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The Effectiveness of Several Plants as Anesthetics in the Wet Transport System for *Oreochromis niloticus* Tilapia Fry

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Abstract

Tilapia (*Oreochromis niloticus*) is a highly valuable aquaculture species, yet handling and transportation processes often induce stress that can reduce survival. Anesthetics are commonly used to minimize stress, but synthetic agents may leave residues and potentially harm both fish and consumers. Therefore, natural plant-based anesthetics are increasingly explored as safer alternatives. This study aimed to evaluate the effectiveness of galangal (*Alpinia galanga*), bandotan (*Ageratum conyzoides*), and frangipani flower (*Plumeria acuminata*) extracts as natural anesthetic agents for tilapia fry during transport. The experiment used a completely randomized design with three treatments and five replications. Tilapia fry (3–5 cm) were exposed for six hours to plant extracts at predetermined doses. Measured parameters included induction time, recovery time, and survival rate. Data were analyzed using ANOVA followed by Tukey's test. The results indicated significant differences among treatments. Galangal extract produced the fastest induction (3.9 minutes), quickest recovery (5.5 minutes), and highest survival rate (99.6%). Bandotan extract showed moderate performance, while frangipani flower extract produced the slowest induction, longer recovery, and lowest survival (82%). Overall, galangal extract proved to be the most effective and safest natural anesthetic. The study demonstrates that plant-based anesthetics can reduce transport-related stress without leaving harmful residues, offering practical benefits for sustainable aquaculture, especially in wet transport systems.

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INTRODUCTION

Tilapia (*Oreochromis niloticus*) is an important commodity in aquaculture and is a type of fish with high economic value. As the projected demand and consumption of tilapia increase, various studies have been conducted to improve its quality. These efforts are inseparable from the handling and transportation processes, which are prone to causing stress in fish.

Stress in tilapia can be minimized using anesthesia. According to Opiyo et al. (2013), anesthesia is a chemical substance that has a calming effect, reduces balance, and decreases fish movement. The use of anesthesia in aquaculture is also reported to be beneficial in reducing stress and the risk of illness during procedures that have the potential to cause pressure on fish. In aquaculture, anesthesia is used to stun fish during weighing, sorting, tagging, administering medication through injections, live transportation, and spawning activities.

In general, anesthesia is divided into two categories, namely synthetic anesthesia and natural anesthesia. According to Hasan (2018), synthetic anesthetics such as MS-222/triacine methanesulfonate, benzocaine, quinaldine sulfate, metomidate, and propoxate are quite effective in inducing anesthesia in fish. However, the use of these synthetic chemicals raises concerns because they can leave residues on exposed fish, potentially endangering human health if the fish are consumed.

According to Souza et al. (2019), several compounds in synthetic anesthetics such as MS-222, metomidate, phenoxyethanol, benzocaine, and quinaldine sulfate can cause stress in fish. In addition, a number of synthetic anesthetics have also been reported to have negative effects on fish health. In contrast, natural anesthetics contain active compounds that can provide anesthetic effects on fish. Yudhistira et al. (2020) explained that natural ingredients with anesthetic potential are generally rich in secondary metabolites, such as saponins, flavonoids, and terpenes. Natural ingredients with anesthetic potential, as described by Adewale et al. (2017), which use *Ocimum gratissimum* extract on *O. niloticus* juveniles; Imjai et al. (2018) with their research on *Piper betle* extracts on Nile tilapia *O. niloticus;* Netto et al. (2017) on *Ocimum basilicum* and *Cymbopogum flexuosus* for Nile tilapia juveniles; Rezende et al. (2017) in essential oils: tea tree, clove, eucalyptus, and mint oils; and Ventura et al. (2020) which use essential oil of *Ocimum basilicum* and eugenol for Nile tilapia.

Tiexeira et al. (2016) stated that a decrease in cortisol hormone is associated with the blocking of sensory information transmission to the hypothalamus, thereby inhibiting a series of hormonal systems that indicate stress. This physiological defense response is associated with the release of cortisol and catecholamine hormones. Increased blood glucose levels are not directly related to the release of cortisol, but cortisol can cause hyperglycemia (high blood glucose) due to glucose synthesis in the liver (gluconeogenesis). Furthermore, Hohlenwerger et al. (2017) explain that anesthesia administered to fish is also thought to reduce catecholamine hormone levels when fish experience stress. These hormones are related to respiratory activity and metabolism in fish.

The inhibition of anesthetic compounds on the activity and consciousness of fish is related to the transmission of impulses through the nerves. Callier et al. (2003), stated that in the process of nerve impulse transmission, there are neurotransmitters or receptors between neurons. One such neurotransmitter is dopamine, which is a neurotransmitter in vertebrates that functions as a modulator of nerve activity and regulates various functions in the central nervous system. Dopamine receptors are located in both presynaptic and postsynaptic cells. During impulse or signal transmission, the release of dopamine hormone in the presynaptic terminal is inhibited by dopamine receptors by reducing Ca^{2+} ion activity, while postsynaptic dopamine receptors can activate K^{+} channels, causing the channels to open and depolarization to occur. Generally, dopamine receptors are the target of anesthesia.

The condition of fish seeds needs to be taken into consideration when transporting them from the hatchery's location to the buyer's location, because long travel times can cause stress to the fish and result in the death of the fish being shipped. Therefore, to minimize the effects caused by these conditions, the fish need to be rendered unconscious or stunned.

The use of galangal, bandotan, and frangipani flowers as fish anesthetics has been done, but there is no specific information comparing the three substances when used on Tilapia. The purpose of this study was to determine the effectiveness of several plants as anesthetic agents in the fish seed transportation system.

METHODS

This study is an experimental study using a completely randomized design consisting of three treatments with five replicates. Treatment A used galangal extract (*A. galanga*) at a dose of 9 mg/L, treatment B used bandotan leaf extract (*A. conyzoides*) at a dose of 4 mg/L, and treatment C used frangipani flower extract (*P. acuminata*) at a dose of 6 mg/L. The doses were determined based on previous studies (Aini et al., 2014; Ilhami et al., 2015). The study was conducted by placing 10 Nile tilapia fry, size 3-5 cm, for each replication, in the tanks that had been mixed with anesthetic substances derived from plant extracts for 6 hours. The parameters observed were: (A) induction time, calculated from the moment the extract was added to the aquarium until the fish appeared to lose their balance. (B) Recovery time after six hours, the water in the maintenance aquarium was replaced with new water, and the recovery time was calculated. (C) Survival rate, which was calculated by dividing the number of fish still alive by the initial number of fish and multiplying by one hundred. The research results were analyzed using ANOVA to determine whether there were differences between treatments using R software and followed up with further tests (Tukey test) to determine the best treatment.

RESULT AND DISCUSSION

Induction Time

The results of the study on induction time, recovery time, and survival rate are presented in Table 1. Induction time was the time needed for the fish fry to reach an unbalanced state due to the addition of anesthetic agents. Based on the analysis results, the induction time of fish showed a very significant difference between treatments ($p < 0.01$). Treatment which used Galangan produced the fastest induction time with an average of 3.96 ± 0.57 minutes, followed by treatment B, which used bandotan with an average of 22.46 ± 0.68 minutes, and treatment C (Frangipani) with an average of 28.10 ± 0.83 minutes.

Table 1. The mean and SD of induction time, recovery time, and survival rate of several anesthetic agents

Treatment	Induction	Recovery	Survival
Galangal	3.96 ± 0.57	5.50 ± 0.81	99.6 ± 0.55 %
Bandotan	22.46 ± 0.68	11.14 ± 0.94	94.8 ± 1.10 %
Frangipani	28.10 ± 0.83	10.63 ± 0.54	81.8 ± 0.45 %

Tukey's post hoc test showed that each treatment was significantly different from the others. Treatment using galangal indicated that the average was the best and significantly different from other treatments. Treatment with bandotan leaves had an induction time slower than with galangal, but still significantly different from the treatment using frangipani flowers. The treatment using frangipani flowers indicated the longest induction time and a significant difference from the other two treatments. Thus, the treatment with galangal statistically provided the fastest anesthetic effect. This indicates that the type of plant extract used had a significant effect on the speed at which the fish reached the anesthetic state. Overall, the treatment that uses galangal oil was the best treatment for the induction time parameter because it significantly reduced the induction time compared to the other treatments. The effect of galangal, bandotan, and frangipani on induction time is also illustrated in the boxplot diagram (Fig. 1.)

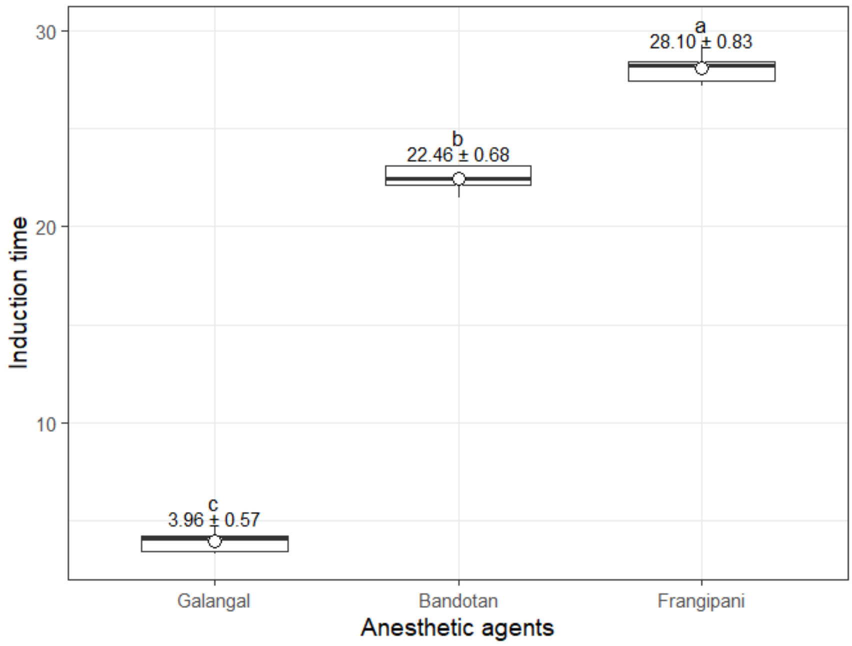

Figure 1. Induction time per treatment (mean ± SD, 5 replications)

The treatment that uses galangal oil showed the fastest induction time. This is closely related to the main component of galangal rhizome, eugenol, which is known as a natural anesthetic with a mechanism of action that inhibits nerve impulse transmission by blocking ion channels in the neuron membrane, thereby reducing the motor response activity of fish (Pikulkaew et al., 2017).

Recent research also reinforces the effectiveness of eugenol: for example, Molina-López, Lora-Benítez, and Moyano-Salvago (2024) found that eugenol induces anesthesia faster than MS-222 in zebrafish, albeit with a longer recovery time, but still within a safe range. Analysis of the molecular mechanism through network pharmacology and molecular docking shows that eugenol targets neuroactive receptors and calcium signaling pathways, which contribute to its depressant effects (Zeng et al., 2024). Eugenol supplemented with clove oil can increase eugenol penetration through the skin and gills of fish, as well as significantly increase binding activity to GABA_A receptors, which supports the mechanism of anesthesia through GABA modulation (Kheafwu et al., 2022). The neurological effects of eugenol on fish were also found in a study of *Apteronotus leptorhynchus*, where eugenol decreased the frequency of electric organ discharge, indicating a strong effect on the central nervous system (Eske et al., 2023).

Treatment using bandotan leaf extract showed a slower induction time compared to other treatments. Bandotan leaves are known to contain flavonoids, saponins, and phenols (Aini et al., 2014), which are bioactive compounds with physiological activity, but their anesthetic potential is relatively weaker than eugenol, so they do not provide a rapid central nervous system depressant effect.

Flavonoids and phenols are known to have antioxidant and anti-stress activities that are important in reducing free radicals and maintaining the physiological stability of fish (Zhou et al., 2021; Rahat et al., 2020). However, these two groups of compounds are not strong neurodepressants, so they are unable to trigger the inhibition of nerve impulse transmission as eugenol does (Pikulkaew et al., 2017; Molina-López et al., 2024). Therefore, fish treated with bandotan extract tend to enter the anesthetic phase more slowly because the mechanism of action of the active compounds does not directly target ion channels or central nervous system receptors (Zeng et al., 2024).

Additionally, the saponin content in bandotan leaves is known to cause mild irritation to the gills and mucous membranes (Rahat et al., 2020), triggering a physiological stress response and increasing ventilation rate. This condition can slow down the induction process as the fish activate a protective response to the irritation.

Treatment using frangipani flower extract resulted in the slowest induction time among all treatments. Frangipani flowers are known to contain eugenol and linalool (Ilhami et al., 2015), but the concentration of these active compounds is much lower than the eugenol content in galangal rhizomes. In addition, linalool has mild anesthetic properties, works slowly, and generally produces low-level sedation in aquatic organisms.

In several phytochemical studies, linalool is categorized as a mild sedative compound, with weaker central nervous system depressant activity than eugenol (Zhou et al., 2021; Rahat et al., 2020). The mechanism of linalool is more related to mild modulation of GABA receptors and decreased locomotor activity, but it is not strong enough to induce deep anesthesia quickly (Kheafwu et al., 2022). This explains why fish treated with frangipani flower extract take longer to enter the anesthetic phase.

Although frangipani flowers also contain eugenol, its concentration in the flower tissue is relatively low, and its bioactivity is not as strong as that of eugenol from galangal, which has been proven to have a fast and stable anesthetic effect (Pikulkaew et al., 2017; Molina-López et al., 2024). The combination of eugenol–linalool in frangipani flowers does not produce a significant synergistic effect, but rather results in weak and slow anesthesia, mainly due to the high content of non-bioactive components in the extract, which can increase the metabolic load on fish.

Ilhami et al. (2015) stated that the inhibition of activity by anesthetic compounds in fish begins when the fish are placed in a medium containing anesthetic compounds. When the fish breathe, the anesthetic compounds are absorbed along with water, oxygen, and anesthetic substances captured by the gills and then enter the blood vessels. The anesthetic compounds carried by the blood to the brain block post-synaptic dopamine receptors and inhibit dopamine release, suppressing the central nervous system and causing sedation, relaxation, and a decrease in spontaneous fish activity, a decrease in respiration rate, and a decrease in metabolic activity, which can ultimately cause the fish to lose consciousness.

Recovery Time

Recovery time is calculated from the moment the fish begin to move after the water in the treatment tank containing an anesthetic is replaced with freshwater. The analysis of variance showed a significant difference in recovery time between treatments ($p < 0.01$). Treatment that used galangal showed the fastest recovery time with an average of 5.50 ± 0.81 minutes, followed by treatment that used frangipani with an average of 10.63 ± 0.54 minutes, and treatment that used bandotan with an average of 11.14 ± 0.94 minutes. Tukey post hoc test illustrated that galangal treatment is the most superior because it not only produces the fastest induction time (as shown in the previous analysis) but also allows the fish to recover the fastest. A short recovery time is very important because it reduces post-anesthesia stress on the fish. Treatments that use bandotan dan frangipani, although different in induction time, have a similar impact on recovery time. A boxplot diagram was performed to visualize the differences in the results of these treatments and is shown in Figure 2.

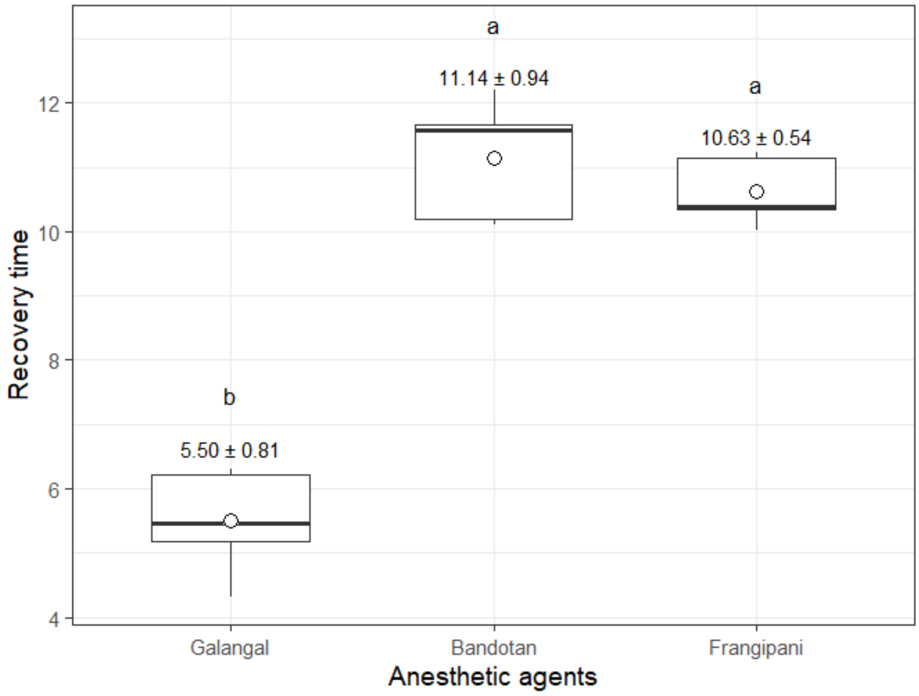

Figure 2. Recovery time per treatment (mean ± SD, 5 replications)

The treatment that used galangal oil restored the fish most quickly among all treatments. Treatment with bandotan leaf extract resulted in a longer recovery time. The physiological response to mild irritation of the gills and mucous membranes caused by saponins in bandotan leaves required greater metabolic effort both during anesthesia and during recovery, resulting in a longer recovery time. These results are consistent with the literature, which shows that plant extracts with flavonoid-phenol content are more antistress than primary anesthetics, so their effectiveness as natural anesthetic agents tends to be lower than compounds such as eugenol, which have a strong neurodepressant mechanism (Eske et al., 2023; Soto & Burhanuddin, 1995). Treatment using frangipani flower extract resulted in a relatively long recovery time. Non-active compounds such as resin, minor terpenes, and other volatile components can cause fish to undergo greater physiological compensation, especially in the gills and osmoregulatory system. This condition can slow down recovery because fish require higher energy for detoxification and homeostasis (Zeng et al., 2024; Eske et al., 2023).

Survival Rate

The survival rate was obtained by counting the fish that were still alive after being left for 24 hours, dividing this number by the total number of fish at the start of the treatment, and multiplying by 100.

Based on the ANOVA results, the survival rate also showed a very significant difference between treatments ($p < 0.01$). Treatment using galangal provided the highest survival rate with an average of 99.6 ± 0.55%, followed by treatment using bandotan with 94.8 ± 1.10%, and frangipani treatment with 81.8 ± 0.45%. Based on the results of the Tukey post-hoc test, the treatment that used the galangal has the highest percentage and is significantly different from the treatment that used bandotan dan frangipani. Treatment that used bandotan has a lower percentage of survival rate and is significantly different from treatments that used galangal and frangipani. Meanwhile, treatment that used frangipani has the lowest survival rate and is significantly lower than all treatments. This result pattern confirms that galangal provides the best results; frangipani, despite producing a long induction time, also produced the lowest survival rate, indicating higher potential toxicity or greater stress. These results indicate that the galangal has a safer anesthetic effect during the fish seed transportation process. Bar chart diagram visualisation shown in Figure 3.

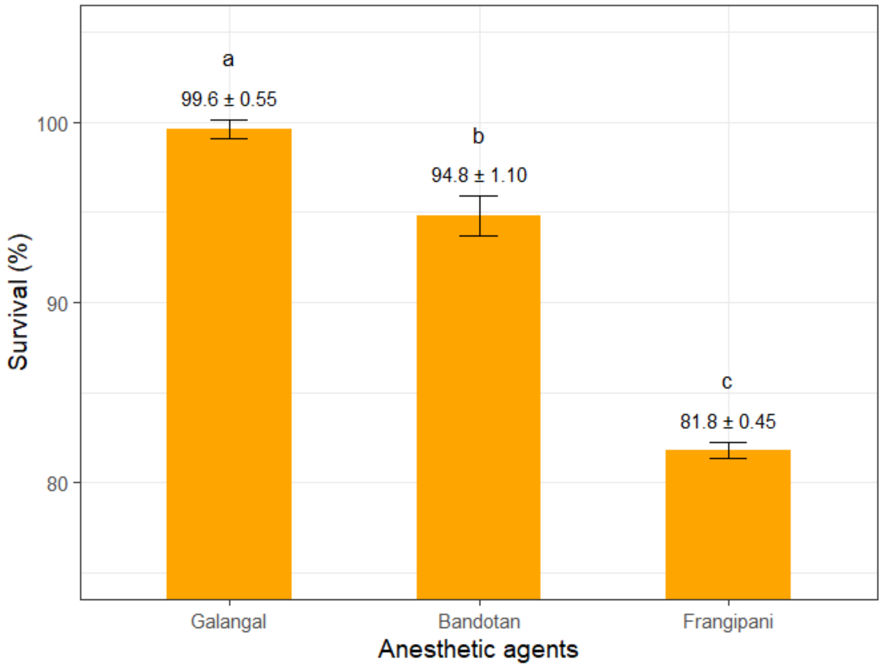

Figure 3. Survival rate per treatment (mean ± SD, 5 replications)

The effectiveness of eugenol as a natural anesthetic is also reflected in the survival rate of fish after transport: research on gurami fry shows that the use of eugenol can maintain high survival rates while controlling metabolic stress (Midihatama, Subandiyono & Haditomo, 2018). The previously mentioned increase in physiological load from bandotan leaves may also explain the lower survival rate in treatment used bandotan compared to galangal. When the administered compound does not act specifically on the nervous system, fish must perform greater metabolic compensation, especially under transport conditions that already cause oxidative stress (Kheafwu et al., 2022; Midihatama et al., 2018). The combined effects of slow induction, gill irritation by saponins, and strenuous recovery efforts contributed to the decline in fish survival, although it was still in the medium category. The metabolic burden caused by inactive compounds such as resins, minor terpenes, and other volatile components that require more energy to achieve homeostasis is also thought to contribute to lower survival rates in frangipani treatment.

During the anesthesia process, compounds that do not specifically target the nervous system have the potential to cause greater physiological stress (Midihatama et al., 2018). Because linalool is unable to trigger nerve impulse suppression as quickly as eugenol, fish tend to experience a long transition period before entering anesthesia, which increases the stress response. This may explain the very slow induction, longer recovery, and lowest survival rate.

The results of this study are consistent with the literature, which states that compounds such as linalool are only effective as mild sedatives but are not suitable for use as the primary anesthetic for transporting live fish, which requires rapid induction and stable recovery (Soto & Burhanuddin, 1995; Zhou et al., 2021). The research results also indicate that eugenol is a fast-acting, effective, and relatively safe natural anesthetic agent for use in fish seed transportation.

This study also observed residues remaining on tilapia meat that had been anesthetized naturally through sensory testing. As in previous studies using plants as anesthetics, the use of mastrante essential oil (*L. alba*) (Hohlenwerger et al., 2016); clove oil (*S. aromaticum*) (Simoes et al., 2010), and lemon verbena essential oil (*A. triphylla*) (Tiexeira et al., 2016) showed that fish meat that had been anesthetized did not show any differences in taste and smell through sensory testing. Similarly, the results of this study indicate that the three substances do not leave residues on fish that have been immersed in the anesthetic. This shows that the use of natural anesthetics is safe for consumption in Tilapia fish.

CONCLUSION

This study concludes that all three substances have the potential to be used as anesthetic agents that can be utilized for transporting fish seeds using a wet system. Galangal is the anesthetic substance that most quickly induces unconsciousness in fish, has the fastest recovery time, and has the lowest negative effects with the highest survival rate. In addition, anesthesia from plant materials does not leave residues in fish seeds, making it safe for cultivation and consumption in the future.

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