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An overview of the potential of *Citrus hystrix* (kaffir lime) essential oil as mosquito repellent

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ABSTRACT

A chemical-based insect repellent such as N, N-diethyl-3-methylbenzamide (DEET) is commonly used against all insects despite its toxicity in humans. Hence, a repellent agent from natural sources of plant essential oil was introduced. Some of the plants which have the repellent potential are catnip, hairy basil, citronella, vetiver, clove, and citrus. Citronellal, citronellol, geraniol, eugenol, eugenol-acetate, and beta-caryophyllene are among the repellent active components in these plants. *Citrus hystrix* (kaffir lime) was known for several applications in food, health, and skincare, but its potential as mosquito repellent is still new. This overview paper highlights the potential of kaffir lime essential oil as a mosquito repellent and the encapsulation of essential oil related to mosquito repellent. Several studies on kaffir lime essential oil as mosquito repellent was conducted and revealed that the major repellent active components are citronellol and limonene. Pure kaffir lime essential oil provides one-hour repellent protection against the mosquito. However, an addition of 5% vanillin prolongs the protection time to three hours. Essential oil is easily vaporized at room temperature which shortens its effectiveness time. Encapsulation of the essential oil would control the vaporization process and hence prolong its efficiency. Nonetheless, almost no research work reported on the encapsulation of kaffir lime essential oil and the release mechanism of the encapsulated kaffir lime.

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1. INTRODUCTION

Mosquitoes like Aedes aegypti spread diseases such as chikungunya, dengue, and yellow fever. World Health Organization (WHO) reports that dengue remains an as big threat in many countries such as Southeast Asia region and Western Pacific region [1]. A chemical-based insect repellent such as N, N-diethyl-3-methylbenzamide (DEET) is commonly used against all insects although the effectiveness time is short, has an unpleasant odor, and may irritate the skin. Hence, research on green and safe natural-based mosquito repellent made from plant essential oil was introduced.

Some effective mosquito-repellent plants are catnip, hairy basil, citronella, vetiver, and clove [2]. Furthermore, essential oils from *Citrus aurantifolia*, *Citrus aurantium*, *Citrus hystrix*, *Citrus maxima*, *Citrus medica*, *Citrus reticulate*, *Citrus sinensis*, and *Citrus microcarpa* have the repellent potential against *Aedes aegypti* and *Cx. quinquefasciatus* [3]. *Citrus hystrix* (kaffir lime) is mostly found abundant in tropical

South-Eastern Asia countries [4]. Citronellol, a major component of kaffir lime leaf, and limonene, a major component of kaffir lime peel oil, show strong repellent activity against mosquitoes [5].

Essential oils can be extracted from plants that contain valuable compounds with various biological activities such as phenols, aldehydes, ketones, mono, and sesquiterpenes [6]. Essential oil is volatile and very sensitive to temperature change. Encapsulation of essential oil facilitates controlled release to reduce volatilization and degradation of its efficiency [7]. Several papers reported on mosquito repellent from the encapsulated essential oil of various plants [8], [9], but almost no work was found on the encapsulation of kaffir lime essential oil as a mosquito repellent. This paper highlights the potential of kaffir lime essential oil as a mosquito repellent and the encapsulation of essential oil related to mosquito repellent.

2. SPECIALTY FUNCTION OF *CITRUS HYSTRIX*

Kaffir lime is a tropical fruit that originated from South-Eastern Asia countries. The special characteristic of kaffir lime is determined by its precious compound, which can be extracted using various extraction methods. Extraction methods gave an important effect on the yield and composition of the extract [10]. Conventional methods such as hydro-distillation, steam distillation, organic solvent extraction, and cold pressing techniques are cost-effective and simple to implement. Then again, high costs and technical expertise are required for innovative techniques such as supercritical fluid extraction, subcritical extraction, ultrasound-assisted extraction, microwave-assisted extraction, microwave hydro-diffusion, and gravity extraction methods [11]. These modern methods have been carried out on an industrial scale [12]. However, conventional methods are usually used since they are less expensive and easy to handle.

McNeil *et al.* [13] found that the main composition of hydro-distillation extracted kaffir lime leaf were monoterpenes with terpinen-4-ol (13.0%), b-pinene (10.9%), a-terpineol (7.6%), 1,8-cineole (6.4%), citronellol (6.0%), and p-cimene (5.6%). Meanwhile, Srifuengfung *et al.* [14] reported that the composition of steam distillation extracted kaffir lime fruit peel was mainly of monoterpene hydrocarbons (65.98%) followed by oxygenated monoterpenes (20.68%) and sesquiterpene hydrocarbons (3.32%). D-limonene (25.28%), b-pinene (21.10%), and sabinene (14.99%) were the major components of monoterpene hydrocarbons, while citronellal (7.63%) and terpinen-4-ol (5.06%) were the major components of oxygenated monoterpenes. The specialty function of kaffir lime extract is very much designed by their composition.

Several scientific research reports on the potential of kaffir lime in different fields are shown in Table 1. The strong kaffir lime flavor from fruits and leaves is popular in Asian cuisines. Besides, the antioxidant property of kaffir lime juices shows that they can be a natural source of daily supplements [15]. On the other hand, the interaction effect of limonene and other minor components in the extract was found as an antibacterial agent towards a wide range of bacterial organisms [14], [16]. In the medical field, work on a mixed extract of kaffir lime leaves, rhizomes of lemon grass, and galangal discovered that the mixed extract which consists of citronella, linalool, 1,8-cineole (eucalyptol), and acetophenone, has valuable effects on blood cholesterol [17]. Furthermore, the kaffir lime extract was identified to reduce the viability of cervical and neuroblastoma cells which reveals its potential as an anticancer [18], however, the respective active compounds were yet to be investigated.

Several research reported that kaffir lime can act as a natural insect repellent [1]–[3], [19]. Moreover, it was reviewed that b-pinene, d-limonene, and terpinene-4-ol in kaffir lime peel essential oil were the active repellent compounds against *Aedes aegypti* [1]. The specialty functions of kaffir lime in different fields are investigated; however, for certain applications, extensive research work including artificial (in vitro) and field trials (in vivo) should be conducted before commercialization.

Table 1. Specialty function of *Citrus hystrix*

Application	Functions	References
Food and supplement	Antioxidant drink	[15]
Antibacterial	Antibacterial oral spray	[14]
	Antimicrobial-resistant bacteria	[16]
Medical field	Antioxidative and immunomodulatory	[17]
	Anticancer	[18]
Insect repellent	Mosquito repellent	[1], [2], [3], [19]

3. *CITRUS HYSTRIX* AND MOSQUITO REPELLENT TEST METHOD

Table 2 shows the test methods which measure the efficiency of kaffir lime as a mosquito repellent. The potential of turmeric, citronella, hairy basil and kaffir lime as mosquito repellent was reported [20]. The four volatile oils and DEET were subjected to the human-bait test method (mosquito cage) with and without vanillin against *Aedes aegypti*, *An. Dirus*, and *C. quinaquefasciatus*. Turmeric and kaffir lime provided one-

hour protection against the mosquito. However, with the addition of vanillin, the repellency against *Aedes aegypti* was greatly improved to 4.5 and 3 hours, respectively. Although kaffir lime alone shows less repellency against *Aedes aegypti*, it still can be used as a potential mosquito repellent due to its abundant availability, especially in Southeastern Asia countries.

A similar finding was reported on the repellent activity of 8 citrus plants including *Citrus hystrix* DC, *Citrus aurantium* L., *Citrus maxima* (Burm. f.) Merr., *Citrus medica* L. var sarcodaclylis Swingle, *Citrus reticulate* Blanco, *Citrus sinensis* Osbeck, and *Citrofortunella microcarpa* (Bunge) Wijnands against female adult of *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say) by human-bait technique (mosquito cage) [3]. The essential oils were dissolved in ethyl alcohol and kept at room temperature before the test. They found that the mean protection of *Citrus hystrix* DC against female adults of *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say) was 20.95 ± 8.67 and 45.0 ± 8.7 minutes with the protection of 98.3% and 98.3%, respectively.

Besides, the repellency of 38 essential oils including kaffir lime against mosquito bites was investigated. The essential oils were diluted in 70% alcohol. Three different concentrations (10%, 50%, and undiluted) of essential oils were applied against *Aedes aegypti* by the human-bite technique (mosquito cage). The result revealed that the repellency of kaffir lime with different concentrations of 10%, 50%, and undiluted was 10 min, 30 min, and 60 min, respectively [19].

Other than the human-bait technique, the excito-repellency (ER) test system is another technique that can be used to understand the behavioral responses of the mosquito population towards the contact and non-contact exposure to the mosquito repellent compound [21]. For contact exposure, pretreated paper (impregnated with the mosquito repellent agent) is placed inside the test chamber, while for non-contact exposure, the pretreated paper is located outside the chamber. The test reveals the repellent behavior of the mosquito towards the repellent compound, which acts as either contact excitation (contact) and/or spatial repellent (non-contact).

Earlier, the use of the ER test system in investigating the repellent behavior of *Aedesaegypti* L. and *Anophelesminimus* against different concentrations of kaffir lime oil in absolute ethanol was reported [21]. Both mosquitoes demonstrated excellent spatial repellency behavior and non-contact excitation against the kaffir lime essential oils. They stated that the repellent active compounds in the leaf oil are mainly citronellal, citronellene acetate, isopulegenol, and geranyl acetate; while the peel oil is predominately citronellal, limonene, and b-pinene.

The human-bait technique (mosquito cage) test reveals that pure kaffir lime essential oil can protect the human skin within 60 min. However, dilution of the essential oil in alcohol reduces the protection duration. On the other hand, an addition of other essential oils such as vanillin oil enhances the protection duration significantly. Combining several essential oils with repellent properties produces a better natural mosquito repellent [2]. The ER test reveals the spatial repellency behavior of the mosquito towards the kaffir lime essential oil. Based on the behavior, a suitable repellent product can be developed which considers the release of kaffir lime volatile compound into the air.

Table 2. *Citrus hystrix* essential oil and mosquito repellent test methods

Test method	Ingredients	Repellent protection/ behavior	References
Human-bait	Kaffir lime	60 min	[20]
	Kaffir lime+vanillin	180 min	
	Kaffir lime in ethyl alcohol	20.95 min 98.3% protection	[3]
	Kaffir lime in alcohol with different concentrations 10%, 50%, 100%	10 min 30 min 60 min	[19]
Excito-repellency	Kaffir lime leaf oil in ethanol– 1-5% concentration	Spatial repellency 78-87%	[21]
	Kaffir lime peel oil in ethanol – 2.5% and 5% concentration	Spatial repellency 78%	

Essential oil is volatile and very sensitive to heat, light, and oxygen [22]. Hence, the appropriate handling method of the essential oil after extraction and product formulation is very important. Degradation of essential oil through oxidation can be reduced by proper storage in dark bottles, away from direct sunlight and sources of heat. Encapsulation of essential oil is one of the current techniques that can be used to control vaporization and prolong its effectiveness.

4. ENCAPSULATION OF ESSENTIAL OIL FOR MOSQUITO REPELLENT

Encapsulation of essential oil produces micro or nano-size sphere capsule with a uniform wall around it. The material inside the capsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating, or membrane. The most commonly used encapsulation processes include coacervation, spray drying, centrifugal extrusion, fluidized bed coating, freeze drying, emulsion, liposome entrapment, spray cooling, solvent evaporation, and in situ polymerization [7]. Emulsion and coacervation are among the popular encapsulation techniques in mosquito repellent studies.

Table 3 shows a list of the previous study on the encapsulation of essential oil for mosquito repellent. Sakulku *et al.* [23] worked on the encapsulation of citronella oil by nanoemulsion technique. Citronella oil, glycerol, and surfactant (Montanov®82) were dispersed under the stirring condition at 200 rpm, 50 ºC for 5 min. The mixture was emulsified by a high-speed homogenizer at 16500 rpm for 3 min. Subsequently, this pre-emulsion was passed through the high-pressure homogenizer for five cycles at a pressure of 1500 bars before cooling down to room temperature and stored at 25 ºC. Nanoemulsion is recommended for stability during storage and ability to control the release of essential oil. On the other hand, encapsulated Apiaceae oil by microemulsion technique was also investigated [24]. In the emulsion preparation, distilled water was added to a mixture of essential oil, polysorbate 80, glycerol, and ethanol. They claimed that the microencapsulated essential oil was able to exert toxicity against mosquito larvae.

Furthermore, the investigation was conducted on the repellent efficiency of complex coacervation encapsulated citronella essential oil on cotton fabric [25]. Gelatin and citronella oil were homogenized at 40 °C, 18500 rpm for 5 minutes. Coacervation was accomplished by dilution with distilled water. The temperature was then reduced to below 10 °C and the pH was adjusted to pH 8 with sodium hydroxide. Glutardialdehyde aqueous solution was slowly added upon mechanical stirring. The suspension was kept overnight with continuous stirring at room temperature before spray drying.

Moreover, the microencapsulation of citronella oil by simple coacervation technique was studied [26]. Gelatin aqueous solution and essential oil were stirred and emulsified at 50 °C. Coacervation was done with an addition of sodium sulfate solution. The temperature of the suspension was reduced to 5 °C and formaldehyde solution was added. Eventually, the microcapsules were harvested and washed with ethanol and water before freeze-drying. Microencapsulation offers a promising alternative to prolong the repellent action duration of citronella oil. Similarly, [27] reported on the encapsulation of *Zanthoxylum limonella* oil by simple coacervation technique. Gelatin and the essential oil were emulsified under high agitation. Coacervation was done by the addition of sodium sulfate solution at 40 °C before the temperature was brought down to about 5 °C and glutaraldehyde solution was added as a crosslinking agent. The suspension was then heated up to 40 °C and stirred for about 3–4 h. The suspension was cooled to room temperature and the microcapsules were filtered, washed with Tween 80 solution, dried, and stored in a glass bottle.

Table 3. Encapsulation of essential oil for mosquito repellent

Encapsulation methods	Essential oil	References
Nanoemulsion	Citronella oil	[23]
Microemulsion	Apiaceae oil	[24]
Complex coacervation	Citronella oil	[25]
Simple coacervation	Citronella oil	[26]
Simple coacervation	*Zanthoxylum limonella*	[27]

The micro and nanoemulsion techniques produce stable emulsion that can be stored in the form of emulsion itself at 25 °C. In contrast, the coacervation techniques require secondary processes such as capsule collection, filtration, washing, spray drying or freeze drying, and finally, the capsules are stored in powder form.

5. RELEASE RATE AND MECHANISM OF ENCAPSULATED ESSENTIAL OIL

The release rate, mechanism, and kinetic of encapsulated essential oil in the respective media are other important factors to be explored to study the behavior of the final product formulation. The release mechanism of encapsulated essential oil through controlled, continuous, or focused release is very much affected by its final application. The encapsulated essential oils are released from the wall of the microcapsule through rupture, dissolution, and diffusion.

The release behavior of encapsulated citronella essential oil which was produced by simple and complex coacervation techniques using Arabic gum and gelatin as wall material was studied [28]. The citronella essential oil released from the fresh wet microcapsule by diffusion following the Fickian diffusion mechanism and the release of the core material is significant with time. On the other hand, the release of

complex coacervation encapsulated citronella essential oil in Tween 80 solution was found to follow the Korsmeyer-Peppas controlled release model of super case II prediction and the release is by wall erosion [29]. An earlier study also reported that microencapsulation using the complex conservation method on citronella essential oil using cotton and polyester also followed the Korsmeyer-Peppas release model for these two matrices [30]. As for the release mechanism, it can follow Fickian and anomalous diffusion depending on the hydrophobicity properties of the materials used.

The release kinetics of Azadirachtin from microencapsulated *Azadirachta indica* A. Juss (Meliaceae) bioinsecticide was reported [31]. Several factors such as the physical properties and differences due to particle factors were found to affect the active compounds' release kinetics. Stability during storage and release mechanism of encapsulated kaffir lime essential oil in the targeted environment are important factors to be comprehended as the efficiency of mosquito repellent can be determined.

6. CONCLUSION

Kaffir lime leaf extract consists of monoterpenes with terpinen-4-ol, b-pinene, a-terpineol, 1.8-cineole, citronellol, and p-cimene as principal compounds. Kaffir lime fruit peel extract consists mainly of monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpene hydrocarbons. Meanwhile, D-limonene, beta-pinene, and sabinene were the major components of monoterpene hydrocarbons, while citronellal and terpinen-4-ol were the major components of oxygenated monoterpenes. They are rich in functional components which support their application as an antioxidant, antimicrobial, anti-cancer, anti-inflammatory, and mosquito repellent. The compounds in kaffir lime such as limonene and citronellal are unappealing to most insects and mosquitoes.

Pure kaffir lime essential oil is capable to protect the human skin within 60 min. However, dilution of the essential oil in alcohol reduces the protection duration. On the other hand, the addition of other essential oils such as vanillin oil to the kaffir lime essential oil enhances the protection duration significantly. Essential oil is volatile and very sensitive to heat, light, and oxygen; hence, reducing its efficiency. Consequently, encapsulation of essential oil can be used to control vaporization and prolong its effectiveness. Emulsion and coacervation are among the popular encapsulation techniques for mosquito repellent study. The wet encapsulated essential oil releases the core material by wall diffusion and wall rapture following the Fickian diffusion mechanism and the Korsmeyer-Peppas controlled release model of super case II prediction, respectively. Almost no work reported on the encapsulation of kaffir lime for mosquito repellent, hence the potential should be explored and established.

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