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Antifungal Activity Test of The Aqueous and Ethanolic Extracts of Strawberry Leaves Against *Absidia corymbifera*

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ABSTRACT

Absidia corymbifera is an opportunistic fungal pathogen that can cause serious infections, particularly in immunocompromised individuals. However, studies on natural antifungal agents effective against this species remain limited. This study investigates the antifungal activity of strawberry (*Fragaria spp.*) leaf extracts as a potential natural treatment against *A. corymbifera*. Aqueous and ethanol extracts were prepared using cold extraction methods—blending and maceration, respectively. Strawberry leaf extract was subjected to a qualitative phytochemical screening test and a quantitative total phenolic content test using the Folin-Ciocalteu method, then an antifungal activity test was carried out using the diffusion method. Phytochemical screening revealed the presence of tannins, flavonoids, and alkaloids in the aqueous extract, while the ethanol extract contained tannins, flavonoids, and steroids. The total phenolic content of ethanol extract of strawberry leaves is greater than that of strawberry leaf aqueous extract. Aqueous and ethanol extracts of strawberry leaves showed fungistatic antifungal inhibition against *A. corymbifera* with a larger inhibitory zone diameter in strawberry leaf ethanol extract. These findings suggest that strawberry leaf extracts, particularly ethanol-based, hold promise as a natural antifungal agent for controlling infections caused by *A. corymbifera*.

Keyword: absidia corymbifera, antifungal, strawberry leaves

1. INTRODUCTION

Indonesia's tropical climate, characterized by high humidity and warm temperatures, creates a favorable environment for the proliferation of fungi, bacteria, and parasites. These conditions frequently lead to various skin infections, particularly among individuals with compromised immune systems, poor hygiene, or predisposing health conditions (Murbiah, Romadoni, & Rini, 2019).

One of the more severe fungal infections affecting the skin is zygomycosis or mucormycosis, which is caused by several species of Mucorales fungi, including *Lichtheimia sp.*, *Rhizopus sp.*, *Mucor sp.*, *Cunninghamella sp.*, *Rhizomucor sp.*, *Saksenaea vasiformis*, and *Apophysomyces elegans* (Petrikkos, Skiada, & Drogari-Apiranthitou, 2014) Among these, *Absidia corymbifera* has been identified as a significant pathogenic species associated with cutaneous mucormycosis (Rosa, Wahyuni, & Murdiyah, 2020). Current treatment of mucormycosis relies primarily on antifungal drugs such as amphotericin B, which serves as the first-line therapy. However, its use is often limited by severe side effects and toxicity. In cases of intolerance, posaconazole may be administered as an alternative, although its effectiveness is relatively lower and its bioavailability is limited (Abeyakirthi, Piran, Tilakaratne, & Mitchell, 2021). However, the efficacy rate of posaconazole is lower than amphotericin B and the use of this drug is also

limited because it has limited bioavailability (Lewis & Kontoyiannis, 2013), In addition, it is not recommended for patients with neutropenia, cancer, or patients at higher risk of malabsorption (Spellberg & Ibrahim, 2010). Therefore, plant extracts are starting to be widely used to help treat several skin diseases caused by fungi to prevent intolerance and resistance to synthetic antifungal drugs.

Strawberry (*Fragaria spp.*) leaves are a promising source of bioactive compounds with antifungal properties. They are known to contain euscaphic acid, myrianthic acid, and tormentic acid—triterpenoids with potential antifungal activity (Gleńsk, Dudek, Rybacka, Włodarczyk, & Fecka, 2021). Additionally, strawberry leaves are rich in polyphenolic metabolites, particularly ellagitannins, galloyl esters of quinic acid, and glucuronosyl flavonols (Fecka, Bednarska, & Włodarczyk, 2022). The highest concentrations of phenolic compounds have been reported in non-fruit parts of the plant such as stolons, crowns, and leaves (Zhu, Nakagawa, Kishikawa, Ohnuki, & Shimizu, 2015). These secondary metabolites contribute to antimicrobial, antioxidant, and anti-inflammatory activities. However, information on the chemical content of aqueous extract and ethanol extract of strawberry leaves is still very limited.

Strawberry leaf extract is rich in triterpenoid compounds that are involved in antifungal activity (Gleńsk et al., 2021). Previous studies have demonstrated the antifungal potential of various strawberry leaf extracts. The ethyl acetate and methanol fractions of the leaves have shown activity against several fungal and bacterial strains (Lee, Kim, & Yook, 2018; El-Mesallamy, Hussein, Gerby, & Abd El Azim, 2013). The presence of tannins, flavonoids, and polyphenols in both aqueous and ethanol extracts suggests that these solvents are effective in extracting antifungal compounds (Bayat, Zargar, Astarkhanova, Pakina, Ladan, Lyashko, & Shkurkin, 2021); Auliya, Suhartinah, & Ansory, 2022). However, despite growing evidence of the antifungal potential of strawberry leaf extracts, no studies to date have specifically examined their activity against *Absidia corymbifera*. This research aims to address this gap by evaluating and comparing the antifungal activity of aqueous and ethanol extracts of strawberry leaves against *A. corymbifera*. In addition, phytochemical profiles and total phenolic contents of the extracts will be analyzed. The findings of this study are expected to contribute to the development of natural antifungal formulations, such as herbal soaps or topical agents, to help prevent fungal skin infections caused by *A. corymbifera*.

2. RESEARCH METHOD

2.1. Strawberry Leaf Extract Manufacturing

Strawberry leaves are washed thoroughly with running aqueous and dried in the oven for 2.5 hours at a temperature of 55 ºC. Extraction is carried out with a solvent of aqueduct and 96% ethanol. Extraction using aqueducts is carried out by weighing 60 grams of dried strawberry leaves and adding 500 mL of aqueducts then blended for 5 minutes, then filtered using a cloth strainer. The extracts and filtrates that are still left in the blender are filtered back by adding aquades, the total result of strawberry leaf aqueous extract is 550 mL. Extraction using 96% ethanol was carried out with *a shaker* at a speed of 102 rpm for 1 hour, as much as 60 grams of coarse powder of dried strawberry leaves was macerated using 400 mL of 96% ethanol. The residue from the filtering was then remacerated using 96% ethanol as much as 300 mL for 1 hour, remaceration was carried out twice. All macerated filtrates are united in one container as ethanol extract filtrate and concentrated using *a vacuum rotary evavorator* at a speed of 140 rpm and a temperature of 50 ◦C for 4 hours, then assisted by the evaporation process using *a hot plate* at 70◦C to obtain a thick extract.

2.2. Phytochemical Screening of Strawberry Leaf Extract

Phytochemical screening of aqueous extract and ethanol extract of strawberry leaves was carried out to identify the content of secondary metabolites, including several qualitative tests, including:

a. Alkaloid test

   A total of 2 mL of extract samples were added with 2 mL of concentrated HCl. Furthermore, it can be tested with Mayer reagent or Wagner reagent as many as 2-3 drops through the side of the test tube. The presence of alkaloid compounds is indicated by the formation of white or cloudy yellow

deposits in the Mayer test and brown deposits in the Wagner test (Julianto, 2019) (Melati & Parbuntari, 2022).

b. Tannin test

A total of 2 mL of extract samples were added with 3 drops of 5% $FeCl_3$ reagent, positive test results were shown by a change in color to deep green or blackish blue (Julianto, 2019).

c. Saponin test

A total of 2 mL of extract sample is added with 4 mL of aquadest then shaken for 1 minute then a foam will form, the foam will be stable after being added with a few drops of HCl (Nurani, Edityaningrum, Guntarti, & Zainab, 2024).

d. Flavonoid test

A total of 2 mL of extract samples are added 2-3 drops of 10% NaOH solution. The presence of flavonoid compounds is indicated by the change in color to deep yellow (Julianto, 2019).

e. Steroid and terpenoid tests

A total of 5 mL of extract samples were added with 3 drops of Lieberman-Burchard reagent and then beaten until homogeneous. The presence of terpenoids is indicated by the occurrence of a brownish-orange color while the greenish-blue color indicates the presence of steroids (Melati & Parbuntari, 2022).

2.3. Total Phenolic Content Test of Strawberry Leaf Extract

The testing of the total phenolic content of aqueous extract and ethanol extract of strawberry leaves was carried out quantitatively with the Shimadzu UV-2450 UV-Vis spectrophotometer using the Folin-Ciocalteu method as follows:

a. Manufacture of galic acid parent solution (0.05%)

A total of 50 mg of galic acid was dissolved in 0.5 mL of 96% ethanol, then diluted with aquadest to a volume of 100 mL, and obtained a 500 ppm master solution (Andriani & Murtisiwi, 2018).

b. Determination of maximum wavelength (λmaks)

A 30 ppm concentration galic acid solution was measured in the wavelength range of 400-850 nm using a UV-Vis spectrophotometer (Andriani & Murtisiwi, 2018) (Dewantara, Agus, & Andayani, 2021).

c. Measurement of galic acid standard solution

Standard solutions are made in a series of concentrations of 10, 20, 30, 40 and 50 ppm. Each concentration of gallic acid solution was taken as much as 0.8 mL and 4 mL of Folin-Ciocalteu reagent (1:10) was added, the solution was shaken and left for 3 minutes. Then a solution of 7.5% Na2CO3 of 3.2 mL is added and the solution is boiled until homogeneous and left for 45 minutes at room temperature. Next, the absorbance of the solution is measured at the maximum wavelength of the galic acid and a calibration curve of the galic acid is created (Dewantara et al., 2021).

d. Determination of the total phenolic content of strawberry leaves

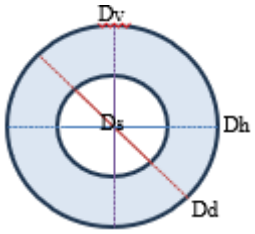
A total of 50 mg of aqueous extract and 10 mg of strawberry leaf ethanol extract were each dissolved with an aquaades:ethanol solvent (1:1) in a measuring flask to the limit mark and a sample was obtained with a concentration of 1000 ppm each. The sample solution is then reacted with the same procedure as the manufacture of a standard gallic acid solution. The aqueous extract and ethanol extract test solutions are made triplo respectively, the absorbance of the solution is measured at the maximum wavelength in 3 repetitions (Andriani & Murtisiwi, 2018). The total phenolic content was determined by entering the absorbance data of aqueous extract samples and strawberry leaf ethanol extract in the equation of the raw curve of gallic acid as a value of y, then the x-value obtained was the equivalent of milligrams of gallic acid in each gram of extract sample (GAE). The calculation of the total phenolic level is determined by the following formula:

$$KTFe = \frac{c \,.\, v \,.\, fp}{g}$$

where, c = sample concentration, v = volume of extract used, fp = dilution factor, and g = sample weight.

2.4. Antifungal Activity Test of Strawberry Leaf Extract

Antifungal activity testing was carried out to find out whether the test sample used was able to inhibit the fungus by marking the formation of an inhibition zone. Testing of the antifungal activity of aqueous extract and ethanol extract of strawberry leaves against *A. corymbifera* was carried out using the diffusion method with replication 3 times. The test samples of strawberry leaf aqueous extract (0.5 mL/mL) and ethanol extract (0.5 g/mL) of strawberry leaves were made at a concentration of 50% with aquades solvent and 2.5% ethanol. A 5.5 mm diameter well is made in the medium so that *Potato Dextrose Agar* (PDA) has been inoculated with 100 µL of *A. corymbifera suspension* using a cylindrical cup *or* cork borer and dripped with a test sample of 20 µL. Then incubated at a room temperature of 20-25 ºC for 66 hours. The diameter of the barrier zone around the wells is measured every 6 hours using a caliper. The positive control of ketoconazole (0.5 g/mL) was also used in a concentration of 50% and the negative control used was aquades and 2.5% ethanol. The calculation of the diameter of the inhibition zone is determined by the following formula:



$$\frac{(Dv - Ds) + (Dh - Ds) + (Dd - Ds)}{3}$$

Dv = diameter vertical
Dh = diameter horizontal
Dd = diameter diagonal
Ds = diameter of the shaft

3. RESULTS AND ANALYSIS

The results of this study present a comprehensive evaluation of the phytochemical composition, total phenolic content, and antifungal activity of aqueous and ethanol extracts of strawberry (*Fragaria spp.*) leaves against *A. corymbifera*. The discussion focuses on comparing the bioactive compounds identified in both extracts, their quantified phenolic contents, and the degree of fungal inhibition observed at various time points. By correlating the chemical profiles with the antifungal performance, this section aims to provide insight into the potential of strawberry leaf extracts—particularly the ethanol extract—as a natural alternative for controlling fungal infections caused by *A. corymbifera*. The findings are discussed in the context of previous studies on plant-derived antifungal agents and highlight the relevance of solvent polarity in extracting active compounds.

3.1. Phytochemical Screening of Strawberry Leaf Extract

The sample used in this study was 3-month-old strawberry leaves planted in the Magetan, East Java is seen in Figure 1. Based on the results of the determination test conducted at the Plant Systematics Laboratory, Faculty of Biology, Gadjah Mada University with letter number 00873/S.Tb./IV/2025, strawberry leaves are derived from the strawberry plant of the species *Fragaria x ananassa Duchesne* with the local name garden strawberries.

Figure 1. *Strawberry leaves*

The resulting strawberry leaf aqueous extract is in the form of a brownish-yellow suspension with a distinctive leafy aroma. A thick extract of blackish-green strawberry leaves of 9.3 grams is produced from the maceration of *shakers* with 96% ethanol solvent. The yield from the extraction of strawberry leaves produced is 15.5%. In this study, aqueous extract and ethanol extract of strawberry leaves were phytochemical screening for the presence of tannins, flavonoids, saponins, steroids/terpenoids, and alkaloids with the test results in Table 1.

Table 1. Phytochemical screening results

Parameter	Reagents	Test Results	
		EA	EE
Tannin	FeCl3 5%	+	+
Flavonoid	NaOH 10%	+	+
Saponin	Aquades + HCl	-	-
Steroid	Liberman-Burchard reagent	-	+
Terpenoid		-	-
Alkaloid	HCl + Wagner reagent	+	-
	HCl + Mayer reagent	+	-

Description: EA= Strawberry leaf aqueous extract and EE= Strawberry leaf ethanol extract

In this study, the tannins detected in the strawberry leaf extracts were identified as hydrolyzable tannins, as evidenced by the appearance of a blue-black coloration upon the addition of ferric chloride, consistent with previous findings (Arviani et al., 2023). This is due to the formation of the khelat complex compound between tannins and ferry chloride seen in Figure 1., the presence of Fe3+ ions as the central atom and tannins that have oxygen atoms with free electron pairs can coordinate with the central atom as ligands. The oxygen atom in the orthohydroxy position of the tannins has the lowest energy so that it is possible to become a ligand in the formation of complex compounds (Sulasmi, Saptasari, Mawaddah, & Zulfia, 2019). This is in accordance with the results of the research Fecka et al. (2022) that ellagitanin is one of the main components present in strawberry leaves which is included in the type of hydrolyzed tannin. Ellagitanin may provide protection against the development of metabolic syndrome and some microbial diseases or infections (Muthukumaran et al., 2017).

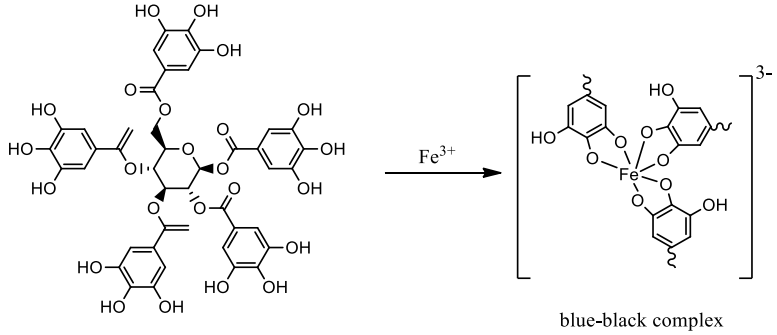


Figure 2. Reaction of the formation of tannin complex compounds with ferri chloride

The presence of flavonoid compounds in aqueous extract and ethanol extract of strawberry leaves in this study was characterized by a change in color to deep yellow after 10% NaOH was added. This is due to the metathesis of salts producing flavonoid derivatives with altered properties. The reaction that occurs between flavonoid compounds and bases can be seen in Figure 2. This mechanism involves the deprotonation of acids by bases, that is, in the hydroxyl group of flavonoid compounds forming phenolic ions that increase the delocalization of electrons, then followed by the resulting anion reaction to resonating to form quinoids producing a deep yellow color (Nurani et al., 2024). These results are in line with research Salas-Arias, Irías-Mata, Sánchez-Kopper, Hernández-Moncada, Salas-Morgan,

Villalta-Romero, & Calvo-Castro (2023) that strawberry leaf ethanol extract based on *High Performance Liquid Chromatography-Mass Spectrometry* (HPLC-MS) testing contains nine subclasses of flavonoid compounds. Flavonoid compounds can bind to phospholipids of fungal cell membranes so that they can interfere with cell membrane permeability and fungal growth will be inhibited (Arifin, Khotimah, & Rahmayanti., 2018).

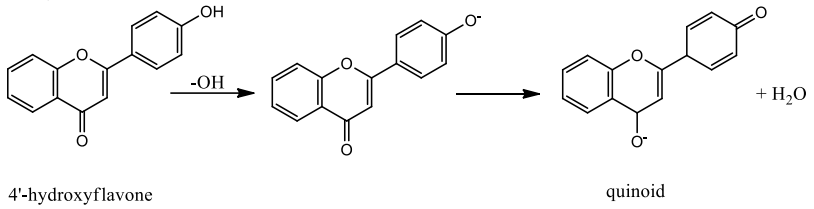


Figure 3. Reaction to the formation of quinoids

Terpenoids have various biological activities, one of which is antimicrobial so that it is widely used in medicine, agriculture, and industry (Nugroho, 2017). Triterpenoid compounds can be used as a remedy for skin disorders (Heliawati, 2018). This group of terpene compounds consists of various substances that are generally insoluble in aqueous (Arviani et al., 2023). In this study, terpene compounds were only found in strawberry leaf ethanol extract in the form of steroids which are triterpenoid compounds derived from squaleen triterpenes (Julianto, 2019). This is in accordance with the results of isolation and identification in the study Gleńsk et al. (2021) which reported the presence of six triterpenoid compounds in strawberry leaves. Changes in the green test solution indicate the presence of steroid compounds. Reactions between steroids and Liberman-Buchard reagents, according to Zaini & Shofia (2020) predicted as in Figure 3. A dehydration reaction occurs those releases aqueous molecules in an acidic medium, resulting in the double bond being transferred. The compound undergoes resonance acting as electrophiles and carbocation. The carbocation attack causes electrophilic addition, followed by the release of the hydrogen group and its electrons, so that the compound undergoes conjugation extension (Nurjannah, Mustariani, & Suryani, 2022).

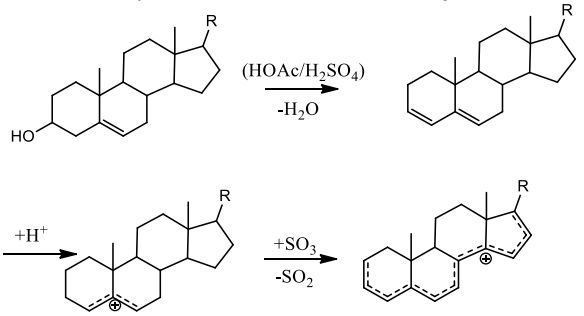


Figure 4. Steroid reactions with Liberman-Buchard reagents

In general, alkaloids in plants bind to organic acids to form salts that can be extracted with appropriate organic solvents (Wahyuningsih, Yunita, Sundari, Pagalla, Kalalinggi, Alpian, Nurmalasari, Suryandani, Ramlah, & Nasrullah, M 2024). Alkaloid bentuk garamnya mudah larut dalam air (Heliawati, 2018). Alkaloid compounds are antifungal because they can prevent fungal DNA replication (Komala, Yulianta, & Siwi., 2019). The presence of alkaloid compounds in this study was only found in strawberry leaf aqueous extract. The results of this study have similarities with the research Kusuma, Djuhariah, & Desi (2023) that in dried powder and infusion of strawberry leaves the presence of alkaloid compounds was detected, in addition to the study Salas-Arias et al. (2023) Negative phytochemical screening results were also obtained in the alkaloid test of strawberry leaf ethanol extract. According to Marliana et. al, (2005) in (Hanifa, Wirasisya, Muliani, Utami, & Sunarwidhi, 2021) The reaction that occurred in the alkaloid test is seen in Figure 4. The yellowish-white precipitation is formed due to the presence of a coordinate covalent bond between the free electron pairs on the alkaloid

nitrogen atom with the K^+ ion of the potassium tetraiodomercurate (II) in the Mayer reagent. As for the Wagner test, reddish-brown deposits were formed because the K^+ ions in potassium iodide formed a coordinate covalent bond with nitrogen in the alkaloids, the reddish-brown color arose due to the presence of I^{3-} ions resulting from the reaction of iodine with I^- of potassium iodide.

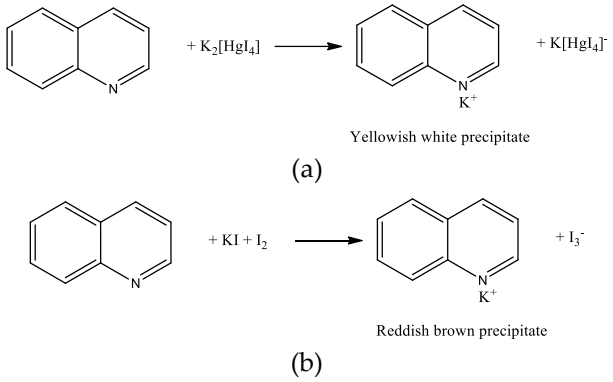


Figure 5. Alkaloid identification reaction by reagents (a) = Mayer and (b) = Wagner

Saponins are made up of sugars such as glucose, galactose, glucuronic acid, or xylose bound to hydrophobic aglicons that may be triterpenoids or steroids (Moghimipour & Handali, 2015). The results of phytochemical screening of aqueous extract and ethanol extract of strawberry leaves in this study did not detect the presence of saponins. In this study, the extract was made by cold extraction method without heating. While saponins are one of the glycoside groups (Arviani et al., 2023), glycoside compounds are more easily soluble in aqueous solvents by heating (Badan Pengawas Obat dan Makanan, 2023). So it is suspected that the saponin levels in this study are very small and not detected in phytochemical screening. These results are different from studies Salas-Arias et al. (2023) Where ethanolic extract of strawberry leaf showed positive saponin content in the foam test. This can possibly be caused by the difference in the ethanol solvent used, in the study the extraction used 70% ethanol solvent which has a greater aqueous content than 96% ethanol. While saponins are more easily dissolved in aqueous (Nugroho, 2017).

3.2. Total Phenolic Content Test of Strawberry Leaf Extract

Phenolic compounds are organic compounds that contain benzene rings with one or more hydroxyl groups bonded (Nurani et al., 2024). Based on the results of phytochemical screening that has been carried out, strawberry leaf extract contains phenolic compounds in the form of tannins and flavonoids. Phenolic compounds have pharmacological effects as antimicrobials (Wahyuningsih et al., 2024), This pharmacological effect is expected to provide an antifungal effect against *A. corymbifera*.

The determination of the total phenolic content of strawberry leaf extract was carried out using the Folin-Ciocalteu method with gallic acid as the standard solution. Gallic acid is reacted with Folin-Ciocalteau reagents in an alkaline atmosphere. The added 7.5 % Na_2CO_3 solution is used as a alkaline atmosphere because phenolic compounds react with Folin-Ciocalteau reagents only in the alkaline atmosphere to cause proton dissociation into phenolate ions. During the reaction, the hydroxyl group of the phenolic compound as a reducing agent reacts with the Folin-Ciocalteau reagent forming a blue molybdenum-tungsten complex that will become more concentrated if the phenolic content in the sample is greater (Mukhrian, Sugiarna, Farhan, Rusdi, & Asrul., 2019). The exact chemical structure of the Folin Ciocalteu reagent is unknown, but it is described as a complex mixture of phosphotungstic acid and phosphomolybdate reduced during testing resulting in blue chromophores (Pérez, Dominguez-López, & Lamuela-Raventós., 2023). According to Munteanu & Apetrei (2021) The phenolic compound reaction with Folin-Ciocalteu that occurs can be seen in Figure 5.

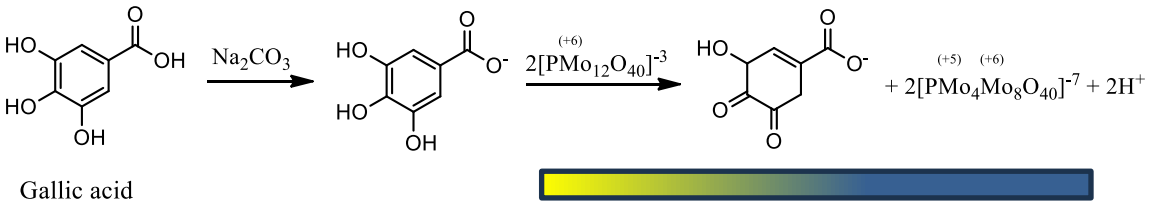


Figure 6. Gallic acid reaction with Folin-Ciocalteu reagent

The maximum wavelength obtained in this study is 758 nm. The results of the measurement of the standard solution of gallic acid can be seen in Table 2. with the calibration curve of the relationship between concentration and absorbance in Figure 6. and obtained the results of the total phenolic content of strawberry leaf extract in Table 3.

Table 2. Absorbance data of standard galic acid solution

Concentration (ppm)	Absorbance
0	0
10	0,13
20	0,166
30	0,328
40	0,455
50	0,58

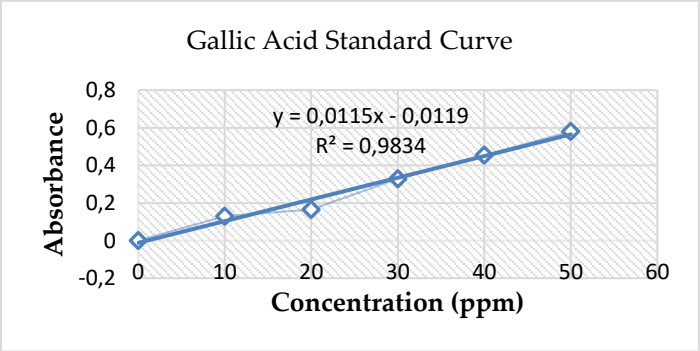


Figure 7. Gallic acid standard curve

Table 3. Total phenolic content test results

Sample	EA			EE		
Absorbansi	0,05176	0,06116	0,06787	1,44157	1,44844	1,59637
X concentration (mg/L)	5,528	6,345	6,929	126,361	126,958	139,822
KTFe (mg GAE/g)	5,528	6,345	6,929	126,361	126,958	139,822
Average (mg GAE/g)	6,267			131,047		
SD	0,70			7,61		
KTFe ± SD (mg GAE/g)	6,267 ± 0,70			131,047 ± 7,61		

Description: EA= Strawberry leaf aqueous extract, EE= Strawberry leaf ethanol extract, and KTFe= Total phenolic content

Based on the graph in Figure 6. The regression equation for gallic acid absorbance is Y = 0.0115x – 0.0119. In the equation of the raw curve of gallic acid, a linear relationship between absorbance and concentration is obtained with a correlation coefficient value of >0.98 which is close to one. The total phenolic concentration of the sample solution of aqueous extract and ethanol extract of strawberry leaves was calculated based on the standard linear equation of gallic acid obtained the results were 6.267±0.70 mg GAE/g and 131.047±7.61 mg GAE/g, respectively.

The total phenolic content of strawberry leaf ethanol extract in this study was greater than the total phenolic content of the study Zhu et al. (2015) which shows that the results of ethanol extract and strawberry leaf aqueous extract respectively have a total phenolic content of 117.1±2.7 mg GAE/g extract and 129.6±4.2 mg GAE/g extract, while the total phenolic content of strawberry leaf aqueous extract in this study is smaller. This difference can most likely be suspected due to slight differences in extraction methods, length of time, and concentrations of ethanol solvents used. In research Zhu et al. (2015) The ethanol solvent used is more polar, namely 99.5% ethanol, and the extraction of the maceration shaker is carried out for 24 hours, and for aqueous extract, a freeze-drying procedure is carried out. The total phenolic content of aqueous extracts in this study was also smaller than the total phenolic levels of aqueous extracts in the study of Dias, Barros, Fernandes, Ruphuy, Oliveira, Santos-Buelga, Barreiro, & Ferreira., (2015), where the total phenolic content of strawberry leaf aqueous extract by infudation method was 43.99±0.37 mg GAE/g extract and strawberry leaf aqueous extract by decocation method was 45.38±0.80 mg GAE/g extract. This shows that different extraction methods can affect the chemical compound content of the extracted chemicals.

Phenolic compounds can be polar or non-polar so that phenolic compounds and polyphenols will be extracted in solvents according to polarity (Nurani et al., 2024). The total phenolic content of strawberry leaf ethanol extract in this study was greater than that of strawberry leaf aqueous extract. This can be caused because the phenolic compounds in strawberry leaves are more easily soluble in ethanol solvents compared to aqueous solvents. Ethanol solvents can extract phenolic compounds with a wider polarity range, while aqueous solvents can only extract highly polar phenolic compounds and glycoside-bound phenolic compounds such as flavonoid glycosides and coumarin glycosides but need to be assisted by heating (Badan Pengawas Obat dan Makanan, 2023). Phenolic compounds that bind sugars will increase their solubility in polar solvents (Nurani et al., 2024).

3.3. Antifungal Activity Test of Strawberry Leaf Extract

The antifungal activity test of strawberry leaf extract with a concentration of 50% against *A. corymbifera* was determined using the well diffusion method. The average result of the inhibition zone diameter of aqueous extract and ethanol extract of strawberry leaves with a concentration of 50% is shown by the formation of an inhibition zone around the well with the results collected in Table 4.

Table 4. Average diameter of strawberry leaf extract inhibition zone (mm)

Hour	EA	EE	C +	C -
6	0 ± 0	0 ± 0	0 ± 0	0 ± 0
12	0 ± 0	0 ± 0	11,633 ± 6,561	0 ± 0
18	1,31 ± 2,269	0 ± 0	22,13 ± 3,132	0 ± 0
24	6,9 ± 3,154	9,245 ± 1,157	19,187 ± 1,435	0 ± 0
30	8,345 ± 1,959	10,055 ± 1,791	17,773 ± 2,122	0 ± 0
36	6,765 ± 0,332	4,22 ± 1,499	16,143 ± 1,957	0 ± 0
42	3,13 ± 0,760	11,495 ± 0,0495	18,92 ± 2,037	0 ± 0
48	1,733 ± 0,085	13,065 ± 0,516	16,95 ± 0,980	0 ± 0
54	0 ± 0	1,23 ± 1,087	16,42 ± 1,512	0 ± 0
60	0 ± 0	1,44 ± 1,247	17,83 ± 3,987	0 ± 0
66	0 ± 0	0,789 ± 0,800	15,41 ± 1,383	0 ± 0

Description: EA= 50% strawberry leaf aqueous extract= strawberry leaf ethanol extract 50% C+= positive control (ketoconazole), and C+= negative control (aquades and ethanol 2.5%)

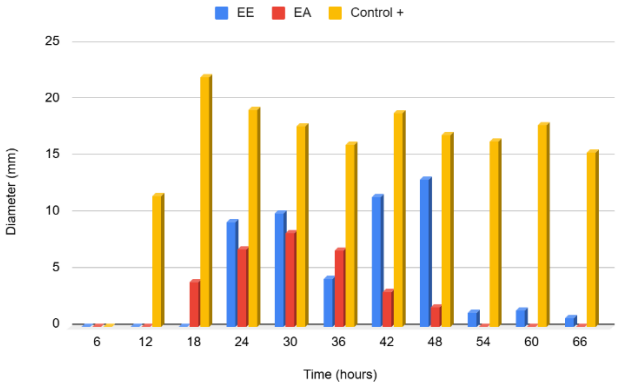


Figure 3. Graph of the antifungal activity of strawberry leaf extract

Based on the graph in Figure 3. The diameter of the antifungal inhibition zone of strawberry leaf extract tends to increase along with the increase of certain incubation times, then decrease. At the 6th hour, the fungal growth inhibition zone was not visible for all positive test and control solutions because it was still the early phase of fungal spore growth in the new environment. The diameter of the highest positive control inhibition zone at the 18th hour was 22.13 ± 3.132 mm. As for the negative control, there is no indication of an inhibitory zone against the fungus. Based on these results, it was shown that the test procedure performed correctly and the solvent used did not have antifungal activity (Novia Putri, Nuriyah Wahidah, Hosiyah, Taufiq Al Hafidz, & Faisal., 2023). The highest antifungal activity in inhibiting the growth of *A. corymbifera* for strawberry leaf aqueous extract at the 30th hour, while strawberry leaf ethanol extract at the 48th hour, this is indicated by the largest inhibition zone diameter at the clock which is 8.345 ± 1.959 mm and 13.065 ± 0.516 mm, respectively. Based on the category of inhibition response in the study of Katili, Wewengkang, & Rotinsulu (2020) In this study, the diameter of the aqueous barrier zone of strawberry leaf extract has moderate inhibition and ethanol extract of strawberry leaf has strong inhibition against *A. corymbifera*.

The antifungal activity of strawberry leaf ethanol extract with a concentration of 50% against *A. corymbifera* in this study had a small inhibition when compared to the inhibition of methanol extract and strawberry leaf hydromethanol extract with a concentration of 1% (10 mg/mL) in the study of El-Mesallamy et al. (2013) terhadap *Fusarium oxysporum, Aspergillus niger, penicillium sp., Cladosporium sp.*, & *Aspergillus flavus*. The diameter of the inhibition zone of strawberry leaf aqueous extract with a concentration of 50% is close to the diameter of the inhibition zone of methanol extract and strawberry leaf hydromethanol extract with a concentration of 1% against *penicillium sp.* and *F. oxysporum* respectively, namely 8 mm and 9 mm. Meanwhile, the maximum inhibition zone diameter of ethanol extract of strawberry leaves with a concentration of 50% is not far from the diameter of the inhibition zone of methanol extract and hydromethanol extract of strawberry leaf with a concentration of 1% against *A. niger*, which is 10 mm. This can occur allegedly due to the difference in the solvent in the test solution used to dissolve the extract and the type of fungus.

In this study, strawberry leaf ethanol extract has a stronger inhibition than strawberry leaf aqueous extract in inhibiting *A. corymbifera.* This is because the content of active compounds in ethanol extract is greater than in aqueous extract, as stated in Table 3. The content of one of the active compounds that has antifungal activity in the form of total phenolic content in strawberry leaf ethanol extract is greater than that of strawberry leaf aqueous extract. Polyphenol compounds such as tannins and flavonoids are antifungal because they have hydroxyl groups that can interact with biological targets such as enzymes, receptors, and DNA so that they can inhibit fungal growth (Nurani et al., 2024). The type of solvent, the compound content, and the concentration of the sample can affect the antibacterial activity (Hafizah, Permatasari, & Muchlishah, 2024) So these factors are also suspected to affect the antifungal activity of strawberry leaf extract against *A. corymbifera*. In addition, strawberry leaf aqueous extract has a high moisture content. Enzymatic reactions in extracts that have a high

moisture content can cause the active compound to be unstable or degraded so that it can reduce antimicrobial activity (A. A. Setiawan, Aditama, & Yusransyah, 2018). The diameter of the antifungal inhibition zone can also be influenced by technical factors such as ambient temperature, pH, and media, as well as biological factors such as biofilm formation, phenotype changes, hydrolysis enzyme secretion, adhesin expression and fungal cell invasion during the research process (Arifin et al., 2018).

The antifungal activity of strawberry leaf extract with a concentration of 50% against *A. corymbifera* in this study was fungistatic, characterized by the presence of cloudy inhibition zones due to less fertile fungal growth around the test sample compared to areas outside the influence of strawberry leaf extract *samples.* This suggests that the growth of the fungus is inhibited by the test compound but does not kill the test fungus. Antifungal with the sense of fungistatic is as a compound that can inhibit the growth of fungi without killing them (Herkamela, 2022). According to Mutschler (1999) in (Queendy & Roza, 2019), The criteria for antifungal inhibition zones are divided into two, namely fungistatic and fungicidal. Where fungistatics have a way of working as antifungal compounds that inhibit the growth of fungi without killing them, it is characterized by a cloudy inhibition zone, while fungicides can kill test fungi, characterized by a clear inhibition zone.

Overall, the results demonstrate that ethanol extract of strawberry leaves exhibits stronger antifungal activity against *A. corymbifera* than the aqueous extract, likely due to its higher content of phenolic compounds such as flavonoids and tannins. These bioactive constituents, along with the influence of solvent polarity and extract stability, play a key role in determining the inhibitory capacity of the extracts. The antifungal activity observed was classified as fungistatic, as evidenced by the cloudy inhibition zones, indicating growth suppression without fungal cell death. These findings suggest that strawberry leaf ethanol extract has promising potential as a natural, plant-based antifungal agent, particularly for applications where fungistatic activity is sufficient, such as in topical formulations or preventive skincare products. Future studies are encouraged to explore formulation stability, broader-spectrum antifungal efficacy, and potential synergistic effects with other natural compounds to further support its use in antifungal therapeutics.

4. CONCLUSION

Based on the results of the study, positive strawberry leaf aqueous extract contains tannins, flavonoids, and alkaloids compounds. Meanwhile, strawberry leaf ethanol extract contains tannins, flavonoids, and steroids. However, saponin compounds were not detected in phytochemical tests for both types of extracts. The total phenolic content of aqueous extract and ethanol extract of strawberry leaves were 6.267 ± 0.70 mg GAE/g and 131.047 ± 7.61 mg GAE/g. The antifungal activity of aqueous extract and ethanol extract of strawberry leaves at a concentration of 50% against *A. corymbifera* showed fungistatic antifungal properties with the highest inhibition zone diameter in order, namely 8,345 ± 1,959 mm at the 30th hour which was classified as moderate and 13,065 ± 0.516 mm at the 48th hour which was classified as strong.

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