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Short Communication: Evaluation of antibacterial activities and phytochemical composition of ethanolic extract of *Diplazium esculentum*

FESKAHARNY ALAMSJAH[1], SASQIA FANDINI[1], MILDAWATI MILDAWATI[2,♥]

[1]Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Science, Universitas Andalas. Jl. Unand, Limau Manis, Padang 25167, West Sumatra, Indonesia

[2]Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Science, Universitas Andalas. Jl. Unand, Limau Manis, Padang 25167, West Sumatra, Indonesia. Tel.: +62-751-777427, Fax.: +62-751-71343, ♥email: mildawati@sci.unand.ac.id

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Abstract. *Alamsjah F, Fandini S, Mildawati M. 2024. Short Communication: Evaluation of antibacterial activities and phytochemical composition of ethanolic extract of* Diplazium esculentum*. Biodiversitas 25: 937-941.* Antibacterial agents are crucial in fighting infections by stopping or inhibiting pathogenic bacteria growth. *Diplazium esculentum* (Retz.) Sw., is a natural resource with great potential in traditional medicine. This study aimed to investigate the antibacterial effectiveness of ethanol extract of *D. esculentum* against two common bacteria, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. This research was done using disc diffusion technique with comprehensive phytochemical screening. The results revealed antibacterial properties of the ethanolic extract of *D. esculentum* against *P. aeruginosa* and *S. epidermidis*. The most potent antibacterial activity was observed at 80 and 60% concentrations against *P. aeruginosa*, resulting in inhibition zones with 5.90 mm and 5.47 mm diameters. A similar pattern was also observed against *S. epidermidis*, where 80% concentration showed significant efficacy, with an inhibition zone of 5.69 mm diameter. Phytochemical analysis result revealed the presence of various secondary metabolites, including alkaloids, steroids, polyphenols, tannins, flavonoids, and saponins. These phytochemical compounds contribute antibacterial properties and have promising potential for further research to develop natural antibacterial agents.

Keywords: *Diplazium esculentum*, ethanol, inhibition zone, phytochemicals, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*

INTRODUCTION

Antibacterials are medications designed to combat harmful bacteria by eliminating and inhibiting their proliferation. These agents are highly effective treatments for many infections (Theuretzbacher et al. 2020). The sources of antibacterial compounds encompass a diverse spectrum, including microorganisms, animals, and plants (Silva et al. 2020). Notably, there is an increasing emphasis on exploring plant-derived antibacterial compounds, which offer promising therapeutic alternatives (Santos et al. 2016). It's worth mentioning that these plant-derived compounds have a long-standing history of application in traditional medicine (Fridlender et al. 2015). Their utilization of traditional healing practices attests to the rich repository of plants' antibacterial chemicals.

Diplazium esculentum (Retz.) Sw., a natural substance deeply rooted in traditional medicine, has emerged as a vital resource for the Himalayan community. People of this region have used the versatile properties of *D. esculentum* for the prevention or treatment of several diseases such as diabetes, smallpox, asthma, diarrhea, rheumatism, dysentery, headache, fever, wounds, pain, measles, hypertension, constipation, oligospermia, bone fracture, and glandular swellings. Various extracts of *D. esculentum* were evaluated to elucidate their phytochemical and pharmacological activities. It has a wide array of pharmacological properties, such as antioxidant, antimicrobial, antidiabetic, immunomodulatory, CNS stimulant, and anti-anaphylactic activities (Semwal et al. 2021). The Kuala Kapuas community uses a decoction of *D. esculentum* to ease pain. Moreover, they used plant extract for the treatment of body swellings (Saputri and Putri 2017; Awang-Kanak et al. 2021; Rankoana 2022). Moreover, the ethanol extract of *D. esculentum* has demonstrated potential in treating infectious wounds, further highlighting its role as an alternative therapy. It has exhibited efficacy in promoting the healing of infected wounds on rat skin (Priya et al. 2022). These remarkable properties of *D. esculentum* can be attributed to several bioactive compounds, including flavonoids, phenols, steroids, flavones, and triterpenoids (Watanabe et al. 2021). Some bioactive compounds have shown promise in treating various infectious diseases in living organisms. However, it is essential to emphasize that further scientific validation is needed to ensure the safety and efficacy of these traditional uses (Zaini et al. 2016)

Infections are common daily, often stemming from microorganisms such as bacteria, parasites, viruses, and fungi. Notably, some bacteria frequently implicated in human infections include *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (Sarmah et al. 2018). The ethanol extract of *Diplazium esculentum* has a rich reservoir of active compounds. These compounds exhibit

considerable potential as antibacterial agents for inhibiting the growth of *P. aeruginosa* and *S. epidermidis*, providing valuable insights into their therapeutic potential. As such, there is a compelling need for further, in-depth investigation in this area. Therefore, the objective of the present study was to evaluate the antibacterial activity of ethanolic extract of *D. esculentum*, against *P. aeruginosa* and *S. epidermidis.*

MATERIALS AND METHODS

Sample preparation

The fresh green healthy leaves of *D. esculentum* were obtained from the environment around Universitas Andalas Reservoir, Limau Manis, Pauh, Padang City, West Sumatra, Indonesia. Plant samples were put in plastic and then taken to the laboratory.

Fresh collected samples were washed with clean water. Then wiped with tissue and dried without direct sunlight. After drying, the sample was pulverized using a grinder (Sulaiman et al. 2017). Furthermore, the extraction process was carried out using a modified method by Sulaiman et al. (2017). The dried powder of *D. esculentum* weighed as much as 500 g was put into a maceration container, then extracted by the maceration method using 96% ethanol solution. Then, the maceration container was allowed to stand for 3x24 hours at room temperature while stirring repeatedly so that the active substance was perfectly extracted. After that, extract was filtered using filter paper. Then, the obtained extract was concentrated with a rotary evaporator to separate the solvent from the active substance.

Distillation of ethanolic extract concentrations

The ethanolic extract was initially obtained and subsequently prepared at various concentrations, namely 20, 40, 60, and 80%. For 80% concentration, 1.6 mL of 100% concentration extract was taken and mixed with 0.4 ml of 10% DMSO as a diluent, followed by homogenization. Similarly, for 60 and 40% concentration, 1.2 and 0.8 mL of 100% concentration extract was combined with 0.8 and 1.2 mL of 10% DMSO diluent, respectively. Last, for 20% concentration, 0.4 mL of 100% concentration extract was mixed with 1.6 mL of 10% DMSO diluent, and the mixture was homogenized.

Inhibition testing using the disc diffusion method

The two test bacteria, namely *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* were obtained from the Sumatran biota laboratory, Universitas Andalas. The culture was taken with a sterile ose needle and then suspended in a tube containing sterile 0.9% physiological NaCl solution until the turbidity was the same by the Mc. Farland 0.5 standard turbidity (Mpila et al. 2012). Paper discs were dipped in each ethanolic extract concentration of *D. esculentum*. Tetracycline was used as a positive control by weighing 3 mg dissolved in 10 mL of sterile distilled water; Dimethyl Sulfoxide (DMSO) was used as a negative control. After dipped, the paper discs were placed on the surface of the Nutrient Agar (NA) medium that had been applied with bacterial inoculum using sterile tweezers and incubated at 37°C for 24 hours until the inhibition zone formed. The observation and measurement of the inhibition zone diameter formed around the disc after 24 hours using a caliper.

Phytochemical test of ethanol extract of *D. esculentum*

Various phytochemical tests of ethanolic extract of *D. esculentum* were carried out as follows (Harborne 1998):

Alkaloids

One mL of ethanolic extract solution of *D. esculentum* was added to 1.5 mL of 2N HCl, then heated for 5 minutes and filtered. Furthermore, 5 drops of Dragendorff reagent were added to the filter. The orange precipitate formed by the Dragendorff reagent indicates the presence of alkaloids.

Triterpenoid/Steroid

A solution of 1 mL of ethanol extract of *D. esculentum* was added with 5 drops of anhydrous acetic acid and then shaken until mixed. Furthermore, two drops of H2SO4 2N were added and then shaken. The formation of a blue-green precipitate indicates the presence of steroids, and an orange-brown or red solution indicates the presence of triterpenoids.

Polyphenols/Tannins

One mL of ethanolic extract of *D. esculentum* was added with 2 drops of 1% $FeCl_3$ reagent. The formation of green, purple, dark blue, or greenish black indicates the presence of phenolic compounds.

Flavonoids

One mL of ethanolic extract of *D. esculentum* was added with 0.1 g Mg powder and 2 mL of 2N HCl. The formation of yellow, orange, red, or blue indicates the presence of flavonoid compounds.

Saponins

One mL of ethanolic extract of *D. esculentum* was added to 2 mL of hot water, shaken vigorously, and given one drop of 2N HCl. The formation of permanent foam characterized the presence of saponin.

RESULTS AND DISCUSSION

Antibacterial activity test of ethanolic extract of *D. esculentum* by disc diffusion method

The results of antibacterial activity test against bacteria *P. aeruginosa* and *S. epidermidis* are shown in Tables 1 and 2. The results unambiguously showed that ethanolic extract inhibited the growth of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* growth. The inhibition was characterized by the formation of discrete inhibition zones. This showed that ethanolic extract of *D. esculentum* had strong antibacterial activity (Table 1, Table 2 and Figure 1).

The average inhibition zone against *P. aeruginosa* was 5.90 mm at 80% and 5.47 mm at 60% concentration. The average inhibition zone at 80% concentration for *S. epidermidis* was 5.69 mm. The inhibition zone was clear, colorful region generated around the paper disc indicating that extract had suppressed the growth of bacteria. This occurrence directly interprets the extract's inhibitory effect on bacterial growth (Figure 1).

The antibacterial activity test results showed that ethanolic extract obtained from *D. esculentum* was found effective against *P. aeruginosa* and *S. epidermidis*. At 40% and 20% concentrations, the ethanolic extract exhibited a zone of inhibition of 4.26 and 3.84 mm against *P. aeruginosa*, respectively: at 60% concentration, ethanolic extract showed a substantial antibacterial effect against *S. epidermidis*, resulting in a 4.44 mm inhibition zone. At 40 and 20% concentrations, the inhibition zones measured were 2.45 and 2.09 mm, respectively.

The ethanolic extract of *D. esculentum* inhibited *P. aeruginosa* and *S. epidermidis* with distinct categorizations based on the inhibition zones. (i) At 80% ethanolic extract concentration displayed a moderate inhibitory effect against *P. aeruginosa* and *S. epidermidis*. (ii) The 60% ethanolic extract concentration showed moderate inhibition against *P. aeruginosa*, but weak against *S. epidermidis*. (iii) The 40 and 20% concentrations were categorized as weak regarding their inhibition effect on bacterial growth.

Phytochemical test of ethanol extract of *D. esculentum*

The phytochemical analysis of the ethanol extract from *D. esculentum* has been conducted shown in Table 3.

Table 3. Phytochemical test results of ethanolic extract of *D. esculentum*

Component	Phytochemical tests
Alkaloids	+
Triterpenoids	-
Steroid	+
Tannins	+
Polyphenols	+
Flavonoids	+
Saponins	+

Note: +: Presence of compound groups, -: Absence of compound groups

Table 1. Inhibition zone diameter of ethanolic extract of *D. esculentum* against *P. aeruginosa*

Concentrations	Inhibition zone diameter repetitions (mm)			Sum	Mean± St.dev
	1	2	3		
80%	5.26	7.64	4.81	17.71	5.90±1.52
60%	4.28	7.4	4.72	16.4	5.47±1.68
40%	3.95	5.29	3.55	12.79	4.26±0.91
20%	3.3	4.92	3.31	11.53	3.84±0.93
K(-) DMSO	0	0	0	0	0
K(+) (Tetracycline)	17.53	25.26	17.1	59.89	19.96±4.59

Table 2. Inhibition zone diameter of ethanolic extract of *D. esculentum* against *S. epidermidis*

Concentrations	Inhibition zone diameter repetitions (mm)			Sum	Mean± St.dev
	1	2	3		
80%	4.87	4.75	7.46	17.08	5.69±1.53
60%	4.25	4.15	4.92	13.32	4.44±0.41
40%	2.36	2.4	2.59	7.35	2.45±0.13
20%	1.87	2.04	2.36	6.27	2.09±0.25
K(-) DMSO	0	0	0	0	0
K(+) (Tetracycline)	18.1	17.61	17.83	53.54	17.84±0.24

A **B**

Figure 1. Antibacterial activity of ethanolic extract of *D. esculentum* against *P. aeruginosa* (A) and *S. epidermidis* (B). a. control (+) tetracycline; b. control (-) DMSO; c. 20% ethanol extract; d. 40% ethanol extract e. 60% ethanol extract; f. 80% ethanol extract

Discussions

The antibacterial activity of an ethanolic extract obtained from *D. esculentum* varied against *P. aeruginosa* and *S. epidermidis*, indicating moderate and weak inhibitory categories. This is consistent with the findings of Oroh et al. (2015), who investigated the methanol extract of *Diplazium dilatatum* in a similar setting. The methanol extract of *Diplazium dilatatum* inhibited *Staphylococcus aureus* and *Escherichia coli* at 30 and 60% concentrations. However, at 90% concentration, the methanol extract of *Diplazium dilatatum* exhibited significant antibacterial activity, resulting an average inhibition zone diameter of 6.70 mm against *Staphylococcus aureus* and 8.00 mm against *E. coli*.

These findings show the heterogeneity of antibacterial activity of plant extracts. The secondary metabolite chemicals found in ethanolic extract of *D. esculentum* fern were alkaloids, steroids, polyphenols, tannins, flavonoids, and saponins. The ability of ethanolic extract of *D. esculentum* to inhibit *P. aeruginosa* and *S. epidermidis* may be due to the presence of these active chemicals. The impact of these active compounds is pivotal in their role as antibacterial agents. The antibacterial mechanism used to prevent bacterial growth vary depending on the numerous active chemicals in the extract. Secondary metabolite compounds can inhibit bacterial growth by damaging the cell wall, changing the permeability of the cytoplasmic membrane, disrupting protein synthesis, and destroying the metabolic system in the cell by inhibiting intracellular enzyme activity (Igbinosa et al. 2020). According to Hayat et al. (2020), phytochemical screening of *D. esculentum* water extract revealed the presence of flavonoids, polyphenols, alkaloids, terpenoids, tannins, and saponins.

Alkaloid works as antibacterial agents by disturbing the structural integrity of bacterial cells, specifically the peptidoglycan component, which is a critical component of the bacterial cell wall. This disruption results in inadequate development of the cell wall layer, which leads to cellular malfunction and, eventually, cell death (Yan et al. 2021). Steroid, on the other hand, have antibacterial effects via a different mechanism. They are known to cause liposome leakage by damaging the lipid membrane of bacterial cells. Furthermore, because of their permeability to lipophilic substances, steroids can interact with phospholipid membranes, resulting in diminished membrane integrity and a deformed cell membrane shape. These effects ultimately lead to cell lysis and increased cell fragility (Sudarmi et al. 2017).

Tannins have a diverse action mode as antibacterial agents, involving numerous ways to inhibit bacterial growth. They have antibacterial activity via inactivating microbial cell adhesins, stimulating enzymes, and interfering with protein transport within the inner layer of the cell. Furthermore, tannins affect the target sites of the polypeptides that make up the bacterial cell wall, resulting in the defective synthesis of this key structural component (Lim et al. 2013). As a result, bacterial cells undergo lysis due to osmotic and physical pressure, culminating in cell death (Valon and Levayer 2019).

Polyphenols carry out their mechanism by acting as toxins within the bacterial protoplasm. They achieve this action by destroying and penetrating the bacterial cell wall, inducing protein precipitation within the bacterial cell. Surprisingly, phenolic substances with large molecular sizes can activate critical enzymes within bacterial cells even in very low concentrations. The consequences of polyphenol activity on bacterial cells include damaging cell integrity, leading to protein denaturation, enzyme activation, and the subsequent leakage of cellular contents, as Shamsudin et al. (2022) elucidated.

Flavonoids enact their mechanism through several critical pathways. They form complex compounds with extracellular and soluble proteins, damaging the bacterial cell membrane and the subsequent release of intracellular compounds (Donadio et al. 2021). Furthermore, flavonoids play a pivotal role in hindering DNA and RNA synthesis by intercalating or forming hydrogen bonds with nucleic acid base stacking. Saponins initiate their mechanism through protein denaturation. Their surface-active properties, similar to detergents, enable them to reduce the surface tension of the bacterial cell wall, thereby compromising the permeability of the bacterial membrane. This disruption of the cell membrane interferes with the bacteria's survival. Subsequently, saponins diffuse through the cytoplasmic membrane, destabilizing the membrane and causing cytoplasm leakage from the cell (Gilabert-Oriol 2013).

The antibacterial activity of ethanolic extract of *Diplazium esculentum* (Retz.) Sw against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* bacteria was found best at 80% concentration, with the moderate category. The ethanolic extract effectively suppressed the growth of *P. aeruginosa* and *S. epidermidis*. Furthermore, secondary metabolite compound analysis revealed the presence of numerous antibiotic-active components, such as alkaloids, steroids, polyphenols, tannins, flavonoids, and saponins. These findings shed more light on *Diplazium esculentum's* potential utility in developing natural antibacterial medicines.

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