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Antibacterial Activity of *Drosera* Sp. Ethanolic Extract Against *Staphylococcus Aureus* ATCC 25923

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Abstract – ***Drosera sp.* is an insectivorous plant with secondary metabolites, including naphthoquinones which can be used as antibacterial agents. *Staphylococcus aureus* is a bacterium that often causes nosocomial infections, such as ARI. This research aims to determine the antibacterial activity of four Drosera species, namely *D. burmannii, D. sessilifolia, D. capillaris*, and *D. filiformis*, against *Staphylococcus aureus* ATCC 25923. The agar diffusion method was employed in antibacterial testing by measuring the inhibition zone. The inhibition zone measured using a 7,000 ppm concentration of *Drosera sp.* ethanolic extract was 6.74 ± 0.21 mm, 9.21 ± 0.32 mm, 18.18 ± 0.60 mm, and 15.67 ± 0.37 mm for *D. burmannii, D. sessilifolia, D. capillaris,* and *D. filiformis,* respectively. The four Drosera sp. extracts were proven to have antibacterial potential against Staphylococcus aureus ATCC 25923. The largest value was produced by D. capillaris which was close to 19.73 ± 0.55 mm from the positive control (chloramphenicol).**

Keywords *– Antibacterial activity, Drosera* sp., *Staphylococcus aureus*

INTRODUCTION

Nosocomial infections initiate various diseases presented in almost all healthcare facilities, specifically in hospitals, both in Indonesia and worldwide. These are caused by pathogenic microorganisms, including *Staphylococcus aureus* which has developed resistance to certain antibiotics, including penicillin, ampicillin, and vancomycin [1], leading to treatment difficulty. *Staphylococcus aureus* causes an acute respiratory infection (ARI) [2], with symptoms such as fever, cough, and sore throat. ARI prevalence in Indonesia is 4.4% and the highest, 12.8%, occurs in children below five years [3].

Infectious diseases are generally managed with antibiotics, but in some cases of bacterial resistance, alternative treatments are needed, such as using herbs against gram-positive and gram-negative bacteria. Drosera is a species of carnivorous herbal plant that can be used as an alternative, containing secondary metabolites applied traditionally as raw materials for pharmaceutical preparations [4], In Indonesia, the extract has been used as a mixture of active ingredients in formulating cough medicines. Drosera contains phenols, flavonoids (Quercetin), anthocyanins, and naphthoquinones, namely plumbagin and 7-methyljuglone [5], [6], which are used to treat respiratory tract infections, such as bronchitis and tuberculosis [7], some of its pharmacological activities include antioxidant, anti-inflammatory, and anticancer effects [8].

The application of *D. rotundifolia's* extract against gram-negative bacteria, such as *Escherichia coli, Salmonella enteritidis, Yersinia enterocolitica*, and gram-positive bacteria such as *Bacillus thuringiensis, Clostridium perfringens, Listeria*

monocytogenes, and *Staphylococcus aureus* [9], [10], the combination of *D. binata* extract with silver nanoparticles (AgNPs) increases the activity against *Staphylococcus aureus* [11], in this research, the antibacterial activity of *D. burmannii, D. sessilifolia, D. capillaris*, and *D. filiformis* in Indonesia was determined against *Staphylococcus aureus.* These four species have similarities, a tendency to grow in the southern hemisphere [12], and contain compounds including phenols, flavonoids, alkaloids, and anthraquinone with antioxidant effects [13], [14].

METHODS

This study is an in vitro experimental study using a Post-Test Only Control Group Design conducted at the Phytochemical and Microbiology Laboratory, Faculty of Pharmacy, University of Surabaya for 5 months.

Materials

The herbal plant samples used were four *Drosera* species, namely *D. burmannii, D. sessilifolia, D. capillaris, and D. filiformis*. The plants were grown on sphagnum-moss media in Surabaya-East of Java, Indonesia. The test bacterium employed was *Staphylococcus aureus* ATCC 25923 which was grown at the Faculty of Pharmacy, Surabaya University.

Preparation of *Drosera sp.* Herb Extract

Dry Simplicia powder of *Drosera sp.* was extracted by maceration method using 96% ethanol as solvent. Maceration was carried out for 24 hours with 6 hours of immersion and stirring. The filtrate was separated from the residue which was macerated again twice. This was combined with the second filtrate and processed into a concentrated extract, then the yield of each sample was determined [15].

Preparation of Test Solution

Approximately 70 mg of the concentrated extract was weighed and dissolved in 10 ml of 10% DMSO. Therefore, a concentration of 7,000 ppm was obtained and graded from 3,000 ppm to 6,000 ppm. The 10% DMSO was applied as negative control while the positive was chloramphenicol. *Staphylococcus aureus* ATCC was cultured on Nutrient Agar slanted (NA) media and incubated at 37°C for 24 hours. This was suspended in liquid media with the addition of 3 mL of 0.9% NaCl and homogenized, then the turbidity of the bacterial suspension was measured using a 0.5 McFarland densitometer [16].

Antibacterial Activity Test

A petri dish containing liquid agar and 100 L of *Staphylococcus aureus* suspension was allowed to solidify. Afterward, wells were made using a cork bore with a 6 mm diameter and each was filled with *Drosera sp.* at five different concentrations, negative control, and positive control. The treatment was incubated at 37°C for 24 hours and then a clear inhibition zone was marked on the agar medium to indicate the absence of bacterial growth [17].

RESULTS AND DISCUSSION

Drosera sp. Herb Extract

The maceration method used 96% ethanol solvent to extract the active compound content of *Drosera sp.* herb powder. This allows the obtained compounds including plumbagin, anthraquinone, and flavonoid to be undamaged and more stable than the extraction method conducted with high temperatures which can affect antibacterial activity [18], in this research, the ethanol extract of *D. spatulata* var. *bakoensis* produced better antibacterial activity than aqueous and methanol extracts [7], the yield obtained from the four *Drosera sp.* ranged between 23 - 66%, meaning many compounds were extracted from the herb with ethanol solvent. It is also a Simplicia's non-specific parameter that can be used as quality control of a traditional medicinal raw material [19], and the data are presented in Table 1.

Table 1. The yield of *Drosera sp.* Extract

Drosera Species	Yield (%)
D. burmannii	24.51
D. sessilifolia	23.73
D. capillaris	37.30
D. filiformis	66.81

Antibacterial activity of *Drosera sp.*

Antibacterial activity was determined through the ability of *Drosera sp.* to hinder bacterial growth on solid NA media which was characterized by an inhibition zone. The four species at concentrations of 3,000, 4,000, 5,000, 6,000, and 7,000 ppm produced inhibition zones against *Staphylococcus aureus*, and the results are presented in Table 2.

Table 2. Determination of Inhibition Zone of *Drosera sp.* Extract against *Staphylococcus aureus*

Spesies Drosera	Diameter of Inhibition Zone (mm)						
	a	b	c	d	e	Chloramphenicol	DMSO 10%
D.burmannii	0	0	7.32 ± 0.5	7.81 ± 0.3	8.19 ± 0.16	19.64 ± 1.25	0
D. sessilifolia	0	0	8.02 ± 0.69	8.82 ± 0.33	9.13 ± 0.49	20.71 ± 0.9	0
D. capillaris	9.90 ± 0.31	11.02 ± 0.38	12.24 ± 0.31	14.21 ± 0.4	17.70 ± 0.61	19.37 ± 1.09	0
D. filiformis	8.97 ± 1.2	9.68 ± 0.3	10.58 ± 0.29	14.04 ± 0.37	16.78 ± 0.39	20.30 ± 0.65	0

Description: a) 3,000 ppm, b) 4,000 ppm, c) 5,000 ppm, d) 6,000 ppm, e) 7,000 ppm

Based on the antibacterial test, the concentration of *Drosera sp.* used ranged from 3,000 to 7,000 ppm. *D. burmannii* and *D. sessilifolia* extracts did not produce inhibition zones at concentrations of 3,000 ppm and 4,000 ppm, but they began at 5,000 ppm – 7,000 ppm. Meanwhile, *D. capillaris* and *D. filiformis* extracts produced inhibition zones from concentrations of 3,000 to 7,000 ppm. Greater extract concentration generates a higher inhibition zone. From the four samples, 7,000 ppm led to a high inhibition zone as demonstrated in Fig. 1. Similarly, Rita et al. (2018) explained that elevations in both the inhibition zone and extract concentration were directly proportional [20].

Also, the content of active compounds in the extract and antibacterial activity increased concurrently. In the comparison of antibacterial activity between samples at a 7,000 ppm concentration, the *D. capillaris* extract produced the largest inhibition zone diameter of 17.70 mm±0.61 mm. This was followed by *D. Filiformis*, *D. Burmannii*, and *D. sessilifolia* with values of 16.78 mm ± 0.39 mm, 8.19 mm ± 0.16 mm, and 9.13 mm ± 0.49 mm, respectively. The chloramphenicol positive control had 19.37–20.71 mm, while two *Drosera sp.* which had values close to this range were retested simultaneously and they produced inhibition zones as presented in Table 3.

Table 3. Inhibition Zone Test for 7000 ppm concentration of *Drosera sp.* against *Staphylococcus aureus*

Drosera Species	**Diameter of Inhibition Zone (mm)**
D. burmannii	6.74 ± 0.21
D. sessilifolia	9.21 ± 0.32
D. capillaris	18.18 ± 0.60
D. filiformis	15.67 ± 0.37
Chloramphenicol	19.73 ± 0.55

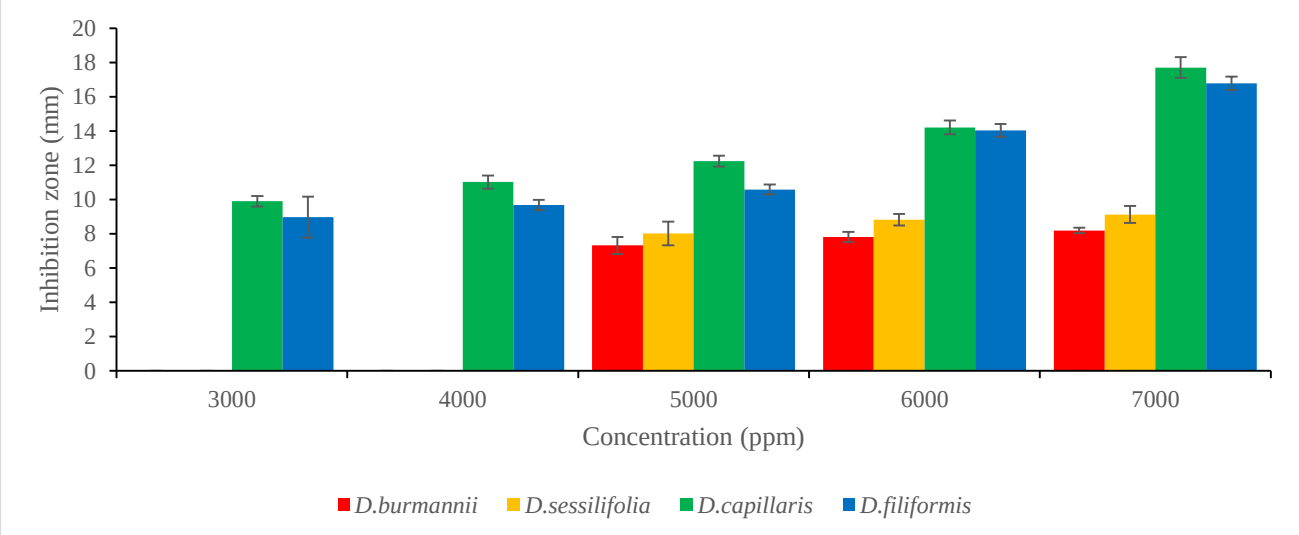

Figure 1. Inhibition zone of *Drosera sp.* extract against *Staphylococcus aureus*

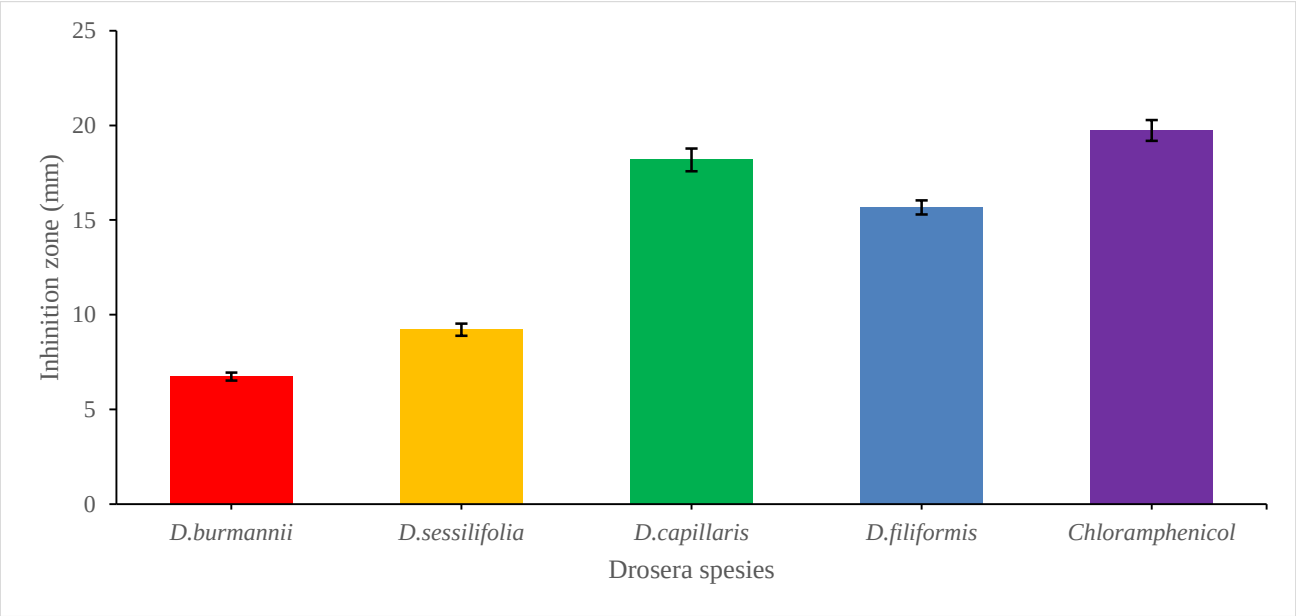

Figure 2. Inhibition zone of *Drosera sp.* extract at 7000 ppm against *Staphylococcus aureus*

Another species, *D. magnifica*, has antibacterial activity that is synergistic with antibiotics and can suppress bacterial virulence factors in *in vitro* tests [21], the compound in *Drosera sp.* which acts as an antibacterial against gram-positive bacteria is plumbagin from the naphthoquinone group [11], other compounds such as alkaloids, anthraquinones, and flavonoids also have antimicrobial effects against microbes causing respiratory tract infections [7], the mechanism of antibacterial action is by inhibiting nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, and porins in cell membranes, as well as changing membrane permeability [22].

D. capillaris and *D. filiformis* extracts at 7,000 ppm had antibacterial activity close to the positive control, namely chloramphenicol with an inhibition zone of 19.73±0.55 mm as demonstrated in Fig. 2. Natural ingredients can be classified as strong antibacterials once their inhibition zone value is equal to or better than the positive control. Compounds with an inhibition area diameter of 10-20 mm or more are claimed to contain strong antibacterial potential [23], the extracts of *D. capillaris, D. filiformis, D. burmannii,* and *D. sessilifolia* can have antibacterial potential at higher concentrations. However, their toxicity effect needs to be considered, because it increases alongside the concentration. In 2019, Lee did not reported death and toxicity in mice but obtained an LD_{50} value of 4,800 mg/kg by using single (300-1,200 mg/kg) and repeated doses (150-600 mg/kg) of *D. rotundifolia* for 4 weeks [24].

CONCLUSION

The 96% ethanol extract of four *Drosera sp.* produced antibacterial activity against *Staphylococcus aureus* ATCC 25923 as indicated by the inhibition zones, hence they are potential antibacterial agents. *D. burmannii* and *D. sessilifolia* as well as *D. capillaris* and *D. filiformis* produced an inhibition zone at concentrations of 5000ppm and 3000 ppm, respectively. At a 7000 ppm concentration, the inhibition zones of each sp. extract were 6.74±0.21 mm, 9.21±0.32 mm, and 15.67±0.37 mm for *D. burmannii, D. sessilifolia,* and *D. filiformis,* respectively. Meanwhile, *D. capillaris* had the largest inhibition zone, which was 18.18±0.60 mm, and the activity closest to 19.73±0.55 mm from the positive control (*chloramphenicol*).

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