

Acanthus ilicifolius Leaf Extract Potential as an Alternative Functional Food Beverage

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Received: February 26, 2024. Accepted: March 15, 2024. Published: March 28, 2024

Abstract: This study aimed to identify the active compounds, antioxidants, and antibacterial effectiveness of *Acanthus ilicifolius* leaves against microbial food-borne diseases as an alternative to functional food beverages. This study was an experimental study using a completely randomized design on a laboratory-scale experiment (in vitro), and the data obtained in this study were analyzed by analysis of variance (ANOVA). The results showed that the phytochemical screening of *Acanthus ilicifolius* leaves contained metabolite compounds of flavonoids, phenols, and tannins of 1072.44 mg/100g, 2619.64 mg/100 g, and 3205.66 mg/100g, respectively. The IC₅₀ value of *Acanthus ilicifolius* leaf extract, namely 221.67 ppm, has fragile antioxidant activity. At the same time, the highest inhibition was produced at an extract concentration of 100%. The concentration of *Acanthus ilicifolius* leaf extract at a concentration of 100% resulted in the highest average inhibition on bacteria of 9.67 ± 0.36 mm and 10.12 ± 1.12 mm, respectively, against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* sp. of 6.82 ± 0.15 mm, 6.18 ± 0.02 mm, and 6.19 ± 0.07 mm.

Keywords: *Acanthus ilicifolius*; Active Compound; Antibacterial; Antioxidant; Functional Food.

Introduction

Food-borne disease is one of the food-borne diseases that is still a threat to global health today. Contamination of food by microorganisms or other hazardous substances, both at the production and consumption stages, can trigger the spread of this disease. Fung et al. (2018) [1] stated that environmental pollution, food deviation processes, and poor food processing are triggers for contamination. Food-borne microbial disease will enter the body along with the food consumed and be digested and absorbed by the body. The body will experience symptoms ranging from mild to fatal if the infection is severe. Cases of food-borne diseases due to microbiological contamination, namely bacteria, viruses, and parasites, account for more than 90%. Cases of bacterial contamination account for only around 30% of cases of food-borne diseases [2]. Although the percentage is not very high, several research reports have stated that the number of extraordinary events and death rates caused by food-borne diseases from bacterial contamination is the highest [3]. Various types of microbial food-borne disease can cause food-borne disease.

Food-borne pathogenic microorganisms are responsible for many diseases affecting human health and the economy [4]. Bacteria that can usually cause it include *Salmonella*, *Campylobacter*, *Shigella*, *E. coli*, *Vibrio*, *Yersinia*, *Staphylococcus*, and *Listeria*. In addition, microorganisms that cause food infection also come from viruses such as Norovirus and parasites such as *Giardia*, *Taenia*, *Cyclospora*, and *Toxoplasma* [5], [6]. In addition, molds belonging to the mold group are one of the pathogens that can cause food-borne diseases. The fungus *Aspergillus* sp. is dominant and commonly found in the air,

causing food contamination. This fungus can produce aflatoxin, a toxic substance that can cause food spoilage, decrease body immunity, and trigger cancer [7].

The treatment of infectious diseases involves the administration of synthetic chemical drugs. However, long-term use can have an impact on the body. However, long-term use can affect the body. Currently, the development of herbal medicine is widely carried out. This aims to reduce the risk of using synthetic drugs in the long term. Herbal medicines are used not only in the treatment but also in the prevention and cure phases of infectious diseases. Herbal medicines can be made from developing plants or food products that contain bioactive compounds. Developing food products such as functional beverages containing antibacterial compounds is significant in overcoming microbial infections in the body. Functional beverages are one type of food that can fulfill two functions: providing nutritional intake and sensory satisfaction. Beverages are a form of practical food and contain more active substances than processed foods. One of the potential functional beverages has health and fitness benefits [8].

Acanthus ilicifolius is a plant classified as a mangrove that can grow in areas with low salinity. This plant grows to form shrubs around the pond area [9]. This plant is an annual shrub, wet-trunked, and grows upright or lying down at the base, 0.5-2 m high, in many clumps. Stem: round, cylindrical, slightly limp, smooth surface, brownish, and long thorns. This is a single leaf, located across, with pinnate leaves and a dark green color.

Acanthus ilicifolius leaf extract contains alkaloids, flavonoids, polyphenols, phenylethanoid glycosides, and coumarins [10]. *Acanthus ilicifolius* is widely distributed in Southeast Asia and is traditionally used in China as an anti-inflammatory and anti-hepatitis drug. *Acanthus ilicifolius* is

How to Cite:

Wulansari, N. T., Padmiswari, A. I. M. ., Harditya, K. B., & Sintyadewi, P. R. (2024). *Acanthus ilicifolius* Leaf Extract Potential as an Alternative Functional Food Beverage. *Jurnal Pijar Mipa*, 19(2), 331-336. <https://doi.org/10.29303/jpm.v19i2.6590>

also known to contain antioxidants that can benefit the skin. Several studies state that the bioactive compounds in this plant can fight disease. The content of chemical compounds in *Acanthus ilicifolius* functions as neuralgia, analgesics, anti-inflammatory, antioxidants, antifertility, hepatoprotective, antitumor, antileukemia, anticancer, antimicrobials, antiviral, antifungal [11-13]. In the NTB area, these leaves have begun to be used as tea, cultivated by the Emang Lestari Village PKK women's group. They can potentially become typical souvenirs from Emang Lestari Village [14].

However, the potential and utilization of *Acanthus ilicifolius* leaves are still not widely known. This study aimed to identify the active compounds, antioxidants, and antibacterial effectiveness of *Acanthus ilicifolius* leaves against food-borne microbial diseases as an alternative to functional food beverages. In addition, the results of this study are used as primary data in the development of innovative functional beverage products that are beneficial to health.

Research Methods

Tools and Materials

This research uses tools such as an Erlenmeyer flask, filter paper, evaporator, incubator, spectrophotometer, cork borer, petri dish, autoclave, masks, gloves, and vernier callipers. Meanwhile, the ingredients in this research include *Acanthus ilicifolius* leaves, ethanol, aluminium chloride reagent, gallic acid, tannic acid, PA methanol, DPPPH solution, distilled water, and ampicillin.

Research Design

This experimental study used a completely randomized design in a laboratory-scale experiment (in vitro).

Preparation of Extract

The maceration process makes the extract. The maceration stage of the extract began with sifting the dried *Acanthus ilicifolius* leaves and weighing 50 grams of simplicia. In addition, 250 ml of ethanol solvent and simplicia were added to the Erlenmeyer flask in a ratio of simplicia to ethanol solvent of 1:5. The maceration method was carried out for 1 x 24 hours at a temperature of $28 \pm 1^\circ\text{C}$. For 5 minutes every 12 hours, the maceration process was carried out manually so that the extract was still mixed with the solvent. The extract was filtered using filter paper. After that, the filtered extract was placed in the evaporator flask to remove the solvent in the extract and obtain a thick extract. This extract was evaporated at a temperature of 50°C and stopped when the vapor droplets from the solvent disappeared. In addition, the concentrated extract was put into a sample bottle, and phytochemical screening was carried out.

Analysis Phytochemical

Calculation of total flavonoids and using the spectrophotometric method with aluminium chloride reagent [15]. The absorbance was read using a

spectrophotometer at a wavelength of 510 nm, and Quercetin was used as a standard. As for phenols using the *folin-ciocalteau* method, the absorbance is read using the standard spectrophotometry of gallic acid [16]. Total tannins with spectrophotometric principles are those carried out by [17]. Absorbance was measured at a wavelength of 755 nm with a spectrophotometer using the standard tannic acid.

IC 50 Antioxidant Activity Test

A 100 ppm solution of *Acanthus ilicifolius* was obtained by dissolving 10 mg of extract in 100 millilitres of methanol PA. Further dilution can be achieved by varying concentrations utilizing PA methanol solvent. 5 mg of DPPH solids were dissolved in 100 ml of PA methanol to create the DPPH stock solution. A control solution comprising 1 ml of 50 ppm DPPH solution and 2 ml of PA methanol was also designed to compare. Samples of *Acanthus ilicifolius* containing up to 2 ml and 2 ml of DPPH solution were incubated at 27°C for 30 minutes or until the DPPH activity changed color. After incubation, samples are measured for absorbance at 517 nm wavelength with a Uv-vis spectrophotometer [18].

Antibacterial Test of *Acanthus ilicifolius* Leaf Extract

Testing plant extracts used the well diffusion method. NA media containing bacterial isolates was made into diffusion wells using a 5 mm cork borer. In the diffusion well, 20 μL of *Acanthus ilicifolius* leaf extract was added with each concentration. The negative control was added with distilled water, and the positive control was added with the antibiotic ampicillin. Then incubated at room temperature for 24 hours. After the incubation period, observations were made of the formed inhibition zones, and their diameters were measured.

Data analysis

Analysis of variance (ANOVA) was used to examine the data from this investigation [19]. Duncan's Multiple Range Test (DMRT) at the 5% significance level will continue the test if there is a difference at $p < 0.05$.

Results and Discussion

Phytochemical Screening

This study was initiated by screening the phytochemicals of *Acanthus ilicifolius* leaf extract before carrying out antibacterial tests on *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*. The extract phytochemical screening results of *Acanthus ilicifolius* leaves are shown in Table 1 below.

Table 1. Extract Phytochemical Screening Results
Metabolite Compounds *Acanthus ilicifolius* leaves

No.	Metabolite Compounds	Rate (mg/100g)
1	Flanovoid	1072.44
2	Phenol	2619.64
3	Tannins	3205.66

Table 1. shows the phytochemical screening of *Acanthus ilicifolius* leaves containing secondary metabolites of flavonoids, phenols, and tannins of 1072.44 mg/100g, 2619.64 mg/100g, and 3205.66 mg/100g, respectively. Similar research by [20] Suryati et al. (2019) showed that *Acanthus ilicifolius* leaf extract contains alkaloids, flavonoids, phenolics, terpenoids, and tannins. Differences in the content of secondary metabolites in a plant are influenced by the habitat where it grows, genetic diversity, harvesting, and post-harvest processing [21].

Flavonoid Compound Content

Quantitative analysis of total flavonoid compounds using UV-Vis spectrophotometry was carried out to determine how much total flavonoid content was contained in the ethanol extract of *Acanthus ilicifolius* leaves extract.

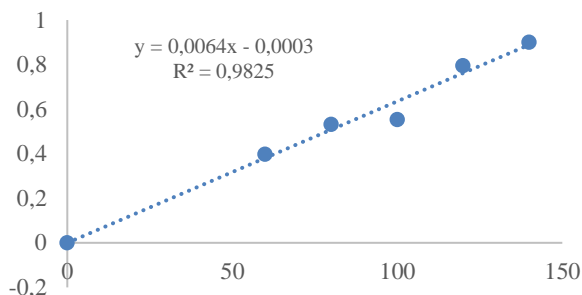


Figure 1. Flavonoid Linear Regression Equation Curve

This study's flavonoid content was determined using a Quercetin standard solution. The Quercetin standard results obtained were plotted between the levels and absorbance so that a linear regression equation was obtained, namely $y = 0.0064x - 0.0003$; $R^2 = 0.9825$, as shown in Figure 1. Based on the regression equation, the calculation of total flavonoid content in *Acanthus ilicifolius* leaves shows that the total flavonoid content is 1072.44 mg/100g. Flavonoids in natural materials have benefits such as analgesics, antitumors, antioxidants, anti-inflammation, antibiotics, anti-allergies, and anti-diuretics [32].

Phenol Compound Content

Phenol is a secondary metabolite with at least one atomized ring (C6) and one or more hydroxyl groups. The phenol linear regression equation curve is shown in Figure 2 below.

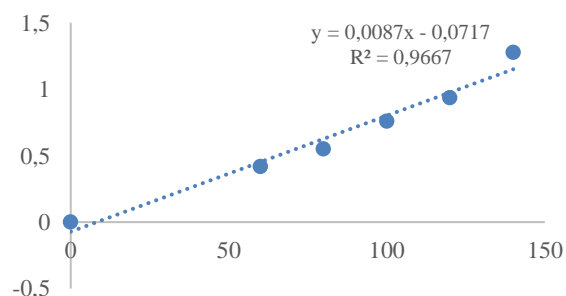


Figure 2. Phenol Linear Regression Equation Curve

Figure 2 shows that the linear equation on the Galat Acid Equivalent standard calibration curve is $y = 0.00087x - 0.0717$ with $R^2 = 0.9667$ with a phenol content of 2619.64

mg/100g. Phenol acts as an antioxidant related to its ability to capture DPPH, hydroxyl radicals, and superoxide radicals [33].

Tannin Compound Content

Tannin is one of the secondary metabolites of the polyphenol class that is widely found in plants. The Tannin Linear Regression Equation Curve is shown in Figure 3 below.

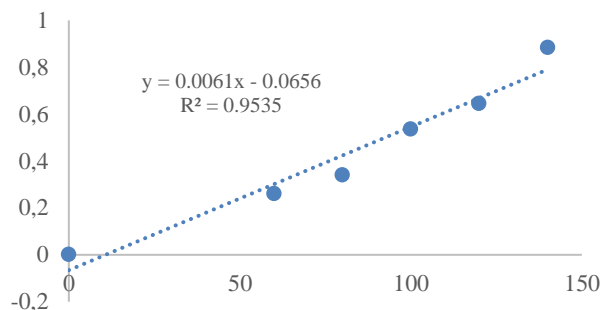


Figure 3. Tannin Linear Regression Equation Curve

In determining quantitative tests, tannin levels were obtained with the regression equation that can be seen in Figure 3, namely $y = 0.0061x - 0.0656$; $R^2 = 0.9535$. Based on the regression equation, the calculation of tannin content in *Acanthus ilicifolius* was carried out so that the tannin content was 3205.66 mg/100g. Tannin is also a polyphenolic compound rich in health benefits such as astringent, anti-diarrhea, antibacterial, and antioxidant [34].

Antioxidant Compounds

Compounds known as antioxidants can give one or more electrons to free radicals, thereby preventing the free radical process. The absorbance level and percentage of antioxidant activity in *Acanthus ilicifolius* leaf extract are shown in Table 2 below.

Table 2. Antioxidant Test Results

Concentration (mg/ml)	Absorbance	Antioxidant Activity (%)	IC 50 (ppm)
0.00	0.578	0.000	
0.02	0.542	6.228	
0.05	0.514	11.073	221.67
0.07	0.486	15.917	
0.10	0.445	23.010	
0.12	0.418	27.682	

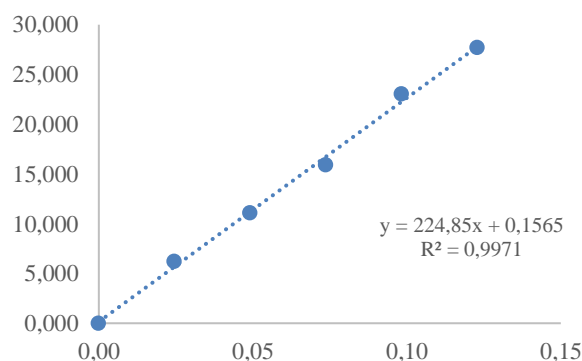


Figure 4. Antioxidant Linear Regression Curve

The greater the IC50 value, the weaker the antioxidant activity of the sample. In other words, the smaller the concentration needed, the better a compound inhibits free radicals. This study showed an IC50 value of 221.67 ppm (Table 2), so *Acanthus ilicifolius* leaf extract was categorized as having weak antioxidant activity [22]. Antioxidant levels were obtained with the regression equation that can be seen in Figure 4, namely $y = 224.85x + 0.1565$; $R^2 = 0.9971$.

Antioxidants help counteract free radicals. Free radicals can come from food, excessive sun exposure, air pollution, chemical compounds, and certain drugs [23]. Antioxidants are compounds that can counteract the harmful effects of free radicals, produced as a byproduct of oxidative metabolism or the body's metabolic and chemical reactions [24].

The heating process influences antioxidant activity in a sample. The leaves of *Acanthus ilicifolius* are dried using a food dehydrator involving a heat temperature of around 75°C. Damage due to heating can be controlled by temperature and duration of the heating process. Even though the temperature is correct, the thermal process can still damage the antioxidant compounds contained in the material [25]. Therefore, it is estimated that the heating

process affects the antioxidant activity of *Acanthus ilicifolius* leaves. Based on these results, the leaf extract of *Acanthus ilicifolius* has the potential to be developed as a drink containing antioxidants that can counteract free radicals in the body.

Average Inhibition of *Acanthus ilicifolius* Leaf Extract Against Bacterial Growth

Table 3 shows that *Acanthus ilicifolius* leaf extract can inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* sp. An extract concentration of 100% produced the highest inhibition. The concentration of *Acanthus ilicifolius* leaf extract at 100% concentration resulted in the highest average inhibition on bacteria of 9.67 ± 0.36 mm and 10.12 ± 1.12 , respectively, against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* sp. respectively of 6.82 ± 0.15 mm, 6.18 ± 0.02 mm and 6.19 ± 0.07 mm. *Acanthus ilicifolius* extract effectively inhibits the growth of *Vibrio harveyi* bacteria [9]. In addition, root, stem, and leaf extracts of *Acanthus ilicifolius* have antibacterial activity against *Klebsiella*, *Staphylococci*, *Escherichia coli*, *Enterobacter*, and *Pseudomonas* sp. [35].

Table 3. Average Inhibition of *Acanthus ilicifolius* Leaves Against Bacterial Growth

Extract concentration	Average inhibition of <i>S. aureus</i> (mm)	Average inhibition of <i>E. coli</i> (mm)	Average inhibition of <i>Salmonella</i> sp. (mm)
50%	$6.07 \pm 0.01bc$	$6.08 \pm 0.00bc$	$5.76 \pm 0.09b$
100%	$6.82 \pm 0.15d$	$6.18 \pm 0.02c$	$6.19 \pm 0.07c$
Control (+)	$13.14 \pm 0.24g$	$11.60 \pm 0.39e$	$12.15 \pm 0.77f$
Control (-)	$0.00 \pm 0.00a$	$0.00 \pm 0.00a$	$0.00 \pm 0.00a$

Description: The table showed the difference in letters in the treatment, which shows significantly different results ($p < 0.05$)

The inhibition of *Acanthus ilicifolius* leaves is caused by the content of metabolites such as flavonoids, phenols, and tannins. By rupturing cell membranes, inhibiting enzymes, attaching to adhesins, and destroying cell walls, flavonoids prevent the growth of bacteria. The beta ring and -OH group in flavonoids are believed to be the structural components that give them their antibacterial properties [26].

This compound is polar and acts as an antibacterial. The mechanism of action of phenolic compounds in killing bacterial cells is by denaturing bacterial cell proteins. Purwantiningsih et al. (2014) [27] that at high concentrations, the phenol content penetrates and disrupts the bacterial cell wall and precipitates protein in the bacterial cell. In lower concentrations, phenol inactivates essential enzyme systems in bacterial cells.

Tannins are polar polyphenolic compounds. Tannins act as antibacterial agents by inhibiting bacterial extracellular enzymes and taking over the substrates needed for bacterial growth [28]. Tannic acid inhibits the attachment of bacteria to the surfaces [29]. A lack of bacterial adhesion to the surface results in bacterial cell death. Moreover, the sugar and amino acid uptake is inhibited by tannic acid, which limits the growth of bacteria [30]. Tannins have several mechanisms of inhibiting growth: cell wall synthesis, disrupting cell membranes, and inhibiting fatty acid biosynthetic pathways. Furthermore, tannins suppress the expression of multiple virulence

factors' genes, including adhesins, biofilms, enzymes, motility, and toxins, and they also function as quorum-sensing inhibitors [31]. Due to the content of these secondary metabolites, *Acanthus ilicifolius* leaves can potentially be developed as a functional drink that can inhibit bacterial growth.

Conclusion

Acanthus ilicifolius leaf extract contains active compounds such as flavonoids, phenols, and tannins, and the IC50 value of *Acanthus ilicifolius* leaf extract is 221.67 ppm. *Acanthus ilicifolius* leaf extract effectively inhibits the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* sp. Therefore, due to the presence of these active compounds and antibacterial activity, *Acanthus ilicifolius* leaf extract has the potential to be an alternative functional food beverage.

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