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RESEARCH ARTICLE

Analysis of total flavonoid content and antibacterial activity of the ethanolic extract of Apu-apu herb (*Pistia stratiotes*) against *Salmonella typhimurium*

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Abstract

Background: There is a need to develop new anti-infective compounds from natural ingredients that have minimal side effects. *Pistia stratiotes* (apu-apu) is an example of a natural plant from the Araceae family, **Aim:** This study aims to determine the total flavonoid content of the ethanolic extract of apu-apu herb and its antibacterial activity against *Salmonella typhimurium*. **Methods:** Total flavonoid content was determined by using the AlCl_3 method and the antibacterial activity test was done using the disc diffusion method, divided into four test concentrations, namely 100, 200, 300, and 400 mg/mL. **Results:** The results showed that the total flavonoid content of the ethanolic extract of apu-apu herb was 0.353 ± 0.060 mg QE/g extract. The results of the antibacterial activity test showed that the inhibition zones at concentrations of 100, 200, 300, and 400 mg/mL were 8.2 ± 0.1 mm; 9.2 ± 0.15 mm; 9.9 ± 0.4 mm; and 11.2 ± 0.25 mm respectively. **Conclusion:** It can be concluded that the ethanolic extract of apu-apu herb contains a total flavonoid of 0.353 ± 0.060 mg QE/g and has an antibacterial activity against *Salmonella typhimurium*.

Introduction

Diarrhoea is one of the top ten high-prevalence infectious diseases. According to 2013 data from the World Health Organisation (WHO), diarrhoea is the number two killer disease in children after acute respiratory infection (ARI) (WHO, 2013). There are various causes of diarrhoea, such as bacterial, fungal, protozoal, etc., and *Salmonella typhimurium* is one of the most common bacterial causes of diarrhoea.

S. typhimurium produces enterotoxins that act on the small intestines, resulting in excess fluid secretion into the small intestine cavity, which in turn causes diarrhoea and vomiting. In addition, these bacteria also produce endotoxins that can attack the body's defence system causing fever, inflammation, and decreased iron (Arisman, 2009).

To control infectious diseases, drug development and the search for new sources of anti-infective compounds from natural ingredients with minimal side effects need to be carried out to support the health level of the community. In this article, a plant from the Araceae family of the *Pistia* genus, namely *Pistia stratiotes*, the Apu-apu plant, is being studied for its potential antibacterial activities. (Figure 1).

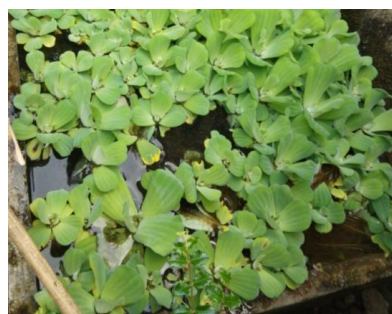


Figure 1: *Pistia stratiotes* (personal documentation, 2019)

Apu-apu contains some flavonoid compounds, alkaloids, steroids, and glycosides (Tripathi *et al.*, 2010). It is often used as a medicine for treating gout (Jha *et al.*, 2010), tuberculosis, dysentery, eczema, leprosy, ulcer, piles, syphilis, and parasitic worms (Kumar *et al.*, 2010), and fever (Kumar *et al.*, 2011). In previous studies, the effectiveness of the methanolic extract of apu-apu leaves was tested as an antibacterial at concentrations of 25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL against *Pseudomonas aeruginosa*, *Shigella sp*, *Serratia sp*, *Salmonella sp*, and *Klebsiella sp* bacteria. It was observed that the methanolic extract of the apu-apu leaf has an inhibitory effect on the growth of each bacterium (Abraham, 2014). To further gather more evidence on the apu-apu herb's antibacterial properties, it had to be tested on *S. typhimurium* bacteria and its total flavonoid content.

Methods

Materials

The materials used in this research were the apu-apu herbs dried into simplicial. The chemicals used to determine the total flavonoid content included 96% ethanol, quercetin, aluminium chloride (AlCl₃), and Potassium acetate (CH₃COOK). For the antibacterial testing, the chemicals used were sterile distilled water (Otsuka), physiological NaCl (Widatra), dimethyl sulfoxide (DMSO) (Emsure), and the sample bacteria was *S. typhimurium* with the Nutrient agar (Merck) as the pure culture. The bacterial medium used in the diffusion method (disc) was Mueller Hinton Agar (MHA) (Himedia). The positive control used in this experiment was chloramphenicol 30 µg.

Extract preparation

Apu-apu herb Simplicia powder was macerated for three days using 96% ethanol (1:10). The macerate was concentrated using a rotary evaporator to get a thick extract (Ministry of Health Republic of Indonesia, 2000).

Antibacterial activity test

The antibacterial activity test using the disc diffusion method is based on Balouri and the authors (Balouri *et al.*, 2016). Antibacterial tests of extracts and fractions were carried out on Muller Hinton agar (MHA) media, which had been poured into sterile Petri dishes and then solidified. A total of 1 mL of bacterial culture was added to the petri dish and spread evenly with a spreader. Each petri dish was divided into positive control (chloramphenicol 30 µg/ paper discs), negative

control (DMSO 10%) paper, and test discs saturated with 100 µL extract solution at different concentrations. paper discs were placed in each section and incubated at 37°C for 24 hours. It was repeated three times for this antibacterial activity test. A clear area around the disc indicated the absence of bacterial growth. Then, the diameter of the transparent zone was measured using a caliper.

Determination of total flavonoid content

The method used to determine the total flavonoid content was AlCl₃ (Woisky and Salatino, 1998). 0.5 mL of the sample solution was pipetted and reacted with 1.5 mL of ethanol, 1 mL of AlCl₃ reagent (10% v/v distilled water), 1 mL of CH₃COOK, and 2.8 mL of distilled water. It was allowed to stand for 25 minutes in a cuvette. Next, the absorbance of the solution was measured using a spectrophotometer UV-Vis at the wavelength of 433 nm. The value of total flavonoid content was expressed in mg QE (quercetin equivalent) in 1 gram of extract.

Results

Antibacterial activity test

The results of the antibacterial activity test (Table I) showed that the ethanolic extract of apu-apu herb demonstrated antibacterial activity against *S. typhimurium*, which was indicated by forming an inhibitory zone at all tested concentrations. The negative control dimethyl sulfoxide (DMSO 10%) showed no inhibition zone detected around the discs tested toward *S. typhimurium*. The chloramphenicol antibiotic disc as positive control showed the largest average diameter of the inhibition zone compared to the tested solution. The results of the antibacterial activity test of the ethanolic extract of apu-apu herb from the one-way ANOVA and LSD tests at each test concentration had significant differences ($p < 0.05$), as shown in Table I.

Determination of total flavonoid content

The total flavonoid content was determined at 50 minutes at a relatively stable incubation which was calculated by entering the absorbance value of the sample at a wavelength of 433 nm in the curve equation $y = 0.0594x + 0.3328$ ($r = 0.995$; $r^2 = 0.992$). Average extract content of 0.353 mg QE/g extract \pm 0.060 was obtained.

Table I: Antibacterial test result

Test Groups		Average \pm SD diameter of inhibition zone (mm)
Ethanollic extract	10mg/disc	8.20 \pm 0.1*
	20mg/ disc	9.20 \pm 0.15*
	30mg/ disc	9.90 \pm 0.4*
	40mg/disc	11.20 \pm 0.25*
	Negative (DMSO 10%)	0.00 \pm 0*
Control	Positive (Chloramphenicol 30 μ g/ disc)	11.90 \pm 0.15*

SD= Standard Deviation; *significant differences between groups ($p < 0.05$).

Discussion

The antibacterial activity test, carried out using the diffusion method, aims to determine the sensitivity of a microorganism to certain antibiotics, which is characterised by forming a clear zone around the disk (Balouri *et al.*, 2016). The antibacterial activity test on *S. typhimurium* bacteria was divided into the test group, the positive control group, and the negative control group. The positive control for antibacterial testing was chloramphenicol antibiotic disk 30 μ g, using four tested concentrations of 10, 20, 30, and 40 mg/disc.

The results of the antibacterial activity test of the ethanolic extract of the apu-apu herb from the one-way ANOVA and LSD tests at each test concentration had significant differences, as shown in Table I. The table shows that at a concentration of 40 mg/disc, the antibacterial inhibition was 11.2 \pm 0.25 mm, showing the most excellent inhibitory power than the other extract concentration. However, as a positive control, chloramphenicol still has the greatest inhibitory activity.

The results of this study indicated that the ethanolic extract of the apu-apu herbs had antibacterial activity against the test bacteria, which was suggested by the formation of an inhibition zone around the disc, as seen in Table I. From the research results, it can be inferred that the greater the test concentration, the greater the inhibitory power. In other words, the test concentration and antibacterial activity are directly proportional. This can be due to the high concentration, which eventually leads to a high level of active compounds in the test solution (Brook *et al.*, 2005).

Compared with the results of the previous study Abraham performed in 2014 using the methanolic extract of apu-apu leaves against *Salmonella sp.* at concentrations of 75 mg/mL and 100 mg/mL showed an inhibition zone of both 10mm and 13mm,

respectively. Meanwhile, the ethanolic extract of apu-apu at a 100mg/mL concentration showed an inhibition zone of 8.2mm. These results show that the ethanolic section of the apu-apu herbs has a lower inhibitory power than the methanolic extract of the Apu-apu leaves at the same concentration.

The results of the determination of the total flavonoid content of the ethanolic extracts obtained an average range of 0.353mg QE/g section \pm 0.060. Based on the results of this study, it is suspected that the flavonoid has antibacterial properties (Tripathi 2010) also shows that the apu-apu plant contains flavonoids, alkaloids, steroids, and glycosides.

The action of flavonoids as an antibacterial is to form complex compounds with extracellular and dissolved proteins so that they can damage bacterial cell membranes and are followed by the release of intracellular compounds (Cowan,1999). According to Cushnie and Lamb, flavonoids also play a role in inhibiting DNA-RNA synthesis by intercalation or hydrogen bonding with the accumulation of nucleic acids and in energy metabolism (Cushnie & Lamb, 2005). These compounds will interfere with energy metabolism and similarly inhibit the respiratory system because sufficient energy is required for the active absorption of various metabolites and the biosynthesis of macromolecules.

Thus, the extract can inhibit the growth of *S. typhimurium* at all tested concentrations, and it is suspected that flavonoids play a role in the antibacterial activity. Moreover, further research is recommended to separate specific bioactive compounds with antibacterial activity as a candidate for antibacterial herbal products.

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