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ANTIFUNGAL STUDY OF *UNCARIA GAMBIR* ROXB. (UGR) EXTRACT AGAINST *GANODERMA* SP. IN VITRO

STUDI ANTIFUNGI EKSTRAK *UNCARIA GAMBIR* ROXB. (UGR) TERHADAP *GANODERMA* SP. SECARA IN VITRO

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Abstract

Ganoderma sp. is a pathogenic fungus that causes basal stem rot in oil palms, leading to significant yield losses. This study explores the antifungal potential of *Uncaria gambir* Roxb. extract against *Ganoderma* sp. using in silico and in vitro methods. In silico analysis from the KNApSAcK and PASS Online databases identified 18 secondary metabolites with probability activity (Pa) values ranging from 0.5 to 0.7, indicating high potential. Cinchonain Ia, an alkaloid with the highest Pa value of 0.638, has poor solubility. In vitro tests on *U. gambir* extract at various concentrations (0%, 1.5%, 2%, 2.5%, and 3%) showed that the extract was ineffective in inhibiting the growth of *Ganoderma* sp. Further research with variations in solvents, extraction methods, and formulations is needed to optimize the antifungal activity of this extract.

Keywords: *Antifungal, Bioinformatics. Ganoderma sp., Uncaria gambir Roxb, In Vitro,*

Abstrak

Ganoderma sp. adalah jamur patogen penyebab busuk batang basal pada kelapa sawit, yang menyebabkan kerugian hasil besar. Penelitian ini mengeksplorasi potensi antifungal ekstrak *Uncaria gambir* Roxb. terhadap *Ganoderma* sp. dengan metode *in silico* dan *in vitro*. Analisis in silico dari database KNApSAcK dan *PASS Online* mengidentifikasi 18 metabolit sekunder dengan nilai probabilitas aktivitas (Pa) antara 0,5 hingga 0,7, menunjukkan potensi tinggi. Cinchonain Ia, alkaloid dengan Pa tertinggi 0,638, memiliki kelarutan yang buruk. Uji in vitro pada ekstrak *U. gambir* di berbagai konsentrasi (0%, 1,5%, 2%, 2,5%, dan 3%) menunjukkan bahwa ekstrak tidak efektif menghambat pertumbuhan *Ganoderma* sp. Penelitian lebih lanjut dengan variasi pelarut, metode ekstraksi, dan formulasi diperlukan untuk mengoptimalkan aktivitas antifungal ekstrak ini.

Kata Kunci : *Ganoderma sp.*, Antifungi, *Uncaria gambir* Roxb, In Vitro, Bioinformatika.

INTRODUCTION

Oil palm plantations are currently facing the problem of low productivity, mainly caused by stem rot (BPB) disease caused by Ganoderma sp. This disease can result in losses of up to 50%, around 250 million dollars annually (Susanto, 2011; Darmono, 2011 in Salsabila *et al*.,

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Vol. 2, No. 1, Thn. 2025
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2022). A decrease in productivity occurs when the oil palm population is only 50% (Priwiratama & Susanto, 2020), and around 54% of old plants (21-24 years) are infected with BPB (Evizal & Prasmatiwi, 2022).

Various efforts to control *Ganoderma* sp. have been carried out, including technical culture and the use of chemical pesticides. Still, these methods are less effective and have the potential to cause resistance and pollution. The use of biological agents such as Trichoderma sp. and Stenotrophomonas hemophilia has also been tried, but organic (plant) pesticides are still rarely used. Gambir plants (*Uncaria gambir* Roxb.) have high economic value and are rich in catechins (Santoso & Pangawikan, 2022) and other components such as catechu acid, quercetin, and tannins (Rahmawati et al., 2012). Gambir is thought to have properties as an antioxidant, anticancer, antibacterial, and antifungal (Iskandar *et al*., 2022; Osipova *et al*., 2020; Desai & Joshi, 2019; Alagumuthu *et al*., 2018; Alhadrami *et al*., 2021). Gambir extract shows various pharmacological effects, including antifungal (Santoso & Pangawikan, 2022). Research by Nasrun & Nurmansyah (2015), showed that the botanical fungicide formula with citronellal, geraniol, eugenol, and catechin effectively suppresses the growth of R. *microporus mycelium*, with inhibition reaching 81.39%. Therefore, *Uncaria gambir* Roxb. is thought to have the potential to be a botanical pesticide to control *Ganoderma* sp. The purpose of this study was to determine the secondary metabolite compounds contained in the Uncaria gambir plant and to determine the effectiveness of Uncaria gambir extract in inhibiting the growth of *Ganoderma* sp.

RESEARCH METHODS

The research was conducted from June to July 2024 at the Plantation Plant Science Laboratory, Department of Agricultural Technology, Pontianak State Polytechnic. This study used a non-factorial Completely Randomized Design (CRD) with 5 treatments with 6 replications so that 30 experimental units were obtained. Each treatment used a test fungus, namely Ganoderma sp. The treatment is coded as follows:

K_0 = Without gambir extract 0% (Control with aquadest)
K_1 = 1.5% gambir extract
K_2 = 2% gambir extract
K_3 = 2.5% gambir extract
K_4 = 3% gambir extract

In this study, the tools used include beakers, autoclaves, hammermills, spray bottles, porcelain cups, Erlenmeyer flasks, measuring cups, vernier calipers, cork borers, laminar airflow, microscopes, Bunsen burners, tweezers, cutters, volume pipettes, rotary evaporators, glass jars, and analytical scales. The materials used in this study were distilled water, ethanol, ethyl acetate, *Ganoderma* sp. mushrooms, PDA media, 10% $FeCl_3$, 10% HCl, *Uncaria gambir* leaves, filter paper, sterile cotton, coulee reagent (a mixture of 0.5 ml of chloroform, 0.5 ml of acetic anhydride and 2 ml of concentrated sulfuric acid), Mayer reagent, Wagner reagent, tissue paper, 10% NaOH, aluminum foil, and the Illustrated General of Imperfect Fungi Book.

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In Silico Test Based on Informatics

This study refers to a study conducted by Yasmin *et al*., (2022) which uses data from knapsackfamily.com and analyzes the potential of compounds using PASS ONLINE from Way2Drugs. The initial stages include visiting the KNApSAcK site and searching for the scientific name of *Uncaria gambir* to see a list of active compounds. These compounds (http://www.knapsackfamily.com/kNApSAck_Family/) are then recorded and the SMILES (Simplified Molecular Input Line Entry System) code is taken for further analysis. The next step is to analyze these compounds using Way2Drug Software (http://www.way2drug.com/passonline/), where the SMILES code of each compound is entered. These predicted results are used to identify antifungal biological activities, with prediction accuracy evaluated based on information such as activity probability (Pa) and inactivity probability (Pi) values (Filimonov *et al*., 2014 in Yasmin *et al*., 2022).

Sterilization

The tools to be used are ensured to be sterile first to prevent possible contamination. Glassware is heated in an oven at 170ºC for about 2 hours. Meanwhile, the ose needle and tweezers are sterilized by burning them over a Bunsen burner. The media to be used in the study are also prepared by a sterilization process using an autoclave at 121ºC for 15 minutes (Sirait *et al*., 2016).

Making Simplisia

Simplisia is a natural material that has been dried and used for medicine but has not undergone further processing (Ministry of Health, 2017). Samples of *Uncaria gambir* Roxb. leaves collected were taken from Jalan Bina Jaya Gang Berkah Jaya, Kota Baru, Pontianak, West Kalimantan. One kilogram of Gambir leaves collected was then cleaned of dirt using running water in a wet sorting process. After that, the leaves were filtered and dried in the air or using a drying cabinet until they reached the desired dryness and hardness. The weight of the dried material was measured and ground using a blender. The ground simplisia was then made into powder and stored in a container protected from sunlight (Karnirius, 2022).

Making *Uncaria gambir* Extract

Making gambir leaf extract using the maceration method. The choice of the maceration method is due to its ability to extract active compounds through soaking without heating. So that it can avoid damage to compounds that are labile or not resistant to heat. The extraction process is carried out using an organic solvent of ethyl acetate (semi-polar) as much as 100 grams of dry powder of the simplicia, divided into 3 macerator containers with a ratio of 1: 6 (Desta *et al*., 2014). The mixture is stirred for 15 minutes and left for 3 days. After that, the macerate is filtered to separate the solids from the filtrate and then evaporated with a Rotary evaporator at a temperature of 40-50°C to obtain a thick extract (Sani *et al*., 2014). The thickened extract is

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Vol. 2, No. 1, Thn. 2025
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weighed, and the percentage yield is calculated during the process for each variation of maceration time. The yield is calculated using the formula:

$$Yield = \frac{Final\ Weight}{Initial\ weight}\ x\ 100\ \%$$

The best results will be used at the next stage.

Dilution of Uncaria gambir Extract

Thick extract from the leaves of *Uncaria gambir* Roxb. (with a concentration of 100%) dilute using distilled water, the dilution method used is to dissolve 10 ml. The *Uncaria gambir* extract was then weighed using an analytical balance for each concentration (0.15 g, 0.2 gr, 0.25 gr, and 0.3 gr). Then add each solvent slowly while stirring until the extract is homogeneous, the solvent is added continuously to 10 ml. Homogenization was carried out using a vortex to reach a concentration of 1.5%; 2%; 2.5% and 3%.

Pengenceran Ekstrak *Uncaria gambir*

The thick extract of *Uncaria gambir* Roxb. leaves (with a concentration of 100%) was diluted using distilled water, the dilution method used was to dissolve as much as 10 ml. The Uncaria gambir extract was then weighed using an analytical balance for each concentration (0.15 g, 0.2 g, 0.25 g, and 0.3 g). then each solvent was added slowly while stirring until the extract was homogeneous, the solvent was added continuously up to 10 ml. Homogenization was carried out using a vortex until it reached a concentration of 1.5%; 2%; 2.5% and 3%.

Making PDA Media

Potato Dextrose Agar (PDA) media as much as 39 grams was weighed and dissolved in 1 liter of distilled water, then homogenized using a magnetic stirrer and heated until the solution was clear. The PDA solution was put into an Erlenmeyer flask, covered with sterile cotton and aluminum foil, then sterilized by autoclaving at a temperature of 121ºC and a pressure of 1 atm for 15 minutes. After that, the PDA was cooled to a temperature of 10-20°C, before being poured into a petri dish (Putri *et al*., 2019).

Isolation and Identification of Ganoderma sp.

Isolation and identification of *Ganoderma* sp. were carried out based on research (Maryono, 2020) on infected oil palm plants around the Faculty of Agriculture, Tanjungpura University. Ganoderma sp fruit body. Photographed using a Samsung Galaxy A15® LTE smartphone, then taken using a cutter and wrapped in transparent plastic for isolation in the laminar airflow ITP 2 Laboratory, Pontianak State Polytechnic. The fruit bodies were cleaned, cut into small pieces, dipped in 10% HCl for 5-10 minutes, and planted in a petri dish. Next, incubate at a temperature of 28-30°C.

https://jurnal.rocewisdomaceh.com/index.php/roce e-ISSN : 3032-4505
Vol. 2, No. 1, Thn. 2025
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Purification of Ganoderma sp. Isolate.

Purification is carried out to ensure that the cultured isolate is active again and undergoes metabolism after storage. This process is carried out by taking the isolate using a cork borer and replanting it on PDA media with slight pressure. The samples were then incubated at 28°C using an incubator for 18-24 hours to obtain fungal colonies.

In Vitro Antifungal Activity Test

The medium poisoning method was used to test the inhibitory power of *Uncaria gambir* extract on PDA media, according to research by Wahyuni *et al*., (2022) which stated that antifungals were added to PDA media. The extract was taken in concentrations of 3%, 2.5%, 2%, 1.5%, and negative control (-) using 1 ml of distilled water, mixed with 10 ml of PDA medium, then poured into a petri dish. Ganoderma sp. taken with a cork borer and planted in PDA media, then incubated for 7 days at 28°C. According to Priyanka *et al*., (2014), the inoculated media was aseptically incubated at 37°C before measuring inhibition. The collected data was used to calculate the percentage of extract inhibition using the formula from Hardani *et al*., (2020).

$$\%DH = \frac{Dk - Dp}{Dk}\ x\ 100\%$$

DH = Inhibitory Power
Dk = Control Diameter
Dp = Treatment Diameter

The assessment of the zone of inhibition refers to the classification of Davis and Stout (1971), which is divided into categories weak (> 5 mm), moderate (5-10 mm), strong (11-20 mm), and very strong (> 20 mm).

Phytochemical Test

Qualitative tests in phytochemical screening include alkaloid, flavonoid, phenol, and saponin tests, referring to the methodology described by Williams *et al*., (2021). This phytochemical test aims to identify active compounds in gambier leaf extract. The test procedures carried out are:

a) Alkaloid Test: The thick extract of gambier leaves (2 ml) is evaporated to form a residue, then dissolved in 5 ml of 2N HCl and divided into 3 test tubes. A yellow precipitate in the second tube (Mayer's reagent) and a brown or red precipitate in the third (Wagner's reagent) indicate the presence of alkaloids.
b) b) Flavonoid Test: Gambir leaf extract (2 ml) was added with 3 drops of 10% NaOH solution. The color change to yellow indicates the presence of flavonoids.
c) Phenol Test: Gambir leaf extract (2 ml) is divided into 2 test tubes. Tube B, which was reacted with 10% FeCl3 solution, showed the presence of phenol with a bluish-black color
d) Saponin Test: Gambier leaf extract (2 ml) was added to 10 ml of distilled water and shaken. The formation of foam indicates the presence of saponin.

https://jurnal.rocewisdomaceh.com/index.php/roce e-ISSN : 3032-4505
Vol. 2, No. 1, Thn. 2025
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Data Collection and Analysis

The data collected is original data which was measured using a caliper to determine the diameter of the inhibition zone (mm). Data were processed with Minitab and analyzed using Analysis of Variance (ANOVA) to evaluate significant differences in antifungal activity between various extract concentrations. If ANOVA shows a significant difference, continue with the Duncan Test at the 5% level.

RESULTS AND DISCUSSION

Probability Activity Test of *Uncaria gambir* Antifungi In Silico

Table 1 shows the results of in silico tests using computer software, in Table 1. There are 18 secondary metabolite compounds identified with a range of Pa (Probability Activity) and Pi (Probability Inactivity) values in the range 0.5 < Pa < 0.7. This shows that the compounds contained in Uncaria Gambir have a fairly high chance of being used as active antifungal ingredients. This is supported by the statement of Malikhana *et al*., (2021) which states that if the Pa value is in the range 0.5 < Pa < 0.7, it indicates that the compound has a fairly high level of bioactivity which makes it have a high potential to be successfully tested. Both in vivo and in vitro experiments. Table 1 shows that the Cinchonain Ia compound has the highest pa value, namely 0.638. As an alkaloid compound, Cinchonain Ia has high potential as an antifungal, by the opinion of Sari *et al*., (2022) who stated that alkaloid compounds can disrupt the peptidoglycan in fungal cells, causing failure in cell wall formation and ultimately cell death.

Table 1. Probability Value of Antifungal Activity of Secondary Metabolites of *Uncaria Gambir* Roxb Plants

No.	Metabolit	Antifungi	No.	Metabolit	Antifungi	No.	Metabolit	Antifungi
1	(+)- Catechin	0,552	7	Gallic acid	0,398	13	Gambiriin B3	0,497
2	(-)- Epicatechin	0,552	8	Cinchonain Ia	0,638	14	Procyanidin B3	0,534
3	(+)- Epicatechin	0,552	9	Gambiriin C	0,534	15	Procyanidin B1	0,534
4	Mitraphylline	0,109	10	Gambiriin A1	0,528	16	Uncarine B	0,109
5	Roxburghine B	0	11	Gambiriin A3	0,549	17	Gambiriin A2	0,528
6	Catechol	0,376	12	Gambiriin B1	0,565	18	Gambiriin B2	0,565

Table 2. Metabolite Solubility Data in Water

No.	Metabolic	Solubility	Log S	Class
1	(+)-Catechin	1.66e+00 mg/ml ; 5.72e-03 mol/l	-2.24	Soluble
2	(-)-Epicatechin	1.66e+00 mg/ml ; 5.72e-03 mol/l	-2,24	Soluble
3	(+)-Epicatechin	1.66e+00 mg/ml ; 5.72e-03 mol/l	-2,24	Soluble
4	Mitraphylline	8.11e-01 mg/ml ; 2.20e-03 mol/l	-2.66	Soluble
5	Roxburghine B	1.63e-03 mg/ml ; 3.32e-06 mol/l	-5.48	Moderately soluble
6	Catechol	5.34e+00 mg/ml ; 4.85e-02 mol/l	-1,31	Very Soluble
7	Gallic acid	7.86e-01 mg/ml ; 4.62e-03 mol/l	-2,34	Soluble
8	Cinchonain Ia	3.05e-03 mg/ml ; 6.75e-06 mol/l	-5,17	Moderately soluble
9	Gambiriin C	1.46e-04 mg/ml ; 2.60e-07 mol/l	-6,58	Poorly Soluble
10	Gambiriin A1	3.58e-05 mg/ml ; 6.17e-08 mol/l	-7,21	Poorly Soluble

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Vol. 2, No. 1, Thn. 2025
https://doi.org/10.71275/roce.v2i1.52

No.	Metabolic	Solubility	Log S	Class
11	Gambiriin A3	4.40e-04 mg/ml ; 7.59e-07 mol/l	-6,12	Poorly Soluble
12	Gambiriin B1	2.38e-05 mg/ml ; 4.23e-08 mol/l	-7,37	Poorly Soluble
13	Gambiriin B3	2.38e-05 mg/ml ; 4.23e-08 mol/l	-7,37	Poorly Soluble
14	Procyanidin B3	1.31e-04 mg/ml ; 2.26e-07 mol/l	-6,65	Poorly Soluble
15	Procyanidin B1	1.31e-04 mg/ml ; 2.26e-07 mol/l	-6,65	Poorly Soluble
16	Uncarine B	8.11e-01 mg/ml ; 2.20e-03 mol/l	-2,66	Soluble
17	Gambiriin A2	3.58e-05 mg/ml ; 6.17e-08 mol/l	-7,21	Poorly Soluble
18	Gambiriin B2	2.38e-05 mg/ml ; 4.23e-08 mol/l	-7,37	Poorly Soluble

Information : Log S scale Insoluble < -10 < Poorly < -6 < Moderately < -4 < Soluble < -2 Very < 0 < Highly.

Table 2 shows that water-soluble compounds, such as (+) -catechin, (+) -epicatechin, Mitraphylline, gallic acid, and uncarine B, have solubilities ranging from 1.66 ml/ml to 0.81 mg/ml with a Log S scale ranging from -2.24 to 2.66. These compounds are classified as soluble with good solubility, although not as high as catechol. In contrast, roxburghine B and cinchonain Ia have lower solubility, ranging from 0.00163 mg/ml to 0.00305 mg/ml with a Log S scale ranging from -5.48 to -5.17, so they can still be categorized as moderately soluble. Meanwhile, gambierin C, gambierin A_1, gambierin A_3, gambierin B_3, procyanidin B_1, gambierin A_2, and gambierin B_2 are classified as less soluble in water, with solubility between 0.0000238 mg/ml to 0.00146 mg/ml and Log S scale between -6.58 to -7.37.

Uncaria gambir extract yield

The data in Table 3 shows that the Uncaria gambir extract produced a good yield, namely 54.5 grams with a yield percentage of 5.45%.

Table 3. Percentage Yield

Information	Total	Amount (Isromarina, dkk. 2019)
Initial Weight (gr)	1000	500
Final Weight (gr)	54,5	25,39
% Yield	5,54	5,08

Research by Isromarina *et al*., (2019) recorded the yield of *Uncaria gambir* extract using ethyl acetate solvent of 5.08%. The extract yield is considered good if the value is less than 50% (Evitasari & Susanti, 2021).

Phytochemical Test

Based on the data from the phytochemical test results carried out on *Uncaria gambir* extract, the results of secondary metabolites contained in the extract were phenolics and terpenes/steroids.

Table 4. Phytochemical Test Results

No.	Metabolic	Phytochemical Test Results	Phytochemical Test Results Isromarina *et al.*, (2019)
1	Flavonoid	-	+
2	Alkaloid	-	+
3	Terpenoid	++	-
4	Saponin	-	+
5	Fenolik	+	+

Compounds suspected of containing antifungal in *Uncaria gambir* Roxb plants such as flavonoids, saponins, and alkaloids are not dissolved in the solvent. However, in the study of Isromarina *et al*., (2019) *Uncaria gambir* extract with ethyl acetate solvent contained alkaloids, flavonoids, phenols, and saponins.

Antifungal Activity Test of *Uncaria gambir* Extract In Vitro

Data from the results of the antifungal activity test on Ganoderma boninense isolates in vitro can be seen in Table 5. The measurement results obtained showed that the control treatment (K0) experienced slower growth compared to the *Uncaria gambir* extract application treatment.

Table 5. *Ganoderma* sp Diameter Measurement Data

Treatment an	Observation Time (mm)			
	Day 2	Day 3	Day 4	Day 5
K0	16,03	27,42	44,43	50,82
K1	18,53	31,93	60,38	71,46
K2	16,09	30,81	51,23	63,60
K3	16,26	32,07	53,24	67,75
K4	16,90	32,03	52,13	64,79
Average	**16,762**	**30,852**	**52,282**	**63,684**

Table 5 shows that the average diameter in the control treatment on day 5 was 50.82 mm, while the K1 treatment with an extract concentration of 1.5% reached 71.46 mm. This is thought to be due to the absence of compounds that inhibit the growth of *Ganoderma* sp. fungi, as stated in the phytochemical test results (Table 5). The results of the in silico test (Table 1 and Table 2) showed that only cinchonain Ia was suspected of being soluble in ethyl acetate, with an antifungal pa value of 0.638. This compound comes from the phenolic and terpenoid groups, which have higher antioxidant and antibacterial activities than their antifungal properties. Pambayun *et al*., (2007) stated that extraction with ethyl acetate on gambir leaves produces an extract with the greatest inhibitory power against gram-positive bacteria. According to Indriyah *et al*., (2023), the ethyl acetate fraction has very strong antioxidant activity, with an LC50 value close to ascorbic acid. Mahardani & Yuanita, (2021) reported that Uncaria gambir Roxb extract positively contained phenolics with a level of 31.73 mg GAE/gr sample.

Previous research by Alviodinasyari *et al*., (2015) showed that the use of biological agents *Trichoderma* spp. SBJ8 can control Ganoderma boninense fungi quite well. In addition, the administration of Ganofend in nurseries can reduce the death rate of oil palm seedlings due to

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Vol. 2, No. 1, Thn. 2025
https://doi.org/10.71275/roce.v2i1.52

Ganoderma boninense attacks. Many other studies have shown that the use of resistant pathogens and biological control agents, including *Aspergillus* spp., *Trichoderm*a spp., *Penicillium* spp., and *Henderson*, is effective in controlling *Ganoderma* sp. (Alexander *et al*., 2014; Dahang & Munthe, 2019; Munthe, 2018; Peng et al., 2020; Kim-Phin & Arnnyitte, 2016 in Dahang *et al*., 2021).

CONCLUSION

1. In silico analysis identified 18 secondary metabolites in *Uncaria gambir* with Probability Activity (Pa) values between 0.5 and 0.7, indicating high potential antifungal activity. The compound with the highest Pa was Cinchonain Ia (0.638), which is an alkaloid with a high potential as an antifungal agent. Although catechin and epicatechin also showed potential, their solubility in water was good, while Cinchonain Ia had lower solubility, so the compound extracted in ethyl acetate was a minor compound.
2. The results of the antifungal activity test of *Uncaria gambir* Roxb extract against Ganoderma sp. showed that this extract was not effective in inhibiting fungal growth. In vitro testing showed no significant decrease in fungal growth compared to the control. This may be due to the insolubility or inactivity of the active compounds in the extract. In addition, active compounds such as Cinchonain Ia do not dissolve well in the aquadest used for extract dilution.

Suggestion

1. To improve understanding of the antifungal potential of *Uncaria gambir*, it is recommended to conduct further testing with various types of solvents and extraction methods. The use of alternative solvents such as methanol or water may be more effective in dissolving active compounds that have the potential to be antifungal.
2. Adjusting the concentration and formulation of the extract can increase its effectiveness. Testing various concentrations and better formulations is needed to find the optimal conditions that can increase antifungal activity.
3. This study shows that although *Uncaria gambir* has potential, its effectiveness as an antifungal agent against Ganoderma sp. still needs to be improved through further research and development approaches.

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