ANTIMICROBIAL ACTIVITY OF *Zebrina pendula* Schnizl. EXTRACT AGAINST *Staphylococcus aureus, Candida albicans* AND *Bacillus subtilis*

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ABSTRACT

Zebrina pendula Schnizl., known for its medicinal properties against dysentery, contains chemical compounds like polyphenols, flavonoids, quinones, and terpenoids. Despite its potential, research on *the Zebrina pendula* schnizl. remains limited. This study aimed to assess the antimicrobial activity of *Zebrina pendula*. extracts against *Staphylococcus aureus*, *Candida albicans*, and *Bacillus subtilis* using the microdilution method. The stages of the study include determination, phytochemical screening of *Zebrina pendula*, standardization, multilevel extraction using n-hexane, ethyl acetate, and 96% ethanol, and antimicrobial activity testing via the microdilution method to determine minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC). The MIC activity for *Staphylococcus aureus*, ethanol extract, and water fraction exhibited an MIC value of 10,000 µg/mL, whereas the n-hexane fraction presented a value of 5000 µg/mL, and the ethyl acetate fraction had an MIC of 2,500 µg/mL. *Candida albicans* tests yielded identical MIC values of 10,000 µg/mL for each solvent. When examining *Bacillus subtilis*, the ethanol extract showcases an MIC value of 39.1 µg/mL, the n-hexane fraction registers 39.1 µg/mL, the ethyl acetate fraction reports 2,500 µg/mL, and the water fraction indicates an MIC of 10000 µg/mL. The MBC activity against *Staphylococcus aureus*, with the ethanol extract, n-hexane fraction, and water fraction all sharing an MBC value of 10000 µg/mL, while the ethyl acetate fraction records an MBC of 2500 µg/mL. The MBC for *Candida albicans* was 10,000 µg/mL for each solvent. For *Bacillus subtilis*, the MBC value for the ethanol extract is 78.1 µg/mL, n-hexane fraction reports 312.5 µg/mL, and ethyl acetate fraction displays and MBC of 2,500 µg/mL, respectively.

Keywords: *Zebrina pendula* Schnizl. microdilution, *Staphylococcus aureus, Candida albicans, Bacillus subtilis*.

INTRODUCTION

Indonesia has significant potential for the development of herbal medicines, boasting over 30,000 plant species. However, approximately 9,700 of these species have been recognized for their medicinal properties.

The emergence of the COVID-19 pandemic, which confines individuals to their homes, has prompted a shift in people's interests, leading to the exploration of new hobbies. One such pursuit is gardening, encompassing a diverse array of plants, particularly ornamental species that are abundant in Indonesia (Asnahwati, 2021).

As horticultural enthusiasts continue to swell, the cultivation of ornamental plants is on the rise. Their allure lies in their vibrant array of colors, suitability for indoor placement, and maintenance simplicity. Beyond their ornamental value, many of these plants also confer health benefits, serving in the treatment of conditions such as bloody cough, chronic dysentery, boils, vaginal discharge, and gonorrhea (Hariana, 2006).

Within specific plant components, such as bark, roots, and leaves, healing and pain-reducing properties define the concept of medicinal plants. Conversely, ornamental plants are predominantly cultivated for their aesthetic value and decorative appeal (Kartika 2018). However, it is worth noting that beyond their decorative role, ornamental plants can also possess medicinal attributes, and they can often be found in the vicinity of residences (Sumiyati, 2021).

Zebrina pendula Schnizl. is a noteworthy example of an ornamental medicinal plant. It contains various chemical compounds including polyphenols, flavonoids, quinones, and terpenoids. Polyphenols are compounds with multiple phenol groups that contribute to the prevention of degenerative ailments and cardiovascular disorders. These polyphenolic compounds can be classified into two distinct categories: flavonoids (flavones, flavanols, flavanones, isoflavones, anthocyanidins, and chalcones) and tannins (phenolic acid polymers, catechins, and isocatechins) (Proklamasiningsih et al., 2019).

Earlier investigations of the antimicrobial efficacy of *Zebrina pendula* Schnizl. employed natural drying techniques and maceration extractions. This research encompassed the evaluation of the impact on bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Shigella dysenteriae*. The diffusion method was employed to assess antimicrobial properties, yielding findings indicating that the extract was derived from *Zebrina pendula* Schnizl. could impede microbial proliferation, albeit without ascertaining the specific minimum inhibitory concentration or minimum bactericidal concentration (Puspadewi et al., 2016).

RESEARCH METHODS

Materials and Equipments

This study necessitated several materials, including *Zebrina pendula* Schnizl. leaves, distilled water, 96% ethanol (PA), n-hexane (PA), ethyl acetate (PA), toluene, DMSO (Smart-Lab®), Nutrient Agar (NA) medium (Oxoid®), Mueller Hilton Agar (MHA) medium (Oxoid®), Potato Dextrose Agar (PDA) medium (Merck®), Mueller Hilton Broth (MHB) medium (Himedia®), Potato Dextrose Broth (PDB) medium (Himedia®), and various reagents for phytochemical screening (including ammonia, chloroform, 2N hydrochloric acid, Mayer reagent, Dragendorf reagent, $FeCl_3$ solution, 1% gelatin solution, Magnesium powder, amyl alcohol, 5% KOH solution, dilute hydrochloric acid, ether, 10% vanillin solution, sulfuric acid, Lieberman Bouchard. Additional materials encompassed $BaCl_2$, H_2SO_4, 0.9% NaCl solution (Otsuka®), tetracycline, chloramphenicol (Colsancetin®), and ketoconazole (Solinfec®). The microbial strains used were *Staphylococcus aureus* ITBCCB90, *Candida albicans* ITBCCR66, and *Bacillus subtilis* ITBCCB111.

Research Procedure

1. Sampling

Zebrina pendula Schnizl. utilized in this study was sourced from the Begonia House at Kebun Haji Dayat, situated in Jalan Lembang Asri. The leaves used in this study were approximately four months old. Authentication was performed by the Resources Division of the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran (UNPAD), Sumedang Regency, West Java, Indonesia.

2. Preparation of raw material (leaves powder)

In this study, only *Zebrina pendula* Schnizl. leaves were selected. The leaves were weighed and cleaned to remove impurities. Weighed and cleaned *Zebrina pendula* Schnizl. leaves that were subsequently dried in a drying cabinet. After complete drying, *Zebrina pendula* Schnizl. leaves were pulverized using a blender, after which their weights were measured again.

3. Extraction of *Zebrina pendula*

A maceration method was employed for the extraction process. First, 1 kg of *Zebrina pendula* Schnizl. simplicia was weighed and placed into a macerator containing 5 L of 96 % ethanol solvent (sufficient to submerge the material), with the volume being accurately measured. The mixture was then allowed to stand for 24 hours with intermittent stirring. This extraction cycle was repeated at least twice until the color of the solvent had faded or reached near clarity. The resulting macerate was concentrated using a rotary evaporator to yield a dense extract (Indarto et al., 2019).

4. Fractionation of *Zebrina pendula*

A thick extract of *Zebrina pendula* Schnizl. was weighed to approximately 10 g and dissolved in 300 mL water. Subsequently, the sample was transferred to a separating funnel, to which 300 mL of n-hexane was added. After thorough shaking to induce separation into distinct layers, fractions were partitioned into separate containers. The aqueous layer was then subjected to the same process using 300 mL ethyl acetate solvent. The multistep separation procedure was repeated until the color of the solvent faded or became transparent. The obtained fractions were concentrated using a rotary evaporator (Akhsanita, 2012).

5. Characterization of *Zebrina pendula*

Several examinations were conducted using Z. *pendula* Schnizl. simplicia, including an organoleptic assessment involving observation of shape, color, and odor. Further examination encompassed characteristics such as water-soluble extract content, ethanol-soluble extract content, water content, total ash content, and loss on drying Additionally, phytochemical screening involved examining alkaloids, flavonoids, saponins, polyphenols, tannins, monoterpenoids, and sesquiterpenoids (Kesehatan, 2023).

6. Activity testing of *Zebrina pendula* Schnizl. Extract

Antimicrobial activity of Z. pendula ethanol extract leaves was performed using the microdilution technique. This methodology utilized a 96-well microplate organized into 12 columns and 8 rows, totaling 96 wells, as shown in Figure 1. The first column served as a negative control, housing only 100 µL of the test extract solution pre-dissolved in Mueller Hinton Broth (MHB) growth medium. Columns 2-12 contained a mixture of 50 µL of the test solution and 50 µL of the microbial suspension. Column 12 functioned as the positive control, harboring growth medium and microbial suspension, while columns 2-11 consisted of growth medium, test microbes, and test samples. Column 1 contained 50 µL of test sample, which was thoroughly mixed. Successive transfers of 50 µL from one column to the next were performed, starting from column 1 and progressing to column 11. Subsequently, 50 µL of Column 11 was drawn and discarded. In each well designated for testing, 50 µL of microbial suspension was added.

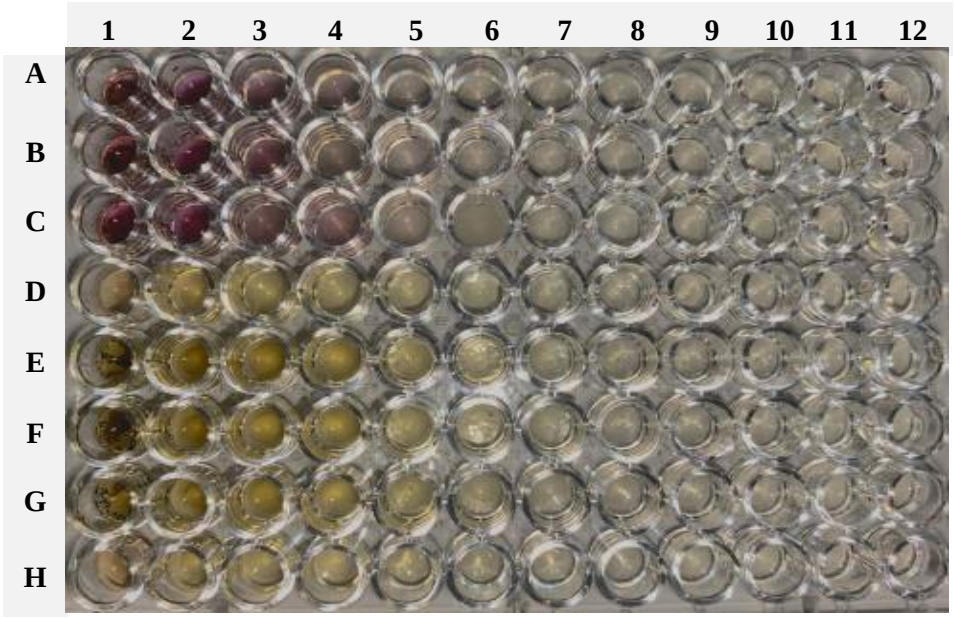

Figure 1. Arrangement of Tested and Control Samples Wells on Microplate

Note :
1- (A,B,C) : the extract solution and medium (negative control)
1- (D,H) :antibiotics and medium
2 -11 (A,B,C) : medium, the extract solution and the microbial suspension
12 (A,B,C) : medium and the microbial suspension (positive control)
2-11 (D) : antibiotics, medium and the microbial suspension
2 -11 (E,F,G) : medium, the extract solution and the microbial suspension
2-11 (H) : antibiotics,medium and the microbial suspension
12 (A-H) : medium and the microbial suspension

The microplate was then incubated at 37°C for 24 hours for bacteria and at 28°C for 48 h for yeast. During the incubation period, observations were made to ascertain the clarity or presence of turbidity within each well.

To determine the Minimum Inhibitory Concentration (MIC) score, if well 4 displayed clarity while well 3 exhibited turbidity, the MIC score was considered to be represented by well 4. In the context of wells 4 through 12 exhibiting color clarity, a volume of 5 µL was drawn from each of these wells and deposited into individual Petri dishes containing Nutrient Agar (NA). These Petri dishes were subsequently incubated at 37°C for 24 hours (bacteria) and 28°C for 48 hours (yeast). If no microbial growth occurred, the Minimum Bactericidal Concentration (MBC) was determined.

To gauge the equivalence of the activity of the test substance against reference antibiotics, a comparative evaluation was carried out involving tetracycline, chloramphenicol, and ketoconazole.

RESULTS AND DISCUSSION

The test plants utilized in this study were identified by verifying the true identity of *Zebrina pendula* Schnizl. An analysis of records maintained by the Plant Taxonomy Laboratory, Faculty of Biology, Universitas Padjadjaran (UNPAD) conclusively confirmed the utilization of *Zebrina pendula* Schnizl. were used as the test plants. The results of determination with number 59/HB/11/2022 show the species *Zebrina pendula* from the Commelinaceae family. Based on macroscopic observations, the color of *Zebrina pendula*

Schinzl. leaves is purple. This distinct coloration is attributed to the presence of anthocyanins, which are known for their potential to confer resistance to microbes (García-Varela et al., 2015). The appearance of *the Zebrina pendula* Schinzl plant is shown in Fig.Figure 2.

Figure 2. Photographs of *Zebrina pendula* Schinzl.

Subsequent weighing of *Zebrina pendula* Schnizl. Dry leaves resulted in a total of 1.12 kg, yielding a score of 90.169%. The results of the simplicia characterization are as follows:

Table I. Results of Characterization of *Zebrina pendula* Simplicia

Characteristic	Result
Water content	5.37 ± 2.38 % v/w
Water soluble extract content	45.70 ± 1.13 % w/v
Ethanol soluble extract content	11.55 ± 0.77 % w/v
Loss on drying	6.85 ± 1.62 % w/w
Total ash content	13.56 ± 0.46 % w/w
Acid insoluble ash content	4.85 ± 0.71 % w/w
Water soluble ash content	10.91 ± 0.03 % w/w

Water content determination was conducted using the toluene distillation method to establish a minimum threshold or range for water content within the material. It is imperative for the water content to remain below 10.00%, as surpassing this threshold may trigger enzymatic processes and microbial deterioration (Manoi et al., 2006). The obtained results for water content determination reflected 5.37 ± 2.39 % v/w.

The quantification of the extract levels encompassed both water-soluble and ethanol-soluble extracts. This quantitative assessment aimed to determine the compounds present in *Zebrina pendula* Schnizl. leaves were dissolved in water and ethanol, respectively. The water-soluble extract content was 45.70 ± 1.13 % w/v, while the ethanol-soluble extract content was 11.55 ± 0.78 % w/v. This observation suggested that more compounds from *Zebrina pendula* Schnizl. dissolved in water than in ethanol.

Determination of ash content spanned total ash, acid-insoluble ash, and water-soluble ash. This evaluation aimed to identify the presence of mineral or metal impurities in the crude sample. The total ash content was found to be 13.56 ± 0.46 % w/w. The total ash content not only signals the physiological ash stemming from the plant itself but also non-physiological ash originating from external pollution sources such as air, soil, and water contaminants.

The acid-insoluble ash content was at 4.85 ± 0.71 % w/w. This indicates the presence of acid-resistant compounds, including sand and silica, in the crude sample (Kementerian Kesehatan Republik Indonesia, 2017). Water-soluble ash content was determined at 10.91± 0.03 % w/w, showing the notable mineral content within the simplicia.

Maceration was used as the extraction method in this study. This technique involves cold phytochemical screening of crude samples and the extract obtained to determine the secondary metabolite content. The results of the phytochemical screening Table II.

Table II. Results of Phytochemical Screening

Chemical constituents	Reagents	*Tested samples*				
		crude sample	ethanol extract	n-hexane fraction	ethyl acetate fraction	water fraction
Alkaloids	Dragendorf	-	-	-	-	-
	Mayer	-	-	-	-	-
Polyphenols	$FeCl_3$	+	+	-	+	+
Tannins	Gelatin 1%	+	+	-	+	+
Flavonoids	Amyl Alcohol	+	+	-	+	+
Quinones	KOH 5%	+	+	-	+	+
Saponins	Dilute HCl	-	-	-	-	-
Monoterpenoids/ Sesquiterpenoids	Vanillin SO_4	+	+	-	-	-
Steroids/ Triterpenoids	Lieberman Bouchard	+	+	+	+	-

Note (+) :
alkaloids – Mayer : white precipitate
alkaloids – Dragendorff : brick red precipitate
polyphenols : green-blue-black color
tannins : white and blackish green deposits
flavonoids : yellow-red color (amyl alcohol layer)
quinone : yellow color
saponin : forms stable foam
monoterpenoids/sesquiterpenoids : colors are formed
steroids/triterpenoids : green-blue color

Flavonoids, polyphenols, tannins, quinones, and terpenoids are secondary metabolites that exhibit antimicrobial potential. For instance, flavonoids exert antibacterial effects by disrupting bacterial cell membranes, resulting in the discharge of intracellular components. This disruption hinders the synthesis of nucleic acids (Puspadewi et al., 2016) and curtails energy production and aggregation within bacterial cells (Hariyati et al., 2015).

Polyphenolic compounds exert an inhibitory influence on bacterial growth by oxidizing phenolic compounds and consequently inhibiting enzymes. Quinones function by adhering to microbial cell surfaces and binding to polypeptide cell walls and enzymes within cell membranes, thereby eliminating substrates essential for microbes. Given their lipophilic properties, terpenoid compounds disrupt cell membranes. This disruption leads to respiration inhibition, increased cell membrane permeability, and cellular membrane damage (Cowan, 1999).

Subsequent to establishing the Minimum Inhibitory Concentration (MIC) score, the subsequent endeavor involves ascertaining the Minimum Bactericidal Concentration (MBC) score, which signifies the lowest concentration capable of terminating bacterial growth. MBC determination involves transferring the results of the MIC test to agar media. The MBC score was defined as the minimum concentration at which bacterial growth was absent. Both MIC and MBC assessments employed test samples encompassing the ethanol extract, ethyl acetate fraction, n-hexane fraction, and water fraction. The MIC and MBC scores are presented in Table III.

Table III. Results of Microdilution Testing

Test Sample	Test Microbes	MIC (µg/mL)	MBC (µg/mL)
Ethanol extract	*Staphylococcus aureus*	10,000	10,000
	Candida albicans	10,000	10,000
	Bacillus subtilis	39.1	78.1
N-hexane fraction	*Staphylococcus aureus*	5,000	10,000
	Candida albicans	10,000	10,000
	Bacillus subtilis	39.1	312.5
Ethyl acetate fraction	*Staphylococcus aureus*	2,500	2,500
	Candida albicans	10,000	10,000
	Bacillus subtilis	2,500	2,500
Water fraction	*Staphylococcus aureus*	10,000	10,000
	Candida albicans	10,000	10,000
	Bacillus subtilis	10,000	-
Tetracycline	*Staphylococcus aureus*	19.5	19.5
Ketoconazole	*Candida albicans*	78.1	78.1
Chloramphenicol	*Bacillus subtilis*	19.5	19.5

The process of determining the MIC revealed that all four extracts of *Zebrina pendula* had an inhibitory effect on the growth of *Staphylococcus aureus* at different MIC. Notably, the ethanol extract and water fraction shared identical MIC values of 10,000 µg/mL, whereas the n-hexane fraction displayed a value of 5000 µg/mL, and the ethyl acetate fraction had a value of 2,500 µg/mL. The ethyl acetate fraction outperformed the other extracts in terms of inhibiting *Staphylococcus aureus* growth.

MBC determination revealed varying bactericidal activities against *Staphylococcus aureus* among the four *Zebrina pendula* Schnizl. extracts and the standard, each exhibiting distinct MBC values. The ethanol extract, n-hexane fraction, and water fraction had MBC values of 10,000 µg/mL. Meanwhile, the ethyl acetate fraction showed superior bactericidal efficacy against Staphylococcus aureus, which was marked at 2500 µg/mL. For *Candida albicans*, both the MIC and MBC values were uniform at 10,000 µg/mL, analogous to the comparison used, ketoconazole, with an inhibition of 78.1 µg/mL.

In the context of *Bacillus subtilis*, the MIC value is 39.1 µg/mL for the ethanol extract and n-hexane fraction, whereas the ethyl acetate fraction registers a MIC of 2,500 µg/mL, and the water fraction reports a MIC of 10,000 µg/mL. The MBC values for the ethanol, n-hexane, and ethyl acetate fraction were 78.1 µg/mL; 312.5 µg/mL; and 2,500 µg/mL, respectively. The water fraction did not have an MBC value because it was tested at a concentration of 10,000 µg/mL. Water fractions from *Zebrina pendula* Schnizl. leaves did not exhibit the ability to eliminate *Bacillus subtilis*. Thus, further testing at higher concentrations is necessary to ascertain whether the water fraction has an MBC value. In testing with *Bacillus subtilis*, the results showed that the ethanol extract from *Zebrina pendula* Schnizl leaves had better antibacterial activity than the other fractions because it was capable of inhibiting and terminating at lower concentrations.

The outcomes obtained from the MIC and MBC assessments underscored the latent potential of *Zebrina pendula* Schnizl. leaves. However, it is noteworthy that the administered doses were relatively substantial for achieving the desired resistance. These results further suggested that the ethanol extract of *Zebrina pendula* Schnizl. showed promising effects on gram-positive bacteria. This could potentially be attributed to the presence of anthocyanins, phenolic compounds known for their antimicrobial properties. It is estimated that gram-positive bacteria might be more susceptible to anthocyanins, and that the extract could employ a mechanism that directly interferes with bacterial membrane structures.

This disparity is linked to the simpler structural makeup of gram-positive bacteria, which is characterized by a singular plasma membrane and a significant proportion (90%) of peptidoglycan. In contrast, Gram-negative bacteria possess a more intricate structure, including a double membrane encompassed by an outer membrane composed of three layers: lipoprotein, lipopolysaccharide, and peptidoglycan (Silhavy et al., 2010).

CONCLUSION

Zebrina pendula Schnizl. leaves exhibited Minimum Inhibitory Concentration (MIC) activity against *Staphylococcus aureus*, *Candida albicans*, and *Bacillus subtilis*. The ethanol extract and water fraction of *Zebrina pendula* Schnizl. leaves exhibited an MIC value of 10,000 µg/mL, whereas the n-hexane fraction presented a value of 5,000 µg/mL, and the ethyl acetate fraction had an MIC of 2,500 µg/mL. *Candida albicans* tests yielded identical MIC values of 10,000 µg/mL for each solvent. When examining *Bacillus subtilis*, the ethanol extract showcases an MIC value of 39.1 µg/mL, the n-hexane fraction registers 39.1 µg/mL, the ethyl acetate fraction reports 2,500 µg/mL, and the water fraction indicates an MIC of 10000 µg/mL. *Zebrina pendula* Schnizl. leaves also exhibited Minimum Bactericidal Concentration (MBC) activity against *Staphylococcus aureus*, with the ethanol extract, n-hexane fraction, and water fraction sharing an MBC value of 10,000 µg/mL, while the ethyl acetate fraction had an MBC of 2,500 µg/mL. *Candida albicans* displayed uniform MBC values of 10000 µg/mL for each solvent. For *Bacillus subtilis*, the MBC value for the ethanol extract is 78.1 µg/mL, n-hexane fraction reports 312.5 µg/mL, and ethyl acetate fraction display and MBC of 2,500 µg/mL, respectively.

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