

Identification of compounds isolated from a methanolic extract of *Acanthus ilicifolius* leaves and evaluation of their antifungal and antioxidant activity

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Abstract. Andriani D, Revianti S, Prananingrum W. 2020. Identification of compounds isolated from a methanolic extract of *Acanthus ilicifolius* leaves and evaluation of their antifungal and antioxidant activity. *Biodiversitas* 21: 2521-2525. *Acanthus ilicifolius* L. (Acanthaceae) is commonly found in mangroves along the east coast of Surabaya. It can be used as an indicator of environmental pollution and damage in mangrove ecosystems. Studies have reported that *A. ilicifolius* has antimicrobial, antifungal, antiviral, anti-inflammatory, analgesic, antioxidant, anticancer, antileishmanial, and hepatoprotective activity due to the chemical compounds in the plant. This study aimed to determine the phytochemical compounds in methanolic extracts of *A. ilicifolius* and their antifungal and antioxidant activity. The study involved laboratory experiments with a post-test only control group design. Antifungal activity against *Candida albicans* biofilm was determined using microtiter plates. Antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Phytochemical screening used colorimetric methods. Methanolic extracts of *A. ilicifolius* at 16% and 20% concentration had the same inhibitory effect as nystatin against *C. albicans* (about 70% inhibition of biofilm). Chemical compounds identified in the extract included flavonoids, alkaloids, glycosides, polyphenols, tannins, and steroids. Methanolic extracts of *A. ilicifolius* have strong antioxidant and antifungal activity, and the plant's phytochemical compounds are potential candidates for antifungal therapy.

Keywords: *Acanthus ilicifolius*, *Candida albicans* biofilm, antioxidant, phytochemical compound

INTRODUCTION

Acanthus ilicifolius L. (common names jeruju or holly-leaved *Acanthus*) is a member of the Acanthaceae (Velmani et al. 2016; Ragavan et al. 2015). Its leaves are green and highly variable in shape, from lanceolate to obovate; its leaf margins are entire to spiny and dentate. The stem is thick, and green, purple, or colors in-between, with axial spines present or absent, and if present, always facing upwards (Ragavan et al. 2015). *A. ilicifolius* var. *xiamenensis* is a mangrove species, and has long been used to treat various diseases (Chi et al. 2019). In traditional medicine, it is used to treat allergies, helminthiasis, dyspepsia, paralysis, asthma, headache, rheumatism, and skin diseases (Sardar et al. 2018; Singh and Aeri 2013). In Indonesia, *A. ilicifolius* is used as a phytoremediation agent for copper (Wahwakhi et al. 2017)

Acanthus ilicifolius has bioactive compounds that have the potential to be antibacterial, antiallergic, anthelmintic, anti-inflammatory, antioxidant, antileishmanial, osteoblastic, hepatoprotective, anticancer, antiulcer, and antimicrobial (Sardar et al. 2018; Ganesh and Vennila 2011; Chi et al. 2019). The antimicrobial activity of *A. ilicifolius* leaf extracts is higher than that of root, fruit, and flower extracts (Khajure and Rathod 2010). Ethanolic extracts of *A. ilicifolius* leaves from Mahakam, Indonesia are reported to have the best minimum inhibitory concentration against *Vibrio harveyi*, *Escherichia coli*,

Staphylococcus aureus, *Aeromonas hydrophila*, and *Saprolegnia* sp., compared to extracts of *Avicennia marina*, *Sonneratia alba*, and *Rhizophora stylosa* (Saptiani et al. 2018). Alkaloids, steroids, terpenoids, saponins, flavonoids, tannins, and glycosides are reported to be in ethanolic extracts of *A. ilicifolius*. This result is different from those of other extraction methods. Ganesh and Vennila (2011) reported that *A. ilicifolius* leaf methanolic extracts contained proteins, resins, steroids, tannins, glycosides, sugars, carbohydrates, saponins, terpenoids, phenols, alkaloids, cardiac glycosides, and catechol. The methanolic extract of *A. ilicifolius* exhibited the highest antioxidant activity compared to other solvent extracts, i.e., ethanol, chloroform, and ethyl acetate (Sofia and Marlee 2016).

Acanthus ilicifolius leaf extracts showed promising activity against *Candida albicans*, with alcoholic, butanolic, and chloroformic extracts showing strong inhibitory action. Previous studies have reported that chloroformic and methanolic extracts of *A. ilicifolius* have antifungal activity against *C. albicans* in oral candidiasis immunosuppressed model; 8% and 16% chloroform extracts could increase the expression of TLR2 and IL-22 that play a role in immunity against *C. albicans* (Andriani et al. 2017; Andriani and Pargaputi 2018, 2019). The MIC value of *A. ilicifolius* methanolic extract was 0.416 mg/ml against *C. albicans* (Sofia and Marlee 2016). In oral candidiasis immunosuppressed model, 16% and 20% methanolic extracts of *A. ilicifolius* had the ability to

increase macrophages, which are known to be the innate immune cells involved in the response against *C. albicans* infection (Setyawan et al. 2019). Therefore, this study is aimed at determining the phytochemical compounds with antifungal and antioxidant activity in methanolic extracts of *A. ilicifolius*.

MATERIALS AND METHODS

Sampling

Acanthus ilicifolius leaves were collected from the Wonorejo Mangrove Forest, Surabaya, East Java, Indonesia. *A. ilicifolius* leaves were identified based on the description in a guide issued by the Plant Bioscience and Technology Laboratory, Department of Biology, Institute Technology Sepuluh Nopember, Surabaya, Indonesia, i.e.: leaf shape highly variable, lanceolate to obovate; acute to acuminate or rounded with or without spiny edge in the apex, leaf base attenuate and leaf margin entire to spiny and dentate.

Extract preparation

The methanolic extraction of *A. ilicifolius* leaves was based on Gayathri and Gayathri (2014), with a slight modification of the maceration technique. We made the *A. ilicifolius* methanolic extract with leaves oven-dried at 45°C and then ground to a coarse powder. About 1000 g of the coarse powder was soaked in 96% methanol for 48 h in an Erlenmeyer flask covered with aluminum foil, with occasional shaking and stirring. The mixture was then filtered with Whatman Grade 1 filter paper. The solvent was evaporated at low pressure by using a rotary evaporator to obtain a viscous mass, which was evaporated again in a water bath to separate the extract from the solvent to yield as much as 121.5 g of pure methanolic extract of *A. ilicifolius* leaves.

Phytochemical examination

The total phenolic concentration in the plant extract was determined using a spectrophotometric method; the Folin-Ciocalteu assay was used for determining total phenols at a wavelength of 765 nm. The extract was dissolved in 96% ethanol and made up to a concentration of 100 mg/10 mL (Nurhasnawati 2019). This study performed phytochemical screening to assess the qualitative chemical composition of methanolic extracts using commonly employed precipitation and coloration reactions to identify the major secondary metabolites like alkaloids, flavonoids, glycosides, phenols, and tannins. The phytochemical analyses of the methanolic extract of *A. ilicifolius* leaves were carried out using standard procedures and screened for the presence of the secondary metabolites. The observations were verified for flavonoids and tannins using the ferric chloride test, for steroids using the Salkowski test, for alkaloids by Mayer's test, and for glycosides by the biuret and Legal's tests (Senthilkumar 2013).

Antioxidant activity

Testing for antioxidant activity was carried out according to Handayani (2018), with modifications. A 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was made by dissolving DPPH in methanol to obtain a 0.06 mM solution. Sample solutions were prepared by dissolving 10 mg of each sample in methanol. Then the samples were serially diluted by adding methanol p.a. A quercetin solution was made up of use as the standard. Then, 3.5 mL of the DPPH solution was added to 1 mL of methanol. The absorbance of the solution was measured by a UV-Vis spectrophotometer at a wavelength of 517 nm. The antioxidant activity of the samples was determined by adding 4 mL of DPPH solution to 1 mL of each concentration of the sample. This solution was then measured for its absorbance at the maximum wavelength.



Figure 1. Leaves of *Acanthus ilicifolius* plants from the Wonorejo Mangrove Forest, Surabaya, Indonesia. Bar = 2 cm

The percentage reduction of DPPH was determined by the following formula:

$$\% \text{SCV} = \frac{A1 - A2}{A1} \times 100,$$

Where: %SCV = percentage of DPPH radical scavenging activity; A1 = absorbance of the control; and A2 = absorbance of the sample.

The IC₅₀ value is the concentration of the sample that is capable of inhibiting or reducing DPPH by 50%. It is determined by the linear relationship between the concentration of the test solution (x-axis) and % damping (y-axis) from the equation $y = a + bx$, and is calculated using the following formula:

$$\text{IC}_{50} = \frac{(50 - a)}{b}$$

Antifungal activity

The antifungal activity of the *A. ilicifolius* methanolic extract was determined by the biofilm inhibition test using the microtiter plate method (Dhanasekaran et al. 2014). The results of the biofilm inhibition test in the form of optical density values (OD) were read using an ELISA reader (wavelength 509 nm). Inhibition of biofilm formation was calculated using the following formula (Pratiwi et al. 2015):

$$\% \text{inhibition} = (1 - \frac{(x\text{ODs} - x\text{ODbs})}{x\text{ODp}}) \times 100,$$

where ODs = optical density (509 nm) of samples tested; ODbs = optical density of sample blank; and ODp = (OD test solvent – OD solvent blank).

RESULTS AND DISCUSSION

Current research demonstrates that polyphenols contribute to the total antioxidant activity of fruits and vegetables (Poornaa et al. 2011). The level of polyphenols in the study was 1.18% w/w. Polyphenols can be categorized based on the number of phenol rings (Li et al. 2014). Phenolic compounds derived from plants include simple phenols, benzoquinones, phenolic acids, acetophenones, naphthoquinones, xanthenes, bioflavonoids, coumarins, stilbenes, tyrosine derivatives, hydroxy acids, cinnamic acids, flavonoids, lignans, and tannins. They are compounds containing hydroxyl groups (-OH) which are bound directly to the ring group of aromatic hydrocarbons (Dhianawaty and Panigoro 2013). These compounds have high antioxidant ability and free radical scavenging capacity by inhibiting the enzymes responsible for reactive oxygen species (ROS) production and reducing highly oxidized ROS (Li et al. 2014). The polyphenols in this study indicate that flavonoids and

tannins are important components in the *A. ilicifolius* extract (Table 1).

Antioxidant capacity is derived not only from the phenolic content, but also from several other phytochemicals. The methanolic extract of *A. ilicifolius* in this study contained flavonoids, alkaloids, glycosides, polyphenols, tannins, and steroids (Table 1). This result is similar to that obtained by Gayathri and Gayathri (2014) who found that methanolic extracts of *A. ilicifolius* leaves contained flavonoids, glycosides, phenols, tannins, steroids, saponins, and terpenoids. Avijit et al. (2012) also reported that crude methanolic extracts of *A. ilicifolius* contained alkaloids, steroids, flavonoids, tannins, reducing sugars, and glycosides. Poornaa et al. (2011) reported tannins, flavonoids, glycosides, and alkaloids in methanolic extracts *A. ilicifolius*.

Table 1. Phytochemicals detected in a methanolic extract of *Acanthus ilicifolius* leaves.

Metabolites	Results
Alkaloids	+
Flavonoids	+
Glycosides	+
Polyphenols	+
Steroids	+
Tannins	+

Note: (+) = presence; (-) = absence

Table 2. Antioxidant activity of a methanolic extract of *Acanthus ilicifolius* leaves

Sample	Sample absorbance	% SCV	IC ₅₀ (µg/mL)
<i>A. ilicifolius</i> 8%	0.446	41	
<i>A. ilicifolius</i> 12%	0.43	44	17.51
<i>A. ilicifolius</i> 16%	0.378	51	
<i>A. ilicifolius</i> 20%	0.375	51	
Blank	0.774		

Table 3. Antifungal activity of a methanolic extract of *Acanthus ilicifolius* leaves against *Candida albicans* biofilm.

Group	Replicate	Inhibition against <i>C. albicans</i> biofilm (%)
<i>A. ilicifolius</i> 8%	4	50.97 ± 1.81 ^{a,c,d,e}
<i>A. ilicifolius</i> 12%	4	54.81 ± 2.82 ^{b,c,d,e}
<i>A. ilicifolius</i> 16%	4	73.63 ± 3.62 ^{a,b,c}
<i>A. ilicifolius</i> 20%	4	75.69 ± 7.26 ^{a,b,d}
Nystatin	4	77.58 ± 2.79 ^{a,b,e}

Note: Antifungal activity is given as mean ± standard deviation; mean antifungal activity with a different superscript letter was significantly different ($p < 0.05$) in one-way ANOVA with the LSD post hoc test.

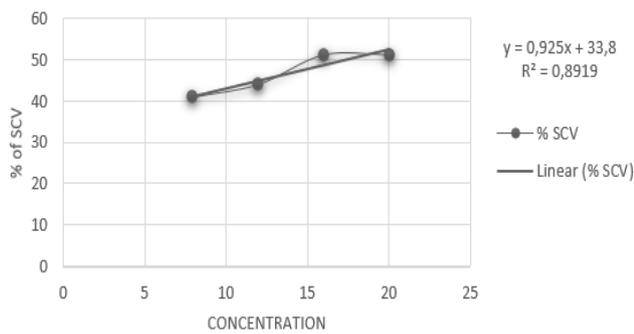


Figure 2. DPPH scavenging activity of a methanolic extract of *Acanthus ilicifolius* leaves

The DPPH method was used in this study because DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule; it produces a violet solution in methanol (Poornaa et al. 2011). According to Phongpaichit et al. (2007), a compound's antioxidant activity is very strong if its IC_{50} value is $< 10 \mu\text{g/mL}$, strong if IC_{50} is $10\text{-}50 \mu\text{g/mL}$, medium if IC_{50} is $50\text{-}100 \mu\text{g/mL}$, and weak if IC_{50} is $100\text{-}250 \mu\text{g/mL}$, and it is not active if IC_{50} is $> 250 \mu\text{g/mL}$. The results of this study indicate strong antioxidant activity of the methanolic extract of *A. ilicifolius* ($IC_{50} = 17.51 \mu\text{g/mL}$). Our findings differ from Avijit et al.'s (2012) who obtained $IC_{50} = 5.1 \mu\text{g/mL}$ indicating very strong antioxidant activity. This strong activity may be due to the presence of flavonoids which have the ability to scavenge free radicals and superoxide and hydroxyl radicals by single-electron transfer (Poornaa et al. 2011)

The potential antifungal activity of different plant sources against *C. albicans* is well known. Nevertheless, studies on the antifungal activity of methanolic extracts of *A. ilicifolius* are limited. *C. albicans* is the third most common pathogen causing superficial infections such as oral candidiasis. The antifungal activity of the methanolic extracts of *A. ilicifolius* in various concentrations in this study is shown in Table 3. All concentrations showed an antifungal effect against *C. albicans* culture growth. The highest activity against *C. albicans* was observed for the 20% concentration of *A. ilicifolius* methanolic extract, while the 16% extract showed no significant difference compared to 20% ($p > 0.05$). The antifungal activity of 8% and 12% *A. ilicifolius* extracts was significantly different ($p < 0.05$) from that of the positive control (nystatin). On the other hand, there was no significant difference ($p > 0.05$) between 16% and 20% methanolic extracts of *A. ilicifolius* and nystatin in their activity against *C. albicans*. These results show that all concentrations of methanolic extracts of *A. ilicifolius* in this study had an antifungal effect against *C. albicans*. Poornaa et al. (2011) also reported that the methanolic fraction of *A. ilicifolius* is active against *C. albicans*, and that it has a stronger and broader spectrum of antimicrobial activity compared to petroleum ether or hexane extracts.

The antifungal activity of the methanolic extract of *A. ilicifolius* may be due to the active components identified

by the phytochemical analysis. The antibacterial mechanisms of alkaloids involve inhibition of nucleic acid synthesis and enzyme activity and compromise of outer membrane and cytoplasmic membrane integrity. Several classes of alkaloids, including imidazoles, isoquinolines, piperidines, pyrrolidines, pyrrole-imidazoles, and cinchona alkaloids, inhibit the formation of bacterial biofilms (Cushnie et al. 2014). The antimicrobial mechanism of tannins involves protein complexing through covalent and non-covalent interactions as well as polysaccharide complexing (Othman et al. 2019). This mechanism may also occur in fungal inhibition. John et al. (2012) reported that most of the active antimicrobial complexes dissolve in polar solvent better than in water. The strong antifungal activity in this study may be due to the presence of alkaloids and tannins in the methanolic extract of *A. ilicifolius*. Saranya et al. (2015) suggested that the presence of 2-benzoxazolinone (BOA) and benzoxazinoids in methanolic leaf extracts of *A. ilicifolius* may promote antifungal and antioxidant activity. Therefore, more detailed investigations are necessary to determine the active compounds in these extracts, which may improve the analysis and help develop the potential of methanolic extracts of *A. ilicifolius*.

In conclusion, methanolic extracts of *A. ilicifolius* from Surabaya, East Java have antifungal activity against *C. albicans*, high antioxidant activity, and phytochemical compounds that can be considered candidates for antifungal therapy. Further studies on methanolic extracts of *A. ilicifolius* are required to explore active compounds and the antifungal effect in vivo.

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