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The Effectiveness of Antifungal Emulgel Containing Ethanol Extract of Ketepeng Cina Leaves (*Cassia alata* L.) in Inhibiting the Growth of Dermatophyte Fungi

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Abstract: *The leaves of Cassia alata L. (Ketepeng cina) are known to have antifungal properties and can be used to treat fungal skin infections such as tinea versicolor and ringworm. The leaf extract of Cassia alata contains antimicrobial compounds that inhibit fungal growth. The aim of this study was to determine the effectiveness of an antifungal emulgel containing ethanol extract of Cassia alata leaves in inhibiting the growth of dermatophyte fungi. This research was conducted from May to December 2024 at the Pharmacy Laboratory and Microbiology Laboratory of Cendrawasih University Jayapura. The plant samples were collected from Koya Barat, Jayapura City, and extracted using the maceration method with 96% ethanol. Phytochemical screening tests showed that Cassia alata leaves were positive for alkaloids, saponins, tannins, flavonoids, and phenols. The antifungal activity test of the ethanol extract emulgel formulation against Trichophyton rubrum, Microsporum canis, and Epidermophyton floccosum showed significance values lower than the commonly accepted level ($\alpha = 0.05$), indicating that the null hypothesis (H_0) was rejected and the alternative hypothesis (H_1) was accepted. Based on these findings, it can be concluded that the antifungal emulgel formulation containing ethanol extract of Cassia alata leaves is effective against Epidermophyton floccosum at a concentration of 1000.*

Keywords: *Cassia alata L. leaves, Ethanol extract, Emulgel, Dermatophyte fungi*

1. INTRODUCTION

Indonesia, located in the tropical region and crossed by the equator, has high temperature and humidity characteristics. These conditions are highly conducive to the growth of various microorganisms, including pathogenic fungi. One of the most commonly encountered fungal infections is caused by dermatophytes. Dermatophytes are a group of fungi that infect keratinized tissues such as skin, nails, and hair. This infection, known as tinea, is estimated to affect around 20 to 25% of the global population (Muhammad & Nur, 2023).

Dermatophytosis, a fungal infection of the skin, is a significant global health concern. Hay et al. (2014) reported that tinea corporis, one form of dermatophytosis, has the highest prevalence in Asia, reaching 35.4%. In Indonesia, dermatophytosis ranks second after pityriasis versicolor, with the most common cases being tinea cruris and tinea corporis (Agustine, 2012; Pravitasari et al., 2019).

Various plants in Indonesia are known to have medicinal properties, one of which is the leaf of Ketepeng Cina (Cassia alata L.). This plant contains several secondary metabolites, including alkaloids, flavonoids, saponins, terpenoids, steroids, and tannins, which are known to have antifungal potential (Fajri, Marfu'ah, & Artanti, 2018).

Emulgel, as a topical dosage form, offers several advantages that support improved therapeutic effectiveness and patient comfort in the treatment of dermatophytosis. According to

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Rowe et al. (2009), emulgel exhibits high stability, good spreadability, and more effective skin penetration compared to conventional topical formulations. Its stability is supported by the gel network, which prevents the separation of oil and water phases, while its good spreadability enhances application comfort. Furthermore, the combination of oil and water phases in the emulgel system can enhance the penetration of active ingredients into the skin. Therefore, an emulgel formulated with Ketepeng Cina (Cassia alata L.) leaf extract has the potential to provide more effective antifungal therapy in inhibiting the growth of dermatophyte fungi.

2. RESEARCH METHOD

Time and Place

This research was conducted from May 2024 to December 2024 at the Pharmacy Laboratory of the Health Polytechnic of the Ministry of Health Jayapura and the Microbiology Laboratory of Cendrawasih University.

Research Instruments

Tools

A set of maceration tools, measuring cylinders, beakers, Erlenmeyer flasks, volumetric flasks, stirring rods, porcelain dishes, analytical balance, water bath, microscope, oven, incubator, laminar air flow (LAF), petri dishes, emulgel containers, inoculating loop, L-shaped spreader, caliper, ruler, emulgel molds, fruit dryer.

Materials

Aluminum foil, filter paper, cover glass and object glass, 96% ethanol, 1% ketoconazole, DMSO (Dimethyl Sulfoxide), Sabouraud Dextrose Agar (SDA) media, 0.5 McFarland standard, 0.85% NaCl, sterile distilled water, spiritus, aquadest, glycerin, propylene glycol, TEA, carbopol, *Cassia alata* L. (Ketepeng Cina) leaf simplicia, and dermatophyte fungi including *Trichophyton rubrum*, *Microsporum canis*, and *Epidermophyton floccosum*.

Research Design

This study was designed using a Completely Randomized Design (CRD) with two tested factors. The first factor is the ethanol extract of *Cassia alata* leaves, tested at six different concentration levels. The second factor is the emulgel formulation of the same ethanol extract, tested at five different concentrations. Each combination of these two factors was repeated three times to ensure the validity and reliability of the research results.

Preparation of Simplicia

Fresh *Cassia alata* leaves (5 kg) were collected and sorted to remove dirt and damaged leaves. The leaves were washed, chopped into smaller pieces, and dried using a fruit dryer at 40°C. After drying, a second dry sorting was done, followed by grinding into a fine powder using a blender.

Preparation of Cassia alata Leaf Extract

The simplicia was extracted using maceration with a 1:5 ratio — 450 g of powdered leaves soaked in 2,250 mL of 96% ethanol for 3 days with occasional stirring. The extract was filtered and concentrated using a water bath. The thick extract was prepared into six concentrations: 50, 100, 250, 500, 750, and 1000 ppm.

Phytochemical Screening of Cassia alata Leaves

Phytochemical tests were conducted to identify secondary metabolites:

- **Flavonoids:** 1 g of powder extracted with ethanol, heated, then tested with HCl and magnesium; red precipitate indicates positive.
- **Alkaloids:** 4 g of sample treated with chloroform and ammonia, filtered, and tested with Meyer's reagent; white precipitate indicates positive.
- **Saponins:** 10 g of sample extracted with hot methanol, tested with Libermann-Burchard and foam formation test; stable foam indicates positive.
- **Tannins:** 20 mg of sample mixed with ethanol and $FeCl_3$ 1%; a bluish-black or green color indicates positive.

Preparation of Extract Treatment Concentrations

Each concentration was prepared by dissolving 1–6 grams of *Cassia alata* ethanol extract into 10 mL of 5% DMSO (prepared by mixing 5 mL DMSO into 100 mL distilled water).

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Dermatophyte Culture Quality Test

- **KOH Staining:**
  Fungal samples were placed in 10–20% KOH on a slide, covered with a cover glass, and left for 10–30 minutes to dissolve keratin. Microscopic examination was performed at 10x and 40x magnifications to observe fungal elements like septate hyphae or spores.
- **Lactophenol Cotton Blue (LPCB) Staining:**
  A drop of LPCB was placed on a clean slide. Fungal samples were taken aseptically and spread over the drop. After covering with a cover glass, observation was done under a microscope at 10x and 40x. Blue-colored fungal structures such as hyphae and spores indicated a positive result.

Preparation of Dermatophyte Fungal Suspension

Trichophyton concentricum, *Candida albicans*, and *Microsporum gypseum* were regenerated on SDA medium and incubated at 37°C for 24 hours. Colonies (about 1 mm in diameter) from five sites were suspended in 5 mL of 0.85% NaCl. The suspension was adjusted to match a 0.5 McFarland standard (λ 520–625 nm), resulting in a fungal concentration of 1×10^6 to 5×10^6 CFU/mL.

Working Procedure

Preparation of Materials and Equipment

- **Oil Phase Preparation**
  Oleic acid (2.5 g) was heated at 60°C, followed by the addition of Span 80 (0.56 g) and stirred until homogeneous.
- **Aqueous Phase Preparation**
  15 mL of distilled water was heated at 60°C. Methylparaben (0.05 g), *Cassia alata* extract (2.5 g), Tween 80 (1.44 g), PEG 400 (7.5 g), and glycerin (2.5 g) were added sequentially, stirring after each addition.
- **Primary Emulsion Formation**
  The oil phase was slowly added to the aqueous phase while homogenizing at high speed for 5–10 minutes until a stable, milky emulsion formed. The emulsion was cooled to room temperature with occasional stirring.
- **Preparation of Carbopol Gel Base**
  Carbopol 940 (0.9 g) was slowly dispersed into 10 mL of distilled water with continuous stirring, allowed to swell for at least 30 minutes.
- **Combining Emulsion and Gel Base**
  The cooled emulsion was added to the swollen Carbopol gel with gentle stirring. TEA (2.7 g) was added drop by drop until a uniform and stable emulgel formed.
- **Final Volume and pH Adjustment**
  The emulgel was transferred to a 50 mL measuring cylinder, and distilled water was added to reach exactly 50 mL. The pH was adjusted to 5.5–7.0 and recorded.

Antifungal Activity Test

- *Trichophyton rubrum*, *Microsporum canis*, and *Epidermophyton floccosum* were streaked onto solid media.
- 100 mg of emulgel was mixed with 1 mL of distilled water and applied onto a sterile paper disc.
- The disc was placed on the inoculated media and incubated at 37°C for 24–48 hours.
- The diameter of the inhibition zone was measured using a caliper.

Data Analysis

This study employed quantitative analysis by measuring the inhibition zone to indicate antifungal activity. Observation data from the ethanol extract gel concentration test and the emulgel formulation were analyzed using the SPSS application with analysis of variance (ANOVA), followed by the Honestly Significant Difference (HSD) test or Tukey's test.

3. RESULTS AND DISCUSSION

RESULT

Table 1. Yield of Ethanol Extract of *Cassia alata* Leaves

Observation	Initial Weight of Simplicia	Final Extract Weight	% Yield
Ethanol Extract	1000 grams	140 grams	14%

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Table 2. Phytochemical Screening Results of *Cassia alata* Leaf Extract

Compound Group	Reagent/ Test	Observation Result	Outcome
Alkaloids	1. Extract + Mayer's 2. Extract + Dragendorff's 3. Extract + Boucardat's	1. Precipitate formed 2. Precipitate formed 3. No precipitate formed	(+)
Saponins	Extract aquadest	Foam formed within approximately 10 minutes	(+)
Tanins	$FeCl_3$ 1%	Greenish-black color with precipitate formed	(+)
Flavonoids	Extract + Mg + HCl	Orange	(+)
Terpenoids	Acetic Acid + H_2SO_4	Negative	(-)
Phenols	$FeCl_3$ 1%	Dark bluish color	(+)

Results of Antifungal Activity Test of *Cassia alata* Leaf Ethanolic Extract

Table 3. Antifungal Effectiveness Test Results of *Cassia alata* Ethanolic Leaf Extract Against *Trichophyton rubrum*

Extract Concentration (ppm)	Replication (mm)			Average (mm)
	1	2	3	
500	8,61	9,26	9,68	9,18
750	9,95	9,27	11,18	10,13
1000	11,26	11,14	11,20	11,20

Table 4. Results of Antifungal Effectiveness Test of *Cassia alata* Ethanolic Leaf Extract Against *Microsporum canis*

Extract Concentration (ppm)	Replication (mm)			Average (mm)
	1	2	3	
500	6,05	6,34	6,15	6,18
750	7,12	7,51	7,43	7,23
1000	8,35	8,43	8,21	8.33

Table 5. Results of Antifungal Effectiveness Test of *Cassia alata* Ethanolic Leaf Extract Against *Epidermophyton floccosum*

Extract Concentration (ppm)	Replication (mm)			Average (mm)
	1	2	3	
500	7,22	7,14	8,11	7,49
750	8,63	8,18	9,13	8,64
1000	9,17	9,91	10,05	9.71

Results of Antifungal Activity Test of *Cassia alata* Leaf Emulgel Formulation

Table 6. Results of Antifungal Effectiveness Test of *Cassia alata* Leaf Emulgel Formulation Against *Trichophyton rubrum*

Extract Concentration (ppm)	Replication (mm)			Average (mm)
	1	2	3	
500	6,78	7,04	7,13	6,98
750	9,11	9,24	8,72	9,02
1000	11,03	10,71	11,40	11,04
K+	37,20	37,20	37,20	37,20
K-	0,0	0,0	0,0	0,0

Table 7. Results of Antifungal Effectiveness Test of *Cassia alata* Leaf Emulgel Formulation Against *Microsporum canis*

Extract Concentration (ppm)	Replication (mm)			Average (mm)
	1	2	3	
500	6.03	6.10	6.04	6.04
750	6,64	6,81	6,33	6,59
1000	7,82	7,30	7,57	7,56
K+	37,20	37,20	37,20	37,20
K-	0,0	0,0	0,0	0,0

Table 8. Results of Antifungal Effectiveness Test of *Cassia alata* Leaf Emulgel Formulation Against *Epidermophyton floccosum*

Extract Concentration (ppm)	Replication (mm)			Average (mm)
	1	2	3	
500	6,23	6,13	6,12	6,16
750	8,03	7,27	7,04	7,44
1000	8,15	8,93	8,07	8,38
K+	37,20	37,20	37,20	37,20
K-	0,0	0,0	0,0	0,0

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Table 9. Results of Antifungal Effectiveness Test of *Cassia alata* Leaf Emulgel Formulation Against *Trichophyton rubrum*. BNJ Test Result at 5% = 0.60

Treatment	*Mean*	*Interval*	*Notation*	*Category*
K+	37,20		a	SK
1000	11,04	26.16	b	K
750	9,02	2.02	c	S
500	6,98	2.04	d	S
K-	0.00	6.98	e	L

Description : L = Weak (≤ 5mm); S = Moderate (> 5-10 mm); K = Strong (> 10-20 mm); SK = Very Strong (> 20mm) (David et al., 1971)

DISCUSSION

In this study, maceration was chosen as the extraction method for *Cassia alata* leaves due to its simplicity, low cost, and ability to preserve heat-sensitive (thermolabile) compounds, as it is conducted at room temperature. Prolonged soaking in ethanol allows optimal penetration into plant cells and efficient extraction of polar compounds. According to Nurhasnawati (2017), maceration offers key advantages such as ease of use, no need for complex equipment, and no heating, thus minimizing the risk of degrading natural substances.

Phytochemical screening of *Cassia alata* leaf extract showed positive results for alkaloids, saponins, tannins, flavonoids, and phenols.

The results of the study demonstrated that *Cassia alata* leaf extract effectively inhibited the growth of *Trichophyton rubrum*, *Microsporum canis*, and *Epidermophyton floccosum*. The antifungal compounds present in the extract may act synergistically, as suggested by Goodman & Gilman (2008), where overlapping or enhanced inhibition zones indicate combined activity. This synergistic approach aims to improve treatment efficacy while minimizing resistance and side effects.

The antifungal activity of the extract was evident from the clear zones formed around the discs, indicating areas where fungal growth was inhibited. These effects are attributed to the presence of active compounds such as flavonoids, tannins, saponins, phenolics, and alkaloids. The average inhibition zones ranged from moderate to strong, suggesting that the extract acts as a fungistatic agent — inhibiting fungal growth by disrupting enzymatic activity and hyphal elongation. This disruption prevents hyphal fragmentation and inhibits fungal reproduction.

Hujjatusnaini (2007) reported that *Cassia alata* leaf extract at various concentrations significantly inhibited the growth of *Trichophyton* species within 24–48 hours of treatment, with 60% concentration being the most effective. Similarly, Fajri et al. (2018) found that ethanolic fractions of *Cassia alata* extract exhibited the highest antifungal activity against *Microsporum canis*, especially at a concentration of 42.5%. Additionally, Sule *et al.* (2010) noted strong antifungal effects of *Cassia alata* against *Trichophyton verrucosum* and *Epidermophyton floccosum* as well as other microbes.

The study demonstrated that the emulgel formulation of *Cassia alata* leaf extract was effective in inhibiting the growth of *Trichophyton rubrum*, *Microsporum canis*, and *Epidermophyton floccosum*. Among the tested concentrations, 1000 ppm showed the strongest antifungal activity (excluding the positive control), with an average inhibition zone of 11.04 mm against *T. rubrum*. ANOVA analysis indicated a significant effect ($p < 0.05$), confirming that extract concentration significantly influences antifungal activity. These findings are consistent with Bayuaji et al. (2012), who reported that *Cassia alata* extract cream had optimal antifungal activity at 1.5%.

For *Microsporum canis*, the 1000 ppm emulgel also yielded the highest inhibition zone (7.56 mm), with a significance value of p = .000. This supports findings by Nutrisia (2015), who demonstrated that a gel containing 2% *Cassia alata* extract effectively inhibited *Candida albicans*, *M. canis*, and *T. mentagrophytes*, comparable to 2% ketoconazole.

Against *Epidermophyton floccosum*, the 1000 ppm emulgel produced an average inhibition zone of 8.38 mm, again with significant results (p = .000). The antifungal activity of the emulgel was attributed to the presence of active phytochemicals—alkaloids, saponins, tannins, and flavonoids—which are known for their antifungal properties.

Ketoconazole was used as the positive control in this study. It acts by inhibiting ergosterol synthesis—an essential component of fungal cell membranes—by blocking the enzyme lanosterol-14-α-demethylase in the cytochrome

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P450 system. This disrupts membrane integrity and leads to leakage of intracellular contents (Bindusari, 2001; Katzung, 2004). Additionally, ketoconazole suppresses the biosynthesis of triglycerides and phospholipids and inhibits oxidase and peroxidase activity (Katzung, 2004).

The results of this study were analyzed using ANOVA followed by Tukey's Honest Significant Difference (HSD) test. Findings indicated that the emulgel formulation of ***Cassia alata*** leaf extract exhibited significant antifungal activity against ***Trichophyton rubrum***. The 5% HSD test confirmed a dose-dependent relationship: higher extract concentrations (500, 750, and 1000 ppm) led to significantly larger inhibition zones. This effect is attributed to the presence of secondary metabolites—such as alkaloids, flavonoids, saponins, and tannins—which are known to exert antifungal effects by damaging fungal cell walls, inhibiting protein synthesis, or disrupting essential metabolic processes.

The significant difference between all treatment groups and the negative control (emulgel without extract) confirms that the inhibitory activity originated from the active compounds in the extract. These findings support the potential development of ***Cassia alata*** leaf extract as an effective topical antifungal agent.

Inhibition zone size was influenced by both extract concentration and the type of fungal strain tested (Bibliogr et al., 2023). Higher extract concentrations generally produced larger inhibition zones due to increased levels of antifungal compounds (Yacob et al., 2010).

4. CONCLUSION

The ethanol extract of *Cassia alata* leaves has been proven effective in inhibiting the growth of *Trichophyton rubrum*. Its formulation into an antifungal emulgel also demonstrated significant inhibitory activity. The highest antifungal effectiveness was observed at a concentration of 1000 ppm, indicating a dose-dependent relationship. These findings suggest that *Cassia alata* extract has strong potential as a topical antifungal agent.

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