A Novel Green Technology Kaffir Lime Extract as Lizard Repellent

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Abstract. The ubiquity of lizard in homes and has caused huge problem to human. They are one of the very important agents to transmit Salmonella that cause food poisoning towards human being. Through this research the potential of Kaffir lime peel toward lizard was reported. The process to produce Kaffir lime extract is hydrodistillation extraction method. The repellency test of essential oil will be assess at different concentration (0%, 25%, 50%, 75% and 100%). The duration of observation will be test from 3 to 6 hour duration. The results has shown that the concentration up to 50% and above of essential oil from Kaffir lime peel extraction may reflect the lizards in a state of discomfort.

1. Introduction
Kaffir lime (Citrus hystrix) is a very common herb that was use as ingredients in the dishes in South East Asian country such Thailand, Laos, Malaysia, Vietnam and Indonesia. It has distinctive aroma which believe can aid to reduce stress. In fact, not many studies were reported on utilization of Kaffir lime peel as repellent. Kaffir lime consist of several part which is leaves, stem, and peels. For all of that oil extract obtained from peel is higher compare to oil obtained from leaves. Literally, fruit peels is consider as waste for user. Henceforth, this research will mainly focus on implement the citrus products and application of citrus by products [1]. Kaffir lime extract product have huge potential to be use as repellent which is applicable to lizard. Lizard have potential to mechanically carry and transmit salmonella bacteria to human being. Salmonellosis was a type of food poisoning caused by Salmonella bacteria .Salmonella disease can cause someone to feel irksome and if the person was infected during pregnancy the consequence can become worst [2] Through this study researcher will focusing on applying Kaffir lime extract as repellent towards common house gecko (Hemidactylus frenatus). Its commonly can be seen around the house and annoyingly this kind of lizard will leave its dropping. Generally, house geckos are insectivores and as for adults its going to eat spiders and other invertebrates.
They are quite active at night and will produce vexatious sound that will disturb your sleep. This lizard has been considered as a pest in prevalent of country. Several concentration were studied to test the repellency effect of Kaffir lime extract toward lizard which is 0%, 25%, 50%, 75%, and 100%, v/v respectively. Research about repellents derived from Kaffir lime extract is quite limited at this time. Recently there was a study of repellents effect of Kaffir lime peel against cockroaches [3]. Until now, no studies have shown to directly focusing on application of Kaffir lime extract as lizard repellent. From, this research the Kaffir lime essential oil will be extract by using two methods which is Soxhlet extraction method and Steam distillation method which the aim is to observe which method is more effective. This extraction oil then will be test on lizard from Hemidactylus frenatus species under specific condition.

1.1. Research Motivation
One way to repel Lizard or other pest is either by using chemical insect repellent or hire pest Control Company. This chemical repellent and pesticide were used to control, repel or kill pest, for example lizard are considered as disturbance to human whether from its dropping or its presence that can scare children or their annoying sound that can disturb our sleep. Without consumer realise when they consume chemical repellent for a long period of time it will cause bad complication to their own health and even environment. Due to this issue they need to spend a lot of money to cure their health and even government need to add additional budget to solve environment issue that has been caused by this pesticide and repellent.

Toxic repellent spray not only kills pests but also effects the non-targets such as human being and pets. This repellent can be inhaled, ingested or absorbed through skin. Approximately 16 million people living in the United States of America (USA) are sensitive to pesticides. This is because their immune systems are already damaged by pesticides in their living environment. Studies to this illnesses such as asthma, thyroid disorder, and possible fatal illnesses such as cancer has been done by [4]. Furthermore, is not only effect human being but also effect the aquatic life which this repellents substance are highly toxic to fish and other aquatic life [4]. Consumer used toxic chemicals because they was unaware of alternative solution which we have safer repellent which derive from natural ingredients. Generally, this research was aim to provide alternative solution to consumer who are allergic to chemical repellents by developing a more safe and environmental-friendly lizard repellent that was derived from Kaffir lime extract.

2. Methodology
This topic includes detail process flow and methodology that will be apply in this research including the extraction method for kaffir lime peel as well as the methodology to test the repellent activity of kaffir lime extract against lizard (Hemidactylus frenatus). It is essential to have high understanding of the process from first process until the last process to avoid any unexpected error in research. Basically, from this research we going to clarify which is the best extraction method that act the best as lizard repellent. Furthermore, we also want to elucidate which concentration is the greatest to use for lizard repellent. For this current study, about six different concentration of kaffir lime extract will be tested which are 0%, 25%, 50%, 75%, and 100% respectively. In addition, this manuscript only describe one method of Kaffir lime extraction only, which steam distillation method.

Besides, to test repellency efficiency for the lizard same method from previous research [3] will be apply. Which we going to use 3 container to test the efficiency. In generating the best outcome of this research all the preparations are made according to the research order to get the optimum result. All the necessary information will be recorded carefully for further analysis that will be use to achieve research objective.

2.1. Material, chemical and equipment
This are the following material that will be used in this experiment from extraction process until efficiency test of repellent.
Table 1. Materials involved.

| Material and chemical          | Purpose                                      |
|-------------------------------|----------------------------------------------|
| Kaffir lime peel              | Starting material                            |
| Water                         | Medium for steam distillation process        |
| Dichloromethane               | Solvent                                      |
| Acetone                       | Solvent                                      |
| Heptane                       | Solvent                                      |
| Anhydrous sodium sulphate     | To dry the solution                          |

Table 2. Equipment involved.

| Equipment                     | Purpose                                      |
|-------------------------------|----------------------------------------------|
| Steam distillation apparatus  | Extract kaffir lime peel                     |
| PVC tubes                     | Link                                         |
| Container                     | Channel for lizard path                      |

2.2. Extraction of Kaffir lime peel
In this sub-section there was three important step that become the most important thing to extract the Kaffir lime peel. Analysis of Kaffir lime peel and repellency of oil extract against lizard. All of this thing will be explained in this sub-topic.

2.2.1. Hydrodistillation extraction.
As for the second extraction method, Kaffir lime essential oil will be extract by using steam distillation process. In general, steam distillation process consist of three main thing which are. Distillation column, a condenser and heating system that illustrated in Figure 1. About, 10 litre of filtered water will be fill inside the column and a packed bed contained of Kaffir lime peels is located at the upper compartment of the column. To heat the system immersion type of heater will be use to raise up the water temperature. Moreover, the water level of the tank will keep unchanged. In other to reduce loss of water during the process the water coolant will recycle until it reached desired water level Henceforth, it will reduce wasted water and also minimize the temperature difference between water tank and water flow-in. Steam will passes through Kaffir lime peel and transport small particles of extract to the condenser. Next, it will change again the liquid mixture that is actually a mixture of oil and water. The mixture will fully occupy the separator that separate between oil and water. The advantage of this automated steam distillation process is to provide proper temperature control to meet user requirement and selection of suitable temperature profiles for associated plant during extraction process with reasonable cost operation. The temperature used to for heating process was 80 celcius. The production yield will be obtain approximately 50 minutes after process started. Two layers will be form, the top layer corresponds to oil while bottom layer is water. The oil sample will be transfer into separating funnel. Petroleum ether will be add into the funnel to form immiscible solvent where the oil and dichloromethane solvent at the bottom layer and water at the upper layer. The oil at lower layer was taken and the liquid at the upper layer will be remove. Then, the evaporating process will be continue by dry up remaining water contained in oil layer.
2.2.2. Repellency activity.
The lizard use is from *Hemidactylus frenatus* species which will be collected around college area. Previous developments [3] in the fields of repellent have led to a different application. A required number of lizard will collect and keep in a container, which was perforated for aeration. Same method as [3] has been applied in the current study. These lizard were starved for 1 day for experiment. For the present study, an apparatus was improvised outside the laboratory with the following compartments: middle plastic chamber from which projects two PVC tubes (15 cm each) as shown in Figure 2. The end of each plastic tube was fitted to two containers and marked as A and B, respectively. The five (5) starved lizard has been introduced into the middle plastic chamber. Container A was taken as the treated side while container B as the control side. Biscuit powder will act as food for the lizard was coated with 1 mL of different concentrations of the essential oil extracts, i.e., 0-100% (v/v) oil/ethanol, and placed in container A, while biscuit powder in container B was not treated [3].

2.2.3. Percentage repellency.
The lizard located in the treated and control areas were carefully observed and counted 6 hours after treatment. The extent of repellency of essential oil extracted from the Kaffir lime peel was calculated using the following relationship [5]. From this formula the percentage repellency of Kaffir lime extract against lizard will be obtain. Henceforth, the results from both of this equation is really important because it was interrelated with the objective which is to produce an effective repellent from bio-based product.

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PC = \left[1 - \frac{NT}{NT+NC}\right] \times 100
\]

\(NT\) : Number of insects trapped in the chemical treated test chamber
\(NC\) : Number of insects trapped in the control test chamber
\(PC\) : Percentage repellency (Percentage of lizard trapped in control test chamber)
3. Result and discussion

Figure 3 shows the increases of the Kaffir lime peel oil concentration in line with the values of the percentage repellency increased. Based on previous study [3] the main reason that this green substance have a strong repellent effect due to the main composition of the oil itself. This will make the lizard is in a state of discomfort. According to [6] the Kaffir lime peel oil has major citronellal component. The citronellal is also the main component in plant-based insect repellent. However, others researcher was claimed that the sense of smell stimulus has been one of the factor disrupt the insect behaviour. And more, the presence of volatile compounds having strong odour could block the tracheal respiration of the lizard leading to their death [6].

![Figure 2. Repellency test setup.](image)

![Figure 3. Percentage repellency of Lizard toward Kaffir lime peel oil.](image)
4. Conclusion
As conclusion, the concentration of 50% and above of essential oil derived from Kaffr lime peel have given a direct impact on insect and that’s what happened to the lizard in this study. Effect on the lizard due to the different concentration may be considered as novel findings in the potent botanical insecticides against the insect. The result in the current study of Kaffr lime peel would provide an information and knowledge about the effectiveness of insect repellent.

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