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RESEARCH ARTICLE

REVISED Antibacterial activity of Betadine (*Jatropha multifida* L.)stem extract on *Pseudomonas aeruginosa* growth *in vitro*

[version 2; peer review: 2 approved]

Hendry Rusdy, Diah HI Damanik

Department of Oral and Maxillofacial Surgery, Faculty of Dentistry Universitas Sumatera Utara, Medan, Sumatera Utara, 20155, Indonesia

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Abstract**Background**

Today, people use plants to treat various types of diseases and improve human health. One of the medicinal plants is the Betadine plant (*Jatropha multifida* L.). Betadine plants have many functions, especially the sap, leaves, fruit and seeds. The compound contents in Betadine stem sap, which is efficacious as an antimicrobial, are saponins, tannins, flavonoids and labaditin. One of the bacteria that cause infection is *Pseudomonas aeruginosa*. These bacteria can cause opportunistic and nosocomial infections.

Methods

This study was a true experimental laboratory with a post-test only control group design. This study used Betadine stem sap extract with concentrations of 25%, 50%, 75%, 100%, gentamicin cream 10% as positive control, and dimethyl sulfoxide (DMSO) solution as negative control. This study used the Kirby-Bauer diffusion method and the bacterium *Pseudomonas aeruginosa* was grown on nutrient agar media, then incubated for 24 hours and calculated using calipers. Research data were analyzed using one-way ANOVA test.

Results

The highest inhibition zone was group 50% (12.725 ± 0.2500 mm) while the lowest inhibition zone was group 100% (8.675 ± 0.5620 mm).

Conclusions**Open Peer Review****Approval Status** ✓✓

	1	2
version 2 (revision) 02 Apr 2024	 view	 view
version 1 27 Oct 2022	 view	 view

1. **Khaga Raj Sharma** , Tribhuvan University, Kirtipur, Nepal

2. **Siti Nur Hazwani Oslan** , Universiti Malaysia Sabah, Kota Kinabalu, Malaysia

Any reports and responses or comments on the article can be found at the end of the article.

Betadine stem extract had antibacterial activity in inhibiting the growth of *Pseudomonas aeruginosa* bacteria, with the 50% concentration being the most effective in inhibiting the growth of *Pseudomonas aeruginosa* bacteria.

Keywords

Betadine, sap, *Jatropha multifida* L., *Pseudomonas aeruginosa* L., Infection, Antibacterial, Nosocomial, Opportunistic.



This article is included in the **Plant Science** gateway.

Corresponding authors: Hendry Rusdy (hendry.rusdy@usu.ac.id), Diah HI Damanik (irenedamanik2697@gmail.com)

Author roles: **Rusdy H:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Damanik DH:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

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REVISED Amendments from Version 1

We added new figure of Betadine plant with its morphology parts such as leaves, flower and fruit. We revised petri plates figures by typing text over the plates. We added the voucher specimen number of *Jatropha multifida*. This new version more in-depth interpretation of the result and the relation to the previous study. We also added the novelty of this research that used different extract (Betadine sap) and bacteria (*Pseudomonas aeruginosa*). We clearly mention step by step Betadine sap extract preparation. We clearly mention DMSO 100% solution as negative control. We added 3 new references for the voucher specimen number, Betadine figure and the relation of the previous study explanation.

Any further responses from the reviewers can be found at the end of the article

Introduction

In Padang, West Sumatra Province, people use Betadine stem sap (*Jatropha multifida* L.) to heal and eliminate external wound infections by applying Betadine stem sap on the injured body part. Indonesian people call this plant a Betadine plant because it has the same potential as an external wound medicine like Betadine in duration to heal an infected external wound.¹⁻³

According to Ivan *et al.* 2019, the Betadine plant (Figure 1) can inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.⁴ Betadine plant sap consists of multifidol, biobollein, multifidone, multifidil, flavonoid, labaditin, saponin and tannin.⁵ In a study conducted by Aransiola *et al.* 2014 about the antibacterial and antifungal activity of Betadine plant sap, they found that the minimum inhibitory concentration (MIC) of Betadine plant sap gram negative bacteria (*P. aeruginosa*, *E. coli*, and *Salmonella typhi*) was 66 mg/ml. These results indicate that the antibacterial activity found in the Betadine plant sap is lower in gram negative bacteria because gram negative bacteria are more resistant to antibacterials.⁶

Based on WHO data in 2013, the mortality rate due to resistant bacterial infection is 700,000 people per year.⁷ *P. aeruginosa* is an anaerobic, nosocomial and opportunistic bacterium. *P. aeruginosa* bacteria live in humid environments such as surgical instruments, 74% in dental water and dental disinfecting units used for more than 24 hours.

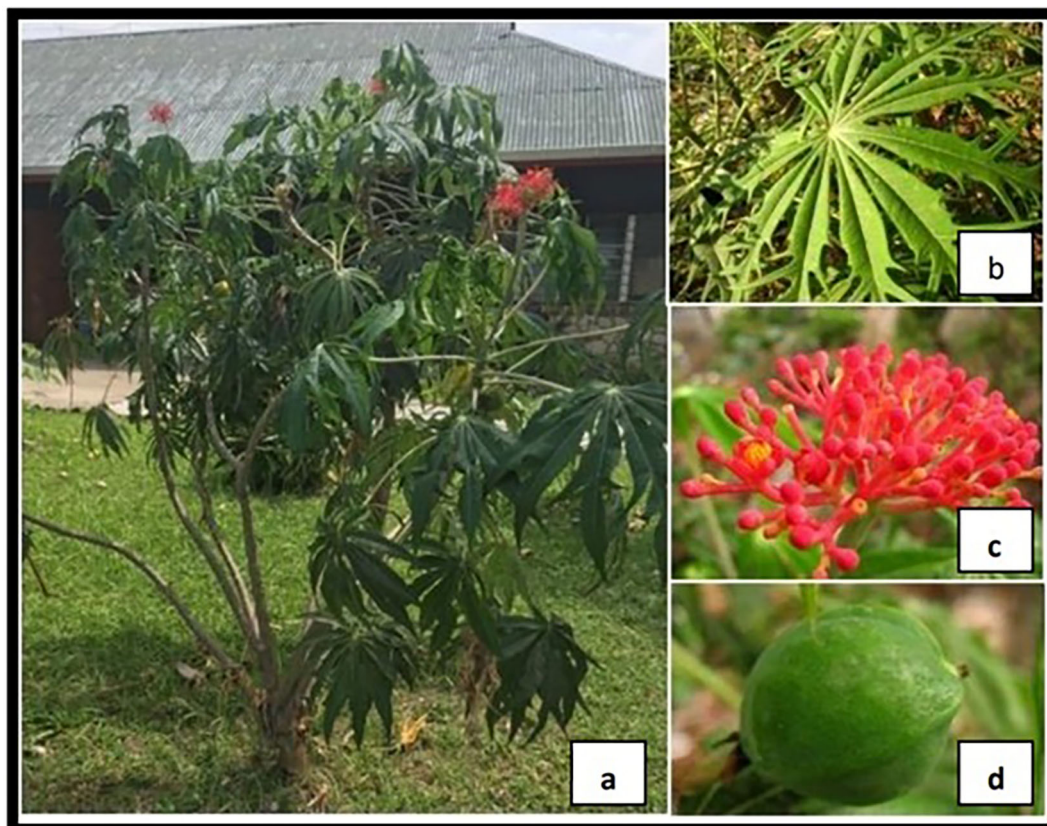


Figure 1. (a) Betadine Plant (*Jatropha multifida* L.); (b) Leaves; (c) Flower; (d) Fruit.¹¹

P. aeruginosa was isolated in maxillary osteomyelitis and brain abscesses in patients who had dental infections. This bacteria can cause meningitis, endocarditis, pneumonia and sepsis with an average mortality of 12–25%. This bacteria is resistant to many antibacterial including amoxicillin. Antimicrobial ingredients that are effective against *Pseudomonas aeruginosa* are hard to find, so natural resources such as Betadine plants can be used to suppress bacterial growth.^{5,8,9}

Based on the explanation above, the authors are interested in conducting research on “Antibacterial activity of Betadine stem (*Jatropha Multifida* L.) extracts on *Pseudomonas aeruginosa* bacteria growth by *in vitro*”.

Methods

Study type

This study was a true experimental study with a post-test only control group design. True experimental study measured or observed data after the treatment was given.

Location

Laboratory of Traditional Medicine and Microbiology, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia.

Duration

Study duration was $\pm$ 2 month, from December 2019 to January 2020.

Sample size

In calculating the size of experimental research samples, we used the Federer formula:

$$(t-1)(r-1) \geq 15$$

where t = number of treatment and r = number of repetition. The study used six treatment group: group 1 (25% Betadine stem sap extract), group 2 (50% Betadine stem sap extract), group 3 (75% Betadine stem sap extract), group 4 (100% Betadine stem sap extract), group 5 (gentamicin as a positive control), group 6 (dimethyl sulfoxide or DMSO as a negative control)

The number of repetitions (r) are four. So, 24 samples were obtained.

Dependent variable

The dependent variable in this study was the sensitivity test growth of *Pseudomonas aeruginosa* that measured by the diameter of the inhibitory zone.

Independent variable

The independent variable in this study was the concentration of Betadine stem sap extract used, which were 25%, 50%, 75% and 100%.

Controlled variable

There were six controlled variable such as the growth media used for the bacterium *Pseudomonas aeruginosa* was nutrient broth agar, the incubation temperature of *Pseudomonas aeruginosa* is 37°C, the incubation time of *Pseudomonas aeruginosa* was 24 hours, the isolation and culture technique of *Pseudomonas aeruginosa* bacteria, sterilization of tools, the materials and media used, and operator skills.

Uncontrolled variable

The vary betadine stem morphology such as diameters and length. The vary growth time of betadine stem, mature betadine stem has higher and browner sap. The vary nutrients of the soil and rainfall rate may contribute the different sap. Uncontrolled condition of laboratory storage such as power outage.

Instruments

Autoclaves (Tomy ES 315, Japan), UV-VIS spectrophotometers (Orion Aquamate 8000, Germany), ovens (Mettler UN55, Germany), micropipets (Dragon Lab, China), Laminar air flow cabinet (Astec HLF I1200L), glass cuvettes, refrigerators (Thermo Scientific), incubators (Mettler, Germany), vortex mixers (Biosan, Latvia), glass tubes, glass petri dish, test tubes, inoculating loops, bunsen, measuring cups 20 ml, spatula, Vernier calipers (Electric Digital Capliner), analytical scales (Sartorius BSA323S-CW) and Erlenmeyer.

Materials and Method

Materials

Betadine stem sap extract 100% concentration (the voucher specimen number FHI 109458, FHI 108012, FHI 96651, FHI 31400, FHI 109872),¹⁰ bacterial culture of *Pseudomonas aeruginosa* (ATCC® 140218, pure culture stock Faculty of Pharmacy, University of Sumatera Utara, Indonesia), DMSO solution 100%, gentamicin ointment 10% (IKAGEN®), aquadest, nutrient agar powder (Oxoid), nutrient broth agar powder (Oxoid), label paper, and 6 mm sterile paper discs.

Method

First, the process of collected Betadine stem sap involved cut the stem and collected the sap in a sterile container. To purify the sap from impurities, a maceration method was used. Soaked 10 ml sap with 1 L DMSO 100% and continuous stirred for the first 6 hours, followed by occasional stirred for the next 18-hour. Then filtered to obtain the macerate. This macerate was evaporated using a rotavapor device at a temperature of 40°C to obtain a thick extract. Mixed the extract and DMSO 100% using a micropipette. This process resulted in extracts with concentrations of 25%, 50%, and 75%.

Next, all the instruments were sterilized with the autoclave at 121°C for 15 minutes and dried with an oven 170°C for 1–2 hours. Inoculating loop and pinset were sterilized with the bunsen flame. After that, took dilution of Betadine stem sap extract 100% with DMSO using a micropipet into a glass tube and measured with sartorius analytical scales (1 ml). The nutrient agar powder (28 g) was mixed with 1000 ml aquadest in an erlenmeyer flask with a spatula. This was heated for 2 hours at 100°C and after that saved in autoclave. Then, sterilized inoculating loops were used to take the isolated *P. aeruginosa* colonies and these were then placed into test tubes each containing the tilt media (contained 3 ml nutrient agar). The tube was put in an incubator at 37°C for 24 hours. Took *P. aeruginosa* colony using sterilized inoculating loops and than put into a tube that contain 10 ml nutrient broth agar. The tube was put on vortex mixers for 1 minute. Took 3 ml bacteria and put into glass cuvetts, then put into UV-VIS spectrophotometers with 560 nm wavelength. The result must be 25% transmittance. In laminar airflow cabinet took 20 ml nutrient agar using measuring cups and 1 ml bacteria suspense using micropipets into glass petri dishes. The petri dishes were shaken slowly in a figure of eight movement until homogeneous. Paper discs were soaked with 25%, 50%, 75%, 100% Betadine stem sap extract, gentamicin and DMSO 100% for 3 minutes. Then these were placed on the surface of the nutrient agar and a little pressure was applied. The petri dishes were labelled with label paper and placed in the refrigerator for 1 hour. Then the petri dishes were put into an incubator for 24 hours at 37°C. The diameter of inhibitory zone was clear zone in the petri dish. Measured it with caliper.

Data analysis

Data was analyzed using software IBM SPSS Statistics Desktop 20.0 Windows Multilingual eAssembly (CRG2LML). The data was processed and analyzed with one way ANOVA test, and post hoc LSD (least significant difference).

Results

From this study, the lowest inhibition zone was produced by the Betadine stem sap extract group 100% (8.675 ± 0.5620 mm), while the highest inhibition zone was produced by the 50% (12.725 ± 0.2500 mm) Betadine stem sap extract group (Figures 2 and 3). The Saphiro-Wilk test with a significance of $p > 0.05$ was used for the normality test and the data were found to be normally distributed ($p > 0.05$) (Table 1). The data was distributed normally, so statistical analysis was continued using a one-way ANOVA analysis test and post hoc LSD to see whether there was a significant difference in the diameter of the inhibition zone between group 25%, 50%, 75%, 100%, K+, and K-. The ANOVA test showed a significant difference ($p < 0.05$) in the effect of the inhibition zone diameter between group 25%, 50%, 75%, 100%, K+ and K- (Table 2). The LSD post hoc test was used to determine the significance of the inhibition of the growth of *Pseudomonas aeruginosa* between all groups in this study. The results of the LSD post hoc test are shown in Table 3.

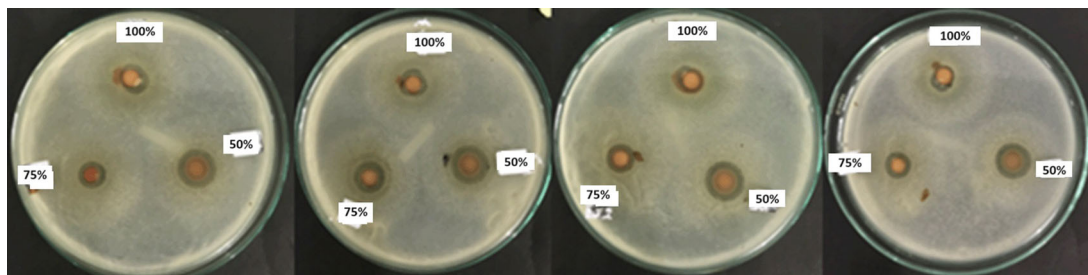


Figure 2. Inhibition zone group 100% (upper paper discs), group 75% (bottom left paper discs), group 50% (bottom right paper discs) with four repetitions.

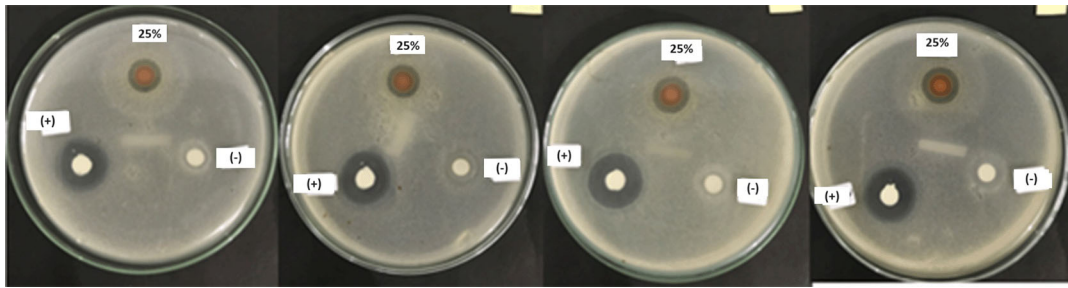


Figure 3. Inhibition zone group 25% (upper paper discs), group positive control (bottom left paper discs), group negative control (bottom paper discs) with four repetitions.

Table 1. Results for measurement of inhibition zone diameter.

Repetitions	Inhibition Zone Diameter (mm)					
	100%	75%	50%	25%	K+	K-
1	8.2	8.6	12.4	11.9	17.8	-
2	9.3	9.4	12.7	12.1	17.9	-
3	9.0	9.1	12.8	12.5	18.6	-
4	8.2	8.6	13.0	12.6	18.8	-
Mean	8.675	8.925	12.725	12.275	18.275	-
SD	0.5620	0.3948	0.2500	0.3304	0.4992	0.00

Table 2. One-way ANOVA test results.

Treatment	Number of repetition	The average $\pm$ standard deviation	P-Value ANOVA
100%	4	8.675 $\pm$ 0.5620	p = 0.000
75%	4	8.925 $\pm$ 0.3948	
50%	4	12.725 $\pm$ 0.2500	
25%	4	12.275 $\pm$ 0.3304	
K+	4	18.275 $\pm$ 0.4992	
K-	4	-	

Table 3. LSD post hoc test results.

Konsentrasi	100%	75%	50%	25%	K+	K-
100%	-	0.371	0.000	0.000	0.000	0.000
75%	-	-	0.000	0.000	0.000	0.000
50%	-	-	-	0.116	0.000	0.000
25%	-	-	-	-	0.000	0.000
K+	-	-	-	-	-	0.000
K-	-	-	-	-	-	-

Discussion

The study used Kirby-Bauer's method.⁹ This method is fast, easy, simple and produces effective results to show the antibacterial activity of a substance.⁹ The solvent was DMSO which is able to dissolve polar and nonpolar compounds and does not have antibacterial activity so it does not change the results.¹² Factors that influence the diffusion of extracts into the media are the extent of the concentration gradient, solubility, diffused molecular mass, temperature and density of the solvent.⁶

Inhibition zone group 25% (12.275 mm) smaller than group 50% (18.275%).¹³ This is because the group 50% had higher active ingredients than the group 25%. This is in line with previous research conducted by Brooks *et al.*³ and Anggita *et al.*¹⁴ in 2018 which states that the effectiveness of an antibacterial agent is influenced by the concentration of a given substance, the higher concentration has the higher active ingredient as an antibacterial, thus increasing the inhibition ability against microbes.

The inhibition zone group 75% the was 8.925 mm and group 100% was 8.675 mm.¹³ The decrease in inhibition zone diameter due to the minimum diffusion power of the dense viscous extract is because it had a high solution density. The group 75% had minimum penetration power because the minimal amount of solvent. The group 100% had minimum penetration power because there was no solvent. Molecules moved slowly because it is more difficult to pass through denser media so it takes longer and results in smaller inhibitory zones.⁹ This is similar to a study conducted by Komariah *et al.* 2013, where there was a decrease in antibacterial activity along with an increase in the concentration of the extract due to the higher concentration, the viscosity would increase so that the extract would be more difficult to diffuse into the agar media.¹⁵

The antibacterial activity of the Betadine stem sap extract is related to its antibacterial content, namely phenols, tannins, saponins and labaditin.³ Large molecule of phenol are able to activate essential enzymes in microbial cells even at low concentrations.¹⁶

Phenol compounds contained in Betadine stem sap are flavonoids and tannins.¹² Flavonoids work by inhibiting nucleic acid synthesis, inhibiting cytoplasmic membrane function and inhibiting energy metabolism. Flavonoids can damage the outer membrane and cytoplasm of gram-negative bacteria, disturb the exchange of nutrient and metabolite and inhibit the energy supply for bacteria.^{17,18}

Tannins can cause lysis of bacterial cell membranes due to the differences in bacterial cells osmotic pressure. Tannins damage cell membranes by breaking the phosphate group H^+ so the phospholipid molecule breaks down into glycerol, carboxylic acid and phosphoric acid. Saponins are also contained in Betadine stem sap. Its mechanism of action is by interfering with bacterial cell membrane stability, causing bacterial cell lysis, damaging the cell membrane and releasing various important components of microbial cells, namely proteins, nucleic acids, nucleotides and others.^{17,18} According to a study conducted by Barbosa *et al.* 2019, Betadine stem sap contains labaditin which is effective in gram-positive bacteria but less effective in gram-negative bacteria because of the more complex structure of gram-negative bacterial cell walls.¹⁹

This study similar to Wayan *et al.* research 2015 that used betadine leaves extract for *Staphylococcus aureus* growth inhibition. The concentration group that they used was 25%, 50%, 75% and 100%. The group 25% was 11.2 mm, the group 50% was 13.8 mm, the group 75% was 11.8 mm, and the group 100% was 7.2 mm. Their result showed lower antibacterial activity than this study, however both showed there was a decrease inhibition zone in group 75% and 100%.⁹

The result of this study was vary form Darmawi *et al.* 2013 that used betadine stem sap for *Staphylococcus aureus* growth inhibition. The concentration group that they used was 25%, 50%, 75% and 100%. The group 25% was 13 mm, the group 50% was 13.5 mm, the group 75% was 14.6 mm, and the group 100% was 15.7 mm. The vary of the result due to swab method bacteria cultivation. Darmawi *et al.* used swab method for bacteria cultivation, that made the bacteria grown only on the surface.²⁰

From the four group of Betadine stem sap extract that were tested, the strongest antibacterial activity found the group 25% and group 50%.¹³ However, when compared with the positive control antibiotic gentamicin, Betadine stem sap extract was not more effective than gentamicin. This is due to the fact that many gram-negative bacteria contain lipids and little peptidoglycan. The outer membrane of the *Pseudomonas aeruginosa* is a bilayer that serves for the selective defense of compounds entering and exiting cells. The outer membrane consists of phospholipid (inner layer) and lipopolysaccharide (outer layer). This makes it difficult for active compounds to enter the cell so antibacterial activity of Betadine stem sap extract is smaller than gentamicin.^{19,21}

The novelty from this research is that not only betadine stem leaf extract can be used as an antibacterial material, but also betadine stem sap extract. Betadine stem sap extract is not only effective on gram-positive bacteria, but also effective on gram-negative bacteria.

This research proves that Betadine (*Jatropha multifida* L.) stem sap extract has antibacterial activity on inhibiting the growth of *Pseudomonas aeruginosa* *in vitro*. This result is the first step in the possibility of utilizing papaya leaf extract as an alternative natural ingredient for antibiotics in dentistry with the need for a series of tests such as clinical trials, toxicity and side effects so that this research can be utilized by the public.

Conclusions

Based on the results, Betadine stem sap extract has an antibacterial activity on *Pseudomonas aeruginosa* growth *in vitro*. The most effective concentration to inhibit the growth of *Pseudomonas aeruginosa* is the group 50%, with the largest inhibitory zone diameter of 12.725 ± 0.2500 mm. This study has limitations, so further research is recommended to investigate using concentrations below 50% and using dilution methods to obtain the MIC (minimum inhibitory concentration) and MKC (minimum kill concentration).

Data availability

Underlying data

Figshare: Antibacterial Activity of Betadine (*Jatropha multifida* L.) Stem Extract on *Pseudomonas aeruginosa* Growth In-Vitro. <https://doi.org/10.6084/m9.figshare.20359653.v2>.¹³

This project contains the following underlying data:

- Data Inibition Zone of Each Repetition of Betadine Extract.csv (the file contains plain data of each concentration of Betadine stem sap extract with four repetitions).
- Test of Normality.csv (The file contains normal data distribution).
- Descriptives.csv (The file contains median, variance, standart deviation, minimum, maximum, range, inter-quartile range, skewness and kurtosis each concentration of Betadine stem sap extract).
- Case Processing Summary.csv (file contains of summary valid and missing antibacterial activity each concentration).
- Oneway Anova Test.csv (The file contains of data which is distinguish the antibacterial activity of each concentration of Betadine stem sap extract against *Pseudomonas aeruginosa* bacteria).
- Post Hoc Test.csv (The file contains of data which is show the significant difference between each concentration of Betadine stem sap extract).

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Acknowledgements

An earlier version of this article can be found at: <https://repositori.usu.ac.id/handle/123456789/25701?show=full>.

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Open Peer Review

Current Peer Review Status:  

Version 2

Reviewer Report 20 May 2024

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Siti Nur Hazwani Oslan 

Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia

The manuscript is scientifically sound and well-structured. The authors have presented their results effectively and responded comprehensively to previous comments. The revisions have significantly improved the clarity and overall quality of the manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microalgae; functional food; Food microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 15 April 2024

<https://doi.org/10.5256/f1000research.163442.r261893>

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Khaga Raj Sharma 

Central Department of Chemistry, Tribhuvan University, Kirtipur, Central Development Region, Nepal

Now the paper seems ok and it can be accepted for indexing in the required format of the journal.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Natural products chemistry, extraction, isolation, characterisation of various natural products and to perform the biological activities, molecular docking, green synthesis of nanoparticles and comparison of biological activities.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 15 November 2023

<https://doi.org/10.5256/f1000research.135915.r164184>

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Siti Nur Hazwani Oslan

Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia

Overall, the manuscript presents an interesting study on the antibacterial activity of Betadine stem (*Jatropha Multifida* L.) extracts on *Pseudomonas aeruginosa* bacteria growth by in vitro. The introduction provides good background information about using Betadine stem sap in West Sumatra Province to heal external wound infections and the potential of the Betadine plant as an external wound medicine. The methodology is clearly presented, with an appropriate sample size and controlled variables. However, the uncontrolled variables could be more clearly defined. The instruments and materials used are appropriate for the study. The results are presented in a clear and concise manner, with appropriate statistical analysis. However, the discussion section could be improved by providing a more in-depth interpretation of the results and how they relate to previous studies. Overall, the manuscript has potential, but some revisions are needed to strengthen the discussion and improve clarity.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microalgae; functional food; Food microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 Mar 2024

Diah Harezky Irene Damanik

Thank you for your suggestion and your positive comment. I appreciate it. As your suggestion we already update the new article version.

We already more clearly defined the uncontrolled variables. We added more in-depth interpretation of the results to the discussion section and the relation to the previous studies.

Competing Interests: No competing interests were disclosed.

Reviewer Report 05 June 2023

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Khaga Raj Sharma

Central Department of Chemistry, Tribhuvan University, Kirtipur, Central Development Region, Nepal

I have gone through the manuscript titled **Antibacterial activity of Betadine (*Jatropha multifida* L.) stem extract on *Pseudomonas aeruginosa* growth *in vitro*** and the followings are the queries and the suggestions

1. The preparation of plant extract or the sample preparation is not well mentioned.
2. It will be better to justify using DMSO of high concentration as the negative control while doing the antibacterial activity.
3. In the photographs of the Petri plates used to perform an antibacterial activity, the positive

control, negative control, and the concentrations of the sample needed to be mentioned clearly by typing the text over the plates.

4. Better to write materials and methods instead of procedures.
5. Most of the activities performed in this study have been reported in the literature, it will be better to highlight the novelty of this research.
6. Identification of plant sample is missing. It is needed to mention the voucher specimen number of the plant. It will be clearer if the photograph of the plant is kept in the material and method section.
7. The paper is submitted to the Journal of Food Research, but the manuscript is related to medicinal plants and antibacterial activity. The manuscript seems not suitable for this journal. If the food values and using the plant in food properties makes relevant to this journal.

These are minor revisions and need to be incorporated in the revised manuscript for the acceptance of this paper.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Natural products chemistry, extraction, isolation, characterisation of various natural products and to perform the biological activities, molecular docking, green synthesis of nanoparticles and comparison of biological activities.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 07 Mar 2024

Diah Harezky Irene Damanik

Thank you for your suggestion. I appreciate it.

1. The new article version was clearly mention the preparation of the extract.
2. We used DMSO 100% as negative control.
3. We revised the petri plates figures by typing text over the plates.
4. We revised Procedures section with Material and Method
5. In the new article version, we added the novelty of this research.
6. In the new article version, we added Betadine plane photograph and vocer specimen number from new references.
7. This manuscript is related to Medical Plants Journal because it is explain about antibacterial activity.

Competing Interests: No competing interests were disclosed.

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