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# Evaluation of phytochemical composition and metabolite profiling of macroalgae *Caulerpa taxifolia* and *C. peltata* from the Banda Aceh coast, Indonesia

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**Abstract.** *Akbar SA, Hasan M, Afriani S, Nuzlia C. 2023. Evaluation of phytochemical composition and metabolite profiling of macroalgae* Caulerpa taxifolia *and* C. peltata *from the Banda Aceh coast, Indonesia. Biodiversitas 24: 5283-5292.* The study of seaweeds to extract their bioactive compounds has become increasingly important from the standpoint of broader applications. This study aimed to examine the metabolite profiles and phytochemical content of *Caulerpa peltata* and *Caulerpa taxifolia* along the coast of Banda Aceh in Indonesia. Readers interested in learning more about the biological activities of *C. peltata* and *C. taxifolia* can refer to the material gathered from this study. The three steps proposed by this study were phytochemical composition, antioxidant scavenging, and in vivo metabolite profiling by the use of Gas Chromatography-Mass Spectrometry (GC-MS), Fourier Transform Infrared (FTIR) characterization, and the 2,2-diphenylpicrylhydrazyl (DPPH) test. Using DPPH to measure antioxidant activity, both extracts demonstrated greater scavenging capability, with inhibition values of 42.59% and 39.81%, respectively. In both macroalgae, the GC-MS methodology has shown to be a quick, sensitive, and trustworthy way to monitor individual components and the entire chemical composition. In *C. taxifolia*, there are 29 phytochemical compounds, whereas in *C. peltata*, there are 26 types. The ethanol extract of the macroalgae *C. taxifolia* and *C. peltata* was then subjected to an FTIR spectrum. These findings at 3352 $cm^{-1}$, 1654 $cm^{-1}$, and 1019 $cm^{-1}$ proved that alkanes, alkenes, and hydroxyl groups were present, corresponding to several compounds in the GC-MS measurement results. *Caulerpa taxifolia* and *C. peltata* are potential antibacterial agents; the bioactive compounds for this activity are Heptadecane, Hexadecanoic acid, and other ester derivatives.

**Keywords:** Antibacterial, antioxidant activity, bioactive compounds, *Caulerpa peltata*, *Caulerpa taxifolia*

## INTRODUCTION

The waters around about 70% of Indonesia's landmass have beaches abundant in various biological resources. One of the biological resources found in the Indonesian seas is macroalgae, better known as seaweed. Macroalgae is one type of plant that is large and has a body structure like a thallus. Macroalgae belong to the Protista kingdom, similar to plants because they have different color and pigment characteristics (Moreira et al. 2022). Macroalgae attach themselves to various substrates such as rocks, sandy rocks, wood, mollusk shells, and other epiphytic plants as a place for epiphytic life on other plants (Filote et al. 2021). Macroalgae can live because they stick to a substrate. Attaching macroalgae to the substrate aims to prevent the macroalgae from being carried away by sea currents, waves, or tides (Xiao et al. 2021). Furthermore, macroalgae can also attach to parts of the coral that have experienced weathering (Schmitt et al. 2022).

In general, macroalgae consist of 3 classes, namely brown algae (*Phaeophyta*), red algae (*Rhodophyta*), and green algae (*Chlorophyta*). Green algae have green pigments. The pigment comes from chlorophyll contained in algae (Vahtmäe et al. 2018). Red algae are algae that have a red pigment; this is due to the presence of phycoerythrin pigment reserves contained in the algae. In addition, red algae also contain several pigments, such as chlorophyll, carotenoids, and phycocyanins (El-Shafei et al. 2021). Meanwhile, brown algae is the algae that has the largest size when compared to green algae and red algae. Brown algae have brown pigments; this pigment comes from phycoxanthin, mostly found in algae (Chaldun et al. 2023).

The bioactive compounds contained in macroalgae are responsible for its use as a pharmaceutical agent. This bioactive has great potential to advance the pharmaceutical sector, including anti-tumor, antibacterial, and anticancer treatments, as well as the agrochemical industry, particularly herbicides, antifeedants, and fungicides (Tziveleka et al. 2021; Ferreira et al. 2021). The ability of macroalgae to produce halogenated secondary metabolites that function as bioactive compounds is made possible by the algae's extreme environmental conditions, such as high salinity, or as a means of defense against predators (Bayro et al. 2021).

Banda Aceh, one of the city in Aceh Province, Indonesia has a land area of 61.36 $km^2$ and a vast potential for coastal resources. Macroalgae are a potential marine biodiversity source commonly found along Aceh's coast. Due to the absence of scientific research on the potential of

macroalgae, this plant is not optimally utilized by the coastal communities of Aceh. Marine macroalgae are abundant in the coastal regions of Banda Aceh, where they are predominantly affixed to dead coral rocks, making them susceptible to ultraviolet radiation. The waters of Ulee Lheue Beach are located in Meuraxa Sub-district, which is 3 km from the center of Banda Aceh City. The area of Ulee Lheue village is 725.8 ± 80 hectares and is located at an altitude of 0.8 meters above sea level (masl).

*Caulerpa taxifolia* and *Caulerpa peltata* belong to the same genus, *Caulerpa*, which is included in the Ulvophyceae class. *Caulerpa taxifolia* and *C. peltata* are marine macroalgae that dominate the coastal area of Banda Aceh, specifically on the coast of Ulee Lheue, Banda Aceh City. Many local people do recreation and fishing in the area. Apart from that, Ulee Lheue Beach is one of the beach tourist attractions that local people like. Ulee Lheue Beach also has an embankment made of piles of rocks, which are breakwaters, so big waves are rarely found on this beach. This beach is along the Ulee Lheue port road and is used as a crossing to Sabang; it will find stalls selling various culinary delights along the road to the port. The waters of Ulee Lheue Beach have biodiversity, including coral reefs, fish, mangrove plants, and macroalgae. This study aimed to explore the profile of the macroalgae bioactive compounds *C. taxifolia* and *C. peltata* from the coast of Ulee Lheue Beach, Banda Aceh City, Aceh Province, Indonesia.

## MATERIALS AND METHODS

### Study area

Macroalgae samples were collected from the coastal area of Ulee Lheue Beach, Banda Aceh City, Aceh, Indonesia (Figure 1), using the exploration method. Macroalgae were taken at a depth of approximately ± 2 m in the coastal intertidal zone of Ulee Lheue Beach. The samples were washed with clean water to remove epiphytes, debris, and other objects, and necrotic parts were removed. Algal samples were washed carefully with seawater and fresh water. The macroalgae obtained were then identified morphologically by referring to the morphological descriptions of several previous studies. Furthermore, the research will be conducted at the Marine Chemistry Laboratory, Faculty of Marine Affairs and Fisheries, Universitas Syiah Kuala, Aceh, Indonesia.

### Procedures

*Materials and tools*

The materials used in this research are simplicia *C. taxifolia* and *C. peltata*. The chemicals used for analysis were ethanol, DPPH (2,2-diphenyl-2-picrylhydrazil, 2 N HCl solution, ascorbic acid, Kjeldahl tablets, 2% $H_3BO_3$, bromine cresol indicator, 40% NaOH, concentrated $HNO_3$, $HClO_4$, HF, $NaBH_4$, distilled water, Meyer's reagent dissolved in 60 mL of distilled water, 0.5 g KI B solution, Dragendroff reagent, Wagner reagent, ether, $FeCl_3$. The tools used were a centrifuge, rotary vacuum evaporator (Buchi R-300), microplate (Nunc), glassware, micropipette, water bath, Thermo Scientific ISQ LT Single Quadropole Mass Spectrometer, Thermo Scientific Trace 1310 Gas Chromatograph, and Fourier Transform Infrared (FTIR) (Bruker alpha).

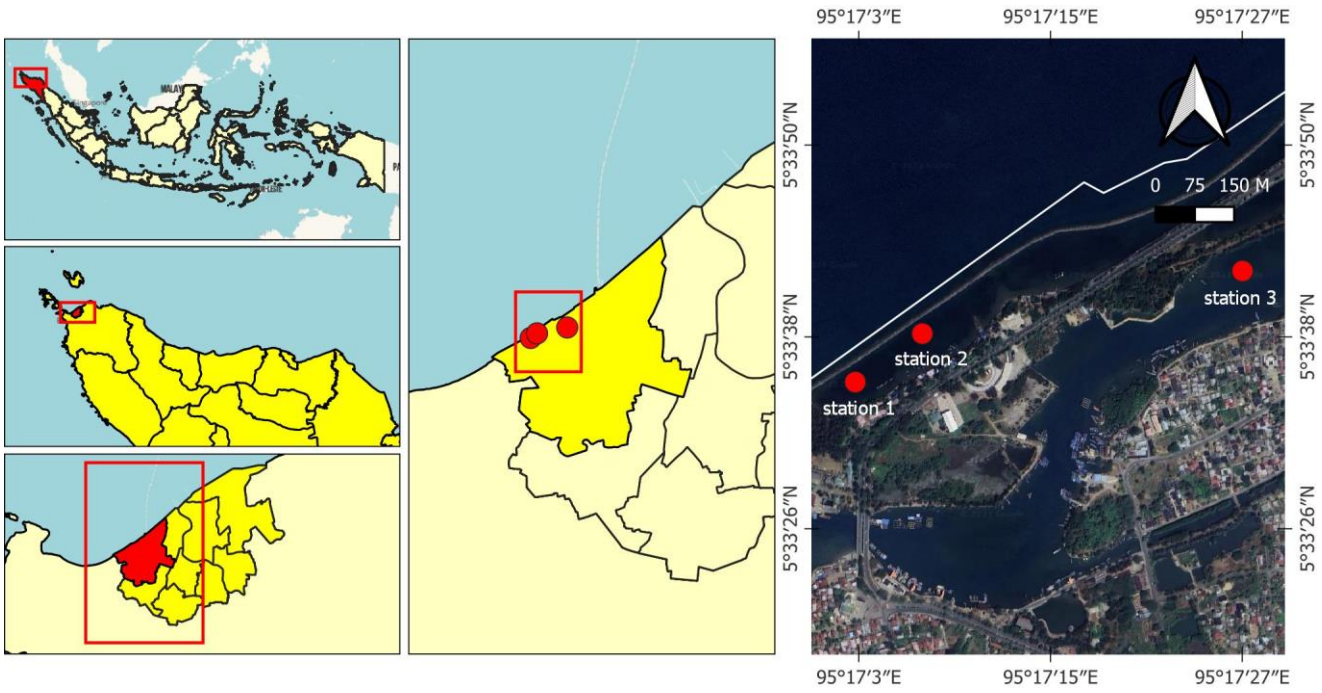


**Figure 1.** Macroalgae sampling locations: station 1 (5°33'35.0"N 95°17'02.9"E), station 2 (5°33'38.0"N 95°17'07.1"E), and station 3 (5°33'41.9"N 95°17'26.9"E) in the Ulee Lheue Beach, Meuraxa, Banda Aceh, Aceh, Indonesia

*Macroalgae sample extraction*

The clean macroalgae were dried in the shade for 2 days. The dried samples were then separated into 2 containers according to the species obtained and then mashed using a blender. Simplicia was weighed as much as 50 g and put into an Erlenmeyer glass. Maceration was carried out with a ratio of 1:5 using ethanol. The mixture is stored for 72 hours; next, the soaking functions to draw out the organic compounds contained in simplicia. Then, the mixture is filtered using ordinary filter paper and concentrated using a rotary vacuum evaporator. The extract paste obtained was then stored at 4°C until further use. The macroalgae extract was then subjected to phytochemical analysis, total phenol content, antioxidant activity, Fourier Transform Infrared (FTIR), and Gas Chromatography-Mass Spectrometry (GC-MS).

*Bioactive components of macroalgae extract*

The phytochemical test was a preliminary test to qualitatively determine the content of active compounds such as alkaloids, flavonoids, phenol hydroquinones, steroids, triterpenoids, saponins, and tannins (Kaushik et al. 2021).

**Data analysis**

*Functional group analysis*

A total of 0.0020 g of sample and 0.1980 g of KBr were weighed and then pulverized and printed to form thin (transparent) plates. Samples were read using the FTIR tool-Bruker alpha. Furthermore, the resulting chromatogram is compared with the IR table.

*Analysis of total phenol content*

With few adjustments, the Starowicz et al. (2021) method was used to measure the total phenol content. Using a 25 mL measuring flask, 5 mg of gallic acid was dissolved in distilled water to create gallic acid standards. Standard samples were then created from this solution at concentrations of 2, 4, 6, 8, and 10 ppm. To determine the total phenol content, 20 mg of the extract was dissolved in ethanol solvent in a 25 mL measuring flask, and the mixture was then shaken to ensure homogeneity. Next, I mL of 50% Follin Ciocalteu reagent was added to 0.5 mL of the extract from the solution, which was then let to stand for 5 minutes. Next, 1 mL of 5% $Na_2CO_3$ was added, and the mixture was homogenized for an hour in the dark. The absorbance value was determined with a UV-VIS spectrophotometer at a wavelength of 725 nm.

*Antioxidant activity by DPPH method*

Antioxidant activity test using the DPPH method with a concentration of 0.1 mM refers to the Munteanu et al. (2021) modified method. Crude seaweed extract samples were dissolved in ethanol at 2, 4, 6, 8, and 10 ppm concentrations. Ascorbic acid was used as a positive control with an absorbance of 0.108. The antioxidant activity of each sample is expressed by the percentage of free radical inhibition calculated by the formula:

$$Inhibition\ (\%) = \frac{Blank\ absorbance - Sample\ Absorbance}{Blank\ absorbance} x\ 100\%$$

*Water quality measurement*

The following characteristics of the water quality were noted: temperature, salinity, pH, dissolved oxygen, phosphate, and nitrate. Seawater samples were collected near the bottom and at the surface layer using a Nansen tube, and pH, salinity, and temperature measurements were done on-site. A GMK-910T thermometer was used to measure the temperature of the seawater, an Atago hand refractometer was used to evaluate salinity, and a HANNA HI9024 series pH meter was used to test pH. It was decided what the amounts of dissolved oxygen were using the Winkler titration method. Using a Shimadzu 1700 UV-VIS spectrophotometer, the spectrophotometric approach was the foundation for the phosphate and nitrate analysis (Pal et al. 2021).

*GC-MS analysis*

GC-MS measurements use the following parameters: initial temp 40°C for 3 min, ramp 3°C/min to 115°C, hold 10 min, ramp 2°C/min to 140°C, hold 8 min, ramp 3°C/min to 210°C, hold 5 min, Inj 210°C, Volume 0 µL, Split 30:1, Carrier Gas He, Solvent Delay 3.00 min, Transfer Temp 210°C, Source Temp 210°C, Scan: 45 to 500 Da, Column 30.0 m x 250 µm. Identification of chemical components was carried out by comparing the fragmentation pattern of the mass spectra of GC-MS results with the reference fragmentation pattern (library) of NIST12.LIB, WILEY229.LIB, and NIST62.LIB. The selected compounds are compounds based on literature searches with a similarity index or SI (Similarity Index) greater than 90, considering these compounds' compatibility with the composition and properties of the original sample. The GC chromatogram's peak area shows a compound's relative concentration to the sample that evaporates during GC-MS operation.

## RESULTS AND DISCUSSION

**Water quality parameters**

The existence of macroalgae is strongly influenced by the quality of the aquatic environment, both physically and chemically. The results of measurements of several environmental parameters, including salinity, temperature, phosphate and nitrate, pH, and dissolved oxygen (DO) in Ulee Lheue coastal waters, are presented in Table 1. These measurements follow the parameters of Minister of Environment Decree No. 51/2004.

**Classification and morphology of macroalgae samples**

The macroalgae samples that have been collected are then identified for species classification and morphology. The results of the identification are presented in Table 2.

**Bioactive components**

Phytochemical analysis is the first step to provide information on the types of bioactive compounds contained in plants. Information about active components is very important to predict active components that benefit the human body. The plants tested can be in fresh, dried,

powder, extract, and dosage forms. All macroalgae samples were subjected to qualitative testing of their bioactive components using color changes or precipitates generated in response to the supplied reagents. The presence of bioactive components of macroalgae is presented in Table 3.

**Antioxidant activity and total phenol**

The $IC_{50}$ value assessed the extract's capacity to block antioxidants. This number represents the sample concentration needed to lower 50% of the DPPH free radical activity (Baschieri and Amorati 2021). Table 4 and Figure 2 exhibit the findings of the antioxidant activity test, which indicated that the 2 macroalgae extracts and standard vitamin C had distinct actions.

**Infrared (IR) spectrum characterization**

FTIR was used to estimate the chemical bonds or functional groups in the extracted ethanol of macroalgae *C. taxifolia* and *C. peltata*. The bonds were found by analyzing the infrared absorption spectra in Table 5. The FTIR spectra of the macroalgae *C. taxifolia* and *C. peltata*, which are ethanol extract, are displayed in Figure 3.

**GC-MS analysis**

The chromatogram results on the ethanol extract of the macroalgae *C. taxifolia* and *C. peltata* showed 29-30 peaks. The GC chromatogram profile of the ethanol extract of the macroalgae *C. taxifolia* and *C. peltata* can be seen in Figure 4, and the chemical component profiles obtained can be seen in Table 6 and Table 7.

**Table 3.** Phytochemical test results for sample ethanol extracts of macroalgae *Caulerpa taxifolia* and *Caulerpa peltata*

| Secondary metabolite | Sample test results | |
|---|---|---|
| | *Caulerpa taxifolia* | *Caulerpa peltata* |
| Flavonoids | + | + |
| Tanin | - | - |
| Polyphenol | + | + |
| Kuinon | - | - |
| Steroid | + | + |
| Triterpenoids | - | - |
| Saponin | - | - |
| Alkaloid | | |
| Mayer | - | - |
| Wagner | + | + |
| Dragendroff | + | + |

**Table 1.** Table of water quality measurements and quality standards

| Parameters | Measurement results | Standard value * |
|---|---|---|
| Temperature (°C) | 29.8-30 | 28-30 |
| Salinity (‰) | 29.4-31.5 | 33-34 |
| pH | 7.81-7.96 | 7-8.5 |
| Dissolved Oxygen (DO) (mg/L) | 7.85-8.2 | >5 |
| Phosphate (mg/L) | 0.1-0.46 | 0.015 |
| Nitrate (mg/L) | 0.2-0.5 | 0.008 |

Note: *Decree of the Minister of Environment No. 51/2004

**Table 4.** Antioxidant activity test results and total phenols of macroalgae extract

| Species | Total phenolic content (mg GAE/g) | Antioxidant activity (IC50)(mg/L)* |
|---|---|---|
| *Caulerpa taxifolia* | 4.48 ± 0.04 | 12.31 |
| *Caulerpa peltata* | 4.87 ± 0.04 | 12.69 |

Note: *$IC_{50}$ vitamin C is 10.58 mg/L

**Table 2.** Classification and morphology of macroalgae samples

| Species | Morphology | Classification |
|---|---|---|
|  | *Caulerpa taxifolia* form flat thallus, branched with upright branches like feathers/ferns with stolons at the bottom. Type of thallus creeping (creeping). The color of the plant is yellow and dark green. Thallus grows bifurcated regularly (pinnate distichous). Roots (Holdfast) are stolon-like (creeping) with an attachment at the bottom, stolons are round (terete), and stipe (leaves) are flat and curved upwards, leaflets (pinnules) are opposite and also flat, crescent-shaped with pointed ends. | Kingdom : Protista<br>Division : Chlorophyta<br>Class : Ulvophyceae<br>Ordo : Bryopsidales<br>Familia : Caulerpaceae<br>Genus : *Caulerpa*<br>Species : *Caulerpa taxifolia* (M.Vahl) C.Agardh, 1817 |
|  | *Caulerpa peltata* is a bright green plant with a creeping part of the thallus called a stolon and an upright part called an assimilator. The form of the thallus is branched (ramiform) and creeping (creeping) and is coenocytic (multiple nuclei). Short erect assimilator, 2-4 cm tall with ramuli (leaves) arranged radially. The ramuli (leaves) are shield-shaped or disc-shaped with slender stalks or stems, 1-2 mm long, ending in a disc 1-2 mm thick and 3-8 mm wide. | Kingdom : Protista<br>Division : Chlorophyta<br>Class : Ulvophyceae<br>Ordo : Bryopsidales<br>Familia : Caulerpaceae<br>Genus : *Caulerpa*<br>Species : *Caulerpa peltata* J.V. Lamouroux, 1809 |

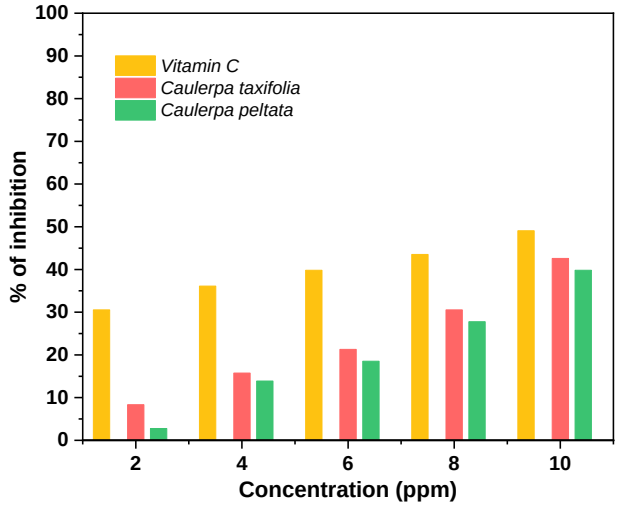


**Figure 2.** *Caulerpa taxifolia* and *Caulerpa peltata* ethanol extracts' capacity to scavenge DPPH radicals

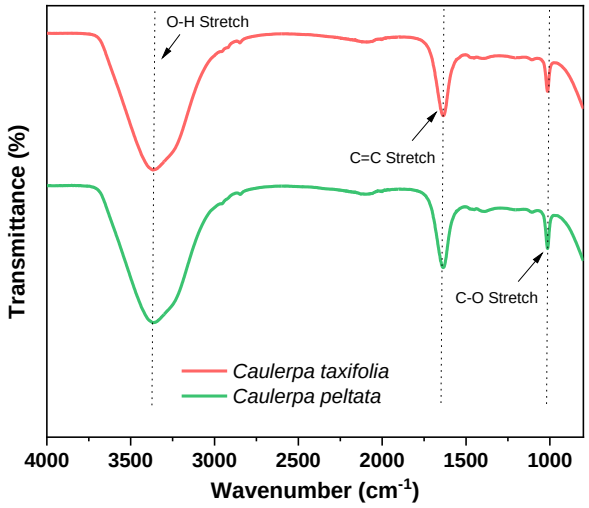


**Figure 3.** FTIR spectrum of *Caulerpa taxifolia* and *Caulerpa peltata* extract

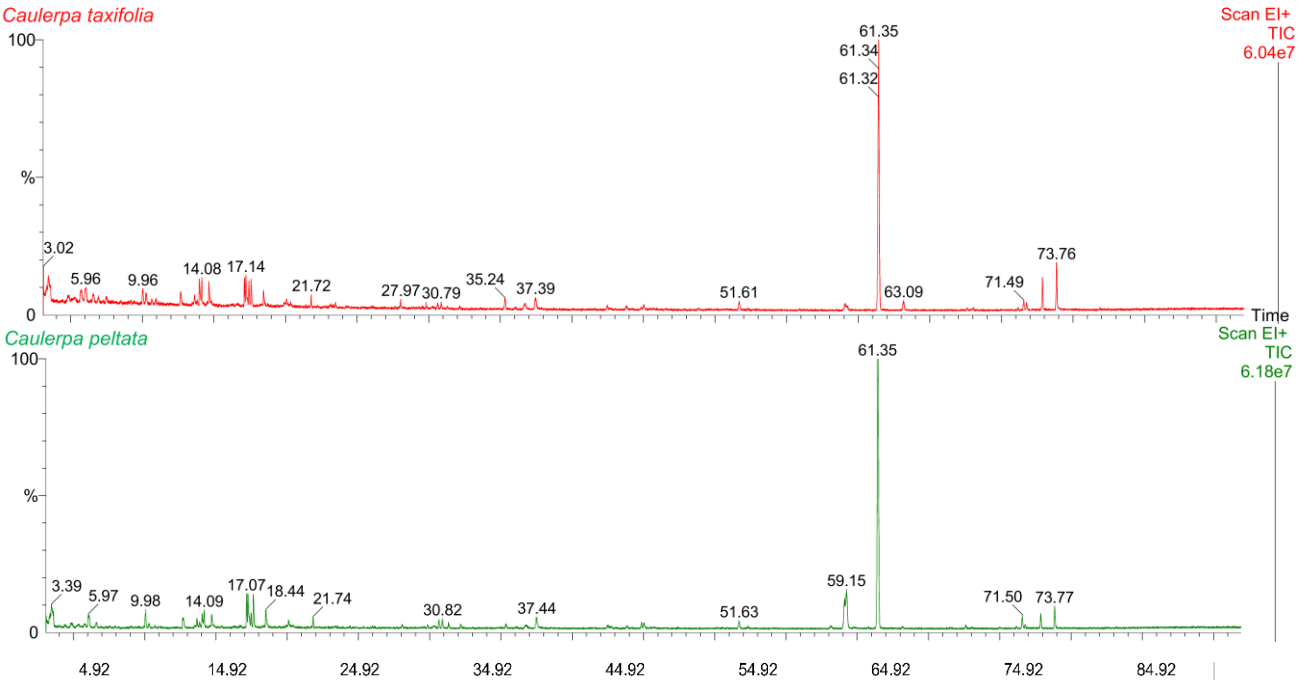


**Figure 4.** Chromatogram profile of ethanol extract of the macroalgae *Caulerpa taxifolia* and *Caulerpa peltata*

**Table 5.** FTIR functional groups and spectral peak values of *Caulerpa taxifolia* and *Caulerpa peltata* extracts

| Frequency range ($cm^{-1}$) | Wave number ($cm^{-1}$) | Possibility functional groups | Compound analysis results | Reference |
|---|---|---|---|---|
| 3677-3012 | 3352 | N-H stretch/ C-O stretch / O-H bend | Alcohol | Patle et al. (2020) |
| 2972-2936 | 2951 | C-H stretch | Alkanes | Parsa (2019) |
| 2878-2831 | 2849 | C-H stretch | Alkanes | Khalid et al. (2023) |
| 1757-1525 | 1654 | C=C stretch | Alkenes | Keke et al. (2023) |
| 1420-1358 | 1396 | C-H bend | Alkanes | Shaheen et al. (2022) |
| 1054-972 | 1019 | C-C(O)-C stretch | C-O from alcohols | Lim et al. (2023) |

**Table 6.** Percentage of chemical composition of ethanol extract from *Caulerpa taxifolia*

| Compounds | Retention Time (min) | Relative area (%) | Molecular formula | Molecular weight | PubChem ID |
|---|---|---|---|---|---|
| rac-(1R,2R)-2-(1H-Imidazol-4-yl)cyclopropane- | 3.244 | 0.698 | $C_6H_{11}C_{l2}N_3$ | 196.07 | 165456611 |
| Furan, 2-ethyl- | 3.361 | 1.344 | $C_6H_8O$ | 96.13 | 18554 |
| Z,E-2,13-Octadecadien-1-ol | 3.437 | 0.937 | $C_{18}H_{34}O$ | 266.5 | 5364462 |
| 2-Butenal, 3-methyl- | 5.660 | 1.154 | $C_5H_8O$ | 84.12 | 61020 |
| Hexanal | 5.964 | 1.259 | $C_6H_{12}O$ | 100.16 | 6184 |
| 1,2-Benzisothiazol-3-amine, TBDMS derivative | 6.483 | 0.545 | $C_{13}H_{20}N_2SSi$ | 264.46 | 91733953 |
| Heptanal | 9.962 | 0.835 | $C_7H_{14}O$ | 114.19 | 8130 |
| 6-(5-Methoxy-4-methylenecyclohex-2-en-1- | 10.178 | 0.672 | $C_9H_{14}O$ | 138.21 | 89925914 |
| Benzoic acid 3-methyl-4-(1,3,3,3-tetrafluoro- | 12.611 | 0.888 | $C_{19}H_{14}F_4O_4S$ | 414.4 | 5292343 |
| 3-Ethyl-3-methylglutaric anhydride | 13.586 | 0.573 | $C_8H_{12}O_3$ | 156.18 | 81433 |
| 1,1-Cyclohexanedimethanol | 13.895 | 1.070 | $C_8H_{16}O_2$ | 144.21 | 250594 |
| Cyclotetrasiloxane, octamethyl- | 14.076 | 0.847 | $C_8H_{24}O_4Si_4$ | 296.61 | 11169 |
| 1,4-Butanediol, 2,3-bis(methylene)- | 14.560 | 1.282 | $C_6H_{10}O_2$ | 114.14 | 548837 |
| Furan, 2-ethyl- | 17.047 | 0.810 | $C_6H_8O$ | 96.13 | 18554 |
| Phenol, 2-ethyl- | 17.362 | 0.842 | $C_8H_{10}O$ | 122.16 | 6997 |
| 6-Methyl-bicyclo[4.2.0]octan-7-ol | 17.525 | 1.104 | $C_9H_{16}O$ | 140.22 | 557202 |
| Decahydronaphtho[2,3-b]furan-2-one, 3- | 18.389 | 0.778 | $C_{19}H_{31}NO_2$ | 305.5 | 9995281 |
| 17-Octadecenal | 19.982 | 0.474 | $C_{18}H_{34}O$ | 266.5 | 41922 |
| Cyclopentasiloxane, decamethyl- | 21.721 | 0.498 | $C_{10}H_{30}O_5Si_5$ | 370.77 | 10913 |
| 14-Methyl-1-pentadecanol | 35.238 | 0.713 | $C_{16}H_{34}O$ | 242.44 | 5283265 |
| Cinnamic acid, 4-hydroxy-3-methoxy-, (5- | 36.638 | 0.528 | $C_{31}H_{40}O_{15}$ | 652.6 | 5369484 |
| 3-Hydroxy-1a,5-bis(hydroxymethyl)-5,6b- | 37.385 | 1.139 | $C_{14}H_{22}O_4$ | 254.32 | 45360340 |
| Hexadecane, 1,1-bis(dodecyloxy)- | 51.608 | 0.568 | $C_{40}H_{82}O_2$ | 595.1 | 41920 |
| Dodecane, 1-cyclopentyl-4-(3- | 59.015 | 0.685 | $C_{25}H_{48}$ | 348.6 | 294711 |
| Heptadecane | 61.355 | 16.594 | $C_{17}H_{36}$ | 240.5 | 12398 |
| Tridecanoic acid, 12-methyl-, methyl ester | 63.088 | 0.697 | $C_{15}H_{30}O_2$ | 242.4 | 21204 |
| 3-Heptadecene, (Z)- | 71.486 | 0.491 | $C_{17}H_{34}$ | 238.5 | 5352250 |
| Nonadecane | 72.788 | 1.463 | $C_{19}H_{40}$ | 268.5 | 12401 |
| Hexadecanoic acid, methyl ester | 73.763 | 2.462 | $C_{17}H_{34}O_2$ | 270.5 | 8181 |

**Table 7.** Percentage chemical composition of ethanol extract from *Caulerpa peltata*

| Compounds | Retention time (min) | Relative area (%) | Molecular formula | Molecular weight | PubChem ID |
|---|---|---|---|---|---|
| 2,3-Epoxyhexanol | 3.303 | 0.637 | $C_6H_{12}O_2$ | 116.16 | 543565 |
| Furan, 2-ethyl- | 3.390 | 1.959 | $C_6H_8O$ | 96.13 | 18554 |
| Tertbutyloxyformamide, N-methyl-N-[4-(1- | 3.513 | 0.531 | $C_{14}H_{24}N_2O_2$ | 252.35 | 550847 |
| Hexanal | 5.970 | 0.401 | $C_6H_{12}O$ | 100.16 | 6184 |
| 9-Acetoxy-1-methyl-8-propyl-3,6- | 5.999 | 0.798 | $C_{15}H_{26}N_2O_2$ | 266.38 | 551609 |
| Cyclotrisiloxane, hexamethyl- | 6.530 | 0.468 | $C_6H_{18}O_3Si_3$ | 222.46 | 10914 |
| Heptanal | 9.979 | 0.999 | $C_7H_{14}O$ | 114.19 | 8130 |
| 2-Heptenal, (E)- | 12.588 | 0.921 | $C_7H_{12}O$ | 112.17 | 5283316 |
| 1-Octen-3-one | 13.603 | 0.476 | $C_8H_{14}O$ | 126.2 | 61346 |
| cis-3-Methylcyclohexanol | 13.959 | 0.686 | $C_7H_{14}O$ | 114.19 | 21599 |
| Cyclotetrasiloxane, octamethyl- | 14.094 | 0.851 | $C_8H_{24}O_4Si_4$ | 296.61 | 11169 |
| 1-Heptadecyne | 14.613 | 0.807 | $C_{17}H_{32}$ | 236.4 | 141274 |
| Furan, 2-ethyl- | 17.070 | 1.041 | $C_6H_8O$ | 96.13 | 18554 |
| Phenol, 2-ethyl- | 17.397 | 0.513 | $C_8H_{10}O$ | 122.16 | 6997 |
| 6-Methyl-bicyclo[4.2.0]octan-7-ol | 17.549 | 1.657 | $C_9H_{16}O$ | 140.22 | 557202 |
| Bicyclo[8.2.0]dodecane, 11,11-dimethyl- | 20.023 | 0.500 | $C_{14}H_{26}$ | 194.36 | 535102 |
| Cyclopentasiloxane, decamethyl- | 21.739 | 0.469 | $C_{10}H_{30}O_5Si_5$ | 370.77 | 10913 |
| 3-Hydroxy-1a,5-bis(hydroxymethyl)-5,6b- | 37.438 | 1.108 | $C_{14}H_{22}O_4$ | 254.32 | 45360340 |
| Pentanoic acid, 5-hydroxy-, 2,4-di-t- | 44.937 | 0.431 | $C_{19}H_{30}O_3$ | 306.4 | 605777 |
| Acetic acid, chloro-, octadecyl ester | 51.631 | 0.642 | $C_{20}H_{39}ClO_2$ | 347 | 79299 |
| 8-Heptadecene | 59.037 | 2.172 | $C_{17}H_{34}$ | 238.5 | 5364555 |
| 9-Heptadecanol | 59.154 | 3.985 | $C_{17}H_{36}O$ | 256.5 | 136435 |
| Heptadecane | 61.348 | 22.236 | $C_{17}H_{36}$ | 240.5 | 12398 |
| Z-5-Nonadecene | 71.497 | 0.690 | $C_{19}H_{38}$ | 266.5 | 5364560 |
| Heptadecane, 9-hexyl- | 72.799 | 0.768 | $C_{23}H_{48}$ | 324.6 | 296566 |
| Hexadecanoic acid, methyl ester | 73.773 | 1.194 | $C_{17}H_{34}O_2$ | 270.5 | 8181 |

## Discussion

*Water quality parameters*

Sea water temperature parameters have a tolerance for macroalgae growth; water temperatures below 25°C will result in decreased growth in the Gracilaria genus (Li et al. 2022), and if the temperature is higher, it will cause the thallus to become pale yellowish and unhealthy. Physiologically, low temperatures cause biochemical activity in the thallus body to stop. At the same time, temperatures that are too high will result in enzyme damage and the destruction of biochemical mechanisms in the macroalgal thallus (Graba-Landry et al. 2020). The water temperature obtained in Ulee Lheue Beach, Banda Aceh City, ranges from 29.8 to 30°C; this temperature is still considered normal for tropical waters. According to Ji and Gao (2021), the optimal temperature for macroalgae growth in the tropics ranges from 15°C to 30°C. The temperature in these waters is also appropriate for supporting marine biota life; it should be between 28-32°C, with variations of up to 2°C from the natural temperature allowed. The temperature of 34.5°C is at which green, brown, and red algae begin to grow (Meier et al. 2022).

Macroalgae depend on salinity for survival; excessive or low salinity will interfere with physiological functions. These waterways have saltwater salinities ranging from 29.4-31.5‰, which is good for macroalgae growth. According to Borburema et al. (2021), macroalgae generally live in the sea with a salinity range of between 30-32‰, but many macroalgae live in a greater salinity range. A salinity range that is too high or too low can disrupt seaweed growth. Nurjanah et al. (2020) said that the range of salinity values for Eucheuma growth ranges from 28-34‰. Furthermore, Muqsith et al. (2022) said that the optimal salinity for Eucheuma growth ranges from 28-33‰. The salinity in these waters corresponds to the average salinity levels for coastal regions. Olli et al. (2023) stated that coastal areas' salinity ranged from 32.0-34.0‰.

These waters have a pH of 7.81-7.96, which is still suitable for macroalgae life. According to Wang et al. (2020), macroalgae may grow continuously in the pH range of 7-8. Even though a pH range of less than 9 is ideal for waterways, a pH of less than 6.5 will slow their growth. Seawater generally has a pH range of 7-8.5, which is acceptable. The pH values within this range are still within these limitations.

Dissolved oxygen is one of the main supports for life in the sea and an indicator of water fertility. Dissolved oxygen levels in water masses are relatively high, usually 6-14 ppm (Correia and Smee 2022). Dissolved oxygen levels in these waters range between 5.57-5.96 mg/L. Farasat et al. (2023) stated that macroalgae can grow at dissolved oxygen levels ranging from 5-6 mg/L. In general, an oxygen content of 5 ppm with water temperatures ranging from 20-30°C is relatively good for fish life (Siddiqua et al. 2022); even if there are no toxic compounds (not polluted) in the waters, an oxygen content of 2 ppm is sufficient to support the life of aquatic organisms (Akbar and Rahayu 2023).

The high and low levels of phosphate and nitrate in a water body are one of the indicators for determining their fertility. Observation showed phosphate and nitrate levels ranged from 0.1-0.46 mg/L to 0.2-0.5 mg/L, respectively; this value is higher than the quality standard. According to Narvarte et al. (2023), phosphate levels in moderately fertile waters range between 0.0021-0.05 mg/L, and fertile waters range between 0.051-0.1 mg/L. There is a suspicion that the elevated phosphate and nitrate levels in the waterways north of Banda Aceh are largely influenced by land-based sources, such as domestic trash. Due to turbulence and resuspension, which cause nutrients in the sediment to be raised into the water column, hydrooceanographic elements like currents also have an impact on the high phosphate and nitrate near the northern waters of Banda Aceh (Kottage and Patrick 2023). Phosphate and nitrate levels in Banda Aceh, which have exceeded the quality standards for marine biota, have implications for the potential for algae blooms (Liu et al. 2023). Phosphate is absorbed by phytoplankton with a maximum limit of 0.27-5.51 $mg.L^{-1}$ (Lin 2023) and enters the food chain.

*Bioactive components*

The macroalgae samples' ethanol extracts yielded good results for alkaloid chemicals in the Wagner and Dragendroff reagents but negative results for the Mayer reagent. Alkaloids have antibacterial and anti-inflammatory properties that help with pain management, blood circulation, postpartum stamina restoration, and uterine infection prevention (Xie et al. 2021). Then, both samples showed positive polyphenol test results. Phenolic compounds can reduce the risk of several chronic diseases because they have inflammatory, antioxidant, carcinogenic detoxification, and anti-cholesterol properties (Wan et al. 2021). Bioactive compounds can be determined through phytochemical tests and are important in antioxidant activity (Widyaningsih et al. 2016; Hasby et al. 2020).

*Antioxidant activity and total phenol*

Ethanol extracts from *C. taxifolia* and *C. peltata* have strong activity with $IC_{50}$ values of 12.31 mg/L and 12.69 mg/L. Analianasari et al. (2022) stated that an $IC_{50}$ value of less than 50 mg/L is classified as having strong antioxidant activity, 50-100 mg/L is moderate, 150-200 mg/L is weak, and more than 200 mg/L is very weak. A low $IC_{50}$ value indicates a strong ability of the extract to act as a hydrogen atom donor (Cao et al. 2023). The high scavenging ability is related to the hydroxyl groups in phenolic compounds (Zhang et al. 2022).

In this study, all two macroalgae extracts showed much lower activity than standard vitamin C at all different concentrations. Macroalgae extract showed higher activity at 10 µg/mL, whereas *C. taxifolia* ethanol extract showed 42.59%, followed by *C. peltata* at 39.81%. Bayro et al. (2021) reported that the ethanol extract of *C. taxifolia* showed 15.88% DPPH scavenging activity at 1000 µg/mL. So far, there has been no study of antioxidant activity in *C. peltata* species.

Phenolic compounds are one type of antioxidant in food. Phenolic compounds are effective sources of antioxidants, restrain free radicals and chelate metal ions. Antioxidant activity is related to phenolic compounds

(Becerril-Sánchez et al. 2021). Phenol compounds are chemical compounds that have the potential to act as antioxidants, but antioxidant activity is not only caused by phenol compounds. Pentacyclic triterpene compounds, vitamin C, and dyes such as chlorophyll, sulfur, and nitrogen act as antioxidants (Tziveleka et al. 2021). Based on Table 4, the total phenolic content of the two types of extracts ranged from 4.48 ± 0.04 to 4.87 ± 0.04. The high antioxidant activity in the ethanol extract indicates the presence of phenolic compounds in the extract.

*Infrared (IR) spectrum characterization*

Strong bonds were found at 3352 $cm^{-1}$, 1654 $cm^{-1}$, and 1019 $cm^{-1}$, while the others varied from weak to medium. These results demonstrated the presence of hydroxyl groups, alkanes, and alkenes. Both extracts showed similar patterns of IR vibration peaks. The peak around 3677-3012 $cm^{-1}$ was assigned as O-H hydroxyl groups (Patle et al. 2020), which may be related to several polyphenol derivative compounds identified in Table 5. In addition, C-H was read as stretching in areas 2972-2936 $cm^{-1}$ and 2878-2831 $cm^{-1}$, as well as bending in areas 1420-1358 $cm^{-1}$. This vibration mode is the alkane chain in the compounds contained in both extracts. Then, the FTIR spectrum at 1654 $cm^{-1}$ was assigned as the C=C from alkenes; this vibrational mode has a medium character, so it can be confirmed that it also contains C=O carbonyls (Keke et al. 2023). This is confirmed by the GC-MS test results in Table 6 and Table 7, which indicate the presence of several carbonyl derivative molecules. The measurable C=C vibrational mode can be derived from flavonoids, polyphenols characterized by two benzene rings joined by a linear carbon chain. The C-H vibration of benzene was also read at around 2950-3180 $cm^{-1}$ (Shaheen et al. 2022). The results of the phytochemical screening, which found the presence of flavonoids and phenols, were corroborated by identifying benzenoid compounds using FTIR spectrophotometry.

*GC-MS analysis*

Both macroalgae extracts showed the same type of chemical component with a larger % area value compared to the others, namely Heptadecane with relative area 22.236%. So, this peak is the main component in the ethanol extract of the macroalgae *C. taxifolia* and *C. peltata*. It has been reported that Heptadecane has antimicrobial activity (Suharti et al. 2023; Kayode et al. 2018). Apart from being an antimicrobial, heptadecane can also be applied to improve the quality of wood biocomposite materials. The results obtained for heptadecane-impregnated wood show good energy storage/release capacity with phase change temperatures suitable for building applications (Can and Žigon 2022). Another study on antioxidant and anti-inflammatory properties was presented by Kim et al. (2013), namely a molecular study of dietary heptadecane for the anti-inflammatory modulation of nf-kb in the aged kidney.

However, several medium-intensity chemical components were identified such us Hexadecanoic acid, methyl ester in *C. taxifolia*, and 9-Heptadecanol in *C. peltata*. Hexadecanoic acid, methyl ester with molecular formula $C_{17}H_{34}O_2$, reportedly possesses nematicides, antimicrobial, pesticide, antioxidant, insecticide, anti-androgenic effects, and hypocholesterolemic (Jabeen et al. 2023; Jiménez-Nevárez et al. 2023). As an antioxidant, studies on the activity of the $IC_{50}$ value against hexadecanoic acid methyl ester have been reported in ethanol extracts of the roots, stems, and leaves of the Song of India plant (*Dracaena reflexa*). The results showed a medium-level IC50 value for leaf extract, 134.62 ± 0.78 (Puspita and Prasetya 2023). Furthermore, it has been reported that 9-Heptadecanol has activity as an antibacterial (Ferdosi et al. 2021; Zhang et al. 2020; Lee et al. 2022). Even though it has a relatively small area (1.194%), it shows good biological activity.

GC-MS analysis of macroalgae *C. peltata* was also reported, collected from the Mandapam coast of Tamil Nadu, and authenticated (Central Marine Fisheries Research Institute, Mandapam) (Hakim and Patel 2020). The results show several chemical components have similar derivatives, namely chloroacetic acid, tetradecyl ester, hexacosanol, and acetate. Both are similar to Acetic acid, chloro-, octadecyl ester, and hexadecanoic acid, methyl ester obtained from measurements in this study. Both components have good antioxidants (Gade et al. 2017).

Apart from the major compounds, there are also minor compounds with relative area 0,5% until 2,5% in the two plant species known to have antimicrobial activity. In *C. taxifolia*, namely Tridecanoic acid, 12-methyl-, methyl ester (Zhang et. 2021), Z,E-2,13-Octadecadien-1-ol (De Lima et al. 2021), and Cinnamic acid (Sova 2012). However, in *C. peltata*, 1-Octen-3-one was recorded (Xiong et al. 2017), Cyclopentasiloxane, decamethyl- (Vaou et al. 2021), and Heptadecane, 9-hexyl- (Chirumamilla et al. 2022).

Based on the IR spectrum test, the ethanol extracts of the macroalgae *C. taxifolia* and *C. peltata* showed a -OH vibrational mode that corresponds to several compounds present in the GC-MS measurement results, namely in *C. taxifolia* recorded rac-(1R,2R)-2-(1H-Imidazol -4-yl)cyclopropan-, Z,E-2,13-Octadecadien-1-ol, 1,2-Benzisothiazol-3-amine, 1,1-Cyclohexanedimethanol, 1,4-Butanediol, 2,3-bis( methylene)-, Phenol, 2-ethyl-, 6-Methyl-bicyclo[4.2.0]octan-7-ol, 14-Methyl-1-pentadecanol, and 4-hydroxy-3-methoxy-, (5-. Meanwhile in *C. peltata* it was recorded 2,3-Epoxyhexanol, cis-3-Methyl-cyclohexanol, 3-Hydroxy-1a,5-bis(hydroxymethyl)-5,6b-, Pentanoic acid, 5-hydroxy-, 2,4-di-t-, and 9-Heptadecanol. Chemical components of macroalgae were identified as containing phenolic groups (Biswas et al. 2023).

In conclusion, our findings showed that *C. taxifolia* and *C. peltata* ethanol extracts showed noteworthy in vitro characteristics. The two extracts were considered to have better performance in terms of their antibacterial and antioxidant properties. The extract's higher biological activity may be attributable to its high phenolic content. Several compounds with potential antioxidant and antibacterial activities were found in the GC-MS study. These compounds could help produce new medications that could help treat or prevent infectious diseases in people and

animals. Plans must be managed for using marine seaweed as an innovative, sustainable natural drug discovery method for treatments, nutraceuticals, and large-scale pharmaceutical industrial uses. Therefore, more thorough research is necessary to fully understand the mechanisms of action of the extracts of *C. peltata* and *C. taxifolia*, as well as their bioactive components, and assess the effects in biological systems in vivo through the use of experimental animal models.

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