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Formulation and Activity Testing of n-Hexane Fraction of Brotowali Leaves (*Tinospora crispa* L.) for a Diabetic Ulcer Treatment

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Abstract. *Diabetes mellitus is one of many degenerative illness with topmost number of cases in the world. One of the complications often created by diabetes mellitus is diabetic ulcer, which is susceptible to bacterial infection, obe of which is Staphylococcus aureus. This research wish to formulate an n-hexane fraction cream preparation from brotowali leaves (Tinospora crispa L.) and evaluate its antibacterial activity. Brotowali leaves were ectracted using 96% ethanol and the maceration method, then fractionates using n-hekxane. Then, antibacterial tests were implemented on the n-Hexane fraction using the disc method and formulates into a cream preparation with concentrations of 12,5%, 25% and 50%. The evaluation of the preparation was implemented by testing its spreadability, pH, homogeneity and organoleptic properties. Derived from the outcome of the study, it was shown that the n-Hexane fraction and Brotowali leaf cream formulation had inhibitory activity against Staphylococcus aureus, with the largest inhibition zone at a concentration of 50%. The n-Hexane fraction Brotowali leaf cream has the potential to be developed as a topical formulation for diabetic ulcers.*

Keywords: *Diabetic Ulcer, Staphylococcus Aureus, Brotowali Leaves, N-Hexane Fraction Cream*

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INTRODUCTION

Diabetes mellitus is one of the many degenerative diseases with the highest prevalence worldwide (Balakumar et al., 2016; Lovic et al., 2020; Liu et al., 2020; Nasrolahi et al., 2019). Indonesia is one of the countries with the highest prevalence of diabetes mellitus. According to the IDF Diabetes Atlas, there were approximately 19.5 million cases of diabetes mellitus in Indonesia in 2021, and this number is predicted to rise to 28.6 million by 2045 (Sun et al., 2022; Darmadi et al., 2025; Hustrini et al., 2025; Rokhman et al., 2022; Tarigan et al., 2024). This data places Indonesia as the fifth-highest contributor to diabetes mellitus cases in the world. Diabetes mellitus is a disease triggered by the pancreas becoming insensitive to insulin due to uncontrolled blood sugar levels (Balaji et al., 2019; Jadon et al., 2024; Manrai et al., 2023; Mohajan & Mohajan, 2023). Diabetes mellitus is divided into type 1 and type 2 diabetes. Type 2 diabetes occurs when the pancreas no longer produces enough insulin to meet the body's needs due to poor habits and the decline in Langerhans cell function (Denggos, 2023).

Diabetic ulcers are one of the many complications. Diabetic ulcers are a series of secondary symptoms that arise from diabetes mellitus (Akkus, G., & Sert, 2022; Mezil, S. A., & Abed, 2021; Vuorisalo et al., 2009; Yang et al., 2022). This is caused by damage to peripheral nerves and blood vessel disorders, which make wounds difficult to heal, susceptible to infection, and increase the risk of amputation (Fadilah et al., 2025). When blood sugar levels are high, bacteria also easily grow. Bacteria are one factor that exacerbates infections in diabetic ulcers, making wounds on the feet of diabetics more severe and widespread. Research conducted by Zuliana et al. (2023) found that the percentage of gram-positive bacteria is greater than gram-negative bacteria, at 22.5% for Staphylococcus aureus in diabetic ulcers. Staphylococcus aureus is a type of bacteria that can worsen wound conditions and is often found in the pores of the skin, the surface of the skin, sweat glands, and the digestive tract (Del, 2020; Linz et al., 2023; Cela et al., 2023). One therapy that can be given to treat bacteria is antibiotics, but therapy with traditional medicines is also starting to be considered in treating bacteria.

Indonesia is one of the many countries with a diverse plant diversity that can be utilized as food and beneficial medicinal ingredients (Kartawinata, 2004; Silalahi et al., 2025; Astutik et al., 2019; Estiasih et al., 2025; Harmayani et al., 2019). Indonesia's tropical forests boast a total of 30,000-50,000 plant species, but only around 7,500 are recognized as useful in traditional medicine. Traditional medicine itself consists of medicines derived from minerals, plants, animals, galenic preparations, or a combination of these elements, with healing effects and has been used for generations (Khafid et al., 2023; Izah et al., 2024). One traditional plant known to have benefits and can be used as an alternative treatment for diabetes mellitus is the Tinospora crispa leaf. Tinospora crispa is known for its bitter taste and various benefits, including antipyretic, analgesic, antiseptic, and antidiabetic properties. The benefits of this plant stem from its constituents, such as flavonoids, alkaloids, tannins, and others (Herdini, 2024; Sharma et al., 2021; Asuk et al., 2015; Soetanm, 2008). Tinospora crispa leaves can be formulated into various dosage forms, such as creams for diabetic ulcers. Creams are semi-solid preparations in the form of emulsions of several medicinal components dispersed in a suitable mixture with a minimum water content of 60% (Tungadi & Pakaya, 2023).

Based on the above background, the purpose of this research is to create a new formulation from the n-hexane fraction of Tinospora crispa leaves that can be used to treat diabetic ulcers and to test its effectiveness for diabetic ulcers. Thus, it is hoped that this research will contribute to a deeper understanding of the potential of the active fraction of Tinospora crispa leaves for diabetic ulcers.

METHODS

The research design for this study was a laboratory experiment and was conducted from May to December 2025 at the Integrated Laboratory of the Clinical Pharmacy Study Program, Faculty of Health Sciences, Universitas Prima Indonesia.

Equipment and Materials

This research used equipment such as an autoclave, separatory funnel, Dean-Stark apparatus, incubator, laminar air flow apparatus, analytical balance, rotary evaporator, and water bath. The materials used in this study were distilled water, amyl alcohol, stearic acid, sulfuric acid, cetyl alcohol, 96% ethanol, FeCl3, glycerin, HCl, paper discs, chloroform, methylparaben, n-hexane, liquid paraffin, Dragendorff's reagent, Lieberman-Bouchard's reagent, Mayer's reagent, propylparaben, magnesium powder, TEA, and toluene.

Preparation of Brotowali Leaf Simplicia

The process of preparing the simplicia began with wet sorting, which was then cleaned with running water and dried in an oven at 50°C until dry. The sample was then ground into a powder (Widayanti et al., 2023).

Making Tinospora crispa Leaf Extract

A total of 580.46 grams of powdered medicinal plants were soaked in 96% ethanol for 3 days, with two remacerations and one maceration, each soaking for 24 hours, and mixing every 6 hours. The maceration was then filtered, and the filtrate was evaporated using a rotary evaporator, then concentrated again in a water bath (Herdini, 2024).

Characterization of Tinospora crispa Leaf Powder

Water Content

2 ml of distilled water and 200 ml of toluene were added to a round-bottom flask, distilled for 2 hours, cooled for 30 minutes, and 5 grams of sample were weighed and heated again for 15 minutes. After the toluene boiled, the drip rate was adjusted to 2 drops per second until half of the water had distilled. Then, the drip rate was increased to 4 drops per second. After all the water had distilled, the inside of the condenser was rinsed with toluene. The distillation process was carried out for 5 minutes, then the receiving tube was allowed to cool to room temperature. After the distilled water and toluene had completely dissolved, the total water content was calculated (Arifin, 2021).

Water-Soluble Extract Content

5 grams of powdered medicinal plants were poured into a glass bottle, then 2.5 ml of chloroform and 97.5 ml of distilled water were added, and shaken occasionally for the first 6 hours, then left for 18 hours. Then, 20 ml of sample filtrate was taken and evaporated to a constant weight. The weight of the extract was then calculated, and the water-soluble extract content of the sample was calculated (Arifin, 2021).

Ethanol-Soluble Extract

5 grams of powdered medicinal plants were placed in a glass bottle, then 100 ml of 96% ethanol solvent was added, shaken occasionally for the first 6 hours, then left for 18 hours. Next, 20 ml of sample filtrate was taken and evaporated to a constant weight. The weight of the extract was then calculated, and the water-soluble extract content of the sample was calculated (Arifin, 2021).

Phytochemical Screening

Alkaloids

0.3 grams of extract was placed in a test tube, followed by Herdini drops of sulfuric acid and Mayer's reagent. The sample was declared positive if a white precipitate formed. In another test tube, 0.3 grams of extract was added, followed by Herdini drops of sulfuric acid and Dragendorff's reagent. The sample was declared positive if the color changed to brick red (Herdini, 2024).

Flavonoids

0.3 grams of extract was placed in a test tube, followed by a small amount of magnesium powder, Herdini drops of HCl, and Herdini drops of amyl alcohol. The sample was declared positive for flavonoids if the color changed to orange (Herdini, 2024).

Tannins

0.3 grams of extract was placed in a test tube, followed by Herdini drops of FeCl3. The sample was declared positive if the color changed to blackish green (Herdini, 2024).

Saponin

0.3 grams of the extract was placed in a test tube, followed by 10 ml of distilled water and 3 drops of HCl. Shake vertically for 10 seconds, then let stand and observe the foam that forms. The sample was declared positive if the foam persisted after 10 minutes (Herdini, 2024).

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Phenol

0.3 grams of the extract was placed in a test tube, then 5 drops of FeCl3 were added. The sample was declared positive if the color changed to blackish green (Herdini, 2024).

Terpenoids and Steroids

0.3 grams of the extract was placed in a test tube, then 5 drops of Lierbman-Bouchard reagent were added. The sample was declared positive for terpenoids if the color changed to purple and positive for steroids if the color changed to blue-green (Herdini, 2024).

Fractionation of Brotowali Leaf Extract

The fractionation process was carried out by dissolving the extract in distilled water (1:10 ratio), then transferring it to a separating funnel placed on a stand and clamp. Next, n-hexane solvent was added (1:1 ratio). Shake several times, then open the tap on the separating funnel and collect the water and n-hexane fractions. This was repeated three times (Sunnah et al., 2024).

Antibacterial Activity Test of Brotowali Leaf Extract Fractions

This test was conducted using the disc diffusion method in NA media. Paper discs were dipped into fractions at predetermined concentrations dissolved in DMSO, then placed on NA media coated with Staphylococcus aureus bacteria. They were then incubated for 24 hours in an incubator (Tarigan & Sembiring, 2025).

Formulation of a Cream Preparation Using Tinospora crispa Leaf Extract Fraction

The cream preparation was formulated by first melting the oil phase (stearic acid, liquid paraffin, cetyl alcohol) in a water bath at a temperature ranging from 70 to 75°C, followed by the water phase (methylparaben, TEA, propylparaben, glycerin) also heated in a water bath. The oil phase was transferred to a heated mortar and then added to the water phase. Both phases were stirred steadily until a creamy mass was formed. The n-Hexane fraction was then added to the finished cream at the specified concentration.

Evaluation of a Cream Preparation Using Tinospora crispa Leaf Extract Fraction

Organoleptic Test

This test was conducted by visually observing the preparation, including color, aroma, and texture.

pH Test

This test was conducted using a pH meter. A good pH value is ideal for skin, which is between 4.5 and 6.5.

Spreadability Test

This test is conducted by placing 0.5 grams of cream on two glass plates, which are then attached together. A 50-gram load is placed on top and left for 1 minute. The same procedure is repeated with a 100-gram load. A good spreadability test requires a spread of between 5 and 7 cm.

Homogeneity Test

This test is conducted by applying a small amount of cream to a glass plate and observing it. A good homogeneity test requires the absence of lumps, coarse particles, or clumps.

Antibacterial Activity Test of Brotowali Leaf Extract Fraction Cream

This test uses the disc diffusion method in NA media. Paper discs are dipped into the cream preparation at a predetermined concentration and then positioned over the NA media, which has been coated with Staphylococcus aureus bacteria. The discs are then incubated for 24 hours in an incubator (Octora et al., 2024).

RESULT AND DISCUSSION

Determination Results

A determination was performed on the Tinospora crispa leaves to ensure the accuracy of the plant's identity and to prevent errors in sample collection. The determination process was carried out at the Medanese Herbarium Laboratory (MEDA), Department of Biology, Faculty of Mathematics and Natural Sciences, University of North Sumatra, with letter number 193/MEDA/2025, which confirms that the plant species in this research is indeed the leaves of the Tinospora crispa L. plant.

Tinospora crispa Leaf Extraction Results

Extraction is one of the initial stages in obtaining active plant compounds. Maceration is a frequently used extraction method. Maceration begins by soaking the sample in a predetermined solvent for 3 x 24 hours at room temperature. Maceration is often used due to its simplicity. In this study, the solvent used was 96% ethanol. This solvent was chosen because it is a universal solvent that can extract both polar and non-polar compounds and is readily available (Qonitah et al., 2022). The results of the extraction of Tinospora crispa leaves using the maceration method and the solvent used were 96% ethanol, as follows:

Table 1. Tinospora crispa Leaf Extraction Results

Sample	Simplisia Weight	Extract Weight	Yield (%)
Brotowali Leaves	580.46 grams	321.74 grams	55.42%

In this study, 580.46 grams of powdered herbal medicine was used, with a total extract weight of 321.74 grams, resulting in a yield of 55.42%. Yield is the ratio of the final result, a thick extract, to the dry herbal medicine used. Therefore, the higher the yield, the more effective the solvent used in isolating active compounds in the plant (Wijaya et al., 2022).

Phytochemical Screening Results

Table 2. Phytochemical Test Results

Phytochemical Test	Reagent	Observation	Result
Alkaloids	Mayer	No white precipitate formed	–
	Dragendorff	Color changed to dark brick red	+
Flavonoids	Mg + HCl	Color changed to orange	+
Tannins	$FeCl_3$	Color changed to greenish-black	+
Saponins	Distilled water + HCl	Foam formation observed	+
Phenols	$FeCl_3$	Color changed to dark green/blackish-green	+
Terpenoids	Liebermann–Burchard	No purple color change observed	–
Steroids	Liebermann–Burchard	Color changed to greenish-blue	+

Description: (+) Positive (-) Negative

The table above shows that the Tinospora crispa leaf extract contains almost all compounds except terpenoids, as the test results show a blue-green color change, indicating a positive result for steroids. In the alkaloid test using Mayer's reagent, a white precipitate forms. This precipitate formation occurs because HgCl2 binds to the N atom in the heterocyclic ring. Based on research, the sample did not show a white precipitate, indicating a negative result in the Mayer's reagent test (Royani & Yuliyanti, 2025). However, based on research conducted by Herdini (2024), the Tinospora crispa leaf sample tested positive for alkaloids.

Tinospora crispa Leaf Powder Characterization Results

Water Content

Table 3. Water Content Test Results

No.	Sample Weight (g)	Water Volume (mL)	Moisture Content (%)
1	5.002 g	0.5 mL	9.99%
2	5.0018 g	0.6 mL	11.99%
3	5.010 g	0.7 mL	13.97%
Average	—	—	**11.98%**

Based on the results of the water content study, it can be concluded that the sample's water content meets the requirements. This is based on the Indonesian Herbal Pharmacopoeia, Edition II, which states that the water content of Tinospora crispa (Brotowali) must be less than 15%, and the resulting water content was 11.98%. Water content can affect sample stability; if the water content is too high, the sample will quickly deteriorate due to the ease of microbial growth within it (Aliifah et al., 2025).

Water-Soluble Essence Content

Table 4. Water-Soluble Essence Test Results

No.	Sample Weight (g)	Extract Weight (g)	Water-Soluble Extract Content (%)
1	5.0012 g	0.2558 g	25.57%
2	5.0027 g	0.2981 g	29.79%
3	5.0021 g	0.2842 g	28.40%
Average	—	—	**27.92%**

Based on the results of the water-soluble extract study, it can be concluded that the sample's water content meets the requirements. This is based on the Indonesian Herbal Pharmacopoeia, Edition II, which states that the water content requirement for Tinospora crispa (Brotowali) is not less than 4.4%, and the water-soluble extract obtained was 27.92%. The water-soluble extract content is necessary because this test reflects the total water-soluble compounds, which can provide an indication of the polar compound content in the sample, thus determining the compounds that play a role in determining certain effects, such as inhibition against Staphylococcus aureus in this study (Romadhani et al., 2025).

Ethanol-Soluble Extract Content

Table 5. Ethanol-Soluble Extract Content Test Results

No.	Sample Weight (g)	Extract Weight (g)	Ethanol-Soluble Extract Content (%)
1	5.0031 g	0.2382 g	23.80%
2	5.0047 g	0.3267 g	32.63%
3	5.0026 g	0.1991 g	19.89%
Average	—	—	**25.44%**

Based on the results of the ethanol-soluble extract study, it can be concluded that the sample's water content meets the requirements. This is based on the Indonesian Herbal Pharmacopoeia II Edition, which states that the water content requirement for Tinospora crispa (Brotowali) must be more than 15.4%, and the total ethanol-soluble extract obtained was 25.44% [16]. Similar to the water-soluble extract test, this test is necessary to determine the total ethanol-soluble compounds, which can provide an indication of the non-polar compounds in the sample, which may play a role in inhibiting Staphylococcus aureus (Romadhani et al., 2025).

Tinospora crispa Leaf Extract Fractionation Results

Table 6. Fractionation Results

Sample	Extract Weight (g)	Fraction Weight (g)	Yield (%)
n-Hexane Fraction of Brotowali Leaves	321.74 g	15.27 g	4.747%

The fractionation process yielded a relatively low total yield of 4.747%. This result is possible because the n-hexane fraction of plants often contains non-polar compounds such as terpenoids and steroids. This theory is supported by research conducted by Purnama et al. (2025). The Tinospora crispa leaf sample predominantly contained polar compounds. This statement is supported by the results of the higher water-soluble extract content compared to the ethanol-soluble extract content. Therefore, when fractionated with a non-polar solvent such as n-hexane, the yield obtained will also be lower.

Antibacterial Test Results of Tinospora crispa Leaf Extract Fractions

Table 7. Antibacterial Test Results of Tinospora crispa Leaf Extract Fractions

Sample	Concentration	Inhibition Zone (mm) – Replicate I	Replicate II	Replicate III	Average (mm)
n-Hexane Fraction of Brotowali Leaves	Positive Control (+)	32.28 mm	–	–	32.28 mm
	Negative Control (–)	0 mm	–	–	0 mm
	30%	11.21 mm	11.51 mm	10.76 mm	11.16 mm
	40%	11.53 mm	11.99 mm	10.90 mm	11.47 mm
	50%	11.65 mm	13.34 mm	13.15 mm	12.71 mm

Description: (+) Positive Control; (-) Negative Control

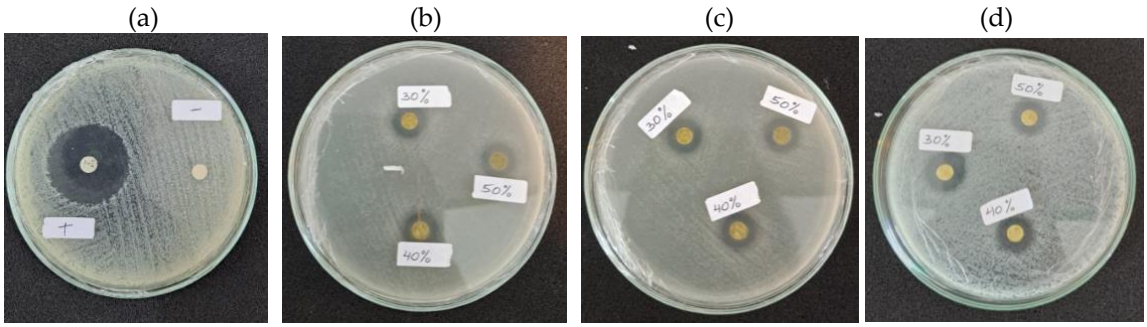

Figure 1. Antibacterial activity of the n-hexane fraction of brotowali leaves using the disc diffusion method (a) Antibacterial test using a positive control (ciprofloxacin) and a negative control (DMSO) (b) First repetition of the antibacterial activity test of the n-hexane fraction of brotowali leaves with concentrations of 30%, 40%, and 50% (c) Second repetition of the antibacterial activity test of the n-hexane fraction of brotowali leaves with concentrations of 30%, 40%, and 50% (d) Third repetition of the antibacterial activity test of the n-hexane fraction of brotowali leaves with concentrations of 30%, 40%, and 50%.

The results of the antibacterial test of the n-hexane fraction of brotowali leaves using the antibiotic ciprofloxacin as a positive control. This antibiotic selection was based on research Pamungkas & Nirwana (2023) which proved that ciprofloxacin is a broad-spectrum antibiotic capable of inhibiting Staphylococcus aureus bacteria. The negative control used was DMSO. DMSO was chosen based on research conducted by Tarigan & Sembiring, (2025), which showed that

DMSO has no antibacterial activity. Three concentrations were used in this study, with the largest inhibition zone at 12.71 mm at the 50% concentration. This indicates a relationship between increasing concentration and antibacterial inhibitory ability.

Cream Preparation Evaluation

Organoleptic Test

Table 8. Organoleptic Test Results

No.	Formula	Form/Consistency	Color	Odor
1	Base	Thick	White	Cream base odor
2	Formulation I	Thick	Pale green	Brotowali leaf extract odor
3	Formulation II	Thick	Light green	Brotowali leaf extract odor
4	Formulation III	Thick	Dark green	Brotowali leaf extract odor

Description: (FI) 12.5%; (FII) 25%; (FIII) 50%

All formulations had a thick, cream-like consistency. The color of each formulation darkened with increasing concentration. This is normal because the higher the concentration, the greater the n-hexane fraction of the Tinospora crispa leaves. The aroma of all three samples was the characteristic bitter aroma of Tinospora crispa leaf extract. This bitter aroma of Tinospora crispa leaves may originate from their phytochemical compounds, as they contain terpenoids and other volatile metabolites, potentially contributing to the characteristic bitter aroma (Anwar & Prasetyo, 2024).

pH Test

Table 9. pH Test Results

No.	Formula	pH Value
1	Base	7.87
2	Formulation I	4.27
3	Formulation II	5.74
4	Formulation III	6.21

Description: (FI) 12.5%; (FII) 25%; (FIII) 50%

The pH requirement for cream preparations is between 4.5 and 6.5, as stated in research conducted by Estefania et al. (2022). Therefore, based on this research, formulations II and III meet the requirements. Formulation I does not meet the requirements. This result may be due to the low fraction concentration, where the pH of the preparation tends to be more acidic due to the influence of weak acid compounds that are not balanced by emulsion stability, making the emulsion system in the cream very likely to become less stable. This theory is supported by research conducted, which states that variations in formulation composition can affect the physicochemical characteristics of cream preparations, one of which is pH. Differences in pH in this formulation illustrate that formulation components play a role in pH stability in topical preparations. The pH of the base can be higher than the required value because the base has not been added to the fraction, only contains distilled water, and several other conditions. Therefore, it is normal for the base pH to be close to the pH of distilled water, which is Arifin.

Spreadability Test

Table 10. Spreadability Test Results

No.	Formula	Load (g)	Spreadability (cm)
1	Base	50 g	3.8 cm
		100 g	4.3 cm
2	Formulation I	50 g	4.0 cm
		100 g	4.5 cm

3	Formulation II	50 g	4.3 cm
		100 g	4.6 cm
4	Formulation III	50 g	4.5 cm
		100 g	4.8 cm

Description: (FI) 12.5%; (FII) 25%; (FIII) 50%

The requirement for the spreadability test is 5-7 cm, as stated in research conducted by Sembiring & Faradina (2024). Therefore, based on the research conducted, no formulation has yet met this requirement. This result may be due to the thick viscosity of the formulation, as stated in research Maleh et al. (2024), which states that increasing viscosity decreases the spreadability test results.

Homogeneity Test

Table 11. Homogeneity Test Results

No.	Formula	Homogeneity
1	Base	Homogeneous
2	Formulation I	Homogeneous
3	Formulation II	Homogeneous
4	Formulation III	Homogeneous

Description: (FI) 12.5%; (FII) 25%; (FIII) 50%

From the results above, it can be seen that the three formulations and bases exhibited homogeneity, as indicated by the absence of lumps, coarse particles, or separation. These results demonstrate that the mixing process for the three formulations successfully produced a physically stable preparation.

Antibacterial Activity Test Results of the Brotowali Leaf Extract Fraction Cream

Table 12. Antibacterial Test Results of the Preparations

Sample	Concentration	Inhibition Zone Diameter (mm) – Replicate I	Replicate II	Replicate III	Average (mm)
Cream of n-Hexane Fraction of Brotowali Leaves	Positive Control (+)	27.05 mm	–	–	27.05 mm
	Negative Control (–)	0 mm	–	–	0 mm
	12.5%	9.55 mm	7.90 mm	7.71 mm	8.39 mm
	25%	11.47 mm	10.74 mm	8.43 mm	10.21 mm
	50%	12.33 mm	12.74 mm	11.96 mm	12.34 mm

Description: (+) Positive Control; (-) Negative Control

The results of the antibacterial test of the n-hexane fraction cream preparation of brotowali leaves show that the n-hexane fraction cream preparation of brotowali leaves has the ability to inhibit Staphylococcus aureus bacteria. The increase in the inhibition zone is also proportional to the increase in concentration, this statement is proven by the largest inhibition zone at a concentration of 50%. Although the inhibition zone created by the cream preparation is smaller than the inhibition zone of the pure fraction, this difference may be caused by the cream base because research Octora et al. (2024) explains that the inhibition zone produced by the preparation is smaller than the pure fraction is something normal, this is because the cream base can inhibit the diffusion of the active substance even though its inhibitory activity is still visible.

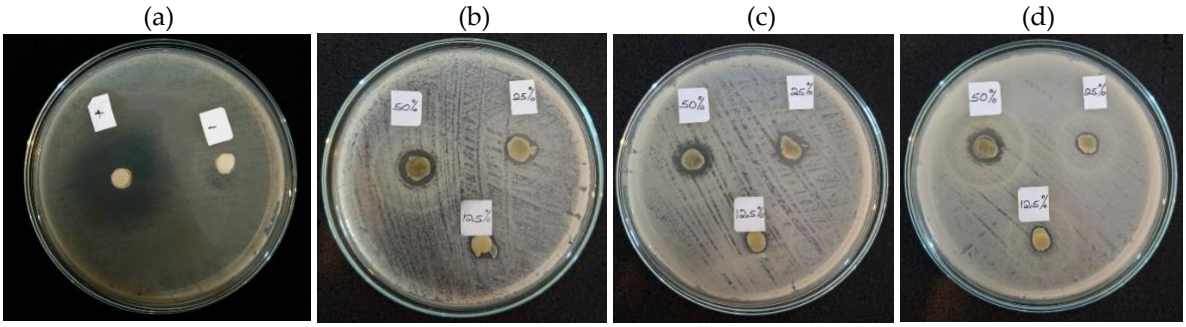

Figure 2. Antibacterial activity of n-Hexane fraction cream of brotowali leaves using disc diffusion method (a) Antibacterial test using positive control (clindamycin) and negative control (base) (b) first repetition of antibacterial activity test of n-Hexane fraction cream of brotowali leaves with concentrations of 12.5%, 25% and 50% (c) second repetition of antibacterial activity test of n-Hexane fraction cream of brotowali leaves with concentrations of 12.5%, 25% and 50% (d) third repetition of antibacterial activity test of n-Hexane fraction cream of brotowali leaves with concentrations of 12.5%, 25% and 50%.

CONCLUSION

Based on this research, it can be concluded that the n-Hexane fraction of Tinospora crispa L. leaves has antibacterial activity against Staphylococcus aureus bacteria, where the largest inhibition zone is at a concentration of 50%, namely 12.71 mm. The most effective formulation of the n-Hexane fraction cream of Tinospora crispa L. leaves in the treatment of diabetic ulcers through antibacterial testing on Staphylococcus aureus is a cream containing a concentration of 50%, namely 12.34 mm and with the high inhibition zone created from the results of the antibacterial test of the n-Hexane fraction cream of Tinospora crispa L. leaves, it can be concluded that there is potential to accelerate the healing of diabetic ulcers, this statement is proven by the large inhibition zone formed by the cream during the antibacterial test.

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