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Exploring The Natural Preservation Potential of Aqueous Guava Leaf Extracts on *Pangasius Pangasius*: An Experimental Study

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Abstract

Fish, being a significant biological indicator in water, serves as a valuable food source once harvested. However, the high protein content in fish makes it an ideal medium for microorganisms, which can lead to spoilage. In areas without access to freezers or ice, preserving fish becomes a challenge. The objective of this study is to investigate the efficacy of Psidium guajava (guava) leaves as a natural preservation method for Pangasius pangasius fish. Key parameters, including gills, eye, texture, odor, and mucilage, were used to evaluate fish quality. Leaf methanolic extract was applied at doses of 0%, 20%, 40%, 60%, and 80%. Data were collected between 1 and 3 days after storage. Results showed that fish quality declined and began to deteriorate after 2 days of storage, particularly in the control treatment (0% extract). However, the fish samples treated with doses of 60% and 80% experienced relatively good quality over the course of 2 days. Although some spoilage occurred in these samples, they remained suitable for consumption. In contrast, fish samples treated with other doses exhibited complete spoilage and were no longer consumable. In conclusion, guava leaf extracts offer a promising alternative for fish preservation.

Keywords: *Guava leaf extract, Fish preservation, P. pangasius fish, Natural preservative.*

1. Introduction

Plants serve as a significant natural source of diverse bioactive compounds. *Psidium guajava*, belonging to the Myrtaceae family, is a prominent fruit species found in tropical regions such as South America, Bangladesh, Pakistan, Indonesia, and India. Extensive research has been conducted on guava plant leaves to explore their potential health benefits, attributed to the presence of various phytochemicals (Kumar *et al.*, 2021). Guava leaves are characterized by their rigid, coriaceous texture, distinct veins, and aromatic scent when crushed (Biswas *et al.*, 2013). Extracts derived from guava leaves exhibit robust antibacterial properties, capable of inhibiting bacterial growth (Riyanto, 2020). These chemicals have demonstrated effectiveness in inhibiting the growth of several microorganisms at different dilutions. Historically, guava leaf preparations have been used in traditional medicine in multiple countries, particularly as an anti-diarrheal treatment (Diaz-de-cerio *et al.*, 2017). Guava leaves possess anti-inflammatory properties, and they are rich in vitamin C, vitamin B, antioxidants, and tannins (Naseer *et al.*, 2018).

Pangasius pangasius, commonly known as Pangasius fish, is considered an ideal species for aquaculture due to its rapid growth rate. However, its population in natural waters has witnessed a

decline over the past two decades due to factors such as overexploitation, habitat degradation, water pollution, and destruction of breeding grounds. It is crucial to focus on captive production to ensure future aquaculture and to meet consumer demands, as well as to aid in replenishing the natural population of *Pangasius pangasius* (Sahoo and Ferosekhan, 2018).

2. Materials And Methods

Guava leaves, Pangasius fishes, Round bottomed flask, Distilled water, Weighing balance, Rotatory shaker, Water bath, Funnels, Whatmann filter paper.

Collection of plant:

Psidium guajava (guava) leaves were collected from the campus area of Adikavi Nannaya University, Rajahmundry, located in Andhra Pradesh state. The plants were brought to the laboratory and cleaned with water to remove soil particles. Subsequently, the leaves were dried at ambient temperature and ground into a powder.

Extraction of crude extract:

Fifty grams of the carefully weighed guava leaf powder was placed into two round-bottomed flasks, each containing 500 ml of distilled water. The flasks were then placed on a rotatory shaker set at a speed of 160. The mixture was boiled for a specified duration at a specific temperature. After cooling, the extract was filtered using Whatmann filter paper and stored for later use.

In this experiment, two factors were considered: guava leaf methanolic extract with doses (K0 = 0%, K20 = 20%, K40 = 40%, K60 = 60%, K80 = 80%) and storage time (D) ranging from 1 day (D.1), 2 days (D.2), to 3 days (D.3).

Fig.1. Freshly collected guava leaves

Fig.2. Sun dried leaves

Fig.3. Dried leaf powder

Fig.4.Weighing 50g powder

Fig.5. 50g guava leaf powder and 500ml distilled water in 2 flasks

Fig.6.Kept on rotatory shaker at 160 range speed

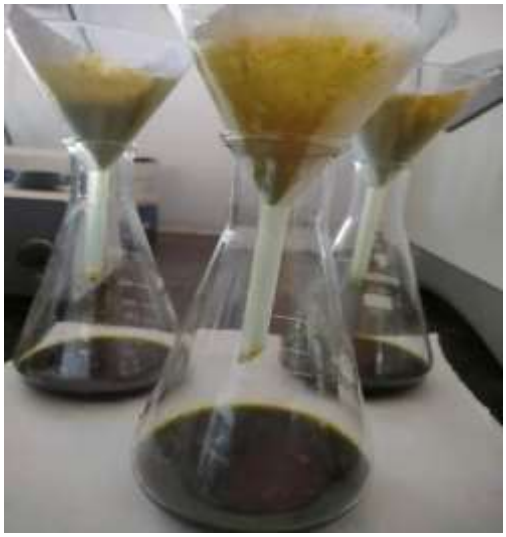


Fig.7. Filtered with whatmann filter paper

Fig.8. Filtered guava leaf extract

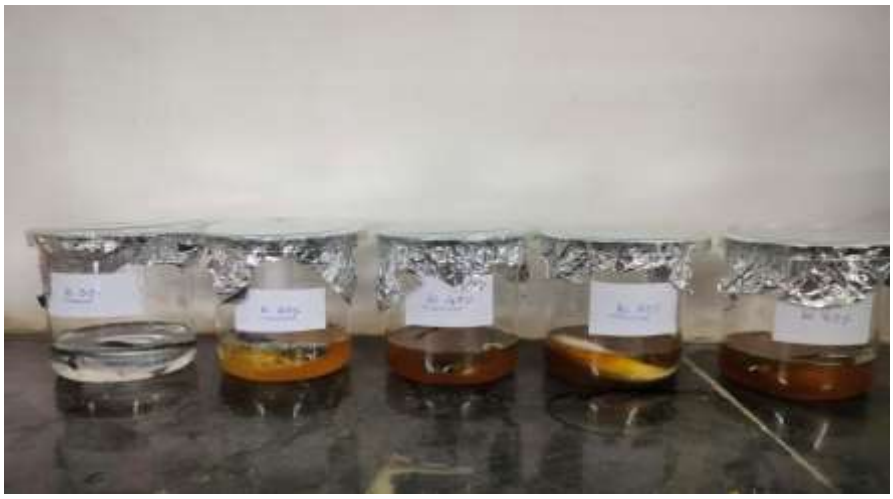


Fig.9. Preservation of fishes in different concetrations

Table 1. Treatment combinations are as follows:

K0 - D.1	K20 - D.1	K40 - D.1	K60 - D.1	K80 - D.1
K0 - D.2	K20 - D.2	K40 - D.2	K60 - D.2	K80 - D.2
K0 - D.3	K20 - D.3	K40 - D.3	K60 - D.3	K80 - D.3

"The evaluation of fish quality preserved with leaf extract was conducted over a span of three days, starting from day one. The physical condition of the fish served as the basis for assigning quality ratings, with a scale ranging from 1 (indicating low/poor quality) to 9 (indicating high/good quality)."

Table 2. Score (1-9) and physical parameters of fish quality were used to determine the score.

FISH PARAMETER	CRITERIA AND SPECIFIC QUALITY	SCORE
Eye	• Bright eye, stick-out pupils, white cornea.	7-9
	• Slightly bright eye, slightly brown pupil, white cornea	5-6
	• concaved eye browned pupil, white cornea.	3-4
	• Very concave pupil, yellow cornea.	1-2
Gills	• Red color sharply, no mucous	9
	• Slightly red color, no mucous	6
	• Red-brown color with thin mucous	3
	• White brown color with thick mucous	1
Aroma	• Specific fresh aroma	9
	• Neutral aroma	6
	• Ammonia and acid aroma	3
	• Strong ammonia and spoiled aroma	1
Texture	• Compact but elastic	9
	• Slightly compact, slightly elastic	6
	• Little bit soft	3
	• Very soft	1
Mucilage and body surface	• Bright thin layer mucosa	9
	• Mucosa began to muddy with white color	6
	• Thick mucosa with white color	3
	• Mucosa will be thick with yellow-brown color	1

3. Results and Discussion

Fishes preserved in different concentrations in different days.


Day 1 (80%)

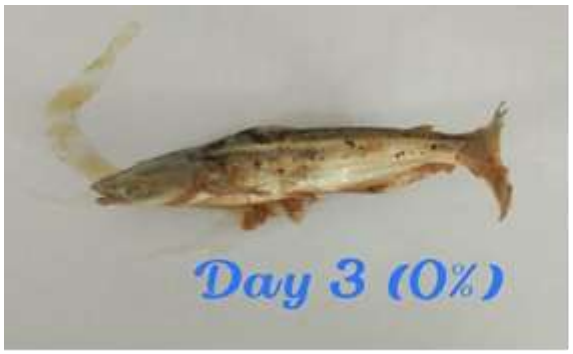

Day 2 (0%)


Day 2 (20%)


Day 2 (40%)


Day 2 (60%)


Day 2 (80%)


Day 3 (0%)


Day 3 (20%)


Day 3 (40%)


Day 3 (60%)

Below table shows the findings of physical fish quality assessments based on observation of 5 parameters. The score ranges from 9 (excellent quality) to 1 (poor quality).

Fig.3. Summary score data observations of 5 parameters

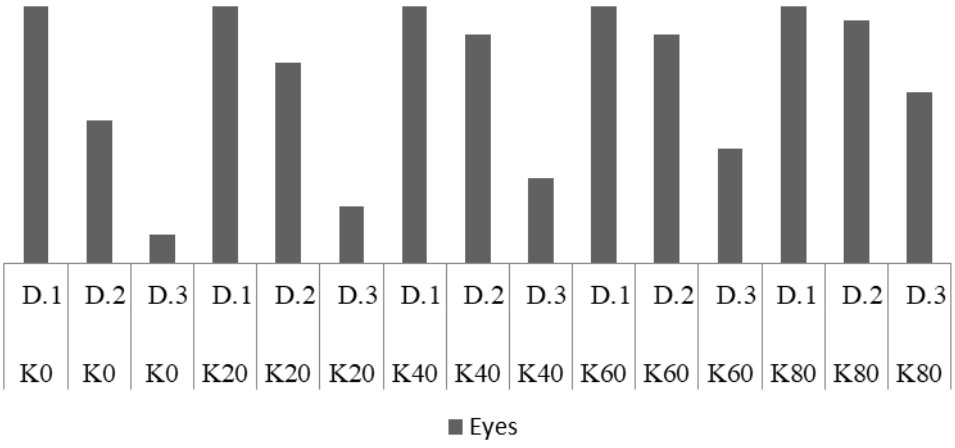
S.NO	Concentration of leaf extract	Days of storage	EYES	GILLS	AROMA	TEXTURE	MUCILAGE
1	K0	D.1	9	7	7	7	6.5
2	K0	D.2	5	6	4	6.5	5.5
3	K0	D.3	1	0	0	1	0
4	K20	D.1	9	9	9	9	9
5	K20	D.2	7	7	5	5	4
6	K20	D.3	2	1	1	2	2
7	K40	D.1	9	9	9	9	9
8	K40	D.2	8	7	5	6	5
9	K40	D.3	3	2.5	2	3	2.5
10	K60	D.1	9	9	9	9	9
11	K60	D.2	8	8	6.5	6	5
12	K60	D.3	4	3	2.5	3	3
13	K80	D.1	9	9	9	9	9
14	K80	D.2	8.5	7.5	7	7	6.5
15	K80	D.3	6	5	4.5	4	3.5

The impact of guava leaf extract on P. *pangasius* fish preservation is discussed in the below graphs

Assessing Fish Quality Based on Eye Appearance:

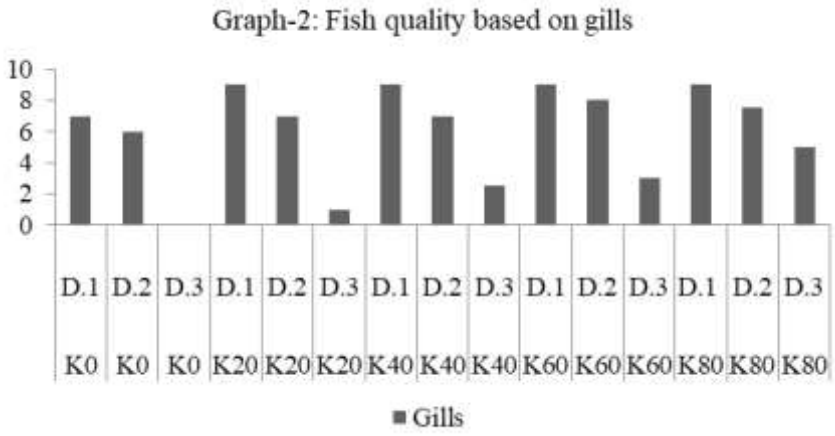
The application of guava leaf extract at 60 and 80 percent concentrations during the second day of preservation resulted in the retention of optimal fish quality, as indicated by consistent quality scores. In contrast, fish samples treated with 0 percent, 20 percent, and 40 percent concentrations showed a rapid decline in quality, suggesting complete spoilage.

Graph-1:Fish quality based on Eye



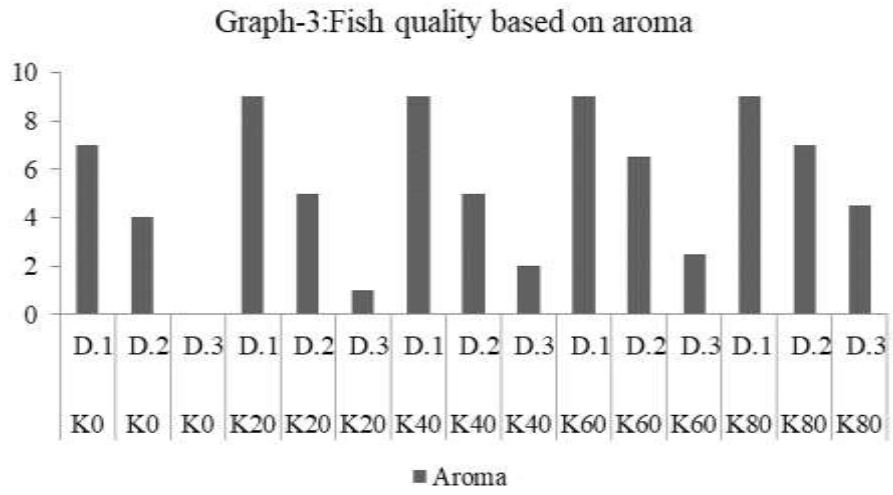
Assessing Fish Quality Based on Gill Parameters:

Graph 2 presents the results of the gill parameter analysis. On the first day of preservation, the application of common guava leaf extract at concentrations of 20%, 40%, and 60% yielded fish quality scores of approximately 9. Treatment with 80% concentration on the second day received a score indicating that the fish remained edible, and preservation on the third day received a score 5. In contrast, the fish quality score dropped to zero on the third day for the control group (0% concentration), indicating spoilage. These findings suggest the potential of common guava leaf extract for fish preservation.



Evaluating Fish Quality Based on Aroma:

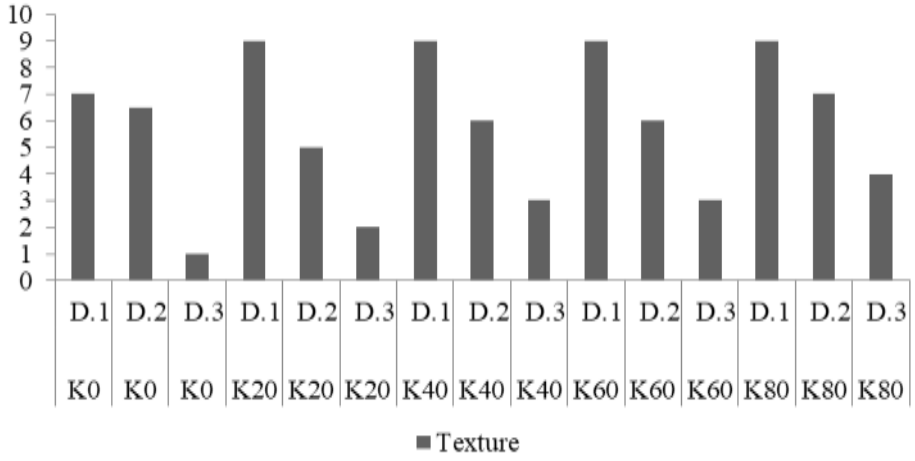
On the third day of preservation, graph 3 demonstrates the quality scores of fish treated with concentrations of 20%, 40%, 60%, and 80%. All treatment groups scored higher than the control group (0%). Notably, the 80% concentration treatment received a score of 4.5, while the remaining treatments achieved a score of 1, 2 and 2.5. These findings highlight the potential aroma-enhancing effects of the 80% concentration treatment compared to the other concentrations and the control group.

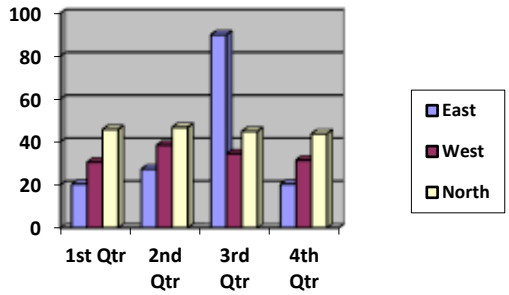


Estimating Fish Quality Based on Texture:

Graph 4 illustrates the fish quality scores during preservation using various concentrations of common guava leaf extract (20%, 40%, 60%, and 80%). The treatment with 80% concentration on the second day received a score of 7 indicating that the fish remained edible. Furthermore, preservation on the third day received a score of 4. In contrast, the control group (0% concentration) experienced a sharp decline in fish quality score, indicating complete spoilage.

Graph-4: Fish quality based on texture

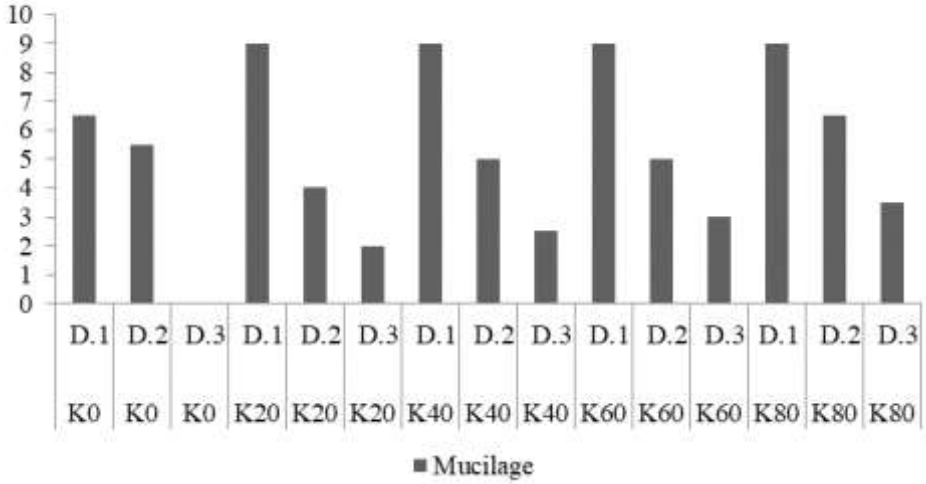




Estimating Fish Quality through Mucilage Evaluation:

The evaluation of guava leaf extract at concentrations of 20%, 40%, 60%, and 80% revealed a consistent quality score for fish preservation over three days. Remarkably, the treatment with 80% concentration showed higher scores than the control group (0%). This suggests that guava leaf extract can effectively preserve fish.

Graph-5: Fish quality based on mucilage



Fish Quality Assessment Within 1 to 3 Days of Storage:

Graphs 1 to 5 demonstrate a decline in fish quality starting from the first day of storage, particularly in the control group without any extract treatment. However, treatments with 60% and 80% concentrations maintained relatively high fish quality scores for over three days of storage, even though the fish samples started to spoil. On the contrary, samples treated with 0%, 20%, and 40% concentrations exhibited spoiling and were unfit for consumption. By the third day of storage, all samples, regardless

of treatment, had spoiled. In conclusion, the guava leaf extract displayed limited effectiveness as a fish preservative.

Drastic Drop in Fish Quality and Effectiveness of Guava Leaf Extract:

Fish quality significantly declined starting from the first day of preservation in the control group without any treatment. However, guava leaf extract at 60% and 80% concentrations demonstrated significant efficacy in extending fish preservation for up to three days. After three days, all fish samples, except in the 80% concentration treatment, had spoiled and were unsuitable for consumption. Overall, it can be inferred that guava leaf extract has the potential to effectively preserve fish, particularly at higher concentrations.

4. Conclusion

Despite challenges such as lack of infrastructure, limited access to ice in remote areas, and a lack of knowledge on fish preservation among traditional fishermen, guava leaf extracts prove to be a promising substitute for other preservatives. This study is particularly valuable for areas with limited processing units and contributes to sustainable aquaculture practices. The findings highlight the potential to extend the shelf life and enhance the quality of fish at ambient temperatures, particularly in regions without refrigeration systems. Guava aqueous extract can effectively prolong fish shelf life and prevent bacterial growth.

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