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Phytochemical Screening and Antifungal Activity of Tropical Almond (*Terminalia catappa*) Leaf Extract Against Fish Pathogenic Fungi

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Abstract Fungal infections are widespread in ornamental fish. Fish keepers use synthetic solutions to eliminate fungi by adding drops to the water, but several studies have shown that such chemicals are harmful to fish. This study aimed to assess the phytochemicals and antifungal activity of Tropical Almond (*Terminalia catappa*) leaf extract to manage fish fungal infection by eliminating external parasites. The research utilized 54 goldfish (*Carassius auratus*) divided into three groups (A, B, and C), with three replicates per dosage. Results showed that the Tropical Almond (*Terminalia catappa*) leaf extract used in this study contains alkaloids, tannins, and saponins. Treatments with Tropical Almond (*Terminalia catappa*) leaf extract in varying doses showed effectiveness in treating fish fungus infections. The highest concentration (12 mg) showed a greater effect. There was a statistically significant difference between the experiments and the control group (p value <0.05). But, results among the experimental groups showed no significant difference. The findings of this study suggest that this plant extract may be useful for treating fungal infections. However, to validate and further develop the findings of fungal inhibition, further research on antifungal activity tests is necessary.

Keywords: *antifungal activity, fungal infection, goldfish (Carassius auratus), tropical almond (Terminalia catappa) leaf extract*

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1. Introduction

Keeping ornamental fish is a trendy hobby throughout the world because aside from it being cheap and available anywhere, goldfish are also easy to maintain [1]. However, due to the changing temperatures and unhealthy water conditions in aquariums, ornamental fish, particularly goldfish, are frequently attacked by fungus. Pathogenic fungi typically start as small, focal infections that can quickly spread throughout the body's surface and do not typically penetrate deeply into muscle [2].

Various solutions, such as Methylene Blue, are currently produced to combat threats like fish diseases. Methylene Blue is frequently used to treat fish eggs in aquaculture to prevent the loss of those eggs to fungal overgrowth because it has antifungal and antiparasitic properties [3]. However, several studies have shown that the use of methylene blue at high concentrations and even at therapeutic levels to treat fish with fungus-related diseases could be harmful to fish [4] [5]. Therefore, developing an alternative substance for this solution to inhibit fish disease without negatively impacting their health is essential to solve this problem.

The extract of Tropical Almond leaves from a species of the family Combretaceae has been proven to have antifungal properties and works as a control of fungal infections [6] [7] [8]. Since Tropical Almond (*Terminalia catappa*) tree grows fast and is native to the Philippines [9], producing a product from its leaf extract could be beneficial considering it has the potential to work against fish fungal infections. Therefore, this study was conducted to assess the phytochemicals and antifungal activity of Tropical Almond (*Terminalia catappa*) leaf extract to manage fish fungal infection by eliminating external parasites.

2. Materials and Methods

2.1. Preparation of Tropical Almond *(Terminalia catappa)* Leaf Powder

Tropical Almond (*Terminalia Catappa*) leaves were thoroughly washed with cold water to remove dirt, debris,

and other impurities. Following the water bath with the leaves, the tropical Almond (*Terminalia catappa*) leaves were rinsed to remove excess water. After being cleansed, it was then exposed to sun-drying for two days to dry them out. The dried leaves of the Tropical Almond (*Terminalia Catappa*) were blended using a blender to make them into a fine powder.

2.2. Preparation of Tropical Almond Leaf *(Terminalia catappa)* Leaf Extract

The pulverized leaves of the Tropical Almond (*Terminalia catappa*) were extracted using ethyl alcohol as its solvent. The leaves were extracted using the maceration method of extraction. In a beaker, 50 grams of Tropical Almond (*Terminalia catappa*) powder and 150 mL of ethyl alcohol were combined. The ethanolic extract was filtered using filter paper. After being filtered, the extract was put into a beaker, wrapped in plastic wrap, covered with aluminum foil, and left for 48 hours at room temperature until it was utilized to treat the infected Goldfish *(Carassius auratus).*

2.3. Collection and Preparation of the Specimen

The Goldfish (*Carassius auratus)* were randomly selected from local fish vendors in Maramag, Bukidnon. A total of 54 goldfish (*Carassius auratus)* were used in the experiment and were divided into three groups (A, B, and C), with three replicates per dosage. Each tank consists of three (3) fish, with a total of nine (9) fish per group. Methylene Blue was used to treat infected fish in Group A. Meanwhile, in Group B, infected fish were treated with an extract from Tropical Almond (*Terminalia catappa*) leaf. Moreover, Group C was the control group, meaning they would receive no treatment.

2.4. Preparation of Fish Tank

In reference to the study of [10] [11], PET plastic will be used as the fish tank of this study. Each group had its PET tanks to separate them. Moreover, eighteen tanks separated the three replicates per group and dose. Every 10-liter PET container was cut to size suitable for 3 liters of water to accommodate three (3) goldfish.

2.5. Preparation of Data Collection

Each treatment was carried out on alternate days throughout the one-week experiment. This study adopted the method of [12]. Therefore, in each study tank, 6 milligrams of methylene blue and 6 milligrams of Tropical Almond leaf extract were added in dosage one on alternate days. In contrast, 12 milligrams of methylene blue and 12 milligrams of Tropical Almond leaf extract were added in dosage two on alternate days.

2.6. Phytochemical Analysis of Tropical Almond *(Terminalia catappa)* Leaf Extract

To identify the presence of Alkaloids, Dragendorff's Test method was used. 1 mL of Dragendorff's reagent was added to 2 mL of extract. The formation of a yellowish precipitate indicates the presence of alkaloids. At the same time, the Froth test will identify the presence of Saponins. 1 mL extract solution was diluted with distilled water to 20 mL and shaken in a graduated cylinder for 15 minutes. The formation of black-green, brownish-green, blue-green, violet, purple, or red-brown solution or precipitate indicates the presence of Saponins. To identify the presence of Tannins, ferric chloride was added. Tannins are indicated by the appearance of black-green, brownish green, blue-green, violet, purple, or red-brown solution or precipitation.

2.7. Statistical Tools

To interpret the results in each group, statistical evidence that the experimental population means are significantly different, data were analyzed using one-way analysis of variance (ANOVA) in p-value <0.05.

3. Results and Discussion

Table 1. Phytochemical Screening Results of Samples

Phytochemical	Test	Sample	Remarks
Alkaloids	1	(+) Formation of a precipitate	Present
	2	(+) Formation of a precipitate	Present
	3	(+) Formation of a precipitate	Present
Tannins	1	(+) Formation of blackish solution	Present
	2	(+) Formation of blackish solution	Present
	3	(+) Formation of blackish solution	Present
Saponins	1	(+) Formation of persistent Froth	Present
	2	(+) Formation of persistent Froth	Present
	3	(+) Formation of persistent Froth	Present

Table 1 shows the Phytochemical screening results of the Tropical Almond (*Terminalia catappa*) leaf extract samples regarding alkaloids, tannins, and saponins. All three (3) samples tested positive. There is a presence of alkaloids due to the formation of a yellow-orange precipitate using Dragendorff's test. In terms of tannins, a Ferric chloride test was used, and the samples had a positive formation of a blackish solution, indicating its presence. Moreover, a froth test was used for saponins, and there was a persistent froth formation, indicating the presence of saponins in the samples. This means that the Tropical Almond (*Terminalia catappa*) leaf extract used in this study contains alkaloids, tannins, and saponins. Similarly, the results are congruent with the study of [13] [14] [15] [16], indicating that the leaves of Tropical Almond (*Terminalia catappa*) contain alkaloid, tannin, and saponins.

Aside from its leaves, Tropical Almond (*Terminalia catappa*) tree also has a presence of alkaloids in its pulp and seeds [17]; tannins from its family Combretaceae, Genus *Terminalia*, and kernels [18] [19]; and saponins in

its bark [20].

Table 2. Effect of Tropical Almond (*Terminalia catappa*) Leaf Extract Against Fish Pathogenic Fungi in Varying Doses

REPLICATE	Dosage 1 (6mg)	Dosage 2 (12mg)
1	3 out of 3 recoveries	3 out of 3 recoveries
2	2 out of 3 recoveries	3 out of 3 recoveries
3	2 out of 3 recoveries	2 out of 3 recoveries
Total No. of recoveries	7	8

Table 2 shows the effect of Tropical Almond (*Terminalia catappa*) leaf extract at varying doses on fish pathogenic fungi. Concerning the analysis results, seven fish in Dosage 1 (6mg) and eight fish in Dosage 2 (12mg) have recovered from fungal infection. Based on the results, fish fungal infections can be treated with 6 mg of extract solution. However, 12 mg of Tropical Almond (*Terminalia catappa*) leaf extract showed greater recovery.

The results are similar to the study of [21], where Tropical Almond *(Terminalia catappa)* leaf extract effectively treats fish with fungal infections regardless of the extract used. The study of [22] revealed that a higher extract concentration would lead to a lower mortality rate and significantly more active fish. However, as asserted by [23], adding more Tropical Almond (*Terminalia catappa*) leaf extracts could endanger the fish by making them hyperactive. The results showed that extracts of 6 mg and 12 mg of Tropical Almond (*Terminalia catappa*) leaf in 3 liters of water are effective in preventing fish fungus infection.

Table 3. Antifungal Effect of Methylene Blue and Tropical Almond (*Terminalia catappa*) Leaf Extract Against Fish Pathogenic Fungi Dosage 1 (6mg)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	10.89	2	5.44	25	0.0012
Within Groups	1.33	6	0.22		
Total	12.22	8			

Table 3 shows the antifungal effects of Methylene Blue and Tropical Almond (*Terminalia catappa*) leaf extract in Dosage 1. The analysis of the variance yielded an F critical value of 5.14 and a computed probability value of 0.001298, which is less than the 0.05 level of significance and led to the rejection of the null hypothesis. This implies that although the Methylene blue and Tropical Almond (*Terminalia catappa*) leaf extract treatments showed recovery among the fish, the number of recovered fish was still significantly different.

This may be because Methylene blue has antifungal properties, yet it can also cause harmful effects on the fish's health [24] and [25]. While the Tropical Almond (*Terminalia catappa*) leaf extract also contains antifungal and antiparasitic properties, it has bioactive compounds that help the fish's growth performance [26]. Furthermore, there was no recovery seen in group C. This may be due to the attack of fungus, which weakens the body systems of fish that would result in death if left untreated [27].

Table 4. Tukey HSD Data on Antifungal Effect of Methylene Blue and Tropical Almond (*Terminalia catappa*) Leaf Extract Against Fish Pathogenic Fungi Dosage 1 (6mg)

	(I) Groups	(J) Groups	Mean Difference (I-J)	Sig.
Tukey HSD	A	B	.00000	1.000
		C	2.33333*	.002
	B	A	.00000	1.000
		C	2.33333*	.002
	C	A	-2.33333*	.002
		B	-2.33333*	.002

The table shows the multiple comparisons, indicating the effect of the experimental and control groups. Comparing the recovery rates of Methylene blue (Group A) and the control group (Group C) revealed a significant difference. Likewise, the comparison between the antifungal effects of Tropical Almond (*Terminalia catappa*) leaf extract (Group B) and the control group (Group C) revealed significant differences in their recovery rates. However, comparing experimental groups A and B revealed no significant difference between their antifungal effects as measured by fungus infection recovery rates. This indicates that both treatments demonstrated efficacy against the fungus infection.

Table 5. Antifungal Activity of Methylene Blue and Tropical Almond (*Terminalia catappa*) Leaf Extract Against Fish Pathogenic Fungi in Dosage 2 (12mg)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	11.55	2	5.78	52	0.0016
Within Groups	0.67	6	0.11		
Total	12.22	8			

Table 5 shows the Antifungal Activity of Tropical Almond (*Terminalia catappa*) leaf extract against fish pathogenic fungi in dosage 2. The analysis of the variance yielded an F critical value of 5.14 and a computed probability value of 0.00162, which is lesser than the 0.05 level of significance and led to the rejection of the null hypothesis. From the data, it can also be ascertained that the effects of different treatments differ in effectiveness at a certain level.

Table 6. Tukey HSD Data on Antifungal Effect of Methylene Blue and Tropical Almond (*Terminalia catappa*) Leaf Extract Against Fish Pathogenic Fungi Dosage 2 (12mg)

	(I) Groups	(J) Groups	Mean Difference (I-J)	Sig.
Tukey HSD	A	B	.00000	1.000
		C	2.33333*	.002
	B	A	.00000	1.000
		C	2.33333*	.002
	C	A	-2.33333*	.002
		B	-2.33333*	.002

Methylene blue showed effectiveness since it can inhibit protozoa and fungi in fish [28] [25]. However, Methylene Blue can also cause a secondary stress

response in fish which could contribute to a wide range of diseases [29] [30]. The Tropical Almond (*Terminalia catappa*) extract showed effectiveness since it has been proven to promote aquarium quality and can be used as a source therapeutic agent [31] [32].

Table 6 indicates the effect of the experimental and control groups using the Tukey HSD. Comparing the Methylene blue (Group A) and control (Group C), recovery rates revealed a significant difference. Similarly, comparing the antifungal effects of Tropical Almond (*Terminalia catappa*) leaf extract (Groups B) and the control group revealed statistically significant differences in the recovery rates. However, comparing experimental groups A and B revealed no significant difference in antifungal effects as measured by recovery rates. This demonstrates that both treatments were effective against the fungal infection.

4. Conclusion

The results of the study revealed that using Dragendorff's test for alkaloids, Froth's test for saponin, and the tannin test with ferric chloride, the Tropical Almond *(Terminalia catappa)* leaf extract has the presence of alkaloids, phenolics, tannins, and saponins.

In addition, different doses of Tropical Almond *(Terminalia catappa)* leaf extract were found to be effective in recovering infected fish. This means that extracts of 6 mg and 12 mg of Tropical Almond (*Terminalia catappa*) leaf in 3 liters of water are effective in preventing fish fungus infection.

Furthermore, results showed a significant difference in the antifungal effects of the experimental and control groups in tanks regarding their fungal infection recovery rate.

5. Recommendations

A recommendation for specie confirmation of fish fungus should be identified to provide a better understanding of the infection and to know whether or not the treatment effectively eliminates specific species of fungi.

Moreover, it is recommended to investigate the varying doses of plant extracts and testing on various fish species to determine the most effective dosage and efficacy against particular fish pathogens. This information is crucial for determining the potential use of the plant extract in aquaculture as a natural and sustainable alternative to synthetic antifungal agents.

Additionally, the Tropical Almond *(Terminalia catappa)* leaf extract has shown antifungal activity against fish pathogenic fungi, suggesting that further research into the plant's bark, stem, and roots would be helpful to determine whether or not these parts also contain bioactive compounds that contribute to this activity. Researchers can find the best sources of bioactive compounds for developing natural antifungal agents for aquaculture by studying various plant parts.

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