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Investigating the bioactive compounds obtained from six seaweeds for food and medical applications

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Abstract
Seaweeds are marine macroalgae has excellent bioactive compounds with biological and pharmacological activities has increased drastically from researchers, food and pharmaceutical companies in the area of medical fields. The diverse, safe and durable natural based antioxidant, antimicrobial agents is gradually gaining attention in recent years to conquer the negative impacts caused by synthetic agents in medical industries. This study investigated the various bioactive compounds present in red, green and brown seaweeds used for medical applications. The preliminary screenings of 15 different bioactive compounds of the six seaweeds were analysed using methanol extracts under the standard qualitative conditions.
The extracts from six seaweeds were tested under *In vitro* conditions, for the minimum inhibitory (MIC), fungicidal (MFC) and bactericidal (MBC) concentrations. The values for the MIC against *Klebsiella Pneumoniae* bacteria were 250 mg/ml, while the MIC against *Candida albicans* fungi ranged from 62.5 mg/ml to 250mg/ml. The MBC of the seaweeds ranged from 250 mg/ml to 1000 mg/ml, and the MFC ranged from 250 mg/ml to 1000 mg/ml. Besides, the total antioxidant activity and DPPH radical scavenging assays were also analysed. Among the five different concentrations of the seaweed extracts, 250 µg/ml showed maximum antioxidant activity than the rest of the concentrations studied. During this analysis, the *Acanthophora spicifera* extracts, showed remarkably higher scavenging activity of 77±0.17% inhibition compared with other seaweed extracts.

Practical applications
Seaweeds are rich source of phenolic compounds, flavonoids, fucoidan and nutritional values. Due to higher antimicrobial, antitumor, anticarcinogenic and antioxidant activity, most of the seaweeds contain high content of vitamins, minerals, proteins and carbohydrates in polysaccharide biochemical structure, which is a natural nontoxic colloidal substance has been used to produce hydrocolloids, alginate, carrageenan and agar are used as thickening and gelling agents. The selected seaweeds are used for the treatment of asthma, goiters, tumour, cancer, cough, ulcers, and urinary diseases.

Keywords: Bioactive compounds, minimum fungicidal concentration, total antioxidant activity, antimicrobial properties, food shell life, nutritional value

Introduction
Many plant extracts having medicinal characteristics, are considered as herbal medicine (Farnsworth, 1990) [9]. The large quantity of population uses many medicinal plants for traditional practice to meet their healthcare needs in recent years. Although some of the diseases are cured only by the herbal medicines and maintained their popularity for historical and cultural reasons (Vishwakarma, Vishwe, Vishwe, & Chaurasiya, 2013) [32].
Natural medicinal products are essential for curing degenerative diseases and are developed from various sources such as plants, microorganisms, marine algae, vertebrates and invertebrates (Newman, Crag, & Snader, 2000, Jones, Chin, & Kinghorn, 2006) [22, 14]. The traditional medicine extracted from plants gained more interest in medical research (Newman, & Crag, 2007) [21].
In recent times, many natural products are attributed by several factors, including distinct therapeutic needs, significant diversity of functional properties occurring naturally by secondary metabolites, effectiveness of active compounds as biochemical probes, characterisation and identification of bioactive compounds from natural products through various novel and sensitive techniques and their application in the medical field (Clark, 1996) [7].
In this study, six seaweed species out of which two green species of *Ulva reticulata, Ulva lactuca;* two red species of *Acanthophora spicifera, Kappaphycus alvarezii* and two brown species of *Sargassum wightii, Padina tetrastromatica* were freshly collected and obtained from

Thonidurai Coast, Mandapam, Tamil Nadu, India. The collected seaweeds were cleaned using fresh water to remove dirt and dust. Fresh cleaned seaweeds were dried at 60 ℃ in oven for 3 days to eliminate impurities and salt. The cleaned and dried seaweeds were extracted using methanol by Soxhlet method.

The seaweeds are excellent sources of vitamins, proteins, minerals and bioactive compounds. Seaweeds acquired major biological properties like antiviral, antioxidant, anti-inflammatory and antibacterial properties. Seaweeds with different active compounds have potential of natural antioxidants with functional properties, in which antibacterial and antioxidants were studied. Many compounds from marine organisms, including seaweeds, have acquired antimicrobial properties.

The different significant bioactive properties of seaweeds, such as bioactive constituents, antioxidant property, minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration of seaweed extracts were evaluated. The bioactive compounds acquired from six seaweeds are attributed to have the antioxidant, antibacterial, antifungal, antiviral and anticancer properties, which were investigated to assess the properties of these compounds constituents, antioxidant and antimicrobial properties.

In the continuing research for the bioactive components of *Ulva lactuca*, *Padina tetrastromatica*, *Ulva reticulata*, *Acanthophora spicifera*, *Sargassum wightii* and *Kappaphycus alvarezii* extracts were evaluated for phytochemical screening, antimicrobial and antioxidant properties using MBC, MFC, MIC, Total antioxidant activity and DPPH assay.

2. Materials and Methods

2.1 Solvent Extraction from Seaweeds

The collected seaweeds were cleaned with fresh water to remove dirt and dust and were dried at 60 ℃ in the hot air oven for 3 days to eliminate impurities and salt. The cleaned and dried seaweeds were extracted using methanol by Soxhlet method [Kou, & Mitra, 2003] [15].

In this method, a Whatman No.1 filter paper thimble containing 20 g of powdered seaweed samples is placed into an extraction chamber. A flask containing 200ml of methanol (1:20w/v) was connected to the extraction chamber. A steady heat source (40-50 ℃) was passed through the condenser flask for 24 hours until the extract was clear. The seaweed extracts obtained in the extraction chamber was kept in rotary vacuum evaporator at 30 ℃ to remove the solvent present in the final extracts and make the end volume to one-fourth of the original volume. After extraction process the final concentrate was dissolved in methanol and stored at 4 ℃ for further process (Maheswaran, Padmavathy & Gunalan, 2013) [17].

2.2 Qualitative determination of bioactive compounds

The bioactive screenings of seaweeds using methanol extracts were carried out using a standard procedure to confirm the presence of various bioactive compounds (Mohammed Deyab, TahaElkatony, & Fatma Ward, 2016, Ponnanikajamideen, Malini, Malarkodi, & Rajeshkumar, 2014) [20, 25]. Each bioactive compound was identified using the standard procedure described in Table 1.

Table 1: Standard qualitative procedure for the identification of bioactive compounds

S. No	Bioactive Compound	Procedure
1.	Saponins	5ml of distilled water was added to 0.5 g of seaweed extract, shaken vigorously and observed for a stable persistent froth which was mixed with 3 drops of olive oil, shaken and observed in the formation of saponins.
2.	Tannins	10ml of distilled water was added to 200mg of plant material and filtered. To 2ml of the filtrate, 2ml of $FeCl_3$ was added, resulting in a blue-black precipitate, indicating the presence of tannins.
3.	Terpenoids	2ml of acetic anhydride and concentrated H_2SO_4 was added to 2ml of seaweed extract and the formation of blue-green rings indicates the presence of terpenoids.
4.	Phytosterols	10ml of chloroform and the same volume of concentrated sulphuric acid was added to 1ml of seaweed extract, in this the top layer turned red and the sulphuric acid layer showed the fluorescence of green colour, which indicated the presence of steroids.
5.	Glycosides	2ml of glacial acetic acid containing one drop of $FeCl_3$was added to 0.5 g of seaweed extract, which was then under laid with 1ml of concentrated H_2SO_4 and the brown ring indicated the presence of glycosides.
6.	Amino acids	A few drops of Ninhydrin reagent was added to 1ml of seaweed extract, showed the purple colour indicating the presence of amino acids.
7.	Alkaloids	10ml of methanol was added to 200mg of seaweed extract and filtered, then a 2ml filtrate +1% HCl + 6 drops of Mayer's reagents, a brownish-red precipitate/orange precipitate occurs and then added with 1ml filtrate indicated the presence of alkaloids.
8.	Flavonoids	A few drops of dilute sodium hydroxide was added to1ml of seaweed extract, the intense yellow colour displayed, which went colourless on the addition of few drops of dilute hydrochloric acid indicated the presence of flavonoids.
9.	Phlorotannins	1ml of Fehling's B reagent was added to 1ml of Fehling's A reagent was added to 1ml of seaweed extract and boiled for few minutes and the appearance of yellow or brownish colour indicated the presence of phlorotannins.
10.	Carotenoids	2ml filtrate was added to 1% concentrated HCl and few drops of magnesium ribbon added to 200mg seaweed extract in 10ml methanol, the formation of pink-tomato red colour indicated the presence of carotenoids.
11.	Phenols	5ml of the extract was pipetted into a 30ml test tube, and then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added and left to react for 30min. Development of bluish green colour was taken as a positive presence of phenol.
12.	Anthocyanins	The presence of anthocyanins has been demonstrated by adding 2ml of the plant extract with 2 ml of 2N HCl. The appearance of a pink-red colour that turns purplish blue after addition of ammonia indicates the presence anthocyanins.
13.	Anthraquinone	0.5 g of the part plant was boiled with 10ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute

		ammonia was added. The resulting solution was observed for colour changes (delicate rose pink colour showed the presence of anthraquinones).
14.	Coumarins	3ml of the diethyl ether extract was evaporated to dryness in a test tube and the residue was dissolved in hot distilled water. It was then cooled and divided into two test portions, one was the reference. To the second non reference test tube, 0.5 ml of 10% NH_4OH was added. The occurrence of an intense/fluorescence under UV light (λmax= 365 nm) is a positive test for the presence of coumarins and derivatives.
15.	Protein	The five test tubes were filled with 0.5 ml of bovine serum albumin (BSA) powder, and the volume brought to 1ml by add distilled water in each test tube. The test tube with 1ml of distilled water serves as blank. Then add 4.5 ml of 48 ml of 2% Na_2CO_3 in 0.1N NaOH, 1 ml of 1% NaK Tartrate in H_2O and 1 ml of 0.5% $CuSO_4.5H_2O$ in H_2O and set aside to incubate for 10 minutes. After incubation, add 0.5 ml of 1 part of Folin-Phenol reagent [2N] mixed with 1 part of water and set aside to incubate for 30 minutes. The standard graph was plotted with the help of measured absorbance at 660 nm using Perkin Elmer's LAMBDA 465 UV-Visible spectrophotometer. Using the standard graph, the amount of protein existence in the given sample was evaluated.

2.3 Determination of minimal inhibitory concentration (MIC)

The seaweed extract was tested using the two-fold serial dilution method to determine the minimal inhibitory level. 2000 µg/ml stock solutions were attained by dissolving the test extract in 5% dimethyl sulfoxide (DMSO). The seaweed extracts were diluted to obtain the final concentrations of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml and 31.25 µg/ml. The test culture contained the bacteria, *Klebsiella pneumoniae* and the fungi; *Candida albicans* were inoculated in tubes of about 100 µl with equal volume of sabouraud dextrose broth for fungi, nutrient broth for bacteria and seaweed extract samples was taken separately in test tubes. The bacteria tubes were incubated at 37 ℃ for 24 hours and fungi tubes were incubated at 30 ℃ for 72 hours. The lowest concentration that produced no visible turbidity after a total incubation period of 48 hours was recorded as the final MIC (Watee Srikong, Pimonsri Mittraparparthorn, Onnicha Rattanaporn, Nutapong Bovornreungroj, & Preeyanuch Bovornreungroj, 2015) [34].

2.4 Determination of minimal bactericidal concentration (MBC)

The test dilution [which showed no visible turbidity] was sub-cultured on freshly prepared nutrient agar media. The test dilution plates were incubated at 37 ℃ for 24-48 hours. The higher dilution concentration was determined as MBC value, where no single bacterial colony on the nutrient agar plates was attained (Watee Srikong, Pimonsri Mittraparparthorn, Onnicha Rattanaporn, Nutapong Bovornreungroj & Preeyanuch Bovornreungroj, 2015) [34].

2.5 Determination of minimal fungicidal concentration (MFC)

The test dilution [which showed no visible turbidity] was sub-cultured on freshly prepared sabouraud dextrose agar media. The test dilution plates were incubated at 30 ℃ for 48-72 hours. The higher dilution concentration was determined as MFC value, where no single fungal colony on the sabouraud dextrose agar plates was attained (Watee Srikong, Pimonsri Mittraparparthorn, Onnicha Rattanaporn, Nutapong Bovornreungroj, & Preeyanuch Bovornreungroj, 2015) [34].

2.6 Determination of Total antioxidant activity

The different concentrations of working standard and extracts of *Ulva lactuca, Acanthophora spicifera, Sargassum wightii, Ulva reticulata, Padina tetrastromatica* and *Kappaphycus alvarezii* was prepared ranging from 50-250µg/ml and 0.1ml of the aliquot were pipette out and poured in a series of test tubes. 1ml of reagent solution contains 0.6M sulphuric acid; 28mM sodium phosphate and 4mM ammonium molybdate were added to the test tubes and incubated at 95 ºC for 90 minutes. The changes in the absorbance of the test samples were recorded at 695nm against blank using a Jusco V-550 UV-VIS spectrophotometer. The total antioxidant capacity of the treated samples was measured in µg/ml to indicate the antioxidant activity (Prieto, Pineda, & Aguilar, 1999) [27].

2.7 Analysis of DPPH scavenging assay

The different concentrations (50, 100, 150, 200 and 250µg/ml) of seaweed extracts were added, at an equal volume, to the methanolic solution of DPPH (0.3mm). The mixture was shaken vigorously and left to stand at room temperature for 35 minutes. Ascorbic acid was considered as standard control solution. Each test sample was made for three replicates (Mensor *et al*., 2001) [19]. After 30 minutes, the changes in the absorbance of the test samples were measured at 518nm using Jusco V-550 UV-VIS spectrophotometer. The ability to scavenge the DPPH radical and the percentage inhibition of antioxidant activity was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{1-(A_{\text{sample}}-A_{\text{sample blank}})}{A_{\text{control}}} \times 100 \quad (1)$$

Where,

A_{control} is the absorbance of the control (DPPH solution without sample).

A_{sample} is the absorbance of the test sample (DPPH solution plus test sample).

$A_{\text{sample blank}}$ is the absorbance of the sample only (sample without any DPPH solution).

2.8 Investigating the bioactive compounds of seaweed extracts using GC-MS analysis

Gas Chromatography-Mass Spectrometry (GC-MS) is more powerful and selective characterization tools for the structure elucidation of components. The biochemical profiles of *Ulva lactuca, Acanthophora spicifera, Sargassum wightii, Ulva reticulata, Padina tetrastromatica* and *Kappaphycus alvarezii* extracts were studied using Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II analysis.

All six seaweed samples were shade dried at room temperature and 5g of each of the seaweed powdered samples were dipped with 98% methanol for 16 hours. Then the seaweed extracts were filtered through Whatman No. 1

filter paper and added with 0.5 g of sodium sulphate solution to remove the sediments present in the solution filtrate. The nitrogen gas was passed through the solution filtrate to attain purified concentrations. An aliquot of 2µl of this solution was used for GC-MS analysis. GC-MS analysis lead to the detection of various active compounds related to the GC fraction of column purified extracts of seaweeds with respect to retention time, chemical nature, molecular weight, peak area (%) and molecular formula are graphically illustrated in the present research (Thirunavukkarasu *et al*., 2014, Idania Rodeiro *et al*., 2015) [30, 11].

The peak points attained at different heights shows the relative concentration of various active compounds as a function of retention time, peak area (%), chemical nature and chemical structure of major compounds attained in seaweed samples. The relative abundance of mass spectra attained at different retention times for various compounds were compared with standard library and identified from National Institute of Standards and Technology data library (Balasubramanian, Ganesh, Shridhar Reddy, & Surya Narayana, 2014) [2].

3. Results and Discussion

3.1 Bioactive Profiles of Six Seaweeds

The preliminary bioactive screening of 15 different bioactive compounds of six seaweeds was assessed using methanol extracts (*Ulva lactuca*, *Acanthophora spicifera*, *Sargassum wightii*, *Ulva reticulata*, *Padina tetrastromatica* and *Kappaphycus alvarezii*) are given in Table 2. The bioactive compounds available in the seaweed extracts are rich in anthocyanins, anthraquinone, flavonoids, phenols, phlorotannins and saponins. The analysis also reveals the additional bioactive compounds available in the seaweed extract namely tannins, terpenoids, coumarins, glycosides, alkaloids, protein and phytosterols.

In ancient years, several plants act as an antioxidant for curing various degenerative diseases. The physiological and metabolic capabilities of marine organisms afford an immense potential for subsidiary metabolite production to live in complex habitat types are not established in terrestrial environments. Therefore, the marine algae are the substantial sources of familiar and novel bioactive compounds (Blunt, Copp, Hu, Munro, Northcote & Prinsep, 2007) [3].

The bioactive analysis of methanol extracts revealed the occurrence of diverse secondary metabolites like flavonoids, phytosterols and saponins with varied quantity. Flavonoids are recognized as a natural world tender class of drug with abundant biological and pharmacological properties. The flavonoid containing plants have gained interest was mainly due to their therapeutic properties like antioxidant, antiallergenic, anti-fungal, anticarcinogenic, anti-inflammatory, antithrombotic and hepatoprotective activities in recent years (Jiang, Zhan, Liu, & Jiang, 2008) [13].

Table 2: Bioactive profiles of six seaweeds

S. No.	Bioactive Compounds	1	2	3	4	5	6
1	Acids	-	-	-	-	-	-
2	Alkaloids	+	+	-	+	-	+
3	Anthocyanins	+	+	+	+	+	+
4	Anthraquinone	+	+	+	+	+	+
5	Coumarin	+	-	-	+	-	-
6	Flavonoids	+	+	+	+	+	+
7	Glycosides	-	-	+	-	+	-
8	Phenol	+	+	+	+	+	+
9	Phlorotannins	+	+	+	+	+	+
10	Phytosterols	-	+	+	-	+	-
11	Protein	+	+	+	+	+	+
12	Reducing sugar	-	-	-	-	-	-
13	Saponins	+	+	+	+	+	+
14	Tannins	+	+	-	+	-	+
15	Terpenoids	+	+	+	+	+	+

(+) - Present, **(-)** - Absent

1. *Ulva lactuca*, 2. *Ulva reticulata*, 3. *Sargassum wightii*,
4. *Padina tetrastromatica*, 5. *Kappaphycus alvarezii*,
6. *Acanthophora spicifera*

The phytosterols and steroids are the active compounds used as intermediate in the bioartificial ancestor for cardenolides and downstream of minor natural products in plants. Marine algae are a rich source of unsaponifiables and non-toxic sterols with medicinal value. The presence of saponins possesses numerous biological properties like haemolytic effects, antimicrobial, anti-inflammatory and antifeedant (Xu, Chen, Liang, Gu, Lui, Leung, & Li, 2000) [35]. The excellent source of active substances, namely anthocyanins, anthraquinone, flavonoids, phytosterols, glycosides, terpenoids and saponins present in crude extracts of *Sargassum Wightii*. It provides biological properties like anti-fungal, antibacterial, antioxidant and anti-carcinogenic (Aseer Manilal *et al*., 2010) [1].

3.2 Minimum Inhibitory and Bactericidal Concentration of Six Seaweeds

MIC and MBC of *Ulva lactuca*, *Acanthophora spicifera*, *Sargassum wightii*, *Ulva reticulata*, *Padina tetrastromatica* and *Kappaphycus alvarezii* extracts are shown in Table 3. The MIC against *Klebsiella pneumoniae* bacteria of six seaweed species exhibited 250mg/ml. MBC results showed good activity against *Klebsiella pneumoniae*. The MBC of six seaweed species ranged from 250mg/ml to 1000mg/ml. MBC of *Ulva reticulata*, *Sargassum wightii* and *Ulva lactuca* are 1000mg/ml, followed by *Kappaphycus alvarezii* and *Acanthophora spicifera* (500mg/ml MBC); and *Padina tetrastromatica* (250 mg/ml MBC).

Table 3: MIC and MBC values in six seaweeds extracts

S. No.	Seaweed Samples	MIC and MBC values (mg/ml) in six seaweed extracts against *Klebsiella pneumoniae*	
		MIC (mg/ml)	MBC (mg/ml)
1.	*Ulva lactuca*	250	1000
2.	*Acanthophora spicifera*	250	500
3.	*Sargassum wightii*	250	1000
4.	*Ulva reticulata*	250	1000
5.	*Padina tetrastromatica*	250	250
6.	*Kappaphycus alvarezii*	250	500

All six seaweed species were exhibiting good antimicrobial properties in the MIC value of 250mg/ml against *Klebsiella pneumoniae*. Furthermore, the minimum bactericidal concentration of 250mg/ml for *Padina tetrastromatica* seaweed, exhibited better antibacterial activities against *Klebsiella pneumoniae* compared to the other seaweeds.

3.3 Minimum Inhibitory and Fungicidal Concentration of Six Seaweeds

MIC and MFC of *Ulva lactuca, Acanthophora spicifera, Sargassum wightii, Ulva reticulata, Padina tetrastromatica,* and *Kappaphycus alvarezii* extracts are shown in Table 4. The MIC against *Candida albicans* fungi of six seaweed species ranged from 62.5 mg/ml to 250 mg/ml. The MIC values of *Sargassum wightii, Kappaphycus alvarezii* and *Acanthophora spicifera* are 62.5mg/ml. The MIC values of *Padina tetrastromatica, Ulva lactuca* and *Ulva reticulata* were 250 mg/ml. The results were identified to coincide well with the anti-fungicidal activity presented in Table 4.

All six seaweed species were exhibiting good antifungal properties against *Candida albicans*. The MFC of six seaweed species ranged from 250mg/ml to 1000mg/ml. The MFC of *Ulva reticulata* and *Sargassum wightii* are 1000mg/ml, followed by *Kappaphycus alvarezii* and *Ulva lactuca* (500 mg/ml), *Padina tetrastromatica* and *Acanthophora spicifera* (250 mg/ml).

Table 4: MIC and MFC values in six seaweeds extracts

S. No.	Seaweed Samples	MIC and MFC values (mg/ml) in six seaweed extracts against *Candida albicans*	
		MIC (mg/ml)	MFC (mg/ml)
1.	*Ulva lactuca*	250	500
2.	*Acanthophora spicifera*	62.5	250
3.	*Sargassum wightii*	62.5	1000
4.	*Ulva reticulata*	250	1000
5.	*Padina tetrastromatica*	250	250
6.	*Kappaphycus alvarezii*	62.5	500

The *Sargassum wightii, Acanthophora spicifera* and *Kappaphycus alvarezii* exhibit minimum inhibitory concentration value of 62.5 mg/ml is highly significant for its antifungal activity. The *Padina tetrastromatica* and *Acanthophora spicifera* showed the minimum fungicidal concentration value of 250 mg/ml has excellent antifungal activity against *Candida albicans*. The *Padina tetrastromatica* showed higher antibacterial and antifungal properties (Padmini Sreenivasa Rao, 1991) [23]. The seaweed extracts are proven to be the excellent antimicrobial properties due to the presence of significant bioactive compounds. Padmini Sreenivasa Rao (1996) and Fernandez *et al*. (1996) suggested that phenolic compounds present in seaweed and other marine plants exhibited good antimicrobial activity (Sreenivasa Rao, & Shelat, 1979; Fernamdez, Garcia, & Saienz, 1996) [29, 10]. Boonchum *et al*. (2011) [4] have reported that aqueous extracts of *Trochomorpha conoides* and the *Halimeda macroloba decaisne* extracts using ethanol shows higher levels of total phenolics (Boonchum *et al*., 2011) [4].

All six seaweed extracts (*Ulva reticulata, Ulva lactuca, Acanthophora spicifera, Sargassum wightii, Padina tetrastromatica* and *Kappaphycus alvarezii*) provided good antimicrobial property against Gram-negative bacteria (*Klebsiella Pneumoniae*) and fungus (*Candida albicans*) which are the common pathogenic organisms create infection on human skin. The organic solvent such as methanol provides higher efficiency for the extraction of bioactive compounds from six seaweeds. The performance of antibacterial activities of six seaweed extracts was also compared to other organic solvents (Inci Tuney, Bilge Hilal Cadirci, Dilek Unal, & Atakan Sukatar, 2006) [12]. Therefore, the methanol extracts of six seaweeds are suitable to develop antimicrobial agents used in pharmaceutical and allied industries.

3.4 Characterising the Antioxidant Properties of Six Seaweed Extracts

It is difficult to measure the various antioxidant compounds in crude extracts of six seaweeds. In recent years, several antioxidant assay methods are developed to screen the antioxidant compounds and also estimate the total antioxidant activity of seaweeds (Prabhakar *et al*., 2006) [26]. DPPH radical-scavenging activity is normally used for analysing the antioxidant compounds, particularly polyphenols and anthocyanins available in marine algae (Duan, Zhang, Li & Wang, 2006; Chandini, Ganesan, & Bhaskar, 2008; Wang, Zhang, Duan, & Li, 2009) [8, 5, 33].

DPPH radical was scavenged by two active compounds, namely polyphenols and anthocyanins for donating the hydrogen atom to bioactive compounds forming the reduced DPPH-H. After reduction, the solution turns from purple colour to yellow colour was quantified based on the absorbance of the spectrum at 517nm wavelength using UV-VIS Spectrophotometer (Chang, Ho, Sheu, Lin, Tseng, Wu, & Huang, 2007) [6]. In this study, the evaluation of antioxidant activities using total antioxidants (Table 5) and DPPH radical scavenging (Table 6) assay for the methanol extracts of six seaweeds such as *Ulva reticulata, Sargassum wightii, Ulva lactuca, Padina tetrastromatica, Kappaphycus alvarezii* and *Acanthophora spicifera* was analysed.

3.5 Total antioxidant activity

All six seaweed extracts were assessed using total antioxidant activity and presented in Table 5. The test result shows that the maximum amount of antioxidant activity was attained at minimum concentration (250µg/ml) of six seaweed extracts. Hence, for the DPPH assay, the same concentration (250µg/ml) was selected for the analysis.

Table 5: Total antioxidant activity assay of six seaweeds

Concentration (µg/ml)	TAC expressed µg/ml of Ascorbic acid					
	Kappaphycus alvarezii	*Sargassum wightii*	*Acanthophora spicifera*	*Ulva lactuca*	*Padina tetrastromatica*	*Ulva reticulata*
50	38.75	59.50	40.00	47.25	28.25	32.00
100	66.00	84.50	54.25	55.50	30.50	38.50
150	98.00	96.75	56.25	103.62	32.50	43.75
200	119.00	108.75	84.75	110.00	37.50	47.75
250	125.75	120.37	88.25	117.5	40.00	62.50

3.6 DPPH radical scavenging assay

DPPH radical scavenging assay of six seaweed extracts was assessed and presented in Table 6. During this analysis, the *Acanthophora spicifera* extract showed remarkably higher scavenging activity of 77±0.17% inhibition followed by *Ulva lactuca* (73±0.08%), *Ulva reticulata* (56±0.03%), *Sargassum wightii* (54±0.08%), *Padina tetrastromatica* (53±0.89%) and *Kappaphycus alvarezii* (39±0.13%).

The inhibition percentage obtained from the DPPH assay of the six seaweeds was dose-dependent. The experimental trials were conducted three times during the study to achieve an effective inhibition percentage of antioxidant activities. However, these extracts showed a lower DPPH scavenging assay compared to standard ascorbic acid (ASCA). The result coincides with previous studies of the variety of plants, including brown algae and red algae (Park, Shahidi & Jeon, 2004; Kuda, Tsunekawa, Goto, & Araki, 2005) [24, 16].

Table 6: DPPH radical scavenging assay (Inhibition %) of six seaweeds

S. No.	Seaweeds	Ascorbic acid (AscA)	DPPH (Inhibition %)
1.	*Kappaphycus alvarezii*	95 ± 0.13	39 ± 0.13
2.	*Ulva lactuca*	95 ± 0.13	73 ± 0.08
3.	*Padina tetrastromatica*	95 ± 0.13	53 ± 0.89
4.	*Ulva reticulata*	95 ± 0.13	56 ± 0.03
5.	*Sargassum wightii*	95 ± 0.13	54 ± 0.08
6.	*Acanthophora spicifera*	95 ± 0.13	77 ± 0.17

(Concentration of extracts used = 250 µg/ml)

3.7 Identification of Bioactive Compounds of Seaweed Extracts using GC-MS analysis

The extracts of the six algae under study, using GC-MS analysis revealed through the spectrum lines, and confirm the presence of major bioactive compounds in each seaweed species that attribute to the antioxidant, antibacterial, anti-cancer and antifungal properties.

3.7.1 GC-MS analysis of Ulva Lactuca

GC-MS analysis of *Ulva Lactuca* extract reveals the retention time corresponding to the compounds is shown in Figure 1. The retention time of each bioactive compound attributed to the antifungal, anti-cancer, antibacterial and antioxidant properties.

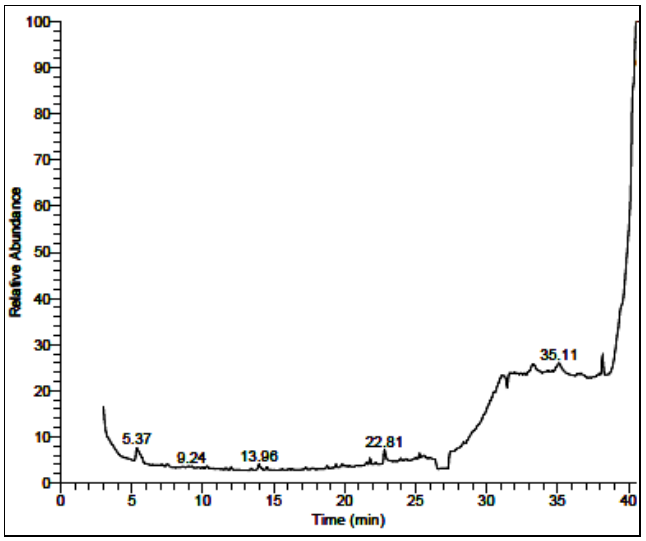

Fig 1: GC-MS analysis of *Ulva lactuca*

The significant compounds identified during the analysis were butyl hydroquinone, methyl pentadecanoate, octadecanoic acid, chloro diphenylquinoline and benzamide. The chemical nature corresponding to the obtained compounds attributed to antifungal, anti-cancer, antibacterial and antioxidant properties is benzoic acid, decanoic acid, stearic acid, quinolone and methoxybenzamide respectively (Mayer, & Hamann, 2004) [18]. The vibrant principles in *Ulva Lactuca* with their chemical nature, retention time, peak area (%) and activities are presented in Table 7.

Table 7: Chemical nature, retention time, peak area (%) and activity of bioactive compounds identified in *Ulva Lactuca* extract by GC-MS analysis

S. No.	Name of the compound	Molecular formula	Chemical nature	Retention time	Peak area (%)	Activity
1.	Butylhydroquinone	$C_{13}H_{18}O_2$	Benzoic acid	13.96	1.67	Antioxidant, Antibacterial, Antifungal.
2.	Methyl pentadecanoate	$C_{16}H_{32}O_2$	Decanoic acid	22.84	20.15	Antioxidant, Antibacterial, Antifungal.
3.	Octadecanoic acid	$C_{19}H_{38}O_2$	Stearic acid	26.52	18.21	Antioxidant, Antibacterial Antifungal.
4.	Chloro diphenylquinoline	$C_{21}H_{14}ClN$	Quinolone	35.11	2.55	Antioxidant, Antimicrobial.
5.	Benzamide	$C_{18}H_{21}NO_4$	Methoxy benzamide	36.46	1.20	Antioxidant, Antibacterial, Anti-cancer

3.7.2 GC-MS analysis of Ulva Reticulata

GC-MS analysis of *Ulva Reticulata* extract reveals the retention time corresponding to the compounds is shown in Figure 2. The retention time of each bioactive compound attributed to the antifungal, anti-cancer, antibacterial and antioxidant properties. This was evident from the retention time peaks from *Ulva Reticulata* and *Ulva Lactuca* seaweeds that are given in Figure 1 and Figure 2 respectively.

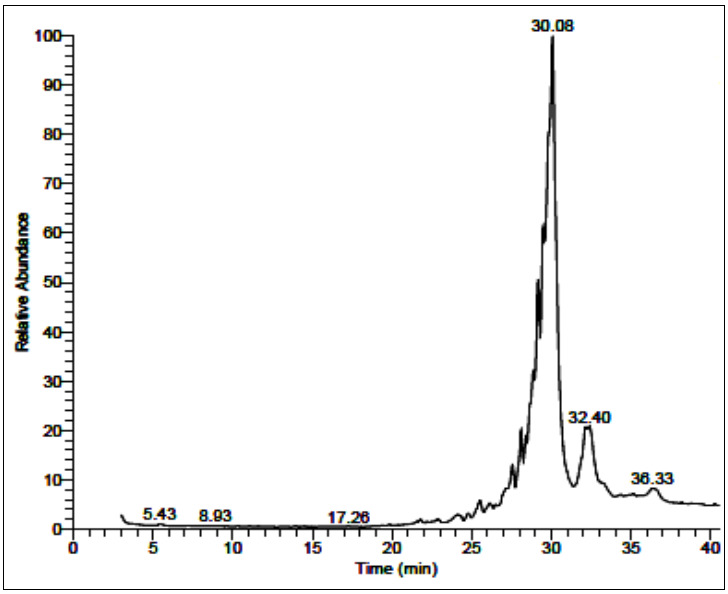

Fig 2: GC-MS analysis of *Ulva reticulata*

During the analysis, ester compounds of tetradecanoic acid, hydroxy vitamin, benzoyl-dihydro-hydroxy-furo benzopyran one, difluoro-pentafluorophenyl-dimethylsilyloxy-benzene and dinitro-bis-fluorene were identified for *Ulva Reticulata* extracts.

Table 8: Chemical nature, retention time, peak area (%) and activity of bioactive compounds identified in *Ulva Reticulata* extract by GC-MS analysis

S. No.	Name of the compound	Molecular formula	Chemical nature	Retention time	Peak area (%)	Activity
1.	Tetra decanoic acid	$C_{14}H_{28}O_2$	Myristic acid, ester	17.26	7.46	Antioxidant, Antibacterial, Antifungal
2.	Hydroxy vitamin	$C_{27}H_{44}O_2$	Vitamin D_3	28.98	16.34	Antioxidant, Anti-inflammatory
3.	Benzoyl-dihydro hydroxy flurobenzo pyranone	$C_{27}H_{22}O_6$	Benzyl pyranone	30.08	23.34	Antioxidant, Antibacterial Antifungal
4.	Fluoro Penta Fluoro Phenyl dimethyl silyloxy benzene	$C_{14}H_9F_7OSi$	Methyl silyloxy benzene	32.40	1.75	Antioxidant, Anti-cancer, Anti-inflammatory, Anti-androgenic
5.	Dinitro-bis-fluorene	$C_{25}H_{18}N_4O_4$	Fluorene	36.33	0.52	Antioxidant, Antibacterial, Anti-cancer

It can be seen from the Table 8, indicated that more ester groups obtained from *Ulva Reticulata* seaweed extract attributing to the antifungal, antioxidant, anti-cancer and antibacterial properties (Mayer & Hamann, 2004) [18]. The chemical nature corresponding to the obtained compounds was myristic acid, vitamin D_3, benzyl pyranone, methyl silyloxy benzene and fluorine.

3.7.3 GC-MS analysis of Sargassum Wightii

GC-MS analysis of *Sargassum Wightii* extract reveals the compounds attributing to antifungal, anti-cancer, antibacterial and antioxidant properties with corresponding retention time is shown in Figure 3.

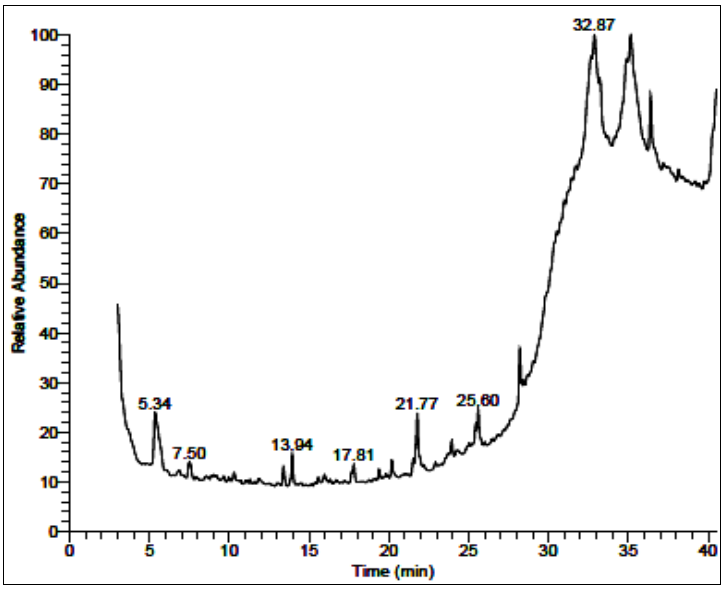

Fig 3: GC-MS analysis of *Sargassum wightii*

During the GC-MS analysis, only three ester compounds representing the antioxidant activity were obtained. The compounds identified during the analysis are the tetradecanoic acid-methyl ester, hexadecanoic acid-methyl ester and octadecanoic acid-methyl ester. The vibrant principles in *Sargassum Wightii* with their chemical nature, retention time, peak area (%) and activity are presented in Table 9.

Table 9: Chemical nature, retention time, peak area (%) and activity of bioactive compounds identified in *Sargassum Wightii* extract by GC-MS analysis

S. No.	Name of the compound	Molecular formula	Chemical nature	Retention time	Peak area (%)	Activity
1.	Tetradecanoic acid-methyl ester	$C_{14}H_{28}O_2$	Myristic acid, ester	17.81	5.94	Antioxidant, Antibacterial, Antifungal.
2.	Hexadecanoic acid-methyl ester	$C_{16}H_{32}O_2$	Palmitic acid, ester	21.77	5.33	Antioxidant, Antibacterial Antifungal.
3.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	Stearic acid, ester	25.60	2.84	Antioxidant, Anti-cancer, Anti-inflammatory

The chemical natures of the compounds obtained are attributed to antifungal, anti-cancer, antibacterial and antioxidant properties are stearic acid, myristic acid and palmitic acid.

3.7.4 GC-MS analysis of *Padina tetrastromatica*

GC-MS analysis of *Padina tetrastromatica* extract, and the corresponding retention time of the compounds is shown in Figure 4. The retention time of each bioactive compound attributed to the antifungal, anti-cancer, antibacterial and antioxidant properties. During the analysis, three ester compounds of tetradecanoic acid-methyl ester, hexadecanoic acid-methyl ester and octadecanoic acid-methyl ester representing the antioxidant activity were identified. The chemical nature corresponding to the obtained compounds was palmitic acid, stearic acid, and myristic acid (Reichelt, & Borowitzka, 1984) [28].

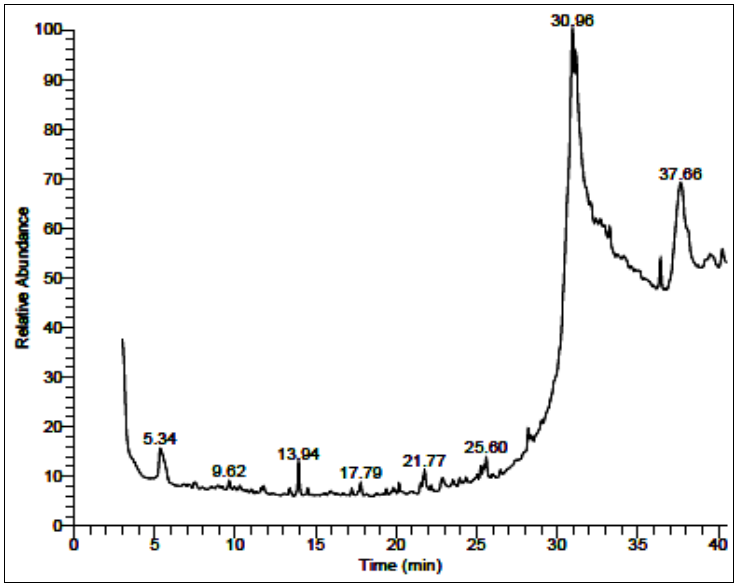

Fig 4: GC-MS analysis of *Padina tetrastromatica*

The vibrant principles in *Padina tetrastromatica* with their chemical nature, retention time, peak area (%) and activity are presented in Table 10.

Table 10: Chemical nature, retention time, peak area (%) and activity of bioactive compounds identified in *Padina tetrastromatica* extract by GC-MS analysis

S. No.	Name of the compound	Molecular formula	Chemical nature	Retention time	Peak area (%)	Activity
1.	Tetradecanoic acid-methyl ester	$C_{14}H_{28}O_2$	Myristic acid, ester	17.79	5.94	Antioxidant, Antibacterial, Antifungal.
2.	Hexadecanoic acid-methyl ester	$C_{16}H_{32}O_2$	Palmitic acid, ester	21.77	4.05	Antioxidant, Antibacterial Antifungal.
3.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	Stearic acid, ester	25.60	2.29	Antioxidant, Anti-cancer, Anti-inflammatory, Anti-androgenic

Usha & Rani (2015) [31] reported the presence of similar chemical compounds attributed to be antifungal, antioxidant, anti-cancer and antibacterial properties obtained from *Padina pavonia (Linnaeus) Lamour* seaweed. The earlier research work indicates the presence of antifungal, anti-cancer, antibacterial and antioxidant compounds like tetradecanoic acid-methyl ester, n-hexadecanoic acid and octadecanoic acid in the seaweed extracts are similar to that of the present analysis (Usha, & Rani, 2015) [31].

3.7.5 GC-MS analysis of *Acanthophora spicifera*

GC-MS analysis of *Acanthophora spicifera* extract revealed the retention time corresponding to the compounds is shown in Figure 5.

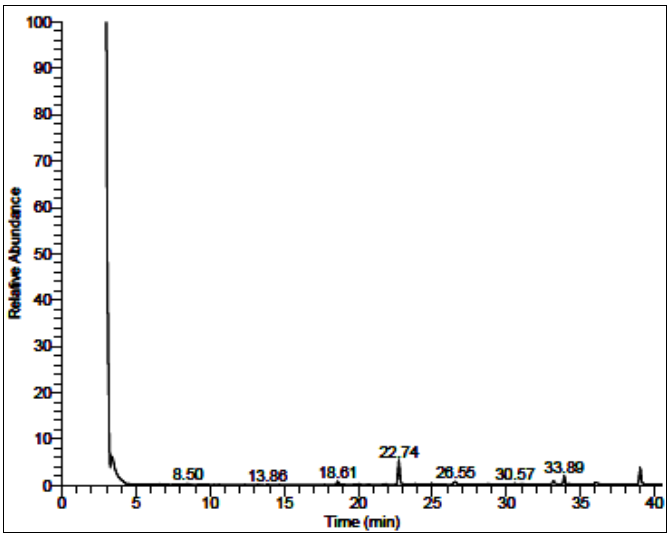

Fig 5: GC-MS analysis of *Acanthophora spicifera*

The compounds identified during the analysis were butyl hydroquinone, tetradecanoic acid, methyl pentadecanoate, octadecanoic acid and tetrahydroxy dimethylanthracene. The vibrant principles in *Acanthophora spicifera* with their chemical nature, retention time, peak area (%) and activities are given in Table 11.

The chemical nature corresponding to the obtained compounds attributed to antifungal, anti-cancer, antibacterial and antioxidant properties are benzoic acid, myristic acid, decanoic acid, stearic acid and imidazole.

Table 11: Chemical nature, retention time, peak area (%) and activity of bioactive compounds identified in *Acanthophora spicifera* extract by GC-MS analysis

S. No.	Name of the compound	Molecular formula	Chemical nature	Retention time	Peak area (%)	Activity
1	Butylhydroquinone	$C_{13}H_{18}O_2$	Benzoic acid	13.86	0.67	Antioxidant, Antibacterial, Antifungal.
2	Tetradecanoic acid	$C_{14}H_{28}O_2$	Myristic acid	18.61	4.37	Antioxidant, Antibacterial, Antifungal.
3	Methyl pentadecanoate	$C_{16}H_{32}O_2$	Decanoic acid	22.74	23.35	Antioxidant, Antibacterial Antifungal.
4	Octadecanoic acid	$C_{19}H_{38}O_2$	Stearic acid	26.55	4.64	Antioxidant, Antimicrobial.
5	Tetrahydroxy dimethylanthracene	$C_{16}H_{14}O_4$	Imidazole	33.89	8.19	Antifungal, Anticancer.

3.7.6 GC-MS analysis of *Kappaphycus alvarezii*

GC-MS analysis of *Kappaphycus alvarezii* extract revealed the compounds attributing to antifungal, anti-cancer, antibacterial and antioxidant properties with corresponding retention time is illustrated in Figure 6. During the GC-MS analysis, the four different bioactive compounds representing the antioxidant, antifungal, antibacterial and anti-cancer activities were observed. The compounds identified during the analysis were the tetradecanoic acid, hexaacetate, hexadecanoic acid, triacontanoic acid, sorbitol, and benzyl mycalamide.

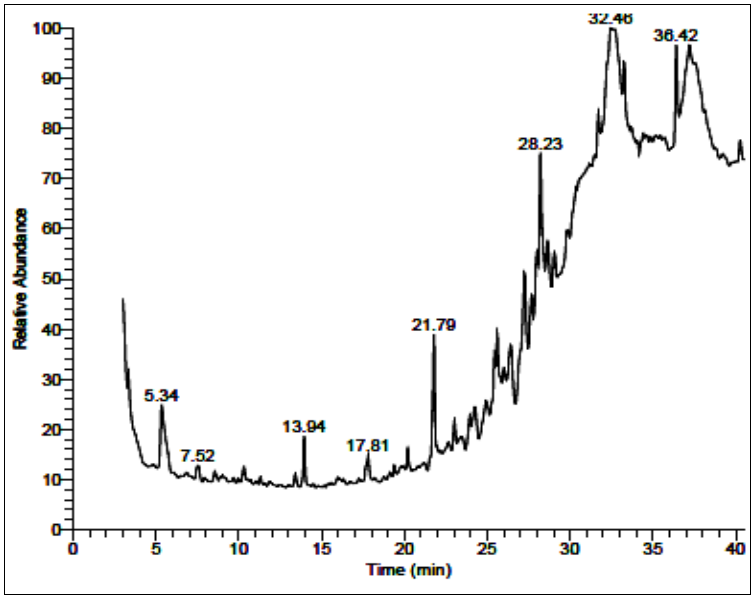

Fig 6: GC-MS analysis of *Kappaphycus alvarezii*

The vibrant principles in *Kappaphycus alvarezii* with their chemical nature, retention time, peak area (%) and activities are presented in Table 12. The chemical natures corresponding to the obtained compounds attributed to antifungal, anti-cancer, antibacterial, and antioxidant properties are myristic acid, palmitic acid, D-glucitol, methyl triacontanoate and benzoic acid respectively.

Table 12: Chemical nature, retention time, peak area (%) and activity of bioactive compounds identified in *Kappaphycus alvarezii* extract by GC-MS analysis

S. No.	Name of the compound	Molecular formula	Chemical nature	Retention time	Peak area (%)	Activity
1.	Tetradecanoic acid	$C_{15}H_{30}O_2$	Myristic acid	17.81	1.57	Antioxidant, Antibacterial, Antifungal.
2.	Hexadecanoic acid	$C_{17}H_{34}O_2$	Palmitic acid	21.79	1.97	Antioxidant, Antibacterial, Antifungal.
3.	Sorbitol hexaacetate	$C_{18}H_{26}O_{12}$	D-Glucitol	28.23	10.16	Antioxidant, Antimicrobial.
4.	Triacontanoic acid	$C_{31}H_{62}O_2$	Methyl triacontanoate	32.46	2.21	Antioxidant, Anti-cancer.
5.	Benzyl mycalamide	$C_{31}H_{47}NO_{10}$	Benzoic acid	36.42	6.02	Antioxidant, Antibacterial.

4. Conclusion

The significant bioactive compounds obtained from six seaweed extracts are attributed to antibacterial, antifungal, anti-inflammatory, anti-androgenic, anti-cancer, and antioxidant properties are identified using GC-MS analysis. The bioactive compounds obtained during this analysis were triggered interest for making natural products using different types of seaweeds in medical applications. The present uses of seaweeds mainly in the area of cosmetics, fertilisers, human foods and used for the extraction of industrial gums and chemicals. It can also used for the treatment of asthma, goiters, tumour, cancer, cough, ulcers, and urinary diseases.

Conflict of Interest

The authors declare that they have no conflict of interest.

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