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## GC-MS and antimicrobial activity analysis of the ethanolic extract of *Caulerpa taxifolia* (M. Vahl) C. Agardh

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**Abstract**

Seaweeds produce various types of secondary metabolites aiding in the protection against several environmental stresses. Many such compounds possess biological activities that have been explored by researchers to develop new drugs. Many macroalgae have been proven as the potential resources for unconventional drugs able to control new diseases or multi-resistant strains of pathogenic microorganisms. To identify new natural resources, their biological properties the chemical composition of *Caulerpa taxifolia*, the microalga was screened by GC-MS technique. GC-MS analysis of the Ethanolic extract of *Caulerpa taxifolia* was carried out and 16 major compounds were found to be present. An *in vitro* anti-bacterial study of ethanolic extract was carried out by agar diffusion method against four microorganisms. Further studies could be continued to check the medicinal potentiality of the compounds available in the extract which can help to treat targeted diseases that could be helpful for mankind.

**Keywords:** *Caulerpa taxifolia*, GC-MS analysis, ethanolic extract and seaweeds

**Introduction**

In recent years, there has been a growing interest in the discovery of new bioactive compounds from marine sources, such as microalgae, seaweeds, and crustaceans. However, the use of novel natural compounds is only conceivable when these are cost-effective and economically viable from an ecological standpoint (Cotas *et al.*, 2020) [20]. In this sense, there is still a long way to go so that new natural products that emerge are economically viable. *Caulerpa* is an edible alga and is used as a salad in Asian countries, especially India and China (Sasikala and Geetha Ramani, 2017) [5]. Among the known *Caulerpa* species, *Caulerpa taxifolia* is an important species and more secondary metabolites producer that protect the plant from herbivores. In addition, it produces di-indole pigments and other compounds with various biological activities (Etcherla, 2014) [6].

Millions of people are being afflicted by different infectious diseases induced by pathogenic bacteria. With the phenomena of high mortality rates and the emergence of new multi-drug resistance, bacterial strains have become one of the threatening health problems worldwide. The antimicrobial activity of microalgae extracts is generally assayed using various organic solvents (Cordeiro *et al.*, 2006) [10]. An organic solvent always provides a higher efficiency in extracting compounds for antimicrobial activity as compared to an aqueous extract (Masuda *et al.*, 1997 & Lima-Filho *et al.*, 2002) [13, 14]. Marine algae are a rich source of novel bioactive compounds that may find several applications (Aziz *et al.*, 2003 & Delattre *et al.*, 2005) [11, 12]. Secondary or primary metabolites from these organisms may be potential bioactive compounds of interest for the pharmacological industry. The cell extracts and active constituents of various algae have been shown to have antibacterial activity against Gram-positive and Gram-negative bacteria. The production of bioactive metabolites is considered to be a response to ecological pressures such as competition, protection from predators and reproduction.

Gas Chromatography-Mass Spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify various substances within a test sample. Applications of GC-MS include identification of unknown samples, virtually leading to drug development. In the last few years, gas chromatography-mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species (Robertson, 2005; Fernie *et al.*, 2004 & Kell *et al.*, 2005) [15, 16, 17].

GC-MS is the best technique to identify the bioactive constituents of long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc. Hence, Gas chromatography (GC) and Mass spectroscopy (MS) associated with particular detection techniques have become sophisticated means for the analysis of various compounds (Vinodh *et al.*, 2013) <sup>[18]</sup>.

### Materials and Methods

Fresh plants of *Caulerpa taxifolia* (M. Vahl) C. Agardh were collected from the Leepuram coastal area in the Kanyakumari district, Tamil Nadu, India. The plants were washed thoroughly in running water to remove soil particles and dust and finally washed with sterile distilled water. The leaves were shade-dried at room temperature. Dried leaves were uniformly pulverized using a mechanical grinder. The extract was prepared with 20.0 g of powdered plant material using 200 ml of ethanol with Soxhlet apparatus and it was used for the following tests and analysis. The images of the plant are shown in Figure 1.

### Antibacterial assay

#### Preparation of plates

The medium was prepared and sterilized as directed by the manufacturer. Defibrinated blood may be necessary for testes on fastidious organisms, in which case the medium should be allowed to cool to 50 °C before 7% of blood was added. Human blood was not recommended as it may contain antimicrobial substances. The medium should be poured into Petri dishes on a flat horizontal surface to a depth of 4mm (25 ml in an 85 mm circular dish; 60 ml in a 135 mm circular dish). Poured plates were stored + 4°C and used within one week of preparation. Before inoculation plates should be dried with lids so that there were no droplets of moisture on the agar surface. The time to achieve this depends on the drying conditions.

The pH of the medium should be checked at the time of preparation and should be 7.2 to 7.4.



**Fig 1:** Showing the image of *Caulerpa taxifolia*

### Preparation of inoculum

At least four morphologically similar colonies from an agar medium were touched with a wire loop and the growth was transferred to a test tube containing 1.5 ml of sterile suitable broth. The tubes were incubated for 2 hours at 35 °C to 37 °C to produce a bacterial suspension of moderate turbidity. The density of the suspension is standardized by dilution with sterile saline or broth to a density equivalent to the barium sulphate standard, 0.5 McFarland units. Before use, the standard should be shaken vigorously.

### Inoculation

Plates were inoculated within 15 minutes of preparation of the suspension so that density does not change. A sterile cotton-wool swab is dipped into the suspension and the surplus was removed by rotation of the swab against the side of the about the fluid level. The medium was inoculated by even streaking of the swab over the entire surface of the plate in three directions.

### Antibiotics discs

After the inoculum had dried, single discs were applied with forceps, a sharp needle or a dispenser and pressed gently to ensure even contact with the medium. When fastidious organisms were to be tested, multiple colonies were touched with a loop and cross-streaked on the appropriate plate for uniform distribution.

Not more than six discs can be accommodated on an 85-mm circular plate and twelve are easily accommodated on a 133-mm circular plate.

Discs were stored at 4°C in sealed containers with a desiccant and were allowed to come to room temperature before the containers were opened. An antimicrobial solution was prepared in the laboratory using the following procedure.

1. A 2.0 mm loopful of the standard antibiotic solution was picked up and lowered carefully onto a paper disc which, when moisture will adhere to the loop.
2. The disc was placed on the surface of inoculated plant in the appropriately labelled segment. Care was taken to avoid inadvertent "contamination" of other discs in the Petri dish of the antibiotic solution.
3. The same procedure was repeated for each antimicrobial agent used, placing the impregnated discs in their respectively labelled segments.

### Incubation

Plates were incubated for 16 to 18 hours at 35 to 37 °C aerobically or in a CO<sub>2</sub> atmosphere for fastidious organisms.

### Reading of zones of inhibition

The diameters of zones were measured to the nearest millimeter with Vernier calipers (preferably) or a thin transparent millimeter scale. The point of abrupt diminution of growth, which in most cases corresponds with the point of complete inhibition of growth, was taken as the zone edge. Organisms showing a film of growth within the susceptible zone were ignored.

### Interpretation

Each zone size was interpreted according to the organism by reference to the tables.

### Gas Chromatography-Mass Spectrometry

#### GC Analysis

The crude plant extract was subjected to centrifuge at about 10,000 rpm for about 30 minutes to remove the particulates.

The clear supernatant was aspirated using a pipette and transferred into a clean vial and labelled. Then the supernatant was subjected to gas chromatography analysis using equipment THERMO GC-TRACE ULTRA version 5.0 and equipped with DB 35 Non-polar capillary column dimension 30 mts, 0.25mm, film 0.25µm. The operation conditions as follows:

**Carrier gas:** He flow: 1.0 ML/Min

**Pressure:** 17 psi for the ware column and 16 psi for the Cp5 column.

**Oven temperature:** 70 °C Raised to 260 °C at 6 °C/min

**Injection volume:** 1 microliter

The peak area calculation was done by star work station and peak identification by comparison with authentic, wherever available calculation of Kovats Retention Index was done.

The Kovats Index system has been widely used in the analysis of food flavours, pesticides and essential oil analysis and essential oil analysis Kovats Retention Index, (I) is defined and calculated by the following equation

$$I = 100N + 100 n \log t' R (N + n) - \log t' R (N)$$

Where,

$t' R(N)$  = Adjusted retention time of n paraffin by hydrocarbon number (N+n) eluting before solution A.

$t' (N + n)$  = Adjusted retention time of n paraffin by hydrocarbon number (N+ n) eluting before solution A.

$t' (A)$  = Adjusted retention time OD solute A

### MS Analysis

Mass spectroscopy analysis was performed on a Shimadzu GC 17 AQP 5,000 MS coupled with a mass detector fitted non-polar DBS (Di-phenyl di benzyl solixane) capillary volume of length 25m × 0.25 mm id

GC MS operation conditions at initial temperature 60° C-300 ° C. The injection volume was 0.1 µl with a helium gas carrier at the flow rate 0.6 ml/minute. Relative Retention time (RRts) of constituents were determined using C5-C6 straight-chain alkanes as standards. Individual constituent of the extract was identified by WILEY and NIST database matching by comparison of mass spectra with published data and by comparison of their RRts.

### Results and Discussion

The antimicrobial activity of the extracts of *Caulerpa taxifolia* was studied against selected bacteria and fungi. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were chosen as the testing organism for bacteria. *Aspergillus niger* and *Candida albicans* were selected as the test organism for fungi. The antibacterial and antifungal potential of extracts was assessed based on the zone of inhibition of microbial growth developed. The results of the antibacterial and antifungal activities are presented in Table 1 & Figure 2.

**Table 1:** Shows the antimicrobial activity of ethanolic extracts of *Caulerpa taxifolia* (S1)

S. No.	Microbes	Stain	S1	Control
1.	Bacteria	<i>Staph aureus</i>	20 mm	17 mm (Amikacin)
		<i>Pseudomonas aeruginosa</i>	21 mm	16 mm (Amikacin)
2.	Fungi	<i>Aspergillus niger</i>	17 mm	14 mm (Nystatin)
		<i>Candida albicans</i>	14 mm	13 mm (Nystatin)

The results of the extracts of *Caulerpa taxifolia* were effective against the selected bacteria and fungi where the inhibitory effect was not much pronounced. In antibacterial activity, when compared with amikacin (standard drugs) the seaweed extracts showed more activity against *Pseudomonas aeruginosa* followed by *Staph aureus*. In antifungal activity, when compared with nystatin (standard drugs) the seaweed extracts showed more activity against *Aspergillus niger* followed by *Candida albicans*. The inhibition zone ranges from 14 to 22 mm for all the sensitive bacterial and fungal strains. The result reveals that the extracts obtained from *Caulerpa taxifolia* have strong activity against selected bacterial and fungal strains when compared with standard antibiotic drugs used in this antimicrobial screening process, extracts of *Caulerpa taxifolia* were found to be more effectively active against both the Gram-positive, Gram-negative, and fungal strains.

Earlier works with the *Ulva* extract was shown to have active against both Gram-negative and Gram-positive bacteria. Comparatively, the Gram-negative was the most resistant to the antimicrobial effect of different extracts tested. Among the specific pathogenic organisms tested, *Pseudomonas* sp. was not inhibited by any seaweed, in contrast to the results where activity was reported in non-Antarctic seaweeds,

including Rhodophyceae and Phaeophyceae (Shannon and Abu-Ghannam, 2016) [27]. Algae showed a minimum inhibitory activity when it was investigated in a study by Kim *et al.* (2013) [28] on ethanol extracts against oral microbial species. In the study conducted by Cai *et al.* (2020) [29], the ethanolic extract of *L. japonica* showed obvious antimicrobial effects against different stains. Among the tested pathogens, the ethanolic extract of *L. japonica* showed the strongest antimicrobial activity against *E. coli*. The antibacterial potential of numerous aqueous extracts from red and brown macroalgae was observed against *S. aureus* (Kamenarska *et al.*, 2009) [30] but to a lesser extent when compared with extracts obtained from different solvents, such as butanol and chloroform. A study by Darah and Lim (2015) [31] using marine algae extract *Enteromorpha intestinalis* against *B. subtilis*, *E. coli*, *P. aeruginosa*, *C. albican* and found only *B. subtilis* and *S. aureus* susceptibility towards the extract. This correlates with the current experiment that showed an inhibition zone against *B. subtilis*. Shanjida *et al.* (2015) [37] reported that, different concentrations of the methanolic extracts of the leaves of *Mangifera indica* exhibited antimicrobial activities against all the isolates of bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*).

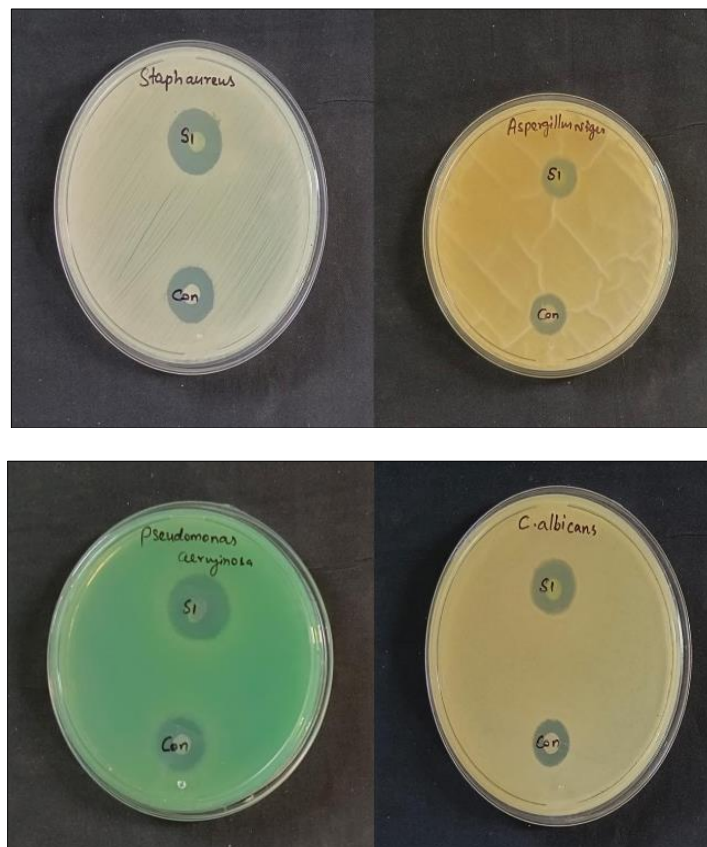


Fig 2: Shows the antimicrobial activity of the ethanolic extracts of *Caulerpa taxifolia* (S1) and control

### Gas chromatography-mass spectrometry

The Ethanolic extract of *Caulerpa taxifolia* was analyzed with GC-MS to detect phytochemicals. GC-MS technique provides the identification and quantification of chemical compounds based on their characteristic fragmentation

patterns at specific retention times. The result of GC-MS revealed a wide range of compounds identified and listed in Table 2. The variety of compounds including their molecular weight and biological activity is shown in Table 3 & Figure 3.

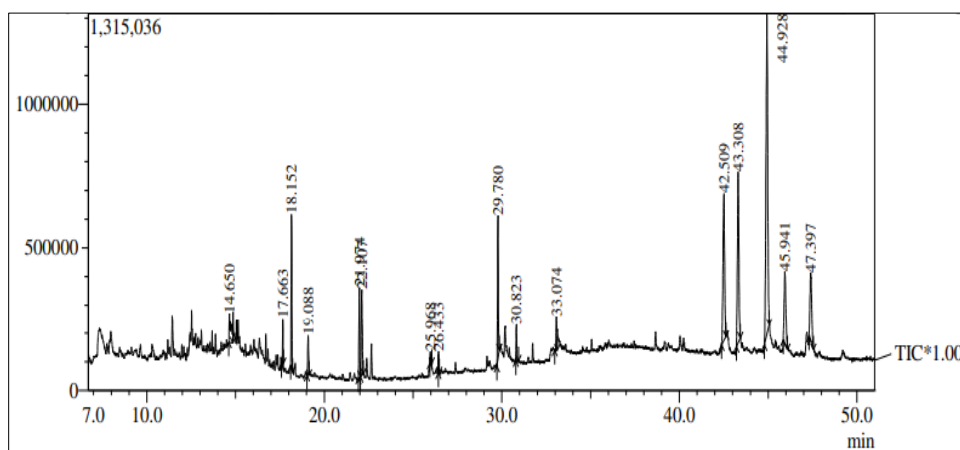


Fig 3: GC-MS chromatogram of ethanolic extracts of *Caulerpa taxifolia*

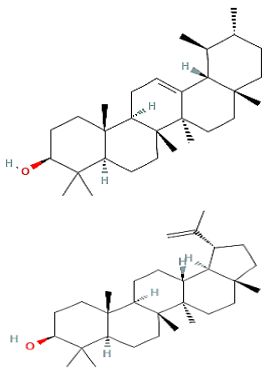
Table 2: Peak report of the Ethanolic extracts of *Caulerpa taxifolia*

Peak	Peak Time	Area	Area%	Height	Height%	Name	Base m/z
1	14.650	702739	2.82	104297	2.02	8-Pentadecanone	57.00
2	17.663	293783	1.18	157904	3.06	Methyl 2-hydroxy pentadecanoate	213.05
3	18.152	1367630	5.48	539145	10.46	Methyl palmitate	74.00
4	19.088	484003	1.94	138443	2.69	Dibutyl phthalate	148.95
5	21.974	936467	3.75	316161	6.13	Methyl Octadeca-9, 12-Dienoate	67.00
6	22.107	1283509	5.14	302114	5.86	9-Octadecenal, (Z)-	55.05
7	25.968	121460	0.49	40894	0.79	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester	58.05
8	26.433	123724	0.50	75548	1.47	2-Octenal, 2-Butyl-	55.05
10	29.780	1506575	6.04	503021	9.76	1-Heneicosanol	55.06
11	30.823	349871	1.04	128323	2.49	Diocetyl Phthalate	148.95

12	33.074	382293	1.53	120503	2.34	1-Hentetracontanol	57.10
13	42.509	3221740	12.91	514217	9.98	Ergost-5-en-3-ol	55.05
14	43.308	3393244	13.60	597928	11.60	Stigmata-5,22-dien-3-ol	55.05
15	44.928	7380641	29.58	1119577	21.72	Stigmast-5-en-3-ol, (3. Beta, 24S)-	57.05
16	45.941	1682820	6.74	254207	4.93	Alpha-amyrin	218.05
17	47.397	1719195	6.89	242713	43.71	Lupeol	218.05

**Table 3:** Physical properties and bioactivity of the compounds present in the ethanoilic extract of *Caulerpa taxifolia*

S. No.	Name of the compound	Mol Formula	Mol. Wt. g/mol	Structure	Biological activity
1	8-Pentadecanone	C <sub>15</sub> H <sub>30</sub> O	226.4		Antimicrobial and antioxidant activity
2	Methyl 2-hydroxy-pentadecanoate	C <sub>16</sub> H <sub>32</sub> O <sub>3</sub>	272.42		Antidyskinetic, Candidapepsin inhibitor and Antibacterial
3	Methylpalmitate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.5		Antieczematic, Antiseborrheic and Antiviral (Rhinovirus)
4	Dibutyl phthlate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278.34		Antiseborrheic and Antiviral (Picornavirus)
5	Methyl Octadeca-9, 12-Dienoate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.5		Antisecretoric, Antimutagenic and Anti-inflammatory
6	9-Octadecenal, (Z)-	C <sub>18</sub> H <sub>34</sub> O	266.5		Anti-infective and Antitoxic
7	3-Cyclopentylchloropropionic acid, 2-dimethylaminoethyl ester	C <sub>12</sub> H <sub>23</sub> NO <sub>2</sub>	213.32		Analeptic and Antitussive
8	2-Octenal, 2-Butyl-	C <sub>12</sub> H <sub>22</sub> O	182.30		Anti-inflammatory and Antiviral (Rhinovirus)
10	1-Heneicosanol	C <sub>21</sub> H <sub>44</sub> O	312.6		Antihypoxic and Antiviral (Picornavirus)
11	Diocetyl Phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390.6		Antisecretoric and Antiviral (Influenza)
12	1-Hentetracontanol	C <sub>31</sub> H <sub>64</sub> O	452.8		Antiinfective and antiviral
13	Ergost-5-En-3-ol	C <sub>31</sub> H <sub>56</sub> OSi	472.9		Antifungal and Antiviral
14	Stigmata-5,22-Dien-3-ol	C <sub>29</sub> H <sub>48</sub> O	412.7		Antiviral (Influenza) and Antifungal
15	Stigmast-S-EN-3-OL, (3.BETA.,24S)-	C <sub>29</sub> H <sub>50</sub> O	414.7		Chemo protectant and Antifungal

16	Alpha-Amyrin	C <sub>30</sub> H <sub>50</sub> O	426.7		Anti-inflammatory and Anti-Fungal
	Lupeol	C <sub>30</sub> H <sub>50</sub> O	426.7		Antiacne, Antithrombotic and Antiprotozoal

Similar to our results, Kumar *et al.* (2008) [32] showed that seaweeds encompass a momentous amount of proteins, vitamins, minerals and trace elements. Many macroalgae contains phenolic complexes and polysaccharides, having various biological activities

The red seaweed *Gracilaria corticata* was subjected to GC-MS analysis by Devi and Kumari (2022) [33] and its Phytochemicals such as Flavonoids, tannin, saponins, terpenoids, glycosides, steroids, fat and fixed oil and several bioactive compounds were observed by using GC-MS analysis. GC-MS analysis was done by Priya and Rajasekaran (2022) [34] where seven components were identified among which has the highest peak and the highest molecular weight. Fatty acids and carotenoid pigments are present at high concentrations in seaweeds, including the species of *U. fasciata* studied by Bhagavathy and P Sumathi (2012) [35]. Carbohydrates, proteins, saponins, alkaloids, and flavonoids were also found to be present. The analysis performed in this study showed palmitic acid to be one of the main components in the fraction analyzed, and the biological importance of this molecule has been highlighted by Stoddart *et al.* (2008) [36]. Similar to our results, the presence of phytochemical compounds such as alkaloids, terpenoids, polyphenols and flavonoids in the ethyl acetate extract of fresh seagrass (Hardoko *et al.*, 2016) [38]. Another study reported the presence of flavonoids, phenols, steroids and glycosides in the *C. serrulata* extracts (Bharathi *et al.*, 2019) [39].

GC-MS results revealed the presence of 16 compounds with different natures present in the ethanolic extract and it also showed significant antimicrobial activity when specific assays were performed. Thus, further study could be continued to check the medicinal potentiality of this extract having important active compounds that can help to treat targeted diseases which could be helpful for mankind.

### Summary and Conclusion

More than 70% of the world's surface is covered by oceans and the wide diversity of marine organisms offers a rich source of natural products. Marine algal invasions have played important roles in community structures around the world. The genus *Caulerpa* is nearly comprised of 60 species which are broadly distributed in tropical and subtropical waters.

Fresh plants of *Caulerpa taxifolia* (M. Vahl) C. Agardh were collected from the Leepuram coastal area in the Kanyakumari district, Tamil Nadu, India.

Standard procedures were used for antimicrobial analysis. Two bacteria (*Staph aureus* and *Pseudomonas aeruginosa*) and two fungal species (*Aspergillus niger* and *Candida albicans*) were taken as test organisms. Ethanolic extracts of *Caulerpa taxifolia* were subjected to GC-MS analysis.

The results showed better antibacterial activity against pathogens used. The results showed that the plant is a potential source of bioactive compounds and could be investigated for natural antibiotics. GC-MS analysis showed the presence of 16 major phytoconstituents with the ethanolic extract. The study has been carried out with the objectives of phytochemical screening, assessing their efficiency as antimicrobial agents. The identified compounds have several biological activities like antimicrobial, antioxidant, anticancer, hypercholesterolemic, anti-inflammatory and other activities. Hence, the presence of phytochemicals could be attributed to their efficiency in their therapeutic effects.

The present work reveals that the extracts obtained from *Caulerpa taxifolia* have strong activity against all the tested bacterial and fungal strains when compared with standard antibiotic drugs used in this screening process. From the results obtained, it could be concluded that *Caulerpa taxifolia* contains various bioactive compounds. However further research should be carried out to identify and purify these antibacterial and antifungal substances. It is a significant source of novel bioactive compounds. Future studies (*in vitro* and *in vivo*) can reveal the potential medicinal properties of the *Caulerpa taxifolia*.

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