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# Identification of Metabolite Compounds from A Ethanol Extract of *Caulerpa racemosa* using LC-MS/MS with Inhibitory Activity of Interleukin-1β and Expression Inhibitory Nitric Oxide Synthase Enzyme; *In Silico* Virtual Screening

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**Abstract**

Osteoarthritis (OA) is the prevailing kind of arthritis, impacting a substantial number of individuals globally. The incidence of OA is on the rise and is projected to further increase due to the aging population and escalating rates of obesity. This study aims to evaluate the anti-OA potential of *Caulerpa racemosa*. The *Caulerpa racemosa* 96 % ethanol extract of the plant was analysed using LC-MS/MS. The metabolite compositions were identified as polyunsaturated fatty acids, terpenes, bisindole alkaloids and diterpenoids. The 18 compounds analysed by LC-MS/MS, and the binding affinity of the compounds to the target proteins Interleukin-1 (PDB ID: 1ITB) was assessed by molecular docking fucosterol (ΔG = –8.29 kcal/mol), variolin A (ΔG = –8.02 kcal/mol), and clionasterol (ΔG = –7.50 kcal/mol) and Nitric Oxide Synthase Inducible (3E7G) was assessed by molecular docking. Brassicasterol (ΔG = –9.25 kcal/mol), fucosterol (ΔG = –9.20 kcal/mol) and 24-methylenecholesterol (ΔG = –9.04 kcal/mol) exhibited the highest docking scores, indicating their strong potential as inhibitors of IL1β and nitric oxide (NO). This knowledge is valuable for the future bioassay investigations about the possible applicability of these medicines as innovative solutions for OA.

**Keywords:** *Caulerpa racemosa*, Inhibitor interleukin-1β, Inhibitory Nitric Oxide Synthase Enzyme, LC-MS/MS, Molecular docking, Osteoarthritis

## Introduction

OA is the predominant kind of arthritis, with a global prevalence that impacts a substantial number of individuals. OA is characterized by the slow degradation of the cartilage that provides cushioning to the ends of bones in joints. This degradation results in symptoms such as pain, stiffness and limited range of motion. OA is more common in older individuals, with the risk increasing with age. The condition predominantly impacts the joints located in the hands, hips and knees [1]. The condition predominantly impacts the joints located in the hands, hips and knees [1]. Common symptoms of OA encompass pain or

hurting, stiffness, limited range of motion and swelling. In some cases, OA can cause reduced function and disability, leading to difficulties in daily tasks or work. Preventive measures for OA include maintaining a healthy weight, controlling blood sugar levels, exercising regularly and protecting joints from injury. Engaging in regular physical activity, such as walking, swimming, or biking, can effectively preserve the health of joints, alleviate stiffness, diminish discomfort and enhance muscle and bone robustness. A healthy diet rich in omega-3 fatty acids and other nutrients can help reduce joint inflammation and slow the progression of OA [2]. Recent research indicates that OA is a common ailment globally. According to the Global Burden of Disease research, 595 million people worldwide or 7.6 % of the total population had OA in 2020 [3]. The incidence of OA is on the rise and is projected to further increase due to the aging population and escalating rates of obesity. Knee OA is the prevailing kind of OA, impacting 16.0 % of adults aged 15 and above worldwide [4]. OA has a substantial role in the number of years individuals live with impairment when it comes to musculoskeletal diseases. Public knowledge of changeable risk factors and educational initiatives for prevention are crucial to mitigate the significant impact of OA.

Interleukin-1 beta (IL-1β) is a key factor in the development of cartilage injury and degradation in OA. The involvement of synovial-derived IL-1β has been linked to the disease process in noninflammatory arthropathies such as OA. Human cartilage afflicted by OA exhibits an increased expression of IL-1β messenger RNA (mRNA), which is not observed in normal cartilage. The autocrine release of IL-1β by the cartilage affected by OA is present in adequate amounts to regulate the generation of NO and prostaglandin E2 (PGE2), both of which are known to contribute to cartilage destruction and inflammation. The primary methods by which IL-1β operates in OA comprise; Deterioration of cartilage IL-1β is a powerful stimulator of cartilage breakdown, triggering the production of mRNA and regulating the presence of disease-related proteases. Synovitis refers to inflammation of the synovial membrane, which lines the joints. IL-1β induces synovitis, a condition marked by inflammation and swelling of the lining of the joint. Activation of genes associated with pain: IL-1β can stimulate the production of nerve growth factor, a crucial molecule that increases sensitivity to pain in OA [5]. In chondrocyte metabolism, the presence of IL-1β causes a shift towards catabolism, leading to death and destruction of the extracellular matrix [6]. The regulation of chondrocyte function involves the stimulation of chondrocytes by IL-1β, which leads to an increase in the expression levels of bone morphogenetic protein 2 (BMP2) and matrix metalloproteinase 13 (MMP13). This is achieved by targeting the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase/specificity protein 1 (MEK/ERK/SP1) pathway, which enhances cartilage breakdown. Mechanical strain also plays a role in this process. Mechanical strain can stimulate the expression of IL-1β in chondrocytes, which may contribute to the development of OA [7] starting and advancing OA by increasing the activity of pathways that break down tissues. The pathophysiology of OA has been associated with the impairment of mitochondrial function in human chondrocytes, mediated by IL-1β [8]. NO is recognized as a prominent inflammatory mediator in OA and plays a significant role in several pathological alterations that occur with the progression of the disease [9]. NO has a catabolic role in the development of OA and is responsible for mediating the inflammatory response. It participates in the breakdown of matrix metalloproteinases, hinders the production of collagen and proteoglycans and aids in the regulation of apoptosis. NO and its redox products play a role in the detection and alleviation of pain in OA, making them potential targets for pain treatment in the illness [10]. NO facilitates the reduction of the matrix through many routes and has a crucial function in the creation and breakdown of proteoglycan and collagen in cartilage [11]. Recent research indicates that NO and its redox products may possess beneficial properties for cartilage protection. Further investigation is required to clarify the function of NO and its compounds on both healthy and OA cartilage [12]. Efforts have been made to develop therapeutic methods that can limit the activity of IL-1β and disrupt its signal transmission to treat OA. The use of IL-1β inhibitors may

be beneficial in slowing down the advancement of the illness [13]. Furthermore, NO has been shown to mediate crosstalk between IL-1β and WNT signalling in primary human chondrocytes by reducing the expression of certain proteins involved in cartilage homeostasis. IL-1β and NO play crucial roles in the pathophysiology of OA, contributing to cartilage damage, inflammation and the auto destructive pathway in OA. Understanding these pathways is essential for developing targeted therapeutic interventions for OA [14].

*Caulerpa racemosa* is a green seaweed that is endemic to the Indo-Pacific region, including Southeast Asia. It is frequently observed thriving on rocky surfaces coated with sand, deceased coral colonies, and coasts that are fairly exposed, with many other types of seaweed [15]. *Caulerpa racemosa* has been utilised in traditional medicine and as a dietary source throughout the Indo-Pacific region for an extended period. The species *Caulerpa racemosa*, also known as sea grapes, is a popular local delicacy in coastal areas in southeast Asian countries like Indonesia, the Philippines and Malaysia (Sabah). It is usually consumed as a salad or vegetable [16]. *Caulerpa racemosa*, possesses many secondary metabolites. Several chemicals have been identified in *Caulerpa racemosa* through research, including caulerpin, caulerpicine and caulerpenyne. Caulerpin is recognised for its ability to induce a slightly anesthetic effect, respiratory difficulties, sedation and impaired equilibrium, whilst caulerpicine is thought to possess neurotrophic properties. In addition, *Caulerpa racemosa* has yielded 3 diterpenoids and 12 previously identified compounds, including racemobutenolids A and B [17-19]. These compounds have attracted attention because of their potential pharmacological activity, including antiproliferative and apoptotic effects, as well as their potential anti-obesity and anti-aging capabilities [20]. Caulerpa species, such as *Caulerpa racemosa*, possess chemical defences that are mostly due to terpenoid metabolites. These metabolites consist of aldehyde, enol acetate, or other active groups [21]. *Caulerpa racemosa* has been found to inhibit cancer cell migration and alter the expression of epithelial-mesenchymal transition proteins, indicating its potential as an anticancer agent [22]. An aqueous extract of *Caulerpa racemosa* has been shown to ameliorate cardiometabolic syndrome markers and modulate the gut microbiome in mice [23]. *Caulerpa racemosa* many have potential therapeutic properties. It is known for its antioxidant, antibacterial and anticancer potential. Studies have shown that *Caulerpa racemosa* synthesizes a variety of metabolites, including polyunsaturated and monounsaturated fatty acids, terpenes, bisindole alkaloids and diterpenoids [24,20]. The compound caulerpin, a bisindole alkaloid, has been identified in *Caulerpa racemosa* and is believed to play a role in its antibacterial and anticancer effects. *Caulerpa racemosa* exhibits promising bioactive properties, making it a subject of interest for further research and potential therapeutic applications [24,22].

Different analytical techniques, including infrared spectroscopy (IR), liquid chromatography-mass spectrometry (LC-MS) and gas chromatography (GC) with or without mass spectrometry (MS), are used to identify the phytochemical substances present in herbal products [25]. Furthermore, the combination of analytical techniques with *in silico* tools and public web servers is frequently employed to forecast the inhibitory efficacy of various phytochemical compounds against enzymes and proteins associated with diseases [26].

The objective of this study is to evaluate the capacity of *Caulerpa racemosa* to function as an anti-OA agent. Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analysis was conducted to identify the chemicals present in the 96 % ethanol extract of *Caulerpa racemosa*, and subsequently analyse these compounds. In molecular docking, the ligand compound LC-MS/MS will be utilized to determine whether the metabolite constituents have an affinity for the target proteins Nitric Oxide Protein Data Bank archive (PDB ID: 3E7G) and Interleukin-1β (PDB ID: 1ITB).

## Materials and methods

### Preparation of plant material and samples

*Caulerpa racemosa* marine biota was collected from the ocean deeps of Gili Iyang Island, Dungkek District, Sumenep City, East Java, Indonesia. Plant determination have been carried out at the Faculty of Fisheries and Marine Sciences, Airlangga University. The newly harvested seaweed was meticulously organized, thoroughly cleansed, thinly cut and subsequently dehydrated using aeration. Freshly harvested seaweed is used as the entire plant, which is the stem known as the thallus, thoroughly cleaned with running water to remove salt from the surface of the plant, carefully separated between all parts of the plant and impurities (coral), then cut into thin slices, and then dried using aeration. Subsequently, the substance was pulverized using a grinder known as Fomag FCT-Z200, resulting in the production of a finely ground green powder. Procedure for extracting the extraction process for this plant begins by measuring the amount of grinding powder, which is then soaked in a 96 % ethanol solvent at a ratio of 1:2 for a duration of 3 days. The liquid that passed through the filter was then separated by filtration, and the solid remaining was soaked in liquid twice. The obtained filtrates were evaporated using a Buchi rotary evaporator. The extracts from the Rotary Evaporator were frozen at –20 °C for 48 h under vacuum conditions (0.0 mmHg) in a (Lab freeze Dryer (lyophilizer) Instrument of type FD-12-MR from China) until a consistent moisture content was reached for freeze-drying. The room temperature was 27.5 °C and the ice condenser temperature was –70.3 °C. The powder obtained was kept in a refrigerator for later use.

### LC-MS/MS analysis

Initially, in the liquid chromatogram method, chromatograms containing chemicals with high polarity would be seen, whereas those having less polar molecules would be observed subsequently. The separation findings were analysed using the Xevo G2-S Quadrupole time-of-flight (QTof) mass spectrometer detector (Waters, USA) to generate a chromatogram peak. The LC-MS/MS instrument was employed to identify the chemicals present in the stems of *Caulerpa racemosa*, an ultrasound-assisted method was used to extract 100 mg of plant material dry weight using 2 mL of methanol/water (80/20; v/v) for 20 min with an Ultrasons device from JP Selecta in Abrera, Spain. The sample was centrifuged at 13,000 rpm for 10 min using a Micro Star 12 centrifuge (VWR; Radnor, PA, USA), and the resulting liquid was passed through a 0.45 µM microfilter (Chromservis; Prague, Czech Republic). The methanol was evaporated from the filtered supernatant using a vacuum rotary evaporator (Heidolph; Schwabach, Germany) at 100 mbar and 25 °C. The clear water solution was diluted with water and either acetonitrile or methanol in specified volumes and proportions, and then applied to a SPE column. After sample preparation, the specimen will be inserted into the liquid chromatography (LC) system using a micro syringe with a volume of 5 µL. It will then be inserted into the UPLC (Ultra Performance Liquid Chromatography) column, specifically the ACQUITY UPLC BEH (Ethylene Bridge Hybrid) C18 column with dimensions of 2.1×50 $mm^2$ and a particle size of 1.8 µM. The LC system used is the ACQUITY UPLC® H-Class System manufactured by Waters in the United States. The liquid samples will undergo droplet formation and subsequently be subjected to electrospray ionization (ESI) with a positive (+) charge using a needle. The mass range will be from 50 to 1,200 m/z, with the source temperature set at 100 °C and the desolvation temperature set at 350 °C. Furthermore, we employed conical gas flow rates of 0 L/h and desolvation gas flow rates of 793 L/h, together with impact energy varying from 4 to 60 eV. Furthermore, the Q-ToF analyser separated the ions produced by the detector. The elution system employed a gradient elution system with 2 solvents: Water containing formic acid and acetonitrile containing formic acid. The eluents were used at a flow rate of 0.2

mL/min. The MestReNova, and MassLynx V4.1 application was utilized to acquire and analyse the data from the chromatogram peaks.

**Molecular docking simulation**

***Instrument in silico studies***

The hardware utilized was the Xiaomi RedmiBook 15 laptop, including a 15.6-inch Full HD display equipped with 8 GB of DDR4 RAM running at a frequency of 3200 MHz. It was powered by the 11th Generation Intel® Core™ i3-1115G4 processor, capable of reaching speeds up to 4.1 GHz. The laptop also featured Intel® Iris® UHD Graphics Utilized. The laptop was connected to an internet network (Wifi). The operating system is Windows 11 Home software, Discovery Studio Visualizer®. The software tools used in this study include AutoDock Tools® 1.5.6, AutoDock 4.2.6, Autogrid 4.2.6, PyMOL®, and Open Babel®, Marvin Sketch® (ChemAxon), Notepad++ and LigPlot+ are used, together with the Protein Data Bank site and the PubChem site.

**Ligand and target preparation**

Eighteen chemicals derived from the ethanolic extract 96 % of *Caulerpa racemosa* were utilised as test ligands based on LC-MS/MS data analysis. The complete structure is initially depicted in a 2D format using ChemDraw Ultra 12.0. It is subsequently transformed into a 3D representation and refined using the molecular mechanical parameters (MM2 force field) algorithm. The target protein utilised for the study was the Type-1 Interleukin-1 Receptor Complexed with Interleukin-1 beta protein (PDB ID: 1ITB), together with the human inducible Nitric Oxide Synthase (iNOS) protein (PDB ID: 3E7G), all of which were obtained from the Protein Data Bank via the website https://www.rcsb.org/. The Kollman charges were assigned to the receptors, while the Gasteiger charge was assigned to the ligands.

**Molecular docking modelling**

To validate the molecular docking, the process of redocking was performed. This involved moving the initial ligand to the target pocket using accurate grid coordinates. AutoDock tools (version 4.2) were utilized for this purpose. The ligand's position must exhibit a root-mean-square deviation (RMSD) of less than 2.0 Å following the redocking procedure. The settings for docking and the grid box were established using the docking validation findings described in **Table 2**. The ultimate configuration of each docking's conformation was stored in a *dlg file. An investigation of ligand-receptor interactions was performed using Discovery Studio 2016.

The semi-flexible docking method involves placing the ligand in a flexible manner while keeping the protein's stiff shape. The Lamarckian Genetic Algorithm was utilized to perform molecular docking, with a population size of 150 people and a maximum evaluation count of 2,500,000 for every 100 independent iterations. The best pose was established by assessing the lowest binding energy score (ΔG) along with the inhibition constant (Ki) value. Additionally, Discovery Studio Visualizer discovered the functional essential amino acid that played a role in the docking interaction.

## Results and discussion

**Identification of compounds contained by LC-MS/MS**

One effective analytical method for identifying chemicals is LC-MS/MS. Target chemicals are physically separated before being detected using MS. This method is commonly used to identify and measure a wide range of substances, such as the bioactive compounds found in the marine algae Ulva sp.,

Caulerpa sp. and Codium sp [27]. The Compound identification inside the 96 % ethanol extract of *Caulerpa racemosa* using LC-MS/MS. **Figure 1** displays the results of the collected chromatogram data and with best peak intensity (BPI). The MassLynx V4.1 SCN884 program developed by Waters Inc is used to analyze the main ions and molecular formulae of chemicals. The main ions and molecular formulae are utilized as data for chemical analysis via the online library available at http://www.chemspider.com/Default.aspx. Interpretation of compounds can also be supported based on previous research that has identified the chemical content contained in the green algae *Caulerpa racemosa*. The outcomes of compound interpretation are visible in **Table 1**. According to the data analysis, a total of 18 substances were discovered. Including, the chemical observed at a retention time (Rt) of 11.3 is identified as caulerpin. This is corroborated by a study undertaken by [28], which found that *Caulerpa racemosa* contains bioactive chemicals, including caulerpin. Caulerpine has diverse biochemical and pharmacological properties that make it promising for therapeutic applications. It has demonstrated antinociceptive and anti-inflammatory effects, and has shown potential as an antiviral agent against Bovine Viral Diarrhea Virus (BVDV) replication [29,30]. Additionally, it exhibits spasmolytic effects by blocking the influx of Ca2+ in the *guinea pigileum*, which can be beneficial in treating various infections. These compounds have garnered significant interest in recent decades, and additional research is necessary to ascertain the potential of caulerpins as therapeutic candidates. Cytotoxicity experiments were conducted to assess the cytotoxic effects of caulerpine [30]. The analysis revealed the presence of caulerpenyne and fucosterol, which had a retention duration of 17.18. This finding aligns with previous studies undertaken by [31,32]. The search results indicate that Caulerpa species possess a diverse range of bioactive chemicals, such as polysaccharides, terpenoids, alkaloids, sterols, fatty acids and bisindole alkaloids [21].

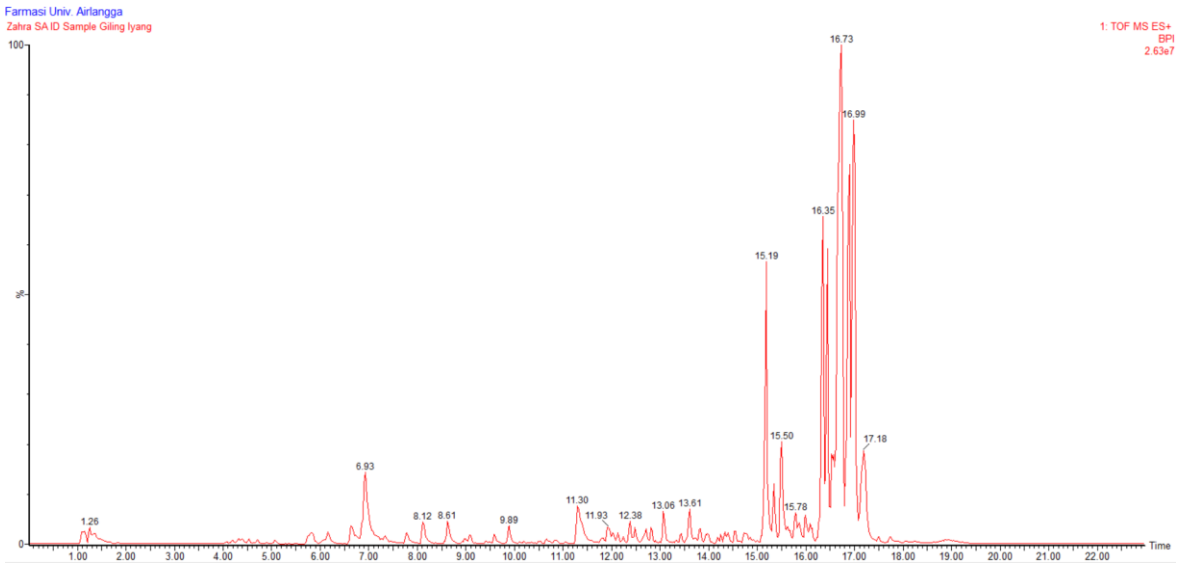


**Figure 1** Chromatogram of 96 % ethanol extract of *Caulerpa racemosa* using LC-MS/MS.

**Table 1** The metabolite profile of *Caulerpa racemosa* was observed using LC-MS/ESI-MS analysis.

| No | RT | Mass m/z | Callculated m/z | Fragmentation (m/z) | Molecular Formula | Tentative Compound | References |
|---|---|---|---|---|---|---|---|
| 1 | 1.23 | 118.0863 | 118.0873 | 110.0873; 104.1080; 97.0766; 82.0150 | $C_5H_{11}NO_2$ | Betaine | [43] |
| 2 | 1.26 | 143.0707 | 143.0829 | 110.0098; 97.0771; 84.0457 | $C_6H_{10}N_2O_2$ | 2,4-imidazolidinedione, 3,5,5-trimethyl | [44] |
| 3 | 5,831 | 197.1181 | 197.1178 | 179.1076; 133.1019, 105.0710 | $C_{11}H_{16}O_3$ | Loliolide | [45] |
| 4 | 8.12 | 385.1188 | 385.1193 | 371.1029; 285.1250; 1650913; 128.0631 | $C_{23}H_{16}N_2O_4$ | (5aZ,7Z,12aZ,14Z)-13-(methoxycarbonyl)-5,12-dihydrocycloocta[1,2-b:5,6-b']diindole-6-carboxylic acid/ Monomethyl caulerpinate | [46] |
| 5 | 8.61 | 371.1026 | 371.1034 | 327.1136; 309.1029; 165.1282; 125.9874 | $C_{22}H_{14}N_2O_4$ | Caulerpinic acid | [28] |
| 6 | 9.58 | 324.1209 | 324.1214 | 291.1962; 275.2014; 233.1543; 158.1553 | $C_{15}H_{13}N_7O_2$ | Variolin A | [47] |
| 7 | 11.3 | 399.1339 | 399.1362 | 367.1097; 340.1223; 279.0930; 215.1444 | $C_{24}H_{18}N_2O_4$ | Caulerpin | [28] |
| 8 | 11.3 | 399.3621 | 399.1362 | 367.1097; 340.1223; 279.0930; 215.1444 | $C_{28}H_{46}O$ | 24-methylenecholesterol | [48] |
| 9 | 11.3 | 399.3621 | 399.1362 | 367.1097; 340.1223; 279.0930; 215.1444 | $C_{28}H_{46}O$ | Brassicasterol | [48] |
| 10 | 12.64 | 279.2327 | 279.2324 | 235.1703; 193.1593; 118.0655 | $C_{18}H_{30}O_2$ | Linolenelaidic acid | [49] |
| 11 | 12.66 | 447.4197 | 447.2901 | 396.1424; 319.2243; 301.2163; 279.2325 | $C_{29}H_{50}O$ | Clionasterol | [48] |

| No | RT | Mass m/z | Callculated m/z | Fragmentation (m/z) | Molecular Formula | Tentative Compound | References |
|---|---|---|---|---|---|---|---|
| 12 | 12.66 | 447.4197 | 447.2901 | 396.1424; 319.2243; 301.2163; 279.2325 | $C_{29}H_{50}O$ | Sitosterol | [28] |
| 13 | 15.50 | 609.2716 | 6.092.713 | 609.2716; 591.2604; 489.3288; 443.3509 | $C_{35}H_{36}N_4O_6$ | Harderoporphyrin | [50] |
| 14 | 15.74 | 427.3576 | 427.358 | 425.3799; 336.1233; 3132742; 165.0916 | $C_{29}H_{46}O_2$ | 10,12,14-nonacosatriynoic acid | [51] |
| 15 | 17.18 | 413.1361 | 413.2675 | 396.1475; 324.1210; 301.1421; 236.1498 | $C_{21}H_{26}O_6$ | Caulerpenyne | [52] |
| 16 | 17.18 | 413.3778 | 413.2675 | 396.1475; 324.1210; 301.1421; 236.1498 | $C_{29}H_{48}O$ | Fucosterol | [48] |
| 17 | 17.73 | 565.4069 | 565.4046 | 485.3617; 463.3797; 413.2684; 271.2639 | $C_{40}H_{52}O_2$ | Canthaxanthin | [15] |
| 18 | 18.06 | 401 | 401.3412 | 373.3459; 324.1202; 279.1671; 213.1273 | $C_{28}H_{48}O$ | 22,23-dihydrobrassicasterol | [48] |

**Table 2** Validation of molecular docking simulation.

| No | Receptor for Drug Target | PDB ID | Docking Site (x; y; z) | Docking area (x; y; z) | ΔG (kcal/mol) | RMSD (Å) | Number in Cluster |
|---|---|---|---|---|---|---|---|
| 1 | IL-1β | 1ITB | x = 48.729; y = 3.25; z = 16.546 | 60×60×60 | –8.15 | 2.5 | 20 |
| 2 | iNOS | 3E7G | x = 92.041; y = 22.956; z = 78.193 | 40×40×40 | –6.67 | 0.989 | 98 |

**The anti-OA potential of *Caulerpa racemosa* is revealed through molecular docking testing**

One valid method that helps visualize the main 'binding interactions' between the ligand and the identified '3-dimensional structure' of the protein is called molecular docking. The study concentrated on interaction modes that were necessary for significant "structural interactions" and offered insightful knowledge for the development of inhibitors. One of the most researched methods for "finding" new ligands

for well-known targets is molecular docking. A 'free energy binding' estimate can be used to analyze the majority of compounds. The drug's 'affinity' for the targets is indicated by the value of free energy binding. Similarly, the 'promising' compound is indicated by the molecule with the lowest inhibition constant [33]. The 96 % ethanol extract of *Caulerpa racemosa* shown significant action in inhibiting 2 proteins: IL1β (PDB ID: 1ITB) and PDB ID: 3E7G. Several phytochemical components were found in this extract. The compounds fucosterol, variolin A and clionasterol are present. The binding energy scores of –8.29, –8.02 and –7.5 (kcal/mol) were supplied, which surpass and closely resemble the control compound meloxicam's binding energy of –8.15 (kcal/mol) in IL1β inhibition. Several phytochemical components were found in this extract. The compounds 22,23-dihydrobrassicasterol, fucosterol and variolin A are present. Subsequently, we conducted molecular tethering to evaluate the NO inhibitory activity at the 3E7G receptor. Three compounds, namely brassicasterol, fucosterol and 24-methylenecholesterol, exhibited the most favorable binding energy scores of –9.25, –9.20 and –9.04 (kcal/mol), respectively, at the active site of the 3E7G receptor, which surpass and closely resemble the control native ligand Ethyl 4-[(4-methylpyridin-2-yl) amino] piperidine-1-carboxylate binding energy of –6.17 (kcal/mol) in NO inhibition (**Table 3**). A low negative value of the free binding energy (ΔG) indicates a spontaneous influence on the binding of the protein and ligand, leading to the stabilization of their connections. Furthermore, they exhibit exceptional efficacy as molecular docking prediction inhibitors [34]. The binding scores with the lowest values are directly related to the inhibition constant (Ki) values with the lowest values.

Fucosterol demonstrates anti-inflammatory qualities and has the potential to suppress IL-1β, a cytokine known for its pro-inflammatory effects. Multiple studies have shown that fucosterol has the capacity to hinder inflammation and oxidative stress induced by different factors, such as particulate matter and cobalt chloride. For instance, a study shown that fucosterol suppressed the generation of NO and the production of pro-inflammatory cytokines, such as IL-1β, is observed, in macrophages. Furthermore, it was discovered that fucosterol provides protection against cobalt chloride-induced inflammation by suppressing hypoxia-inducible factors through the PI3K/Akt pathway. These data indicate that fucosterol has the capacity to hinder the generation of IL-1β and NO, and also regulate inflammatory responses [31,35].

Brassicasterol has been reported to possess inhibitory properties on NO generation. A study has shown that brassicasterol has strong inhibitory effects on the formation of NO, with the potency being depending on the dose [36,37]. Furthermore the inhibiting action of brassicasterol on the production of NO has been linked to its anti-inflammatory characteristics [38]. Brassicasterol, a sterol derived from plants, has been identified as the primary sterol has potential consequences for modulating the expression of the induction of the NO synthase gene [39].

In the marker compound caulerpin, a bisindole alkaloid, it has been extracted from the lipoid extract of *Caulerpa racemosa*. This compound it demonstrates anti-inflammatory properties via regulating the production of cytokines and blocking pro-inflammatory agents. Caulerpin therapy in a mouse model of colitis was observed to decrease the levels of pro-inflammatory cytokines, including IL-1β, IL-6, IL-12, TNF-α and IFN-γ, while simultaneously boosting the levels of the anti-inflammatory cytokine IL-10 in the colon tissue afflicted by the disease [40].

The molecular docking study showed fucosterol, 22,23-dihydrobrassicasterol and 24-methylenecholesterol exhibited the highest docking scores, indicating their strong potential as inhibitors of IL-1β. Similarly, brassicasterol, fucosterol and 24-methylenecholesterol demonstrated significant chemical potential as inhibitors of NO production. The molecular docking study showed fucosterol, variolin A and clionasterol exhibited the highest docking scores, indicating their strong potential as inhibitors of IL-1β. Similarly, brassicasterol, fucosterol and 24-methylenecholesterol demonstrated significant chemical potential as inhibitors of NO production.

Hydrogen bonds are non-covalent interactions that significantly influence docking scores, complex formation and the intensity of binding modes [41]. The hydrophobic interaction is a significant force that plays a crucial role in the protein folding process. Its energy has been extensively investigated and described in several research studies [42]. In **Figure 2** illustrates the identify the crucial amino acid residues that have a role in the interaction between the hydrogen-active sites THR300, SER238, ASN204, GLU105, LYS103 and the hydrophobic sites ASN299, ASP239, HIS301, ASN108, THR147 on the IL-1β receptor. The interaction between the hydrophobic-active sites ASN299, ASP239, HIS301, ASN108 and THR147 on the NO receptor, which is derived from the control drug meloxicam, involves native ligand amino acid residues. The binding of IL1β receptors to the test compounds fucosterol and variolin A resulted in the formation of hydrogen bonds with the comparison substances meloxicam, specifically THR300 and SER238, which also participate in the tethering of the molecules. The hydrophobic bonds present in the test substances fucosterol, variolin A and clionasterol share multiple amino acid residues with the meloxicam comparator compound. These residues include THR147, ASN108, ASP239, ASN299 and HIS301. In addition, when NO receptors interact with the test compounds brassicasterol, fucosterol and 24-methylenecholesterol through molecular tethering, the active site of the receptor is primarily influenced by interactions with hydrophobic amino acids, resulting in hydrophobic bonds playing the most significant role. The amino acid residues ASN370, ALA351, TYR347, ARG266, GLY371, GLN263, ASP382 and ARG388 exhibit hydrophobic interactions similar to those of the natural ligand ethyl 4-[(4-methylpyridin-2-yl)amino]piperidine-1-carboxylate.

**Table 3** The molecules from LC-MS/MS were docked against 1ITB and 3E7G.

| No | Compound | ΔG (kcal/mol) | | Ki | |
|---|---|---|---|---|---|
| | | **1ITB** | **3E7G** | **1ITB** | **3E7G** |
| | Control | | | | |
| 1 | Meloxicam | –8.15 | –7.47 | 1.06 µM | 3.35 µM |
| 2 | Ethyl 4-[(4-methylpyridin-2-yl) amino] piperidine-1- carboxylate | - | –6.67 | - | 29.82 µM |
| | *Caulerpa racemosa* | | | | |
| 1 | Fucosterol | –8.29 | –9.20 | 836.04 nM | 180.75 nM |
| 2 | 22,23-dihydrobrassicasterol | –7.37 | 8.90 | 299.15 nM | 299.15 nM |
| 3 | 24-methylenecholesterol | –7.33 | –9.04 | 4.27 µM | 235.19 nM |
| 4 | Brassicasterol | –7.18 | –9.25 | 5.43 µM | 165.00 nM |
| 5 | Variolin A | –8.02 | –7.12 | 1.31 µM | 6.03 µM |
| 6 | Clionasterol | –7.5 | –8.97 | 3.18 µM | 268.19 nM |
| 7 | Sitosterol | –7.13 | –8.99 | 5.98 µM | 259.01 nM |
| 8 | Caulerpinic acid | –7.26 | –7.43 | 4.81 µM | 3.60 µM |
| 9 | Caulerpin | –6.35 | –7.58 | 22.00 µM | 2.80 µM |
| 10 | Caulerpenyne | –7.33 | –6.12 | 4.21 µM | 32.65 µM |
| 11 | Loliolide | –6.03 | –6.15 | 37.89 µM | 31.05 µM |
| 12 | Monomethyl caulerpinate | –5.39 | –5.68 | 111.31 µM | 68.07 µM |
| 13 | Linolenelaidic acid | –5.44 | –4.3 | 103.20 µM | 700.59 µM |
| 14 | 2.4-Imidazolidinedione, 3,5,5- trimethyl | –4.24 | –3.99 | 780.67 µM | 1.19 mM |
| 15 | 10,12,14-Nonacosatriynoic Acid | –3.73 | –4.29 | 1.84 mM | 712.17 µM |

| No | Compound | ΔG (kcal/mol) | | Ki | |
|---|---|---|---|---|---|
| | | 1ITB | 3E7G | 1ITB | 3E7G |
| 16 | Betaine | –3.19 | –3.04 | 4.60 mM | 5.88 mM |
| 17 | Harderoporphyrin | 10.96 | –6.49 | - | 17.48 µM |
| 18 | Canthaxanthin | 346.55 | 1.17e + 006 | - | - |

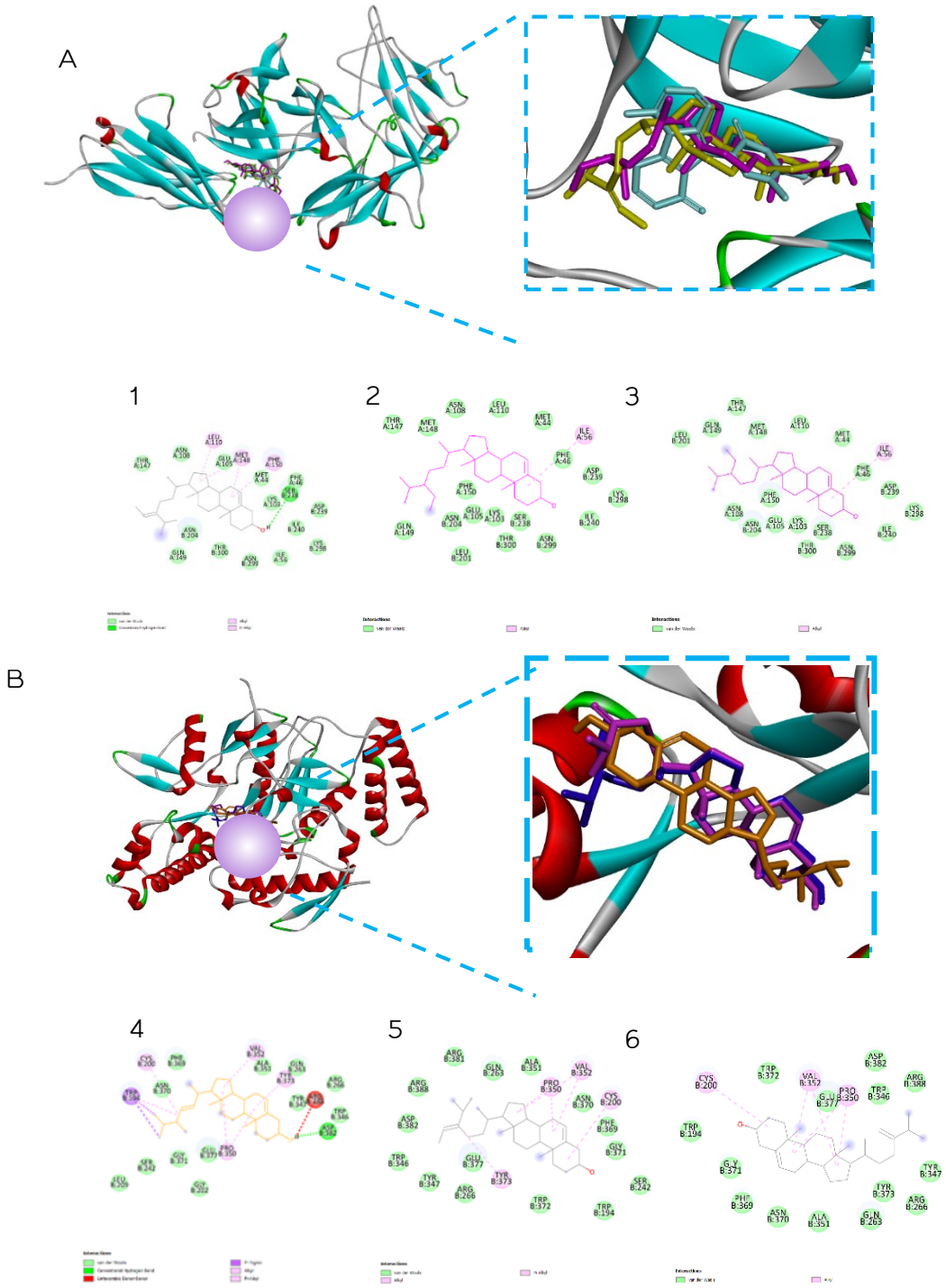


**Figure 2** Visualization of interaction between fucosterol[1], variolin A[2] and clionasterol[3] on the 1ITB (A) Receptor and brassicasterol[4], fucosterol[5] and 24-methylenecholesterol[6] on the 3E7G (B) receptor active.

## Conclusions

The investigation of the 96 % ethanol extract of *Caulerpa racemosa* using LC-MS/MS revealed the presence of 18 components, which included polyunsaturated and monounsaturated fatty acids, terpenes, bisindole alkaloids and diterpenoids. The IL1β receptor (PDB ID 1ITB) showed the greatest molecular docking score for fucosterol, followed by variolin A and clionasterol. During the *in silico* molecular docking assay at the NO inhibitory receptor (PDB ID 3E7G), brassicasterol obtained the highest molecular docking score, followed by fucosterol and 24-methylenecholesterol. This knowledge is valuable for future bioassay investigations about the possible applicability of these medicines as innovative solutions for OA. It is imperative to separate the molecules acquired from the docking process outlined in this investigation.

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