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Application of *Aglao E Unhas* Compound from *Aglaophenia cupressina* Lamoureoux against *Colletotrichum gloesporioides* on Strawberry and *Aspergillus niger* on Mango

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This study aimed to extract and characterize the main bioactive compound of *Aglaophenia cupressina* Lamoureoux using UV, IR, and NMR spectroscopy. This compound was then tested *in vitro* using the agar diffusion method at a concentration of 15, 30, and 45 ppm against two fungi isolated from the spoiled strawberry and mango. The efficacy of *Aglao E Unhas* was tested in fully ripe strawberries and mangoes. The number of fungi that grew during storage was counted using the standard plate count method. The results of the isolation of hydroid *A.cupressina* yielded the pure compound *Aglao E Unhas* derivative of alkaloids, which is a new compound, in the form of white crystals, melting point at 55–56 ˚C, which has 15 carbon atoms and 39 hydrogen atoms, one NH group in a heterocyclic ring. *Aglao E Unhas* at 45 ppm has antifungal properties against *Colletotrichum gloesoporioides* and *Aspergillus niger*. The largest inhibition zone against *Colletotrichum gloesoporioides* was 18.20 mm at 48 h and increased to 18.70 mm at 72 h of incubation. The inhibition zone against *Aspergillus niger* at 48 hours of incubation resulted in 17.35 mm and 72 h of incubation increased to 18.00 mm.

Keywords: *Aglao E Unhas*, *Aglaophenia cupressina* Lamoureoux, antifungal, bioactivity, fresh-cut fruits, hydroid

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INTRODUCTION

Food quality and safety are among the main problems in the production and marketing of fresh fruits such as strawberries and mangoes. Currently, strawberries are highly susceptible to anthracnose disease caused by several species of *Colletotrichum* fungi, including *Colletotrichum acutatum*, *Colletotrichum fragariae*, and *Colletotrichum gloeosporioides* (Ciofini *et al*. 2022). Likewise, black spots on the skin surface of mangoes are caused by *Aspergillus niger*, which accelerates spoilage (Alkan and Kumar 2018). *Colletotrichum* and *Aspergillus* can produce pectolytic enzymes that soften fruit tissue, especially cell walls composed of polysaccharides and pectate (Santi *et al*. 2021). In addition, the level of fruit spoilage is also influenced by the diffusion of carbon dioxide and oxygen gas into and outside the fruit, which occurs through the lenticels and fruit surface.

Naturally, these gaseous exchanges depend on the thickness of the skin layer, which is easily decomposed during postharvest handling, especially when the fruit starts rotting and the ethylene gas production becomes high. The fruit spoilage can spread quickly to other fruits in the same storage room (Strano *et al*. 2022). The existence of this disease causes the quality and income of farmers to decrease (Widiastuti *et al*. 2015). Commonly, farmers use synthetic pesticides to control this disease. However, residues from synthetic pesticides could harm the environment and human health. Therefore, a solution for handling the spread of fungal infections using natural antifungal compounds is needed (Benbrook and Davis 2020).

One of the natural antifungal compounds is derived from a group of marine invertebrates. *Aglaophenia cupressina* Lamoureoux is a marine hydroid that belongs to the phylum Cnidaria and is abundantly found in Samalona Island, South Sulawesi, Indonesia. According to Putra *et al*. (2017), marine hydroid has promising bioactive compounds for various industries including the agricultural industry. *A. cupressina* Lamoureoux *was* reported to have high antibacterial activity against pathogenic bacteria such as *Escherichia coli* and *Salmonella typhi* (Johannes *et al*. 2013; Johannes and Litaay 2016). The results of Johannes' research (2013), Hydroid *A. cupressina* L. extract succeeded to isolate a new compound from an alkaloid derivative named *Aglao E Unhas*. This compound exhibited antifungal activity against *Candida albicans* and *Malassezia furfur*. Thus, this present research aimed to [1] isolate and re-characterize the compound of *Aglao E Unhas* from the hydroid *A. cupressina* L., as well as evaluate the antifungal activities of *Aglao E Unhas* against *Aspergillus niger* and *Colletotrichum gloesporioides*.

MATERIALS AND METHODS

Sample Preparation of *A. cupressina* Lamoureoux

Approximately 20 kg of wet *A. cupressina* Lamoureoux samples were collected from Samalona Island, South Sulawesi. It was dried in an oven at 50 °C for 24 h and then mashed using a mortar and pestle to obtain a 6 kg powder sample.

Extraction, Partition, and Isolation of Hydroid *Aglaophenia cupressina* Lamoureoux

The extraction process was carried out using the maceration method with methanol for 24 hours three times. The extraction solution was filtered and evaporated to obtain a thick macerate. The liquid-liquid partitioned (1:1 ratio) with n-hexane. The filtrate or n-hexane layer was evaporated to obtain a thick extract of n-hexane and transferred for vacuum column chromatography and compressed column chromatography to obtain pure compounds. Furthermore, analyzed by thin-layer chromatography (TLC) and melting point measurements. Structural analysis of spectroscopic data was performed by measuring pure compounds using IR and NMR spectrophotometer at 500.1 MHz (^{1}H) and 125.7 MHz (^{13}C).

Isolation of Fungi from *Fragaria* x *ananassa* Dutch and *Mangifera indica*

One fully ripe *Fragaria x ananassa* Dutch and *Mangifera indica* was obtained from the market. Washed for 30 min, dried using air-dried for 1 h, and stored at room temperature (25 °C) for 3 d to decay the fruits. The isolation was carried out by cutting the spoiled fruit into small segments (0.5 cm) with a sterile scalpel, aseptically placing it on potato dextrose agar, and incubated at 37 °C for 3 x 24 h.

Fungal Identification Using Morphological Characteristics

Fungal isolates obtained from spoilt fruit were identified based on their macroscopic morphology (mycelium color and mycelium structure) and microscopic morphology (hyphae color, hyphae structure, conidiophores, and conidia). Based on macroscopic morphology, a fungus was selected from each type of fruit. Selected fungi from *Fragaria x ananassa* Dutch and *Mangifera indica* were coded as FAS-01 and MF-01, respectively. A small portion of selected fungal isolates was picked using a sterile swab, inoculated on a slide containing a fraction of a prepared solidified potato dextrose agar, and incubated for 2 x 24 h at 37 °C. Fungal preparations were viewed under the microscope (IS1153Pli Euromex iScope Mikroskop Trinokuler) with a magnification of 400x to detect hyphae color, hyphae structure, conidiophores, and conidia.

Preparation of *Aglao E Unhas* Treatment for *In Vitro* Test

Aglao E Unhas (0.45 mg) was dissolved in 10 ml of DMSO (dimethyl sulfoxide) to obtain a solution with a concentration of 45 ppm. The 45 ppm solution of *Aglao E Unhas* was poured into dilution bottles of 700 and 300 µL, respectively, and subsequently added with DMSO until each solution amounted to 1 mL. Therefore, the solution obtained had concentrations of 30 and 15 ppm, respectively. Ketoconazole at 45 ppm was used as a positive control, and DMSO at 0.5% served as a negative control.

Fungal Preparation

The selected fungal codes FAS-01 and MF-01 were suspended or diluted using a sterile 0.9% NaCl solution, and then homogenized. The turbidity of the suspension was measured using a spectrophotometer to obtain a transmittance of 75%.

***In Vitro* Antifungal Test of *Aglao E Unhas* against Fungi Codes FAS-01 and MF-01**

The test was performed using the agar diffusion method (triplicate) with an inner diameter of 6 mm, an area diameter of 8 mm, and a height of 10 mm. The sterile PDA medium was cooled to 40–45 °C. Then, it was poured aseptically into a 10-mL Petri dish and allowed to solidify as a base layer. After solidification, 1 mL of each test fungal suspension was added to 5 mL of PDA medium. They were then homogenized, poured on top of the base layer, and left semi-solid as a seed layer. The backup was then placed aseptically with sterile tweezers on the surface of the medium at a distance of 2–3 cm from the edge of the Petri dish and left at room temperature. Each reservoir was filled with 0.25 mL of *Aglao E Unhas* with concentrations of 15, 30, and 45 ppm. Ketoconazole and DMSO were poured at 0.25 mL each using a micropipette and incubated at 37 °C for 48 and 72 h. The diameter of the fungal growth inhibition was measured using a caliper.

Resistance Test of Strawberries and Mangoes to Fungal Growth

The concentration of *Aglao E Unhas* used was 45 ppm with a solution volume of 500 mL for soaking one fully ripe strawberry. For the ripe mango fruit, the volume of *Aglao E Unhas* solution was as high as 500 mL. The treatment consisted of control (without immersion) and *Aglao E Unhas* immersion for 2 min.

Standard Plate Count of Fungi from Strawberries and Mangoes

The SPC test was carried out by taking a sample of each strawberry and mango with a cotton swab and putting it into 9 mL of sterile distilled water, homogenizing to obtain a dilution of 10^{-1}, 1 mL of the solution was inserted into 9 mL of sterile distilled water so that a dilution of 10^{-2} was obtained, dilutions of 10^{-3}, and so on were made from the same stock. 1 mL of suspension from each dilution was placed into a Petri dish, and PDA media was added to the fungi, which were then incubated at 37 °C. The SPC test was performed on Days 0, 4, and 8 (T0, T4, and T8) by counting the number of colonies.

Data Analysis

Data were analyzed using a one-way ANOVA test (IBM SPPS Statistics 25) with a 5% confidence interval.

RESULTS

Structure Analysis of *Aglao E Unhas*

The weight of the *Aglao E Unhas* compound is 22.4 mg, and the form is of white crystals. Also, the melting point is 55–56 ºC. The character of this compound fluoresces under a UV lamp, but it is not visible by the TLC color test. The UV spectrum (CH_3OH) showed maximum absorption at λ_{max} 292 (455) nm and λ_{max} 220.8 (142) nm. IR spectra (KBr) vmax 3327, 3396, 3246 cm^{-1} (secondary N-H), 2920 cm^{-1} and 2848 cm^{-1} (CH_2 and CH_3), 1735 cm^{-1} and 1670 cm^{-1} (C-N), 1462 cm^{-1} (C-H), and 1128 cm^{-1} and 1049 cm^{-1} (C-O-C). NMR spectrophotometer analysis includes ^{13}C-NMR, heteronuclear multiple bond correlation (HMBC), and heteronuclear multiple quantum coherence (HMQC), the results are shown in Figure 1 and Table 1.

Analysis of the ^{13}C-NMR spectrum showed that there were two carbon groups, notably the group with a signal that appeared in the range of 64.46–72.09 with a further chemical shift and a signal group in the range of 14.32 to a lower chemical shift. The four signals in the first group δ_C 72.69, 72.03, 70.57, and 64.46 indicate four carbons in the heterocyclic ring – namely, C-1, C-2, C-3, and C-4.

The six signals in the second group represent 15 carbons; five consecutive signals δ_C 32.10, 29.54, 28.25, 22.87, and 14.32 indicate C-13′; C-2′, C-1′; C-14′ and C-15′. A signal with high intensity at a chemical shift of δ_C 29.87, 29.84, 29.79, and 29.76 shows 10 symmetrical carbons, the carbon being C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-9′, C-10′, C-11′, and C-12′. Analysis of the ^{1}H-NMR signal spectrum showed the same profile as the ^{13}C-NMR signal, indicating that there were also two groups of hydrogen spectrum signals – notably δ_H 0.87–1.56 Hz. The characteristics of protons in the first group were identified: δ_H 3.84 (1H, *m*, *J* = 4.25 Hz) indicates one proton at C-3, the signal at δ_H 3.69–3.72 (2H, *dd*, *J* = 11.6; 3.65 Hz),

Table 1. The data of ^{1}H-NMR and ^{13}C-NMR from *Aglao E Unhas* compound.

No.	^{13}C-NMR δc (ppm)	^{1}H-NMR δH: ppm (multiplicity, *J* dlm HZ)	HMBC
1	72.69	3.52 (1H, *dd, J* = 9.75; 3.65)	2, 4
		3.48 (1H, *dd, J* = 9.80; 6.15)	
2	72.03	3.45 (2H, *m, J* = 4.30)	1′, 2′, 3
3	70.57	3.84 (1H, *m, J* = 4.25)	4
4	64.46	3.72 (1H, *dd, J* = 11.6; 3.65)	2
		3.69 (1H, *dd, J* = 11.6; 3.65)	
5	–	3.62–3.64 (1H, *dd, J* = 11.6; 4.85)	4
6	29.54	1.56 (2H, *m, J* = 7.35)	2′
7	26.63	1.24–1.34 (2H, *m*)	3′
8	29.87; 29.84	1.24–1.34 (20H, *m*)	–
	29.79; 29.76		
9	32.10	1.24–1.34 (2H, *m*)	12′
10	22.87	1.24–1.31 (2H, *m*)	13′
11	14.32	0.87 (3H, *t, J* = 7,30)	13′,14′

identified in that group. Furthermore, several signals in the second group were identified as many as 31 hydrogens with the following chemical shift: a signal at δ_H 1.56 (2H, *m*, *J*= 7.35 Hz) indicates a proton at C-1′, a signal appearing at δ_H 1.24–1.34 (2H, *m*) indicates a C-2′, a signal at δ_H 1.24–1.34 (2H, *m*) indicates a C-2′ signal the signal appearing at δ_H 1.18–1.34 (2H, *m*) indicates C-3′–12′, the signal appearing at δ_H 1.24–1.34 (2H, *m*), and the signal at δ_H 0.87 (3H, *t*, *J* = 7.30 Hz). Based on the above analysis, it is known that the compound has 39 hydrogens.

The elution of the compound structure was amplified by the HMBC spectrum, showing the long-range correlation between the proton signal and the carbon signal as follows: proton signal δ_H 3.52 (H-1) with carbon signals δ_C 72.03 (C-2) and 64.46 (C-4); a correlation was also seen between proton signal δ_H 3.45 (H-2) with carbon signal δ_C 29.54 (C-1), 26.63 (C-2), and 70.60 (C-3); correlation of proton signal δ_H 3.84 (H-3) with carbon signal δ_C 64.5 (C-4); the correlation of the proton signal δ_H 3.71 (H-4) with the carbon signal δC 26.63 (C-2′). There is also a long-range correlation between the proton signal δ_H 1.56 (H-1′) and the carbon signal 26.63 (C-2′), as with the correlation of

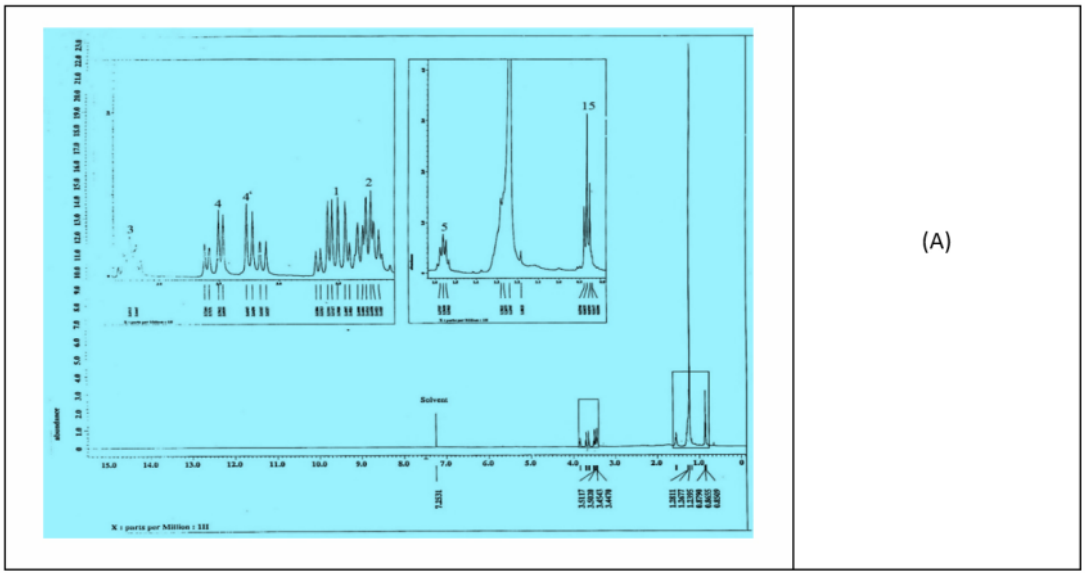

Figure 1. The analysis of NMR spectrophotometer.

indicates two protons at C-4, signals at H 3.62–3.64 (1H, dd, J = 11.6; 4.85 Hz) indicate one proton bound to a nitrogen hetero atom as a secondary amine, signals at δ_H 3.48 and 3.52 [2H, *dd*, *J* = 9.80; 6.15 and 9.75 (3.65 Hz)] represents two protons C-1, the signal at δ_H 3.45 (2H, *m*, *J* = 4.30 Hz) shows a proton at C-2, so eight hydrogens are

the proton signal δ_H 1.24 (H-2′) and the carbon signal δ_H 29.87 (C-3′) and the correlation of proton signal δ_H 0.87 (H-15′) with carbon signals δ_C 22.9 (C-14′) and 32.1 (C-13′).

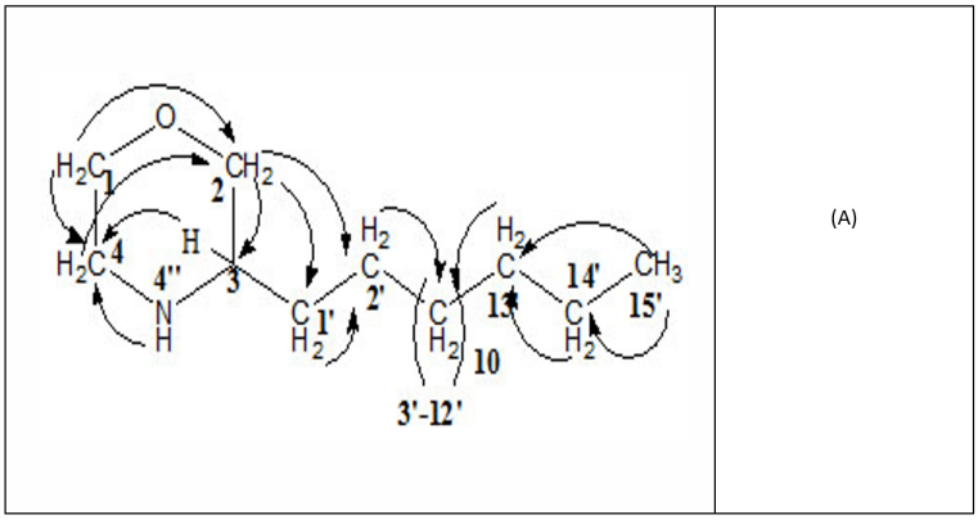

Figure 2. The correlation between heteronuclear multiple bond correlation (HMBC) and *Aglao E Unhas*.

Identification of Fungi Isolate FAS-01 from Strawberries

The fungus that grows is then identified based on macroscopic and microscopic characteristics. The results of the observations can be seen in Table 2 and Figure 3.

Based on macroscopic identification, the mycelium color characteristics indicate that the fungus has a white to greenish (olive) color and a smooth texture. Microscopic identification revealed that the fungus had transparent hyphae, insulated hyphae, branching conidiophores, and cylindrical conidia with blunt ends.

Identification of Fungi Isolate MF-01 from Mangoes

The results of morphological observations of fungi that

Table 2. The result of macroscopic and microscopic identification of fungi from strawberries.

Characteristics of morphology	Result	
	Macroscopic	Microscopic
Mycelium color	White to greenish (olive)	–
Mycelium structure	Smooth	–
Hyphae color	–	Transparent
Hyphae structure	–	Insulated hyphae
Conidiophores	–	Branching conidiophores
Conidia	–	Cylindrical conidia with blunt ends

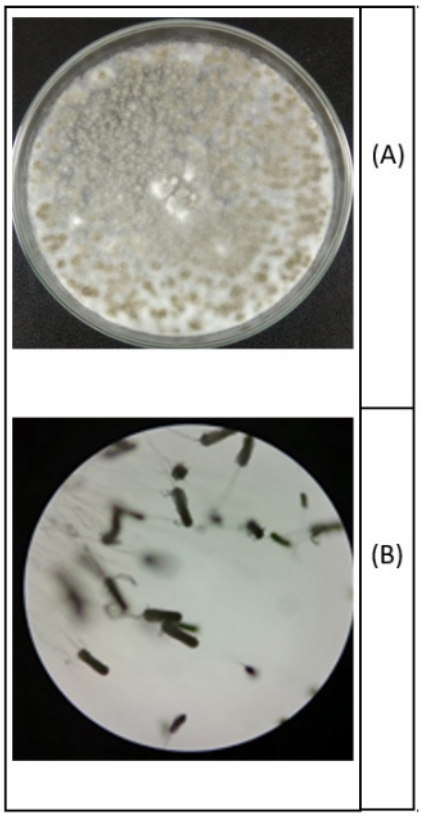

Figure 3. The result of characteristics of isolated fungi M-01 from strawberries: [A] macroscopic identification; [B] microscopic identification (400x magnification).

were isolated from mango fruit showed in Table 3 and Figure 4. Based on macroscopic observations, the fungal mycelium color was white at the periphery of the colony. A brownish-black mycelium was found in the center of the colony with a rough mycelium structure. Colonies of *Aspergillus niger* on isolation media were initially white to yellow, yellow-brown, and then turned blackish brown. Conidia that were dark brown to black will began to appear after an incubation period of 48 h.

Table 3. The result of macroscopic and microscopic identification of fungi from mango.

Characteristics of Morphology	Result	
	Macroscopic	Microscopic
Mycelium color	White (margin) Brownish-black (center)	–
Mycelium structure	Rough	–
Hyphae color	–	Transparent
Hyphae structure	–	Branched and partitioned
Conidiophores	–	Thick brownish black
Conidia	–	Dark brown to black, clumps consists of one cell

Based on observations, it was found that *Aspergillus niger* has branched and insulated hyphae with a conidia globe to subglobose shape that is black to dark brown; the conidia head of the fungus is shaped like a transmitter and is large with a thick conidiophore.

Antifungal Activities of *Aglao E Unhas*

The result of *Aglao E Unhas* antifungal activities against *C. gloeosporioides* and *A. niger* showed in Tables 4 and 5.

Based on the results of the antifungal test in Table 4, *Aglao E Unhas* against *C. gloeosporioides* at 48 h of incubation showed that at a concentration of 45 ppm, the largest inhibition zone was 18.20 mm and at 72 h of incubation, it increased to 18.70 mm. Also, at a concentration of 30 ppm, the length of the inhibition zone increased from 17.40 to 18.60 mm, and the concentration of 15 ppm of the inhibition zone increased from 17.40 to 17.60 mm. This indicated that *Aglao E Unhas* is fungicidal against *C. gloeosporioides*. While the positive control using ketoconazole was fungistatic because the inhibition zone formed at 48 h decreased from 18.50 to 16.70 mm. The negative control did not form any inhibition zone, indicating that DMSO was used as the solvent.

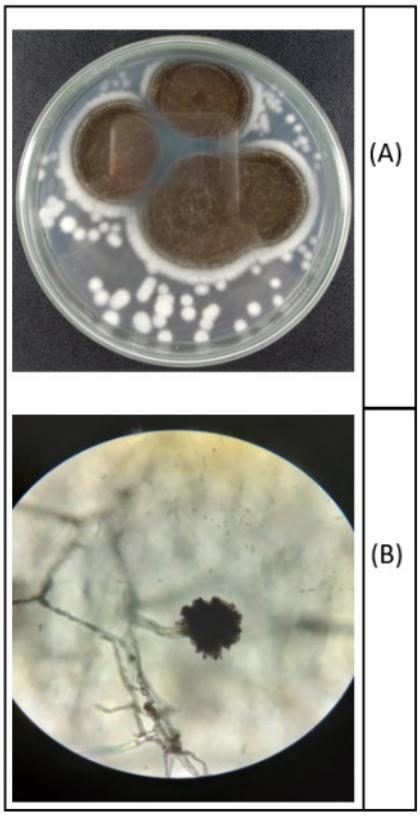

Figure 4. Characteristics of *Aspergillus niger*: [A] macroscopic identification; [B] microscopic identification (400x magnification).

Table 4. The result of inhibition zone of *Aglao E Unhas* against *C. gloeosporoides*.

Concentration of *Aglao E Unhas* (ppm)	Inhibition zone of *Aglao E Unhas* against *C. gloeosporoides* (mm)	
	48 h	72 h
15	17.40 ± 0.00^{b}	17.60 ± 0.08^{c}
30	17.40 ± 0.14^{b}	18.60 ± 0.00^{d}
45	18.20 ± 0.08^{c}	18.70 ± 0.08^{d}
Ketokonazol (positive control)	18.50 ± 0.00^{d}	16.70 ± 0.00^{b}
DMSO (negative control)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Table 5. The result of inhibition zone of *Aglao E Unhas* against *Aspergillus niger*.

Concentration of *Aglao E Unhas* (ppm)	Inhibition zone of *Aglao E Unhas* against *Aspergillus niger* (mm)	
	48 h	72 h
15	15.80 ± 0.08^{c}	16.10 ± 0.06^{c}
30	16.80 ± 0.04^{d}	17.20 ± 0.00^{d}
45	17.35 ± 0.04^{e}	18.00 ± 0.08^{e}
Ketokonazol (positive control)	15.40 ± 0.00^{b}	15.10 ± 0.08^{b}
DMSO (negative control)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

In Table 5, it can be seen that the *Aglao E Unhas* treatment of *Aspergillus niger* at 48 h incubation resulted in the largest inhibition zone at a concentration of 45 ppm, notably 17.35 mm, and 72 h incubation increased to 18.00 mm. A concentration of 30 ppm also increased from 16.80 to 17.20 mm, and the concentration of 15 ppm also increased from 15.80 to 16.10 mm. This indicates that *Aglao E Unhas* is fungicidal against *Aspergillus niger*. Ketoconazole as a positive control was fungistatic against *Aspergillus niger* because there was a decrease in the inhibition zone at 48 h of observation from 15.40 to 15.10 mm. In this case, the antifungal ketoconazole only inhibited the growth of *Aspergillus niger* but was not lethal. Similarly, DMSO only functions as a solvent because it does not form an inhibition zone.

Standard Plate Count (SPC) of Microbes (Bacteria and Fungi) from Strawberry

The average number of colonies growing at T0 with treatment was 1.1×10^3 CFU/mL, whereas at T4 with treatment, the average number of fungal colonies growing was 1.2×10^4 CFU/mL, and at T8, the average number of the fungal colonies in the treatment was 1.1×10^6. CFU/

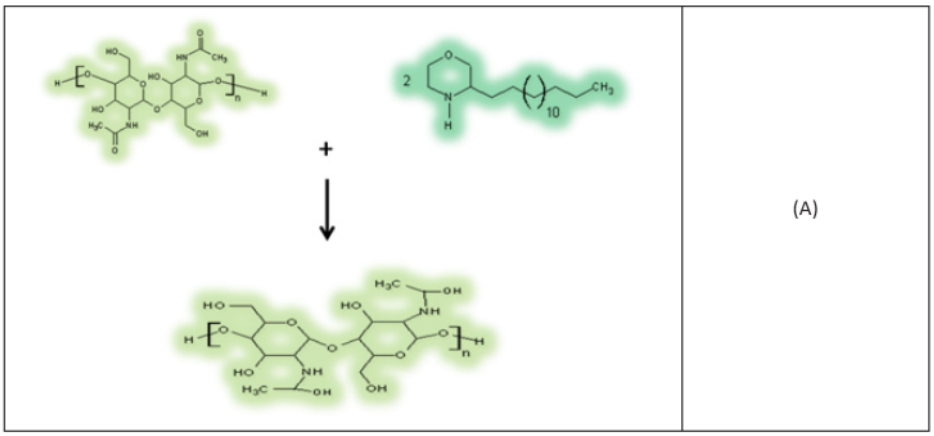

Figure 5. The mechanism of interaction between fungi cell wall compound and *Aglao E Unhas*.

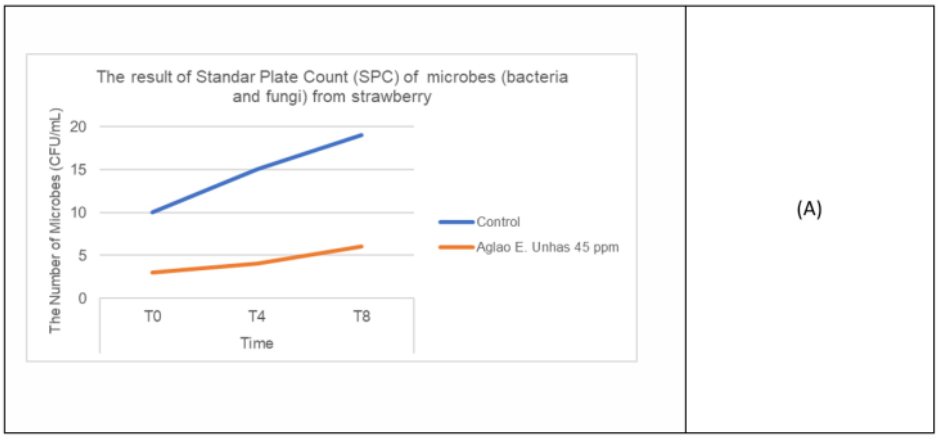

Figure 6. The result of standard plate count (SPC) of microbes (bacteria and fungi) from strawberries.

Table 6. The result of standard plate count (SPC) of microbes (bacteria and fungi) from strawberries.

Treatment	Time		
	T0	T4	T8
Control (untreated strawberry)	1.1×10^{10} CFU/mL	3.5×10^{15} CFU/mL	2.7×10^{19} CFU/mL
Aglao E Unhas (45 ppm)	1.1×10^{3} CFU/mL	1.2×10^{4} CFU/mL	1.1×10^{6} CFU/mL

Table 7. The result of standard plate count (SPC) of microbes (bacteria and fungi) from mango.

Treatment	Time		
	T0	T4	T8
Control (untreated mango)	1.1×10^{7} CFU/mL	3.7×10^{10} CFU/mL	2.7×10^{15} CFU/mL
Aglao E Unhas (45 ppm)	1.1×10^{2} CFU/mL	1.1×10^{4} CFU/mL	1.1×10^{5} CFU/mL

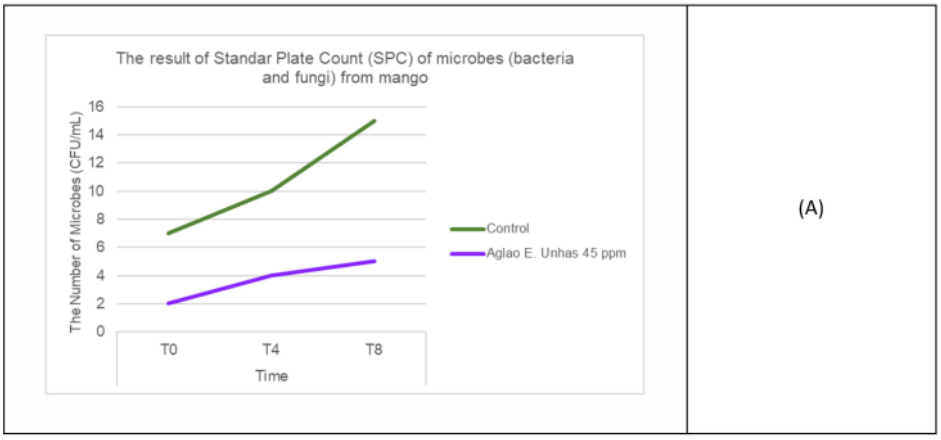

Figure 7. The result of standard plate count (SPC) of microbes (bacteria and fungi) from mango.

mL. In control or without treatment, the average number of fungal colonies growing at T0 was 1.1×10^{10} CFU/mL, and at T4, it was 3.5×10^{15} CFU/mL; for T8, the average number of fungal colonies was 2.7×10^{19} CFU/mL. This indicated that the total number of fungi growing on the treated strawberry had much lower than the number of colonies growing on the control or untreated strawberry.

Standard Plate Count (SPC) of Microbes (Bacteria and Fungi) from Mango

Based on the results of colony calculations, at the beginning of storage (T0), the total number of fungi in mango without treatment (control) was 1.1×10^{7} CFU/mL, and at T4, the total number of fungi without treatment was 3.7×10^{10} CFU/mL, and at T8 or Day 8 of fungal growth, it was 2.7×10^{15} CFU/mL. For the immersion treatment with *Aglao E Unhas* at T0, the number of fungi in mango was 1.1×10^{2} CFU/mL, and T4 had a total number of fungi at 1.1×10^{4} CFU/mL, and for T8, the total number of fungi was 1.1×10^{5} CFU. /mL, a significantly different amount compared to no immersion or the control.

DISCUSSION

Identification of Fungi from Strawberries

The result of macroscopic identification is in line with the result of previous research that fungi from the *Colletothrichum* genus have green, white, yellowish-white, white-brown, and yellow-brown colonies, and the colony texture is smooth like cotton (Marin-Felix *et al.* 2017; Patricia *et al.* 2021).

Fungi of the genus *Colletothrichum* have insulated hyphae and transparent and elongated conidia with rounded ends (Damm *et al.* 2014; Zheng *et al.* 2022). Based on the study by Kimaru *et al.* (2018), *C. gloeosporioides* has a cylindrical spore shape with a blunt spore tip. Based on the observed macromorphological and micromorphological characters, the obtained *Colletothrichum* fungus is suspected to have similar characteristics to *C. gloeosporioides*. *C. gloeosporioides* caused anthracnose disease in strawberries appeared the symptoms in blackish-brown spots on the fruit and soft rot (Serrato-Diaz *et al.* 2020).

Identification of Fungi from Mangoes

According to Ijadpanahsaravi *et al*. (2021), a fungus that has a black surface and a white to yellowish underside is *Aspergillus niger* (Murray *et al*. 2021), whereas a fungus that has white hyphae and dark brown to black conidia covering the white hyphae are morphological characteristics of *A. niger*. *A. niger* also has characteristics of flat margins and umbonate-shaped elevation with a rapid growth period (Tambuwal *et al*. 2018). These characteristics are following those observed on agar media.

Antifungal Activities of *Aglao E Unhas*

Aglao E Unhas, at concentrations of 15, 30, and 45 ppm showed fungicidal against *C. gloeosporioides*. In the present study, a pronounce inhibition zone formed at 72 h of incubation. *Aglao E Unhas* compounds from the alkaloid group were able to kill the fungus of *C. gloeosporioides.* These alkaloid compounds would bind to the ergosterol of the fungus to form holes that caused cell membrane leakage that lead to cell death (Lin *et al*. 2021). Wong-Deyrup *et al*. (2021) also reported alkaloid compounds to have antifungal activities by damaging fungal cell membranes.

The mechanism of antifungal activity of the alkaloids works by inserting it between the cell wall and DNA, thereby preventing fungal DNA replication so that fungal growth will be disrupted, which will result in death (Wong-Deyrup *et al*. 2021). The reaction between chitin and the active group of *Aglao E Unhas* formed a complex of *Aglao E Unhas* and chitin (Johannes 2013). This reaction appears to be effective in destroying fungal cell walls because the ring structure of *Aglao E Unhas* causes the chitin fibril structure to stretch due to condensation through the amide group of chitin with *Aglao E Unhas*, which affects the backbone structure of the chitin molecule, resulting in cell wall damage.

Standard Plate Count (SPC) of Microbes (Bacteria and Fungi) from Strawberry

Based on the results, it can be seen that the number of fungi that grew on untreated strawberries was high in terms of the number of colonies compared to that of strawberries soaked in *Aglao E Unhas* at 45 ppm. This indicates that *Aglao E Unhas* can suppress the number of. In addition, soaking with *Aglao E Unhas* can act as a natural preservative to extend the shelf life of strawberries by 8 d at room temperature. According to Maraei and Elsawy (2017), strawberries can last only 3–4 d at room temperature.

Standard Plate Count (SPC) of Microbes (Bacteria and Fungi) from Mango

This study proves that *Aglao E Unhas* can extend the shelf life of the fruit because it has antifungal properties that can be seen from the total of fungi, which is lower from the first day to the eighth day. According to Thawabteh *et al*. (2019), compounds from the alkaloid group generally exhibit antimicrobial and anticancer activities. The mechanism of alkaloid antifungal activity is by inserting it between the cell wall and DNA, preventing fungal DNA replication, and disrupting fungal growth (Yusoff *et al*. 2020). According to Lima *et al.* (2019), the denaturation of fungal cell wall proteins causes the fragility of fungal cell walls, allowing other active substances that are fungistatic to penetrate easily. If the denatured protein is an enzyme protein, the enzyme cannot function, causing the metabolism and nutrient absorption process to be disrupted (Sim *et al*. 2021).

CONCLUSION

Aglao E Unhas extract has fungicidal effects on *C. gloeosporioides* and *A. niger* of strawberries and mangoes, respectively. Furthermore, the *Aglao E Unhas* compound extended the shelf life of strawberries and mangoes for 8 d of storage at room temperature.

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STATEMENT ON CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

REFERENCES

ALKAN N, KUMAR P. 2018. Postharvest storage management of mango fruit. In: Achieving Sustainable Cultivation of Mango. Galán Saúco V, Lu P eds. p. 377–402.

BENBROOK CM, DAVIS DR. 2020. The dietary risk index system: a tool to track pesticide dietary risks. Environmental Health 19(1): 1–8.

CIOFINI A, NEGRINI F, BARONCELLI R, BARALDI E. 2022. Management of Post-harvest Anthracnose: Current Approaches and Future Perspectives. Plants 11(14): 1856.

DAMM U, O'CONNELL RJ, GROENEWALD JZ, CROUS PW. 2014. The *Colletotrichum destructivum* species complex-hemibiotrophic pathogens of forage and field crops. Studies in Mycology 79(1): 49–84.

IJADPANAHSARAVI M, PUNT M, WÖSTEN HA, TEERTSTRA WR. 2021. Minimal nutrient requirements for induction of germination of *Aspergillus niger* conidia. Fungal Biology 125(3): 231–238.

JOHANNES E. 2013. Utilization of Bioactive Compound Isolated Hydroid *Aglaophenia cupressina* Lamoureoux as Sanitizer in Fresh Fruits and Vegetables [Doctoral Dissertation]. Universitas Hasanuddin, Sulawesi, Indonesia.

JOHANNES E, LITAAY M. 2016. The bioactivity of hexadecanoic acid compound isolated from hydroid *Aglaophenia cupressina* Lamoureoux as antibacterial agent against *Salmonella typhi*. International Journal of Biological and Medical Research 7: 5469–5472.

KIMARU SK, MONDA E, CHERUIYOT RC, MBAKA J, ALAKONYA A. 2018. Morphological and molecular identification of the causal agent of anthracnose disease of avocado in Kenya. International Journal of Microbiology.

LIMA SL, COLOMBO AL, DE ALMEIDA JUNIOR JN. 2019. Fungal cell wall: emerging antifungals and drug resistance. Frontiers in Microbiology 10: 2573.

LIN LB, GAO YQ, HAN R, XIAO J, WANG YM, ZHANG Q, ZHAI YJ, HAN WB, LI WL, GAO JM. 2021. Alkylated salicylaldehydes and prenylated indole alkaloids from the endolichenic fungus *Aspergillus chevalieri* and their bioactivities. Journal of Agricultural and Food Chemistry 69(23): 6524–6534.

MARAEI RW, ELSAWY KM. 2017. Chemical quality and nutrient composition of strawberry fruits treated by γ-irradiation. Journal of Radiation Research and Applied Sciences 10(1): 80–87.

MARIN-FELIX Y, GROENEWALD JZ, CAI L, CHEN Q, MARINCOWITZ S, BARNES I, BENSCH K, BRAUN U, CAMPORESI E, DAMM U, DE BEER ZW. 2017. Genera of phytopathogenic fungi: GOPHY 1. Studies in Mycology 86: 99–216.

MURRAY RP, ROSENTHAL KS, PFALLER MA. 2021. Fungal Classification, Structure, and Replication, Medical Microbiology, 9th ed. Elsevier Health Sciences.

PATRICIA LC, DEL SOCORRO RG, IVÁN RH, ERIKA DL, KEIKO S, ALBERTO NZ. 2021. Occurrence and infective potential of *Colletotrichum gloeosporioides* isolates associated to *Citrus limon* var. Eureka. Biotechnology Reports 31: e00651.

PUTRA MY, WIBOWO JT, MURNIASIH T. 2017. A review of chemistry and biological activities of the Indonesian octocorallia. Journal of Applied Pharmaceutical Science 7(5): 219–227.

SANTI L, BEYS-DA-SILVA WO, BERGER M, YATES JR, BRANDELLI A, VAINSTEIN MH. 2021. Penicillium oxalicum secretomic analysis identify plant cell wall degrading enzymes important for fruit juice extraction. Journal of Food Science and Technology 58(5): 1764–75. DOI: 10.1007/s13197-020-04688-9

SERRATO-DIAZ LM, MARIÑO YA, BAYMAN P. 2020. Pathogens causing Anthracnose and fruit rots of coffee associated with the coffee berry borer and the entomopathogenic fungus *Beauveria bassiana* in Puerto Rico. Phytopathology (9): 1541–1552.

SIM SY, SRV A, CHIANG JH, HENRY CJ. 2021. Plant proteins for future foods: a roadmap. Foods 10(8): 1967.

STRANO MC, ALTIERI G, ALLEGRA M, DI RENZO GC, PATERNA G, MATERA A, GENOVESE F. 2022. Postharvest Technologies of Fresh Citrus Fruit: Advances and Recent Developments for the Loss Reduction during Handling and Storage. Horticulturae 8(7): 612.

TAMBUWAL AD, MUHAMMAD IB, ALHAJI S, MUHAMMAD S, OGBIKO C. 2018. Morphological and biochemical characterization of isolated *Aspergillus niger*, *Saccharomyces cerevisiae*, and *Zymomonas mobilis* from local indigenous sources. GSC Biological and Pharmaceutical Sciences 5(3): 78–85.

THAWABTEH A, JUMA S, BADER M, KARAMAN D, SCRANO L, BUFO SA, KARAMAN R. 2019. The biological activity of natural alkaloids against herbivores, cancerous cells, and pathogens. Toxins 11(11): 656.

WIDIASTUTI A, NINGTYAS OH, PRIYATMOJO A. 2015. Identification of fungus causing postharvest disease on several fruits in Yogyakarta. Jurnal Fitopatologi Indonesia 11(3): 91–96.

WONG-DEYRUP SW, SONG X, NG TW, LIU XB, ZENG JG, QING ZX, DEYRUP ST, HE ZD, ZHANG HJ. 2021. Plant-derived isoquinoline alkaloids that target ergosterol biosynthesis discovered by using a novel antifungal screening tool. Biomedicine & Pharmacotherapy 137: 111348.

YUSOFF SF, HARON FF, TENGKU MUDA MOHAMED M, ASIB N, SAKIMIN SZ, ABU KASSIM F, ISMAIL SI. 2020. Antifungal activity and phytochemical screening of *Vernonia amygdalina* extract against *Botrytis cinerea* causing gray mold disease on tomato fruits. Biology 9(9): 286.

ZHENG H, YU Z, JIANG X, FANG L, QIAO M. 2022. Endophytic *Colletotrichum* species from aquatic plants in southwest China. Journal of Fungi 8(1): 87.

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