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Identifikasi, Karakterisasi dan Uji Aktivitas Antifungi Senyawa Kimia dari Ekstrak Metanol Makroalga *Padina australis* Terhadap Jamur Ketombe *Malassezia globosa*

Identification, Characterization and Antifungal Activity Test of Chemical Compounds from Methanol Extract of Macroalgae Padina australis Against Dandruff Fungus Malassezia globosa

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Abstract

Padina australis is a species of brown macroalgae with potential as a natural source of bioactive compounds, including antifungal agents. This study aimed to identify the functional groups present in the methanolic extract of Padina australis using Fourier Transform Infrared (FTIR) spectroscopy and to evaluate its antifungal activity against Malassezia globosa through the disk diffusion method. FTIR analysis revealed the presence of hydroxyl (3433.41 cm^{-1}), aliphatic C–H (2916.47 cm^{-1}), carbonyl (1735.99 and 1643.41 cm^{-1}), amine (1573.97 and 1242.20 cm^{-1}), C–O, and carboxylate (COO^{-}) groups, indicating key secondary metabolites such as flavonoids, phenols, esters, amines, and polysaccharides. Antifungal testing showed that the extract at a concentration of 100 ppm produced the largest inhibition zone (16.58 $\pm$ 4.71 mm), while the minimum inhibitory concentration (MIC) was determined at 12.5 ppm with an inhibition zone diameter $\geqslant$12 mm. These findings suggest that Padina australis holds promising potential as a natural source for developing antifungal therapies against Malassezia globosa.

Keywords: *Padina australis, FTIR, bioactive compounds, antifungal, functional groups.*

1. INTRODUCTION

Dandruff is a common dermatological condition characterized by abnormal desquamation of scalp skin cells, accompanied by itching, flaky appearance, and white scales, typically without severe inflammation.or only mild erythema [1,2,3]. Approximately 50% of the adult population worldwide is affected by dandruff, with a higher prevalence in males [1,4]. One of the primary causative agents is *Malassezia globosa*, a lipophilic yeast that is part of the normal skin microbiota, but can proliferate under certain conditions such as excessive sebum production and hormonal changes [3,5].

Current treatments for dandruff generally rely on topical antifungal agents such as ketoconazole prolonged use may cause adverse effects including skin irritation, xerosis (dry skin), and disruption of the skin microbiota balance [2,6]. Consequently, the search for natural alternatives with antifungal activity is becoming increasingly important.

Padina australis, a species of brown macroalgae commonly found in tropical regions, including Indonesia , has been reported to exhibit antibacterial[7], antifungal[8], and antioxidant[10]. Previous s properties against various microorganisms[9]. Previous studies have shown that methanolic extracts and n-hexane fractions from the genus Padina contain various classes of compounds such as alkaloids, flavonoids, steroids, terpenoids, and saponins,

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which demonstrate significant antibacterial activity against pathogenic bacteria such as *Salmonella typhi* and *Escherichia coli.* [13,14]. In addition, antifungal activity against *Aspergillus flavus* has also been reported [8,15].

Nonetheless, studies on its antifungal activity specifically against *Malassezia globosa*, as well as the characterization of its bioactive compounds through spectroscopic approaches, remain limited. This study aims to evaluate the antifungal activity of methanolic extract of *Padina australis* against *Malassezia globosa* using the disc diffusion method, and to identify the functional groups of its bioactive compounds through Fourier Transform Infrared (FTIR) analysis.

2. METHODS

Equipment and Materials

The primary equipment used in this study included a rotary evaporator (Eyela N-1300, Eyela Co. Ltd., Japan), an ultrasonic water bath (Bransonic 1510E-MT, Branson Ultrasonics Corp., USA) for assisted extraction, an incubator (Memmert UF55, Memmert GmbH + Co. KG, Germany), and an analytical balance. Sterilization and aseptic procedures were carried out using a laminar airflow cabinet (Horizontal Laminar Flow Cabinet BBS-H1100, China) and an autoclave (Hirayama HVE-50, Hirayama Autoclave Co., Ltd., Japan). Functional group analysis was conducted using a Fourier Transform Infrared (FTIR) spectrophotometer (Shimadzu FTIR-8400, Shimadzu Corporation, Japan) equipped with a DTGS detector. Sample homogenization was performed using a standard laboratory blender. Additional laboratory tools included general-purpose glassware and a laboratory drying oven.

The chemical reagents and biological materials used consisted of methanol (pro analysis, Merck KGaA, Germany), dimethyl sulfoxide (DMSO, Merck, Germany), Sabouraud Dextrose Agar and Broth, ketoconazole disks (30 µg/disk, Oxoid Ltd., UK) as the positive control, and blank filter paper disks (6 mm, Oxoid Ltd., UK) as the negative control. The macroalgae *Padina australis* were collected from Cibuaya Beach, West Java, and subsequently authenticated at BRIN (National Research and Innovation Agency, Jakarta). The fungal test organism *Malassezia globosa* strain YP3003 was obtained from PT. Indinesia Paramartha Laboratories. All solutions and culture media were prepared using sterile distilled water.

Procedural

1. Sampling of *Padina australis*

Fresh specimens of *Padina australis* were collected randomly from Cibuaya Beach, Ujung Genteng, West Java (7° 34′ 69″ S, 106° 40′ 23″ E) at 15:00 WIB on dry season. Entire thalli (including blades, stipes, rhizoids, and spores) were placed in polyethylene bags containing seawater and transported in a cooler box to the Research Laboratory, Faculty of Pharmacy, Universitas 17 Agustus 1945 Jakarta. A portion of the collected sample was preserved in 70 % ethanol for morphological identification at BRIN Apriliani Soegiharto Science Area, East Ancol, Jakarta, Indonesia

Figure 1. Cibuaya Beach, Ujung Genteng, West Java

2. Extraction of Padina australis

Fresh samples of Padina australis were washed under running water and shade-dried to prevent degradation of heat/light-sensitive compounds. Moisture content was analyzed gravimetrically using a Moisture Content Analyzer (Mode A60/1 g, 100 °C) based on SNI 2354-2:2015, and only samples with maximum of 30% moisture were processed further. The dried samples were ground into fine powder and stored in airtight containers protected from light.

Extraction was carried out using ultrasound-assisted extraction (UAE) following previous methods [11,12] A total of 30 g of simplicia powder was macerated in 300 mL methanol (1:10 w/v), then treated ultrasonically at 42 kHz and 50% amplitude for 20 min, while maintaining bath temperature at 60–70 °C to optimize release of bioactive compounds. After extraction, the mixture was filtered to separate the liquid extract from the solid residue. The methanolic filtrate was then concentrated using a rotary evaporator at 60 °C under reduced pressure until a viscous crude extract was obtained. The final crude extract was weighed to determine the extraction yield and stored in a sealed container at 4 °C for further analysis and testing.

3. Yield and Phytochemical Screening

The yield of the extract was calculated at two stages: (1) the simplicia yield from fresh Padina australis biomass, and (2) the methanol extract yield from the dried powder. The simplicia yield was determined by comparing the weight of fresh seaweed to the final weight after drying. The extract yield was calculated by comparing the weight of the crude extract to the weight of the dried simplicia used in the extraction process [8]. These calculations were used to evaluate the efficiency of drying and extraction procedures.

Phytochemical screening was carried out qualitatively to detect the presence of major secondary metabolites in the methanol extract. The tests were conducted using standard reagents and procedures. For alkaloid detection, the extract was dissolved in 2 N hydrochloric acid and tested with Dragendorff's and Wagner's reagents, which produced orange and reddish-brown precipitates, respectively, if alkaloids were present. Flavonoids were tested by adding 2–3 drops of 10% potassium hydroxide (KOH) solution, followed by dilute hydrochloric acid; the appearance of a yellow color indicated the presence of flavonoids. Saponins were identified by shaking the extract with distilled water (1:10 ratio) for 30 seconds; the formation of stable foam indicated a positive result. Steroids and triterpenoids were tested using the Liebermann–Burchard reaction, where the extract was dissolved in chloroform and carefully layered with concentrated sulfuric acid; the appearance of bluish-green or violet colors indicated a positive result. The presence of tannins or polyphenols was detected by adding 5% ferric chloride ($FeCl_3$) solution to the extract; a dark green color indicated the presence of hydrolyzable tannins. The outcomes of these qualitative assays confirmed the existence of several key bioactive constituents in the methanol extract of Padina australis, which were hypothesized to contribute to its antifungal potential [16].

4. Antifungal Activity Assay

The antifungal activity of Padina australis methanolic extract was evaluated against Malassezia globosa using the disk diffusion method. A stock solution was prepared by dissolving 100 mg extract in DMSO (100 ppm), followed by serial dilutions (50, 25, 12.5, 6.25, and 3.125 ppm).

The fungal strain Malassezia globosa (YP3003) was cultured in Sabouraud Dextrose Broth (SDB) at 37 °C for 72 hours [17]. After incubation, 600 µL of suspension was spread on Sabouraud Dextrose Agar (SDA) plates, and sterile 6 mm paper disks were placed on the surface. Each disk was loaded with 100 µL extract solution. Ketoconazole (30 µg/disk) served as positive control, while DMSO was used as negative control. All treatments were prepared in triplicate (n=3). Plates were incubated at 37 °C for 24 hours [18].

Antifungal activity was determined by measuring inhibition zone diameters (mean ± SD). The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration producing an inhibition zone ≥12 mm, according to interpretive criteria for Malassezia spp.[19,20].

5. FTIR Analysis

Two grams of thick extract were subjected and analyzed using a Shimadzu FTIR-8400 spectrophotometer equipped with a Deuterated Triglycine Sulphate (DTGS) detector. The spectral analysis was conducted over a wavenumber range of 4000–400 cm^{-1}, using the instrument's standard resolution settings. The analysis was carried out at the Instrumental Chemistry Laboratory, Faculty of Mathematics and Natural Sciences Education (FPMIPA), Universitas Pendidikan Indonesia, Bandung.

Infrared spectra revealed functional groups such as –OH, C=O, C–H, C=C, N–H, COO^-, C–O, and C–N, which were matched with literature data. These indicate the presence of secondary metabolites including flavonoids, phenolics, esters, amines, and polysaccharides, likely contributing synergistically to the extract's antifungal activity.

3. RESULT AND DISCUSSIONS

3.1. *Padina australis* Identification

The macroalgae sample used in this study was morphologically identified by a junior research scientist at the National Research and Innovation Agency (BRIN), located in the Ancol Science Complex, Jakarta. Morphological identification was conducted through visual examination using a stereo microscope and confirmed with relevant taxonomic literature.

The observational results confirmed that the sample belonged to the species *Padina australis*, based on several defining characteristics: [21]

- Color: Yellowish-brown with certain parts appearing whitish due to calcification.
- Form: Fan-shaped thallus with rounded edges, marked by characteristic concentric lines on the surface. Calcareous deposits were present on some parts of the thallus.
- Size: Leaf diameter ranged between 3–4 cm, with the thallus length reaching up to 6–7 cm.

Figure 2. *Padina australis*

3.2. Yield of Simplicia and Methanol Extract *Padina australis*

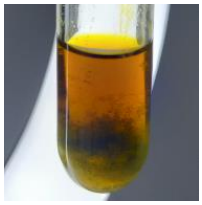
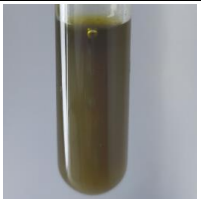
Table 1. Yield Calculation of Dried Simplisia and Methanolic Extract from *Padina australis*

Yield Type	Initial Weight (g)	Final Weight (g)	Yeild (%)
Dried Simplicia	13,000 (wet weight)	1,600	12,31%
Methanolic Extract	570 (dried simplicia)	25	4,89%

3.3. Phytochemical Screening of Methanol Extract of *Padina australis*

Table 2. Phytochemical Screening Results

No	Compound Group	Test Reagent	Result	Description	Test Tube Image
1	Alkaloid	Dragendorff reagent	+	Orange-colored precipitate	

		Wagner reagent	+	Reddish-brown precipitate	
2	Flavonoid	KOH 10% and dilute HCl	+	Yellow color	
3	Saponin	Distilled water	+	Foam height ±0.5 cm	
4	Steroid/Triterpenoid	Liebermann-Burchard reagent	+	Greenish-blue color (triterpenoid) and purple at the bottom layer (steroid)	
5	Tanin/Polifenol	FeCl3	+	Dark green color (tannins are detected)	

3.4. Antifungal Activity Test of Methanol Extract of *Padina australis* against *Malassezia globosa*

The antifungal activity test was conducted using the disc diffusion method against Malassezia globosa.

Table 3. Inhibition Zone Diameter of Padina australis Extract Against Malassezia globosa

Concentration (ppm)	Replicate 1 (mm)	Replicate 2 (mm)	Replicate 3 (mm)	Mean ± SD (mm)
100	11,40	17,74	20,61	16,58 ±4,71
50	9,79	20,61	17,92	16,10 ±5,63
25	15,13	18,78	12,06	15,32 ±3,36
12,5	11,99	12,44	14,35	12,92 ±1,25
6,25	18,03	9,00	12,61	13,21 ±4,55
3,125	14,97	9,66	12,33	12,32 ±2,66

Positive control	11,70	10,90	10,92	11,17 ±0,46
Negative control	0	0	0	0

The relationship between the concentration of Padina australis extract and the average inhibition zone diameter against Malassezia globosa can be seen in Figure 3 below.

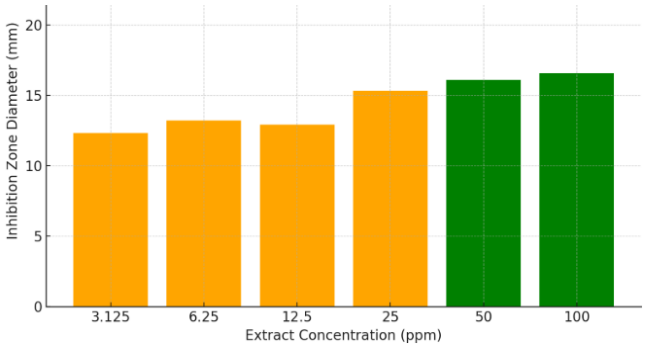


Figure 3. Inhibition Zone Diameter of *Padina australis* Extract Against *Malassezia globosa* at Various Concentrations

In addition, a visual documentation of the inhibition zones formed is also presented to reinforce the quantitative evidence shown in the previous table and graph.

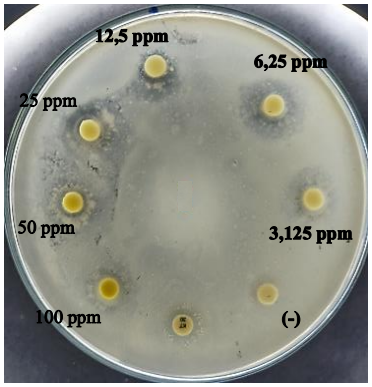


Figure 4. Visual documentation of inhibition zone after 24 hours of incubation (Petri Dish 1)

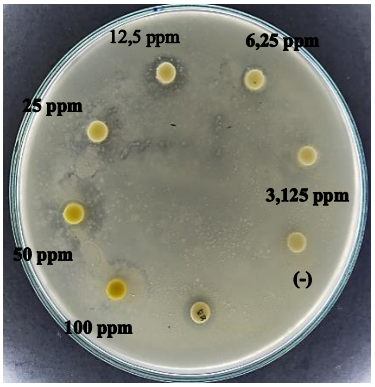


Figure 5. Visual documentation of inhibition zone after 24 hours of incubation (Petri Dish 2)

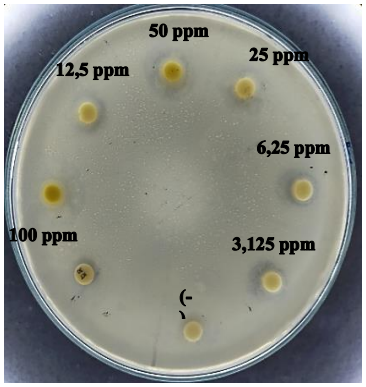


Figure 6. Visual documentation of inhibition zone after 24 hours of incubation (Petri Dish 3)

3.5. FT-IR Analysis of Methanolic Extract of *Padina australis*

FTIR spectrum showed eight dominant peaks corresponding to O–H, aliphatic C–H, C=O, N–H, COO^-, C–N, and alkyne groups.

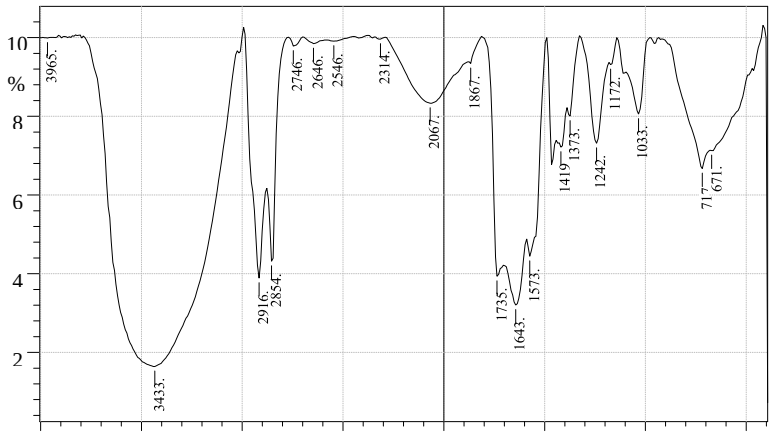


Figure 1. *Padina australis* Methanol Extract FTIR Spectrum

Table 4. Identification of major functional groups from the FTIR spectrum of methanolic extract of *Padina australis* based on literature from other species within the Padina genus

Peak (cm^{-1})	Functional Group and Vibration Type	Possible Compounds	References
3433.41	O–H (Symmetric stretching)	Hydroxyl, alcohol	[28] [36] [29]
2916.47	Aliphatic C–H (Asymmetric stretching)	Lipid, amine salts, alkenes	[34] [35] [30] [31]
1735.99	C=O (Asymmetric stretching)	Carbonyl, aldehyde, amide, aromatic, ketone, ester	[35] [30] [28] [29]
1643.41	C=O (Asymmetric stretching)	Carbonyl, amide, ester	[35] [30] [36] [31]
1573.97	N–H (Primary amine stretching)	Primary amine	[32]
1419.6	COO^- (Symmetric stretching)	Aginate	[36]
1242.2	C–N (Stretching)	Amine	[29]
671.2	-C = C-H:C-H (Bending/Scissoring)	Alkyne, Inorganic content and fatty acid	[35] [29]

4. DISCUSSIONS

The drying process of *Padina australis* yielded 12.31% dried simplicia and 4.89% methanolic extract. Compared to previous studies (16.21–19.32% yield for dried seaweed [22] and and 6.17% methanolic extract yield [13]), the results were slightly lower, possibly due to raw material quality and drying methods.

Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, hydrolyzable tannins, steroids, and triterpenoids. These compounds exhibit notable antimicrobial and antifungal activities. Alkaloids inhibit microbial enzymes, respiration, and DNA function [23], while flavonoids interfere with fungal cell walls, membrane permeability, and key metabolic enzymes [24,25]. Triterpenoids disrupt fungal membranes, and tannins inactivate essential enzymes and hinder microbial adhesion [26,27]. This supports the hypothesis that antifungal activity is due to the synergistic effects of multiple metabolites rather than a single compound.

The antifungal activity test demonstrated that methanolic extract of *Padina australis* produced inhibition zones against *Malassezia globosa* ranging from 12.32 ± 2.66 mm (3.125 ppm) to 16.58 ± 4.71 mm (100 ppm). Based on Sidoretno (2023), this activity can be classified as moderate to strong, with the Minimum Inhibitory Concentration (MIC) determined at 12.5 ppm [19]. The results confirm a dose–response relationship, indicating that increasing extract concentration enhances antifungal activity.

FTIR analysis further revealed functional groups corresponding to bioactive compounds such as flavonoids, phenols, esters, amines, and polysaccharides. In particular, the presence of absorption bands at ~1574 cm^{-1} (N–H stretching of primary amines) and ~1242 cm^{-1} (C–N stretching) may also suggest the occurrence of heterocyclic structures, potentially including an imidazole ring. Imidazole derivatives are well-documented for their antifungal properties, acting by binding to fungal cytochrome P450 enzymes and disrupting ergosterol biosynthesis [37]. Although FTIR cannot definitively confirm the exact heterocyclic structure, these spectral signals support the hypothesis that imidazole-like compounds may be present in the extract and contribute to its antifungal effect.

Overall, the integration of phytochemical screening, antifungal assay, and FTIR analysis strengthens the conclusion that *Padina australis* methanolic extract possesses significant antifungal potential against *Malassezia globosa*. However, as this study is limited to in vitro assays, further research involving compound isolation, advanced spectroscopic techniques (e.g., NMR, LC-MS), and in vivo models is required to confirm the presence of imidazole derivatives and establish their role in therapeutic applicability [33].

5. CONCLUSIONS

FTIR analysis of the methanolic extract of Padina australis confirmed the presence of functional groups such as hydroxyl, carbonyl, aliphatic C–H, N–H, C–N, and carboxylate (COO^-), indicating various secondary metabolites including flavonoids, phenols, esters, amines, and polysaccharides. The antifungal assay demonstrated effective inhibition of Malassezia globosa, with the largest inhibition zone measuring 16.58 ± 4.71 mm at 100 ppm, and a minimum inhibitory concentration (MIC) observed at 12.5 ppm.
These findings support the potential of Padina australis as a promising natural source for the development of antifungal therapies targeting dandruff-causing fungi. However, as this study was conducted in vitro, further research involving compound isolation, advanced spectroscopic analysis, and in vivo validation is needed to confirm its therapeutic applicability.

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