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Purslane Herb (*Portulaca grandiflora*) Ointment Antibacterial Potency against *Staphylococcus aureus*

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ABSTRACT

Recent studies showed that the rose-like purslane (*Portulaca grandiflora*) magenta flower variety herbs ethanolic extract has antibacterial potency. This research aims to investigate the purslane herbs (*Portulaca grandiflora*) extract ointment antibaterial potency against *Staphylococcus aureus*. The ointment base formulation contained vaseline album, cera alba, adeps lanae, paraffin liquidum, cetyl alcohol, nipagin, and nipasol. Extract ointment was prepared by mixing the extract with 10, 20, and 30% concentrations into the ointment base. The antibacterial activity test was conducted in vitro using the disc diffusion method. The antibacterial activity test result of the three extract concentrations of ointments showed that the 30% extract ointment had an antibacterial effect against *Staphylococcus aureus* with 0.81±0.03cm of clear zone. The antibacterial activity test demonstrated that 30% purslane herbs (*Portulaca grandiflora*) extract ointment has potency as an antibacterial against *Staphylococcus aureus*.

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1. INTRODUCTION

Herbal medicine as a source of ethnopharmacology has been widely used comprehensively to prevent and treat diseases in humans since a long time ago. This approach is supported by bioactive compound findings for the development of new drugs as therapeutic tools (Kumar *et al*., 2022; Moorthy *et al*., 2015). Recent research showed that several natural phytochemicals have potential antibacterial properties (de Freitas *et al*., 2020; Singla & Dubey, 2019).

Portulaca grandiflora is an annual plant belonging to the *Portulacaceae* family part. Aqueous extract of *Portulaca grandiflora* toxicity on Wistar rats had been studied (Chavalittumrong *et al*., 2004), and *in vitro* anti-herpes simplex virus and anti-adenovirus activities also had been studied (Chiang *et al*., 2003). In addition, the aqueous extract of *Portulaca grandiflora* has been proven as an immunomodulator in vitro by increasing lymphocyte proliferation. Ethanolic extract of *Portulaca grandiflora* has also demonstrated wound healing effect (Budiawan *et al*., 2024a; Budiawan *et al*., 2024b; Budiawan *et al*., 2023) and alleviate pain (Kirana & Budiawan, 2022a; Kirana & Budiawan, 2022b). Moreover, various fractions of *Portulaca grandiflora* extract are known for their antifungal effects (Purwanto *et al*., 2024).

The research result of antibacterial in vitro activity tests of various variety of *Portulaca grandiflora* and *Portulaca oleracea* ethanolic extract against bacterial test (*Staphylococcus aureus*, *Escherichia coli*, dan *Pseudomonas aeruginosa*) showed antibacterial activity with various inhibition

zones range from 1.56 cm to 2.86 cm. Based on these experiments, various purslane extracts showed that sensitivity to the Gram-positive *Staphylococcus aureus* was higher than Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* (Purwanto, 2021).

Compared to the closest genus (Dkhil *et al*., 2011; Sanja *et al*., 2009; Lim & Quah, 2007), the study of *Portulaca grandiflora* in health benefits and specific characterization is still limited. The ointment of purslane herbs magenta flower variety antibacterial activity has never been reported. Meanwhile, the purslane extract needs to be intact to the wounded skin properly to achieve maximum effect and ointments are an ideal preparation for this purpose.

Ointments are semi-solid preparations used topically. Ease of application and homogeneous active compound dispersion are essential requirements for this formulation (Davis *et al*., 2022). In addition, ointment has the ability to remain intact to the skin. This ability is not found in other preparations such as the cream containing purslane extract as formulated by Kirana *et al*. (2023). Based on the background, it is necessary to investigate purslane herb (*Portulaca grandiflora*) ointment potency as an antibacterial against *Staphylococcus aureus*.

2. METHOD

2.1. Tools and material

Oven, glassware, mortar, stamper, evaporating dish, water bath, paper disk, micropipette, spreader, Eppendorf tube, petri dish, and vernier caliper were used as tools in this study. The materials used in the study were purslane aerial herbs (*Portulaca grandiflora*) magenta flower variety, ethanol 96%, aqua destilata, oxytetracycline ointment, Nutrient Agar (NA), *Staphylococcus aureus* bacteria, vaseline album, cera alba, adeps lanae, paraffin liquidum, cetyl alcohol, nipagin, and nipasol.

2.2. Purslane herb extraction (*Portulaca grandiflora*)

The extraction was conducted by drying the purslane herb *Portulaca grandiflora* magenta flower variety in the oven at 50°C for 5 days (Imawati *et al*., 2023). Dried simplicia was ground to get simplicia powder. Then, simplicia powder was macerated with ethanol 96% with 1:5 ratio. The maceration process was done in 2 days and continued with the re-maceration process twice. The filtration result of the macerate evaporated in the vacuum rotary evaporator until a thick extract was obtained (Indriasari, 2022). The thick extract was then heated in the oven at 50°C to eliminate the remaining ethanol. The result was stored in the refrigerator for the next step (Lolo *et al*., 2017).

2.3. Purslane herb (*Portulaca grandiflora*) ointment preparation procedure

The ointment base was made with the process started by melting the base that contained vaseline album, cera alba, adeps lanae, paraffin liquidum, and cetyl alcohol, and also preservatives which were nipagin and nipasol in the porcelain dish on top of water bath. The melted bases and preservatives were poured into the mortar and stirred until it was cold and homogeneous. The next step was weighing the purslane extract based on the concentration of the ointment which was 10, 20, and 30% of the total ointment weight. Purslane herb extract was then dripped with ethanol 96% until dissolved, then the ointment bases were added gradually and mixed until it was homogeneous.

2.4. Disc diffusion method (*Paper disc*) antibacterial activity test

The disc diffusion method was used to investigate the antibacterial activity of purslane herb ointment. The pure culture of *Staphylococcus aureus* at a concentration of $1{,}5x10^8$ bacteria/ml was poured with a micropipette into the previously prepared Nutrient Agar (NA) medium. Bacteria suspension was then inoculated evenly to the petri dish surface with a spreader aseptically. The paper disc was dipped for 30 minutes in the purslane herb ointment (10%, 20%, dan 30%) which was prepared previously in the Eppendorf tube. For the negative control, paper disc was dipped in the ointment bases. The paper disc which was dipped in the oxytetracycline ointment was used as the positive control. The next step was placing the dipped paper discs on the petri dish surface which was *Staphylococcus aureus* bacteria suspension innoculated. Each petri dish was separated into several quadrants and each was filled with different treated paper discs that had been prepared previously. The petri dish was then incubated for 24 hours at room temperature. The last step was measuring the inhibition zone which was formed around the paper disc with vernier calipers in centimeters.

3. RESULT AND DISCUSSION

Based on the inhibition zone measurement result, which was incubated at room temperature for 24 hours, purslane herb magenta flower variety extract ointment with 30% concentration showed inhibition against *Staphylococcus aureus* bacteria which was signed by clear zone around the paper disc (Figure 1, Table 1).

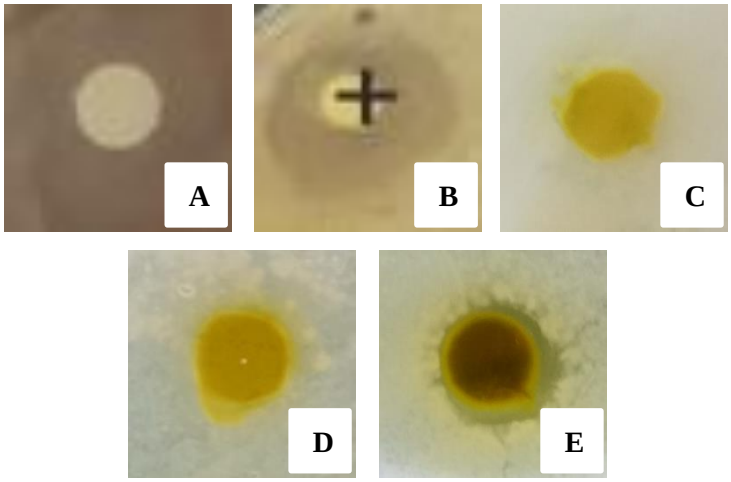

Figure 1. Inhibition Zone of Ointment Base (A), Oxytetracycline Ointment (B), Purslane Herb Extract 10% Ointment (C), Purslane Herb Extract 20% Ointment (D), and Purslane Herb Extract 30% Ointment (E)

Table 1. Antibacterial *Staphylococcus aureus* Activity of Purslane Herb (*Portulaca grandiflora*) Ointment

Bacteria	Inhibition Zone Diameter (cm)				
	Control Ointment		Purslane Ointment		
	Negative	Positive	10%	20%	30%
Staphylococus aureus	0.00 ± 0.00	3.48 ± 0.42*	0.00 ± 0.00	0.00 ± 0.00	0.81 ± 0.03*

Note: *Significantly different ($p<0.05$) with negative control

Purslane herb (*Portulaca grandiflora*) extract which was formulated as an ointment test showed antibacterial activity against *Staphylococcus aureus* in the highest concentration (30%). This antibacterial activity may be due to the high concentration of the extract in the ointment formulation, which allowed it to diffuse well in the agar medium. The purslane herb ointment with 10% and 20% concentration of extract didn't show a clear zone. The base material that was used in the formulation may be affecting the result. This aligns with Pawar and Nabar (2010) research which showed that vaseline bases formulated herbal medicine extract didn't show antibacterial activity because of decreasement in extract diffusion ability. Vaseline has the capability to bind extract, so it decreases the extract's ability to diffuse well in the agar medium. Vaseline is a hydrocarbon base that is intact to the skin in a long time and unease to rinse with water (Sasongko *et al*., 2019).

Figure 2. Purslane (*Portulaca grandiflora*) Magenta Flower Variety

Portulaca grandiflora magenta flower variety (Figure 2) contained various active compounds such as flavonoids, alkaloids, saponins, tannins, and terpenoids (Imawati *et al*., 2023). Flavonoids have

antibacterial activity due to complex forming with bacteria cell membrane wall mechanism (Royani *et al*., 2023). Saponin lowers bacteria cell membrane wall permeability, so bacteria absorb extra cell fluid excessively leading to bacteria death (Wei *et al*., 2021). Tannin is known widely as an antibacterial agent due to disturbing protein transport in the bacteria cell (Rijayanti, 2014). Those various active compounds in the *Portulaca grandiflora* extract may work synergically to inhibit *Staphylococcus aureus* bacteria growth.

4. CONCLUSION

The purslane herb (*Portulaca grandiflora*) ointment antibacterial activity test using the disc diffusion method result showed antibacterial activity against *Staphylococcus aureus* at 30% concentration.

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