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IMPLICATIONS OF *Acanthus ilicifolius* LEAF EXTRACT UTILIZATION TO INHIBIT *Candida albicans* GROWTH TO SUPPORT MANGROVE CONSERVATION

IMPLIKASI PEMANFAATAN EKSTRAK DAUN *Acanthus ilicifolius* UNTUK MENGHAMBAT PERTUMBUHAN *Candida albicans* DALAM MENDUKUNG KONSERVASI MANGROVE

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ABSTRACT

Acanthus ilicifolius, a mangrove species with known therapeutic material, was investigated for its ability to inhibit the growth of *Candida albicans*. This study aimed to analyze the potential inhibitory effect of *A. ilicifolius* leaf extracts on the growth of *C. albicans* yeast. The method used in this study was a completely randomized laboratory experimental design. *A. ilicifolius* leaf and *C. albicans* yeast samples were collected from the mangrove areas in Socah District (Bangkalan Regency) and Paiton District (Probolinggo Regency). Leaf samples were extracted using the 99.98% methanol maceration method, followed by phytochemical analysis and an inhibition test. Qualitative phytochemical analysis of *A. ilicifolius* showed the presence of active compounds, alkaloids, flavonoids, steroids/terpenoids, tannins, and phenols, while saponins were absent. Quantitative tests showed flavonoids as the dominant active compound (162.79 ppm). The *A. ilicifolius* leaf extract exhibited weak to moderate inhibitory effects against *C. albicans*, with each concentration demonstrating significantly different effects. However, there was no statisticaly difference in the active compound content between the two locations. This study highlights the pharmacological potential of *A. ilicifolius* as a plant-based anti-yeast agents for alternative therapies and underscore the importance of mangrove conservation.

Keywords: *A. ilicifolius*, *C. albicans*, conservation, inhibition, phytochemistry

ABSTRAK

Salah satu jenis spesies mangrove yang memiliki manfaat sebagai bahan terapi adalah *Acanthus ilicifolius*. Penelitian ini bertujuan menganalisis potensi daya hambat ekstrak daun mangrove *A. ilicifolius* yang diambil dari 2 lokasi yang berbeda terhadap pertumbuhan khamir *Candida albicans*. Metode dalam penelitian ini menggunakan eksperimen laboratorium terkontrol dengan Rancangan Acak Lengkap. Subjek dalam penelitian ini adalah daun *A. ilicifolius* yang diambil di kawasan mangrove Kecamatan Socah (Kabupaten Bangkalan) dan Kecamatan Paiton (Kabupaten Probolinggo) dalam kondisi segar, serta khamir *C. albicans*. Potensi pemanfaatan *A. ilicifolius* dianalisis melalui ekstraksi, dengan metode maserasi metanol 99,98%, analisis fitokimia, dan uji daya hambat. Hasil analisis fitokimia kualitatif pada *A. ilicifolius* menunjukkan kandungan senyawa aktif alkaloid, flavonoid, steroid/terpenoid, tanin, dan fenol. Namun demikian, tidak ditemukan saponin. Uji fitokimia kuantitatif terhadap A. ilicifolius memperlihatkan kandungan senyawa aktif tertinggi adalah flavonoid (sebesar 162,79 ppm). Ekstrak daun *A. ilicifolius* memiliki daya hambat antikhamir yang dikategorikan lemah hingga sedang, meskipun setiap konsentrasi memiliki daya hambat yang berbeda nyata. Analisis statistik mengindikasikan kandungan zat aktif dalam ekstrak *A. ilicifolius* dari Kabupaten Bangkalan dan Kabupaten Probolinggo tidak menunjukkan perbedaan signifikan. Penelitian ini mendukung konservasi mangrove berbasis manfaat farmakologis dan berkontribusi pada pengembangan agen antikhamir berbasis tanaman untuk terapi alternatif.

Kata kunci: *A. ilicifolius*, *C. albicans*, daya hambat, fitokimia, konservasi

INTRODUCTION

Mangroves are protected ecosystems with a total economic value of IDR 20,290,350,289. This value includes the value of direct use as a traditional medicine ingredient and indirect use as a protector of coastal ecosystems (Hapsari *et al.* 2024). Mangrove forests are tropical coastal forests with several plant species used as unique pharmaceutical therapeutic ingredients (Edu *et al.* 2015). Ethnobotanical studies have shown that mangroves are used in traditional medicine for various diseases, thus strengthening the biopharmaceutical potential of this plant in natural medicine (Prasetyo *et al.* 2023). The use of mangroves as natural medicinal ingredients is carried out through a pharmacological approach by identifying and utilizing their bioactive content. This approach not only provides medical solutions but also increases economic value and supports the conservation of the mangrove ecosystem.

One potential application is the treatment of oral health problems, such as candidiasis or canker sores, that many people often experience. *C. albicans* generally causes this infection, the main pathogen that causes the highest infection among other opportunistic yeasts (Marbun 2020), and is a major factor in invasive candidiasis globally (Parambath *et al.* 2024). Mangrove plants, as a source of bioactive compounds, have shown promising results in inhibiting the growth of pathogens, including *C. albicans*. Mangrove leaf extract of *Avicennia marina* at certain concentrations has been reported to be effective in inhibiting the growth of *C. albicans*, although its effectiveness is lower than that of bacteria such as *Streptococcus mutans* (Rusiaty *et al.* 2018). In addition, the active compound of *Bruguiera gymnorrhiza* extract also has significant inhibitory activity against *C. albicans* as the concentration increases (Khoman *et al.* 2024).

The high need for new anti-yeast agents currently necessitates the exploration of molecules with more effective antimicrobial activity (Armengol *et al.* 2021). The mangrove species *Acanthus ilicifolius* in Ayurveda and Traditional Chinese Medicine (TCM) have long been used to treat various diseases. This mangrove extract has important pharmacological activities and contains various bioactive compounds (Pradnyasuari and Putra 2023). In addition, the methanol extract of *A. ilicifolius* has higher antimicrobial activity against bacteria and yeast than *Rhizophora mucronata* extract (Naidu and Vadlapudi 2010).

A. ilicifolius leaf extract offers promising potential based on its pharmacological value. This study aims to analyze the potential utilization of *A. ilicifolius* mangrove leaves collected from two different locations to inhibit the growth of *C. albicans* yeast. This study contributes to broader efforts to identify plant-based anti-yeast agents that can function as alternatives or complements to existing therapies. These findings are expected to improve our understanding of the therapeutic potential of *A. ilicifolius* and provide a scientific basis for its traditional use in managing yeast infections. This research is in line with the Sustainable Development Goals (SDGs), especially goal 3, namely, good health and well-being. In addition, this research supports the vision of Asta Cita Indonesia in creating sustainable environmental management and improving the quality of human resources based on innovation and explores its implications for mangrove conservation. Therefore, mangrove conservation efforts using an approach based on benefits and medical functions need to be improved. This research contributes to the development of natural pharmacies and the conservation of biological resources. In addition, it supports efforts to improve health, public awareness, and stakeholders in the preservation of biodiversity. Furthermore, this research is in line with global health priorities for combating antimicrobial resistance, optimizing the sustainable use of mangrove biodiversity, and encouraging phytopharmaceutical innovation.

METHODS

Time and location

This study was conducted from October to December 2022 with samples of *A. ilicifolius* mangrove leaves taken from the mangrove area of Socah District (Bangkalan Regency) and Paiton District (Probolinggo Regency). This study was conducted at the Microbiology Laboratory of Trunojoyo University, Madura.

Research tools and materials

The tools used in this study were an oven, blender, scales, Erlenmeyer flask, funnel, measuring cup, beaker, pipette, orbital shaker, test tube, water bath,

dropper plate, distillate flask, condenser, heater, thermometer, spatula, vortex, UV-Vis spectrophotometer, petri dish, autoclave, hot plate, incubator, tweezers, spreader, caliper, micropipette, microtip, needle, and bunsen.

The materials used in this study were *A. ilicifolius* mangrove leaves, *C. albicans* yeast, methanol 99.98%, distilled water, aluminum foil, Dragendorff, Meyer and Wagner reagents, H_2SO_4, $FeCl_3$, NaOH, gallic acid, distilled water, $AlCl_3$, sodium acetate ($C_2H_3NaO_2$), acetic acid, NH_4OH, $K_3Fe(CN)_6$, chloroform, cloth, filter paper, label paper, 1% BaCl, Sabouraud dextrose agar (SDA), and disc paper. In this study, *A. ilicifolius* mangrove leaves were identified using a mangrove species identification book (Primavera *et al.* 2004). The leaves of *A. ilicifolius* were chosen because they contain more bioactive compounds than the stems and roots (Pradnyasuari and Putra 2023). The criteria for *A. ilicifolius* mangrove leaves in this study were taken from natural habitats, namely fresh and healthy leaves, mature (adult), free from damage, disease, or pest attacks, and cleaned before extraction (Figure 1). Young and yellowing leaves were not used in the present study. Yeast isolates were obtained by CV. Wiyasa Mandiri Malang (East Java) was a pure culture.

Mangrove *Acanthus ilicifolius* leaf extraction

The *A. ilicifolius* mangrove leaf samples were cleaned, cut into pieces, dried at a temperature not exceeding 60°C, and ground into powder/flour. The extraction of *A. ilicifolius* mangrove leaves was extracted using the methanol 99.8% maceration method with a ratio of 1:5 and three repetitions (Maharany 2017). The powder of the *A. ilicifolius* mangrove leaf powder (50 g) was added to 250 mL of methanol solution until it was submerged. Maceration was performed at room temperature for 3×24 hours in the dark. Furthermore, the filtration process was carried out using a filter cloth and Whatman paper number 42 to separate the residue from the sample. The filtrate was evaporated to maximize the concentration of the extract in a rotary evaporator at 40°C until a thick extract was obtained (Diana *et al.* 2021). The extract yield was calculated using the following formula:

$$\%Extract\ yield = \frac{Extract\ weight\ (gram)}{Weight\ of\ simplicia\ before\ extraction\ (gram)} \times 100$$

Phytochemical analysis

Phytochemical tests on *A. ilicifolius* mangrove leaf extracts were carried out using qualitative and quantitative methods. The qualitative method was performed by adding reagents to observe changes in the shape and color of the following compounds:

a) Alkaloids

Mangrove leaf extract (10 mg) was taken, 10 ml of HCl was added, and the mixture was heated for 2 min with continuous stirring. Filtration of the leaf extract filtrate and HCl was carried out after cooling, and then 5 ml of HCl and Wagner's reagent were added. A change in color to reddish indicates a positive alkaloid test result (Kumalasari and Andiarna 2020).

(a) (b)

Figure 1. (a) Sample of *Acanthus ilicifolius* mangrove leaves, (b) Reference (Source: Primavera *et al.* 2004).

b) Saponins

Mangrove leaf extract was diluted with distilled water to 20 mL, and vigorous shaking was performed. Foam formation indicates the presence of saponins (De Silva *et al.* 2017).

c) Flavonoids

A small amount of extract was given a few drops of sodium hydroxide, and if the solution turned colorless after adding dilute acid, it indicated the presence of flavonoids (De Silva *et al.* 2017).

d) Phenol

The sample extract was given a few drops of ferric chloride ($FeCl_3$) solution, and the formation of a bluish-black color indicated the presence of phenol (De Silva *et al.* 2017).

e) Tannin

A. ilicifolius leaf simplicia (0.5 g) was boiled with 20 mL of distilled water using a test tube, filtered, and a few drops of 0.1% $FeCl_3$ were added. Brownish-green or blackish-blue colors indicate the presence of tannin content (Kumalasari and Andiarna 2020).

f) Steroid

One gram of simplicia was weighed and macerated using chloroform for 2 hours, then filtered. The filtrate was evaporated until 1 mL remained, then Liebermann-Burchard reagent was added and reheated. The presence of steroids is indicated by a color change to greenish blue in the chloroform layer (Meliala *et al.* 2021).

The quantitative phytochemical test in this study used the following methods:

a) Alkaloids

The alkaloid content test used the gravimetric method by inserting 10 g of the sample, adding 200 mL of 10% acetic acid, and leaving it for 24 hours, followed by filtering. The filtrate was heated until it was reduced to a quarter of the initial volume, and concentrated ammonium hydroxide was added until an alkaloid precipitate was formed. The precipitate was washed with diluted ammonium hydroxide and then filtered. The residue was evaporated and weighed until a constant weight was obtained. The test results are expressed as percentages (%) by weight (Harborne 1978). Analysis of the alkaloid levels was performed using the following equation:

$$\%Active\ Substance\ Content = \frac{W2 - W1}{A} \times 100$$

Description:

W1 = Initial weight of filter paper (g)

W2 = Weight of filter paper and constant residue (g)

A = Weight of sample extract (gram)

b) Flavonoid

Total flavonoid analysis was performed using a quercetin standard with an aluminum chloride reagent. Standard solutions were prepared at concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. Each series solution was added with 3 mL of ethanol 96%, 0.2 mL of $AlCl_3$, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water, then incubated for 30 minutes at room temperature, and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 400-550 nm. Determination of flavonoid levels in samples using 1 mL of sample extract and the same treatment as the standard solution. The absorbance of the solution was measured using a UV-Vis spectrophotometer at the wavelength with the highest absorption obtained (Yulianti *et al.* 2014).

c) Phenol

The phenol content of the sample extracts was analyzed using a standard gallic acid solution. Standard solutions were prepared in a series of concentrations of 1, 2, 3, 4, and 5 ppm. Each solution was added with 0.4 mL of Folin-Ciocalteu reagent, incubated for 8 min, and then 4.0 mL of 7% Na_2CO_3 solution and sterile distilled water were added to 10 mL. The mixture was incubated for 2 hours at room temperature, and the absorbance was measured at a maximum wavelength of 700-900 nm to create a calibration curve. Determination of the phenol content in the sample was carried out using extract filtration (0.5 mL) and was given the same treatment as the standard solution. The absorbance was measured at the maximum absorption wavelength (Sam *et al.* 2016).

d) Tannin

Tannin content was analyzed using a standard solution of tannic acid with Folin-Ciocalteu reagent and Na_2CO_3 solution. The standard solution was prepared in a series of dilutions of 20, 40, 60, 80, and 100 ppm. A total of 1 mL of each solution was placed into a 10 mL measuring flask containing distilled water (7.5 mL). The 0.5 mL of Folin-Ciocalteu reagent was added, and left for 3 min, and then 1 mL of saturated Na_2CO_3 solution

was added and incubated for 15 min. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 400-800 nm. Determination of total tannins in the sample was carried out using 1 mL of extract, then 2 drops of Folin-Ciocalteu reagent, 4 mL of 1 M Na_2CO_3, and distilled water were added to a volume of 10 mL to produce a concentration of 100 mg/mL. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength with the maximum absorption that was previously obtained (Mukhriani *et al.* 2014).

e) Steroids

Quantitative phytochemical test of steroids using standard cholesterol solution and Liebermann-Burchard reagent. Standard solutions were prepared in a series of 40, 60, 80, 100, and 120 ppm. Then, 2 mL of anhydrous acetic acid and 0.1 mL of H_2SO_4, homogenized using a vortex. The outer layer of the tube was covered with aluminum foil to protect it from light. After incubation for 15 min, absorbance was measured at the highest wavelength between 200-800 nm using a UV-Vis spectrophotometer (Meliala *et al.* 2021). Steroid levels were analyzed by inserting 5 g of sample simplicial powder into a 250 mL Erlenmeyer flask, adding 50 mL of chloroform solvent to each sample, and extracting for 1 hour on a shaker machine at a speed of 150 rpm. Filtration and residue were separated using filter paper. Three mL of sample filtrate was put into a test tube, then 0.1 mL of anhydrous acetic acid solution and 0.1 mL of concentrated sulfuric acid were added. The absorption was measured using a UV-Vis spectrophotometer at the maximum wavelength obtained (Ludin and Sakung 2022).

Anti-yeast activity test

The test yeast used in this study was *C. albicans*. *C. albicans* was cultured using the Clinical and Laboratory Standards Institute (2009) standard with Sabouraud dextrose agar (SDA) culture media.

C. albicans suspension was prepared by placing one loop of yeast colony in a test tube containing 3 mL of NaCl 0.9% solution. The mixture was stirred until it was evenly distributed, which was indicated by a change in the color of the liquid to cloudy. The turbidity of the suspension was then compared with the McFarland turbidity standard to ensure that the number of yeast colonies was following the 0.5 McFarland standard, which contains approximately $1–5\times10^6$ CFU/mL. The yeast suspension was grown on 50 µL of SDA medium using a micropipette and leveled with a spreader.

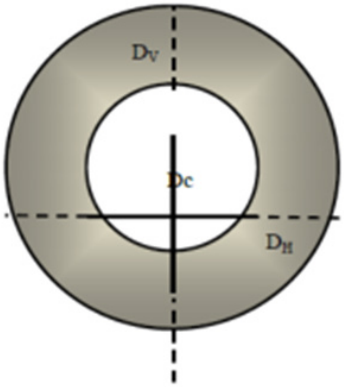
The anti-yeast activity was analyzed using the agar plate diffusion method (Kirby-Bauer) with sterilized, ready-to-use blank discs. The disc paper was dipped in several concentrations of the sample to be tested before being placed in a Petri dish. The concentrations of the *A. ilicifolius* mangrove leaf extract test solutions were 20%, 40%, 60%, 80%, and 100%. The positive control in this study was nystatin because *C. albicans* yeast infection can be overcome using this product (Yandriyan *et al.* 2019). Sterile distilled water was used as a negative control because it had no effect or inhibition on yeast growth. The aqueous solution used was sterile aquades with a pH of 7, so that the yeast was not contaminated with other elements. The test was repeated three times. The Petri dishes were incubated at 37°C for 24 hours.

The level of yeast activity in the agar medium was analyzed after the incubation period by measuring the inhibition zone formed around the disc. The inhibition zone was measured vertically and horizontally using a caliper in mm (Toy *et al.* 2015). A visual representation of the gray zone of inhibition is shown in Figure 2. The diameter of the inhibition zone was calculated using the following formula:

$$Area\ of\ Inhibition\ Zone = \frac{(Dv - Dc) + (DH - Dc)}{2}$$

The diameter of the inhibition zone was measured and combined with the following classification of microbial growth inhibition responses (Puspitasari and Munisih 2025):

Inhibition zone:
≤5 mm = Weak inhibition
>5-10 mm = Moderate inhibition
>10-20 mm = Strong inhibition
>20 mm = Very strong inhibition



Description:
= Disc
= Inhibitory zone
Dv = Vertical diameter
DH = Horizontal diameter
Dc = Disc diameter

Figure 2. Measurement of the diameter of the inhibition zone (grey color) of *C. albicans* yeast vertically (Dv) and horizontally (DH) using a disc (white color Dc) on Saboraud Dextrose Agar culture media (Toy *et al.* 2015).

Inhibition data analysis

This study was a laboratory experiment using a Completely Randomized Design (CRD). The normality test of the data distribution was performed using a normality test with a significance level of 0.05. The one-sample t-test and the independent sample t-test were conducted using data from observations of normally distributed inhibition zones. This test was conducted to identify the differences between concentrations and locations. Statistical tests in this study were performed using SPSS software. The implications of the use of *A. ilicifolius* are described based on the results of the research conducted.

RESULTS AND DISCUSSION

Phytochemical analysis of mangrove leaf extract *Acanthus ilicifolius*

A. ilicifolius leaves were extracted with methanol 99.8%. The extraction of both samples resulted in the percentage yields presented in Table 1.

Mangrove leaf extract from *A. ilicifolius* from the Bangkalan Regency had a higher yield than samples taken from the Probolinggo Regency. The use of methanol at a concentration of 72-96% is very effective in attracting bioactive compounds (Alfauzi *et al.* 2022). The high yield of the resulting extract was due to the longer duration of interaction between the material and the solvent, allowing the solvent to penetrate the material cells more optimally and allowing more active compounds to diffuse out of the cells (Wijaya *et al.* 2018). Research by Puspitasari and Munisih (2022) using methanol solvent with a ratio of 1:4 produced a yield of mangrove extract *A. ilicifolius* of 6%. In addition, Islam *et al.* (2024) showed a yield of 12.38% in *A. ilicifolius* leaf extract weighing 4 g using 100 ml of methanol. The yield was related to the content of active compounds in the sample. The higher the yield, the greater the number of active compounds contained in the sample (Hasnaeni *et al.* 2019). Phytochemical screening identified *A. ilicifolius* mangrove leaves, as shown in Table 2.

Table 1. Yield of *A. ilicifolius* mangrove leaf extract from Bangkalan Regency and Probolinggo Regency.

Sample	Extract Weight (g)		Yield (%)	
	Average	Standard Deviation	Average	Standard Deviation
Bangkalan	4.2	0.7	8.5	1.5
Probolinggo	3.8	1.2	7.6	2.4

Table 2. Results of phytochemical screening of *A. ilicifolius* mangrove extracts taken from Bangkalan Regency and Probolinggo Regency.

No.	Compound	Samples	
		Bangkalan	Probolinggo
1	Alkaloids	+	+
2	Saponins	-	-
3	Flavonoids	+	+
4	Phenol	+	+
5	Tannin	+	+
6	Steroids/Terpenoids	+	+

Table 2 shows the compounds identified in the extract of *A. ilicifolius* mangrove leaves at the Bangkalan Regency and Probolinggo Regency locations. Phytochemical screening in this study did not identify saponins. Saponin compounds were not detected in this study; therefore, it is suspected that these compounds were not dissolved in methanol. The results of research by Islam *et al.* (2024) and Andriani *et al.* (2020) also did not find saponins in *A. ilicifolius* leaf samples extracted using methanol and ethanol, but were detected in samples extracted using acetone and ethyl acetate (Islam *et al.* 2024). Saponin and tannin activities can also be obtained through the boiling process (Nuryani *et al.* 2018). Methanol can dissolve both polar and non-polar analytes, resulting in the production of secondary metabolites such as flavonoids, steroids, alkaloids, and saponins from plants (Thompson 1985). Suryanto and Wehantouw (2009) proved that methanol can attract secondary metabolites such as flavonoids, phenolics, and tannins in *Artocarpus altilis* F. leaves more than ethanol.

The quantitative phytochemical tests in this study involved testing the linearity of the standard solutions to determine the optimal wavelength used in the analysis of the active substance content. The absorbance values of the standard solutions of flavonoids, phenols, tannins, and steroids were 685 nm, 485 nm, 740 nm, and 780 nm, respectively (Figure 3).

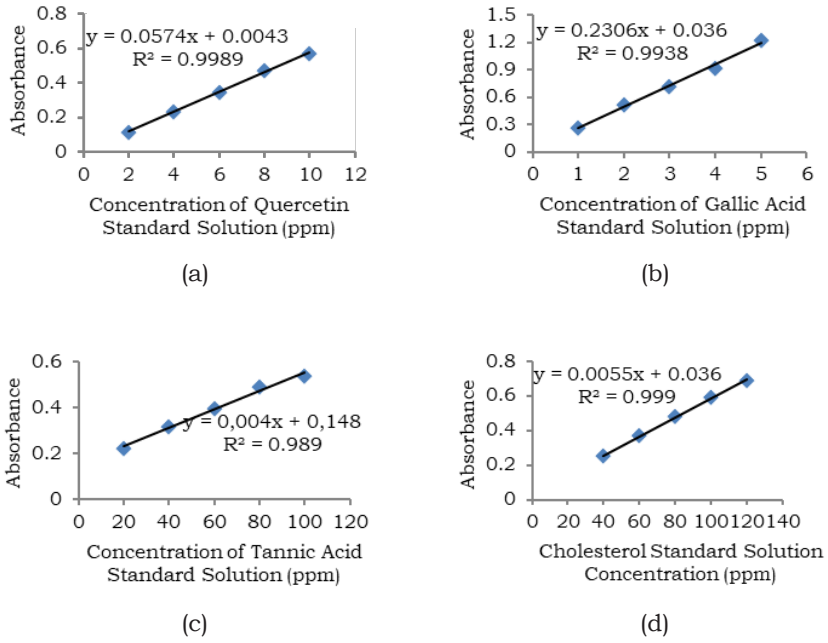


Figure 3. Linearity test of absorbance values of standard solutions of (a) flavonoids, (b) phenols, (c) tannins, and (d) steroids.

Figure 3 shows that all standard solutions have a linear line with a correlation coefficient value on the standard curve approaching 1, meeting the linearity requirements. The linear equation for each curve was used to determine the levels of active substances contained in the *A. ilicifolius* mangrove leaf extract. The results of the analysis of the bioactive content of the samples have varying concentrations and are expressed in grams and parts per million (ppm). Flavonoid compounds in both samples of *A. ilicifolius* mangrove leaves had the highest concentration. The concentrations of compounds contained in *A. ilicifolius* mangrove leaves in both samples are presented in detail in Table 3.

Mangrove leaf extracts from *A. ilicifolius* from both locations had higher flavonoid content than other active compounds. Samples from the Bangkalan Regency have secondary metabolite compounds with higher concentrations than samples from the Probolinggo Regency. This is thought to be due to differences in the yield percentage variations. The difference in the yield produced affects the composition of the active compounds. In addition, the difference in the active substance content between the two samples is thought to be due to the different growth environments. Laoué *et al.* (2022) suggested that the light intensity can affect the production of flavonoids and phenols in response to UV radiation. Flavonoid production tends to increase in plants growing in areas with more intense exposure to sunlight. This study revealed that *A. ilicifolius* mangroves growing in the Bangkalan Regency with high light intensity have higher flavonoid content than those in the Probolinggo Regency.

Flavonoids and phenols synthesized via the phenol pathway are among the largest components of polyphenols. Flavonoid compounds in plants play a role in the characteristic red color. In addition, they protect plants from UV rays from the sun and pathogens. Flavonoids act as competitive inhibitors of tyrosinase. This is because of the influence of the flavonoid structure that matches the form of the substrate, which can compete and is an inhibitor of the tyrosinase enzyme (Prasetyo 2021).

Inhibition of *A. ilicifolius* leaf extract

The results of the inhibition test based on the formation of inhibition zones by *A. ilicifolius* leaf extract on the growth of *C. albicans* are presented in Table 4.

The inhibitory response to *C. albicans* growth by *A. ilicifolius* mangrove extract taken from Bangkalan Regency to *C. albicans* growth is in the low to moderate category. Moderate inhibition was produced by the extracts at concentrations of 60% and 100%. This proves that *A. ilicifolius* mangrove extract has the potential to be an anti-yeast agent. Kalaskar *et al.* (2012) showed that the active compounds in *A. ilicifolius* leaves absorbed through the digestive tract can provide systemic anti-yeast effects on *Aspergillus fumigatus*. *A. ilicifolius* mangrove leaf extract can also have high anti-yeast potential when combined with other substances to increase the activity of its active substances. Andriani *et al.* (2020) revealed that the methanol extract of *A. ilicifolius* taken from Wonorejo Surabaya at concentrations of 16% and 20% had a *C. albicans* biofilm inhibition rate of approximately 70%. The strength of this anti-yeast activity is thought to be influenced by the alkaloid and tannin content in the methanol extract of *A. ilicifolius*, in addition to the presence of 2-benzoxazolinone (BOA) and benzoxazinoid compounds. Prananingrum *et al.* (2022) also explained that effervescent *A. ilicifolius* L made by mixing 4% *A. ilicifolius* L extract and sodium perborate has a better anti-yeast effect in denture care. In addition, oral administration of 16-20% *A. ilicifolius* extract and nystatin in *Rattus novergicus* strain Wistar rats provides effective healing of immunosuppressive oral candidiasis (Setyawan *et al.* 2019). The results of a study by Puspitasari and Munisih (2025) stated that mouthwash preparations given *A. ilicifolius* leaf extract have a higher potential for inhibiting the growth of *C. albicans* than preparations without extract.

Mangrove *A. ilicifolius* leaf extract, administered at various concentrations, caused significant differences in the width of the inhibition zone against the growth of *C. albicans*. This is shown by the statistical tests in Tables 5 and 6.

The results of the test between concentrations at the two locations in this study had a significant value of <0.05, indicating that each concentration of *A. ilicifolius* mangrove leaf extract had a significant effect on the inhibition of *C. albicans* growth. The independent t-test produced a significance value of >0.05, indicating that although the Bangkalan Regency extract sample had a higher active

substance content than the Probolinggo Regency sample, neither sample significantly inhibited the growth of *C. albicans*.

Each secondary metabolite compound has its own mechanism in inhibiting the growth of *C. albicans* (Alfiah *et al*. 2015). Flavonoids can inhibit the growth of conidia in pathogenic yeasts (Nasrul and Chatri 2024). The inhibition of *A. ilicifolius* mangrove leaf extract is mediated by the active flavonoid compound, which has moderate anti-yeast activity (Lee *et al*. 2024). Flavon content (7-104 ppm) can inhibit the growth of *C. albicans* by up to 50% (Ivanov *et al*. 2020). Based on this, flavonoids have the potential to be developed as antifungal agents for the treatment of candidiasis, either alone or in combination with existing anti-yeast drugs (Susilawati *et al*. 2023). Other active compounds, namely alkaloids, have antimicrobial activity through a mechanism that damages the structure of the cell walls of microorganisms. Saponins exhibit anti-yeast activity through their surface-active properties that resemble detergents by reducing the surface tension of the sterol membrane of the yeast cell wall, thereby increasing its permeability. Tannins act as defence agents against yeast by inhibiting the biosynthesis of ergosterol, which is essential for the integrity of the yeast cell membrane (Nasrul and Chatri 2024). The presence of active compounds, tannins, and flavonoids in *A. ilicifolius* mangrove can help in the detection of bioactive compounds that can be used as a basis for drug discovery (Pothiraj *et al*. 2021).

Table 3. Concentration of active compounds in *A. ilicifolius* mangrove leaves taken from Bangkalan and Probolinggo Regencies.

Compound	Concentration	
	Bangkalan	Probolinggo
Alkaloids (%)	5.00	2.00
Flavonoid (ppm)	162.79	153.02
Phenol (ppm)	17.45	6.32
Tannin (ppm)	11.67	17.45
Steroids (ppm)	3.00	2.60

Table 4. Average inhibition zone of *A. ilicifolius* leaf extract against the growth of *C. albicans* at different concentrations and locations.

Samples	Extract Concentration (%)	Average Inhibition Zone (mm)	Inhibition*
Control	Nystatin	17.35	High
	Aquades	0	
Bangkalan	20	2.7	Weak
	40	4.3	Weak
	60	5.7	Currently
	80	5.0	Weak
	100	7.3	Currently
Probolinggo	20	5.0	Weak
	40	4.5	Weak
	60	5.0	Weak
	80	4.0	Weak
	100	5.0	Weak

*Source: Puspitasari and Munisih (2025)

Table 5. Results of the one-sample T test analysis of the inhibition of mangrove extract at each concentration at each sampling location.

	Test Value = 9.5					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Extract Bangkalan	-7.299	14	0.000	-4.5933	-5.943	-3.244
Extract Probolinggo	-10.034	14	0.000	-4.3733	-5.308	-3.439

Table 6. Results of the independent sample T-test analysis of the inhibition of mangrove extract on the growth of *C. albicans* yeast at different locations.

		Levene's Test for Equality of Variances		T-test for Equality of Means						
		F	Sig.	T	Df	Sig (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Mangrove Inhibition	Equal variances assumed	2.294	0.141	-0.287	28	0.776	-0.2200	0.7655	-1.7881	1.3481
	Equal variances not assumed			-0.287	24.918	0.776	-0.2200	0.7655	-1.7968	1.3568

In this study, saponins were not detected in the test samples. Saponins have an inhibitory effect on resistant *C. albicans* strains (Chen *et al.* 2023). In addition, saponins damage cell membranes, which can result in cell death (Setyawan *et al.* 2019). However, the presence of saponins and alkaloids can significantly reduce antioxidant activity (Milugo *et al.* 2013). The description above shows that the extract of *A. ilicifolius* mangrove leaves from both research locations can be said to have good pharmaceutical potential with active compounds that do not reduce each other's effectiveness.

The results of this study show the potential of *A. ilicifolius* mangrove leaves for use in traditional medicine. This will encourage further exploration of the health sector, which can ultimately increase its economic value. In addition, the utilization of *A. ilicifolius* has important impacts on botany, pharmacology, ethnomedicine, and economics, and has positive implications for conservation efforts through the preservation of mangrove ecosystems (Tripathi *et al.* 2024). The development of products from untapped species has the potential to increase production in coastal areas (Pothiraj *et al.* 2021) while increasing the economic value of the community, which ultimately encourages conservation to ensure the sustainability of raw materials. This is in line with the sustainable use-based conservation approach, in which environmental preservation is carried out through the wise use of natural resources. Thus, the development of *A. ilicifolius* as a medicinal plant not only provides health benefits but also contributes to the preservation of mangrove ecosystems.

CONCLUSION

The results showed that mangrove leaf extract from *A. ilicifolius* from Bangkalan and Probolinggo Regencies has potential as an antifungal agent against *Candida albicans*. Phytochemical tests showed the presence of active compounds, namely alkaloids, flavonoids, steroids/terpenoids, tannins, phenols, and no saponin compounds. This potential refers to the inhibitory response of *A. ilicifolius* leaf extract to the growth of *C. albicans* yeast, which is in the weak-to-moderate category, and each concentration has a significantly different inhibition. Although there were differences in the levels of the active compounds, no significant

differences were found in the inhibition of the two samples. The development of *A. ilicifolius* as a medicinal plant can provide multidimensional health, economic, and environmental benefits.

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