



**Comprehensive Biochemical Profiling of *Caulerpa racemosa* from the Libyan Coast: Integrative Analysis of Fatty Acids, Bioactive Metabolites, and Volatile Constituents via GC-MS**

Hana Abohbell

Department of Botany, Faculty of Science, University of Sabratha, Libya.

[hana.abohbell@sabu.edu.ly](mailto:hana.abohbell@sabu.edu.ly)

**ORCID ID:** <https://orcid.org/0009-0001-5746-8304>

**Abstract:**

This GC-MS based study analyzed the ethanolic extract of the *Caulerpa racemosa*, collected from the Libyan coast. Characterization revealed a diverse chemical profile with monounsaturated fatty acids, fatty acid derivatives, terpenoids, steroids and oxygenated lipids of diverse chemistry. The fatty acid profile showed cis-vaccenic acid (1.45%) and oleic acid (0.72%), and cis-11-eicosenoic acid (0.79%), and trans-13-octadecenoic acid (0.53%), suggesting a significant amount of important biological unsaturated fatty acids. The volatile profile was dominated by bioactive constituents such as patchouli alcohol (0.61%), a sesquiterpenoid (5,9-Dimethyl-2-(1-methylethylidene)-1-cyclodecanol) (0.63%) and steroid (Spirost-8-en-11-one, 3-hydroxy-) (0.66%), as well as 11,13-dimethyl-12-tetradecen-1-ol acetate (2.88%). The study shows that *Caulerpa racemosa* is biochemically diverse and The detected metabolites indicate the potential of *C. racemosa* a promising source of bioactive compounds; however, further biological and pharmacological investigations are required to confirm these activities

**Keywords:** *Caulerpa racemosa*; GC-MS; marine metabolites; fatty acid profile; bioactive compounds; Libya; Mediterranean Sea

## الملخص العربي:

تناولت هذه الدراسة، المعتمدة على تقنية الكروماتوغرافيا الغازية المقترنة بمطيافية الكتلة (GC-MS)، تحليل المستخلص الإيثانولي لطحلب *Caulerpa racemosa* الذي جُمع من الساحل الليبي. وقد أظهر التوصيف الكيميائي وجود ملف كيميائي متنوع يضم أحماضاً دهنية أحادية غير مشبعة، ومشتقات الأحماض الدهنية، والتربينويدات، والستيرويدات، إضافة إلى دهون مؤكسجة ذات تراكيب كيميائية متعددة. أظهر تحليل الأحماض الدهنية وجود حمض cis-vaccenic بنسبة (1.45%)، وحمض oleic بنسبة (0.72%)، وحمض cis-11-eicosenoic بنسبة (0.79%)، وحمض trans-13-octadecenoic بنسبة (0.53%)، مما يشير إلى احتواء الطحلب على نسبة ملحوظة من الأحماض الدهنية غير المشبعة ذات الأهمية البيولوجية. أما المكونات المتطايرة، فقد تميزت بوجود عدد من المركبات الحيوية الفعالة، من بينها patchouli alcohol بنسبة (0.61%)، (5,9-Dimethyl-) sesquiterpenoid، (1-cyclodecanol -1-(1-methylethylidene) بنسبة (0.63%)، steroid، (Spirost-8-en-11-one, 3-hydroxy-) بنسبة (0.66%)، إضافة إلى مركب 11,13-dimethyl-12-tetradecen-1-ol acetate بنسبة (2.88%). وتشير المستقلبات التي تم التعرف عليها إلى أن طحلب *Caulerpa racemosa* يُعد مصدراً واعدًا للمركبات الحيوية الفعالة ذات التطبيقات المحتملة. ومع ذلك، فإن إثبات هذه الأنشطة يتطلب إجراء المزيد من الدراسات البيولوجية والدوائية للتحقق من فعاليتها.

الكلمات المفتاحية: *Caulerpa racemosa*; GC-MS؛ المستقلبات البحرية؛ ملف الأحماض الدهنية؛ المركبات الحيوية الفعالة؛ ليبيا؛ البحر المتوسط.



## Study Objectives

The primary objective of this research is to conduct a comprehensive biochemical characterization of the green alga *Caulerpa racemosa* native to the Libyan coastline. Specifically, the study aims to:

1. Identify and quantify the fatty acid composition using GC-MS.
2. Profile the volatile organic compounds and secondary metabolites.
3. Evaluate the nutritional and pharmaceutical potential based on the detected bioactive constituents.
4. Provide a baseline for future pharmacological studies on Libyan marine flora.

## Introduction

Marine ecosystems are centers of bio-diversity with an abundance of complex and structurally rich substances due to the varying ecological and adaptive pressures faced by marine organisms. These adaptations allow marine organisms to synthesize metabolites that differ significantly from land-based organisms in terms of their significant biological activities and chemical properties. Among these marine resources, macroalgae are recognized as vital renewable resources of growing scientific attraction, offering a diverse array of biologically active compounds valuable in health, nutrition, medicine, and industry (Smit, 2004, p. 245; Belkacemi et al., 2020, pp. 87–88; Rangunath et al., 2020, p. 1). *Caulerpa racemosa* is taxonomically classified within the family Caulerpaceae and the order Bryopsidales, and is widely distributed in tropical and subtropical marine environments (Guiry & Guiry, 2025; Klein & Verlaque, 2008, p. 206). The species is commonly consumed in several Asian coastal regions as an edible seaweed due to its high nutritional value, particularly its content of minerals, proteins, essential amino acids, dietary fiber, and a sophisticated distribution of lipids (Palaniyappan et al., 2023, p. 2; Prayogo et al., 2024, pp. 157–159). Previous phytochemical investigations demonstrated that *C. racemosa* contains a broad spectrum of secondary metabolites, including alkaloids, terpenoids, phenolic compounds, and lipid derivatives that contribute to its significant biological activities (Ornano et al., 2014, pp. 1795–1796; Tassakka et al., 2023, pp. 2–8). The pharmacological potential of *C. racemosa* has been increasingly documented during the last decade. Several studies have reported antioxidant and antibacterial activities



associated with crude and solvent extracts of the alga against different pathogenic microorganisms (Belkacemi et al., 2020, pp. 87–93; Sherley et al., 2023, p. 3). Furthermore, metabolites derived from *C. racemosa* exhibit promising cytotoxic effects associated with apoptosis induction and suppression of cancer cell proliferation in HeLa cell lines (Permatasari et al., 2022, pp. 113–118). In recent antiviral research, in silico molecular docking analyses have suggested that selected compounds isolated from *Caulerpa* species possess potential binding affinity toward specific SARS-CoV-2 molecular targets, indicating a possible potential that warrants further experimental validation (Tassakka et al., 2023, pp. 1–10). Anti-inflammatory activities have likewise been associated with several bioactive fractions of the alga through the modulation of inflammatory mediators and oxidative stress pathways (Fernando et al., 2016, pp. 22–25; Prayogo et al., 2024, pp. 159–163).

Central to these biological functions are fatty acids, which play an essential role in the physiological adaptation of algae to competitive marine environments, serving as primary chemical defenses against pathogenic microorganisms and grazers (Pérez et al., 2016, p. 1). The total lipid content in seaweeds typically varies between 0.12% and 6.73% of their dry weight, comprising critical classes such as phospholipids, glycolipids, and neutral lipids (Pérez et al., 2016, p. 5). The chemical profiling of these lipids reveals a complex distribution of saturated and unsaturated fatty acids, including monounsaturated and polyunsaturated fatty acids (Yoon et al., 2018, p. 4). These profiles are often taxonomically specific; for instance, green algae are characterized by a high content of C<sub>18</sub>, whereas red algae are predominantly rich in C<sub>20</sub> (Pérez et al., 2016, p. 5). However, these biochemical compositions are not static and are significantly influenced by environmental factors, including geographical location, salinity, temperature, and seasonality (Pérez et al., 2016, p. 10).

Pharmacologically, algal fatty acids exhibit broad-spectrum bioactivity, acting as effective antimicrobial agents by destabilizing bacterial cell membranes, leading to increased permeability, leakage of cytosolic contents, and eventual cell lysis (Yoon et al., 2018, p. 11). Furthermore, specific saturated fatty acids, such as n-hexadecanoic acid, have been identified as potent anti-inflammatory agents through their ability to act as



competitive inhibitors of the enzyme Phospholipase ‘which initiates the production of inflammatory mediators (Aparna et al., 2012, p. 1). Beyond these effects, unsaturated fatty acids from marine sources have demonstrated the capacity to depress blood cholesterol levels, thereby minimizing the risk of cardiovascular complications (Rout & Kumar, 2015, p. 9). Within the green algae (Chlorophyta), the genus *Caulerpa* stands out as a significant producer of these metabolites. Previous studies demonstrated that *C. racemosa* contains several biologically important unsaturated fatty acids, including oleic acid and cis-vaccenic acid, compounds associated with cardiovascular protection, membrane regulation, and metabolic homeostasis (Magdugo et al., 2020, pp. 3–10; Prayogo et al., 2024, pp. 159–167).

Furthermore, volatile organic compounds and low-molecular-weight metabolites identified through gas chromatography–mass spectrometry (GC-MS) analyses may provide valuable insight into the chemotaxonomic features and bioactive potential of the species. Despite the long-standing presence of *C. racemosa* along the Libyan coastline, comprehensive biochemical characterization studies focusing on Libyan populations remain scarce. Most available investigations have concentrated on ecological distribution or general biological activity, while detailed profiling of fatty acids, volatile constituents, and secondary metabolites under local environmental conditions is still limited. Therefore, the present study aimed to conduct a comprehensive biochemical profiling of *C. racemosa* collected from the Libyan coast through an integrative GC-MS-based analysis of fatty acids, bioactive metabolites, and volatile constituents, in order to evaluate its nutritional significance and explore its prospective pharmaceutical applications.

## MATERIAL AND METHOD

### Algal Material

Fresh samples of the green macroalga *Caulerpa racemosa* (Forsskål) J. Agardh were collected during the spring season (April 2025) from the subtidal zone (3-5 m depth) along the coast near Tripoli, Libya (Approx. 32°54' N, 13°11' E). The algal samples were carefully washed with seawater at the collection site to remove sand, epiphytes, and other debris. Taxonomic identification was confirmed, based on morphological characteristics



### Preparation of Crude Extract

The collected algal samples were thoroughly washed again with fresh water and then shade-dried at room temperature ( $25\pm 2$  °C) for approximately 10-14 days until constant weight was achieved. The dried algal material was ground into a fine powder using an electric blender. The algal powder (100 g) was extracted by maceration with 99.9% ethanol (500 mL) in a conical flask, kept on an orbital shaker (120 rpm) for 7 days at room temperature. The combined filtrates were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator (Buchi Rotavapor R-100) at 40 °C. The resulting crude ethanolic extract was stored at 4 °C in an airtight container until GC-MS analysis.

### GC - MS Analysis

The GC-MS analysis of the ethanolic extract of *C. racemosa* was performed using an Agilent 7890B Gas Chromatograph coupled with an Agilent 5977A Mass Selective Detector. The separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness; Agilent Technologies). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 ml/min. The injector temperature was set at 280 °C, and the injection volume was 1 µl with a split ratio of 20:1. The oven temperature program was initiated at 70 °C (held for 2 min), then ramped at a rate of 10 °C/min to 150 °C (held for 0 min), followed by a ramp of 5 °C/min to 300 °C (held for 10 min). The total run time was 45 minutes. The MS transfer line temperature was 290 °C, ion source temperature was 230 °C, and quadrupole temperature was 150 °C. Mass spectra were recorded in the electron impact (EI) mode at 70 eV, scanning the mass range from m/z 40 to 600.

The identification of the chemical constituents was based on the comparison of their retention times (RT) and mass spectra with those available in the National Institute of Standards and Technology (NIST) Mass Spectral Library (NIST 14). The relative percentage of each component was calculated based on the peak area normalization method.

## Results:

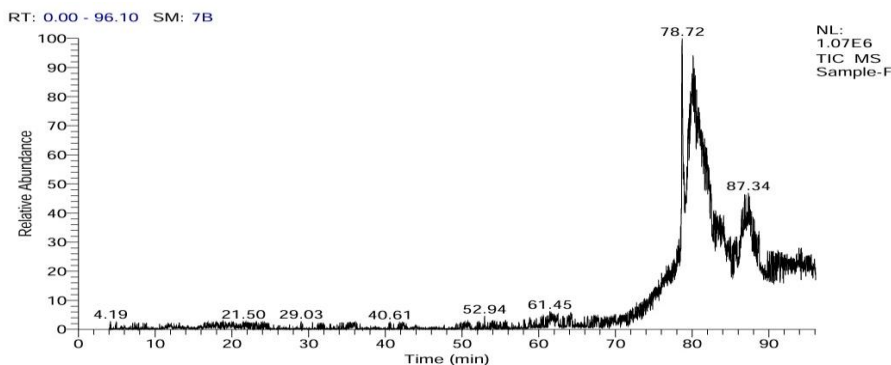


Fig.1 Total Ion Chromatogram (TIC) obtained by GC-MS analysis (Source: Prepared by the author ,2026)

Table 1. Fatty Acid Profile Based on GC-MS Peak Area (%)

Identified Fatty Acid/ Detected Derivative	Retention Time (RT)	Peak Area (%)
cis-Vaccenic acid	82.48	1.45%
Oleic Acid	83.54	0.72%
cis-11-Eicosenoic acid	86.99	0.79%
trans-13-Octadecenoic acid	77.36	0.53%
6-Octadecenoic acid, methyl ester	61.46	0.60%
9-Octadecenoic acid, octadecyl ester	94.31	0.80%

\* Source: Prepared by the author (2026)

Table 2. GC-MS-Identified Antioxidant and Bioactive Compounds

Identified compound	Retention Time (RT)	Peak Area (%)
Patchouli alcohol	74.92	0.61%
Spirost-8-en-11-one, 3-hydroxy-	94.16	0.66%
5,9-Dimethyl-2-(1-methylethylidene)-1-cyclodecanol	66.38	0.63%
11,13-Dimethyl-12-tetradecen-1-ol acetate	80.40	2.88%

\* Source: Prepared by the author (2026)

Table 3. GC-MS-Identified Volatile and Aromatic Compounds

Identified compound	Retention Time (RT)	Peak Area (%)
1,2-Benzenedicarboxylic acid, diisooctyl ester	78.68	10.28%
1H-Indene, 5-butyl-6-hexyloctahydro-	95.95	1.35%
1-Phenyl-3-(2'-cyclohexylethyl)-6-cyclopentylhexane	64.18	0.51%
Decahydro-8a-ethyl-1,1,4a,6-tetramethylnaphthalene	90.39	0.94%
Cyclohexane, 1,3,5-trimethyl-2-octadecyl-	67.18	0.51%

\* Source: Prepared by the author (2026)



## Discussion

The GC–MS profile of the ethanolic extract obtained from Libyan specimens of *Caulerpa racemosa* revealed a metabolome dominated primarily by unsaturated lipid constituents, accompanied by smaller fractions of terpenoid and steroid-related compounds. The chromatographic profile indicated considerable chemical heterogeneity, a feature commonly reported in marine macroalgae exposed to variable environmental conditions (Belkacemi et al., 2020, pp. 91–92).

A substantial proportion of the detected compounds belonged to the monounsaturated fatty acid fraction, particularly oleic acid and cis-vaccenic acid. Their abundance is noteworthy, as both fatty acids have frequently been associated with anti-inflammatory and membrane-related physiological functions in nutritional and biochemical studies (Vassiliou et al., 2009, pp. 7–8; Prayogo et al., 2024, pp. 159–167). Such fatty acids are frequently associated with marine organisms inhabiting environmentally variable coastal systems. Other unsaturated fatty acids, including cis-11-eicosenoic acid and trans-13-octadecenoic acid, were identified at lower relative abundance. The detection of these long-chain unsaturated lipids is consistent with patterns commonly observed in marine organisms inhabiting environmentally variable coastal systems (Belkacemi et al., 2020, pp. 88–92). These metabolites are of interest because related compounds have been investigated in other natural extracts for possible antimicrobial applications and industrial uses, including biofuel-related research (Palaniyappan et al., 2023, pp. 18–20). However, the present investigation was limited to chemical characterization, and no direct biological assays were performed to confirm such activities in the studied extract. Terpenoid and steroid-derived constituents, such as phytol and squalene, were detected within the volatile fraction of *C. racemosa*. These compounds are frequently associated with ecological adaptations and stress-response processes in marine environments (Belkacemi et al., 2020, pp. 88–92). Their detection in the present extract expands the available chemical data for Libyan populations of *C. racemosa*.

Comparison with previous Mediterranean studies, particularly those conducted along the Algerian coast, reveals broad qualitative similarity in fatty acid composition, despite variations in relative abundance patterns (Belkacemi et al., 2020, pp. 92–93). These

differences may reflect environmentally influenced metabolic variation associated with local ecological and physicochemical conditions.

The present investigation focused exclusively on chemical characterization. Nevertheless, the coexistence of abundant unsaturated fatty acids with terpenoid- and steroid-associated metabolites supports continued examination of Libyan *Caulerpa racemosa* as a potential source of chemically diverse marine metabolites. Further investigations based on targeted bioactivity assays remain necessary before attributing definitive pharmacological significance to the detected compounds.

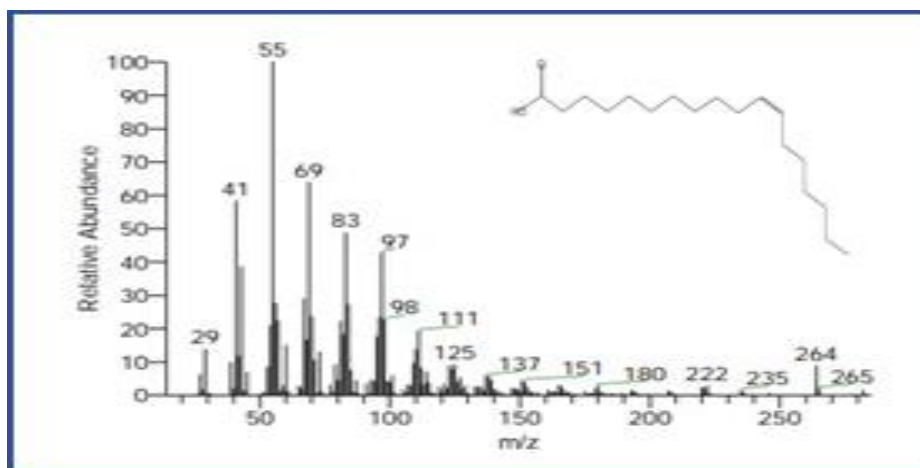
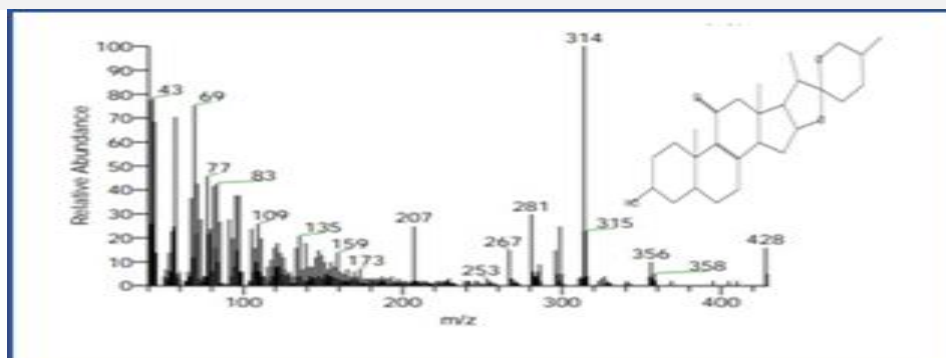
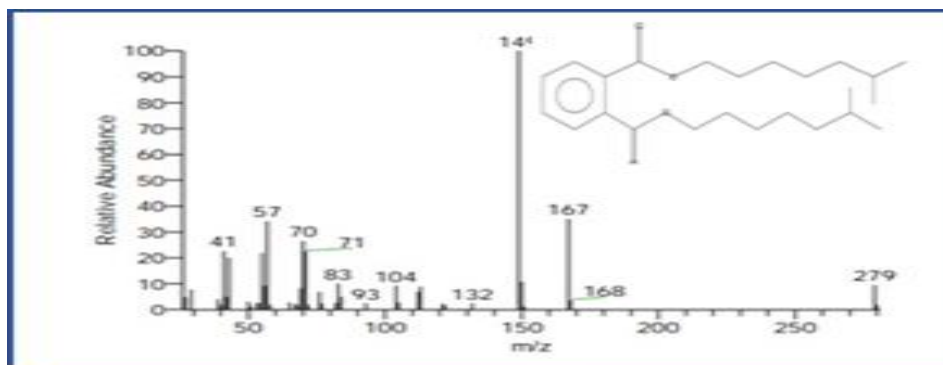


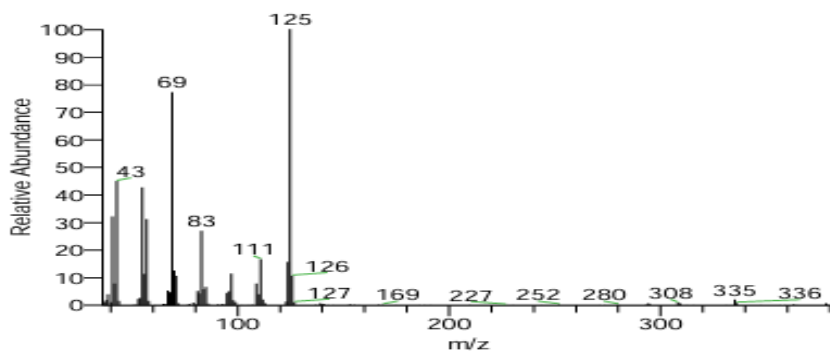
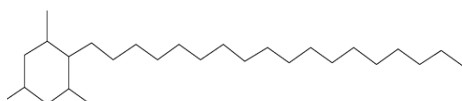
Fig. cis-Vaccenic acid



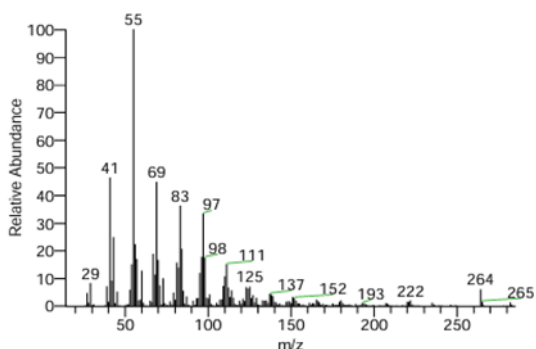
Spirost-8-en-11-one, 3-hydroxy



1,2-Benzenedicarboxylic acid, diisooctyl ester



Cyclohexane, 1,3,5-trimethyl-2-octadecyl-



trans-13-Octadecenoic acid

\* Source: Prepared by the author (2026)



### Conclusion:

- Chemical Diversity: GC-MS analysis revealed a multifaceted chemical profile characterized by a diverse array of unsaturated fatty acids, fatty acid derivatives, terpenoids, steroids, and oxygenated lipids.
- Fatty Acid Composition: The study identified several biologically significant unsaturated fatty acids, notably cis-vaccenic acid (1.45%), oleic acid (0.72%), and cis-11-eicosenoic acid (0.79%).
- Bioactive Constituents: Various compounds with therapeutic potential were characterized, most prominently 11,13-dimethyl-12-tetradecen-1-ol acetate (2.88%), alcohol (0.61%), and specific steroid and sesquiterpenoid derivatives, such as Spirost-8-en-11-one.
- Volatile and Aromatic Constituents: The volatile profile was prominently dominated by 1,2-benzenedicarboxylic acid, diisooctyl ester, which exhibited a significantly high relative abundance of 10.28%.

### Recommendations:

Future studies are recommended to complement the present biochemical characterization with molecular analyses, such as transcriptomic or gene expression profiling, in order to confirm the biosynthetic pathways associated with the detected metabolites and to provide a deeper understanding of their biological functions. Integrating metabolomic and molecular data would strengthen the interpretation of the findings and improve the scientific value of future investigations.

### References:

1. **Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., & Haridas, M.** (2012). Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. *Chemical Biology & Drug Design*, 80(3), 434–439.
2. **Belkacemi, L., Belalia, M., Djendara, A. C., & Bouhadda, Y.** (2020). Antioxidant and antibacterial activities and identification of bioactive compounds of various extracts of *Caulerpa racemosa* from Algerian coast. *Asian Pacific Journal of Tropical Biomedicine*, 10(2), 87–94. <https://doi.org/10.4103/2221-1691.275423>



3. **Fernando**, I. P. S., Nah, J. W., & Jeon, Y. J. (2016). Potential anti-inflammatory natural products from marine algae. *Environmental Toxicology and Pharmacology*, 48, 22–30 .<https://doi.org/10.1016/j.etap.2016.09.023>
4. **Guiry**, M. D., & Guiry, G. M. (2025). *Caulerpa racemosa*. AlgaeBase. National University of Ireland, Galway. Retrieved May 21, 2026, from <https://www.algaebase.org>
5. **Klein**, J., & Verlaque, M. (2008). The *Caulerpa racemosa* invasion: A critical review. *Marine Pollution Bulletin*, 56(2), 205–225 .  
<https://doi.org/10.1016/j.marpolbul.2007.09.043>
6. **Magdugo**, R. P., Terme, N., Lang, M., Pliego-Cortés, H., Marty, C., Hurtado ,A. Q., Bedoux, G., & Bourgougnon, N. (2020). An analysis of the nutritional and health values of *Caulerpa racemosa* (Forsskål) and *Ulva fasciata* (Delile)—Two Chlorophyta collected from the Philippines. *Molecules*, (12)25 ,  
.2901<https://doi.org/10.3390/molecules25122901>
7. **Ornano**, L., Donno, Y., Sanna, C., Ballero, M., Serafini, M., & Bianco, A .(2014) . Phytochemical study of *Caulerpa racemosa* (Forsk.) J. Agarth, an invading alga in the habitat of La Maddalena Archipelago. *Natural Product Research*, 28(20), 1795–1799. <https://doi.org/10.1080/14786419.2014.945928>
8. **Palaniyappan**, S., Sridhar, A., Kari, Z. A., Téllez-Isaías, G., & Ramasamy ,T. (2023). Evaluation of phytochemical screening, pigment content, in vitro antioxidant, antibacterial potential and GC-MS metabolite profiling of green seaweed *Caulerpa racemosa*. *Marine Drugs*, 21(5), 278 .<https://doi.org/10.3390/md21050278>
9. **Pérez**, M. J., Falqué, E., & Domínguez, H. (2016). Antimicrobial action of compounds from marine seaweed. *Marine Drugs*, 14(3), 52.
10. **Permatasari**, H. K., Wewengkang, D. S., Tertiana, N. I., et al .(2022) .Anti-cancer properties of *Caulerpa racemosa* by altering expression of Bcl-2 ,BAX, cleaved caspase 3 and apoptosis in HeLa cancer cell culture. *Frontiers in Oncology*, 12, 964816. <https://doi.org/10.3389/fonc.2022.964816>
11. **Prayogo**, E. W., Sholikhah, I., Suciati, Dej-adisai, S., & Widyowati, R .(2024) . Systematic Review of Green Seaweed *Caulerpa racemosa* as an Anti-Inflammatory



- Agent: Current Insights and Future Perspectives. Jurnal Farmasi dan Ilmu Kefarmasian Indonesia, 11(2),
12. **Ragunath**, C., Kumar, Y. A. S., Kanivalan, I., & Radhakrishnan, S. (2020). Phytochemical screening and GC-MS analysis of bioactive constituents in the methanolic extract of *Caulerpa racemosa* (Forssk.) J. Agardh and *Padina boergesenii* Allender & Kraft. *Current Applied Science and Technology*, 20(3), 380–393.
  13. **Rout**, S., & Kumar, A. (2015). A review on the potentiality of marine seaweeds as a medicinal source. *World Journal of Pharmacy and Pharmaceutical Sciences*, 4(10), 458–476.
  14. **Sherley**, S., Hikmawanti, N. P. E., & Agustin, M. (2023). The effect of extraction methods on total phenolics and antioxidant activities in *Caulerpa racemosa* (Forsskal) J. Agardh extracts. *Vitae*, 30(2), Article e349405
  15. **Smit**, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology*, 16, 245–262.
  16. **Tassakka**, A. C. M. A. R., Iskandar, I. W., Juniyazaki, A. B. A., et al. (2023). Green algae *Caulerpa racemosa* compounds as antiviral candidates for SARS-CoV-2: In silico study. *Narra J*, 3(2), e179.
  17. **Vassiliou**, E. K., Gonzalez, A., Garcia, C., Tadros, J. H., Chakraborty, G., & Toney, J. H. (2009). Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNF- $\alpha$  both in vitro and in vivo systems. *Lipids in health and disease*, 8(1), 25.
  18. **Yoon**, B. K., Jackman, J. A., Valle-González, E. R., & Cho, N. J. (2018). Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing, and therapeutic applications. *International Journal of Molecular Sciences*, 19(4), 1114.