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Original Article

Fungistatic activity and mechanism of *Caulerpa racemosa*, *Caulerpa lentillifera* fractions and caulerpin metabolite against pathogenic fungi

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Abstract

The increasing prevalence of azole resistance in various fungal species presents a significant concern, highlighting the urgent need for new antifungal agents. The aim of this study was to investigate the antifungal activity of fractions from *Caulerpa racemosa, C. lentillifera,* and caulerpin against three species: *Aspergillus flavus, A. niger*, and *Candida albicans*. The *Caulerpa* extracts were obtained through maceration with 96% ethanol, followed by fractionation using vacuum liquid chromatography. Antifungal activity was assessed using the broth microdilution method, while fungal growth kinetics were evaluated through time-kill curves. Bioautography was employed to identify inhibitory compounds, while liquid chromatography high-resolution mass spectrometry (LC-HRMS) was utilized to detect the contents of the extracts and fractions. Scanning electron microscopy (SEM) was used to observe the fungal structure, and the absorbance at 260/280 nm was measured to evaluate the cell leakage. LC-HRMS identified numerous compounds in *C. racemosa* and *C. lentillifera* with antifungal activities, including fatty acids, terpenes, alkaloids, flavonoids, and coumarins. The results indicate that the fractions of both *Caulerpa* did not inhibit the growth of *A. flavus* and *A. niger*, but effectively inhibited *C. albicans*. Among the fractions, F3CR and F4CL exhibited the highest antifungal efficacy against *C. albicans*, with minimum inhibitory concentrations (MICs) ranging from 64 to 128 µg/mL. Caulerpin, the primary metabolite of *Caulerpa*, also demonstrated significant inhibition, with an MIC of 256 µg/mL. The findings suggested that F3CR, F4CL, and caulerpin possessed fungistatic properties. Bioautography results revealed clear zones in the colonies, indicating inhibited fungal growth. The SEM observations showed that fungal cells became rough, perforated, and damaged, which was confirmed by the increase in absorbance at 260/280 nm, suggesting the release of cellular components such as nucleotides and proteins. In conclusion, both *Caulerpa* species and caulerpin are promising candidates for developing new antifungal agents against *C. albicans*.

Keywords: Antifungal activity, *Candida albicans, Caulerpa lentillifera*, *Caulerpa racemosa,* caulerpin

Introduction

The global incidence of invasive fungal infections (IFI) has risen in recent years, particularly affecting immunocompromised individuals who are highly susceptible to life-threatening opportunistic infections [1]. Fungi such as *Candida* spp., which are commonly part of the human

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microbiota, can cause invasive candidiasis in these vulnerable patients [2]. Other fungal pathogens, including *Aspergillus*, *Fusarium*, and *Mucorales*, also pose significant risks, often contributing to healthcare-associated infections (HAI) among patients with underlying conditions [3].

Candidiasis encompasses infections of the skin, mucosa, and internal organs caused by *Candida* spp., with invasive candidiasis involving bloodstream infections (candidemia) and deep-seated infections such as peritonitis and osteomyelitis [1,4,5]. Among the 15 *Candida* species that infect humans, five species account for the most invasive cases: *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* [5]. Notably, *C. albicans* contributes to 75% of vaginal discharge cases in Indonesia, partly due to the country's humid climate, which favors fungal growth [6]. Despite its commensal nature, *C. albicans* poses risks due to virulence factors such as adhesins, hydrolytic enzymes, and biofilm formation, which facilitate host cell invasion and injury [7]. The adaptability of *C. albicans* also enables resistance development through biofilm formation, genetic mutations, and efflux pump expression, posing challenges for antifungal treatment [8]. Resistance to azole antifungals, in particular, is increasingly observed, with *C. albicans* showing the lowest resistance to amphotericin B, nystatin, flucytosine, and caspofungin, but high resistance to miconazole and econazole [9].

In addition to *Candida*, *Aspergillus* species, notably *A. fumigatus*, *A. terreus*, and *A. flavus*, are primary pathogens in immunocompromised hosts, where untreated invasive aspergillosis approaches a 100% mortality rate [10]. Current research highlights marine-derived medicinal plants as potential antifungal agents. The *Caulerpa* genus, or seaweed, contains compounds such as saponins, flavonoids, tannins, sterols, and fatty acids that inhibit *C. albicans* growth [11]. The compound caulerpin, isolated from *Caulerpa*, also demonstrates antifungal properties [12]. The aim of this study was to assess the antifungal activity of extracts from *C. racemosa*, *C. lentillifera*, and caulerpin against *Candida* and *Aspergillus*, investigating their efficacy and potential mechanisms as natural antifungal agents.

Methods

Study design and setting

This experiment was conducted at the Microbiology Laboratory of Institut Teknologi Bandung, Bandung, Indonesia, and Universitas Jenderal Achmad Yani, Cimahi, Indonesia. The study analyzed the activity of *C. racemosa, C. lentillifera*, and caulerpin against *C. albicans, A. flavus,* and *A. niger*. The analysis of antifungal activity was carried out from March to June 2024. The research stages began with the preparation of test fungi, extracts, and fractions of the *Caulerpa*. This was followed by antifungal testing using the microdilution method, evaluation of fungal inhibition properties through the time-kill curve method, bioautography, analysis of fraction components using liquid chromatography high-resolution mass spectrometry (LC-HRMS), and investigation of antifungal mechanisms using scanning electron microscopy (SEM) and cell leakage determination.

Plants and caulerpin preparation

Fresh *C. racemosa* and *C. lentillifera* specimens were collected from coastal areas in Jepara, Indonesia. The collection process was carefully managed to maintain the integrity of bioactive compounds within the fresh seaweed material, which is essential for accurate extract preparation and analysis. Species identification was performed at the Laboratory of Marine Biology, Universitas Padjadjaran, Bandung, Indonesia, with verification documented under reference No. 26/HB/04/2022. For the subsequent steps, a caulerpin of 98% purity was sourced from Anhui Minmetals Development Import and Export (Anhui Minmetals Development I/E, Hefei, China), ensuring the standardized quality needed for reproducible results in compound extraction and analysis.

Extraction and fractionation

The plants were harvested, cut, and dried at 65°C in a cabinet dryer until a constant weight was achieved. A 500-gram sample of the powdered seaweed was extracted with ethanol for three

consecutive 24-hour periods in a closed, dark bottle on a shaker set to 120 rpm at 28°C to obtain ethanolic extract of *C. racemosa* (EECR) and ethanolic extract of *C. lentillifera* (EECL) [13]. Following extraction, the solvent was evaporated to concentrate the extract, which was then redissolved in dimethyl sulfoxide (DMSO) to reach a final concentration of 100 mg/mL. The concentrated extract underwent fractionation using vacuum liquid chromatography (VLC). Silica gel impregnation was performed by adding 20 grams of silica gel to 10 grams of EECR and EECL. A 250 mL eluent was used for each fractionation, with the VLC column packed to a depth of 7 cm. Gradient elution was carried out with a series of solvents: n-hexane-ethyl acetate (Hex-EA) mixtures (9:1, 8:2, 7:3, 5:5, and 3:7), followed by 100% ethyl acetate (EA), chloroform-methanol (Chol-MeOH) mixtures (8:2, 7:3, and 5:5), and 100% methanol (MeOH) [14].

Fungi preparation

The fungal species of *A. flavus*, *A. niger*, and *C. albicans* were sourced from the Microbiology Laboratory at Universitas Jenderal Achmad Yani, Cimahi, Indonesia. Prior to experimentation, each fungal strain was cultured on Sabouraud dextrose agar and incubated at 37°C for 24 hours to ensure optimal growth. Following incubation, the fungi were harvested and suspended in 0.9% saline solution (NaCl) to achieve a 0.5 McFarland standard, ensuring consistency in fungal concentration for subsequent analysis [15].

Microdilution method for antifungal activity evaluation

The antifungal activity against *A. flavus*, *A. niger*, and *C. albicans* was evaluated using the broth microdilution method, employing Sabouraud dextrose broth as the growth medium. This standardized method allows for the determination of the minimum inhibitory concentration (MIC) of test samples. Each well of a 96-well microplate was prepared with a final volume of 100 µL, containing approximately 1.5×10^8 colony-forming units (CFU) of the target fungi.

Control wells were included: negative controls consisted of wells containing the growth medium without antifungal agents, ensuring any observed growth was solely due to the test samples, while positive controls contained 1024 µg/mL of ketoconazole. Test wells contained the extracts (EECL and EECR) or compounds (caulerpin) being evaluated for antifungal activity. The microplates were incubated at 37°C for 24 hours. After incubation, the MIC value was determined by assessing the clarity of the medium in each well. The MIC was defined as the lowest concentration of antifungal agent that inhibited visible microbial growth [16].

Time-kill curve for antifungal activity assessment

The time-kill curve method was employed to evaluate the fungicidal activity of EECL, EECR and caulerpin. An initial microbial inoculum was prepared at a concentration of approximately 0.5 McFarland, ensuring standardized inoculum density for reproducible results across different experimental conditions. Antifungal agent (EECL, EECR, or caulerpin) was then added to the inoculum at final concentrations corresponding to 1, 2, 4, and 8 times the MIC. The inoculum, with the added antifungals, was incubated at 37°C. At predefined time points (0, 4, 8, and 24 hours), 100 µL aliquots were taken from each antimicrobial concentration. The aliquots were serially diluted and plated onto agar media, which were subsequently incubated for 24 hours at 37°C to allow the growth of any surviving colonies. After incubation, the colonies were counted, and a log reduction of colony-forming units per milliliter (CFU/mL) over time was plotted [17].

Bioautography for antifungal evaluation

Bioautography was performed using the contact bioautography method to assess the antifungal potential of the extracts. A thin-layer chromatography (TLC) plate was first preheated at 100°C for 30 mins to prepare it for the analysis. The fraction that showed the best result in MIC test was then applied to the TLC plate using a capillary tube and allowed to dry briefly. Following this, the plate was placed in a developing vessel saturated with the mobile phase consisting of chloroform and methanol (9:1). The plate was allowed to elute until the predetermined chromatographic limit was reached, after which it was removed from the chamber. The TLC results were observed for spot formation, retention factor (Rf) values (the distance of solute/distance of solvent) were calculated, and absorbance was measured at UV light of 245 and 366 nm to identify possible compounds present in the fraction.

The resulting chromatogram was then placed face down onto Sabouraud agar inoculated with the target fungi. The plate was left in contact with the agar for 30 mins to facilitate diffusion of the extract components into the agar. The setup was then incubated for 24 hours. After incubation, the chromatogram was removed, and any inhibition zones observed on the agar surface, corresponding to the spots on the TLC plate, indicated the presence of antifungal compounds [18].

Liquid chromatography high-resolution mass spectrometry (LCHRMS) identification of antifungal compounds

The antifungal compounds were identified using a Thermo Scientific Vanquis UHPLC Binary Pump (Thermo Fisher Scientific, Waltham, USA) coupled with a Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap High-Resolution Mass Spectrometer (Thermo Fisher Scientific, Waltham, USA). The mobile phases consisted of MS-grade water with 0.1% formic acid (phase A) and MS-grade methanol with 0.1% formic acid (phase B). A gradient elution technique was employed with a flow rate set at 0.3 mL/min. Initially, the concentration of mobile phase B was set at 5%, and then gradually increased to 90% over 16 mins. The concentration was maintained at 90% for 4 mins before returning to the initial condition (5% phase B) for the final 5 mins, completing the run in 25 mins.

Intracellular leakage mechanism of action in antifungal activity

The protein and nucleic acid leakage method is a useful approach for evaluating the antimicrobial activity of agents, particularly in terms of their minimum killing concentration (MKC). This technique provides insights into the effects of antifungal agents on microbial cell membranes, specifically their ability to induce leakage of intracellular components.

In this method, *C. albicans* cultures were suspended in 10 mL of nutritional broth and centrifuged for 20 mins at 3500 RPM. After separating the filtrate, the pellet was washed twice in pH 7.0 phosphate buffer and resuspended in the same buffer. The antifungal agent was then added to the resuspended pellet, along with a control group, at concentrations corresponding to the MIC for 24 hours, with incubation in a shaking incubator. After incubation, the supernatant and cell pellet were separated by centrifugation at 3500 RPM for 20 mins. The absorbance of the supernatant was measured at 260 nm and 280 nm using a spectrophotometer to assess protein and nucleic acid leakage. Protein concentrations were quantified at 280 nm, while nucleic acid concentrations were measured at 260 nm. The absorbance values from the leaking cells were compared to the negative control to account for background absorption and determine the extent of leakage induced by the antifungal treatment [19].

Cell and membrane wall disruption mechanism of action in antifungal activity

To assess the antifungal-induced disruption of cell and membrane walls, fungi were prepared for SEM following a series of critical fixation and dehydration steps to preserve cellular structures. The yeast cells were initially treated with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hours at 4°C. Following the primary fixation, samples were further fixed in 2% aqueous osmium tetroxide for 4 hours to enhance membrane preservation. After fixation, the samples were dehydrated through a graded series of alcohols and then subjected to critical point drying using a critical point drying (CPD) unit. The dried samples were mounted onto aluminum studs with double-sided carbon conductive tape. A thin layer of gold was sputter-coated onto the samples for 3 mins to improve conductivity for SEM analysis. The samples were then examined using a Thermo Scientific Prisma E Scanning Electron Microscope (Thermo Fisher Scientific, Waltham, USA) to observe the structural alterations in the cell wall and membrane as a result of antifungal treatment.

Results

Fractionation of vacuum liquid chromatography (VLC) of ethanol extracts of *C. racemosa* (EECR) and *C. lentillifera* (EECL)

The VLC of EECL and EECR yielded ten fractions, as summarized in **Table 1**. The VLC results of the EECR fractions revealed significant differences in polarity based on their Rf values on TLC

(**Table 1**). Fraction 2, obtained using a mixture of n-hexane and ethyl acetate (Hex-EA, 8:2), had an Rf value of 0.96, indicating a compound with low polarity, likely fatty acids and their derivatives. Fraction 4, separated using Hex-EA (7:3), had three Rf values (0.91, 0.88, and 0.56), suggesting the presence of components with varying polarities. Fraction 10 was the heaviest weight at 2.7679 g, likely due to the formation of a salt (**Table 1**).

Table 1. Results of fractionation of vacuum liquid chromatography of ethanol extract of *Caulerpa racemosa* (EECR) and *Caulerpa lentillifera* (EECL)

Extract	Fraction	No. vial	Column mobile phase	TLC mobile phase	Retention factor (Rf)	Weight (g)
EECR	1	1	Hex-EA (9:1)	Hex-EA 5:5	-	0.0054
	2	2	Hex-EA (8:2)	Hex-EA 5:5	0.96	0.0229
	3	3–4	Hex-EA (8:2); (7:3)	Hex-EA 5:5	0.96; 0.91	0.0585
	4	5	Hex-EA (7:3)	Hex-EA 5:5	0.91; 0.88; 0.56	0.033
	5	6–7	Hex-EA (5:5); (3:7)	Hex-EA 5:5	0.49	0.290
	6	8–9	EA 100%	Hex-EA 5:5	0.49	0.066
	7	10	Chol-MeOH (8:2)	Hex-EA 5:5	0.49	0.064
	8	11	Chol-MeOH (8:2)	Hex-EA 5:5	0.21; 0.49	0.1871
	9	12–13	Chol-MeOH (8:2); (7:3)	Hex-EA 5:5	0.49; 0.21; 0.49	0.6178
	10	14–15	Chol-MeOH (5:5), MeOH 100%	Hex-EA 5:5	-	2.7679
EECL	1	1–2	Hex-EA (9:1); (8:2)	Hex-EA 5:5	0.96	0.1512
	2	3	Hex-EA (8:2)	Hex-EA 5:5	0.94	0.0290
	3	4	Hex-EA (7:3)	Hex-EA 5:5	0.89	0.0066
	4	5	Hex-EA (7:3)	Hex-EA 5:5	0.86	0.0087
	5	6	Hex-EA (5-5)	Hex-EA 5:5	0.80	0.0051
	6	7	Hex-EA (3-7)	Hex-EA 5:5	0.65	0.0128
	7	8–10	EA 100%, Chol-MeOH (8:2)	Hex-EA 5:5	0.46	0.1914
	8	11	Chol-MeOH (8:2)	Hex-EA 5:5	0.28; 0.46	0.1871
	9	12–13	Chol-MeOH (8:2); (7:3)	Hex-EA 5:5	0.28	
	10	14	Chol-MeOH (5:5)	Hex-EA 5:5	-	1.4727
	11	15	MeOH 100%	Hex-EA 5:5	-	1.6865

Chol-MeOH: chloroform-methanol; EA: ethyl acetate; Hex-EA: n-hexane-ethyl acetate; MeOH: methanol

Similarly, the EECL fraction exhibited analogous properties (**Table 1**). Non-polar fractions had higher Rf values on TLC, with fraction 1 likely consisting of fatty acids or their derivatives. Fraction 7 had a single Rf value of 0.46, indicating a compound of intermediate polarity with a relatively high weight (0.1914 g). Fractions 4, 5, and 6 had Rf values of 0.89, 0.86, and 0.80, respectively. These fractions likely contained semi-polar compounds such as flavonoids, saponins, tannins, and terpenoids.

Minimum inhibitory concentration based on microdilution

The MIC for EECR and EECL, their respective fractions (F1-F10 for EECR and F1-F11 for EECL), and caulerpin against *A. flavus*, *A. niger*, and *C. albicans* are presented in **Table 2**. The results showed that *C. albicans* was notably inhibited by several extracts, fractions, and caulerpin, with MIC values in the active range (128<MIC≤512 µg/mL). Fractions F3 of EECR (F3CR) and F4 of EECL (F4CL) exhibited the strongest inhibition, with MIC values of 64 µg/mL and 128 µg/mL, respectively, indicating significant antifungal activity. Additionally, caulerpin achieved a good inhibitory effect with a MIC of 256 µg/mL against *C. albicans*. In comparison, the extracts and fractions from both *C. racemosa* and *C. lentillifera* showed minimal inhibitory effects against *A. flavus* and *A. niger* (**Table 2**).

Table 2. Minimum inhibitory concentration (MIC) of ethanol extract of *Caulerpa racemosa* (EECR) and *Caulerpa lentillifera* (EECL) against three pathogenic fungi (*Aspergillus flavus*, *Aspergillus niger*, and *Candida albicans*)

Group	Fraction	Minimum inhibitory concentration (µg/mL)		
		A. flavus	*A. niger*	*C. albicans*
Ketoconazole		32	64	16
DMSO		positive	positive	positive
C. racemosa	Caulerpin	>1024	>1024	256

Group	Fraction	Minimum inhibitory concentration (µg/mL)		
		A. flavus	*A. niger*	*C. albicans*
	EECR	>1024	>1024	256
	F1CR	>1024	>1024	256
	F2CR	>1024	>1024	256
	F3CR	>1024	>1024	64
	F4CR	>1024	>1024	256
	F5CR	>1024	>1024	256
	F6CR	>1024	>1024	512
	F7CR	>1024	>1024	512
	F8CR	>1024	>1024	256
	F9CR	>1024	>1024	512
	F10CR	>1024	>1024	512
C. lentillifera	Caulerpin	>1024	>1024	256
	EECL	>1024	>1024	512
	F1CL	>1024	>1024	512
	F2CL	>1024	>1024	512
	F3CL	>1024	>1024	512
	F4CL	>1024	>1024	128
	F5CL	>1024	>1024	512
	F6CL	>1024	>1024	512
	F7CL	>1024	>1024	512
	F8CL	>1024	>1024	512
	F9CL	>1024	>1024	512
	F10CL	>1024	>1024	256
	F11CL	>1024	>1024	1024

F1CR-F10CR are fraction 1 to 10 of EECR; F1CL-F11CL are the fraction 1 to 11 of for EECL

Time-kill curve for antifungal activity assessment

Since F3CR and F4CL had the strongest inhibition based on the MIC, further tests were only conducted on these fractions, together with caulerpin. The time-kill curve was a kinetic test used to understand the interaction of F3CR, F4CL, and caulerpin against *C. albicans*. This test evaluated whether the antifungal activity was concentration-dependent or time-dependent. It also determined whether the antimicrobial agent was fungistatic or fungicidal. In this study, the time-kill curves demonstrated that antifungal treatments were effective across various dosages and time points. F3CR, F4CL, and caulerpin exhibited fungistatic activity, as indicated by a decrease in log CFU by approximately 0.3$\log_{10}$ or 54% (**Figure 1**).

The results for F3CR showed that the 4×MIC dosage was more effective than the 8×MIC dosage. This was evident from the graph, where 4×MIC resulted in a greater reduction in growth compared to 8×MIC (**Figure 1**). In contrast, for F4CL and caulerpin, the highest dosage (8×MIC) produced a more significant decline in growth, indicating that higher dosages more effectively inhibited *C. albicans* growth (**Figure 1**).

Bioautography for antifungal evaluation

Further antifungal evaluation of F3CR and F4CL against *C. albicans* was conducted by bioautography and the results are presented in **Table 3**. TLC was performed by spotting 5 µL of each extract onto the TLC plate, with a mobile phase of chloroform: methanol (9:1). Active spots were indicated by the formation of a clear zone around the fungal colony, with the Rf value of the clear zone matched to the corresponding chromatogram spot. The bioautography results for F3CR and F4CL showed a single active spot with Rf values of 0.50 and 0.23, respectively (**Figure 2**). The inhibition zones were 15±0.5 mm for F3CR and 20±0.6 mm for F4CL, demonstrating the antifungal activity of the active compounds (**Figure 2**). Additionally, caulerpin, with an Rf value of 0.23, was also tested in bioautography against *C. albicans*; however, no clear zone was observed after 48 hours of incubation, indicating the absence of antifungal activity (**Table 3**). The TLC results for F3CR and F4CL showed green and black spots, indicating areas of weak and strong absorbance (**Figure 2**). Based on observation at 254 and 366 nm, the potential components are suspected to be aromatic group compounds, flavonoids, or phenolics.

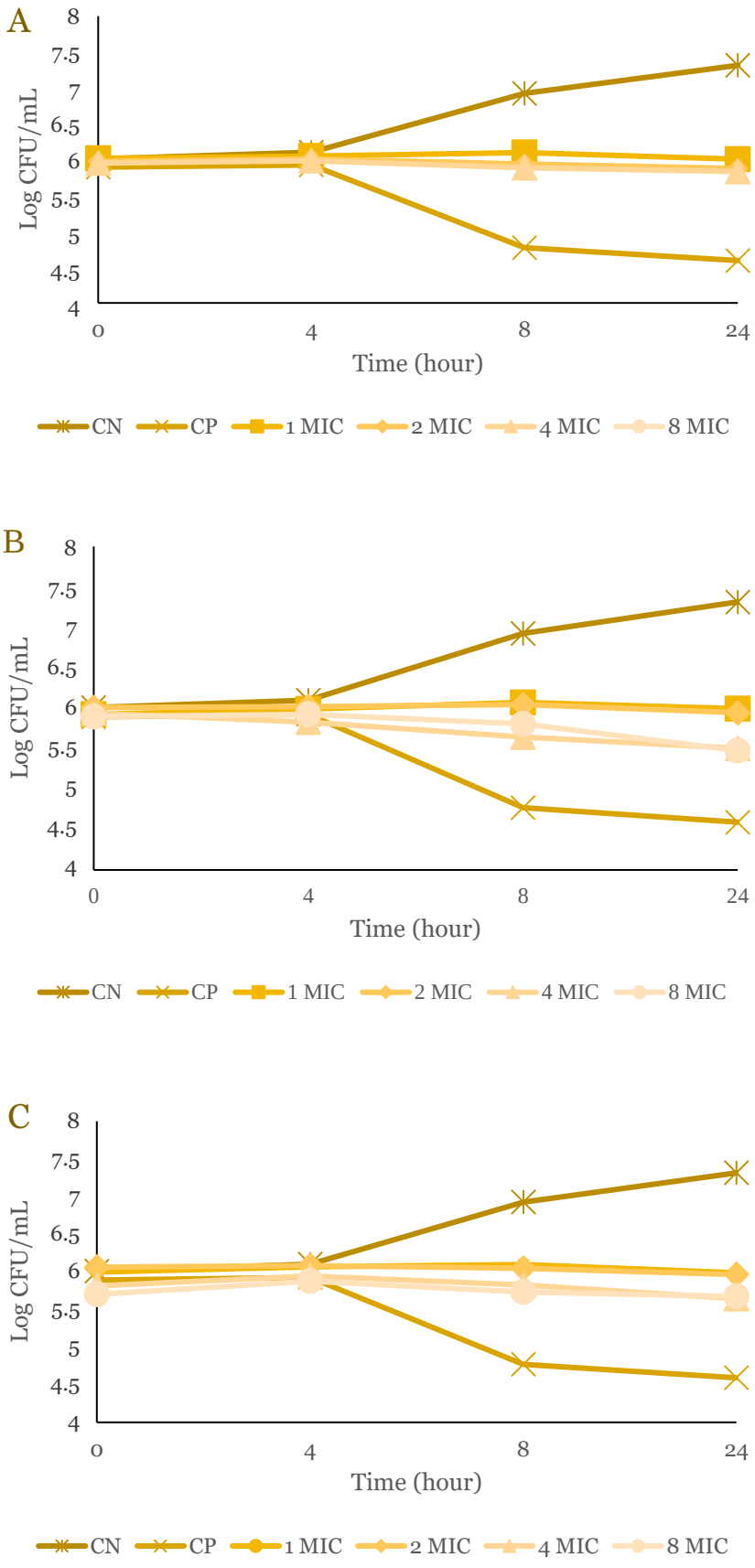


Figure 1. Time-kill curve of *Candida albicans* after incubation for 24 hours for (A) fraction 3 of ethanol extract of *Caulerpa racemosa* (F3CR), (B) fraction 4 of ethanol extract of *Caulerpa lentillifera* (F4CL), (C) caulerpin. CP: positive control (ketoconazole); and CN: control negative, media only.

Table 3. Spots of F3CR and F4CL detected on the thin-layer chromatography (TLC) chromatogram

Fraction	Retention factor (Rf) value	Visible light	UV 254	UV 366
F3CR				
Spot 1	0.27	Green	Green	Red
Spot 2	0.46	Green	Green	Black
Spot 3	0.50	Black	Green	Black
Spot 4	0.62	Grey	Green	Not visible
Spot 5	0.73	Not visible	black	Not visible
F4CL				
Spot 1	0.19	Green	Green	Red
Spot 2	0.23	Green	Green	Black
Spot 3	0.27	Green	Black	Black
Spot 4	0.31	Green	Green	Red
Spot 5	0.38	Black	Black	Black
Caulerpin				
Spot 1	0.23	Green	Green	Black

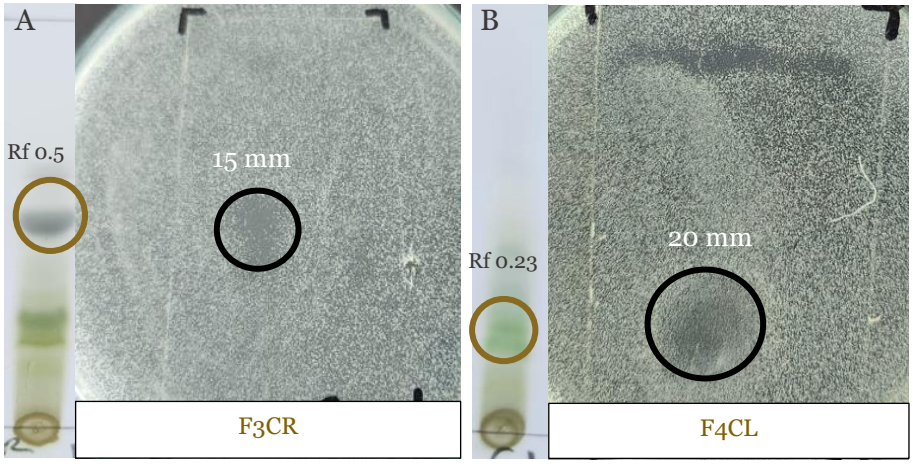


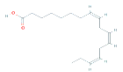
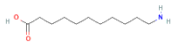
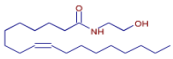
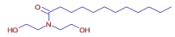
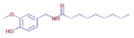
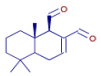
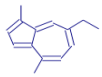
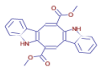
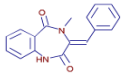
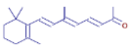
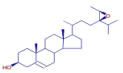
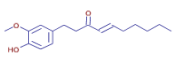
Figure 2. Bioautography result of F3CR (A) dan F4CL (B) against *Candida albicans*. The black circle refers to the inhibited zone caused by the antifungal agent in the thin-layer chromatography (TLC) (gold circle).

LCHRMS identification of antifungal compounds

F3CR and F4CL components were analyzed using LCHRMS. The results of the analysis showed that there were 211 compounds in the F3CR and 210 in the F4CL. Terpenes, phthalates, alkaloids, and fatty acids are the most common compounds identified in both phases. The fraction contained coumarins, vitamins, amides, ketones, and flavonoids (**Table 4**). Among all the categories identified, many compounds possess antifungal properties, as presented in **Table 4**.

Caulerpa is rich in fatty acids, which exhibit antifungal activity. F3CR and F4CL were found to contain terpene compounds, including polygodial, chamazulene, and betulin—a pentacyclic triterpene alcohol detected in F3CR—known for their antifungal properties. Alkaloids were also identified in both F3CR and F4CL. Notably, caulerpin, the primary metabolite of the genus *Caulerpa*, was detected only in F4CL. Another alkaloid, dehydrocyclopeptine, a natural marine-derived compound, was identified in both fractions. Vitamin D was detected in both fractions, while Vitamin A was identified only in F3CR. Fucosterol, a sterol compound commonly isolated from marine plants, was also present in F3CR and F4CL. Coumarin compounds, specifically calephyllolide, were detected in F4CL. Additionally, phenolic compounds, including shogaol, were found in F4CL.

Table 4. Antifungal compounds in F3CR dan F4CL using Liquid chromatography high-resolution mass spectrometry (LCHRMS)

Antifungal compounds	Class	Chemical formula	Structure
Alpha-linolenic acid	Fatty acids and its derivates	$C_{18}H_{30}O_2$	
Arachidonic acid	Fatty acids and its derivates	$C_{20}H_{32}O_2$	
(8Z,11Z,14Z)-heptadecatrienoic acid	Fatty acids and its derivates	$C_{17}H_{28}O_2$	
Eicosapentaenoic acid methyl	Fatty acids and its derivates	$C_{21}H_{32}O_2$	
11-Aminoundecanoic acid	Fatty acids and its derivates	$C_{11}H_{23}NO_2$	
Myristamide	Fatty acids and its derivates	$C_{14}H_{29}NO$	
4-Phenylbutyric acid	Fatty acids and its derivates	$C_{10}H_{12}O_2$	
Oleoylethanolamide	Fatty acids and its derivates	$C_{20}H_{39}NO_2$	
N, N-Bis(2-hydroxyethyl) dodecanamide	Fatty acids and its derivates	$C_{16}H_{33}NO_3$	
Mandenol	Fatty acids and its derivates	$C_{20}H_{36}O$	
Nonivamide	Fatty acids and its derivates	$C_{17}H_{27}NO_3$	
Polygoidal	Terpen and its derivates	$C_{15}H_{22}O_2$	
Chamazullene	Terpen and its derivates	$C_{14}H_{16}$	
Betulin	Terpen and its derivates	$C_{30}H_{50}O_2$	
Caulerpin	Alkaloid	$C_{24}H_{18}N_2O_4$	
Dehydrocyclopeptine	Alkaloid	$C_{17}H_{14}N_2O_2$	
13-apo-beta-carotenone	Vitamin	$C_{18}H_{26}O$	
Cholecalciferol	Vitamin	$C_{27}H_{44}O$	
Fucosterol	Sterol	$C_{29}H_{48}O_2$	
Calophyllolide	Coumarin	$C_{26}H_{24}O_5$	
Shogaols	Phenol	$C_{17}H_{24}O_3$	

Intracellular leakage mechanism of action in antifungal activity

The intracellular permeability assay results showed a significant increase in absorbance at wavelengths 260 and 280 nm in the treatment groups using F3CR, F4CL, and caulerpin against *C. albicans* (**Figure 3**). This increase in absorbance indicated a more substantial release of cellular components compared to the negative control. The combined results for these treatment groups were similar to the positive control, ketoconazole. These findings suggested that the antifungal properties of F3CR, F4CL, and caulerpin were comparable to those of ketoconazole, demonstrating their effectiveness against *C. albicans*. The significant increase in absorbance at wavelengths 260 and 280 nm reflects leakage of essential cellular components, suggesting that these compounds may disrupt the fungal cell wall and membrane, similar to the action of ketoconazole. This result was also supported by SEM images showing fungal cell damages.

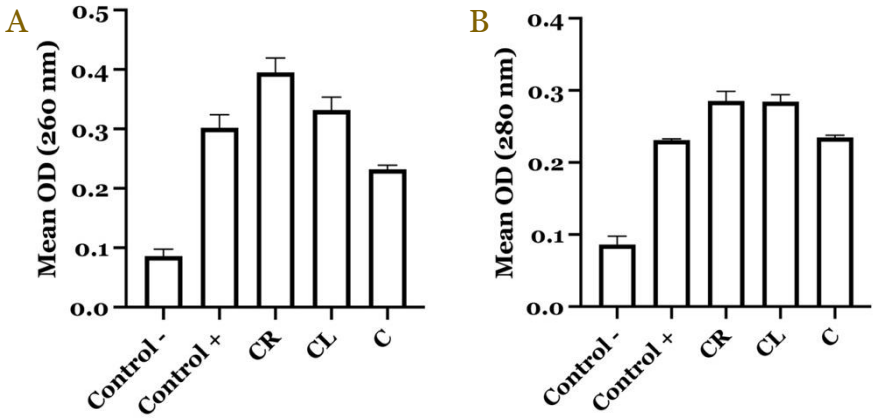


Figure 3. Comparison of absorption of *Candida albicans* cell material contents at 260 nm (A) and 280 nm (B). The optical density (OD) is presented in mean±SD (n=3). Control positive is ketoconazole, while control negative is Sabouraud dextrose broth media and fractions without tested fungi. CR: *Caulerpa racemose*; CL: *Caulerpa lentillifera*; and C: caulerpin.

Cell and membrane wall disruption mechanism of action in antifungal activity

The SEM technique was employed to examine the structural integrity of *C. albicans* and to assess the antifungal effects of compounds from the extract, including F3CR, F4CL, and caulerpin, presented in **Figure 4**. The SEM analysis revealed significant damage to *C. albicans* cells when exposed to 1024 µg/mL concentrations of these compounds. The fungal cell surfaces exhibited prominent alterations, including pitting, roughness, and wrinkling, with some cells showing complete structural collapse. Additionally, the pseudohyphal structures were similarly compromised, presenting visible damage characterized by surface pitting and degradation. These effects were comparable to those observed with ketoconazole treatment, suggesting that F3CR, F4CL, and caulerpin induce a similar mechanism of cell wall disruption as the reference antifungal agent.

Discussion

This study was conducted to assess the antifungal activity of EECR and EECL as well as their fraction against three pathogenic fungi. Our data suggested that the third fraction of *C. racemosa* (F3CR) and the fourth fraction of *C. lentillifera* (F4CL) showed the best inhibition results against *C. albicans*. F3CR and F4CL inhibited the growth of *C. albicans* with an MIC of 64–128 µg/mL, while caulerpin had an MIC of 256 µg/mL. By observing the kinetics of fungal growth using the time-kill curve, F3CR, F4CL, and caulerpin had fungistatic characteristics because the decrease in log CFU was around $0.3\log_{10}$ or 54%. An antifungal is considered to be fungicidal if there is a reduction of CFU/mL of $3\log_{10}$ or about 99.9% [20]. At a concentration of 4 MIC, F3CR had better inhibition results than at 8 MIC within 24 hours. This suggests that an adequate amount of *C. racemosa* is sufficient to inhibit *C. albicans*. Inhibition of *C. albicans* by F3CR, F4CL, and caulerpin may target multiple modes of action.

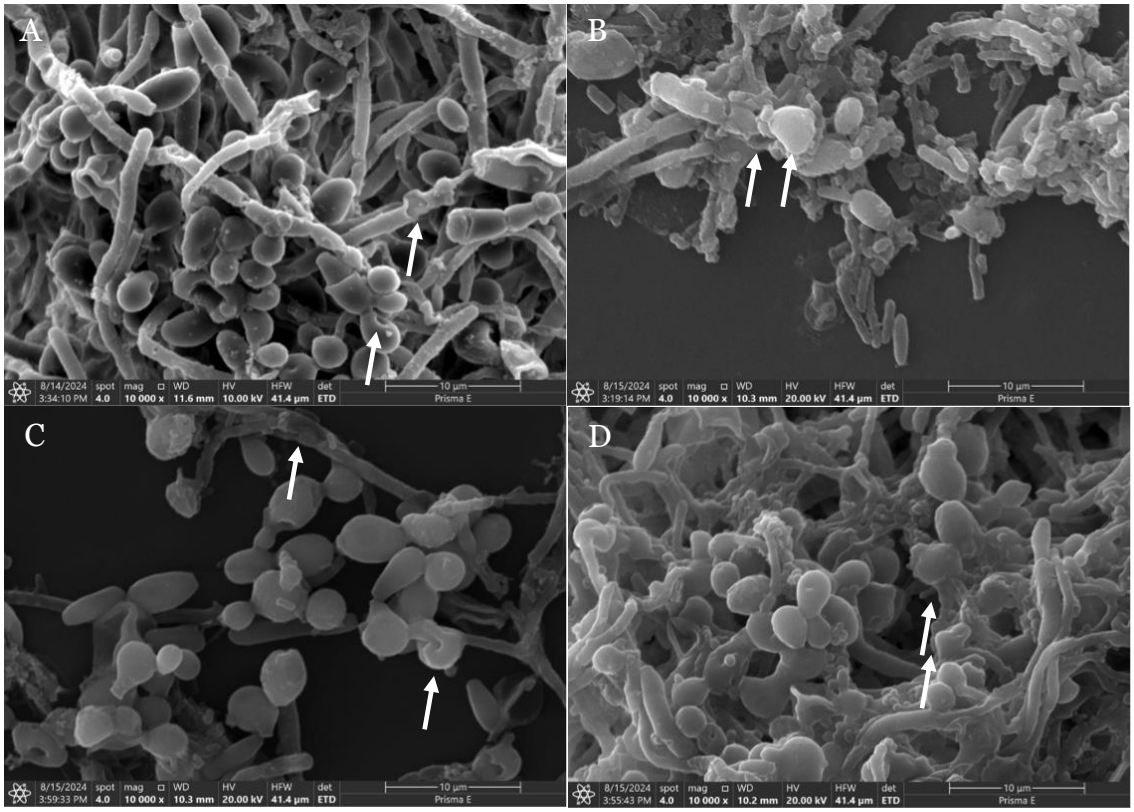


Figure 4. Scanning electron microscopy (SEM) results of ketoconazole (A), F3CR (B), F4CL (C), and caulerpin (D) against *Candida albicans* with magnification 10,000 times. The white arrow refers to the damage of *C. albicans* cells. The cell surface seems rough, central perforation of the cell occurs, and cell surface damage. The pseudo hyphae were also destroyed.

Our results showed both *Caulerpa* species had poor inhibition against *A. flavus* and *A. niger*, this could be attributed to structural differences between *Aspergillus* and *Candida,* suggesting that *Caulerpa* species were more effective against yeast-like fungi than filamentous fungi. Filamentous fungi are more challenging to inhibit extrinsically due to their complex hyphal structures, the ability to produce resistant spores, and the tendency to form biofilms [21]. Resistance to antifungal agents is also more common in filamentous fungi [22].

The results of bioautography showed that the growth of *C. albicans* was inhibited by F3CR and F4CL, suggesting the presence of antifungal in these fractions. The F4CL was suspected to be caulerpin because its Rf value was the same as the caulerpin Rf, which was 0.23. However, in this study, pure caulerpin did not show any inhibition during the bioautography test.

The LCHRMS analysis revealed that *C. racemosa* and *C. lentillifera* contain fatty acids, alkaloids, flavonoids, saponins, terpenes, and phenolic compounds. These bioactive compounds demonstrated antifungal activity against *C. albicans*, potentially through various mechanisms of action. The proposed mechanisms include the disruption of fungal growth by inhibiting DNA replication, thereby preventing cell division and subsequent fungal proliferation. Additionally, the compounds may compromise the integrity of the fungal cell membrane, leading to its destruction and ultimately causing cell death [23]. The antifungal effect of the seaweed extract was also found to inhibit fungal pseudohyphae and zoospores, although this inhibition required a certain period to manifest [24]. Specific metabolites present in *Caulerpa*, such as caulerpin, caulerprenylols A and B, polygodial, and tomentosin, are particularly effective in inhibiting and killing various pathogenic fungal species [25-28]. The F3CR and F4CL fractions predominantly consist of fatty acids and their derivatives, including medium- and long-chain fatty acids and fatty acid esters. Fatty acids are well-documented for their ability to inhibit biofilm formation, a critical virulence factor of *C. albicans*. Biofilm formation enhances the fungus's resistance to conventional antifungal treatments and host immune defenses [28]. The SEM results indicated the absence of biofilm formation in the treated *C. albicans*. This could be caused by the fatty acids in F3CR and F4CL inhibiting biofilm formation.

F3CR and F4CL contained polygodial and chamazulene, which potentially have good antifungal activity against fungi. Polygodial exhibited potent antifungal activity against yeast-like fungi as well as filamentous fungi [26]. Chamazulene is a blue-purple azulene derivative biologically synthesized from the sesquiterpene matricin [29]. At low concentrations, chamazulene showed a good inhibition percentage against fungal mycelium for the growth of *A. niger* [30]. Betulin, a pentacyclic triterpene alcohol identified in the F3CR fraction, has been proposed as an antifungal agent due to its protective effects on cellulose. It has demonstrated antifungal activity against *A. brasiliensis*, remaining effective for several weeks [31]. Betulin had notable antifungal activity when applied as a 1–3% ethanol solution [31]. One of the betulin derivatives, betulinic acid, has also been evaluated for its antifungal properties against a variety of filamentous fungi and yeast strains, including *C. albicans*, *C. krusei*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *Epidermophyton floccosum* [32].

Caulerpin, the main metabolite for the genus *Caulerpa*, was only identified in F4CL, while dehydrocyclopeptine, an alkaloid, was found in F3CR and F4CL. Caulerpin is an alkaloid pigment that has been investigated for its ability to inhibit fungi such as *C. albicans* and C. *neoformans* [12]. In this study, caulerpin showed good inhibition against *C. albicans*, but did not exhibit a clear inhibition zone in the bioautography test. This may be attributed to the low concentration of caulerpin in the TLC, which was insufficient to inhibit *C. albicans* effectively. Dehydrocyclopeptine has been studied for its inhibitory effects against fungi such as *A. ochraceus, A. flavus, Fusarium solani,* and *Alternaria alternata* with MIC<10 µg/mL [33].

F3CR and F4CL contained vitamins, with vitamin D detected in F3CR and F4CL, while vitamin A was only detected in F3CR. Vitamin A is vital as an immunomodulator in the host response to *C. albicans* in human monocytes [34]. Vitamin D3 exhibited broad-spectrum, dose-dependent antifungal activity against *Candida* species [35]. It demonstrated significant effects across various stages of biofilm formation in *C. albicans*, including the initiation, development, and maturation phases [35]. The antifungal mechanisms of Vitamin D3 involved the disruption of ribosome biogenesis, coenzyme metabolism, and carbon metabolism, which collectively hindered fungal growth and viability [35]. Fucosterol, identified in F3CR and F4CL and commonly isolated from marine plants, also potentially has notable antifungal properties [18,21]. It was shown to inhibit the germination of macroconidia of *F. culmorum*, particularly at low concentrations (0.05–0.2%), where growth inhibition and structural degradation of the macroconidia were observed [36]. Additionally, fucosterol induced abnormal morphological changes in the mycelia of *Pyricularia oryzae*. Its antifungal activity extended to other fungi, such as *A. niger* and *C. albicans*, with MICs ranging from 2 to 16 µg/L [37]. Coumarin, identified in F4CL fraction, has antifungal activity against *C. albicans* by damaging cells by forming pores on the cell surface and the cells eventually die due to leakage of cytoplasmic contents [38].

Another compound found in F4CL is shogaol. Shogaol is phenolic compound that exhibits antimicrobial activity [39]. Shogaol significantly inhibited *C. albicans* biofilm formation with a concentration of 10 µg/mL, notably reducing biofilm formation without affecting the growth of planktonic cells [40]. In addition, it could inhibit hyphal growth in mixed colonies and non-living planktonic cells, as well as inhibit cell aggregation [41]. A study indicated that 6-shogaol induced several receptors (CDR1, CDR2 and RTA3) and suppressed the expression of pseudo hyphae/biofilm-related genes (ECE1 and HWP1), supporting the observed phenotypic changes [42]. These results demonstrate the anti-biofilm and antiviral activity of 6-shogaol against drug-resistant *C. albicans* strains [43] .

After exposure to F3CR, F4CL, and caulerpin at a concentration of 1024 µg/mL (1 MKC), fungal cells were examined for damage using SEM. The SEM results showed that the three tested substances caused significant damage to the fungal cells. The damage was observed as surface roughness, perforations, and structural deterioration of the cell surfaces. In addition to the cells, the pseudohyphae were also damaged and destroyed. The most severe damage was observed in cells exposed to F3CR. This damage can have severe consequences for the fungal cells. Fungi with damaged cell walls lose their structural integrity, making them more susceptible to osmotic pressure and physical damage. Damage to the cell membrane disrupts cellular regulation and causes leakage of intracellular contents. Additionally, damage to the cell wall and membrane interferes with nutrient absorption, which is essential for fungal growth [44]. Without an intact

cell wall, fungal cells become more vulnerable to environmental changes, such as pH, temperature, and osmotic pressure, potentially leading to lysis or cell death [45,46]. These findings are supported by an increase in absorbance at wavelengths of 260 nm and 280 nm compared to the negative control. An increase in absorbance at 260 nm indicates the presence of nucleotides, while an increase at 280 nm indicates the presence of proteins [19].

Our study indicated that *C. racemosa* and *C. lentillifera* contain potential antifungal compounds effective against *C. albicans*. These bioactive compounds exhibit diverse mechanisms of antifungal activity. However, this study focused solely on their effects on the fungal cell wall and membrane, limiting insights into other possible mechanisms, such as the inhibition of nucleic acid synthesis, microtubule formation, or enzyme activity. Therefore, further studies are warranted to investigate these additional mechanisms.

Conclusion

Our results indicated that *C. fractions* did not inhibit the growth of *A. flavus* and *A. niger* but effectively inhibited *C. albicans*. F3CR and F4CL fractions were the most effective against *C. albicans*, as indicated by their low minimum inhibitory concentrations (MICs) of 64–128 µg/mL. Caulerpin, the primary metabolite of *Caulerpa*, also demonstrated notable inhibition with an MIC of 256 µg/mL. F3CR, F4CL, and caulerpin exhibited fungistatic activity. These findings were further supported by bioautography results, which revealed clear zones indicating inhibited fungal growth. The antifungal mechanism of the tested agents is believed to involve damage to fungal cell walls and membranes. The SEM observations showed that fungal cells exposed to these compounds exhibited rough surfaces, perforations, and eventual structural destruction. This damage caused leakage of cellular contents, as confirmed by increased absorbance at 260/280 nm, indicating the release of nucleotides and proteins. Based on these findings, it can be concluded that *C. racemosa*, *C. lentillifera*, and caulerpin inhibit *C. albicans* by damaging cell walls and membranes, disrupting pseudohyphae formation, and ultimately preventing cell growth, leading to cell death.

Ethics approval

Not required.

Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

This published paper and its supplementary materials contain all the data created or analyzed during this investigation.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tools for manuscript writing support, specifically ChatGPT. The use of ChatGPT was employed for language refinement. We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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References

1. Pappas PG, Lionakis MS, Arendrup MC, *et al.* Invasive candidiasis. Nat Rev Dis Primers 2018;4:18026.
2. Fang W, Wu J, Cheng M, *et al.* Diagnosis of invasive fungal infections: challenges and recent developments. J Biomed Sci 2023;30(1):42.
3. Reddy GKK, Padmavathi AR, Nancharaiah YV. Fungal infections: Pathogenesis, antifungals and alternate treatment approaches. Curr Res Microb Sci 2022;3:100137.
4. Hussain M, Whitelaw A, Parker A. A five-year retrospective descriptive study on the clinical characteristics and outcomes of candidaemia at a tertiary hospital in South Africa. IJID Reg 2022;3:79-83.
5. Mukaremera L, Lee KK, Mora-Montes HM, *et al. Candida albicans* yeast, pseudohyphal, and hyphal morphogenesis differentially affects immune recognition. Front Immunol 2017;8:629.
6. Armerinayanti NW, Lestari DPO. Risiko kandidiasis serviks pada wanita usia subur akseptor kontrasepsi hormonal. WMJ (Warmadewa Med Journal) 2018;3(1):21-26.
7. Macias-Paz IU, Pérez-Hernández S, Tavera-Tapia A, *et al. Candida albicans* the main opportunistic pathogenic fungus in humans. Rev Argent Microbiol 2023;55(2):189-198.
8. Atriwal T, Azeem K, Husain FM, *et al.* Mechanistic understanding of *Candida albicans* biofilm formation and approaches for its inhibition. Front Microbiol 2021;12:638609.
9. Kessler SQS, Lang PM, Dal-Pizzol TS, Montagner F. Resistance profiles to antifungal agents in *Candida albicans* isolated from human oral cavities: Systematic review and meta-analysis. Clin Oral Investig 2022;26(11):6479-6489.
10. Fosses Vuong M, Hollingshead CM, Waymack JR. Aspergillosis. In: Ackley WB, Adolphe TS, Aeby TC, *et al.*, editors. StatPearls. Treasure Island: StatPearls Publishing; 2024.
11. Novilla A, Nursidika P, Mahargyani W. Komposisi asam lemak minyak kelapa murni (virgin coconut oil) yang berpotensi sebagai anti kandidiasis. EduChemia (J Kim Pendidik) 2017;2(2):161-173.
12. Abdelrheem DA, Abd El-Mageed HR, Mohamed HS, *et al.* Bis-indole alkaloid caulerpin from a new source *Sargassum platycarpum*: Isolation, characterization, in vitro anticancer activity, binding with nucleobases by DFT calculations and MD simulation. J Biomol Struct Dyn 2021;39(14):5137-5147.
13. Manikandan NA, Lens PNL. Sustainable biorefining and bioprocessing of green seaweed (*Ulva* spp.) for the production of edible (ulvan) and non-edible (polyhydroxyalkanoate) biopolymeric films. Microb Cell Fact 2023;22(1):140.
14. Oluah A, Oputa AI, Ndukwe GI, Fekarurhobo GK. Application of vacuum liquid chromatography to the separation of secondary metabolites of *Baphia nitida* Lodd. Stem. J Chem Soc Niger 2020;45(2):220-225.
15. Zia Q, Khan AA, Swaleha Z, Owais M. Self-assembled amphotericin B-loaded polyglutamic acid nanoparticles: Preparation, characterization and in vitro potential against *Candida albicans*. Int J Nanomedicine 2015;10:1769-1790.
16. de-Souza-Silva CM, Guilhelmelli F, Zamith-Miranda D, *et al.* Broth microdilution in vitro screening: An easy and fast method to detect new antifungal compounds. J Vis Exp 2018(132):57127.
17. de Aquino Lemos J, Costa CR, de Araújo CR, *et al.* Susceptibility testing of *Candida albicans* isolated from oropharyngeal mucosa of HIV(+) patients to fluconazole, amphotericin B and Caspofungin. killing kinetics of caspofungin and amphotericin B against fluconazole resistant and susceptible isolates. Braz J Microbiol 2009;40(1):163-169.
18. Suleimana M, McGaw L, Naidoo V, Eloff JN. Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species. Afr J Tradit Complement Altern Med 2009;7(1):64-78.
19. Zorić N, Kosalec I, Tomić S, *et al.* Membrane of *Candida albicans* as a target of berberine. BMC Complement Altern Med 2017;17(1):268.
20. Scorneaux B, Angulo D, Borroto-Esoda K, *et al.* SCY-078 is fungicidal against *Candida* species in time-kill studies. Antimicrob Agents Chemother 2017;61(3):10.1128/aac.01961-16.
21. Latgé JP, Chamilos G. Aspergillus fumigatus and Aspergillosis in 2019. Clin Microbiol Rev 2019;33(1):10.1128/cmr.00140-18.
22. Sharma C, Chowdhary A. Molecular bases of antifungal resistance in filamentous fungi. Int J Antimicrob Agents 2017;50(5):607-616.

23. Hainil S, Mayefis D, Ghiffari HD, *et al.* Antifungal activity test of ethanol extracts and ethyl acetate and n-hexane fraction of sea grapes (*Caulerpa racemosa*) on the growth of the fungi *Trichophyton mentagrophytes*. Open Access Maced J Med Sci 2023;11(A):76-82.
24. Saito H, Tamrin ML. Antimycotic activity of seaweed extracts (*Caulerpa lentillifera* and *Eucheuma cottonii*) against two genera of marine oomycetes, *Lagenidium* spp. and *Haliphthoros* spp. Biocontrol Sci 2019;24(2):73-80.
25. Liu AH, Liu DQ, Liang TJ, *et al.* Caulerprenylols A and B, two rare antifungal prenylated para-xylenes from the green alga *Caulerpa racemosa*. Bioorg Med Chem Lett 2013;23(9):2491-2494.
26. Lee SH, Lee JR, Lunde CS, Kubo I. In vitro antifungal susceptibilities of *Candida albicans* and other fungal pathogens to polygodial, a sesquiterpene dialdehyde. Planta Med 1999;65(3):204-208.
27. Cafarchia C, De Laurentis N, Milillo MA, *et al.* [Fungistatic activity of a sesquiterpene lactone (tomentosin) isolated from fresh *Inula viscosa* (Asteraceae) flowers from the Puglia region]. Parassitologia 2001;43(3):117-121.
28. Wang W, Wang R, Zhang G, *et al.* In vitro antibacterial activities and mechanisms of action of fatty acid monoglycerides against four foodborne bacteria. J Food Prot 2020;83(2):331-337.
29. Clarke S. Chapter 3 - Families of compounds that occur in essential oils. In: Clarke S, editor. Essential chemistry for aromatherapy, second edition. London: Churchill Livingstone; 2008.
30. EL-Hefny M, Abo Elgat WAA, Al-Huqail AA, Ali HM. Essential and recovery oils from *Matricaria chamomilla* flowers as environmentally friendly fungicides against four fungi isolated from cultural heritage objects. Processes 2019;7(11):809.
31. Makarov I, Vinogradov M, Gromovykh T, *et al.* Antifungal composite fibers based on cellulose and betulin. Fibers 2018;6(2):23.
32. Rodrigues GCS, Dos Santos Maia M, de Souza TA, *et al.* Antimicrobial potential of betulinic acid and investigation of the mechanism of action against nuclear and metabolic enzymes with molecular modeling. Pathogens 2023;12(3):449.
33. Abdel-Hadi AM, Awad M, Abo-Dahab N, *et al.* Viridicatin and dehydrocyclopeptine, two bioactive alkaloids isolated from marine derived fungus *Penicillium aurantiogriseum* AUMC 9759. Issues Biol Sci Pharm Res 2015;3:115-122.
34. Klassert TE, Hanisch A, Bräuer J, *et al.* Modulatory role of vitamin A on the *Candida albicans*-induced immune response in human monocytes. Med Microbiol Immunol 2014;203(6):415-424.
35. Lei J, Xiao W, Zhang J, *et al.* Antifungal activity of vitamin D3 against *Candida albicans* in vitro and in vivo. Microbiol Res 2022;265:127200.
36. Meinita MDN, Harwanto D, Tirtawijaya G, *et al.* Fucosterol of marine macroalgae: Bioactivity, safety and toxicity on organism. Mar Drugs 2021;19(10):545.
37. Kumar SS, Kumar Y, Khan MSY, Gupta V. New antifungal steroids from *Turbinaria conoides* (J. Agardh) Kutzing. Nat Prod Res 2010;24(15):1481-1487.
38. Widodo GP, Sukandar EY, Adnyana IK, Sukrasno S. Mechanism of action of coumarin against *Candida albicans* by SEM/TEM analysis. J Math Fundam Sci 2012;44(2):145-151.
39. Ghasemzadeh A, Jaafar HZE, Baghdadi A, Tayebi-Meigooni A. Formation of 6-, 8- and 10-shogaol in ginger through application of different drying methods: Altered antioxidant and antimicrobial activity. Molecules 2018;23(7):1646.
40. Khan A, Azam M, Allemailem KS, *et al.* Coadministration of ginger extract and fluconazole shows a synergistic effect in the treatment of drug-resistant vulvovaginal candidiasis. Infect Drug Resist 2021;14:1585-1599.
41. Mao QQ, Xu XY, Cao SY, *et al.* Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe). Foods 2019;8(6):185.
42. Lee JH, Kim YG, Choi P, *et al.* Antibiofilm and antivirulence activities of 6-gingerol and 6-shogaol against *Candida albicans* due to hyphal inhibition. Front Cell Infect Microbiol 2018;8:299.
43. Elfaky MA, Okairy HM, Abdallah HM, *et al.* Assessing the antibacterial potential of 6-gingerol: Combined experimental and computational approaches. Saudi Pharm J 2024;32(5):102041.
44. Hasim S, Coleman JJ. Targeting the fungal cell wall: Current therapies and implications for development of alternative antifungal agents. Future Med Chem 2019;11(8):869-883.
45. Lorrai R, Ferrari S. Host cell wall damage during pathogen infection: Mechanisms of perception and role in plant-pathogen interactions. Plants (Basel) 2021;10(2):399.
46. Ariño J, Aydar E, Drulhe S, *et al.* Chapter One - Systems biology of monovalent cation homeostasis in yeast: The translucent contribution. In: Poole RK, editor. Advances in microbial physiology. London: Academic Press; 2014.