Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2025 March; 58(1): 66–73

Original article

Fungistatic effect of *Gracilaria verrucosa* on phospholipase enzymes and the cell surface hydrophobicity of *Candida albicans*

Basri A. Gani[1], Ridha Andayani[1], Fitri Yunita Batubara[2], Ifwandi Ifwandi[3], Dharli Syafriza[4], Muhammad Mas'ud Herlambang[1], Shiti Alya Fathirah[1], Ayudia Rifki[4]
[1]Department of Oral Biology, Faculty of Dentistry, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[2]Department of Conservative Dentistry, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia.
[3]Department of Prosthodontics, Faculty of Dentistry, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[4]Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Syiah Kuala, Banda Aceh, Indonesia.

ABSTRACT

Background: *Candida albicans (C. albicans) was reported as a primary cause of oral candidiasis. Phospholipase enzymes and cell surface hydrophobicity (CSH) are involved in the pathogenesis of its infection. Gracilaria verrucosa (G. verrucosa) is reported to contain flavonoids, tannins, and phenolic compounds, which can inhibit the development of C. albicans.* ***Purpose:*** *The study analyzed the effect of the ethanol extract of G. verrucosa on the inhibition of the phospholipase enzyme and CSH of C. albicans.* ***Methods:*** *The G. verrucosa chemical compounds were examined by phytochemical tests and phospholipase enzyme-inhibiting egg yolk media, and a CSH assay was conducted using xylene.* ***Results:*** *Gracilaria verrucosa contains steroids, terpenoids, tannins/phenolics, and flavonoids. The 25%–100% concentrations inhibit the phospholipase enzyme of C. albicans more strongly at 48 hours than at 12 hours and 24 hours ($p < 0.05$). Furthermore, the 100% and 75% concentrations substantially affect the inhibition of CSH of C. albicans, with a strong relationship.* ***Conclusion:*** *Gracilaria verrucosa has a more substantial inhibitory effect in suppressing the phospholipase enzyme and CSH of C. albicans.*

Keywords: *Candida albicans; Gracilaria verrucosa; hydrophobicity; phospholipase enzyme*
Article history: *Received 24 July 2023; Revised 22 January 2024; Accepted 5 February 2024; Published 1 March 2025*

Correspondence: Basri A. Gani, Department of Oral Biology, Dentistry Faculty, Universitas Syiah Kuala. Jl. Hamzah Fansuri Kopelma Darussalam, Banda Aceh, 23111, Indonesia. Email: basriunoe@usk.ac.id

INTRODUCTION

Candida albicans (*C. albicans*) is a common opportunistic fungal pathogen that can cause various human infections, ranging from superficial mucocutaneous infections to severe systemic diseases.[1] One of its virulence factors is the production of phospholipase enzymes, primarily phospholipase A2, which play a crucial role in the degradation of host cell membranes.[2] This enzymatic activity helps the fungus invade host tissues and evade the host's immune response.[3] Increased *C. albicans* can occur using broad-spectrum antibiotics, corticosteroids, cytotoxic agents, and smoker isolates that can trigger oral candidiasis infections.[4]

Phospholipase enzymes are responsible for hydrolyzing phospholipids, the major components of cell membranes.[5] Phospholipase A2 specifically targets the sn-2 position of phospholipids, releasing fatty acids and lysophospholipids.[6] In the context of *C. albicans*, the production of phospholipase enzymes contributes to its pathogenicity by facilitating tissue invasion and nutrient acquisition.[7] The phospholipase enzyme works by hydrolyzing the ester bonds of glycerophospholipids. It tends to contribute to the pathogenicity of *C. albicans* through the destruction of cell membranes to facilitate an invasion of host tissue.[8] Researchers have classified four types of phospholipase in *C. albicans*, namely phospholipase A, B, C, and D. All of these phospholipase types have hydrolase activity in host cells.[9]

Cell surface hydrophobicity (CSH) is considered an essential non-biological factor contributing to hydrophobic interactions related to the adherence of candida cells on the mucosal host surface.[10] It represents the degree to which the surface of *C. albicans* cells interacts with water molecules.

DOI: 10.20473/j.djmkg.v58.i1.p66–73

Fungi with higher hydrophobicity tend to cling to the host tissues and medical devices quickly.[11] This adherence is essential in establishing fungal infections because it enables the pathogen to colonize and multiply inside the habitats of host organisms. *Candida albicans* regulates CSH based on the growth phase, environment, and nutrient availability.[12]

Changes in *C. albicans* pathogens are associated with patients who experience immune disorders.[13] Some studies suggest the treatment of candidiasis depends on the type and virulence of the infection. Fluconazole is an effective drug for oral candidiasis, which has an excellent antifungal effect compared with other antifungal medicines.[14] Unfortunately, fluconazole is not always effective because it has a secondary impact on hosts, such as nausea, vomiting, diarrhea, and stomach pain.[15] Thus, alternatives continue to be sought to reduce these impacts, including using plants or natural materials to be tested for the growth and development of *C. albicans,* as reported by Seleem et al.,[16] who study the types of natural materials for the treatment of candidiasis.

Gracilaria verrucosa (*G. verrucosa)* is a species of red macroalgae, also known as seaweed, prevalent in coastal maritime environments. The substance contains a diverse array of bioactive compounds, several of which have been subject to scientific inquiry into their potential therapeutic use. The bioactive compounds encompass a variety of chemicals, such as polysaccharides, polyphenols, and peptides. There is a potential for specific compounds to exhibit antifungal properties, hence warranting more investigation into their potential impact on *C. albicans*. G*racilaria verrucosa* has been reported as having antifungal potential because it contains phenol compounds widely known as antioxidants.[17]

The hypothesis underlying the research is that *G. verrucosa* may have a fungistatic (inhibitory) effect on *C. albicans* by targeting its phospholipase enzyme activity and altering its CSH. This inhibition could reduce the pathogen's ability to degrade host cell membranes, limiting its invasion and virulence and impeding its ability to establish infections. Measurements of phospholipase enzyme activity and the CSH of *C. albicans* can provide insights into its potential fungistatic. This work aims to assess the inhibitory effects of *G. verrucosa* on the virulence factors of *C. albicans*, specifically the phospholipase enzymes and CSH activity.

MATERIALS AND METHODS

This research has received the ethical clearance 341/KE/FKG/2022 from the Faculty of Dentistry, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia, and the species of *G. verrucosa* was identified by Herbarium Laboratory, Faculty of Mathematics and Natural Science, Universitas Syiah Kuala, No. B/6665/UN11.1.8.4/TA.00.01/2020. This test material was prepared into six different concentrations—100%, 75%, 50%, 25%, 12.5%, and 6.25%—and fluconazole (30 mg/5 mL) (Acme, Indonesia) was determined as a positive control, and the assay material concentration was prepared using the formula V1.C1−V2.V2 (where V is volume and C is concentration).[18] The research materials used were *C. albicans* from smoker isolates obtained from the Microbiology Laboratory, Veterinary Faculty, Syiah Kuala University, Banda Aceh-Indonesia.

The seaweed (*G. verrucosa*) was obtained from the sea of Pulo Aceh, Aceh Besar, Aceh, Indonesia, with a coordinate point of 5° 41' 38.6" N, 95° 03' 06.8" E. The extraction was performed at the Basic Chemistry Laboratory, Faculty of Teacher Training and Education, Syiah Kuala University, Darussalam Banda Aceh, Aceh, Indonesia.

A total of 3 kg of *G. verrucosa* was washed thoroughly with water, drained, cut into small pieces, and dried by airing at room temperature for 3 days to remove the water content so that a constant seaweed weight was obtained. After that, small pieces of *G. verrucosa* seaweed were extracted using the maceration method by placing the seaweed in a jar and soaking it in a 96% ethanol solvent while stirring every day for 2 days. After that, the seaweed extract was filtered using filter paper to obtain the filtrate and residue. The extraction was repeated thrice by soaking the seaweed residue in 96% ethanol to obtain the second and third filtrates. Then, all the filtrate was collected and evaporated using a rotary evaporator at 40°C until the ethanol solvent evaporated and a thick seaweed extract was obtained.[19]

The chemical compounds of *G. verrucosa* were examined using the working principle stated by Soraya et al.[20] Subsequently, the obtained extract was analyzed to determine the presence or absence of chemical constituents that could impede bacterial proliferation (Table 1). The ethanol extract analysis obtained from *G. verrucosa* was conducted using gas chromatography–mass spectrometry with a Shimadzu Japan QP2010 Plus gas chromatography (GC) system. The experimental setup involved the use of a fused GC column, which was coated with polymethyl silicon (0.25 nm 50 m). The following parameters were employed in the experiment: the temperature range was 80°C–200°C; the rate of temperature increase was 5°C per min; the temperature was maintained at 200°C for 20 min; the flame ionization detector temperature was adjusted to 300°C; the injection temperature was set to 220°C; the carrier gas employed was nitrogen, flowing at a rate of 1 mL/min, with a split ratio 1:75; the pressure was 116.9 kPa; the column was 30 m long, with a diameter of 0.25 mm and a 50 mL/min flow rate. The phytochemical screening assay is reported in Table 1.[18]

The *C. albicans* CSH activity was assessed in the first stage. A 10 mL sample of *C. albicans* cultured in peptone media (1.5 10^8 CFU/mL) was poured into a tube and centrifuged at 7,000 rpm for 15 min supernatant. The pellet was then washed with phosphate-buffered saline (PBS)

DOI: 10.20473/j.djmkg.v58.i1.p66–73

pH 7. Hydrophobicity assessment began by inserting 100 µL into a 96-well microplate. The hydrophobicity cell of *C. albicans* was assessed by optical density at 520 nm. Hydrophobicity inhibition was evaluated by adding 5 mL of *G. verrucosa to* each concentration in 1 mL of *C. albicans* suspension, then incubating for 24 hours at 37°C. They were then centrifuged at 7,000 rpm for 20 min. The supernatant was removed, and 1 mL of xylene (Merck KGaA, Darmstadt, Germany) was added and placed in a water bath at 37°C for 10 min. After that, a vortex was used for 30 sec to mix the suspension with xylene, then stored in a water bath at 37°C for 30 min to separate the components. Next, the residue was carefully transferred to another sterile tube, and the remaining xylene in the pipette was resuspended with 2 mL PBS pH 7.0. Then, 150 µL of suspension was applied to the 96-well microplate. The change in absorbance value of hydrophobicity is related to the ability of *C. albicans* to increase virulence to the knowledge of adhesion in mucosal cells. Hydrophobicity percentage index (HI) values were obtained through the following formula, wherein the first stage (the percent value of each liquid [optical density] of the liquid phase and inoculum) was obtained with the formula (percentage value of hydrophobicity OD = [OD value concentration of sample/total OD value all concentrations 100%]): HI = (percent value of OD-inoculum + OD-HI value). The scale used was as follows: strong (65%–100%), moderate (30%–64%), and low (1%–29%).[21]

Sabouraud dextrose agar (Merck KGaA, Darmstadt, Germany) media was coated with 100 µL egg yolk medium and dried in sterile conditions for 30 min.[22] Furthermore, 100µL of *C. albicans* was immersed in 1mL of *G. verrucosa* extract in various concentrations with fluconazole as a positive control and then adapted for 30 min at room temperature and inserted into disc paper. The homogeneity process was carried out on a shaker at a speed of 200 rpm for 15 min. Furthermore, the disc was placed on the yolk media and incubated for 12 hours, 24 hours, and 48 hours at 37°C. The zone of precipitation and the colonies' zone refer to the inhibitory power of *G. verrucosa* extract against the enzyme phospholipase *C. albicans*. The phospholipase activity is the ratio of the colony's diameter to the colony's diameter added to the precipitation zone (mm) and is determined as the ratio of the diameter of the circle to the total diameter of the colony plus the zone of precipitation

Table 1. Phytochemical screening of *G. verrucosa*[18]

Chemical compounds	Material and method
Flavonoid	The examination is carried out by adding Mg, 0.5 ml HCl, and amyl alcohol into a 2 mL extract. The reaction is positive if a red–purple color is formed.
Tannin	Tannin examination is done by mixing a 1% gelatin solution with sodium chloride into 2 mL of extract. The positive reaction wakes up, and a white precipitate is formed.
Saponin	Saponin examination begins by mixing HCI 0.1 into 2 mL of extract and shaking vigorously vertically for 15 sec. If persistent foam forms with a height of approximately 1 cm, then the extract is positive for containing saponin.
Alkaloid	The alkaloid examination is performed by dissolving hydrochloric acid in 2 mL of extract, then filtering, which includes the following: (1) Mayer's test—Mayer's reagent (potassium mercury iodide) is added to the extract. The formation of yellow-colored precipitates contains alkaloids; (2) Wagner's test—Wagner's agent (iodine in potassium iodide) is added to the extract. The formation of brown—reddish brown precipitates indicates positive alkaloids; (3) Dragendroff test—Dragendroff reagent (potassium bismuth iodide) is added to 2 mL of extract. The formation of red precipitates indicates alkaloids.
Steroid	Steroid and terpenoid examination is carried out by adding Carr–Price reagent to 2 mL of extract. The presence of steroid content in the extract forms a green color. Meanwhile, the terpenoid content of the extract is translated into the formation of a red color in the solution.

Table 2. Gas chromatography–mass spectrometry analyses of chemical compounds of *G. verrucosa*

Peak	Retention time (min)	Compounds	Intensity (%)
1	17.345	Oxirane, decyl-	2.023
2	18.187	n-hexadecanoic acid	74.198
3	20.126	Eicosanoic acid	2.262
4	20.306	Nonanoic acid	2.084
5	20.716	Oleic acid	6.609
6	21.731	Pentadecanoic acid	1.176
7	22.862	Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5	2.901
8	25.373	N-(5-chloro-2-hydroxyphenyl)dodecanamide	2.048
9	27.294	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-	1.542

DOI: 10.20473/j.djmkg.v58.i1.p66–73

One-way analysis of variance (ANOVA) was used to analyze the results of hydrophobicity data and phospholipase enzymes from *C. albicans,* and the least significant difference (LSD) method was used for the post hoc test. The Shapiro–Wilk test was used for a normal distribution, and homogeneity values were determined. Data were considered normal and homogeneous if $p > 0.05$. The significance limit for these two data was $p < 0.005$. Pearson correlation was used to determine the relationship between concentration and the time required to assess the effect on hydrophobicity and phospholipase enzymes, with a strong correlation if $r = 0.85–1$, medium if $r = 0.5–0.79$, and low if $r \leq 0.5$.

RESULTS

This study evaluates the fungistatic properties of *G. verrucosa* on the phospholipase enzyme and CSH of *C. albicans*. Furthermore, the chemical compound content of *G. verrucosa* and phytochemical screening are also reported as references for its fungistatic properties. Table 2 reports that there are nine chemical compounds extractable from *G. verrucosa*. Out of these, two chemical compounds exhibited higher values: n-hexadecanoic acid (74.198%) and oleic acid (6.61%). Table 3 shows five chemical compounds with positive (+) values in the phytochemical screening test, such as steroids, terpenoids, and tannin/phenolic compounds.

Table 4 reports the ability of *G. verrucosa* to inhibit the phospholipase enzyme of *C. albicans*. At 12 hours of incubation, none of the concentrations of *G. verrucosa* tested showed significant phospholipase inhibition ($p = 0.069$). Meanwhile, 24-hour incubation showed significant phospholipase inhibition by *G. verrucosa* ($p = 0.047$). Likewise, 48 hours showed strong phospholipase enzyme activity from all concentrations of *G. verrucosa* ($p = 0.029$). There was a significant difference in the inhibition of the *C. albicans* phospholipase enzyme between incubation times of 12 hours, 24 hours, and 48 hours ($p = 0.031$). The assessment limits for the inhibitory categories are strong (>1 mm), moderate (0.75–0.99 mm), weak (0.51–0.74 mm), and no effect (<0.05 mm).[21] The LSD test shows that concentrations of 100% and 75% of *G. verrucosa* have good significance in inhibiting the release of the phospholipase enzyme *C. albicans* (Table 5).

Table 6 reports the hydrophobic index of *G. verrucosa* towards the cell surface of *C. albicans*. Based on the assessment scale, 100% and 75% concentrations strongly influence changes in the hydrophobicity of the *C. albicans* cell surface, including positive controls. In contrast, the other concentration groups are moderate. Based on the one-way ANOVA of the hydrophobicity index of the *G. verrucosa* between concentration groups, there was a

Table 3. Phytochemical screening of ethanol extract of *G. verrucosa*

Chemical content	Reagent	Ethanol extract	Observation result
Alkaloid	Mayer	-	White sediment
	Wegner	-	Brown sediment
	Dragendroff	-	Red sediment
Steroid	Lieberman-Burchard Test	+	Green / blue color
Terpenoid	Lieberman-Burchard Test	+	Red / purple
Saponin	Shaking	+	Stable foam
Flavonoid	0.5 Mg and HCl	+	Red / purple
Tannin/phenolic	$MgCl_3$	+	Dark green

Tabel 4. Phospholipase inhibition of *C. albicans* by *G. verrucosa*

G. verrucosa (%)	N	Phospholipase inhibition (mm)									*p-value
		12 h			24 h			48 h			
		Mean	SD	Scale	Mean	SD	Scale	Mean	SD	Scale	
C_{100}	3	1.02	0.00	Strong	1.11	0.00	Strong	1.28	2.12	Strong	
C_{75}	3	1.00	0.49	Strong	1.00	0.14	Strong	1.40	0.14	Strong	
C_{50}	3	0.91	0.70	Moderate	1.02	0.70	Strong	1.20	0.35	Strong	
C_{25}	3	0.97	0.70	Moderate	0.97	0.56	Moderate	1.15	0.42	Strong	0.031
$C_{12.5}$	3	0.96	0.91	Moderate	0.84	0.70	Moderate	0.95	0.00	Moderate	
$C_{6.25}$	3	0.95	0.28	Moderate	0.93	0.58	Moderate	0.81	0.70	Moderate	
Fluconazole	3	0.95	0.77	Moderate	0.94	1.13	Moderate	1.04	0.84	Strong	
*p-value	21		0.069			0.047			0.029		

* One-way ANOVA

DOI: 10.20473/j.djmkg.v58.i1.p66–73

Table 5. The least significant difference (LSD) test on the effect of *G. verrucosa* in inhibiting the phospholipase enzyme *C. albicans*

G. verrucosa (%)		*p-value		
		12 h	24 h	48 h
C_{100}	C_{75}	0.046	0.001	0.001
	C_{50}	0.041	0.021	0.210
	C_{25}	0.439	0.011	0.471
	$C_{12.5}$	0.416	0.001	0.011
	$C_{6.25}$	0.430	0.001	0.002
	Fluconazole	0.420	0.001	0.046
C_{75}	C_{50}	0.416	0.021	0.001
	C_{25}	0.402	0.001	0.010
	$C_{12.5}$	0.416	0.001	0.001
	$C_{6.25}$	0.339	0.001	0.001
	Fluconazole	0.391	0.001	0.350
C_{50}	C_{25}	0.675	0.001	0.101
	$C_{12.5}$	0.729	0.001	0.001
	$C_{6.25}$	0.728	0.001	0.001
	Fluconazole	0.748	0.000	0.370
C_{25}	$C_{12.5}$	0.805	0.000	0.001
	$C_{6.25}$	0.871	0.512	0.001
	Fluconazole	0.871	0.571	0.429
$C_{12.5}$	$C_{6.25}$	0.717	0.012	0.617
	Fluconazole	0.615	0.017	0.017
$C_{6.25}$	Fluconazole	1.015	0.612	0.001

*Post hoc LSD ($p < 0.05$ is significant)

Table 6. Hydrophobicity index of *C. albicans* influenced by *G. verrucosa*

G. verrucosa (%)	*n*	Hydrophobicity index (520 nm)					*p-value
		Mean	SD	Frequency	Index	Scale	
C_{100}	3	2.681	0.176	26%	68%	Strong	
C_{75}	3	2.259	0.263	22%	64%	Strong	
C_{50}	3	1.301	0.967	13%	55%	Moderate	
C_{25}	3	1.364	0.346	13%	55%	Moderate	0.041
$C_{12.5}$	3	0.311	0.053	12%	53%	Moderate	
$C_{6.25}$	3	0.301	0.054	13%	55%	Moderate	
Fluconazole	3	2.035	0.054	20%	62%	Strong	

* One-way analysis of variance

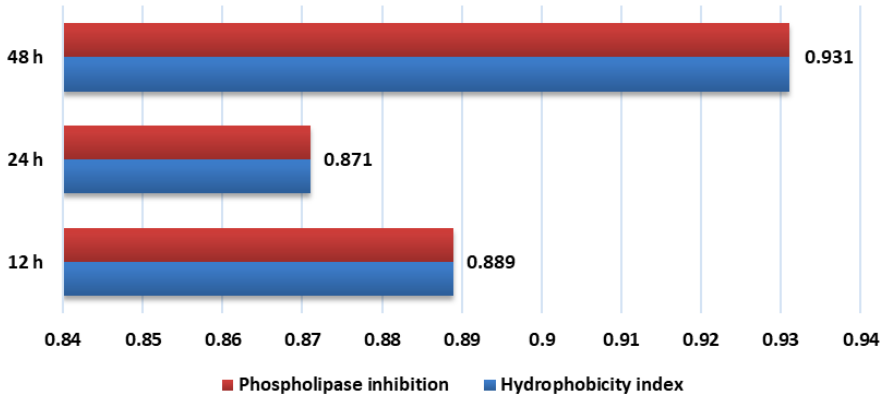

Figure 1. Pearson correlation coefficient between inhibition of phospholipase enzyme and hydrophobicity index. The three treatment times of phospholipase enzyme inhibition strongly correlate with the hydrophobicity index of the *C. albicans* cell surface.

DOI: 10.20473/j.djmkg.v58.i1.p66–73

Table 7. The least squares difference (LSD) test on the effect of *G. verrucosa* in inhibiting hydrophobicity of *C. albicans* cell surface

G.verrucosa (%)		*p-value
C_{100}	C_{75}	0.010
	C_{50}	0.001
	C_{25}	0.001
	$C_{12.5}$	0.000
	$C_{6.25}$	0.001
	Fluconazole	0.031
C_{75}	C_{50}	0.010
	C_{25}	0.012
	$C_{12.5}$	0.000
	$C_{6.25}$	0.001
	Fluconazole	0.041
C_{50}	C_{25}	0.575
	$C_{12.5}$	0.019
	$C_{6.25}$	0.011
	Fluconazole	0.041
C_{25}	$C_{12.5}$	0.011
	$C_{6.25}$	0.017
	Fluconazole	0.019
$C_{12.5}$	$C_{6.25}$	0.711
	Fluconazole	0.015
$C_{6.25}$	Fluconazole	0.001

*Post hoc LSD ($p < 0.05$ is significant)

significant difference ($p < 0.05$:0.041). Based on the LSD test, Table 7 shows that concentrations of 100% and 75% have a better ability to inhibit the hydrophobicity of *C. albicans* cells surface.

Figure 1 reports Pearson's correlation of the role of *G. verrucosa* in inhibiting the formation of the phospholipase enzyme with the ability of *C. albicans* to form CSH. These three treatment times have a strong relationship between the inhibitory power of the phospholipase enzyme and the hydrophobicity of the *C. albicans* cell surface. The direction of the positive ridges indicates that *G. verrucosa* has a similar effect in inhibiting the phospholipase enzyme and the formation of CSH. It means that *G. verrucosa* can work together to cause changes in the virulence of *C. albicans.*

DISCUSSION

This study uses the ethanol extract of *G. verrucosa* as a test material to measure the inhibitory power of the enzyme phospholipase and CSH of *C. albicans* isolated from smokers' saliva. One of the smoker isolates' selections is expected to have high virulence properties tested for sensitivity with *G. verrucosa*. *Candida albicans* from smokers isolates had faster morphological transitions from blastospores to pseudohypha and truly hypha than *C. albicans* ATCC 10231 isolates.[1]

The chemical compounds of *G. verrucosa,* such as n-hexadecanoic acid and oleic acid, are reported to contain antioxidant and antifungal properties (n-hexadecanoic acid and oleic acid).[23] The activities were targeted inhibitors for phospholipase A2 of *C. albicans*. This approach could facilitate a potential anti-inflammatory drug. The findings from the investigation on enzyme kinetics provide evidence that n-hexadecanoic acid acts as a competitive inhibitor of phospholipase A2.[24] *Gracilaria verrucosa* contains steroid compounds, terpenoids, and tannins/phenolics, which can act as antifungal agents that can suppress the growth and development of *C. albicans*.[25] Their compounds have different roles and properties as *C. albicans* virulence inhibitors. This study examined the effects of the three compounds in *G. verrucosa* as inhibitors of phospholipase enzyme and CSH of *C. albicans* smoker isolates.

Furthermore, *G. verrucosa* can suppress the phospholipase enzyme of *C. albicans*. It still provides optimal effects at a concentration of 25%, especially at 48 hours of incubation time. The fact that *G. verrucosa* inhibits *C. albicans* phospholipase activity is encouraging for fungal infection treatment. The pathogenicity of *C. albicans* depends on its phospholipase enzyme, which breaks down host cell membranes.[13] *Gracilaria verrucosa* at 25% is the minimum dose to block this enzyme, primarily when incubated for 48 hours.

Gracilaria verrucosa may include antifungal secondary metabolites or phytochemicals. These chemicals are abundant at 25%, allowing them to interact with fungal cells. These drugs may inhibit phospholipase, preventing *C. albicans* from breaking down host cell membranes and reducing its pathogenicity.[26] The 48-hour incubation period matters, too. Enzyme and fungal growth take time to climax. *Gracilaria verrucosa* can decrease phospholipase activity for a longer time by incubation for 48 hours with *C. albicans*. This prolonged exposure may explain the remarkable inhibitory effectiveness. Moreover, a 25% concentration may be enough to override fungal defenses. One potential approach involves the inhibition of efflux pumps or disrupting essential metabolic pathways in *C. albicans*.[27] Implementing these measures can potentially enhance the inhibition of phospholipase in *G.verrucosa*.

The highest concentration (100%) of G. *verrucosa* is most effective at inhibiting the enzyme phospholipase of *C. albicans*. It can be assumed that a concentration of 100% signifies the use of *G. verrucosa* at its full potency, hence delivering the highest quantity of bioactive components and possible inhibitory agents for combating *C. albicans*. The high concentration of the substance is expected to impact the fungal cells significantly, impeding their ability to evade or develop resistance against the inhibitory effects.[28] Consequently, the enzyme phospholipase, which plays a pivotal role in the pathogenicity of *C. albicans*, experiences enhanced inhibition.

Moreover, the length of incubation is of utmost importance in this procedure. Extended incubation periods offer *G. verrucosa* prolonged exposure to *C. albicans*. The long duration of exposure facilitates a continuous suppression of phospholipase activity.[29] The attainment

DOI: 10.20473/j.djmkg.v58.i1.p66–73

of maximum enzyme activity frequently necessitates a certain amount of time, and the inhibitory effect can be intensified by prolonging the presence of *G.verrucosa*. The concentration, contact time, and surface area influence natural materials, such as nanoparticles, against pathogens.[30] This theory clarifies that a higher concentration of the test material used can lead to a higher ability to prevent the development of pathogens.[31] This ability is correlated with *G. verrucosa*'s ability to disrupt the enzyme synthesis channel in the cytoplasm, thus causing cells to polarize due to the influence of some active compounds possessed by *G. verrucosa*. This polarization causes cells to lack nutrition and have limited oxygen uptake. It is a process to reduce the degree of virulence of *C. albicans*. One of the *G. verrucosa* compounds related to this activity is tannin.[32] This compound is reported as an antifungal related to its ability to activate enzymes and interfere with intra- and extra-membrane transport of proteins.[33]

The highest concentration of *G. verrucosa* can strongly suppress the hydrophobicity activity of the cell surface of *C. albicans*. It can be assumed that *G. verrucosa* can inhibit the signaling system between pathogens when forming biofilms. Hydrophobicity is an early phase carried out by some pathogens before forming biofilms. This signaling is closely related to the degree of formation of hydrophobicity or biofilms in the pathogenesis of infections, both bacteria and fungi, such as *C. albicans*.[34]

High plant extract concentrations may promote bioactive compound–fungal cell membrane contacts, explaining its effectiveness. Many antifungals damage the cell membrane, and leakage of intracellular substances can cause cell death.[35] Plant extracts may also overpower fungal defensive systems, including efflux pumps that remove antifungal drugs at higher doses. Plant extracts may hinder *C. albicans* from releasing antifungal chemicals, making the organism more susceptible.[36] High concentrations of plant extracts may also affect *C. albicans*' metabolic pathways, threatening their survival and growth. Energy generation, protein synthesis, and deoxyribonucleic acid replication can be disrupted, inhibiting fungal cell development.[37] From the perspective of this study, terpenoids contained in *G. verrucosa* can inhibit biofilm formation, prevent adhesion, and inhibit the growth of *C. albicans*. Besides, terpenoids can also disrupt the morphological changes of *C. albicans* from blastospores to hyphae, one of the essential virulence factors in *C. albicans*. The consequence disturbs the synthesis of phospholipids as a source of nutrients and reactive oxygen species.[38]

The fungal cell wall structure's main components are (1,3) β- and 1, 6-β glucan, chitin, and mannoprotein. (1,3) -β-glucans are factors that play a role in CSH[38] and are catalyzed by the enzyme synthase (1,3) β-glucans. 1,3-β glucan synthase is a glycosyltransferase enzyme found in the plasma membrane that is responsible for the construction of the fungal cell wall.[39] Therefore, when the performance of this enzyme is inhibited, the cell wall loses rigidity and causes the attachment of *C. albicans* to epithelial cells to be significantly weakened.[40] These results indicate that the chemical compounds of *G. verrucosa* are more effective in inhibiting the formation of ergosterol *C. albicans*, where ergosterol is a plasma membrane component that plays a role in the construction of chitin, which consists of some polysaccharide components of cell walls and has an essential role in the growth of *C. albicans*.[41] The hydrophobicity of some pathogens is known to have the property of trying to move away from non-polar molecules and approaching polar molecules.[42] Therefore, it can be assumed that besides having opposite properties, *G. verrucosa* also has non-polar properties.[43] The polarity of some compounds contained by *G. verrucosa* can determine the inhibitory power of the enzyme phospholipase and CSH of *C. albicans*.[44]

Both analyses demonstrated that the inhibitory capability of *G. verrucosa* on the hydrophobicity of *C. albicans* cell surfaces correlated with its ability to suppress *C. albicans* phospholipase activity. It was observed that longer incubation times and higher concentrations of *G. verrucosa* resulted in more effective inhibition of CSH and phospholipase enzymes in *C. albicans*. In conclusion, the concentrations of *G. verrucosa* have demonstrated a practical inhibitory impact in suppressing the phospholipase enzyme and decreasing the CSH of *C. albicans*. The level of inhibition is influenced by the concentration of *G. verrucosa* and the incubation period.

REFERENCES

1. Gani BA, Bachtiar EW, Bachtiar BM. The role of cigarettes smoke condensatein enhanced candida albicans virulence of salivary isolates based on time and temperature. J Int Dent Med Res. 2017; 10(Special Issue): 769–77.
2. Lim SJ, Mohamad Ali MS, Sabri S, Muhd Noor ND, Salleh AB, Oslan SN. Opportunistic yeast pathogen Candida spp.: Secreted and membrane-bound virulence factors. Med Mycol. 2021; 59(12): 1127–44.
3. Deng J, Lu Z, Wang H, Li N, Song G, Zhu Q, Sun J, Zhang Y. A secretory phospholipase A2 of a fungal pathogen contributes to lipid droplet homeostasis, assimilation of insect-derived lipids, and repression of host immune responses. Insect Sci. 2022; 29(6): 1685–702.
4. Triawan A, Pudyani PS, HNE SM, - S. The effect of nanochitosan hydrogel membrane on absorbtion of nickel, inhibition of Streptococcus mutans and Candida albicans. Dent J (Majalah Kedokt Gigi). 2015; 48(1): 26.
5. Ali U, Lu S, Fadlalla T, Iqbal S, Yue H, Yang B, Hong Y, Wang X, Guo L. The functions of phospholipases and their hydrolysis products in plant growth, development and stress responses. Prog Lipid Res. 2022; 86(January): 101158.
6. Wilson SK, Knoll LJ. Patatin-like phospholipases in microbial infections with emerging roles in fatty acid metabolism and immune regulation by Apicomplexa. Mol Microbiol. 2018; 107(1): 34–46.
7. Cauchie M, Desmet S, Lagrou K. Candida and its dual lifestyle as a commensal and a pathogen. Res Microbiol. 2017; 168(9–10): 802–10.
8. El-Houssaini HH, Elnabawy OM, Nasser HA, Elkhatib WF. Correlation between antifungal resistance and virulence factors in Candida albicans recovered from vaginal specimens. Microb Pathog. 2019; 128: 13–9.
9. Abraham SB, al Marzooq F, Himratul-Aznita WH, Ahmed HMA, Samaranayake LP. Prevalence, virulence and antifungal activity of

DOI: 10.20473/j.djmkg.v58.i1.p66–73

C. albicans isolated from infected root canals. BMC Oral Health. 2020; 20(1): 347.
10. Jabra-Rizk MA, Falkler WA, Merz WG, Baqui AA, Kelley JI, Meiller TF. Cell surface hydrophobicity-associated adherence of Candida dubliniensis to human buccal epithelial cells. Rev Iberoam Micol. 2001; 18(1): 17–22.
11. Atriwal T, Azeem K, Husain FM, Hussain A, Khan MN, Alajmi MF, Abid M. Mechanistic understanding of Candida albicans biofilm formation and approaches for its inhibition. Front Microbiol. 2021; 12: 638609.
12. Rajkowska K, Kunicka-Styczyńska A, Pęczek M. Hydrophobic properties of Candida spp. under the influence of selected essential oils. Acta Biochim Pol. 2015; 62(4): 663–8.
13. Macias-Paz IU, Pérez-Hernández S, Tavera-Tapia A, Luna-Arias JP, Guerra-Cárdenas JE, Reyna-Beltrán E. Candida albicans the main opportunistic pathogenic fungus in humans. Rev Argent Microbiol. 2023; 55(2): 189–98.
14. Rezeki S, Pradono SA, Subita GP, Rosana Y, Sunnati S, Gani BA. The antifungal susceptibility of Candida albicans isolated from HIV/AIDS patients. Dent J. 2021; 54(2): 82–6.
15. Garcia-Cuesta C, Sarrion-Perez M, Bagan J. Current treatment of oral candidiasis: A literature review. J Clin Exp Dent. 2014; 6(5): e576–82.
16. Seleem D, Pardi V, Murata RM. Review of flavonoids: A diverse group of natural compounds with anti-Candida albicans activity in vitro. Arch Oral Biol. 2017; 76: 76–83.
17. Widowati I, Lubac D, Puspita M, Bourgougnon N. Antibacterial and antioxidant properties of the red alga Gracilaria verrucosa from the North Coast of Java, Semarang, Indonesia. Int J Latest Res Sci Technol. 2014; 3(3): 179–85.
18. Noviyandri PR, , N, Chismirina S. Effect of Nutmeg Flesh (Myristica fragrans Houtt) against Streptococcus mutans growth. J Syiah Kuala Dent Soc. 2021; 5(1): 42–6.
19. Dohude GA, Rusdy H, Hanafiah OA, Johal PK. Inhibitory test of turmeric leaves extract (Curcuma Longa. L) against the growth of Streptococcus mutans bacterial growth in vitro. J Syiah Kuala Dent Soc. 2023; 7(2): 73–7.
20. Soraya C, Mubarak Z, Gani BA. The growth and biofilm formation of Enterococcus faecalis in ethanol extract of Citrus aurantiifolia Indonesian species. J Pharm Pharmacogn Res. 2020; 8(1): 558–68.
21. Gani BA. Role of cigarettes smoke condensate on virulence enhancement of Candida albicans salivary isolate based on times and temperatures. Dissertation: Universitas Indonesia; 2019.
22. Muadcheingka T, Tantivitayakul P. Distribution of Candida albicans and non-albicans Candida species in oral candidiasis patients: Correlation between cell surface hydrophobicity and biofilm forming activities. Arch Oral Biol. 2015; 60(6): 894–901.
23. Duru IA, Maduka TD-O. Profiling and comparison of fatty acids in the oils from the fruits of Dacryodes edulis and Canarium schweinfurthii. J Med Plants Stud. 2020; 8(5): 213–7.
24. Chukwuma IF, Apeh VO, Nworah FN, Nkwocha CC, Emaimo J, Ezeanyika LUS, Nwadiogo V. Inhibition of phospholipase A2 and prostaglandin synthase activities as possible mechanistic insight into the anti-inflammatory activity of Brenania brieyi methanol and chloroform fractions. Thai J Pharm Sci. 2022; 46(1): 75–84.
25. Mubarak Z, Humaira A, Gani BA, Muchlisin ZA. Preliminary study on the inhibitory effect of seaweed Gracilaria verrucosa extract on biofilm formation of Candida albicans cultured from the saliva of a smoker. F1000Research. 2018; 7: 684.
26. Bu Q-R, Bao M-Y, Yang Y, Wang T-M, Wang C-Z. Targeting virulence factors of Candida albicans with natural products. Foods. 2022; 11(19): 2951.
27. Hans S, Fatima Z, Hameed S. Metabolic fitness of Candida albicans is indispensable for functional drug efflux, ergosterol, and chitin biosynthesis. Curr Med Mycol. 2020; 6(3): 9–14.
28. Prabajati R, Hernawan I, Hendarti HT. Effects of citrus limon essential oil (Citrus limon L.) on cytomorphometric changes of Candida albicans. Dent J (Majalah Kedokt Gigi). 2017; 50(1): 43.
29. Santos ALS, Braga-Silva LA, Gonçalves DS, Ramos LS, Oliveira SSC, Souza LOP, Oliveira VS, Lins RD, Pinto MR, Muñoz JE, Taborda CP, Branquinha MH. Repositioning lopinavir, an HIV protease inhibitor, as a promising antifungal drug: Lessons learned from Candida albicans—in silico, in vitro and in vivo approaches. J Fungi. 2021; 7(6): 424.
30. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. Int J Nanomedicine. 2017; 12: 1227–49.
31. Ordoñez R, Atarés L, Chiralt A. Biodegradable active materials containing phenolic acids for food packaging applications. Compr Rev Food Sci Food Saf. 2022; 21(5): 3910–30.
32. Alfarrayeh IIS. Bioactivities and potential beneficial properties of propolis ethanolic extract, caffeic acid phenethyl ester, and Arabic coffee beans extract. Thesis: University of Pécs; 2021. p. 1–14.
33. Salehi, Sharifi-Rad, Seca, Pinto, Michalak, Trincone, Mishra, Nigam, Zam, Martins. Current trends on seaweeds: looking at chemical composition, phytopharmacology, and cosmetic applications. Molecules. 2019; 24(22): 4182.
34. Sebaa S, Hizette N, Boucherit-Otmani Z, Courtois P. Dose-dependent effect of lysozyme upon Candida albicans biofilm. Mol Med Rep. 2017; 15(3): 1135–42.
35. Tyagi S, Lee K-J, Shukla P, Chae J-C. Dimethyl disulfide exerts antifungal activity against Sclerotinia minor by damaging its membrane and induces systemic resistance in host plants. Sci Rep. 2020; 10(1): 6547.
36. Alam M, Bano N, Ahmad T, Sharangi AB, Upadhyay TK, Alraey Y, Alabdallah NM, Rauf MA, Saeed M. Synergistic role of plant extracts and essential oils against multidrug resistance and gram-negative bacterial strains producing extended-spectrum β-lactamases. Antibiotics. 2022; 11(7): 855.
37. Liu X-Y, Huo Y-Y, Yang J, Li T-T, Xu F-R, Wan H-P, Li J-N, Wu C-H, Zhang Y-H, Dong X. Integrated physiological, metabolomic, and proteome analysis of Alpinia officinarum Hance essential oil inhibits the growth of Fusarium oxysporum of Panax notoginseng. Front Microbiol. 2022; 13: 1031474.
38. Kaskatepe B, Aslan Erdem S, Ozturk S, Safi Oz Z, Subasi E, Koyuncu M, Vlainić J, Kosalec I. Antifungal and anti-virulent activity of Origanum majorana L. Essential oil on Candida albicans and in vivo toxicity in the Galleria mellonella Larval model. Molecules. 2022; 27(3): 663.
39. Muszewska A, Piłsyk S, Perlińska-Lenart U, Kruszewska J. Diversity of cell wall related proteins in human pathogenic fungi. J Fungi. 2017; 4(1): 6.
40. Garcia-Rubio R, de Oliveira HC, Rivera J, Trevijano-Contador N. The fungal cell wall: Candida, Cryptococcus, and Aspergillus species. Front Microbiol. 2019; 10: 2993.
41. Bouchelaghem S. Antimicrobial and antibiofilm activities of Hungarian propolis on Candida albicans and Staphylococcus aureus and its mechanism of action. Thesis: University of Pécs; 2022. p. 1–15.
42. Ropponen H-K, Richter R, Hirsch AKH, Lehr C-M. Mastering the Gram-negative bacterial barrier – Chemical approaches to increase bacterial bioavailability of antibiotics. Adv Drug Deliv Rev. 2021; 172: 339–60.
43. Ghedifa A Ben, Vega J, Korbee N, Mensi F, Figueroa FL, Sadok S. Effects of light quality on the photosynthetic activity and biochemical composition of Gracilaria gracilis (Rhodophyta). J Appl Phycol. 2021; 33(5): 3413–25.
44. Hemmati S, Rasekhi Kazerooni H. Polypharmacological cell-penetrating peptides from venomous marine animals based on immunomodulating, antimicrobial, and anticancer properties. Mar Drugs. 2022; 20(12): 763.

DOI: 10.20473/j.djmkg.v58.i1.p66–73