Journal of Health Management and Pharmacy Exploration
https://shmpublisher.com/index.php/johmpe
p-ISSN: 2985-4814
e-ISSN: 2985-5543

Formulation and antifungal activity test of ethanol extract of cashew leaves (anacardium occidentale l.) gel for thrush against candida albicans fungus

Nurfantri Dinar[1*], Himaniarwati[2], Wa Ode Ida Fitriah[1]
[1] Faculty of Science and Technology, Mandala Waluya University, Indonesia
[2] Department of Pharmacy, Mandala Waluya University, Indonesia

Article Info

Article history:
Received April 19, 2026
Revised May 04, 2026
Accepted May 08, 2026

Keywords:
Thrush gel
Cashew leaves
Antifungal
Candida albicans
Ethanol extract

ABSTRACT

Cashew leaves (Anacardium occidentale L.) contain bioactive compounds, including flavonoids, saponins, and tannins, which exhibit potential antifungal activity against Candida albicans. This study aimed to formulate a canker sore gel containing ethanol extract of cashew leaves and to evaluate its antifungal activity against Candida albicans. The extract was obtained by maceration using 96% ethanol. Gel formulations were prepared at concentrations of 5%, 7.5%, and 10%. Physical evaluations included organoleptic properties, homogeneity, viscosity, pH, spreadability, and adhesiveness. Antifungal activity was assessed using the agar well diffusion method, with 2.2% nystatin as the positive control and the gel base as the negative control. All formulations met the requirements for a good gel preparation, exhibiting a semi-solid consistency, brownish-green color, characteristic odor, and homogeneous texture. The viscosity ranged from 120–250 dPas, and the pH was 6, indicating suitability for oral mucosal application. Inhibition zones against Candida albicans were 10.3 mm, 11.6 mm, and 12.3 mm for the 5%, 7.5%, and 10% formulations, respectively. The 7.5% and 10% formulations demonstrated strong antifungal activity. Statistical analysis revealed that concentration variation significantly affected antifungal activity ($p < 0.05$). Significant differences were observed between the 5% formulation and the 7.5% and 10% formulations, whereas no significant difference was found between the 7.5% and 10% formulations. Cashew leaf ethanol extract can be formulated into an effective canker sore gel, with the optimal antifungal activity observed at concentrations of 7.5%–10%.

**Corresponding Author:*
Nurfantri Dinar,
Faculty of Science and Technology,
Mandala Waluya University, Indonesia.
Email: dinarnurfantri@gmail.com
DOI: https://doi.org/10.52465/johmpe.v4i1.676

1. Introduction

Indonesia is a country located on the equator with a tropical climate characterized by high humidity. These high-humidity conditions facilitate the growth and spread of fungal organisms. One of the diseases caused by such fungal infections is oral thrush, which can affect the oral mucosa and cause pain and discomfort [1].

Canker sores remain one of the most common conditions in the general population, including *Recurrent Aphthous Stomatitis* (RAS) and traumatic ulcers. Canker sores are typically accompanied by significant pain due to the loss of epithelial tissue on the soft tissues within the oral cavity; proper management is essential for optimal treatment. Open wounds on the soft tissues of the oral cavity, or traumatic ulcers, are generally caused by mechanical trauma, with the most common cause being a bite [2]. In Indonesia, the recurrence rate of *recurrent aphthous stomatitis* is higher among women than among men, with a rate of 47.6% among women and 21.3% among men. This difference indicates that women tend to experience recurrent aphthous stomatitis more frequently than men [3]. One of the main causes of thrush is a fungal infection, particularly by *Candida albicans*. As an opportunistic fungus, *Candida albicans* not only causes thrush but can also trigger lesions in the oral cavity and gastrointestinal tract, including stomach ulcers (*gastric ulcer*) [4].

Next, to explore potential therapeutic applications, this study collected samples of cashew leaves (*Anacardium occidentale* L.), a rapidly expanding agricultural commodity in Eastern Indonesia. This plant belongs to the Anacardiaceae family and is known for its antimicrobial activity, making it a promising candidate for use in medicine or natural health products. According to Astuty (2022), cashew leaves contain active compounds such as alkaloids, saponins, tannins, flavonoids, and phenolic compounds that function as antifungals. These chemical components make the cashew plant (*Anacardium occidentale* L.) naturally beneficial in treating thrush. [5].

According to a study conducted by Yunita et al. (2024) [6], cashew leaf ethanol extract exhibits antifungal activity against *Candida albicans*, with inhibition zones at concentrations of 5%, 7.5%, and 10%, respectively, and inhibition diameters of 10.35 mm, 11.93 mm, and 14.2 mm [6]. In addition, given the bioactive potential of cashew leaves, developing the appropriate dosage form is crucial to ensure that the active compounds within them can work effectively. One suitable dosage form is a gel, as it has a semi-solid consistency that is easy to apply, provides a cooling effect, and is capable of delivering active ingredients locally, particularly to mucous membranes such as those in the oral cavity.

A gel is a semi-solid preparation consisting of a suspension of small inorganic particles or large molecules dispersed in a liquid. Gels serve as drug carriers specifically designed for the delivery of medication to mucosal surfaces, including the oral mucosa, thereby maximizing the effectiveness of local therapy [7]. The base used in this gel formulation for

the treatment of canker sores is Carbomer 940. The use of Carbomer as a gel base allows for the formation of a gel with good physical properties [8] .

Based on the above discussion, the researchers were interested in developing a formulation by creating a gel preparation for canker sores using ethanol extract of cashew leaves (*Anacardium occidentale* L.) as the active ingredient. This study also aims to evaluate the antifungal activity of the gel formulation against *Candida albicans*, as well as to observe the physical characteristics of the formulation, such as organoleptic properties, homogeneity, viscosity, pH, syneresis, spreadability, and barrier properties. Thus, it is hoped that this gel formulation can serve as an effective, natural-based alternative treatment for thrush.

2. Method

This study is an analytical quantitative study conducted in an experimental laboratory with the aim of comparing the control group to determine the antifungal activity against *Candida albicans* in a gel formulation for canker sores made from ethanol extract of cashew leaves (*Anacardium occidentale* L.).

2.1. Materials and Supplies

This study utilized a population and sample of cashew trees (*Anacardium occidentale* L.) obtained in Tondonggeu Village, Nambo Subdistrict, Kendari City, Southeast Sulawesi. The plant part used was the leaves. The equipment used in this study included an autoclave (Cryste Purister), Erlenmeyer flasks (Pyrex®), a loop, a Bunsen burner, gauze, a tripod, pipettes (1 ml, 5 ml, 10 ml, and 20 ml), an incubator (Memmert-IF30), Petri dishes (Iwaki), porcelain dishes, glass funnels, an analytical balance (Ohaus), an oven (Memmert UN110), stirring rods, measuring cylinders (Iwaki), beakers (Pyrex®), gel containers, a mortar and pestle, pH paper (Nesco), transparent glass, weights (200 grams), glass slides, viscometer (Rion VT-06), aluminum foil, filter paper, rotary evaporator (RE100 Pro), dropper pipettes (Pyrex®), forceps, test tubes (Pyrex®), test tube racks, and maceration vessels.

2.2. Extraction

2.2.1. Sample

The sample used was cashew leaves; sample preparation began with a dry sorting stage to separate the cashew leaves from their stems. Next, wet sorting was performed under running water to remove impurities, followed by draining to reduce excess water. After drying, the sample was weighed and ground using a blender until a fine powder was obtained. A fine powder weighing 1714 g of ground cashew leaves was weighed and placed in a container for maceration. After the sample was submerged, 96% ethanol solvent was added. The jar was covered with aluminum foil and sealed with black tape to avoid direct light. It was stored for three days at room temperature, protected from light, and shaken three times daily: morning, noon, and evening. Subsequently, the maceration product was filtered and concentrated in a rotary evaporator (40–65 °C, 60 rpm) to produce a concentrated extract.

2.2.2. Maceration Method

Extraction of cashew leaves (*Anacardium occidentale* L.) was performed using the maceration method, a technique involving the soaking of crude drug powder to extract active compounds from the natural material. A total of 1,714 grams of crude drug powder was soaked in 96% ethanol at room temperature for at least three days until a liquid extract was obtained. The use of 96% ethanol was chosen due to its effectiveness in dissolving antifungal secondary metabolites, such as flavonoids, tannins, and phenols. This solvent has the ability to extract chemical constituents thoroughly, whether they are non-polar, semipolar, or polar. [9].

2.3. Procedure for Making a Canker Sore Gel

Prepare the equipment and ingredients to be used, then weigh each according to the formulation: cashew leaf extract at concentrations of 5%, 7.5%, and 10% as the active ingredient, Carbopol 940 at 1.5% as the gelling agent, glycerin at 5% as the humectant, triethanolamine at 2% as the pH balancer, sodium saccharin at 0.3% as the sweetener, methylparaben at 0.2% as the preservative, and distilled water to 20 ml as the solvent. Carbopol 940 is dissolved in hot distilled water and stirred until homogeneous to form a gel mass. Next, triethanolamine is added gradually to neutralize the acidity of Carbopol while stirring continuously until homogeneous. Then, methylparaben dissolved in hot distilled water is added to the gel mass, followed by the addition of sodium saccharin dissolved in a small amount of distilled water and glycerin as a humectant, and stirred until homogeneous. The cashew leaf extract, which has been dissolved in glycerin, is then added little by little to the gel base while stirring until evenly mixed, and in the final stage, the homogeneous canker sore gel preparation is transferred into a clean, tightly sealed container.

2.3.1. Organoleptic Test

Organoleptic testing of gel formulations involves observing changes in shape, color, and odor during storage at room temperature. Organoleptic requirements stipulate that gel formulations must remain stable over a specific storage period without undergoing significant changes. The shape or consistency of a gel is greatly influenced by its viscosity. High viscosity indicates strong intermolecular bonds, which prevent structural deformation during storage or clinical use [10].

2.3.2. Homogeneity test

A homogeneity test was conducted by applying the preparation to a transparent glass slide to ensure that all components were evenly dispersed without any clumps. Small, uniform particle size is a key indicator that the preparation has good physical stability [10].

2.3.3. Viscosity Test

To measure viscosity, the evaluation was performed using a Rion VT viscometer by attaching the rotor to the device via a clockwise locking mechanism. After the cup is filled with the gel sample, the rotor is positioned exactly at the center of the container before the device is activated. The measurement is performed using rotor number 2 until

equilibrium is reached, at which point the viscosity value will be displayed in dPaS. The viscosity parameters that meet the ideal standard for gel formulations fall within the range of 50 to 400 dPaS [11].

2.3.4. pH Test

The pH test was conducted using pH paper with an ethanol extract gel of cashew leaves. The procedure began by weighing 0.5 g of the ethanol extract gel of cashew leaves, which was then dissolved in 5 mL of distilled water until homogeneous. After complete dissolution, the pH paper was introduced into the test solution to determine the measured pH value. The purpose of this analysis was to ensure the biocompatibility of the preparation with the skin and oral mucosa, with the target pH range set between 5.5 and 7.9 [7].

2.3.5. Coverage Test

The spread test was conducted by placing 0.5 g of gel between two graduated glass plates, then measuring its diameter after letting it sit for 1 minute, both without a load and with an additional 200 g load. An ideal gel formulation must meet the standard spread diameter of 5–7 cm to ensure ease of use during application [12].

2.4. Antifungal Activity Assay

2.4.1. Instrument Sterilization

The equipment sterilization procedure involves cleaning the instruments, followed by thorough drying. To prevent airborne contamination, the mouths of Erlenmeyer flasks and test tubes are sealed with cotton plugs wrapped in gauze, while other glassware, such as Petri dishes, are protected with tight-fitting paper wraps. The final stage of sterilization uses the dry heat sterilization method in an oven at a controlled temperature of 180°C for a duration of 2 hours, with the aim of achieving sterile conditions before the equipment is used in clinical or laboratory procedures.

2.4.2. Mushroom Substrate Preparation

To prepare the agar medium, 6.825 g of Potato Dextrose Agar (PDA) was weighed and dissolved in 175 mL of distilled water in an Erlenmeyer flask. The mixture was heated on a hot plate. Next, the homogenized PDA medium was sterilized in an autoclave at 121°C for 15 minutes. After the sterilization process is complete, the medium is removed and cooled to a temperature of approximately 45°C, then poured into sterile Petri dishes, 20 ml each. The poured medium is left to solidify at room temperature; the PDA medium is then ready for the cultivation of Candida albicans.

2.4.3. Preparation of Test Mushrooms

Candida albicans was prepared by taking a single loopful of a pure Candida albicans culture using a sterile loop, then streaking it onto slanted Potato Dextrose Agar (PDA) and incubating it at 25–30°C for 24–48 hours until colonies grew well. Next, a loopful of the rejuvenated fungal colony is taken and placed into a test tube containing 9 mL of 0.9% NaCl solution, then shaken until homogeneous to form a fungal suspension. This standardized Candida albicans suspension is then ready for use in antifungal activity testing.

2.4.4. Inhibitory Zone Testing

Antifungal efficacy testing was conducted using the agar diffusion method with the well diffusion technique on Potato Dextrose Agar (PDA) medium. The antifungal potential of an ethanol leaf extract gel from cashew (Anacardium occidentale L.) was evaluated against Candida albicans isolates using the well diffusion method. The procedure began with the inoculation of 1 mL of fungal suspension into 15 mL of liquid Potato Dextrose Agar (PDA) medium at 45°C, which was then transferred to Petri dishes until it reached the solid phase. The solidified medium was then perforated with a 6 mm cork borer to form wells, and each well was filled with 50 µL of gel samples at concentrations of 5%, 7.5%, and 10% to observe the effectiveness of the resulting inhibition zones. Nystatin orabase was used as the positive control, while the negative control consisted of gel base without extract. All petri dishes were left for approximately 30 minutes at room temperature to allow the test material to permeate the medium. Afterward, the petri dishes were incubated upside down at 28–30°C for 48 hours. Antifungal activity was assessed based on the formation of a clear zone (inhibition zone) around the wells, indicating inhibition of Candida albicans growth. The diameter of the inhibition zone was measured using a Vernier caliper in millimeters.

2.5. Data Analysis

This study involved the development of a gel formulation for treating canker sores and the evaluation of the physical quality of the formulation, including organoleptic testing, homogeneity testing, viscosity testing, pH testing, syneresis testing, adhesion testing, and testing of the inhibitory effect against Candida albicans. To determine whether the diameter of the inhibition zone affects the growth of Candida albicans, a statistical analysis was performed using a one-way ANOVA with SPSS version.

2.6. Plant Identification

The identification of Cashew Leaf (Anacardium occidentale L.) samples was conducted at the Pharmacognosy and Phytochemistry Laboratory, Faculty of Pharmacy, Mandala Waluya University. The results of this identification were used to verify and confirm the presence of the species. The results confirmed that the plant used in this study is the cashew leaf (Anacardium occidentale L.). The results of the cashew leaf identification are documented in a letter numbered 127/09.03.01/IV/2026.

3. Results and Discussion

Table 1. Formula design for an ethanol extract of cashew leaf for canker sores

Ingredients	Formula				Function	Range (%)
	0	I	II	III		
Cashew leaf extract	0	5%	7,5%	10%	Active ingredients	
Carbopol 940	1,5%	1,5%	1,5%	1,5%	Gelling agent	0,5-2,0%
Glycerin	5%	5%	5%	5%	Humectan	<30%
Trietanolamin (TEA)	2%	2%	2%	2%	pH balancer	2-4%
Na. Sakarin	0,3%	0,3%	0,3%	0,3%	Sweetener	0,12-0,3%
Methyl paraben	0,18%	0,18%	0,18%	0,18%	Preservative	0,2-0,3%
Aquadest ad	40 ml	20 ml	20 ml	20 ml	Solvent	

The development of this formulation serves as a systematic guideline to ensure the consistency and stability of the resulting gel formulation. By setting the concentrations of cashew leaf extract at 5%, 7.5%, and 10%, this formulation aims to compare the effect of active ingredient dosage on the physical characteristics and efficacy of the formulation.

3.1. Organoleptic Test

Table 2. Organoleptic test

Formula	Examination	Organoleptic Evaluation In Week		
		I	II	III
F0	Color	Clear and transparent	Clear and transparent	Clear and transparent
F1		Brownish-green	Brownish-green	Brownish-green
F2		Brownish-green	Brownish-green	Brownish-green
F3		Brownish-green	Brownish-green	Brownish-green
F0	Smell	Odorless	Odorless	Odorless
F1		The distinctive aroma of cashew leaf ethanol extract	The distinctive aroma of cashew leaf ethanol extract	The distinctive aroma of cashew leaf ethanol extract
F2		The distinctive aroma of cashew leaf ethanol extract	The distinctive aroma of cashew leaf ethanol extract	The distinctive aroma of cashew leaf ethanol extract
F3		The distinctive aroma of cashew leaf ethanol extract	The distinctive aroma of cashew leaf ethanol extract	The distinctive aroma of cashew leaf ethanol extract
F0	Shape	Semi-solid dosage form	Semi-solid dosage form	Semi-solid dosage form
F1		Semi-solid dosage form	Semi-solid dosage form	Semi-solid dosage form
F2		Semi-solid dosage form	Semi-solid dosage form	Semi-solid dosage form
F3		Semi-solid dosage form	Semi-solid dosage form	Semi-solid dosage form

The results of the organoleptic test on the cashew leaf (*Anacardium occidentale* L.) ethanol extract gel formulation for canker sores, based on the formulation′ s odor and color. The results of the organoleptic test on the cashew leaf (*Anacardium occidentale* L.) ethanol extract gel formulation for canker sores at concentrations F1 (5%), F2 (7.5%), and F3 (10%) showed consistent results: color = brownish-green, odor = characteristic of cashew leaf ethanol extract, and consistency = semi-solid formulation from week 1 to week 3. In formula F0 (without extract), the results were color = clear and transparent, odor = odorless, and form = semi-solid preparation from week 1 to week 3. According to Rusli et al. (2021), organoleptic requirements mandate that the gel preparation remain stable over a specific storage period without undergoing significant changes[10].

3.2. Homogeneity Test

Table 3. Homogeneity test

Formula	Homogeneity In Week		
	I	II	III
F0	Homogeneous	Homogeneous	Homogeneous
F1	Homogeneous	Homogeneous	Homogeneous
F2	Homogeneous	Homogeneous	Homogeneous
F3	Homogeneous	Homogeneous	Homogeneous

The results of the homogeneity test on the ethanol extract gel of cashew leaves

(Anacardium occidentale L.) for canker sores, with the test parameters being that the preparation must exhibit a homogeneous composition and contain no coarse particles. In the homogeneity test, homogeneity was confirmed at each concentration–F0 (without extract), F1 (5%), F2 (7.5%), and F3 (10%) from week 1 through week 3. This falls under physical stability; according to Rusli et al. (2021), small and uniform particle size is a key indicator that a preparation has good physical stability.

3.3. Viscosity Test

Table 4. Viscosity test

Formula	Viscosity Measurement (Dpas) In Week			Average Diameter
	I	II	III	
F0	250	250	250	250 ± 0,0
F1	180	180	170	177 ± 5,8
F2	150	150	150	150 ± 0,0
F3	120	140	140	133 ± 11,5

Viscosity tests on the canker sore gel made from ethanol extract of cashew leaves (*Anacardium occidentale* L.) were conducted using a viscometer with rotor number 2. The results showed that the dPas values for formulations F0 and F2 remained unchanged, this was due to the interaction between the Carbopol gel base and the ethanol extract of cashew leaves (*Anacardium occidentale* L.). Carbopol, as a gel-forming polymer, can have its structure disrupted by the presence of active compounds such as flavonoids, tannins, and saponins, which affect the pH and interact with the carboxyl groups, resulting in suboptimal swelling [13]. In formula F1, viscosity increased in the third week, and in formula F3, viscosity increased in the second week. However, the viscosity values in the test still met the criteria established by Higyeungsi *et al.* (2023), who state that the recommended viscosity range for gel formulations is 50–400 dPas.

3.4. pH Test

Table 5. pH test

Formula	pH Measurement In Week			Average Diameter
	I	II	III	
F0	6	6	6	6 ± 0,0
F1	6	6	6	6 ± 0,0
F2	6	6	6	6 ± 0,0
F3	6	6	6	6 ± 0,0

pH testing of the cashew leaf (*Anacardium occidentale* L.) ethanol extract mouth ulcer gel was conducted using a universal indicator in the form of pH paper, with the requirement that the formulation must have a pH consistent with that of the oral mucosa, namely 5.5–7.9. The pH test results showed consistent results for the cashew leaf (*Anacardium occidentale* L.) ethanol extract mouth ulcer gel formulation at concentrations F0 (without extract), F1 (5%), F2 (7.5%), and F3 (10%) from week 1 to week 3, the pH remained at 6 and was still within the range suitable for the oral mucosal membrane, which is 5.5–7.9 [7].

3.5. Coverage Test

Table 6. Coverage test

Formula	Weekly Distribution Observation			Average Diameter
	I	II	III	
F0	5,8 cm	5,7 cm	5,7 cm	5,7 ± 0,06
F1	6 cm	6 cm	5,9 cm	6 ± 0,06
F2	6,6 cm	6,6 cm	6,3 cm	6,5 ± 0,17
F3	6,6 cm	6,3 cm	6,4 cm	6,4 ± 0,15

The results of the spreadability test on the ethanolic extract gel of cashew leaves (*Anacardium occidentale* L.) showed that all gel formulations exhibited relatively stable spreadability during a 3-week storage period. Formula F0 (without extract) had an average spread diameter of 5.7 cm, F1 (5%) was 6 cm, F2 (7.5%) was 6.5 cm, and F3 (10%) was 6.4 cm. In general, formulations with higher concentrations (F2 and F3) exhibited greater spreadability compared to F0 and F1, while still falling within the acceptable spreadability range for gel formulations. According to Mangkey et al. (2023), an ideal gel formulation must meet the standard spread diameter of 5–7 cm to ensure ease of use during application.

3.6. Antifungal Activity of An Ethanol Extract of Cashew Leaves (Anacardium occidentale L.)

The results of antifungal activity testing on a gel formulation for canker sores containing 5%, 7.5%, and 10% ethanol extract of cashew leaves (*Anacardium occidentale* L.) were conducted in the microbiology laboratory to determine the average diameter of the inhibition zone against *Candida albicans*. The average diameters are shown in the following Table 7.

Table 7. Results of antifungal activity testing for *candida albicans*

Treatment	Test mushroom	Average diameter of inhibition zone			Average diameter of inhibition zone	Information
		Replikasi I (mm)	Replikasi II (mm)	Replikasi II (mm)		
K+	*Candida albicans*	21 mm	21,3 mm	22 mm	21,4 mm ± 0,51	Very strong
F0						
F1		10 mm	10,3 mm	10,6 mm	10,3 mm ± 0,30	currently
F2		11 mm	11,3 mm	12,6 mm	11,6 mm ± 0,85	Strong
F3		11,3 mm	12 mm	13 mm	12,3 mm ± 0,85	Strong

Information:
K+ = Positive control of Nystatin orabase drug
F0 = Canker sore gel formula without extract
F1 = 5% concentrated canker sore gel formula
F2 = 7.5% concentration mouth ulcer gel formula
F3 = 5% concentrated canker sore gel formula

Figure 1. Observation of the inhibition zones of Replicas I, II, and III against the positive control (Nyastin) in *Candida albicans*

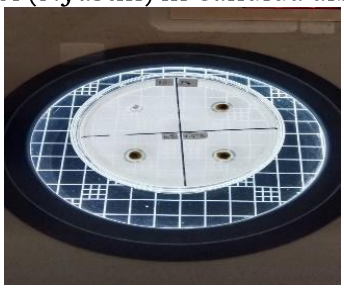

Figure 2. Observation of replication inhibition zone I in the negative control at concentrations of 5%, 7.5%, and 10%

Figure 3. Observation of replication inhibition zone II in the negative control at concentrations of 5%, 7.5%, and 10%

Figure 4. Observation of replication inhibition zone III in the negative control at concentrations of 5%, 7.5%, and 10%

The antifungal activity of cashew leaf (*Anacardium occidentale* L.) ethanol extract was tested using the well diffusion method, which involves making holes in the culture medium and then placing the antifungal sample into the holes. PDA (Potato Dextrose Agar) medium was used for the antifungal testing because it is one of the most commonly used culture media for fungal testing. The experiment was conducted in triplicate to compare the inhibition zones formed; the test measured the diameter of the inhibition zone or the clear area surrounding the sample in the medium.

The results of the antifungal activity test of ethanol extracts from cashew leaves (*Anacardium occidentale* L.) against *Candida albicans* showed that antifungal activity at each concentration exhibited varying levels of inhibitory potency. At a 5% concentration, the average diameter of the inhibition zone was 10.3 mm, which is still categorized as

moderate since inhibition zones typically range from 5 to 10 mm. At a 7.5% concentration, the average diameter was 11.6 mm, and at a 10% concentration, the average diameter was 12.3 mm; these were categorized as strong inhibitory activity because the inhibition zones fell within the 11–20 mm range. This indicates that as the concentration of the ethanol extract of cashew leaves (*Anacardium occidentale* L.) increases, the inhibitory activity against *Candida albicans* also increases. When compared to the negative control (blank without extract), the average inhibition zone was 0 mm, indicating that the solvent and additives used did not affect the antifungal test results. In the positive control (Nystin orabase), the average inhibition zone diameter was 21.4 mm, categorized as very strong, this can be seen in Table 1.

According to a study by Yunita et al. (2024) [6], plant compounds with antifungal activity include saponins, flavonoids, and tannins. Flavonoids act as antifungals through the mechanism of genistein, which inhibits fungal cell division. These compounds work by binding to microtubule proteins, which directly disrupts the mitosis process and ultimately inhibits overall fungal growth. Additionally, tannins inhibit the biosynthesis of ergosterol the primary sterol responsible for fungal cell membrane formation. Meanwhile, saponins exhibit antifungal activity by reducing the surface tension of the sterol membrane in the fungal cell wall, which increases cell permeability. This leads to cellular leakage, characterized by the outflow of intracellular fluid or the inflow of extracellular fluid into the fungal cell, resulting in fungal cell death, as seen in *Candida albicans*.

The active compounds present in the cashew leaf extract obtained can be linked to the results of the antifungal activity test, which showed an increase in the inhibition zone at higher concentrations; the concentrations used in this study were 5%, 7.5%, and 10%. According to Tivani (2020) [14], the higher the concentration of the leaf extract used as a sample to create an antifungal formulation, the larger the inhibition zone against *Candida albicans* [14]. The active compounds saponins, flavonoids, and tannins–have antifungal effects; these active compounds significantly contribute to the size of the resulting inhibition zone..

4. Conclusion

Based on the results of the study titled "Formulation and Testing of the Antifungal Activity of a Cashew Leaf (Anacardium occidentale L.) Ethanol Extract Gel Preparation for Canker Sores Against Candida albicans," it can be concluded that:

1. Ethanol extract of cashew leaves (Anacardium occidentale L.) can be formulated into a gel preparation, and the results indicate that the ethanol extract of cashew leaves (Anacardium occidentale L.) gel preparation for canker sores meets the criteria for a good gel preparation based on organoleptic testing, homogeneity testing, viscosity testing, pH testing, and spreadability testing.
2. The cashew leaf (Anacardium occidentale L.) ethanol extract gel formulation exhibits antifungal activity against Candida albicans, as indicated by the formation of inhibition zones; the average diameter at the optimal concentration was 11.6 mm (strong category) at a concentration of 7.5%, and at a concentration of 10% = 12.3 mm

(strong category), as well as the positive control = 21.4 mm (very strong category) in inhibiting the growth of Candida albicans

3. Variations in the concentration of cashew leaf (Anacardium occidentale L.) ethanol extract significantly affect antifungal activity against Candida albicans, where statistical test results indicate a significant difference (<0.05) at a 5% concentration, compared to the positive control and negative control. However, based on the LSD post-hoc test, no significant difference was found between the 7.5% and 10% concentrations, so the 7.5% concentration is considered insignificant.

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