GC-MS Analysis and In Silico Molecular Docking Study of *Caulerpa racemosa* Microcapsules Under Heat Exposure

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Abstract

Caulerpa racemosa is a nutritionally rich green seaweed with bioactive properties, but its components are sensitive to high temperatures. The application of microencapsulation protects its bioactive compounds during exposure to high temperatures, which can otherwise cause degradation. This study aims to investigate the primary bioactive components in C. racemosa microcapsules subjected to high temperatures (120°C, 140°C, and 160°C) for 5 h. The phytoconstituents were identified using GC-MS. Additionally, an in-silico analysis, including ADME profiling and molecular docking, was conducted using BIOVIA Discovery Studio to assess the pharmacokinetic properties and potential biological interactions of the identified compounds. GC-MS analysis revealed that microcapsules treated at 120°C contained propane 2,2-diethoxy, while at 140°C, two additional compounds, furfural, and 2-furancarboxaldehyde, 5-methyl-, were identified. However, no active compounds were detected in microcapsules treated at 160 °C. Volatile compounds from the alcohol, aldehyde, furan, and ester groups increased with higher temperatures, particularly aldehydes and furans between 120°C and 140°C. The PASS database highlighted the potential of C. racemosa microcapsules in the development of safe, next-generation drugs, that adhere to ADMET properties. Molecular docking studies were performed with NF-κB and MMP-9 receptors. Among the tested compounds, 2-furancarboxaldehyde, 5-methyl- demonstrated the highest affinity for macromolecular targets. This compound's amine group showed strong binding to MMP-9 at Val A:398, suggesting its potential as an antioxidant and anti-inflammatory agent to mitigate illness.

Keywords: *In Silico, microcapsules, high temperature, bioactive compound, Caulerpa racemosa*

Introduction

As an archipelagic nation, Indonesia is rich in marine resources, including *C. racemosa* (commonly known as sea grapes) (Tassakka *et al*., 2023). *C. racemosa* is a marine alga with chlorophyll, giving it its characteristic green color. It grows naturally and is widely used as a vegetable by local communities (Tapotubun, 2018; Tapotubun *et al*., 2018). In various regions, *C. racemosa* is known by different names. For example, in the Kei Islands, it is called lat; in Sulawesi, lawi-lawi; in Jepara, latoh; ar-arosep in the Philippines; umi budo in Japan; and green caviar in Europe and Fiji (Tapotubun *et al*., 2020). This marine alga produces primary and secondary metabolites, which have diverse applications, particularly in the pharmaceutical industry (El-Beltagi *et al*., 2022).

Previous studies have identified various metabolites in *C. racemosa*, including pseudo-ephedrine, 5-butyl-2-methyl-δ1-pyrrolidine, 2-myristynoyl pantetheine, tetracontane, deoxy-spergualin, hexyl octyl ether (Rahul *et al*., 2014), indole derivatives, indane derivatives, sesquiterpenoid derivatives, diphenyl pentadiene derivatives, terpenoids, and fatty acids (Ornano *et al*., 2014). The macro algae also contain phenols, saponins, tannins, flavonoids, reducing sugars, and xanthoprotein (Raj *et al*., 2015). Oxidative stress plays a key role in the pathogenesis of chronic diseases, and antioxidants are commonly used to reduce this stress (Sharifi-Rad *et al*., 2020). The secondary metabolites of *Caulerpa* spp. have demonstrated potential as antioxidants (Susilowati *et al*., 2019), antibacterials (Nurdiansyah *et al*., 2021), immunomodulators (Yoojam *et al*., 2021), antivirals (Tassakka *et al*., 2023), and as anti-inflammatory and analgesic agents (Chowdhury *et al*., 2023).

Microencapsulation is a method used to protect these compounds during storage and

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processing for nutraceutical and pharmaceutical applications. It involves encasing solid, liquid, or gaseous particles within a coating material to isolate them from external conditions. Common microencapsulation methods include spray-drying and freeze-drying (Silva *et al*., 2014). Previous studies have employed microencapsulation to protect *Caulerpa* spp. from damage, using coatings such as maltodextrin (CM), maltodextrin-alginate (CMA), maltodextrin-fish gelatin (CMG) (Kurniasih *et al*., 2018), fish gelatin-Arabic gum (Dewi *et al.,* 2021), and Arabic gum (Damayati *et al*., 2023). The microencapsulation process protects bioactive compounds during thermal processing. Bioactive components protected through microencapsulation have been tested by applying heat treatment. For instance, Purnamayati *et al*. (2018) stated that phycocyanin from *Spirulina* sp., encapsulated using maltodextrin and carrageenan, was able to withstand heating at an inlet temperature of 130℃. Arepally and Goswami (2019) reported that probiotics encapsulated with maltodextrin and Arabic gum could survive heating at an inlet temperature of 150℃. The stability of bioactive components through microencapsulation was also shown by Dewi *et al*. (2022), who stated that chlorophyll from Caulerpa racemosa, encapsulated using fish gelatin and Arabic gum, could endure heating at 160℃. However, to date, changes in the activity effectiveness of bioactive components due to heating—especially in terms of pharmacological evaluation—remain unknown.

The chlorophyll bioactive component in Caulerpa racemosa is known to have antioxidant and anti-inflammatory activities. Lee *et al*. (2017) demonstrated that chlorophyll possesses anti-inflammatory properties capable of reducing tumor necrosis cells and interleukin-6. Zhang *et al*. (2025) stated that chlorophyll exhibits both antioxidant and anti-inflammatory activities in vitro, and its bioactivity increases along with an increase in the radius of gyration, as shown through molecular dynamics simulations. In addition to chlorophyll, Caulerpa also contains caulerpin, an indole alkaloid component. Caulerpin in Caulerpa has also been reported to exhibit anti-inflammatory activity, showing potential for breast cancer therapy, as well as cytotoxic and anti-SARS-CoV-2 activity (Erol *et al*., 2022; Ren *et al*., 2024). However, there is currently no information regarding changes in these bioactive components during heating, especially in the microencapsulation process and treatments involving high temperatures. Several studies have shown that high-temperature processing can induce the Maillard reaction, producing Maillard reaction products (MRPs) with potential antioxidant and anti-inflammatory effects, particularly furfural and its derivatives (Qianqian *et al*., 2020; Nowak *et al*., 2021; Zhao *et al*., 2024). Therefore, a study on the bioactive potential of *Caulerpa racemosa* microcapsule components during heating is necessary. The aim was to explore the anti-inflammatory potential of *C. racemosa* chlorophyll extract through in-silico methods, employing molecular docking simulations. In silico studies are considered an essential step in identifying potential treatments for in vitro and in vivo investigations, providing insight into molecular interactions (Wadood *et al*., 2013; Moelyadi *et al*., 2020). Molecular docking helps predict ligand binding affinities and identifies potential drugs for specific target proteins (Tassakka *et al*., 2023). This study aims to identify bioactive compounds *C. racemosa* microcapsules exposed to high temperatures and evaluated their antioxidant and anti-inflammatory potential using molecular docking.

Materials and Methods

Caulerpa racemosa sample, chlorophyll extraction, and microencapsulation

The methods for chlorophyll extraction and microencapsulation were based on previous research (Dewi *et al*., 2022). *C. racemosa* was sourced from a local market in Karimunjawa Island, Indonesia. Fish gelatin extraction for microencapsulation followed the procedure outlined by (Irwandi *et al*., 2009), and chlorophyll was extracted using 96% ethanol. Arabic gum from Merck (Darmstadt, Germany) was used as the coating material. Freeze-drying was conducted using equipment from Ningbo Yinzhou Sjia Lab Equipment Co., Ltd., with the methodology from Lee *et al*. (2020) and Yamashita *et al*. (2017). Fish gelatin, Arabic gum, and Tween 80 from Merck were used as coating materials. The chlorophyll microcapsules were heated at 120°C, 140°C, and 160°C for five hours in an air-conditioned environment. This specific heating times and temperatures was based on previous research which microcapsules from Arabic gum and fish gelatin could protect chlorophyll from heat damage (Dewi *et al*., 2022).

GC-MS Analysis of active compounds in C. racemosa microcapsules

Gas Chromatography-Mass Spectroscopy (GC-MS) was employed to identify the active compounds present in the chlorophyll microcapsules of *C. racemosa* following the methodology of Tassakka *et al*. (2023). The sample macerated with ethanol to sample ratio 2:1 (v/w) and mixture was then filtered through filter paper to obtain a filtrate. An 0.5 mL of extract was injected into a GC-MS device (QP 2010 Shimadzu Ultra, Kyoto, Japan), with the column length of 30 m and a diameter of 0.25 mm. Helium UHP (He) was used as the carrier gas (1 $mL.min^{-1}$). The column

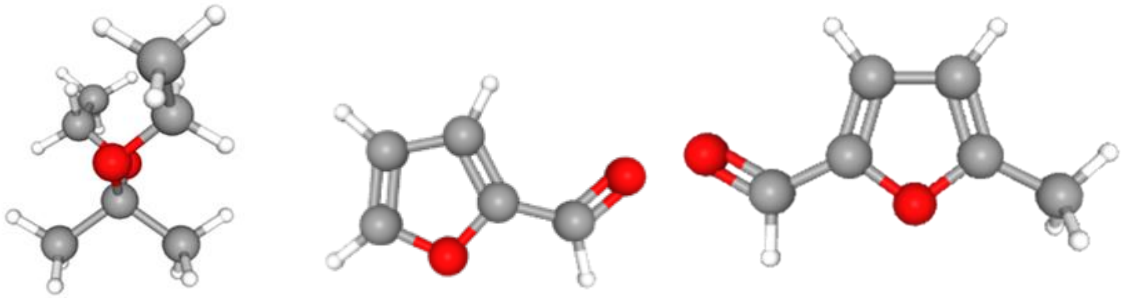
temperature initially set to 70°C for two minutes, then ramped up to 200°C. The interface temperature was maintained at 280°C. Sample dilution ratios were normalized to detect and display all compounds present in the chromatogram.

Protein structure and ligand

The ligands used in this study were the compounds identified from the GC-MS analysis of the chlorophyll microcapsules. The 3D structures of these compounds were obtained by downloading SDF files from PubChem (https://pubchem.ncbi.nlm.nih.gov/), as shown in Figure 1. The 3D structures of NF-κB and MMP-9 were acquired from the Protein Data Bank (https://www.rcsb.org/), with the NF-κB p50-p65 heterodimer complexed to κB DNA (1VKX) and MMP-9 (1GKC). NF- κB is a central mediator of inflammation, response to oxidative stress and DNA damage (Roberti *et al*., 2022). While, recently discovered MMP-9 inhibitors exhibiting notable selectivity and anticancer activity potency (Rashid and Bardaweel, 2023).

Molecular docking

Molecular docking simulations were carried out using AutoDock Vina in the PyMOL program. The docking box was set to precise x, y, and z coordinates (Table 4) to ensure the accurate positioning of ligands within the active site. Docking analysis was used to determine the binding affinity between the ligands and target proteins. The native ligand of each protein was redocked to verify accuracy, with an ideal root-mean-square deviation (RMSD) value of zero (Bell and Zhang, 2019). Protein-ligand interactions were visualized using BIOVIA Discovery Studio Visualizer v21.1.0.20298 (Schrödinger, New York, United States).



(a) [CID:31361] (b) [CID:7362] (c) [CID:12097]

Figure 1. Ligand structures of Propane 2,2-diethoxy (a), furfural (b), and 2-furancarboxaldehyde, 5-methyl (c)

a b

Figure 2. Structure of NF-κB p50-p65 heterodimer complexed with kappa B DNA (PDB ID: 1VKX) (a) and native ligand (b)

ADME analysis

The first step in the ADME analysis involved obtaining the canonical SMILES (Simplified Molecular Input Line Entry System) information from PubChem (Kim *et al*., 2016). The ADME properties of the compounds identified in *C. racemosa* microcapsules were evaluated using the SwissADME platform (http://www.swissadme.ch/) (Lagorce *et al*., 2017) and the ADME-Tlab 2.0 tool (https://admetmesh.scbdd.com/) (Xiong *et al*., 2021).

Results and Discussions

The compounds present in the microcapsules of *C. racemosa* chlorophyll were identified using the GC-MS method. Previous study has been research about evaluation of phytochemical screening, pigment content and metabolite profiling with GC-MS of *C. racemosa* (Palaniyappan *et al*., 2023), molecular docking simulation of anticancer activity (Sanger *et al*. 2023). Other research shows indicated that *Caulerpa racemosa* contains high amount of indolic alkaloid compound caulerpin, which was reported to possess anti-inflammatory activity (Abbot *et al*., 2020). Figure 1 shows that one compound, propane 2,2-diethoxy (64.60%), was identified in microcapsules treated at 120°C. Additionally, two compounds were identified in microcapsules treated at 140°C: furfural (18.08%) and 2-furancarboxaldehyde 5-methyl- (17.75%). However, no active compounds were detected in microcapsules treated at 160°C. Several references have indicated that propane-2,2-diethoxy and furfural components possess anti-inflammatory activity (Nowak *et al*., 2021; Souza *et al*., 2022; Zhao *et al*., 2024).

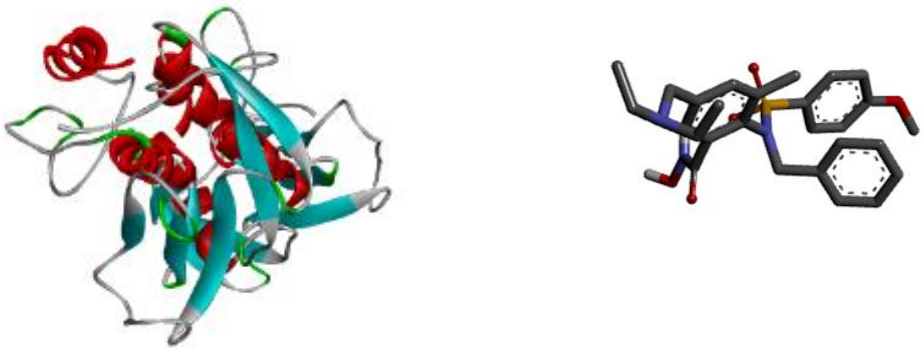


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Figure 3. Structure of MMP-9 (PDB ID: 1GKC) (a) and native ligand (b)

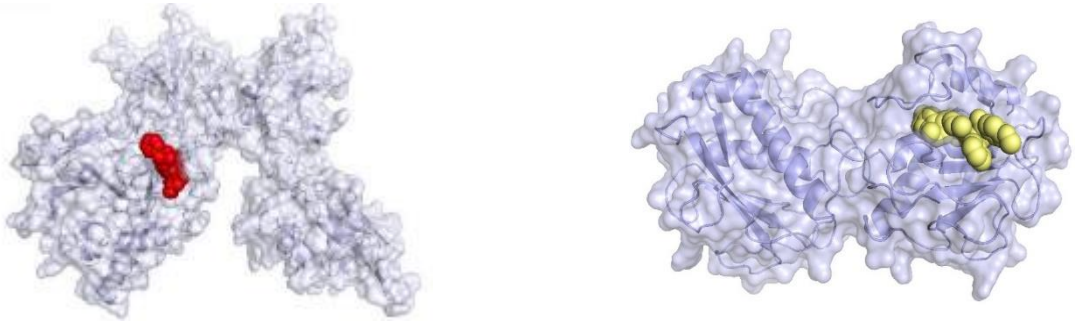

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Figure 4. Visualization of ligand complex binding with NF-κB protein (red) (a) and MMP-9 protein (yellow) (b)

Table 1. Swiss-ADME prediction of pharmacokinetic properties of compounds in *C. racemosa* microcapsules (https://admetmesh.scbdd.com/)

	Model	Compounds			Native ligand NF-kB	Native ligand MMP-9
		Propane, 2,2-diethoxy	Furfural	2-furancarboxaldehyde 5-methyl		
A	Gastrointestinal Absorption	High	High	High	High	High
	Blood-brain barrier permeant	(+) Yes	(+) Yes	(+) Yes	(-) No	(+) Yes
	Bioavailability score	0.55	0.55	0.55	0.55	0.85
D	Plasma protein binding (%)	36.337	74.371	70.804	41.718	98.432
	Volume Distribution ($L.kg^{-1}$)	1.245	1.892	1.703	0.858	0.525
M	CYP3A4 Substrate	(--) No	(--) No	(-) No	(--) No	(+++) Yes
	CYP2D6 Substrate	(--) No	(-) No	(++) Yes	(--) No	(--) No
E	Clearance (Cl) ($mL.min^{-1}. kg^{-1}$)	8.975	8.541	6.582	4.447	1.682
	Half-Life ($T_{1/2}$)	>3h	>3h	>3h	>3h	>3h

Table 2. Swiss-ADME prediction of physicochemical properties of compounds in *C. racemosa* microcapsules (http://www.swissadme.ch/)

Compounds	MW	HA	AHA	RB	HBA	HBD	MR	TPSA
2,2-diethoxypropane	132.20	9	0	4	2	0	37.97	18.46
Furfural	96.08	7	5	1	2	0	24.10	30.21
2-furancarboxaldehyde 5-methyl	110.11	8	5	1	2	0	29.06	30.21
Native ligand NF-kB	475.62	34	6	18	5	3	133.42	113.6
Native ligand MMP-9	511.63	36	18	12	6	2	140.41	107.56

MW: Molecular weight ($g.mol^{-1}$); HA: Heavy atoms; AHA: Aromatic heavy atoms; RB: Rotatable bonds; HBA: H-bond acceptors; HBD: H-bond donors; MR: Molar refractivity ($m3.mol^{-1}$); TPSA: Total polar surface area.

Table 3. Lipinski's rule of five properties of compounds extracted from *C. racemosa* microcapsules

Compounds	A	B	C	D	E	F
Propane 2,2-diethoxy	Yes	Yes	Yes	Yes	Yes	Yes
Furfural	Yes	Yes	Yes	Yes	Yes	Yes
2-furancarboxaldehyde 5-methyl	Yes	Yes	Yes	Yes	Yes	Yes
Native ligand NF-kB	Yes	Yes	Yes	Yes	Yes	Yes
Native ligand MMP-9	No	Yes	Yes	Yes	Yes	Yes

Note: A: Molecular weight < 500 Da; B: Log P < 5; C: H-bond donors < 5; D: H-bond acceptors < 10; E: Molar refraction 40–130; F: Conclusion.

In this study, two proteins associated with inflammation, 1VKX (Figure 2) and 1GKC (Figure 3), were used in docking analysis to predict the potential anti-inflammatory effects of the compounds. The binding affinity, interaction, and responsible amino acids were studied. The binding free energy (ΔG) was calculated in $kcal.mol^{-1}$, and the compound with the lowest ΔG was selected for further investigation.

Table 1 shows the pharmacokinetic properties of the phytoconstituents of *C. racemosa* chlorophyll microcapsules. Pharmacokinetics studies the time course of drug absorption, distribution, metabolism, and excretion. Absorption proved useful in early risk assessment and decision-making during drug development. Distribution showed accurate prediction of Vd, metabolism demonstrated differentiation of CYP450 substrates, and excretion provided accurate information on clearance pathways (Hamidović *et al*., 2021). The gastrointestinal absorption of all *C. racemosa* microcapsules molecules was high, shown that have good bioavailability. Yao *et al*. (2015), explained bioavailability is an essential aspect to consider while developing nutraceuticals.

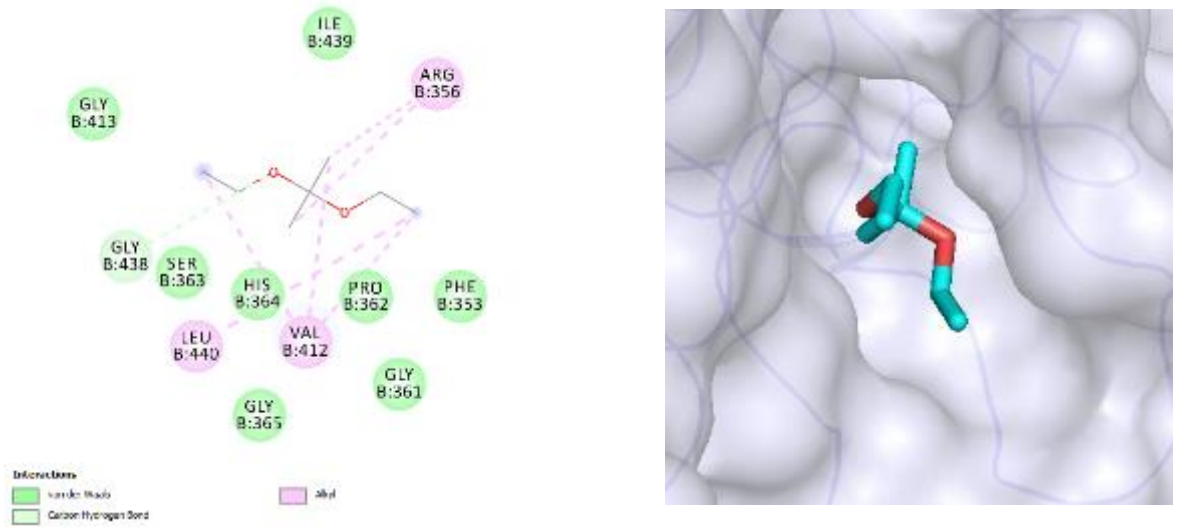

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Figure 5. Visualization of the binding structure of Propane 2,2-diethoxy ligand with NF-κB protein using Discovery Studio Visualizer (a) and PyMOL (b)

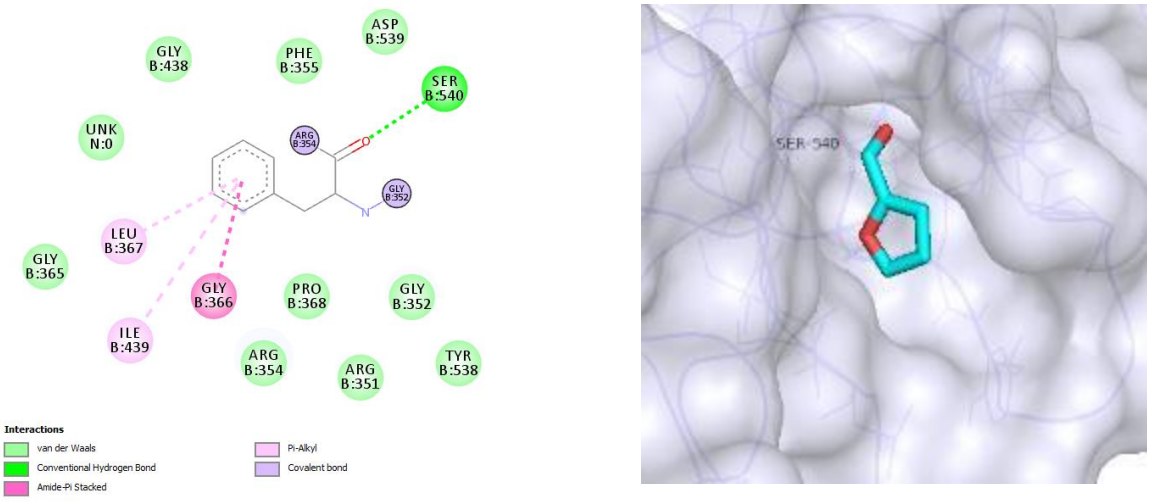

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Figure 6. Visualization of the binding structure of furfural ligand with NF-κB protein using Discovery Studio Visualizer (a) and PyMOL (b)

The physicochemical properties of small molecules in *C. racemosa* chlorophyll microcapsules (Table 2) were analyzed for their role in chemical, biological, and physical processes (Riyadi *et al*., 2022). Factors such as molecular weight, number of heavy atoms, rotatable bonds, H-bond acceptors, H-bond donors, molar refractivity, and total polar surface area were examined. Propane 2,2-diethoxy met the optimum physicochemical criteria.

Table 3 shows the drug-likeness score of the phytoconstituents, with all compounds meeting Lipinski's Rule of Five for drug-likeness. According to Lipinski, an effective drug should have a molecular

Table 4. Coordinate grid box parameters used in molecular docking

	X	Y	Z
NF-kB p50-p65 heterodimer complexed to kappa B DNA (PDB ID: 1VKX)			
Grid center	-3.4309	16.7395	31.6606
Dimension (Angstrom)	20.5295	19.9429	20.6091
MMP-9 (PDB ID: 1GKC)			
Grid center	69.3296	29.4229	112.3185
Dimension (Angstrom)	18.214	14.4629	17.8135

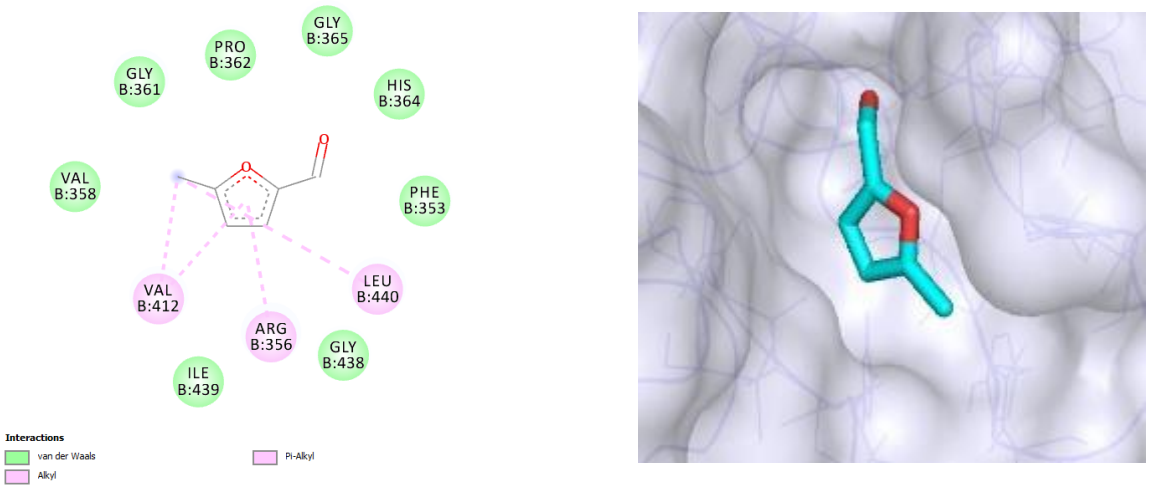

Figure 7. Visualization of the binding structure of 2-furancarboxaldehyde, 5-methyl ligand with NF-κB protein using Discovery Studio Visualizer (a) and PyMOL (b)

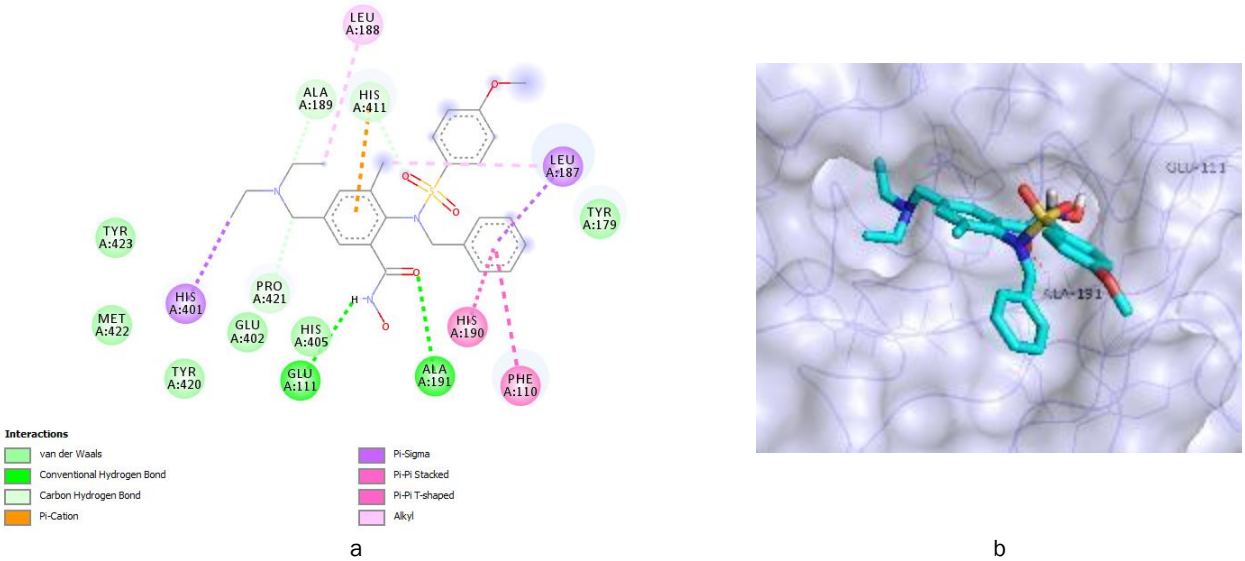

Figure 8. Visualization of the binding structure of native ligand with MMP-9 protein using Discovery Studio Visualizer (a) and PyMOL (b)

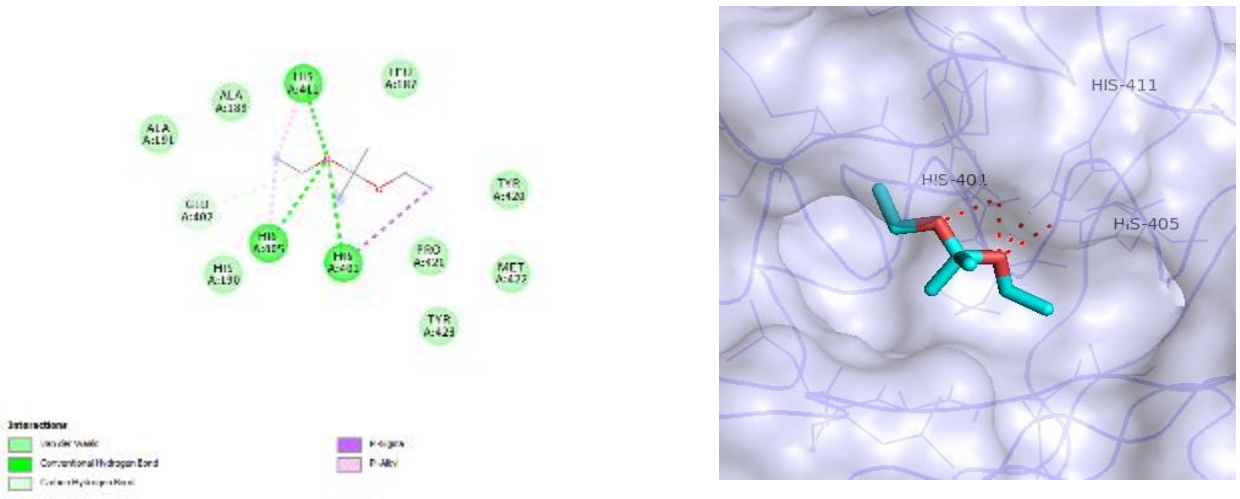

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Figure 9. Visualization of the binding structure of Propane 2,2-diethoxy ligand with MMP-9 protein using Discovery Studio Visualizer (a) and PyMOL (b)

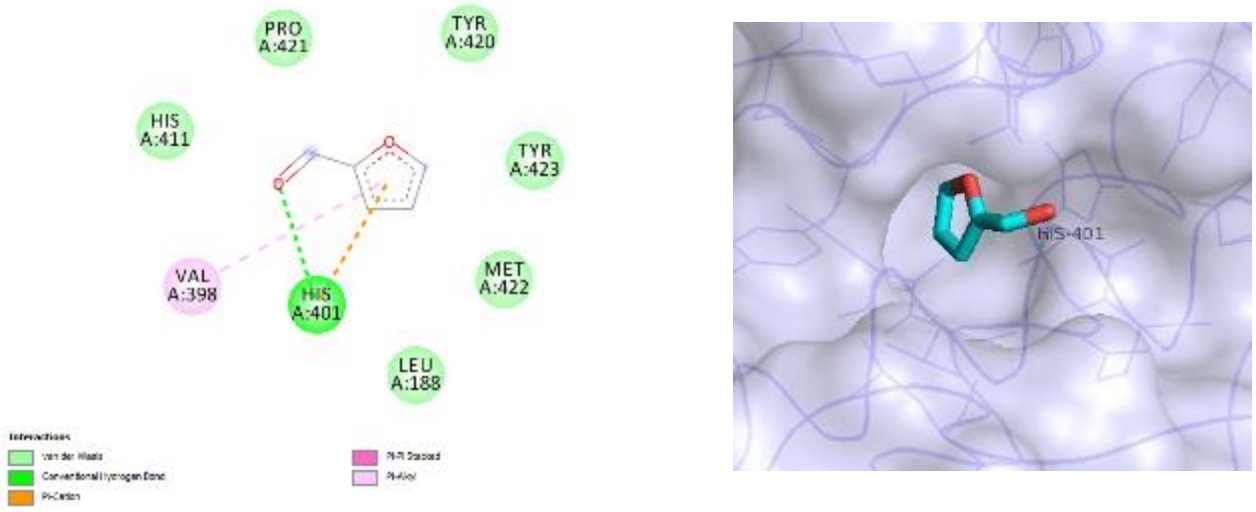

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Figure 10. Visualization of the binding structure of furfural ligand with MMP-9 protein using Discovery Studio Visualizer (a) and PyMOL (b)

weight below 500, MLOGP ≤ 4.15, N or O ≤ 10, and NH or OH ≤ 5. All nitrogen and oxygen with at least one hydrogen are considered H-bond acceptors, while all nitrogen and oxygen with at least one hydrogen are considered H-bond donors. Aliphatic fluorine are acceptors, while aliphatic nitrogen is neither a donor nor an acceptor (Lipinski *et al*., 1997).

The docking results of *C. racemosa* chlorophyll microcapsules with the receptor and native ligand are presented in Table 5. Figure 4 shows the structure of the docked complex, where ligands are docked with target proteins (NF-kB and MMP-9) using PyMOL software to obtain binding affinity values, ensuring RMSD = 0. Based on the results of molecular docking

Table 5. Binding affinity values from molecular docking

Proteins	Compounds	Binding affinity (kcal.mol^{-1})
NF-kB p50-p65 Heterodimer Complexed to kappa B DNA (1VKX)	MG132 (NFkB inhibitor)	-7.6
	2-furancarboxaldehyde 5-methyl	-4.7
	Furfural	-4.3
	Propane 2,2-diethoxy	-4
MMP-9 (1GKC)	MMP-9 inhibitor	-7.5
	2-furancarboxaldehyde 5-methyl	-4.9
	Furfural	-4.5
	Propane 2,2-diethoxy	-4.3

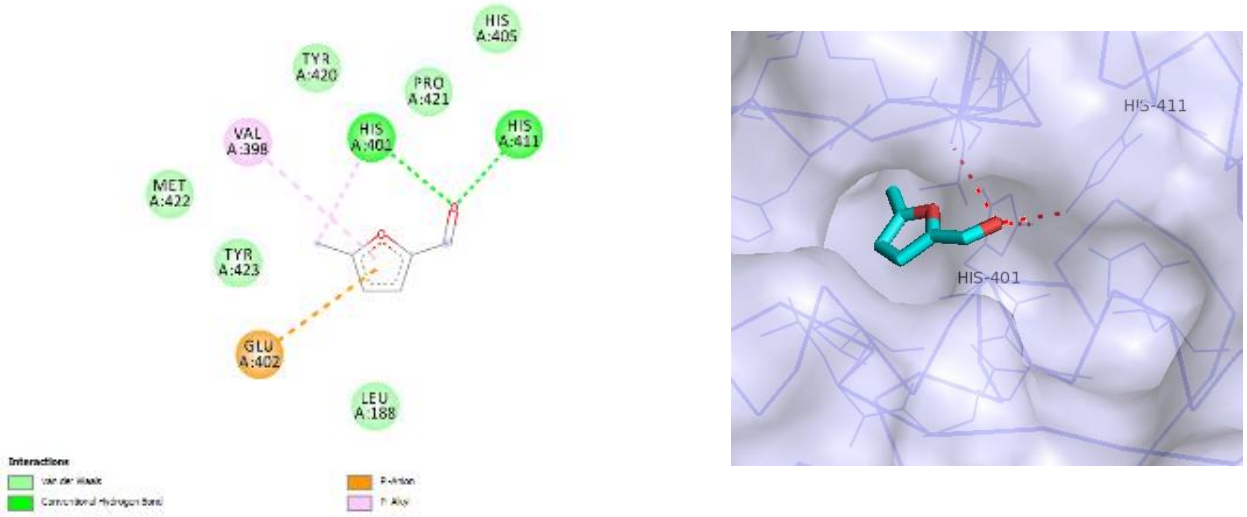

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Figure 11. Visualization of the binding structure of 2-furancarboxaldehyde, 5-methyl ligand with MMP-9 protein using Discovery Studio Visualizer (a) and PyMOL (b)

(Table 5) shows that the compound 2-furancarboxaldehyde 5-methyl has the lowest binding score (most negative) indicating the highest possibility of a stable and strong interaction between the protein and the ligand (Abdulhassan *et al*., 2022). Previous studies have explained that the compound 2-furancarboxaldehyde, 5-methyl-F, can inhibit the formation of sickle cells in the blood (Hamouda *et al*. 2021). The compound is also found in the plants *Artocarpus hirsutus*, *Acmella uliginosa* (Sw.), *Flueggea leucopyrus*, which have opened new perspectives in pharmaceutical research and can be used for the development of new potential antioxidant and anti-inflammatory agents for the treatment of various diseases (Patel *et al*., 2016; Ahmad *et al*., 2020; Mayakrishnan *et al*., 2024).

NF-κB is involved in cellular responses to stimuli like stress, cytokines, and free radicals. The docking of propane 2,2-diethoxy with NF-kB showed a binding energy of ΔG = −4, with the amine group of propane 2,2-diethoxy binding to the NF-kB complex at Arg B:356, Leu B:440, and Val B:412, while the carboxyl group binds at Gly B:438. Figures 5a and 5b illustrate the 2D and 3D interactions of propane 2,2-diethoxy with the NF-kB receptor. Similarly, the docking of furfural with NF-kB revealed binding energy of ΔG = −4.3, with the amine group of furfural binding at Ile B:439 and Leu B:367, and the amide group binding at Gly B:366. Figures 6a and 6b show the 2D and 3D interactions of furfural with NF-kB.

This value of binding affinity (Table 5) was compared with inhibitors of MMP-9 and NF-kB. This data is explained using molecular docking analysis of receptor-ligand binding. These results demonstrated that *C. racemosa* microcapsules molecules are no better than of native ligand. The docking of 2-furancarboxaldehyde 5-methyl with NF-kB displayed a binding energy of ΔG = −4.7. The amine group of 2-furancarboxaldehyde 5-methyl binds to the NF-kB complex at Arg B:356, Leu B:440, and Val B:412.

Figures 7a and 7b show the 2D and 3D interactions of 2-furancarboxaldehyde 5-methyl with NF-kB. MMP-9, a well-studied MMP involved in inflammation (Yabluchanskiy *et al*., 2013), showed a binding energy of ΔG = −4.3 when docked with propane 2,2-diethoxy. The carboxyl group of propane 2,2-diethoxy binds to MMP-9 at Glu A:402, while the amine group binds at His A:405 and His A:411. Figures 9a and 9b display the 2D and 3D interactions of propane 2,2-diethoxy with MMP-9. Furfural showed a binding energy of ΔG = −4.3 when docked with MMP-9, with the amine group binding at Val A:398. Figures 10a and 10b illustrate the 2D and 3D interactions of furfural with MMP-9.

The docking of 2-furancarboxaldehyde 5-methyl with MMP-9 displayed a binding energy of ΔG = −4.9. The amine group binds to MMP-9 at Val A:398. Figures 11a and 11b show the 2D and 3D interactions of 2-furancarboxaldehyde 5-methyl with MMP-9. The binding affinity value significantly affects the stability of the ligand-receptor interaction. The most negative ΔG value indicates the strongest interaction, as the receptor requires less energy to interact with the ligand (Kellenberger *et al*., 2008).

Among the compounds, 2-furancarboxaldehyde 5-methyl showed the highest affinity for the macromolecular targets. The ratio of bond energy between 2-furancarboxaldehyde 5-methyl and its target protein demonstrates its potential. While propane 2,2-diethoxy, furfural, and 2-furancarboxaldehyde 5-methyl exhibited higher energy values than the native ligand during validation, they still showed an affinity for NF-kB, interacting with the protein's active site. 2-Furancarboxaldehyde 5-methyl may serve as a source of antioxidants and anti-inflammatories, reducing inflammation and oxidative stress (Sami *et al*., 2021).

The different amino acid residues obtained from docking, compared to validation, suggest that each compound seeks the most stable conformation at the active site. NF-κB might play a protective role under oxidative stress by suppressing ROS accumulation. Inhibiting NF-κB activation increases TNFα-induced ROS production, lipid peroxidation, and protein oxidation (Djavaheri-Mergny *et al*., 2004). ROS can activate MMP-8 and MMP-9 in periodontal tissues via oxidizing enzymes (Franco *et al*., 2017). Peroxide activation elevates interleukin 6 (IL-6) levels, with MMPs influencing the effects of peroxide (Cavalla *et al*., 2015).

Conclusion

Propane 2,2-diethoxy was identified in microcapsules treated at 120°C, while furfural and 2-furancarboxaldehyde 5-methyl were identified in microcapsules treated at 140°C. Among these, 2-furancarboxaldehyde 5-methyl demonstrated the highest affinity for macromolecular targets. This compound as an antioxidant to reduce the illness and produces anti-inflammatory effects. Furthermore, need for further experimental validation and exploration of bioactive compounds and found optimum temperature to maintain the bioactivity of *C. racemosa* microcapsules.

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