BIOVALENTIA: BIOLOGICAL RESEARCH JOURNAL
***e*-ISSN: 2477-1392**
Vol. 8 No. 1, May 2022

Potential Active Compounds of Mango Parasite *Dendrophthoe pentandra* (L). Miq as an antifungal

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Peer review under responsibility of Biology Department Sriwijaya University

Abstract

The Mango parasite is a parasitic plant that is very detrimental to mango plant owners but has the potential as a producer of secondary metabolites which are expected to inhibit the growth of the fungus Candida albicans. This study aimed to obtain antifungal activity fractions, to obtain pure compound eluates which were active as antifungals, to determine the class of compounds active as antifungals, and to determine the Minimum Inhibitory Concentration (MIC) against the fungus C. albicans. Based on the research that has been done, the fractions that are active as antifungals are the n-hexane and ethyl acetate fractions. The results of purification of the n-hexane and ethyl acetate fractions using gravity column chromatography showed the presence of two pure n-hexane compounds that were active in inhibiting C. albicans, namely N-1 and N4 eluates with inhibition zone diameters of 12 mm and 10 mm, respectively. Purification of the ethyl acetate fraction produced two active compounds, namely eluate E-1 and eluate E-5 which were able to inhibit C. albicans by 10 mm and 12 mm, respectively. The MIC values for N-1, N-4, and E-1 eluates were able to inhibit at a concentration of 500 ppm and were classified as quite strong. The E-5 eluate capable of inhibiting at a concentration of 1000 ppm is classified as weak. Tests for the class of compounds using Thin Layer Chromatography (TLC) and the Rƒ value of the biautography results showed the color spots on the N-1 eluate (Rƒ: 0.9) were blue in the terpenoid group, on the N-4 eluate (Rƒ: 0.6) Alkaloid group is orange in color, the E-1 eluate (Rƒ: 0.8) is yellow in the Phenolic group, the E-5 eluate (Rƒ: 0.9) is purple in the terpenoid group.

Keywords: Dendrophthoe pentandra (L). Miq, Candida albicans, MIC, Terpenoid, Phenolic.

Received: December 22, 2021, Accepted: March 03, 2022

1. Introduction

Infection is a disease that is native to tropical regions such as Indonesia. Infections caused by fungi are among the most common causes of infection. The *Candida* fungus is one of the fungi that causes infection. *Candida* infections are referred to as candidiasis. Candidiasis is a fungal infection caused by *Candida* species, most often *Candida albicans*, that can affect the mouth, vagina, skin, nails, lungs, and digestive tract. *Candida albicans* is a pathogenic fungus that affects humans [13]. Candidiasis is the most frequent systemic fungal infection caused by *Candida albicans* entering the bloodstream, particularly when the host's phagocytic resistance is compromised [22].

Ketoconazole is a topical medication that is commonly used to treat skin candidiasis. However, as fungus resistance to various types of antibiotics became more common, people began to explore other therapies, such as employing plants as antifungals [9]. Plants are frequently utilized as medication to treat disease since they have few negative side effects. According to [17] Antibiotics produced from natural substances are currently commonly utilized since they have moderate adverse effects and can still be digested by the body, according to various studies.

Mango leaves were extracted with methanol to generate methanol extract with a yield of 10.55 percent (b/b) and antifungal activity against *Candida albicans* with the highest inhibition zone at a concentration of 1000 ppm with an inhibition zone of 8.12 mm [10]. The results of the research by [6] the diameter of the inhibition zone against *C. albicans* was measured using the Agar Diffusion method

on the leaves of the Green Champa tree parasite at total flavonoid sample concentrations of 3 %, 6 %, and 9 %, 8.30; 4 and 5.30 mm. This suggests that mango leaves and *D. pentandra* are related (L). Miq. has a high antifungal activity, so the mango parasite's leaves should have the same or higher antifungal activity.

Traditional treatments based on natural plant extracts still use a wide range of doses, so it's important to know the lowest dose or concentration that can inhibit the growth of disease-causing microbes as a starting point for determining the right dose or concentration that's also relatively safe to use. As a result, the Minimum Inhibitory Concentration (MIC) of natural plant extracts must be determined.

The researchers intended to look into the role of secondary metabolites in the mango parasite plant as antifungal and inhibitory to the *Candida albicans* fungus, therefore they looked into the mango parasite *D. pentandra* (L. Miq) as an antifungal.

2. Materials and Methods

2.1. Sample Collection and Processing

The sample of *D. pentandra* (L.) Miq was taken from the Belitang area, Ogan Komering Ulu Timur. Mango parasite leaf sampling was carried out using a purposive sampling method, the part taken was the entire leaf. After that, wet sorting was carried out to separate the leaves from the stems and remove the dirt that was attached to the parasite leaf samples. The leaves of the mango parasite are dried by aerating for several days in the open air without being exposed to direct sunlight. The dried leaves were crushed to form a powder and then sieved using a sieve to obtain *D. pentandra (L)* simplicia powder. Miq.

2.2. Sample Extraction and Fractionation

Extraction was carried out by maceration, namely 500 g of parasite leaf powder was put into a glass bottle container and added with methanol solvent until the simplicia was submerged with a volume of 2000 mL. This mixture is left for 2 x 24 hours. The maceration solution was evaporated using a rotary evaporator at a temperature of 70 ^{o}C. The viscous extract obtained is used in the fractionation stage [23].

The fractionation step was carried out using extracts from maceration added with distilled water in a ratio of 1:1. The extract with a volume of 200 mL was then added with n-hexane (1:1) solvent in a separating funnel and repeated until the n-hexane solution was clear. The methanol-water fraction was continued by adding ethyl acetate solvent and the same treatment was carried out as n-hexane. From the fractionation process, three fractions were obtained, namely the n-hexane fraction, the ethyl acetate fraction, and the methanol water fraction. The three fractions were evaporated using a *rotary evaporator* to obtain an extract in the form of a paste and tested. Furthermore, the three fractions of n-hexane, ethyl acetate, and methanol water will be tested for antifungal activity [14].

2.3. Test the Antifungal Activity of the *Dendrophthoe pentandra* (L). Miq fraction

C. albicans suspension was placed in a 0.1 mL petri plate, 10 mL SDA media was added, and the mixture was homogenized. On the surface of a solid medium containing the fungal culture, 6 mm diameter paper disk was inserted. Each petri dish contained five paper disks, each of which was aseptically dripped with 20 µL of each fraction at a concentration of 10 % in DMSO. All of the tests were done in one petri dish with DMSO as a negative control and ketoconazole as a positive control. The diameter of the inhibitory zone generated was measured after the culture had been incubated for two days. A ruler was used to calculate the antifungal inhibitory zone [20].

2.4. Active Compound Isolation

Silica gel powder was inserted into the column reaching three quarters of the column height, then added with extract and eluted using a gradient solvent system, n-Hexane: ethyl acetate in a ratio of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 as much as 100 mL with an elution rate of 30 drops per minute, the volume of the fraction accommodated is 10 mL gradually with the column valve opened. The compound is then accommodated in a 20 mL vial and evaporated using a *hair dryer* and a concentrated eluate is obtained [4].

2.5. Determination of Minimum Inhibitory Concentration (MIC)

Isolates of antifungal compounds were made with a concentration of 1000; 500; 250; 125; 65.5; 31.25 µL/mL using DMSO as a solvent. The paper disks were placed in a petri dish containing a negative control and 1 positive control of ketoconazole and a paper disk of various concentrations with a volume of 20 µL and then placed into a cup containing the culture medium of the fungus *C. albicans*. The culture was incubated for 2 x 24 hours at 37 ^{0}C and the diameter of the inhibition zone formed was measured [14].

2.6. Analysis with Thin Layer Chromatography (TLC)

Determination of the group of active compounds using a TLC plate with silica Gel254 Merck. Every 1 % pure eluate of *D. pentandra* (L). Miq which has been dissolved with solvent is spotted on the TLC plate using a capillary pipette to form a small circle, then the TLC plate is eluted by placing it in a glass chamber containing solvent. The TLC plate was propped up and left until the solvent rose to the limit line. The eluted TLC plate was sprayed with H_2SO_4

solution and heated using a hot plate, observed the color of the spots formed [21].

The value of the retention factor (R *f*) is determined using the formula:

$$Rf = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent}}$$

2.7. Test of Bioautography

The results of the TLC test with known colored spots of the compound were affixed to a petri dish containing SDA media that had been inoculated with the fungus *C. albicas*. The TLC plate was left attached to the SDA medium for 1 hour so that the active compounds were diffused into the media. After the TLC plate was removed the SDA media was incubated for 48 hours. The clear zone is the area of the active compound [15].

3. Results and Discussion

3.1. Extraction and Fractionation

The result of 500 g extraction with methanol solvent obtained 41g (8.2 %) extract. The fractionation step of 41 g of extract using n-hexane solvent obtained 9.7 g (25.90 %) extract using ethyl acetate solvent obtained 6.87 g (18.34 %) extract while water methanol solvent obtained 20.88 extract g (55.75 %). From the results above, it can be seen that the n-hexane and ethyl acetate fractions get smaller amounts than the methanol water fraction because they depend on the polarity of the compound in the solvent. According to [8] Different solvents will dissolve different compounds depending on the level of polarity.

The results of testing the antifungal activity of extracts of n-hexane, ethyl acetate, and methanol water can be seen in Figure 1. and Table 2.

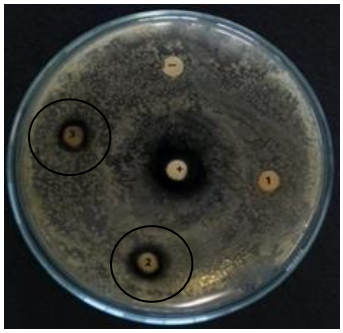

Figure 1. The results of the antifungal fraction activity test.
Description:
1) Methanol, 2) n-hexane, 3) ethyl acetate,
-) DMSO, +) ketoconazole

Table 2. Activity Test Results of the Antifungal Fraction

No	Fraction	Concentration (%)	Inhibitory (mm)
1	Methanol Water	10	0
2	N-heksan	10	12 mm
3	Ethyl acetate	10	10 mm
-	DMSO	10	0
+	Ketokonazole	10	22 mm

Based on Table 2. above, the n-hexane fraction has the largest inhibition zone diameter against *C. albicans*, which is 12 mm, while the ethyl acetate fraction is 10 mm and the methanol fraction has no inhibition zone diameter against *C. albicans.*

The content of compounds that have antifungal activity in *D. pentandra* are compounds that are soluble in n-hexane and ethyl acetate solvents which have antifungal activity, while methanol does not have antifungal activity because it does not contain substances strong enough to inhibit the fungus *Candida albicans*. According to [14] the size of the inhibition zone created as a result of the antifungal activity test is dependent on the amount of active ingredient present in the fraction. Meanwhile, the presence or lack of active chemicals in the fraction determined whether or not an inhibition zone formed around the paper disk.

3.2. Sub-faction Refining

The fractionation step was carried out using extracts from maceration added with distilled water in a ratio of 1:1. The extract with a volume of 200 ml was then added with n-hexane (1:1) solvent in a separating funnel and repeated until the n-hexane solution was clear. The results of the sub-fraction obtained using gravity column chromatography were 13 cups of n-hexane solvent isolate using a ratio of n-hexane: ethyl acetate (7:3) 3 cups, (8:2) 4 cups, (9:1) 4 cups, 1 cup 100% n-hexane, and 1 cup methanol. And vice versa with the ethyl acetate subfraction. The antifungal test results can be seen in Figure 2 and Figure 3.

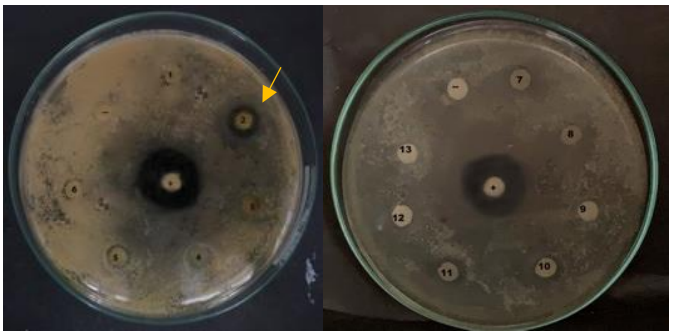

Figure 2. Test of n-hexane Sub Fraction.
Note: ↙ : No 2) Active Antifungal *C. albicans*

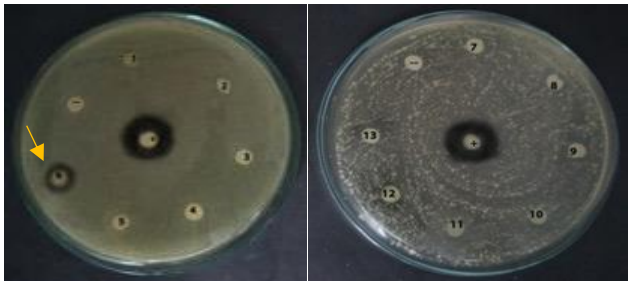

Figure 3. Test of Ethyl acetate Sub Fraction.
Note: ↘: No 6) Active Antifungal *C. albicans*

The inhibition zone diameter of *C. albicans* on isolate No. 2 with an inhibition zone of 10 mm was found in the test findings of the n-hexane subfraction as an antifungal isolate. Isolate No. 6 with an inhibition zone diameter of 10 mm in the ethyl acetate subfraction isolate with an inhibition zone diameter of *C. albicans*. The presence of chemicals in the isolate that inhibited fungus development resulted in the establishment of an inhibition zone in this test. In line with the opinion of [14] The formation of an inhibitory zone around the paper disk indicates that the extracts/fractions from plants contain antimicrobial compounds.

3.3. Purification and Anti Activity Test Fungi Isolate No. 2 N-hexane

Isolate No. 2 from the n-hexane sub-fraction was known to be active, so it was continued with purification using gravity column chromatography. The results of the purification of the n-hexane sub fraction No. 2 were obtained as many as 12 eluates. All eluates purified by column were tested for their antifungal ability (Table 3).

Table 3. *C. albicans* antifungal test results Isolate Sub Fraction n-hexane No 2

No	Eluate	Color of eluate	Zone of inhibition
1	1	Yellow	12 mm
2	2	Yellow	0
3	3	Yellow orange	0
4	4	Yellow orange	10 mm
5	5	Yellow	0
6	6	Yellow orange	0
7	7	Yellowish green	0
8	8	Greenish yellow	0
9	9	Blackish green	0
10	10	Blackish green	0
11	11	Blackish green	0
12	12	Blackish green	0
+	Ketokonazole		27 mm
-	DMSO		0

Based on Table 3. above, each eluate has a different color, according to the compound content of the eluate. The secondary metabolites contained in the extract have been separated according to the polarity of the compound towards each eluate. According to [18], the separation of compounds by column chromatography method aims to separate compounds based on their level of polarity, namely through stationary and mobile phases.

In Table 3. the eluates which have antifungal compounds are No. 1 and No. 4 with the largest diameter found in eluate No. 1 which is 12 mm and eluate No. 4 has an inhibition zone of 10 mm. This is because the eluates No. 1 and No. 4 have active substances as antifungals that can inhibit the fungus *C. albicans.* According to [1] the results of different diameters of the inhibitory zones showed different eluate abilities in inhibiting the growth of the test fungi, this difference in the diameter of the inhibition zones could be caused by differences in the content of secondary metabolites contained.

3.4. Purification and Antifungal Activity Test of Isolate No. 6 Ethyl Acetate

Isolate No. 6 from the ethyl acetate sub-fraction was known to be active as an antifungal, so it was continued with purification using gravity column chromatography to obtain pure compounds in isolate No. 6. The results of gravity column chromatography for isolate No. 6 ethyl acetate were obtained as much as 10 eluate. All eluates purified by column were tested for their antifungal ability (Table 4).

Table 4. Results of C. albicans Anti antifungal test Isolate Sub Fraction No. 6 Ethyl acetate

No	Eluate	Color of eluate	Zone of inhibition
1	1	Greenish yellow	10 mm
2	2	Greenish yellow	0
3	3	Green	0
4	4	Dark chocolate	0
5	5	Dark chocolate	12 mm
6	6	Yellowish green	0
7	7	Yellowish green	0
8	8	Chocolate	0
9	9	Chocolate	0
10	10	Chocolate	0
+	Ketokonazole	1000	23 mm
-	DMSO	100%	0

Table 4. Above isolates No. 6 ethyl acetate which is antifungal, namely eluate with No. 1 and 5 with the largest diameter found in eluate No. 5 which is 12 mm and eluate No. 1 has an inhibition zone of 10 mm. From the data above, it can be seen that the pure eluate compounds that are active as antifungals are in No. 1 and No. 5 which means that the other eluates cannot inhibit the test fungi due to the ability of the compounds in the eluate not to be able to inhibit the test fungi.

3.5. Minimal Inhibitory Concentration (MIC)

Determination of the minimum inhibitory concentration (MIC) N-1, N-4, E-1, and E-5 against the

fungus *C. albicans* produced different MICs. The smaller the MIC, the higher the antifungal activity.

Table 5. Minimum Inhibitory Concentration Eluate N-1, N-4, E-1, and E-5

No	Eluate	Concentration	Inhibitory zone
1	N-1	1000	11 mm
2		500	9 mm
3		250	0
4		125	0
5		65,5	0
6		31,25	0
7	Control (+)	1000	20 mm
8	Control (-)	100%	0
1	N-4	1000	13,8 mm
2		500	13,1 mm
3		250	0
4		125	0
5		65,5	0
6		31,25	0
7	Control (+)	1000	20,1 mm
8	Control (-)	100%	0
1	E-1	1000	13,8 mm
2		500	13 mm
3		250	0
4		125	0
5		65,5	0
6		31,25	0
7	Control (+)	1000	23 mm
8	Control (-)	100%	0
1	E-5	1000	12 mm
2		500	0
3		250	0
4		125	0
5		65,5	0
6		31,25	0
7	Control (+)	1000	24 mm
8	Control (-)	100%	0

Based on Table 5. MIC eluate N-1 against the fungus Candida albicans was 500 μg/mL with an inhibition zone diameter of 9 mm. The MIC produced by the N-1 eluate terpenoid group compounds can be categorized as strong antifungal groups. This is in accordance with the opinion of [14] Active compounds that have a MIC of less than 100 μg/mL are classified as compounds that have a very strong level of activity. Active compounds that have MIC values between 100-500 μg/mL are classified as compounds that have strong enough activity. Compounds that have a MIC value between 500-1,000 μg/mL are classified as compounds that have weak activity, and active compounds that have a MIC of more than 1,000 μg/mL are classified as compounds that do not have antifungal activity.

Based on Table 5. The MIC eluate N-4 against the fungus *C. albicans* was 500 g/mL with an inhibition zone diameter of 13.1 mm. The MIC produced by the eluate alkaloid group N-4 can be categorized as quite strong antifungals. This is because the higher the concentration of compounds in the eluate, the more inhibited the growth of *C. albicans.* This is in line with the opinion of [12] which states that the higher the concentration of an anti-microbial substance, the faster the microorganism cells die or their growth is inhibited.

Based on Table 5. The MIC eluate E-1 against the fungus Candida albicans was 500 μg/mL with an inhibition zone diameter of 13 mm. The MIC produced by the E-1 eluate phenolic group compounds can be categorized as quite strong antifungal groups. Meanwhile, the variation of concentration below 500 ppm could not inhibit the growth of fungi.

Based on Table 5. The MIC of E-5 eluate against the fungus Candida albicans was 1000 μg/mL with an inhibition zone diameter of 12 mm. The MIC produced by the E-5 eluate terpenoid group compounds can be categorized as weak antifungal groups. According to [2], The ability and rate of diffusion of the active components in the medium, the sensitivity of microorganisms to the active substances, and the thickness and viscosity of the medium all determine the size of the inhibitory area.

3.6. Biautographic Identification and Test Antifungal Active Compound

Table 9. Thin Layer Chromatography Test (TLC)

No	Eluate	RF Value	Color	Compound Group
1	N-1	0,9	blue	Terpenoid
2	N-4	0,6	orange	Alkaloids
3	E-1	0,8	yellow	Phenolic
4	E-5	0,9	purple	Terpenoid

Based on Table 9. The results of the TLC that have been carried out can be seen that there is a single color spot, namely the pure compound N-1 eluate on the TLC plate. According to [11], the single spot that results shows that the isolate being tested is pure or a single compound. The stain's visible hue is blue, which is a terpenoid group. The appearance of a blue color with an R*f* value of 0.9 with a ratio of 8:2 (n-hexane: ethyl acetate) is presented in Table 9. and Figure 4. The blue color is produced after being sprayed with 10% H_2SO_4 and heated on a hot plate. The blue color seen is thought to be a group of terpenoid compounds. This is in accordance with the opinion of [24].

Based on Table 9. the results of the TLC that have been carried out can be seen that there is a single color spot, namely the pure compound N-4 eluate on the TLC plate. The color of the stain that appears is an orange color, which is an alkaloid group. The appearance of orange color with an R*f* value of 0.6 with a ratio of 9:1 (n-hexane: ethyl acetate) is presented in Table 9. and Figure 5. Orange colored spots are produced after being sprayed with H_2SO_4 and heated on a hot plate. The orange color seen is thought to be an alkaloid

compound. This is in accordance with the opinion of [5] The emergence of an orange color indicates significant alkaloid compound values.

Table 9 shows that there is a single color spot, namely the pure compound E-1 eluate on the TLC plate, as a result of the TLC that was conducted. The phenolic group is shown by the yellow color of the stain that emerges. Table 9 and Figure 6 show the appearance of a yellow hue with an R*f* value of 0.8 and a 7:3 (n-hexane: ethyl acetate) ratio. After being sprayed with H_2SO_4 and heated on a *hot plate*, yellow spots appear. A phenolic substance is thought to be the source of the yellow color. This is in accordance with the opinion of [16] a phenolic compound is responsible for the yellow color.

Using Table 9 as a basis. The TLC results indicate that there is only one spot color on the TLC plate, and is the pure compound E-5 eluate. Purple is the color of the stain, which belongs to the terpenoid category. Table 5 and Figure 7 show the appearance of purple color with an R*f* value of 0.9 and a 6:4 (n-hexane: ethyl acetate) ratio. After being sprayed with H_2SO_4 and heated on a *hot plate*, purple images formed. A group of terpenoid chemicals is assumed to be responsible for the blue/purple color noticed. This is in accordance with the opinion of [24], after being sprayed with H_2SO_4 and heated, hotspots containing terpenoid chemicals will turn blue-green or purplish-red. The results of the bioutographic test of active eluate as antifungal can be seen in Figures 4, 5, 6, 7.

Figure 4. Results of Biautography Test for Eluat N-1 Active As Antifugal

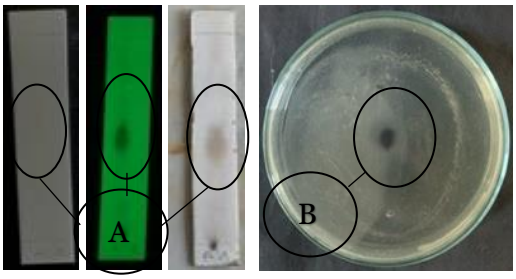

Figure 5. Results of Biautography Test for Eluat N-4 Active As Antifugal

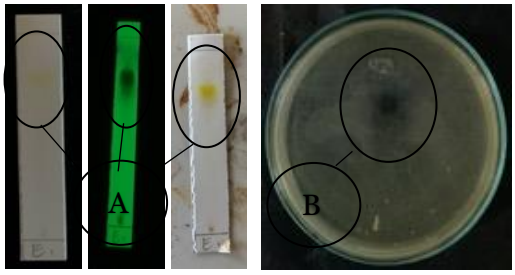

Figure 6. Results of Biautography Test for Eluat E-1 Active As Antifugal

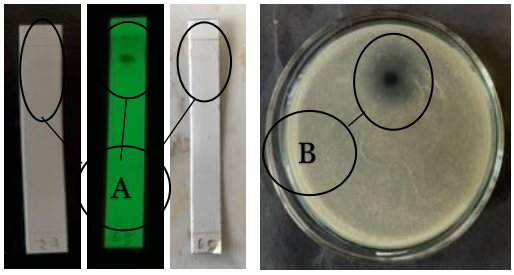

Figure 7. Results of Biautography Test for Eluat E-5 Active As Antifugal

Note:
A: TLC and Rf plate spot color
B: Biautographic test active compound

It can be seen in Figures 4, 5, 6, 7. The same distance between the TLC plate and the plate which was active as antifungal indicated that the compounds were the same between the TLC plate and the plate in each eluate.

In Figure 4. The N-1 eluate shows the location of the compound spots on the TLC plate and the clear zone that appears has the same distance of 4.5 cm or R*f* 0.9, which is a terpenoid compound. Terpenoids have strong antifungal activity against various pathogens, including *C. albicans*, with the mechanism of action of making non-specific membrane lesions on the cell wall of *C. Albicans* [19].

In Figure 5, the compound spots on the TLC plate are shown, and the clear zone that appears has the same distance, namely 3 cm or R*f* 0.6, which is an alkaloid compound. The mechanism of action of alkaloids is a substance that inhibits esterase, DNA, and RNA polymerase, as well as decreasing cellular respiration and playing a function in DNA intercalation [26].

Figure 6 shows this. Eluate E-1 shows the location of the compound spots on the TLC plate and the clear zone that appears has the same distance, namely 4 cm or R*f* 0.8, indicating phenolic compounds. Senyawa fenolik bekerja terutama dengan cara mendenaturasi protein sel dan membran sel. Phenolic compounds work mainly by denaturing cell proteins and cell membranes. At a concentration of 0,1-2% phenol damages the cytoplasmic membrane leading to leakage of important metabolites [25].

Figure 7 shows it. The molecules zones on the TLC plate are shown in the N-5 eluate, and the clear zone that appears has the same distance of 4.5 cm or R*f* 0.9, indicating

that it is a terpenoid compound. Terpenoids work by destroying fungal cell organelles, either through the cytoplasmic membrane or by interfering with the formation and development of fungal spores, to restrict the growth of pathogenic fungus [3].

4. Conclusion

Based on the results of the research that has been done, it can be concluded that.

Mango parasite (*Dendrophthoe pentandra* (L). Miq) fraction which was active as antifungal was N-hexane and ethyl acetate fraction.

The purification results of the n-hexane and ethyl acetate fractions produced two eluates that were active in inhibiting *C. albicans*, namely N-1 and N-4 eluates and E-1 and E-5 eluates.

The MIC values for eluates N-1, N-4, and E-1 indicated that they could inhibit up to a concentration of 500 ppm and were categorized as strong enough to inhibit the fungus *C. albicans.* Meanwhile, the MIC value in the E-5 eluate was a concentration of 1000 ppm which was categorized as weak in inhibiting the fungus *C. albicans*.

The secondary metabolite group of N-1 eluates are terpenoids, N-4 eluates are alkaloids, E-1 eluates are phenolic and E-5 eluates are terpenoids which are active as antifungal *C. albicans.*

5. Acknowledgement

The author also expresses many thanks to Dr. Salni, M.Si. As a lecturer and head of the phytochemistry lab who has helped a lot during the research.

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