

ANTIFUNGAL ACTIVITY *SARGASSUM CRASSIFOLIUM* AND *SARGASSUM POLYCYCTUM* AGAINST *CANDIDA ALBICANS*

OOM KOMALA, TRIASTINURMIATININGSIH, RINA YULIANTI

Department of Biology, Faculty of Mathematics and Natural Sciences, Pakuan University, Bogor,
Indonesia

Jalan Pakuan P.O Box 452 Telp/Fax. (0251) 8375547 Bogor

<http://www.unpak.ac.id>, oom.komala@unpak.ac.id, triasti_nur@unpak.ac.id

e-mail address of corresponding author: oomkomala@unpad.ac.id

ABSTRACT

Introduction: *Sargassum crassifolium* and *Sargassum polycystum* are brown algae species and contain active compounds such as flavonoids, alkaloids, saponins, phenols, and triterpenoids that act as antibacterial, antiviral and antifungal. **Aims** of study was to determine antifungal *Sargassum crassifolium* and *Sargassum polycystum* against *Candida albicans*. **Methods:** Antifungal activity of *Sargassum crassifolium* and *Sargassum polycystum* extract were examined using the disc diffusion methods with concentrations of 250 mg/ml and 500 mg/ml compared to Ketoconazole 50 µg/ml against *Candida albicans* through measurement of the width of the inhibition zone. Qualitative phytochemical tests to know compound of alkaloids, triterpenoid, saponin, flavonoid, and tannin. **Results:** 500 mg/ml *Sargassum crassifolium* and *Sargassum polycystum* exhibited promising fungistatic agent against *Candida albicans* with diameter of inhibition zone i.e 22 mm for *Sargassum crassifolium* and 21.6 mm *Sargassum polycystum* and the its power of the antifungal is lower than Ketoconazole 50 µg/ml. *Sargassum crassifolium* and *Sargassum polycystum* contained triterpenoids, saponins, and flavonoids. **Conclusion:** 96% ethanol extract of *Sargassum crassifolium* and *Sargassum polycystum* have antifungal activity of *Candida albicans*. Antifungal effect *Sargassum crassifolium* (22 mm) not real different with *Sargassum polycystum* (21.6 mm) against *Candida albicans*.

KEYWORDS : Antifungal, *Candida albicans*, *Sargassum*

KEY MESSAGES :

Sargassum crassifolium and *Sargassum polycyctsum* contain alginate, flavonoids, alkaloids, saponins, phenols and triterpenoids are useful as an antifungal. Antifungal compounds proven in *Sargassum* can be used by the community as an alternative in treating Candidiasis.

INTRODUCTION

Seaweed secondary metabolite has potential as producer of bioactive as antibacterial, antiviral, and antifungal ^[1]. Seaweed extract can be used to treat candidiasis in patients with vaginal infections (vaginosis) ^[2]. Seaweed in some coastal waters Indonesia has potential for alternative medicine^[3]. *Sargassum crassifolium* and *Sargassum polycystum* contain active compounds such as flavonoids, alkaloids, saponins, phenols, and triterpenoids that act as antibacterial, antiviral, and antifungal ^[4]. Seaweeds from Bayah beach Lebak Banten has the potential to be developed as an alternative treatment ^[5,6]. *Candida albicans* become dominant and cause states pathological when the immune system declines both locally and systemically ^[7]. This study was conducted to find alternative medicine to treat candidiasis. Therefore, it is necessary for natural controlling material that does not cause adverse impact to human health. This research is determine the most effective concentration of extract as antifungal of *Candida albicans*.

MATERIALS AND METHODS

Equipment used include laboratory glasses, autoclave, incubator, laminar Air Flow Cabinet, analytical scale, ose, measuring flask, petri dish, 5 mL pipette, hot plate, micropipet, sieve no 20,

reaction tube, tube rack, Whatman filter paper, oven, plastic silk, measuring cup, calipers, desiccator, grinder, rotary evaporator. Ingredients used *Sargassum crassifolium* and *Sargassum polycystum* taken from Bayah Beach, Banten, *Candida albicans* come from LIPI, 96% ethanol, sterile aquadest, Potato Dextrose Agar (PDA), H₂SO₄ 2 M, HCl, Mg, reagent (Mayer, Dragendorf, and Wagner), Ketoconazole 50 µg/ml, and FeCl₃ 5%.

Preparation of Sargassum crassifolium and Sargassum polycystum extract

Each 200 g *Sargassum crassifolium* and *Sargassum polycystum* powder was maceration in 96% ethanol with a ratio of 1: 10 for 3 x 24 hours. The powder of simplicia was immersed in 1500 mL of 96% ethanol for 3 x 24 hours, and filtered to obtain the filtrate. The obtained residue was soaked with 500 mL of 96% ethanol for 3 x 24 hours, then filtered to obtain the filtrate. Next, the combined filtrate is evaporated using a evaporator at 50°C. Furthermore, reheat again for 3 hours at 50° C so obtained a viscous extract, the goal is to remove the solvent that is still present at the active compound ^[8].

Preparation of Antifungi Activity Test

A total of 0.5 ml of 100% extract dissolved with 0.5 ml sterile aquadest so that in get 50% concentration. Paper discs 6 mm were immersed in each 250 mg/ml and 500 mg/ml extract, as well as a positive control of ketocenazole 50 µg/ml and dried at 40°C to dry. *Candida albicans* isolates were obtained from the Microbiology Laboratory (InaCC), Indonesian Center for Sciences (LIPI) - Cibinong, Bogor. *Candida albicans* cultures were prepared in concentration 10⁴ colonies / mL.

Phytochemical Test

Phytochemical tests are qualitatively performed to determine the bioactive components contained in each *Sargassum crassifolium* and *Sargassum polycystum* extract. Phytochemical analyzes included the test of alkaloids, flavonoids, triterpenoids, saponins, and tannins^[9].

Antifungi Activity Test

The method used for activity *Sargassum crassifolium* and *Sargassum polycystum* extract to the growth of *Candida albicans* is a agar diffusion method^[10]. Paper discs containing *Sargassum crassifolium* and *Sargassum polycystum* extracts with concentrations of 250 mg/ml and 500 mg/ml are placed over Potatos Dectrosa Agar medium. Next, the petri dish is wrapped using a silk plastic and stored at 37° C for 1 x 24 hours. Obstacles growth of microorganisms is seen around the disc paper on the media.

Statistical Analysis

The data were analyzed by Multi Analysis of Variance (ANNOVA) method followed by Duncant observation using SPSS 16.0 application. The result were considered to be statistically significant when the $P < 0.05$.

RESULTS AND DISCUSSION

RESULTS

The Yield of Extract

Rendement of *Sargassum crassifolium* and *Sargassum polycystum* extract and powder were influenced by amount of solvent used and length of maceration time (Table 1). The rendement of extract obtained from this research are 1.2 % and 1.5% (Table 1).

“ Table 1: Rendement analysis of *S. crassifolium* and *S. polycystum*”

Species	Wet Weight	Simplicia Weight	Rendement (%)	
			Extract	Powder
<i>Sargassum crassifolium</i>	7,800 g	420.77 g	1.2	5.3
<i>Sargassum polycystum</i>	6,700 g	350.60 g	1.5	5.2

Phytochemical Test

Phytochemical tests of *Sargassum crassifolium* and *Sargassum polycystum* extracts were performed in order to determine the content of the compounds in both extracts, which were associated with antibacterial activity. Based on the qualitative analysis results known *Sargassum* extract has a flavonoid, saponin and triterpenoid compound (Table 2).

“Table 2: Phytochemical Analysis of *S. crassifolium* and *S. polycystum*”

Identification compound	Test Result	
	<i>Sargassum crassifolium</i>	<i>Sargassum polycystum</i>
- Alkaloids		
- Dragendorf	-	-
- Mayer	-	-
- Wagner	-	-
Flavonoid	++	++
Saponin	++	+
Triterpenoid	++	++
Tanin	-	-

Description: (-) compounds undetectable ,
 (+) there are compounds,
 (++) there are compounds

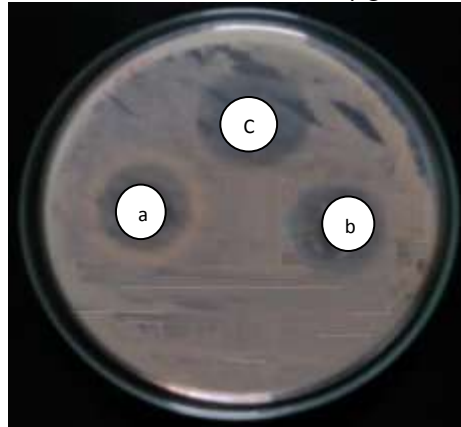
Antifungi Activity Test

Test of antifungal activity of 96% ethanol extract *Sargassum crassifolium* and *Sargassum polycystum* on *Candida albicans* growth showed diverse inhibitory diameter result. Diameter of inhibitory area of *Sargassum crassifolium* and *Sargassum polycystum* extract respectively are 20.3 ± 0.58 mm and 20 ± 1 mm as in Table 3 and Fig. 1 and 2 follows.

“Table 3: Diameter zone Average of *S. crassifolium* and *S. polycystum*”

Species	Repeatation	Treatment (mm)		
		250 mg/ml	500 mg/ml	C (+)
<i>Sargassum crassifolium</i>	1	20	22	25
	2	20	21	24
	3	21	23	27
	Total	61	66	76
	Average	20.3±0.58^{ab}	22±1^a	25,3±1.53
<i>Sargassum polycystum</i>	1	20	23	23
	2	19	20	24
	3	21	22	25
	Total	60	65	72
	Average	20±1^{ab}	21.6±2.16^a	24±1

Description: The numbers followed by the letters show the real difference based on the test Duncan at 99% confidence level. C+ : Ketoconazole 50 µg/ml



“Figure 1. Inhibition zone of *Sargassum crassifolium* Extract (Concentration 250 mg/ml (a), 500 mg/ml (b), C+) against *Candida albicans*”. C+ : Ketoconazole 50 µg/ml



“Figure 2. Inhibition zone of *Sargassum polycystum* Extract (Concentration 250 mg/ml, 500 mg/ml, C+) against *Candida albicans*” C+ : Ketoconazole 50 µg/ml

DISCUSSION

Water content in fresh seaweed is generally about \pm 80 - 90%. The moisture content of drying simplicia using parsley oven from *Sargassum crassifolium* was obtained at 3.01% and the water content of *Sargassum polycystum* was 2.87%, still complying with Directorate General of Food and Drug Control^[10] for the brown algae 5%. Water content is very influential on the material quality. Lower the water content in the seaweed, it is the better quality^[11], and can be stored for a longer time period.

According to Table 2, phytochemical analysis of *Sargassum crassifolium* and *Sargassum polycystum* extracts contain active compounds of flavonoids, triterpenoids and saponins. Saponin in the *Sargassum crassifolium* and *Sargassum polycystum* extract showed positive result marked with stable foam form for 15 minutes after shaking. Foams contained in saponin compounds test show the presence of glycosides capable of forming foam in water. Saponin compounds are soluble in water and contain hydroxyl functional groups making it easier to enter in cells and form complexes with cell membrane proteins. The damage can cause membrane permeability changes, resulting in lysis of the fungal cell membrane^[12]. Secondary metabolites of *Halimeda macroloba* have bioactive antifungal compounds^[13].

The triterpenoid test of *Sargassum crassifolium* and *Sargassum polycystum* extracts showed positive results (+) characterized by violet color change to dark green. The color change occurs after the addition of concentrated H_2SO_4 as much as ten drops. The widely used test is the Lieberman-Burchard reaction which, with most triterpene and sterol, gives a green-blue color^[9]. In low plants are usually found on the leaves (thallus) that serves to resist insects and microbes. The active compound is mainly found also in low plants, but sometimes there are in high plants such as triterpenoids in brown algae and coconut. Triterpenoid compounds are lipophilic which can cause disruption of fungi cell membranes and dissolve lipids contained in cell membranes,

can inhibit fungal growth by destroying wall and cell membranes structures in order to increase antifungal activity. Compounds contained in the methanol extract have the ability to break down the cytoplasmic membranes of *Candida albicans* cells. Such compounds may inhibit the synthesis of cell wall polymers by inhibiting the action of the synthase enzyme (1,3) -B glucans essential for the normal growth and development of the fungus^[14].

Category of resistance zone of a test material is determined to be weak if ≤ 5 mm, strong enough if 6 to 10 mm, strong if 11 - 20 mm, and very strong if > 20 mm^[15]³. The higher the concentration used, the larger the drag zone will be formed. The activeness of an inhibition is a selection criterion for an antimicrobial compound for the fungicide. Damage caused by antimicrobial components may be micosidal (permanent damage) and micostatic (temporary damage). Based on Tables 3 and Figures 1 and 2, the *Sargassum crassifolium* and *Sargassum polycystum* extracts have strong antifungal activity because they inhibit *Candida albicans* growth with an average diameter of more than 20 mm^[15]. *Sargassum crassifolium* and *Sargassum polycystum* extracts showed the largest inhibitory zone of 500 mg/ml concentration of 22 mm and 21.6 mm respectively. Ethanol extract 96% *Sargassum* has antifungal activity as well as *fucoidan* *Sargassum wightii* has pathogenic antibacterial effect^[16]. *Sargassum polycystum* extracts exhibited higher bacteriostatic activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus* (MIC = 0.065 mg/ml) lower than the standard MIC of potential antimicrobial drug (0.100 mg/ml). Antibacterial activity of *S.cinereum* extract against *S.epidermidis* bacteria has bacteriocidal, bacteriostatic and inhibitory inhibition mechanism^[17]. The antifungal substances in *Sargassum crassifolium* and *Sargassum polycystum* extracts are mono (2-ethylhexyl) phalates, polysaccharides and polyphenols. These compounds can suppress the growth of pathogenic fungi. *Sargassum* sp. containing the active compounds of steroids, alkaloids, phenols, and triterpenoids function as antibacterial, antiviral, and anti-fungal^[18]. The

mechanism of microorganisms resistance by antimicrobial compounds is caused by several factors, The first line of defense is biofilms, which can be formed by most bacteria to overcome the action of antimicrobial agents. Some other bacteria employ the second line of defense, the cell wall, cell membrane, and encased efflux pumps. When antimicrobial agents permeate the first two lines of defense and finally reach the cytoplasm, many bacteria will make use of the third line of defense, including alterations of intracellular materials and gene regulation to protect themselves from harm by bactericides ^[19]. Based statistical and Duncant analysis of 99% confidence level, it can be seen that between treatment of concentration 250 mg/ml, 500 mg/ml, and positive control of ketoconazole 50 µg/ml showed significant different effect (P <0.01). Inhibitory zone diameter of *Sargassum crassifolium* and *Sargassum polycystum* extracts lower than the ketoconazole 50 ppm as a comparison. Ketoconazole is a commercial antimicrobial as a positive control that can inhibit the entire test fungus with a larger inhibitory zone diameter than the *Sargassum crassifolium* and *Sargassum polycystum* extracts. Ketoconazole is a pure antimicrobial agent while *Sargassum crassifolium* and *Sargassum polycystum* extracts are still in the extract containing organic ingredients other than antimicrobials. Ketoconazole is a stable antifungal and diffuses well in agar medium. The antibiotic ketoconazole may work by inhibiting protein synthesis^[19]. The difference of this inhibitory zone may be due to differences in the concentration of the active compound in in the *Sargassum crassifolium* and *Sargassum polycystum* extracts. The size of the inhibitory zone diameter is influenced by the sensitivity of the test organism, culture medium and incubation period, the diffusion rate and the concentration of the antifungal active compound^[12]. **Conclusion,** Extract ethanol 96% *Sargassum crassifolium* and *Sargassum polycystum* have antifungal activity of *Candida albicans*. Antifungal effect *Sargassum crassifolium* (22 mm) not real different than *Sargassum polycystum*

(21.6 mm) to *Candida albicans*. Phytochemical compound of *Sargassum crassifolium* and *Sargassum polycystum* extract contain flavonoid, saponin, and triterpenoid compound.

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