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Phenolic Profile and Antimicrobe of the *Asplenium Nidus* L. from Mount Gumitir, Jember, East Java, Indonesia

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ABSTRACT
Mount Gumitir, Jember, East Java has a high number of wild flora for new medicinal resources. *Asplenium nidus* is a medicinal ferns that abundant in the area. This research aimed to measure the phenolic compound of the lamina and midrib sample of *A. nidus* from two different locations and the antimicrobial activity of the crude extract of fresh and dry sample. The phenolic compounds of flavonoid and phenol were detected using quercetin and Folin-Ciocalteu reagent respectively. The quantitative analysis of phenolic compounds was conducted based on absorbance detection using a spectrophotometer. The antimicrobial assay was applied with the agar diffusion method against the gram-positive bacterium *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli*. The result indicated that all samples of *A. nidus* contained flavonoid and phenol with a higher concentration in the lamina. The crude extract of *A. nidus* from Gumitir has higher phenolic compounds compared to the sample from Garahan. The crude extract presented bactericide activity against the two pathogenic bacteria. The higher antimicrobial activity was observed from the dry leaves sample. The phenolic compound of *A. nidus* might become a potential resource for the treatment of multidrug-resistant bacteria since their bactericide activities differ from traditional antibiotics.

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1. INTRODUCTION

Indonesian flora with a total of 2,500 to 7,500 plant species recorded has high potency as a medicinal resource (Cahyaningsih et al., 2021). The bioactive substances of plants that are often used in medicine are secondary metabolites, especially from phenolic and flavonoid groups (Velu et al., 2018). The bioactive can be used for the treatment of microbe resistance to drugs or Multi-Drug Resistance (MDR) 3 (Lim et al., 2021; Mukherjee et al., 2022).

Our previous study recorded the medicinal potencies of wild plants from the Mount Gumitir area (Setyati et al., 2020). Asplenium nidus L. was one of the abundant ferns surrounding the Jember district (Ulum and Setyati, 2015, Ulum and Setyati, 2017, Setyati, 2021, Ulum et al. 2023). This fern has been studied for its medicinal potency in other Asian countries (Jarial 2018, Tahir 2015). The major metabolite components of this species are phenol and flavonoids (Hammami et al. 2016) but different geographical origins might influence the metabolite content of this species (Sampaio et al., 2016). In this study, we aim to observe the medicinal potency of A. nidus from Mount Gumitir based on the antimicrobial activity of leaf extract against gram-positive and negative pathogen bacteria and phytochemical characterization, especially the phenolic compound. This study provides information on the phenolic content and the antimicrobial activities of the extract against pathogenic bacteria which will be beneficial for new medicine resources from local flora.

2. RESEARCH METHOD

Sample location and collection

The leaves samples of *Asplenium nidus* L. were collected from two areas in Mount Gumitir, Jember District, East Java, Indonesia. The first location was an agroforestry mix of coffee plantation (698-703 asl) with

the abiotic condition: humidity 76.6 % rh, temperature 25.7 °C, soil pH 6.8, wind speed 0.3, light intensity 3708.9 lux. The second location was a pine plantation in Garahan village (535-538 asl) with abiotic conditions: humidity 78.5 % rh, temperature 24.7 °C, soil pH 6.9, wind speed 0.7, light intensity 4576.6 lux.

Sample extraction

The leaves of *A. nidus* were sorted into stems and leaves. The samples were cleaned with running tap water, air-dried, separated the lamina and midrib, and then the fresh sample and the dry sample were crushed with a commercial blender. The crude extract was prepared from 1 gram of *A. nidus* crude and powder diluted with 90 ml methanol dan macerated for 3 days with a short stirring every 24 hours. Whatman filter paper No. 1 was used for the extraction of the extract and the filtrate was concentrated using a rotary evaporator for 20 minutes. The concentrated crude extract obtained was used for phenol and flavonoid detection and antimicrobial tests.

Metabolites Analysis

Metabolite detection:

Flavonoid

The presence of flavonoid was detected following methods by Ratnasai et al. (2022). 1 mL crude extract was diluted into 1 mL ethanol 70%, then 0,1 g Mg was added followed by 10 drops of HCl. After shaking the mixture, the colour changes were observed. The positive flavonoid presence was indicated by the colour red, yellow, or orange.

Phenols

The presence of phenols was detected following methods by Ratnasari et al. (2022). A total of three drops of 3 FeCl3 1% were applied to the 1 mL crude extract. The positive content of phenol was indicated by the colour change in the extract with the colour of green, red, purple, blue or dark.

Phenol and flavonoid quantification:

Flavonoid

Quantitative analysis of flavonoid content followed the method by Setyati et al. (2020). Quercetin was used as standard. A total of 10 mg of crude extract dissolved in 10 mL of methanol 95 % (1000 ppm). 0.5 mL of the mixture was added with 1.5 mL of 95 % methanol, 0.1 mL of 10% AlCl3, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. Then incubated for 30 min at room temperature. Absorbance was determined using UV-Vis spectrophotometry at 435 nm. Measurement was triplicate and the average absorbance value was calculate for the flavonoid content.

Phenol

Quantitative analysis of phenol content followed the method by Setyati et al. (2020). A total of 10 mg crude extract was mixed with 10 ml (1000 ppm). 0,1 mL mixture was added with 1,2 mL aquadest and 0,1 mL Folin-Ciocalteu reagent, then incubated for 5 minutes. Afterward, 0,3 mL Na2CO3 20% and 0,3 mL aquadest were added to the mixture and incubated again for 30 minutes at room temperature. Absorbance was determined using UV-Vis spectrophotometry at 743 nm. Measurement was triplicate and the average absorbance value was calculated for the flavonoid content.

Antimicrobial assay

The antibacterial activity of the crude extract was performed with agar well diffusion methods (Balouiri et al., 2016) against a gram-positive bacterium *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli*. Before the antimicrobial test, all bacteria were subculture and incubated until they were grown into the mid-logarithmic phase (Kim et al., 2017). For the positive control we used chloramphenicol 1% and the negative control was methanol absolute. The crude extract was assayed in a series of concentrations, i.e., 10 %, 20 %, 30 % 40 %, and 50 %.

Each bacteria culture was dissolved in 1 mL of nutrient agar (NA) media and poured into petri dishes then left to condense. A total of four wells were made with a cork borer (5 mm diameter). Each diffusion well was filled with 20 µL of crude extract and the control liquid. The assay was four plicates. The observation was conducted by measuring the diameter of the inhibition zone formed around the well.

3. RESULT AND DISCUSSION

Phytochemical analysis

The phytochemical analysis of *Asplenium nidus* from Garahan and Gumitir revealed that the methanolic extract of the lamina and midrib sample contained phenol and flavonoids (Table 1). The yield extract of flavonoid was in the range of 1.2 % to 5.83 %, while the phenol was in the range of 6.33 % to 17.96 %. The lamina of *A. nidus* had a higher yield of flavonoid and phenol (c.a. 3-fold higher) compared to the midrib. Furthermore, the sample originating from Gumitir yielded a higher flavonoid and phenol compared to the sample from Garahan.

Table 1. Flavonoid and phenol content of the *A. nidus* from Gumitir

Organ	Origin	Flavonoid (%)	Phenol (%)
Lamina	Gumitir	5.83 ± 0.06	17.96 ± 0.02
	Garahan	3.35 ± 0.00	15.07 ± 0.02
Midrib	Gumitir	1.51 ± 0.04	6.47 ± 0.01
	Garahan	1.20 ± 0.01	6.33 ± 0.03

The ethnomedicine of *A. nidus* had been reported with the utilization of the frond organ to treat soreness, asthma, and halitosis (Jarial 2018, Tahir 2015). Here we reported that among the frond structure of *A. nidus*, a higher yield for phenolic compound was at the lamina part rather than the midrib. Phenol and flavonoids are two of the main groups of seven phenolic compounds. Those metabolites had biological activities such as antioxidant, anticancer, antimicrobe, and anti-inflammatory (Zhang et al., 2022). The organ part and sample origin influenced the content of total phenol had been reported in a study of Ferns *Blechnum orientale* (Jasim et al, 2015). An environmental condition such as temperature, solar radiation, humidity, and soil nutrient influences the growth, metabolites and development of plants (Sampaio et al, 2016). The alternation of phenolic compound might related to the plant's adaptation to the stress. Under environmental stress, epiphytic ferns alter their photosynthesis performance (Ulum et al, 2023). The higher solar radiation increases the reactive oxygen species (Ulum et al. 2021), therefor plant produces phenolic compounds as antioxidant properties to protect the leaves against the deleterious effect of UV radiation (Klatt et al., 2018).

Antimicrobial activity

The methanolic extract *A. nidus* presented an antimicrobial activity against *E. coli* and *S. typhi* (Figure 1). The fresh and dry leaf samples with the lowest concentration of 50 % positively inhibited the two pathogenic bacteria. The higher antibacterial activity was observed from dry leaves crude extract (Table 2). The highest diameter of the inhibition zone was observed from dry leaf extract with a concentration of 100 % against *E. coli* (4.6 cm) and S. typhi (3.1 cm) (Table 2).

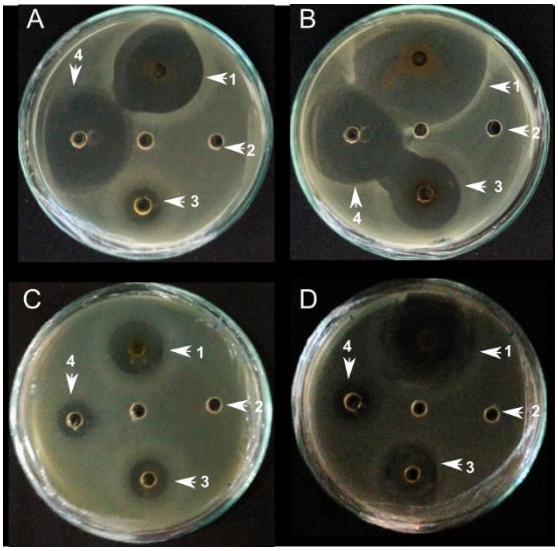

Figure 1. Antimicrobial activity of methanolic extract from fresh and dry leaves of *A. nidus* with agar diffuse method. The character represents the microbes: A – B: *E. coli*; C – D: *S. typhi*; The number represent the sample, 1: *A. nidus* (concentration of 100 %); 2: Negative control (methanol 98%); 3: *A. nidus* (concentration of 50 %); 4: Control positive (Antibiotic 50 mg/ ml)

The global rise of infection by multidrug-resistant (MDR) bacteria required the discovery of new antimicrobial resources (Uddin et al., 2021). The plant produces a high concentration of phenolic compound as a defence mechanism against microbial attack and can be used to treat human bacterial pathogens (Živković et al., 2020). Several studies reported the antimicrobial potencies of ferns (e.g. Živković et al., 2020, Jarial 2018). Nevertheless, our experiment on the methanolic extract of *A. nidus* presented relatively equal activities of antibacterial against gram-positive and gram-negative bacteria which are potentially useful for natural medicine for treating multidrug-resistance bacteria. The antibacterial activities of the *A. nidus* crude extract might related to their phenolic content. The inhibitory activity of the phenolic compounds through bacterial pathogens can be in various modes of action. The review of Takó et al. (2020) reported the mechanism i.e. inhibition of biofilm

formation, altering the membrane structure, destroying the synthesis of nucleic acid, cell wall, cytoplasmic membrane, enzyme and energy production. Thus, mechanism is different from the activity of traditional antibiotics which might potentially treat MDR.

Table 2. Inhibition zone of methanolic extract from fresh and dry leaves of *A. nidus*

Leaves Sample	Concentration (%)	*Diameter Of Inhibition Zone (Cm)*	
		E. coli	*S. typhi*
fresh	100	2.86	2.26
	50	1.28	1.35
dry	100	4.65	3.47
	50	2.42	1.88

4. CONCLUSION

The crude extract of *Asplenium nidus* from Gumitir and Garahan contained flavonoid and phenol with a higher concentration in the lamina rather than midrib. The sample of *A. nidus* from Gumitir presented a higher falvonoid and phenol compared to the sample from Garahan. The crude extract indicated antibacterial activity against gram *E. coli* and *S. typhi*. The higher antimicrobial activity was observed from the dry leaves sample compared to the fresh leaves. The phenolic compound of *A. nidus* might had bactericide activities that differ from traditional antibiotics which beneficial potency for multidrug-resistant bacteria therapeutics.

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