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[florea] Submission Acknowledgement

1 message

Marheny Lukitasari <bot_smtpojs@unipma.ac.id> Wed, Dec 4, 2024 at 12:25 PM
To: Antonius Budiawan <antonius.budiawan@ukwms.ac.id>

Antonius Budiawan:

Thank you for submitting the manuscript, "Potensi Salep Ekstrak Herba Krokot (Portulaca grandiflora) sebagai Antibakteri Staphylococcus aureus" to Florea : Jurnal Biologi dan Pembelajarannya. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Marheny Lukitasari
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3 messages

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To: Agus Purwanto <agus.purwanto@ukwms.ac.id>, Antonius Budiawan <antonius.budiawan@ukwms.ac.id>, Christianto Adhy Nugroho <christianto.adhy.n@ukwms.ac.id>, Christina Indriasari <christina.indriasari@ukwms.ac.id>

Dear Agus Purwanto, Antonius Budiawan, Christianto Adhy Nugroho, Christina Indriasari:

We have reached a decision regarding your submission to Florea : Jurnal Biologi dan Pembelajarannya, "Potensi Salep Ekstrak Herba Krokot (Portulaca grandiflora) sebagai Antibakteri Staphylococcus aureus".

Our decision is: Revisions Required

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3 attachments

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Potensi Salep Ekstrak Herba Krokot (*Portulaca grandiflora*) sebagai Antibakteri *Staphylococcus aureus*

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ABSTRACT

Recent research showed that the rose-like purslane (*Portulaca grandiflora*) magenta flower variety herbs ethanolic extract has antimicrobial potency. This research aims to determine the purslane herbs (*Portulaca grandiflora*) extract ointment antimicrobial potency against *Staphylococcus aureus*. Ointment base formulation contained of 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 antimicrobial activity test conducted in vitro using the paperdisk difussion method. The antimicrobial activity test result of the three extract concentrations of ointments showed that the 30% extract ointment had antimicrobial effect on *Staphylococcus aureus* with 0.81±0.03cm of clear zone. The antimicrobial activity test demonstrated that 30% purslane herbs (*Portulaca grandiflora*) extract ointment has a potency as antimicrobial against *Staphylococcus aureus*.

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1. PENDAHULUAN

Tumbuhan obat dengan pemanfaatan bahan mentah atau senyawa murni secara etnofarmakologi telah diterapkan secara komprehensif untuk mengobati dan mencegah penyakit manusia sejak dahulu kala. Pendekatan tanaman tradisional ini telah didukung untuk menghasilkan senyawa bioaktif untuk obat terbaru sebagai alat terapi (Kumar *et al*., 2022; Moorthy *et al*., 2015). Sejumlah penelitian telah menunjukkan bahwa senyawa fitokimia alami memiliki aktivitas antijamur potensial (de Freitas *et al*., 2020; Singla & Dubey, 2019).

Portulaca grandiflora adalah tanaman herba tahunan kecil termasuk anggota familia *Portulacaceae*. Penelitian sebelumnya, ekstrak air *Portulaca grandiflora* digunakan untuk mempelajari toksisitasnya pada hewan uji tikus Wistar (Chavalittumrong *et al*., 2004), *in vitro* anti-herpes virus simplex dan aktivitas anti- adenovirus (Chiang *et al*., 2003). Selain itu, ekstrak air *Portulaca grandiflora* ditemukan untuk meningkatkan proliferasi limfosit in vitro, menunjukkan peran dalam imunomodulasi. Ekstrak etanol Portulaca grandiflora juga memiliki kemampuan sebagai penyembuh luka (Budiawan *et al*., 2024a; Budiawan *et al*., 2024b; Budiawan *et al*., 2023) dan menurunkan rasa nyeri (Kirana & Budiawan, 2022a; Kirana & Budiawan, 2022b). Selain itu, berbagai fraksi ekstrak *Portulaca grandiflora* diketahui memiliki aktivitas antifungi (Purwanto *et al*., 2024).

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Hasil pengamatan hasil pengujian aktivitas antibakteri *in-vitro* ekstrak etanol beberapa kultivar tanaman *Portulaca grandiflora* dan *Portulaca oleracea* terhadap bakteri uji (*Staphylococcus aureus*, *Escherichia coli*, dan *Pseudomonas aeruginosa*) mampu menunjukkan aktivitas antibakteri dengan zona penghambatan yang beragam berkisar antara 1.56 cm dan 2.86 cm. Berdasarkan hasil pengujian aktivitas antibakteri beberapa kultivar tanaman krokot menunjukkan bahwa kepekaan bakteri uji Gram-positif *Staphylococcus aureus* lebih tinggi dibandingkan dengan bakteri uji Gram-negatif *Escherichia coli* dan *Pseudomonas aeruginosa* (Purwanto, 2021).

Dibandingkan dengan genus kerabat dekatnya (Dkhil *et al*., 2011; Sanja *et al*., 2009; Lim & Quah, 2007), studi tentang manfaat kesehatan dan karakterisasi rinci *Portulaca grandiflora* masih sedikit dan terbatas. Hasil-hasil penelitian sebelumnya tentang aktivitas sediaan salep ekstrak herba krokot (*Portulaca grandiflora*) varietas bunga magenta terhadap aktivitas antibakteri belum dilaporkan. Aplikasi ekstrak herba krokot *Portulaca grandiflora* membutuhkan bentuk sediaan salep yang dapat meningkatkan waktu kontak ekstrak dengan kulit yang terluka.

Salep merupakan sediaan semi padat yang digunakan secara topikal sehingga kemudahan dalam pengolesan serta dispersi bahan aktif yang homogen menjadi persyaratan sediaan tersebut (Davis *et al*., 2022). Salep juga mampu menempel dengan baik di kulit. Kemampuan ini tidak dimiliki oleh sediaan lain seperti krim berbahan aktif ekstrak krokot yang diformulasikan Kirana *et al*. (2023). Berdasarkan latar belakang tersebut, maka perlu dilakukan penelitian terkait potensi salep ekstrak herba krokot (*Portulaca grandiflora*) sebagai antibakteri *Staphylococcus aureus*.

2. METODE

2.1. Alat dan bahan

Oven, alat gelas, mortar, stamfer, cawan porselen, *waterbath*, paperdisk, mikropipet, *spreader*, tabung eppendorf, cawan petri, dan jangka sorong digunakan sebagai alat dalam penelitian ini. Bahan menggunakan herba aerial krokot (Portulaca grandiflora) varietas bunga magenta, etanol 96%, aqua destilata, salep oksitetrasiklin, Nutrient Agar (NA), bakteri *Staphylococcus aureus*, vaseline album, cera alba, adeps lanae, paraffin liquidum, cetyl alcohol, nipagin, dan nipasol.

2.2. Ekstraksi herba krokot (*Portulaca grandiflora*)

Ekstraksi dilakukan menggunakan bagian aerial tanaman krokot *Portulaca grandiflora* varietas bunga magenta yang dikeringanginkan selama 5 (lima) hari pada suhu 50°C dengan oven (Imawati *et al*., 2023). Simplisia kering yang diperoleh dihaluskan sehingga diperoleh serbuk simplisia. Kemudian serbuk simplisia dilakukan maserasi dengan etanol 96% dengan perbandingan 1 gram serbuk simplisia dan 7 (tujuh) ml etanol. Maserasi dilakukan selama 2 hari dan dilakukan remaserasi sebanyak 2 kali. Hasil penyaringan maserat kemudian diuapkan dengan *vaccum rotary evaporator* sampai mendapatkan ekstrak kental (Indriasari, 2022). Selanjutnya ekstrak kental dituang ke cawan petri dan dipanaskan pada suhu 50°C untuk menghilangkan sisa etanol dan selanjutnya disimpan di lemari es sampai dengan penggunaan selanjutnya (Lolo *et al*., 2017).

2.3. Prosedur pembuatan salep ekstrak herba krokot (*Portulaca grandiflora*)

Basis salep dibuat dengan tahapan melebur basis yang terdiri dari vaseline album, cera alba, adeps lanae, paraffin liquidum, dan cetyl alcohol dan pengawet yang terdiri dari nipagin dan nipasol dalam cawan porselen diatas waterbath. Basis dan pengawet yang sudah lebur dimasukkan ke dalam mortir dan diaduk hingga dingin dan homogen. Salep mengandung ekstrak herba krokot dibuat dengan menimbang ekstrak sesuai konsentrasi 10, 20, dan 30% dari total salep. Ekstrak herba krokot ditetesi ethanol 96% hingga larut, kemudian ditambahkan basis salep sedikit demi sedikit dan dicampur hingga homogen.

2.4. Pengujian aktivitas antibakteri metode difusi cakram (*Paperdisk*)

Metode difusi cakram digunakan untuk uji antimikroba (Baur *et al*., 1966). Penuangan suspensi kultur murni bakteri *Staphylococcus aureus* sebesar $1{,}5x10^8$ bakteri/ml dengan mikropipet ke medium Nutrient Agar (NA) yang disiapkan sebelumnya. Suspensi bakteri tersebut kemudian diinokulasikan secara merata menggunakan *spreader* secara aseptis di seluruh permukaan cawan petri. Kemudian menyiapkan sediaan salep krokot berdasarkan perbedaan konsentrasi (10%, 20%, dan 30%) yang sudah disiapkan dalam tabung eppendorf untuk untuk merendam paper disk selama 30 menit. Langkah berikutnya, untuk uji aktivitas antibakteri dilakukan menggunakan cawan petri

yang telah diinokulasi dengan suspensi bakteri uji *Staphylococcus aureus*. Setiap cawan petri dibagi menjadi beberapa kuadran yaitu berisi *paperdisk* yang telah direndam dengan salep ekstrak tanaman krokot yang berbeda konsentrasinya, kontrol negatif dengan perendaman basis salep, dan kontrol positif yang menggunakan salep oksitetarasiklin 3%. Kondisi inkubasi semua lempeng agar dilakukan pada suhu kamar selama 24 jam. Langkah terakhir adalah mengamati zona hambat yang terbentuk di sekitar *paperdisk* dengan mengukur besarnya zona hambat yang diukur dalam milimeter dengan jangka sorong.

3. HASIL DAN PEMBAHASAN

Berdasarkan hasil pengukuran LDH yang didapatkan bahwa sediaan salep ekstrak etanol herba krokot mawar varietas bunga magenta dengan masing-masing konsentrasi ekstrak (10%; 20%, dan 30%) yang diinkubasi pada suhu 37°C selama 24 jam menunjukan hasil dapat menghambat bakteri *Staphylococcus aureus* ditandai dengan timbulnya zona bening di sekitar paper disk (Tabel 1).

Tabel 1. Aktivitas Antibakteri *Staphylococcus aureus* Salep Herba Krokot *Portulaca grandiflora*

Bakteri Uji	Diameter Zona Hambat (cm)				
	Kontrol		Salep		
	Negatif	Positif	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*

Keterangan: *Berbeda signifikan (p<0,05) dengan kontrol negatif

Hasil uji ekstrak herba krokot (*Portulaca grandiflora*) yang diformulasikan dalam bentuk salep dengan basis kombinasi menunjukkan aktivitas antibakteri *Staphylococus aureus* pada konsentrasi ekstrak 30%. Aktivitas antibakteri tersebut kemungkinan disebabkan karena tingginya konsentrasi ekstrak dalam salep sehingga mampu berdifusi dengan baik dalam media agar. Salep dengan konsentrasi ekstrak 10% dan 20% tidak menunjukkan zona hambat. Hasil ini kemungkinan disebabkan oleh kandungan basis salep vaselin dalam formula. Hasil tersebut sejalan dengan penelitian Pawar dan Nabar (2010) yang menunjukkan bahwa ekstrak tanaman obat yang diformulasikan dengan basis vaselin tidak menunjukkan aktivitas antibakteri karena menurunnya kemampuan difusi dari ekstrak. Vaselin memiliki kemampuan untuk mengikat ekstrak sehingga mengurangi kemampuannya untuk berdifusi menembus media agar. Vaselin merupakan basis salep hidrokarbon yang mampu bertahan di kulit dalam jangka waktu lama dan tidak mudah dicuci dengan air (Sasongko *et al*., 2019).

Portulaca grandiflora mengandung berbagai kandungan senyawa aktif seperti flavonoid, alkaloid, saponin, tanin, dan terpenoid (Imawati *et al*., 2023). Flavonoid memiliki aktivitas antibakteri melalui mekanisme pembentukan kompleks dengan dinding sel bakteri (Royani *et al*., 2023). Saponin menurunkan permeabilitas dinding sel bakteri sehingga menyerap cairan ekstra sel secara berlebihan dan mengakibatkan kematian bakteri (Wei *et al*., 2021). Tanin dikenal secara luas sebagai antibakteri dengan melalui gangguan transport protein dalam sel (Rijayanti, 2014). Berbagai senyawa yang terkandung di dalam *Portulaca grandiflora* tersebut kemungkinan bekerja secara sinergis untuk menghambat pertumbuhan bakteri *Staphylococcus aureus*.

4. KESIMPULAN

Hasil pengujian aktivitas antibakteri sediaan salep herba krokot (*Portulaca grandiflora*) yang dilakukan menggunakan metode difusi cakram (*paperdisk*) menunjukkan adanya aktivitas antibakteri *Staphylococcus aureus* pada konsentrasi 30%.

UCAPAN TERIMAKASIH

Penulis mengucapkan terimakasih kepada Universitas Katolik Widya Mandala Surabaya yang telah memberikan dukungan dana sehingga penelitian ini dapat terlaksana.

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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*,

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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).

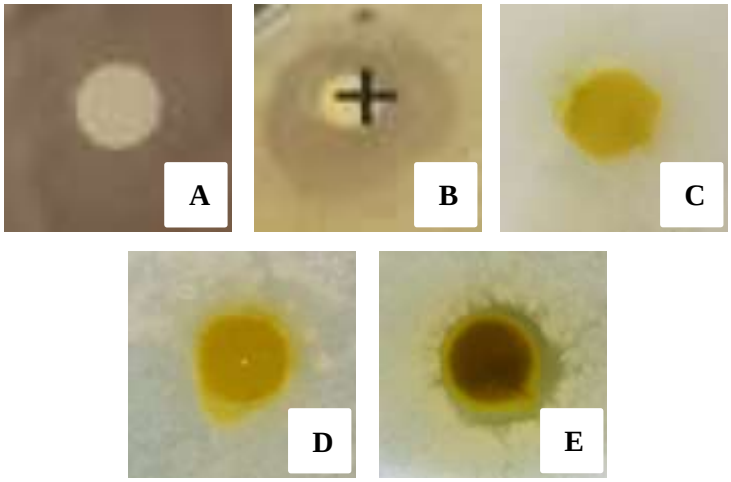

Figure 1. Inhibition Zone of Ointment Base (A), Oxytetracycline Ointment (B), Purslane Herb Extract 10% Ointment (C), Purslane Herb Extract 20% Ointment (D), and Purslane Herb Extract 30% Ointment (E)

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

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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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