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The Potentials of Isolated Hexadecanoic Acid of Hydroid *Aglaophenia cupressina* Lamoureoux as an Antifungal Compound on the Rotten Strawberries *Fragaria x ananassa* Dutch. and Mango *Mangifera indica* L

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Abstract*— Overcoming microbial contaminants in fresh fruits is not merely by recognizing the level of contamination. Still, it requires another effort, such as applying a compound of natural and effective ingredients proven effective in reducing microbial contaminants and safe for health. The hexadecanoic acid used in this study was isolated from the tropical marine hydroids *Aglaophenia cupressina* Lamoureux. This study aimed to analyze the ability of bioactive compounds acid from the hydroid *Aglaophenia cupressina* Lamoureoux to inhibit the growth of fungi that cause rotten strawberry *Fragaria x ananassa* Dutch and mango *Mangifera indica.* Hexadecanoic acid was obtained by isolating it from the hydroid *Aglaophenia cupressina* Lamoureoux through the maceration, fractionation, and purification stages. Isolating fungi was done by using the PDA (Potato Dextrose Agar) medium to characterize macroscopically and microscopically and to test the inhibition using the diffusion method, which was incubated for 48 hours and 72 hours at the hexadecanoic acid concentrations of 15 ppm, 30 ppm, and 45 ppm. The results showed the hexadecanoic acid concentration of 45 ppm in the 72-hour incubation could inhibit the growth of two fungal isolates on strawberries, *Fragaria x ananassa* Dutch, i.e., *Botrytis cinerea* and *Rhizopus stolonifer,* for successive concentration, 24.00 mm and 22.75 mm. Meanwhile, the growth of *Aspergillus niger*, fungi from mangoes, could be inhibited by the hexadecanoic acid by 14.75 mm, 18.25 mm, and 23.50 mm, respectively, for the concentration of 15 ppm, 30 ppm, and 45 ppm with the 72-hour incubation.

Keywords*— *Aglaophenia cupressina* Lamoureoux; acid, antifungal *Fragaria x ananassa* Dutch; antifungal *Mangifera indica.

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I. INTRODUCTION

Aglaophenia cupressina Lamoureoux is a marine hydroid abundantly living in the Spermonde islands, South Sulawesi, Indonesia. We investigated the potential of this marine hydroid as an antibacterial ingredient on *Escherichia coli* [1] and the *Salmonella thypi* [2]. The isolation of hydroid *A. cupressina* reveals three types of pure compounds; one of them is hexadecanoic acids in the form of white crystals with a melting point of 43º-44ºC [1]. Hexadecanoic acid is a fatty acid that has antimicrobial properties by damaging the structure of microbial cells through synergistic working mechanisms with various active compounds; thus, the influence of antimicrobial activities increases [3], [4].

The microorganism is a major limiting factor in extending the shelf life of fruits, especially fruits high in water content, such as strawberries and mango. This factor inhibits the production and marketing of fresh fruit, especially regarding food quality and safety. Strawberry is one of the fruit products of which the storage is rapidly damaged and decayed by mechanical influences. Thus, strawberries' shelf life is relatively short. Decay of fruit can be caused by a pectolytic enzyme produced by microorganisms that can soften the fruit tissues, especially the cell wall composed of *polysaccharide pektat*, such as mushroom *botrytis cinerea*, *rhizopus stolonifer,* and *Colletotrichum fragariae* [5]. Strawberry is infected due to damages in the post-harvest handling during the process of transportation and storage in supermarkets or traditional markets [6]-[9]. The damage level to fruit is

influenced by gas diffusion into and out of the fruit, occurring through lenticels scattering on the surface of the fruit. The gas diffusion is naturally inhabited by a quickly rotting layer of skin when handling the post-harvest; when the fruit begins to rot, the rot can spread very quickly to other fruit stored simultaneously [10].

The best strawberry storage technique at a cool temperature is between 0-1ºC, requiring a considerable cost. Similarly, mango becomes agribusiness commodities of which the storage is easily decayed by fruit flies frequently tacking mango. This condition gives rise to fungi *Aspergillus niger* causing anthracnose in the form of black spots on mango's skin surface [11]. The damage of anthracnose attack can significantly degrade mango quality [12].

Various studies indicate that the microbial contamination on fresh fruits is more than the required provisions for 10^3 cell/g of a sample [13]. The industry generally uses a commercial synthetic antimicrobial product that necessarily risks human health because chemical compounds endangering health are accumulated [14]. Currently, the synthetic antimicrobial product is most widely used to control microorganisms on fruits. Although it is relatively effective, many synthetic antimicrobial products have significant weaknesses, such as reduced efficacy in the long term due to the development of pathogenic microbes resistance and their adverse effects on human health and the environment [15]. Moreover, synthetic antimicrobial products raise the resistance of fungal pathogens to antifungal compounds that can create new types of pathogens [16]. Therefore, an alternative compound is necessarily found as a safe antifungal compound for health that does not cause pathogenic microbe resistance. An applicable business is to isolate bioactive compounds from natural materials safe for health and inactivate bacteria and fungi that contaminate food ingredients [16], [17].

II. Materials and Method

This study employed an experimental method using hydroid *A. cupressina* Lamoureoux, methanol, n-hexane, hexadecanoic acid, strawberry, mango, acid compounds, physiological NaCl, medium potato dextrose agar (Merck), ketoconazole (PT Alpharma), Dimethyl sulfoxide (Merck), 70% of alcohol, distilled water, lactophenol, and aluminum foil.

A. Extraction, Partition and Isolation of Hydroid Aglaophenia Cupressina Lamoureoux

The collected samples were washed clean from their substrate, macerated with methanol three times for 1 x 24 hours, filtered, and then evaporated to obtain thick *maserat*. The collected samples were then partitioned to be liquid 1:1 with n-hexane. The filtrate or layer of n-hexane was evaporated to obtain a thick extract of n-hexane and then continued in the vacuum chromatography column and press chromatography column to obtain pure compounds. The next step was analyzing the collected sample using the thin-layer chromatography (TLC) and the melting point measurement using the comparison examples of hexadecanoic acid [1].

B. Isolating Decaying Fungus of Strawberry and Mango

Strawberry and mango from markets were selected uniformly in terms of fruit ripeness, colors, and sizes. The fruits were then washed clean, dried, stored, and left to rot at the temperature of 25ºC. The fungi isolation was done by using a sterile swab to take fungus that grows on strawberries and mango. Then, the fungus was placed on the growing potato dextrose agar medium in Petri dishes and incubated at a temperature of 37ºC for 1-3 x 24 hours.

C. Identifying Fungi

Fungal isolates were collected aseptically using a swab. Then, they were put on top of the sterile object-glass spilled with medium PDA liquid until it became solid. The prepared fungi were then put in a petri dish lined with sterile filter paper and moistened with little sterile distilled water. Then the prepared fungi were incubated in an incubator for 2 x 24 hours at a room temperature of 37º C. Once the growth emerged, the prepared fungi were observed under a microscope with 400 times magnification.

D. Preparing Test Solutions and Control Solutions

Hexadecanoic acid compounds were weighed as much as 0.45 mg and dissolved in 10 ml of dimethyl sulfoxide (DMSO). Moreover, thus, a solution with a concentration of 45 ppm was obtained. The next step was making a testing solution with 30 and 15 ppm concentrations. The positive control used ketoconazole with a concentration of 45 ppm, while the negative control used DMSO.

E. Preparing the Suspension of the Testing Fungus

The isolated testing fungus was suspended or diluted using the 0.9% sterile NaCl solution and then homogenized. The turbidity of suspension was measured using a spectrophotometer to obtain the 75% of transmittance values.

F. Testing the Obstacle of Hexadecanoic Acid Compound

Testing was conducted using the agar diffusion method with a reservoir with an inside diameter of 6 mm, a wide diameter of 8 mm, and a height of 10 mm. The sterile potato dextrose agar medium was cooled at the temperature of 40-45ºC. Then, it was poured aseptically into Petri dishes as much as 10 ml, allowed to be solidified as the base layer. After being solidified, put 1 ml of testing fungal suspension in 5 ml of medium potato dextrose agar. Then it was homogenized and poured over the base layer. Leave it half dense as the seed layer. After the reservoir was placed aseptically with sterile tweezers on the surface of the medium, the distance of each reservoir was 2-3 cm from the edge of the petri dish. Leave it at room temperature. Each reservoir was filled with 0.25 ml of hexadecanoic acid compounds isolated hydroid *Aglaophenia cupressina* Lamoureoux with concentrations were respectively 15, 30, and 45 ppm. Each of the ketoconazole solution as the positive control and DMSO as a negative control was poured as much as 0.25 ml using a micropipette. The next step was incubating the ketoconazole solution and DMSO at a temperature of 37º C for 48 and 72 hours.

G. The Measuring the Diameter of Inhibition

The observations were conducted by measuring the diameter of inhibition growth of fungi around the reservoir by using a caliper. The measurement was made after the

incubation process for 48 and 72 hours to determine the ability of the bioactive hydroid compound that inhibits the growth of fungi.

H. Data Analysis

The results of measuring inhibition based on the sensitivity of the fungus to hexadecanoic acid compound isolates of hydroid *A. cupressina* Lamoureoux based on the zone of inhibition for 48 and 72 hours of incubation were tabulated and analyzed. The potentials of antifungal compound acid isolates of hexadecanoic acid *A. cupressina* Lamoureoux were discovered by observing the clear zone formed and surrounding the reservoir. The inhibition zone was measured in the hexadecanoic acid compound isolated from the hydroid with concentrations of 15 ppm, 30 ppm, and 45 ppm. The measurement results were analyzed by comparing the inhibition zone diameter of positive and negative controls with the inhibition zones from all samples and each type of sample concentration. Similarly, the growth of the inhibition zone was analyzed for 48 and 72 hours to determine the bioactivity of the hexadecanoic acid compound in inhibiting the growth of decaying fungus of strawberry and mango.

III. Results and Discussion

A. The Structure Analysis of the Hexadecanoic Acid Compound

This study analyzed 70 mg of yellowish-white crystal with a melting point of 43°-44°C. The characteristics of the compound are glowing under UV and having blue color. However, these characters did not appear using the TLC color test. Spectrum UV (CH3OH) shows absorption maximum at λmaks 212 (164) nm, the spectrum of IR (KBr) vmaks 3472 cm-1 (OH), 2921, 2855 cm-1 (C-H aliphatic), 2672 cm-1 (C-H aliphatic), 1707 cm-1 (C-O), 1466 cm-1 and 1415 cm-1 (CH2 and CH3), and 1299 cm-1 (C-O). The analysis of an NMR spectrophotometer included 1H-NMR, 13C-NMR, and HMBC and reveals the results presented in Figure 1 and Table 1.

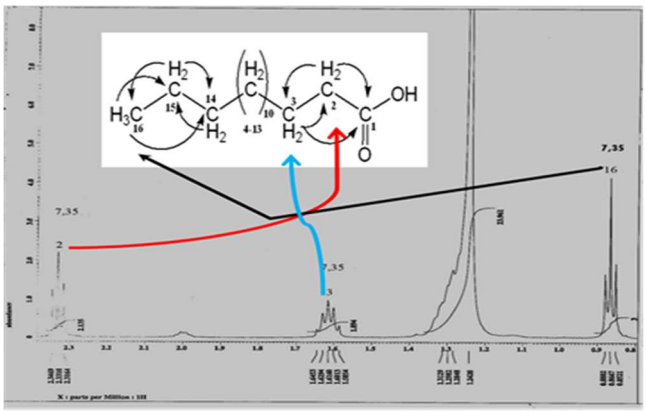

Fig. 1 The analysis of NMR spectrophotometer.

The hexadecanoic acid compound has 16 carbons, identified through the appearing signals as δC 180.64, with the most remote chemical shift indicating carbons in the carboxyl group. The signal on the δC 34.30 and 32.12 shows the C-2 and C-14. There are six carbons in symmetrical positions; they are C-7, C-8, C-9, C-10, and C-11. The carbon gave one signal with high intensity at δC 29.89. Moreover, the very close visible signal was at δC 29.89, 29.79, 29.56, 29.44, and 29.25. These numbers show the C-4, C-5, C-13, C-6, and C-12 signals at δC 24.85, 22.88, and 14.31. The three numbers show C-13, C-15, and C-16.

TABLE I
THE DATA OF 1H-NMR AND 13C-NMR OF HEXADECANOIC ACID COMPOUND

No	13C-NMR δ_C (ppm)	1H-NMR δ_H: ppm (multiplicity, *J* in Hz)	HMBC
1	180.64	-	-
2	34.30	2.33 (2H, *t J* = 7.35)	1.3
3	24.85	In 1.62 (2H, m, *J* = 7.35)	1.2
4-13	29.89	1.24 – 1.28 (20 H, *m*)	
	29.79	1.28 to 1.29 (2H, *m*)	
	29.56	1.31 – of 1.33 (2H, *m*)	
14	29.44	0.87 (3H, *t*, *J* = At 6.70)	
15	29.25	3.75 (1H, s)	15
16	32.12		14.16
OH	22.88		14.15
	14.31		
	-		

The spectrum analysis of 1H-NMR revealed several signals. The signal at δH 2.33 (2H, t, J = 7.35) showed 2 protons at C-2. The signal at δH 1.62 (2H, m, J = 7.35) showed 2 protons at C-3. The signal at δH 1.24 - 1.28 (20 H, m) showed 20 hydrogens in the C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-12, and C-13. The signal at δH 1.28 to 1.29 (2H, m) showed 2 protons at C-14. The signal at δH = 1.31 - 1.33 (2H, m) showed 2 protons at C-15. The signal at δH = 0.87 (3H, t, J = 6.70) showed 3 protons at C-19. The signal at 3.75 (1H, s) showed the proton on the hydroxyl group. The amount of hydrogen in the compound is 32.

Eluding the compound structures reinforced by spectrum HMBC shows the correlation distance between the proton signals and carbon signals. The proton signal δH 2.33 (H-2) had a far distance correlation with the carbon signal δC 180.64 (C-1) and 24.85 (C-3). The proton signal δH 1.28 to 1.29 (H-14) was correlated with the signal δC 22.88 (C-15). The proton signal δH 1.31 - 1.33 (H-15) was correlated with the carbon signal δC 32.12 (C-14) and δC 14.31 (C=16). Meanwhile, the proton signal δH 0.87 (H-16) was correlated with the carbon signals δC 32.12 (C-14), δC 32.12 (C-14), and δC 22.88 (C-15). The hexadecanoic acid had the molecular formula $C_{16}H_{30\ 2}O_{2.}$ According to [18] and [19], acid or palmitic acid has antifungal properties.

B. The Microscopic Observation of Fungal Isolates Decaying Strawberries

Fungi isolated from a strawberry are included in the genus *Botrytis* and *Rhizopus* (Table 2). The fungi causing rotten strawberry *Fragaria x ananassa* Dutch. are *Botrytis cinerea* and *Rhizopus stolonifer* [20].

TABLE II
THE MICROSCOPIC OBSERVATION RESULTS OF TWO FUNGAL ISOLATES CAUSING ROTTEN STRAWBERRY

No	Isolates	Hyphae	Hyphal pigmentation	Asexual spores	Forms of asexual spore	Genus
1	A1	Septate	Colorless (hyaline)	Conidiospores	Conidia with oval and oblong shapes, a cluster, and multi-cellular	Botrytis
2	A2	Aseptate	Black	Sporangiospores	Rounded black Spores	Rhizopus

A1 fungi had insulated hyphae that do not have pigmentation (hyaline). The fungus proliferated, and the hyphae covered the Petri dish's surface within 2 x 24 hours. Hyphae have an asexual reproduction in the form of oval and clustered conidiospores.

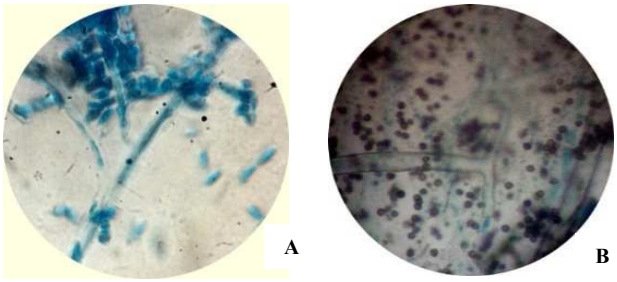

Fig. 2 Fungus A (A) and fungus B (B) at 400 X magnification.

Fig. 2 A2 fungus had black and non-insulated hyphal pigmentation. A2 fungus had an asexual reproduction in the form of rounded sporangiospores, a rhizoid as a tool to adhere to the substrate, and rounded columella.

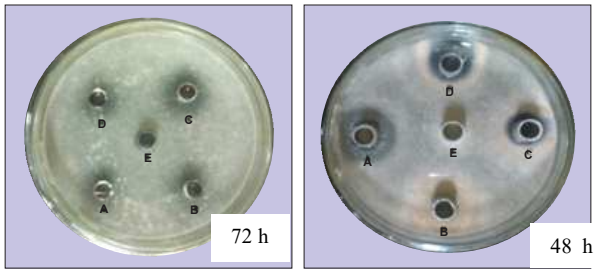

Fig. 3 Antifungal bioactivity of inhibition zone of hexadecanoic acid compounds in A1 isolates at 48 and 72 hours.

TABLE III
MEASUREMENT OF INHIBITION ZONE OF A1 AT 48 AND 72 HOURS.

No	The concentration of the hexadecanoic acid	The average diameter of the inhibition zone (mm)	
		48 hours	72 hours
1	A = 15 ppm	13.75	13.25
2	B = 30 ppm	20.53	20.00
3	C = 45 ppm	23.25	24.00
4	D = Control (+)	20.00	21.25
5	E = Control (-)	0.00	0.00

The results of measuring the inhibition zone on A1 (genus botrytis fungi) show that the bioactivity of hexadecanoic acid at a concentration of 15 and 30 ppm is fungistatic because the inhibition zone on the 48-hour measurement reduced on the 72-hour measurement: from 13.75 mm and 20.53 mm to 13.25 mm and 20.00 mm. However, the bioactivity of hexadecanoic acid at a concentration of 45 ppm was fungicidal. This result was observable from the inhibition zone formed on the 48-hour measurement that increased on the 72-hour measurement: from 23.25 mm to 24.00 mm. This result deploying that a compound will be microcidal or microstatic depending on the compound's concentration [21].

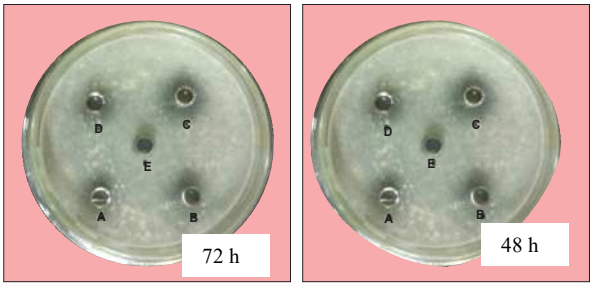

Fig. 4 Antifungal bioactivity of inhibition zone of hexadecanoic acid compounds in A2 isolate at 48 and 72 hours.

TABLE IV
MEASUREMENT OF INHIBITION ZONE OF A2 AT 48 AND 72 HOURS.

No	The concentration of the hexadecanoic acid	The average diameter of the inhibition zone (mm)	
		48 hours	72 hours
1	A = 15 ppm	12.75	11.50
2	B = 30 ppm	16.00	13.00
3	C = 45 ppm	22.25	22.75
4	D = Control (+)	20.50	21.00
5	E = Control (-)	0.00	0.00

The results of measuring the inhibition zone of A2 (genus botrytis fungi) show that the bioactivity of hexadecanoic acid at a concentration of 15 and 30 ppm is fungistatic because the inhibition zone on the 48-hour measurement reduced on the 72-hour measurement: from 13.75 mm and 20.53 mm to 13.25 mm and 20.00 mm. An antimicrobial is fungistatic if an antifungal compound can inhibit the growth of microbes [22]-[24]. If the administration of the compound is given continuously while the addition of the compound is discontinued or runs out, the growth will increase. The acid with a concentration of 45 ppm was fungicidal. It was proven that the formed inhibition zone on the 48-hour measurement increased on the 72-hour measurement: from 22.25 mm to 22.75 mm. The fungistatic nature can be changed and provide fungicidal effects if the concentration of the compound is used more greatly [25]- [26].

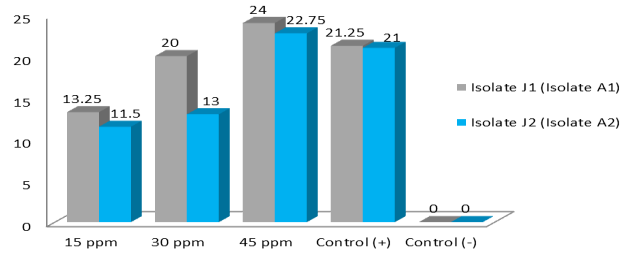

Fig. 5 The histogram of the inhibition diameter of J1 (A1 isolate) and J2 (A2 isolate) acid compound during the 72-hour incubation.

Figure 6 shows that the concentration of 30 ppm and 45 ppm of the hexadecanoic acid compound in A1 likely had effective antifungal bioactivity on the growth of fungi based on 14 mm of the inhibition diameter. The sensitive and effective inhibition diameter to employ is ≥14 m [27]. Meanwhile, the 15-ppm concentration of the hexadecanoic acid compound in A1 and A2 isolates were considered to be less effectively inhibiting the growth of fungi. The difference in the inhibition zone was caused by various concentrations given to the fungus [16], [28]. The inhibition diameter of the hexadecanoic acid compound in the A1 isolate is larger than in the A2 isolate. This condition was caused by differences in the cell wall of both fungi. The cell wall of the A1 isolate was more subtle than that of the A2 isolate, of which the cell wall was likely rough. this finding agrees with the statement that a factor possibly causing differences in the inhibition of a fungal test is the different cell walls of testing fungi [29], [30].

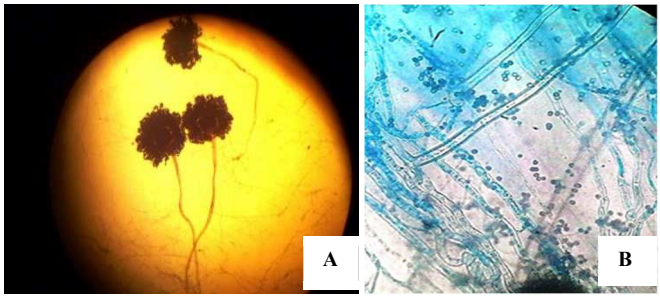

fig. 6 the macroscopic observation results of the colony morphology of the fungal isolates causing rotten mango *mangifera indica* l. the fungal colony (a) insulated hyphae (b) (400 x magnification).

b fungi had insulated hyphae that did not have pigmentation (hyaline). the spores had a straight and long form with a large-sized conidial head and field covering almost the entire surface of the vesicle. moreover, the spores had an asexual reproduction in the form of conidiospore. the observed isolates were under the microscopic characteristics of the fungus aspergillus niger [31]. he deploys that the fungus *aspergillus niger* has smooth upright conidiospore shaping globus [32]. the upper side of the conidia of *aspergillus niger* is blackish, brown, and black [16]. these are the entire colors of aspergillus niger's conidia. the top of the conidia enlarged and formed *globusa*, while the tip was similar to small trunks. the presence or absence of trace elements highly influenced pigment production [33].

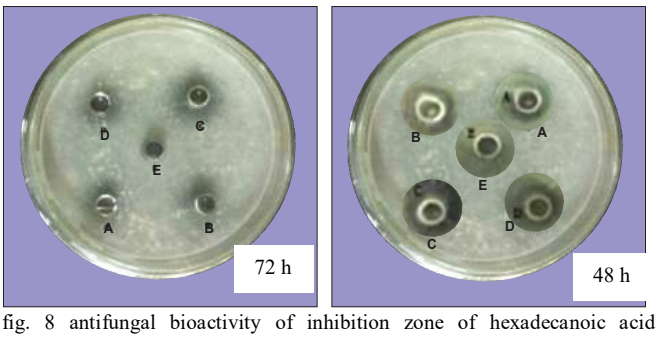

fig. 8 antifungal bioactivity of inhibition zone of hexadecanoic acid compounds in b isolate at 48 and 72 hours.

the results of measuring the inhibition zone b isolate of the fungus *aspergillus niger* showed that the bioactivity of hexadecanoic acid at the concentrations of 15 ppm, 30 ppm, and 45 ppm was fungicidal because the zone inhibition of the 48-hour measurement increased on the 72-hour measurement. fungicidal properties kill the fungus aspergillus niger cells at concentrations of 15 ppm, 30 ppm, and 45 ppm. these conditions are different from *botrytis cinerea* and *rhizopus stolonifer* because *aspergillus niger* has a relatively thinner cell wall. besides, another antimicrobial type has a primary and specific mechanism for testing fungus [34]-[35].

TABLE V
MEASUREMENT OF INHIBITION ZONE OF B AT 48 AND 72 HOURS.

No	The concentration of the hexadecanoic acid	The average diameter of the inhibition zone (mm)	
		48 hours	72 hours
1	A = 15 ppm	13.75	14.75
2	B = 30 ppm	17.25	18.25
3	C = 45 ppm	23.00	23.50
4	D = Control (+)	20.50	21.50
5	E = Control (-)	0.00	0.00

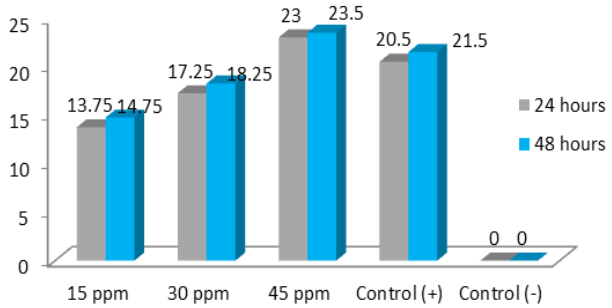

Fig. 9 The histogram of the inhibition diameter of J1 (A1 isolate) and J2 (A2 isolate) acid compound on the 72-hour incubation

Figure 9 shows that the hexadecanoic acid concentrations of 15 ppm, 30 ppm, and 45 ppm are fungicidal. The larger the concentration, the greater the active component substance will be in the concentration; thus, the formed inhibition zones are different [36]. One of the fungicidal properties of killing cells is destroying the fungal cell wall structures by reacting the active antifungal compounds with ergosterol that will form pores in the cell wall; consequently, the cell wall structures are damaged [37]. Ergosterol biosynthesis inhibition in fungal cells is a mechanism

IV. CONCLUSION

The hexadecanoic acid isolated from the hydroid with a concentration of 45 ppm was fungicidal on *Rhizopus stolonifer* (A1) and *Botrytis cinerea* (A2) and caused rotten strawberry *Fragaria x ananassa* Dutch. The hexadecanoic acid with a concentration of 15 ppm, 30 ppm, and 45 ppm are fungicidal on fungus *aspergillus niger* (B) and causes rotten mango *Mangifera indica.*

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