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Research Article
Effects of Gelam, *Melaleuca cajuputi* Methanolic Leaves Extract to the Behavior of Freshwater Prawn (*Macrobrachium rosenbergii*)

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Abstract
Background and Objectives: *Melaleuca cajuputi* is a medicinal plant, containing diverse of phytochemical active substances of several biological properties. Behavioral changes observed in this study somewhat similar to toxic study effect by the phytochemical compounds. The aim of this study was to determine the behavior changes exposed to different concentrations of *M. cajuputi* leaves extract. **Materials and Methods:** Leaf extract of *M. cajuputi* were used for these studies. The concentrations range from 2.0-10.0 g L^{-1} was used for finding range test. Based on the finding range test result, 5 different concentrations (1.5, 2.5, 3.5, 4.5 and 5.5 g L^{-1}) and one control were decided to find LC_{50}. The 96 h LC_{50} of *M. cajuputi* extraction was 2.83 g L^{-1}. Then, the concentrations such as 0, 20 and 30% of LC_{50} of extract *M. cajuputi* and a control were used for behavior study. The behavior changes were recorded in triplicate over a 24 h period and classified into changes, weak, moderate and strong. **Results:** *Macrobrachium rosenbergii* were exposed to *M. cajuputi* extracts and showed several signs of respiratory distress, agitated and abnormal nervous behaviour. Extracts of *M. cajuputi* could have some toxicity effects such as affect the physiological and nervous system. *Macrobrachium rosenbergii* in control group (0.0 g L^{-1}) were active and no behavioural changes throughout the treatment period. There was also no mortality recorded. **Conclusion:** The behavioral study of fish treated with different concentrations are important to know the severity of toxicity effect. The suitable concentration for this medicinal plant can be used for treat fish diseases. Thus, it can help aquaculture industry and give an eco-friendly effect on the ecosystem and agricultural products.

Key words: *Melaleuca cajuputi*, *Macrobrachium rosenbergii*, behavioural changes, mortality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Melaleuca cajuputi Powell (Myrtaceae) or also known as Gelam is one of the most capable medicinal plants that having an extensive spectrum of biological activity. Every part of *M. cajuputi* tree have been known to acquire a broad range of pharmacological properties, especially antibacterial, wound healing, antifungal, antimicrobial and mosquito repellent[1]. It has been popular for medication throughout countries such as India, Indonesia, Vietnam and even Malaysia for many ages. Sahimi[2] found that terpenoids was the major compounds in the methanolic extraction of *M. cajuputi* and important in promoting growth rate and increase immune system in *Macrobrachium rosenbergii*. Recently, the applications of medicinal plants start getting high demand in aquaculture industry due to its safety, effectiveness, inexpensive and widely available across the country.

Macrobrachium rosenbergii is one of the significant species of freshwater prawn that marked for shrimp farming due to its easy reproduction, high fertility rates, fast growth and disease-resistance[3]. These factors enhance management and an economically feasible undertaking[4]. According to Huntingford *et al.*[5], high demands of *M. rosenbergii* exist to maximize profit in all areas in which the organisms are commercially exploited.

Disease can cause economic losses and one of the greatest threats to prawn farming industry. The use of antibiotics and chemotherapeutics agents in aquaculture systems to control and prevent diseases, parasites and other pests, not only leads to high levels of residues in the animals but also they may be toxic and can be harmful to human consumptions[6]. The emergence of antibiotic-resistant and the rising of awareness of consumer health concern resulting of the limiting the accessible variety of antibiotics and chemotherapeutics use in aquaculture[7]. Environment factors such as water quality and physicochemical factors perhaps can contribute to stress incident in prawns and high chances of susceptibility toward microbial infections or disease, due to low immune system[8,9]. Recently, the applications of medicinal plants start getting high demand in aquaculture industry due to its safety, effectiveness, inexpensive and widely available across the country[10]. *Melaleuca cajuputi* is one of the alternative plants used in disease treatment in aquaculture[11].

Behaviour represents the animal's response to physiological and environmental factors and relates one organism to another[12]. In addition, alterations in behavior seem to be major relevant mechanisms by which animals adapt to changes in their environment, including exposure to contaminants[13]. Thus, behavioral endpoints have been considered as much useful as biomarkers to assess chemical exposure effect and even contaminants slightly expose to animals[14].

The prawns behaviorally change from suspended in the water column to principally bottom dwelling, crawling individually and swimming in a forward direction. They are also territorializes, aggressive and cannibalistic[15] which foreseen as an important contribution in research on behavioral activities and the species' best management, behavioral knowledge, physiology and welfare. The study on behavior alternation which caused by the environmental changes is crucial to determine the factors to the changes. The behavioral responses were related to the nervous system and other physiological changes. To date, many studies on applying the plant extraction against pathogenic microorganisms including parasites and fungus could be found effortlessly. Nearly all were recommended to be used on certain dosage for treatment. Nevertheless, the frequency of a dose eliminating the pathogenic microbes in any way possible but they are not concerned of the effect on the host. Thus, this study was conducted to observe the behavioral changes on *M. rosenbergii* with a different dosage of *M. cajuputi* leaves extract.

MATERIALS AND METHODS

Plant extraction: This experiment was conducted on June, 2018. The fresh leaves of *M. cajuputi* were collected from Setiu wetland areas and brought to Institute of Tropical Aquaculture and Fisheries Research (AKUATROP), Universiti Malaysia Terengganu. *Melaleuca cajuputi* leaves were washed individually under running tap water to remove any traces of soil particles and other dirt. The samples were shade-dried 72 h for the leaves to completely dry. The leaves were pulverized into fine powder by using mechanical grinder. About 35 g of the plant sample (powder) were mixed in 1000 mL of 80% methanol until the powder was fully immersed, incubated and shakes 24 h, with 200 rpm and 27°C by using incubator shaker (Max Q Mini 4000). The methanol extract was filtered by using vacuum pump (Rocker 300, Taiwan) with Whatman No. 1 filter paper and the residues were removed.

After that, the filtered solution was concentrated by using rotary vapor (Buchi, Switzerland) with water bath at 35-38°C, velocity of the rotation was 150 rpm and the pressure need to be adjusted. The extraction process obtained the dark brown viscous concentration. Keep it in the eppendorf tube and freeze dry by using freeze dryer (Buchi, Switzerland) until it becomes parched. The powder was stored in the freezer -20°C to evade microbe activity and to maintain the quality of powder for further used.

Acute toxicity bioassay: Healthy juvenile of *M. rosenbergii* (5.98±0.54 cm length, 2.45±0.75 g weight) were used in this experiment. In the hatchery, *M. rosenbergii* (n = 210) were placed in the tank containing 500 L of clean water and acclimatized for 14 days under hatchery conditions (salinity at 0.05ppt, pH 6.6-7.2, dissolved oxygen 6.0-7.5 mg L^{-1} and water temperature at 26°C) prior to commencement of toxicity bioassay. *Macrobrachium rosenbergii* were fed with commercial pellets daily at 3% of their body weight and 80-90% of water was changed when the water turned slightly cloudy. Feeding was stopped 24 h prior to and during the 96 h exposure period so as to prevent interference with stomach contents[16].

Preliminary screening was carried out to determine the use of extract concentrations along with the control as described by Fafioye[17]. In preliminary finding range test (FRT) for 24 h, one hundred and fifty *M. rosenbergii* (n = 150) were separated into 5 groups and each group was exposed to the different concentration of *M. cajuputi* leaf extract (2.0, 4.0, 6.0, 8.0 and 10.0 g L^{-1}). However, the results from FRT showed highest mortality in 6.0, 8.0 and 10.0 g L^{-1} concentration. So that, in acute toxicity test (96 h LC_{50}) of *M. cajuputi* leaf extract was done according to Nagi[11] with slightly modification of concentration. Six concentrations were used in this experiment; one control and five different concentrations of *M. cajuputi* leaf extract. The toxicants were exposed at concentration; 1.5, 2.5, 3.5, 4.5 and 5.5 g L^{-1} with a control 0.0 g L^{-1} of *M. cajuputi* extract. Ten *M. rosenbergii* were put inside the glass aquaria containing of 6 L of water. The dead *M. rosenbergii* were removed from the glass aquaria to avoid the deterioration of water quality. Probit analysis method[18] was u sed to determine the median lethal concentration (LC_{50}) of the extract in the treatment. Then, *M. rosenbergii* were randomly separated into groups in the glass aquaria containing of 6 L of water with 10 prawns/tank. The totals of one hundred and twenty *M. rosenbergii* (n = 120) were used in this experiment for treatment. Four group of *M. rosenbergii* were exposed to 10, 20 and 30% of LC_{50} of extract *M. cajuputi* and a control in triplicate over a 24 h period. The treatments of *M. cajuputi* extracts were given and the behavioral changes of *M. rosenbergii* were recorded at 24 h.

Behavioural changes: The behaviour of *M. rosenbergii* was observed and recorded throughout the treatments. The observations were done for 24 h as suggested by Abalaka and Auta[19]. The treatments of *M. cajuputi* extracts were treated to *M. rosenbergii* and the behavioral changes were recorded at 24 h. The behavioral changes of *M. rosenbergii* that were observed are gulping for air at the surface, jumping out of the aquarium, swim in erratically manner, quarrelling, aggression behavior, pleopods are beating frequently, periopods are standing, quiescent behavior, walking movement pattern and dead. Behavioral changes were assessed using a score ranging from 0 (None) to 3 (Strong) depending on the degree and extent of the changes: (0) None, (1) Weak, (2) Moderate and (3) Strong. The behaviors of *M. rosenbergii* were observed for 24 h and the methods were slightly modified from Abalaka and Auta[19].

The data were analyzed using standard analysis Microsoft excel 2007 and the degree of behavior changes were compared between the concentrations of *M. cajuputi* leaf extract.

RESULTS

Acute toxicity bioassay: The effect of different concentrations and exposure time of leaf extract of *M. cajuputi* (0.0-5.5 g L^{-1}) on *M. rosenbergii* are presented in Table 1. No mortality was recorded in the control (0.0 g L^{-1}) during the toxicity test. In

Table 1: Different concentration of *M. cajuputi* leaf's extract with exposure time on *M. rosenbergii*

Concentration (g L^{-1})	Number of sample	12 h	24 h	48 h	72 h	96 h	Total of dead prawns	Mortality (%)
0.0	30	0	0	0	0	0	0	0.00
1.5	30	0	1	2	0	1	4	13.37
2.5	30	1	1	2	3	1	8	26.67
3.5	30	4	3	2	2	1	12	40.00
4.5	30	5	4	4	3	0	16	53.33
5.5	30	9	5	2	0	0	16	53.33
Total number of dead prawn/time		19	14	12	8	3	56	

Table 2: Respiratory distress of *M. rosenbergii* when treated with different concentration of *M. cajuputi* extracts

	Concentration of *M. cajuputi* extract (g L^{-1})			
Clinical signs	Control	10% of LC_{50}	20% of LC_{50}	30% of LC_{50}
Gulping for air	0	0	1	2
Jumping	0	1	2	3
Erratic swimming	0	0	2	3

Table 3: Agitated behaviors of *M. rosenbergii* when treated with different concentration of *M. cajuputi* extracts

	Concentration of *M. cajuputi* extract (g L^{-1})			
Clinical signs	Control	10% of LC_{50}	20% of LC_{50}	30% of LC_{50}
Quarrel	0	1	2	3
Aggression	0	0	2	3
Pleopod beat	0	0	1	1

Table 4: Abnormal nervous behavior of *M. rosenbergii* when treated with different concentration of *M. cajuputi* extracts

	Concentration of *M. cajuputi* extract (g L^{-1})			
Clinical signs	Control	10% of LC_{50}	20% of LC_{50}	30% of LC_{50}
Periopod stand	0	0	1	1
Quiescent	0	0	1	2
Walking	0	0	2	3
Dead	0	1	2	3

this study, the concentration that affected more than 50% mortality of *M. rosenbergii* was investigated to be 4.5 and 5.5 g L^{-1}. The result showed that the highest number of dead prawn was at 12 h (19 prawns), while the lowest was at 96 h (3 prawns). Based on the data, Probit analysis was used and the result for 96 h was 2.83 g L^{-1}.

Effects on *M. rosenbergii* behaviour: *Macrobrachium rosenbergii* were exposed to *M. cajuputi* extracts and showed several signs of respiratory distress, agitated and abnormal nervous behaviours. From Table 2, *M. rosenbergii* were gulping for air incessantly, jumping out from the aquarium and swimming with erratically manner. These behaviours showed the respiratory distresses were happened in *M. rosenbergii*. At 30% of LC_{50}, *M. rosenbergii* were moderate gulping for air and strong jumping out from the aquarium and swimming inconsistently. The respiratory distress was directly proportional to the concentration of *M. cajuputi* and time of exposure during treatment to *M. rosenbergii*. Besides, *M. rosenbergii* often quarrelling, being aggressively throughout the treatments and beating pleopod intermittently.

At 30% of LC_{50}, *M. rosenbergii* were strong quarrelling and had aggression behaviour, but weak in beating pleopod during the treatments (Table 3). *Macrobrachium rosenbergii* showed abnormal behaviours such as sudden periopod stand, quiescent and strong walking rapidly. Other than that, *M. rosenbergii* were being quiet and not active for some times then, settled at the bottom and eventually died (Table 4). In this study, it showed that *M. rosenbergii* were started to die even at the 10% of LC_{50}. There were not showed any signs of abnormality in *M. rosenbergii* behaviour at 10% of LC_{50}.

DISCUSSION

Many medicinal practitioners from all over the globe have been used a native plants extensively as a traditional medicine, especially related to *Melaleuca* species. In spite of varieties of *Melaleuca* species, constituents found in this study were reported to contain pharmacological activities. There were some research studied that frequent phytochemicals found in *Melaleuca* species were alkaloids, terpenoids, flavonoids, phenolic and aromatic[11,20].

Toxicity test is often applied in aquatic ecotoxicology. The aim of this test is to verify the amount of toxicant or their mixtures that can cause dead for aquatic organisms[21]. The established of LC_{50} value in this study was 2.83 g L^{-1}. From Table 1, the *M. rosenbergii* mortalities were increased with increasing concentration of *M. cajuputi*. While in control group, *M. rosenbergii* were all active and no behavioural changes throughout the treatment period. These conditions might be due to the decomposition of certain active compounds of *M. cajuputi* in water. The value of LC_{50} of *M. cajuputi* methanolic extract in *M. rosenbergii* was much more higher than 127 mg L^{-1} of *M. cajuputi* extract in African catfish, *Clarias gariepinus*[11], 97.61 mg L^{-1} of *Moringa oleifera* extract in *Oreochromis niloticus*[22], 0.033 mg L^{-1} of *Carica papaya* extract in *C. gariepinus*[23]. This showed that the methanolic extract of *M. cajuputi* was less toxic to *M. rosenbergii* compared to the extracts of *M. oleifera* and *C. papaya*. The toxicity is a dose-dependent and varies within the time of exposure of aquatic organisms to toxicants[18]. Thus, it can assume that, *M. rosenbergii* not affected tremendously with *M. cajuputi* extract solution.

The toxicity of *M. cajuputi* extract showed there were no dead of *M. rosenbergii* in Control (0.0 g L^{-1}), 13.37% mortality in 1.5 g L^{-1} concentration and highest mortality (53.33%) in both 4.5 and 5.5 g L^{-1} concentration. These behavioural changes in this study were increased directly with both manipulated variables (concentration of extraction and exposure period). There was also no mortality recorded, which *M. rosenbergii* have to be seen active moving and swimming during the period of treatments. The mortality of *M. rosenbergii* may due to the presence of some toxic compounds in the extract[24]. Although *M. rosenbergii* has been proved to be resistant to various toxicants, *M. rosenbergii* was quite vulnerable to the toxicity of methanolic extract of *M. cajuputi* leaves. Toxicity test is a measure of how poisonous a substance or how large a dose is required to kill or cause damage to an organism. According to Ayoola *et al.*[25], some substances when present in low concentrations may not cause acute detectable effects in organisms, but may in the long run reduce their life span due to the bioaccumulation in organisms.

Behavioural changes are the most susceptible indicator of latent toxic effects. Many researchers have been studied the impact of different plant extracts on the behaviour[20,26]. This study showed that *M. rosenbergii* exposed to *M. cajuputi* extract demonstrated signs of respiratory distress, agitated behaviors and abnormal nervous behaviors. In Table 2-4, *M. rosenbergii* were showed the behavioral changes. The increase of jumping and swimming in erratically manners could be due to sudden response of *M. rosenbergii* to the exposure of *M. cajuputi* extract which supported Maikai *et al.*[27] claimed that there were indicator of physiological stress in aquatic organisms exposed to toxicants. Studies revealed that organisms exposed to toxicants are usually showed signs of changes in erratic, sudden jerky swimming movements and different behavioral activities as shown in this experiments which expressed to be a susceptible indicator of stress in aquatic organisms subjected to sub-lethal concentration of pollutants and toxicants[28,29]. Swimming performance may sturdily influence the ability of an aquatic organism to obtain food, to reproduce or even to avoid unfavorable conditions[30].

The behavioural changes in *M. rosenbergii* occurred as a result of lack of oxygen in the water due to the *M. cajuputi* toxic compounds present. Metabolic activity in aquatic organisms totally depends on the level of dissolved oxygen accessible in environment. Oxygen, therefore, played a vital role in limiting factors such as survival and growth in aquatic organisms. The respiratory distress such as gulping for air frequently, try to jump out from the aquaria many times and erratic swimming were recorded (Table 2). These conditions happened might be due to the consequence of *M. cajuputi* extract on the gills of *M. rosenbergii*. It could enhance the secretion of brown mucous in gills and reduce respiratory activities in *M. rosenbergii*. The mucous secretion found in this study may be a crucial factor to contest the toxic effects of *M. cajuputi* extraction. Mucous acts as a dynamic physical and biochemical barrier, displaying numerous biological and ecological roles such as osmoregulation[31], protection against abrasion[32], protection against environmental toxins and heavy metal toxicity[33], parental feeding[34], protection against pathogens[35] and chemical communication[36]. *Melaleuca cajuputi* compounds that have pharmacological activities were escalated mucous production in *M. rosenbergii*.

Profuse secretion of mucous on the whole body parts of *M. rosenbergii*, more marked in gill region was a protection mechanism of the animals. It is well known that crustacean gills despite the respiration, are also the primary site of osmoregulation. Such type of mucous secretion was quite prominent in *Macrobrahium lamarrei*[37], *Macrobrahium hendersodayanum*[38], *Macrobrahium kistenensis*, *Macrobrahium lamarrei* and *Scylla serrata*[39] and *M. lamarrei*[40] after exposure to various toxicants. These conditions may affect the ability of the gills surface to carry out gaseous exchange actively. Lack of oxygen may cause stress on prawn[41]. Oxygen depletion in water can leads to necrotic, epithelial lifting and abnormal gill tips on the gills[42]. The lifting of epithelial cells may increase the distance of diffusion between water and blood capillaries and might affect the respiration by inhibiting key transport processes[43]. Lack of oxygen in a long run will cause hypoxia and become the main factor of change in behavior of *M. rosenbergii*.

It is well known that the defense and repair mechanisms depend on energy requiring processes such as active transport and synthetic activity. Table 4 showed the abnormal nervous behavior where *M. rosenbergii* tend to be quiescent for some time and settled at the bottom and sooner or later died due to stress. The abnormal nervous behavior usually associated with the failure of nervous system[44]. High mortality happened in *M. rosenbergii* at 30% of LC_{50}. Extracts of *M. cajuputi* perhaps have some toxicity effects that can lead to pathological changes such as severe gill epithelial hyperplasia, usually directly related to gills dysfunction, which may affect the physiological and can cause death[45]. The study conducted by Adesina *et al.*[22] examined the abnormal behaviour in relation to fish stress included erratic swimming, mucus secretion, gasping for air, loss of scales, haemorrhages and stiff fin rays; which are indications of lethargic effect of *M. oleifera* fresh root-bark cold water extract. The abnormality observed could be due to nervous disorder to impaired metabolism, but also because of nervous disorder as earlier reported by Agbon *et al.*[46].

Previous study showed that declination in swimming stamina due to metal exposure indicating a reduction of available energy to detoxification process for survival in aquatic organisms[47]. Decreasing swimming stamina and enhance quarrelling and aggression behavior are showed in Table 3. *Macrobrachium rosenbergii* were continuously quarrelling and in agitated manners as prawns were likely to be stressed and tend to quarrel. The increase of these conditions may be associated with the response of *M. rosenbergii* to the exposure of *M. cajuputi* abruptly. Furthermore, behavior is the final integrated result of biochemical and physiological processes.

CONCLUSION

It could be concluded that methanolic leaf extract of *M. cajuputi* at higher concentration can gives significant effect to the behavioural changes in *M. rosenbergii* compared to the lower concentration. Exposure to *M. cajuputi* was found to result in several changes in the gills of *M. rosenbergii*, thereby affecting the behavior. Hence, further studies need to embark on to ascertain its bioactivity properties that can be used and explored in the future. Furthermore, phytotherapies are cost effectives, environmental friendly and more eco-friendly compared than synthetic molecules. Phytotherapies in medicinal plants are less likely to elicit drug resistance due to the high diversity of plant extract compounds.

SIGNIFICANCE STATEMENT

This study discovered the behavior changes with the different concentrations used. This study will help the researcher to uncover the critical areas of behaviour that many researchers were not able to explore. Thus, a new theory on these compounds combination was triggered the behaviour of prawn.

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