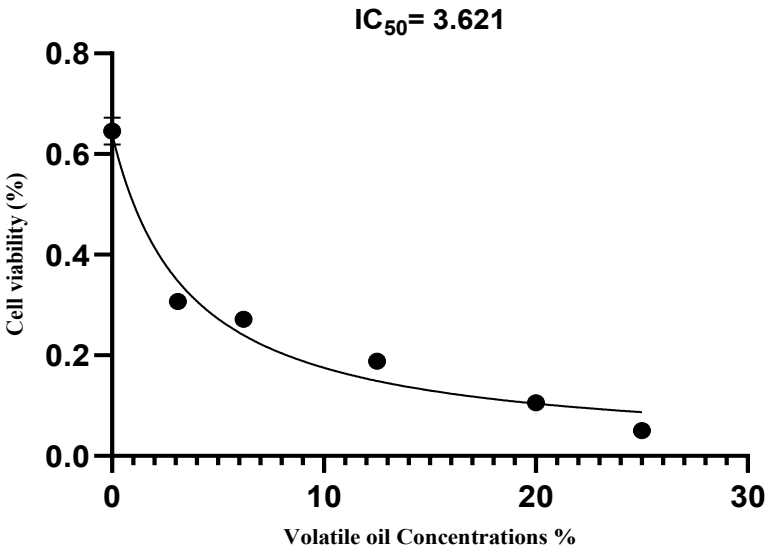

Figure 10. Cytotoxicity evaluation of volatile oil extracted from *Sargassum angustifolium* on human hepatocellular carcinoma cells using the MTT assay.

Toxicity of *Sargassum angustifolium* Volatile Oil on Human Breast Adenocarcinoma Cells (MCF-7).
The apparent reduction of cell viability was also concentration dependent as observed in human breast adenocarcinoma cells (MCF-7) after being exposed to the volatile oil of the Sargassum angustifolium. In a summary provided in Table 5, cell viability had reduced as low as 47.61% at the low concentration up to 11.66% at the high concentration.
The downward shift in the cell viability is depicted in Figure 11 and the respective dose response curve and calculation of the half-maximal inhibitory concentration value is presented in Figure12. The half-maximal inhibitory concentration calculated in MCF-7 cells was 3.333% and this validated the cytotoxic effect of the volatile oil.

Table 5. The MTT assay of cytotoxic effects of volatile oil obtained by the extraction process with *Sargassum angustifolium* on human breast adenocarcinoma cells.

Concentration (%)	OD1	OD2	OD3	Avg OD	Cell Viability (%)
0	0.591	0.596	0.571	0.586	100
3.1	0.277	0.269	0.291	0.279	47.61
6.2	0.255	0.259	0.252	0.2553	43.57
12.5	0.161	0.17	0.15	0.1603	27.36
20	0.104	0.102	0.105	0.1037	17.69
25	0.06	0.07	0.075	0.0683	11.66

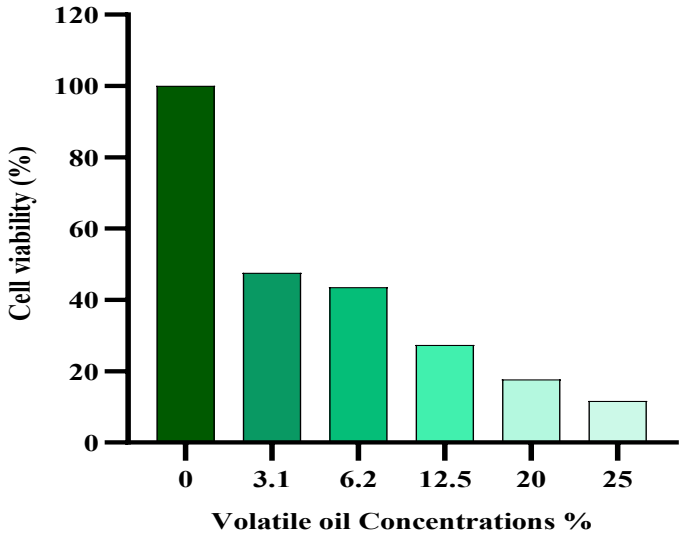

Figure 11. Cell viability response of human breast adenocarcinoma cells to different concentrations of volatile oil extracted from Sargassum angustifolium.

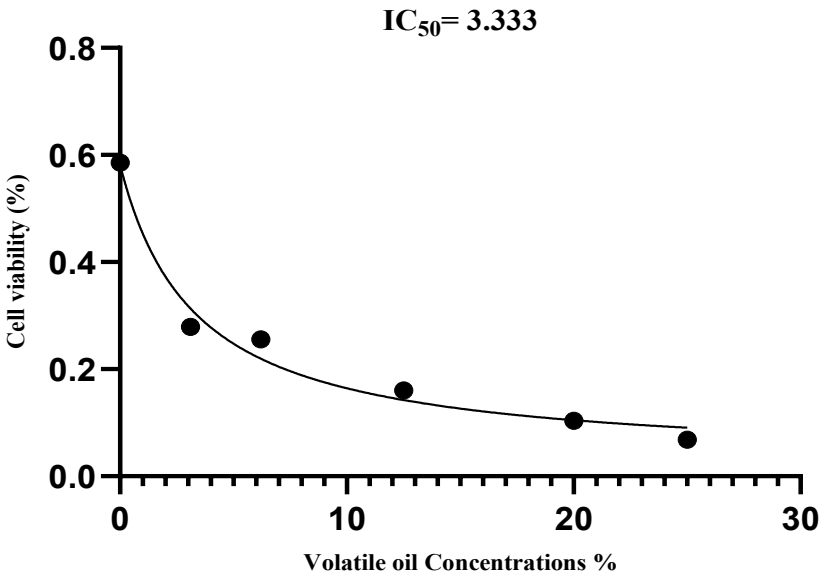

Figure 12. Cytotoxicity evaluation of volatile oil extracted from *Sargassum angustifolium* on human breast adenocarcinoma cells using the MTT assay.

4. Discussion

Volatile oil chemical composition.

The GC -MS analysis proved that the volatile oil of *Sargassum angustifolium* is typified by the preponderance of long-chain aliphatic hydrocarbons including tetradecane, pentadecane, hexadecane, octadecane, and eicosane along with terpenoid hydrocarbons, ketones, fatty acids, and a nitrogen-containing compound. Two Sargassum species (namely, lipophilic or volatile fractions) have also been reported to have similar chemical profiles, with aliphatic hydrocarbons and fatty acid derivatives being significant members of non-polar extracts [3,4,17]. These are usually related to the lipid metabolism and can also be a result of the thermal or oxidative breakdown of the lipids in the membrane during extraction procedures [5].

The oleic acid and palmitic acid identifications are in line with the past studies that determined oleic acid and palmitic acid as the prevalent lipid compounds in the brown algae [16,25]. The two fatty acids have been also reported to have antimicrobial and cytotoxic effects that are associated with destabilizing the membranes, changing the membrane fluidity and causing oxidative stress to microbial and cancer cells [18,26]. Also, the occurrence of terpenoid-associated hydrocarbons and ketones can increase the biological applicability of the volatile oil since terpenoid products of Sargassum spp. have been well-known to possess antimicrobial and anticancer effects [4,6].

Antioxidant activity

The scavenging activity of the volatile oil of S. angustifolium in the DPPH assay had a clear concentration-dependent activity, and moderate antioxidant activity at higher concentrations. The lower radical scavenging activity of the volatile oil can be explained by the fact that the polysaccharide-rich extracts of S. angustifolium reported previously exhibited superior antioxidant activity [9,12], and the volatile oil is mostly composed of lipophilic compounds. Hydrogen or electron donors Polysaccharides and phenolic compounds typically make superior hydrogen or electron donors in DPPH-based systems than hydrocarbons and fatty acids [10,19].

Still, the antioxidant activity identified in this study is also in accordance with the literature of volatile or non-polar fractions of other brown algae wherein moderate DPPH scavenging activity is recent to unsaturated fatty acids and terpenoid compounds [5,16]. Notably, in more generalized reviews of antioxidant capacity determination, lipophilic compounds can be found to have poorer performance on a single-electron transfer or DPPH-based assays, although still capable of binding biologically relevant antioxidant activity, via other pathways, including control of intracellular redox status and prevention of lipid peroxidation [27]. Hence, the intermediate activity of the volatile oil as a DPPH scavenger does not exclude the possibility of its involvement in the effects of the reduction of oxidative stress in living organisms.

Antibacterial activity

The volatile oil of *S. angustifolium* showed rather weak antibacterial effect on the tested bacterial strains as were observed by the first color change during the MIC assay and the few bactericidal activities exhibited only in the highest concentrations. This observation is in contrast with results of fucoidan and crude extracts of the Sargassum species, which often are more active against bacteria at lower concentrations [8,13,18].

The observed low levels of antibacterial effects in the current study can be explained by the fact that saturated aliphatic hydrocarbons were the most common compound in this group, which have lower antimicrobial activity in comparison to polar or amphiphilic compounds [26]. Though the fatty acids like oleic acid have been reported to have the antibacterial effect they highly depend on concentration, level of unsaturation, and bacterial membrane composition [25]. Moreover, lack of strong active phenolic or sulfated polysaccharide

compounds which are major contributors of antimicrobial effect in Sargassum extracts is likely to be the reason of low inhibitory and bactericidal properties of volatile oil fraction.

Cytotoxic activity

There was a strong concentration-related cytotoxic effect of the volatile oil on HepG2 and MCF-7 cancer cell lines but human dermal fibroblast cell-sustained viability was more than 50% in all the different concentrations tested. This selective killing is a sign of some selective cytotoxicity to the malignant cells which is a key criterion in assessing the potential anticancer agent [14,20].

The cytotoxicity of the HepG2 and MCF-7 cells was high; therefore, a combination of these fatty acids, terpenoid-related compounds, and ketones found in the volatile oil could explain the high cytotoxicity. Such classes of compounds have been known to cause apoptosis, dysfunction of mitochondria, and oxidative stress in cancer cells that results in diminished cell viability [6,15,26]. The same selective cytotoxic effect was reported with lipophilic fractions and single glycolipids of Sargassum species with cancer cells being more sensitive than normal cells [11,16].

Conversely, the comparatively low cytotoxicity of human dermal fibroblasts can be compared with other studies indicating that some of the extracts and polysaccharide fractions of Sargassum are non-toxic or even cytoprotective against normal human cells [12,14]. The fact that an IC50 value could not be achieved with fibroblast cells also helps to say that the volatile oil has an attractive selectivity profile, although further mechanistic studies are needed to completely understand the mechanisms of its action which exhibits cytotoxic action.

Conclusion

This paper has examined the chemical profile and in vitro biological properties of volatile oil of Sargassum angustifolium. The volatile oil that is obtained can be analyzed using GC-MS which indicated that long-chain aliphatic hydrocarbons, fatty acids, ketones, and compounds related to terpenoids were the dominant components of the volatile oil, which is indicative of the fact that brown marine algae are lipophilic.

The volatile oil showed a concentration-dependent antioxidant effect in the DPPH assay; however, the oil had an average radical scavenging potential. Antibacterial tests showed poor inhibitory and bactericidal action against the bacterial strains under experiment. Conversely, the volatile oil showed strong cytotoxicity to both HepG2 and MCF-7 cancer cell lines with the capability of remaining relatively viable in normal human dermal fibrous cells thereby showing selective cytotoxicity. The results indicate that S. angustifolium volatile oil has bioactive lipophilic compounds that have anticancer potential and require further research.

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