Research Article

Development of Novel Formulation of Anesthetic from Rosewood (*Aniba rosaeodora*) for Anesthetizing Thai Red-Hybrid Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Using an anesthetic in fish transportation and surgical procedure is an effective way to reduce stress and injuries before surgery and during transportation in fish. Currently, synthetic chemical such as MS-222 is widely used as a fish anesthetic. However, the residue can be found in fish and can harm the consumers. Therefore, natural remedies are of interest in substituting chemical anesthetic. In this study, we used rosewood essential oil (*Aniba rosaeodora* Ducke) as a fish anesthetic due to the property of linalool that has been shown to induce anesthesia. In addition, the essential oil was developed into the emulsion to increase the water solubility and effectiveness as a drug carrier in Thai red-hybrid tilapia (*Oreochromis niloticus*). The rosewood essential oil emulsion was prepared by the catastrophic phase inversion technique. The fish were immersed in an anesthetic solution with different concentrations to determine induction and recovery time. The results showed the best concentration of rosewood emulsion for fish transportation was 0.02 mL/L which can hold the fish at stage 1 anesthesia for 2 hours and the fish recovered after 3 minutes. The best concentration for stage 3 anesthesia to perform surgery was at 0.15 mL/L, the fish were anesthetized to stage 3 within 3 minutes and recovered within 5 minutes. Thus, rosewood essential oil emulsion can be used as an effective anesthetic in fish with good solubility.

Keywords: rosewood essential oil; fish anesthetic; Thai red-hybrid tilapia

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Introduction

Anesthetic agents have been widely used on farmed fish for several activities, such as transportation, vaccination, or surgical practice. Their application can minimize discomfort, injury, or mortality caused by performing those activities in fish [1-2]. In the United States, the safety precautions for anesthesia of fish are imposed by Food and Drug Administration (FDA) and The U.S. Environmental Protection Agency (EPA). For instance, tricaine methanesulfonate (MS-222), which is the only approved chemical anesthetic for use in food fish, necessitates a 21-day withdrawal period before the fish can be reintroduced into natural resources or consumed [3]. Despite that, chemical anesthetics still have a high potential of being accumulated in fish tissue which would subsequently transfer their toxic residues to consumers via the food chain. To avoid the possible drawbacks of applying chemical anesthetics, natural extracts can be utilized as an alternative way to develop a health-friendly anesthetic product as they have a very low-risk impact on both fish and consumers. Moreover, the cost of production tends to be lower than that of synthetic anesthetics.

Rosewood essential oil is extracted from the wood of rosewood trees (*Aniba rosaeodora* Ducke). It is colorless to pale yellow with a pleasant smell. According to the earlier research, rosewood essential oil contains up to 86.23 percent of linalool, a potent anesthesia-induced compound [4]. This chemical functions as a glutamatergic system inhibitor in the central nervous system, causing anesthetic induction in animals [5]. Jankrajang (2019) reported that rosewood essential oil can be administered as an anesthetic for Nile tilapia without causing histopathological effects on the liver [6]. Despite its efficacy, there is increasing concern that dissolving the essential oil in ethanol to increase the water solubility will enable some fish to suffer from alcohol toxicity. For example, it has been reported that being exposed to ethanol, fish undergo severe gill damage [7-8]. Accordingly, this research aims to develop an ethanol-free rosewood essential oil anesthetic to lessen ethanol toxicity by adopting the emulsion formulation method.

The Thai red-hybrid tilapia (*Oreochromis niloticus*) was bred by the Department of Fisheries from the wild-type variant of *O. niloticus.* It is the economically most significant aquatic species in Thailand due to its high demand in the food industry. Thus, some veterinary practices performed with the fish under anesthetics are of the essence to keep the fish healthy and enhance their overall production. Moreover, being very popular fish in numerous Thai cuisines, the fish are frequently transported across the country. Hence, to sedate the fish in storage facilities, anesthetics are required to curtail injury during transportation. Drawing upon the information stated above, this work seeks to investigate the optimal concentration of rosewood essential oil emulsion for simulating the condition of surgery for 10 minutes and for transportation of the Thai red-hybrid tilapia.

Materials and methods

Experimental fish

Thai red-hybrid tilapia (*O. niloticus*) (weight of 9±1.8 g; length of 7±1.8 cm; age of 2-3 months; N = 150 juveniles) was purchased from the fish farm in Bangkok, Thailand. They were reared in a 20-inch (25x51x31 cm) glass aquarium with a dechlorinated tap which was renewed every 2 days. The fish maintaining system was set under the controlled experimental condition with the stability of water temperature, pH of water, dissolved oxygen content, and a 12-h light and dark cycle. The fish were fed commercial dry feed (Thai Spring Day Co., Ltd.) at 5% of body weight once a day; however, they were deprived of food for 24 hours before experiments. The model fish were handled under the best practice of the Committee on Acting on Animal Science, Srinakharinwirot University.

Preparation of rosewood oil emulsion

The emulsion was prepared by the catastrophic phase inversion (CPI) technique. The emulsion was prepared 50 mL/L each time by dividing the emulsion into 2 phases; 1) the water phase was distilled in water and propylene glycol in a 2:1 ratio of 45 mL/L, 2) the oil phase was composed of 2 mL/L of rosewood oil (Chemipan, Bangkok) accounted for 4 percent by volume. Subsequently, the oil phase was mixed with 4 mL/L of Tween 80 to reach the total volume of 8 percent. After that, the water phase solution was dropped into the oil phase solution with a speed of 1 mL/min. The mixture was continually stirred with a magnetic stirrer for 6 hours [9]. To prevent the deterioration of the obtained emulsion, it was stored at room temperature without light exposure.

Determination of anesthetic induction and recovery time for fish surgical performance

Rosewood essential oil was diluted with absolute ethanol in a ratio of 1:4 until it reached a concentration of 0.15 and 0.2 mL/L (at a concentration of 0.1 mL/L, the fish were not anesthetized). To compare the anesthetic efficacy, rosewood essential oil emulsion was also prepared at the same concentrations. Five fish were used at each concentration and the experiments were performed in triplicate. The fish were randomly selected from the glass aquaria and transferred to an anesthetic basin. Anesthesia induction time and fish behaviors were noted. When getting to stage 3 of anesthesia (Table 1), the fish was transferred to a petri dish containing wet cotton. It had remained on the petri dish for 10 minutes to simulate a minor surgery performance. Afterward, it was moved to the anesthetic-free water to observe its recovery behaviors (Table 1).

Determination of anesthetic induction and recovery time for fish transportation

The concentrations of rose essential oil emulsion in this experiment were 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, and 0.07 mL/L. MS-222 (30 mg/L) was used to compare anesthetic efficacy. Ten fish were used at each concentration. The fish were randomly selected from their glass aquaria and immersed in a foam box containing anesthetic added water. After that, the foam box was placed on a shaker with a shaking rate of 450 times per minute to imitate a transportation process. Anesthesia induction time and fish behaviors were noted. Treated fish were expected to stay in stage 1 of anesthesia for 2 hours. After that, they were transferred to a recovery tank to monitor their recovery behaviors (Table 1).

Table 1 Stages of anesthesia and recovery with fish behavior description [10]

Anesthetic stage	Fish behaviors
Stage 1	The sedation stage, responsive to stimuli, reduced the response to physical stimuli
Stage 2	Partial loss of equilibrium, loss of muscle tone, ventilation almost absent
Stage 3	Total loss of equilibrium and responsive to stimuli
Stage 4	Ventilation ceases, cardiac arrest, death
Recovery stage	**Fish behaviors**
Initial recovery	Partial recovery of equilibrium, swimming erratically
Full recovery	Total behavioral recovery, normal swimming

Water quality

Water quality parameters, including water temperature, dissolved oxygen concentrations, and a pH of water, were examined daily and during the surgical and transport experiment. These parameters were assessed three times; before anesthesia added, after anesthesia added, and after experiments.

Statistical analysis

Data were expressed in mean ± SD from three replicate samples of independent experiments. The comparison between means in every experiment was undertaken by one-way analysis of variance (ANOVA) followed by Tukey's test. The statistical significance was achieved with p values less than 0.05.

Results

Anesthetic induction and recovery time for fish surgical performance

The induction and recovery time of rosewood essential oil and its emulsion are displayed in Figures 1 and 2. The findings presented that 0.15 mL/L of rosewood essential oil and its emulsion was the lowest concentration that can induce stage 3 of anesthesia in the fish. The induction time of rosewood essential oil was 173.73±47.4 seconds, while those of its emulsion was 175.93±62.4 seconds. There was no statistically significant difference between the induction time of these two formulas ($p>0.05$). Additionally, at the concentration of 0.15 mL/L, rosewood essential oil, and its emulsion can allow the fish to remain in stage 3 of anesthesia for 10 minutes. This indicated that 0.15 mL/L of rosewood essential oil and its emulsion was an adequate concentration to conduct a minor surgery in the fish. After staying in stage 3 for 10 minutes, the fish receiving 0.15 mL/L of rosewood essential oil and its emulsion can completely recover within 5 minutes. Furthermore, there was no mortality observed in the treated fish after 24 hours of anesthesia exposure.

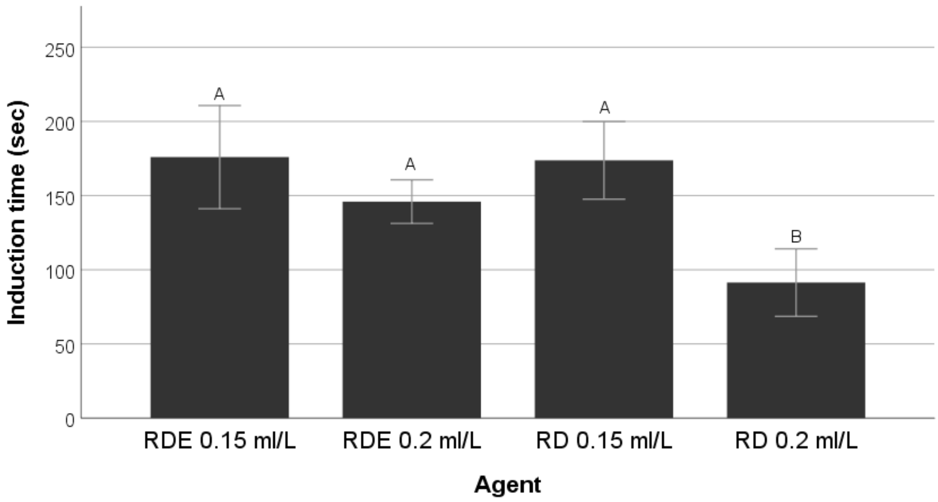

Figure 1 Induction time (second) of different concentrations of rosewood essential oil (RD) and rosewood essential oil emulsion (RDE). Different alphabets indicated a significant difference at 0.05.

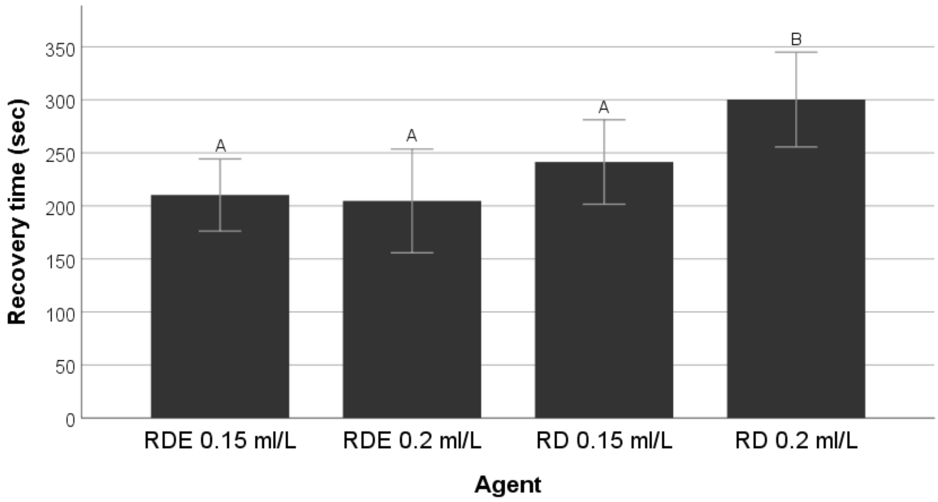

Figure 2 Recovery time (second) of different concentrations of rosewood essential oil (RD) and rosewood essential oil emulsion (RDE). Different alphabets indicated a significant difference at 0.05.

Anesthetic induction and recovery time for fish transportation

As for a short-term transport purpose, the fish are required to be in stage 1 of anesthesia for 2 hours and recover within 10 minutes. As shown in Table 2, 0.01 mL/L of rosewood essential oil emulsion cannot sedate the fish during the transport simulation period. However, this experiment noted that the optimal concentration of rosewood essential oil emulsion that should be used for fish transportation was 0.02 mL/L. This concentration can also enable the fish to fully recover within 5 minutes. Compared with MS-222, the lowest effective concentration of rosewood essential oil emulsion allowed the fish to return to the normal state faster than the recommended concentration of MS-222 (30 mg/L). In addition, no mortality was observed in this experiment.

Table 2 Stages of anesthesia during the transport simulation period

Concentrations (mL/L)	Induction time (min)									Recovery time (min)	Survival rate (%)
	5	10	15	20	25	30	60	90	120		
0 (control)	0	0	0	0	0	0	0	0	0	-	100
30 mg/L MS-222	1	1	1	1	1	1	1	1	1	2.59±0.37	100
0.01	0	0	0	0	0	0	0	0	0	-	100
0.02	1	1	1	1	1	1	1	1	1	2.35±0.17	100
0.03	1	1	1	1	1	1	1	1	1	2.40±0.19	100
0.04	1	1	1	1	1	2	2	2	2	3.03±0.03	100
0.05	1	2	2	2	2	2	2	2	2	3.07±0.05	100
0.06	2	2	2	2	2	2	2	2	2	4.16±0.20	100
0.07	2	3	4	4	4	4	4	4	4	-	0

Note: 0 = Normal, 1 = Sedation, 2 = Loss of equilibrium, 3 = Loss of reflex reactivity, 4 = Stop breathing

Water quality test results

The water quality test results (Tables 3 and 4) were temperature, dissolved oxygen content, and pH were found that no significant difference between before experiments, after adding anesthetic, and after experiments (P>0.05).

Table 3 Water quality parameters in the fish surgery experiment

Anesthetics (mL/L)	Water quality parameters								
	temperature (°C)			DO (mg/L)			pH		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
RD 0.15	24.30±1.37	24.50±0.50	23.90±0.60	6.48±0.25	6.29±0.33	6.20±0.31	7.91±0.27	7.92±0.16	8.03±0.10
RD 0.2	23.70±0.77	23.32±0.47	24.33±0.80	6.43±0.11	6.20±0.33	6.46±0.20	7.80±0.18	7.65±0.15	7.72±0.20
RDE 0.15	24.05±0.53	24.20±0.43	24.40±0.51	6.85±0.33	6.90±0.10	6.70±0.41	8.02±0.21	7.83±0.21	8.10±0.19
RDE 0.2	23.92±0.67	24.27±0.45	24.67±0.81	6.50±0.29	6.42±0.60	6.39±0.57	8.18±0.17	8.19±0.10	8.20±0.15

Note: (1) = before anesthesia added, (2) = after anesthesia added, (3) = after the experiment, RD = rosewood essential oil emulsion, and RDE = rosewood essential oil emulsion

Table 4 Water quality parameters in the fish transportation experiment

Anesthetics (mL/L)	Water quality parameters					
	before experiments			after experiments		
	(1)	(2)	(3)	(1)	(2)	(3)
Control group	25.30±0.92	5.73±0.32	7.94±0.06	25.92±0.34	5.60±0.46	7.89±0.10
30 mg/L MS-222	25.22±0.15	6.13±0.40	8.06±0.42	25.96±0.76	5.97±0.68	8.10±0.45
0.01	25.60±0.26	5.90±0.53	8.20±0.20	25.70±0.53	5.83±0.92	7.87±0.76
0.02	25.0±1.05	5.73±0.32	7.94±0.22	25.95±0.18	5.43±0.21	8.02±0.76
0.03	26.10±1.42	5.93±0.74	8.23±0.65	26.03±0.25	5.50±0.61	7.92±0.27
0.04	26.23±1.08	5.57±0.46	7.88±0.87	26.06±0.64	5.22±0.26	7.83±0.76
0.05	24.80±1.57	6.11±0.25	7.56±0.67	25.20±1.15	6.26±0.85	8.05±0.77
0.06	25.87±0.40	6.02±0.65	8.09±0.78	25.29±0.13	6.09±0.16	8.09±0.27
0.07	25.77±0.45	6.05±0.22	7.92±0.96	25.90±1.22	6.01±0.98	7.86±0.75

Note: (1) = water temperature (°C), (2) = DO (mg/L), and (3) = pH of water

Discussion and Conclusions

As reported by Kizak et al. (2018) [4] that rosewood essential oil is mostly composed of linalool up to 86.23 percent. This chemical behaves as a competitive antagonist of the N-methyl-D-aspartate receptors (NMDARs) in the glutamatergic system of the central nervous system (CNS). When presenting in the CNS, linalool vies with an excitatory neurotransmitter, glutamate, at the NMDARs. This enables calcium ions cannot influx into neurons, leading to the inhibition of nerve impulse conduction. As a result, the state of anesthesia can take place [11].

Propylene glycol and tween 80 were selected as surfactants to allow rosewood essential oil to be in an emulsion state. These two substances, generally used to formulate D-limonene emulsions, can increase the water solubility ability of the oil, and improve its delivery efficiency [12]. It has been found that propylene glycol is less toxic to fish than ethanol [13]. Moreover, tween 80 were used in this anesthetic are safe for fish and humans [19].

Studies have demonstrated that several essential oil emulsions induce anesthesia faster when compared to chemical anesthetics [15-16]. In this present study, the findings revealed that the induction times for surgical anesthesia in Thai red-hybrid tilapia (weight of 9±1.8 g) of rosewood essential oil and the rosewood essential oil emulsion at a concentration of 0.15 mL/L were not statistically different ($p>0.05$). Our results are consistent with the research of Kizak et al. (2018) [4] reporting that rosewood essential oil at a concentration of 0.25 mL/L is the most suitable concentration for the removal of goldfish with an average weight of 7.36±0.77 g. Baldisserotto et al. (2018) [17] also showed that the comparable dose of rosewood essential oil (0.1 and 0.2 mL/L) can induce deep anesthesia in tambaqui *Colossoma macropomum* (weight of 2.0±0.2 g) within 5 minutes. The concentration of essential oils used may depend on the quality and source of the essential oils, or their age, weight, and the type of fish used for the test. In our study, the induction time of rosewood essential oil at a concentration of 0.2 mL/L was statistically different from that of 0.15 mL/L rosewood essential oil, 0.15 mL/L, and 0.2 mL/L rosewood essential oil emulsion. As for fish transportation, the effective lowest concentration of the rosewood essential oil emulsion was 0.02 mL/L, while another work published that rosewood essential oil mixed with ethanol required up to 0.05 mL/L to induce anesthesia in Nile tilapia [18]. More importantly, the employment of the rosewood essential oil emulsion can discard the concern of ethanol application.

In conclusion, the present findings have exhibited that rosewood essential oil and rosewood essential oil emulsion can be applied as an effective anesthetic for Thai red-hybrid tilapia surgery and transportation. As for surgery, the optimum concentration of rosewood essential oil and rosewood essential oil emulsion was 0.15 mL/L, while the optimum concentration of rosewood essential oil emulsion for transportation was 0.02 mL/L. Therefore, owing to its safety and hydrophilicity, rosewood essential oil emulsion is highly recommended to use as a fish anesthetic instead of rosewood essential oil mixed with ethanol or chemical anesthetics, such as MS-222.

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