

PREPARATION OF AN HERBAL MOSQUITO REPELLANT AND ITS STABILITY STUDY

Syrpailin Suchiang¹ and Jyotirmoy Sarma^{1*}

¹Department of Chemistry, Assam Don Bosco University, Tapesia Gardens, Sonapur-781017, Assam, India.

*For correspondence. (jyotirmoy.sarma@dbuniversity.ac.in)

Abstract: Mosquito-borne diseases are a persistent global health concern, necessitating the development of safe and efficient repellents. This work explores the formulation and efficacy of a mosquito repellent liquid derived from natural ingredients, with a focus on essential oils such as lemongrass oil and okra extract used as a surfactant. Lemongrass oil, known for its high citronellal content, provides a potent repellent effect, while okra extracts containing mucilage acts as a natural surfactant, facilitating the homogeneous mixing of essential oils with water, thereby enhancing the formulation's stability and application of the repellent. The preparation process involved extracting essential oil from lemongrass, through reflux extraction method and obtaining mucilage from okra pods. The lemon grass was collected from the Assam don bosco university campus, tapesia gardens, Sonapur-782402, Assam, India. The repellent formulations were then created by emulsifying varying concentrations of lemongrass oil with okra mucilage. The surface active agent present in okra helps in stabilizing the oil/water emulsion. The repellents stability was optimized against the concentration of various components and time. The okra/water extract concentration at 13.3 % v/v was optimized in this present study due to the formulation stability for 20 days. The repellent was found to be stable for 20 days at the LO/water concentration 17 % (v/v), which was also optimized. After 25 days from the day of preparation, the repellent was destabilized due to coagulation. For efficacy testing, proper scientific facility is required concerning the safety aspect. The inclusion of okra mucilage improved the stability of repellent suspension and ease of application.

Keywords: Lemon grass oil; okra mucilage; mosquito repellent; stability.

1. Introduction:

Mosquitoes such as *Aedes*, *Anopheles*, and *Culex* are a serious threat to the public health as they are known vectors for various protozoans, viruses, and bacteria which result in many life-threatening diseases like malaria, filariasis, yellow fever, Japanese encephalitis, chikungunya, and dengue.¹ Thus one of the approaches for control of these mosquito borne diseases is the interruption of disease transmission by either killing the mosquitoes or preventing them from biting individuals.² To anticipate any kind of epidemic diseases or to avoid any discomfort provided by the mosquitoes, a personal protection using repellent products is necessary to minimize the risk of an infection and to reduce the discomfort caused by mosquitoes.³

Commercially available chemical-based insecticides and repellents are known to contain harmful and poisonous compounds which could pose threats to human health and toxicity to non-target organisms. The most commonly used chemical repellents are DEET (N,N- diethyl-meta-toluamide), dimethyl phthalate (DMP), Picaridin (also known as Icaridin) and IR3535(ethyl-butyl-acetyl-amino-propionate).⁴ These compounds are effective but have several drawbacks. However, over time, it became incompetent because mosquitoes acquired immunity to them, and since synthetic repellent are chemicals, causes more harm to the ecosystem by contamination. So, there is a sore need for natural alternatives to synthetic repellents.

To avoid the possible negative effects of synthetic repellents, plant-based natural products such as essential oils could be an alternative to repel mosquitoes. Natural repellents are typically derived from plant extracts and essential oils.⁵ They are considered safe and environmentally friendly compared to synthetic repellents. In light of these challenges, there is growing interest in exploring natural alternatives that offer both efficacy and sustainability.^{4,6}

In the context of herbal repellent formulation, okra derived surfactant and lemon grass oil were excellent candidates. *Cymbopogon citratus* (Lemon grass) oil has been known as effective insect repellent.⁷ Lemongrass oil is the essential oil obtained from the aerial parts of *Cymbopogon citratus* family.⁸ It is composed mainly of citral, a compound known for its strong mosquito repellent properties. Essential oils are volatile naturally occurring, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites.⁹ Biological research has shown that various chemical compounds of lemongrass essential oil exhibit antimicrobial, antioxidant, antiparasitic, insecticidal, and insect repellent activities.¹⁰ Lemon grass oils is considered safe for human use and has a pleasant scent, making it an attractive alternative to synthetic repellents. In recent years, botanical extracts from plants with known insect-repelling properties have also garnered attention, Okra (*Abelmoschus esculentus* L.) extract, also known as a lady finger or gumbo, produces viscous mucilage in

the form of hydrocolloid that contains polysaccharides and protein substances potential that exhibits excellent emulsifying and gelling properties to be used as an emulsifier. ¹¹ Okra mucilage, known for its emulsifying properties is used to stabilize the oil/water mixture which is considered to be safe because it is natural, harmless, safe, biodegradable, and eco-friendly. ¹² Its potential as a natural Surfactant in the formulation of mosquito repellents is of particular interest due to its biocompatibility and biodegradability. It is used in stabilizing essential oil emulsions, showing that it effectively prevents phase separation and degradation of the oil over time. This property is particularly beneficial in formulating mosquito repellents that need to remain effective under varying environmental conditions. ¹³

Incorporating okra mucilage into a mosquito repellent formulation could enhance the stability and efficacy of the product by improving the dispersion of lemongrass oil. The properties and efficacy of lemon grass oil is primarily dependent upon plants geographical location, climate and soil conditions. ^{14,15} These factors affect upon the plants chemical composition. The combination of lemongrass oil's repellent properties and okra mucilage's surfactant properties may lead to an effective and environmentally friendly mosquito repellent.

The combination of two active substances, i.e. lemongrass oils and okra mucilage extracts, is expected to give a synergistic effect as a mosquito repellent. The properties and hence repellent effects of the lemon grass oil are region and concentration specific. The hypothesis of this study is that the essential oils of lemongrass collected from the specified region and okra extract, when combined in specific concentrations, will demonstrate significant mosquito repellent activity and maintain stability over time as the stability of such repellents are crucial factors for their practical application.

2. Materials & methodology

2.1 Materials:

The materials used were Lemon Grass (*Cymbopogon citratus*) for extracting essential oils, collected from Assam Don Bosco university campus situated in Tapesia Gardens, Sonapur-782402, Assam, India, 'okra' for extracting mucilage was purchased from local vendor, distilled water and acetone. Lemon grass oil as well as 'okra' comprises several chemical constituents, each contributing to its unique properties. The major components are summarized in the table 1& 2

Table1: Chemical components of lemon grass oil ¹⁰

Component	Concentration (%)
Citral	65-85
Myrcene	5-15
Geraniol	2-5
Limonene	1-3
citronellal	0.5-1.5

Table2: Components of okra ¹⁴

Component	Component category
Vitamin C	Their structures are combination of hydrophobic and hydrophilic groups so possess surface active property.
Carotene	
Thiamine	
Folic acid	
riboflavin	
Oxalic acid	
Amino acids	

2.2. Methodology

2.2.1 Extraction of essential oils

Essential oil having repellent property was extracted by the reflux extraction or distillation method. ¹⁵ Lemongrass leaves were washed, dried, cut into small pieces, grinded and then quantified. Approximately 50 grams is placed into a round bottom flask with 250 ml of distilled water. The flask is equipped with a rubber stopper connected to the reflux condenser and heated by the use of a heating mantle with temperature control for about 180 minutes. The oil content is extracted in water. When water reaches 100°C, it starts to boil and the steam generated passes through the plant to vaporize the oil, which are then condensed and collected. Then the flask was allowed to cool and filtered. Finally, the oil/water emulsion was transferred into a separating funnel where two layers are separated after some time due to density difference. The oil layer was collected by decantation.



Figure 1 Extraction process of lemongrass oil (LO)

2.2.2 Extraction of Okra mucilage

In our case, conventional method of extraction of okra mucilage is adopted by unripe pods of fresh okra collected from the local market; then the okra pods cut into small pieces by removing the seeds. Minced okra was transferred into 500ml beaker and filled with distilled water, then soak for 12 – 24 h and it was heated in a water bath for 30 - 60 minutes by continuous stirring, and then it was filtered through muslin cloth and the swollen pods were squeezed and get extracted.¹⁵ The mucilage extract was then treated with acetone to wash the mucilage and the extract is again filtered by muslin cloth.

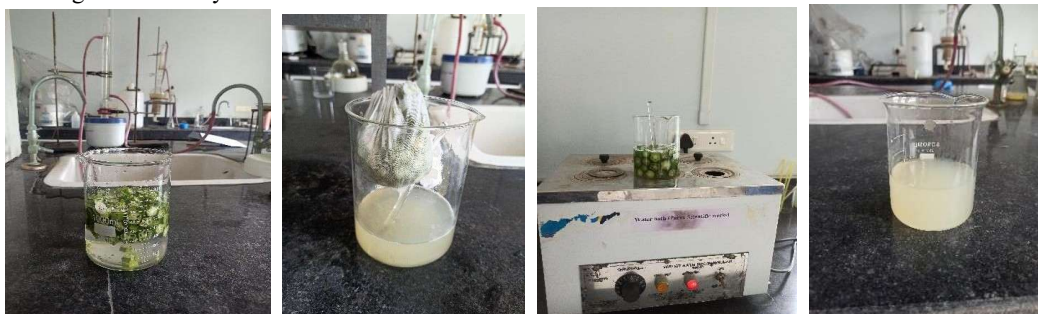


Figure 2 Extraction process of okra mucilage

2.2.3 Repellent Formulation:

The repellent was formulated by mixing lemon grass oil extract in water with the okra extract in various ratios to determine the optimal combination for efficacy and stability. The essential oils and the okra extract were carefully measured and mixed with water in a glass vials using a magnetic stirrer by continuous stirring at a specific temperature to ensure homogeneity until a uniform emulsion was obtained.



Figure 3A typical image of repellent formulation

2.2.4 Stability testing

The stability of a mosquito repellent liquid is crucial to ensure its effectiveness over time. The stability of the formulation was evaluated by visually observing the phase separation of the liquid mixture. The repellent was visually inspected for changes in color and phase separation over a period of maximum 25 days with inspections at different intervals.

3. Results & Discussions:

This section examines the stability of the prepared repellent for optimization of the concentrations of lemon grass oil and okra/water extract. We have done the optimization in a limited concentration range in this particular communication. The stability of the repellent was examined in terms of phase stability over time. The essential oil and the okra mucilage were successfully extracted from their raw materials as discussed in the methodology section. The active components of the lemon grass oil (LO) (table 1) especially *citral* is effective against most of the types of mosquitos in general.¹⁶ Moreover the polyphenolic compounds in okra exhibit surface active property because of the presence of active functional groups as hydrophilic ones in their structure along with the hydrophobic backbone. This further helps in stabilizing the oil/water emulsion.¹⁷

3.1 Optimization of the okra mucilage/water extract concentration:

In this work, a total of seven formulations were prepared in a total volume of 60 ml. The okra mucilage/water extract amounts were varied from 2 to 12 ml as mentioned in table 3. The LO/water amounts were kept fixed at 10 ml (17%v/v) and accordingly the distilled water contents were added. The respective okra/water extract concentrations in these formulations are (3.3%, 5%, 6.7%, 10%, 11.7%, 13.3% and 20% v/v respectively). The formulations are marked as S1 to S7 and shown in figure 4.

Table3 Formulation of repellents with varying okra

Ingredients	S1	S2	S3	S4	S5	S6	S7
LEO/water	10ml	10ml	10ml	10ml	10ml	10ml	10ml
Okra/water	2ml	3ml	4ml	6ml	7ml	8ml	12ml
water	48ml	47ml	46ml	44ml	43ml	42ml	38ml



Figure 4 Repellent formulations with varying okra (S1 to S7)

The okra mucilage concentrations are not kept very high looking into the importance of food conservation. We have selected a limited range of okra/water extract concentrations (S1 to S7), within which the okra concentration alone is definitely less enough. Optimization of the emulsifier concentration was done with respect to the stability of the repellents formulated. The importance of okra based emulsifier concentration upon the stability of repellent was also studied and found responsible to the surface active nature of the chain groups.^{14,16} In fig. 5, a bar diagram is presented showing the stability of the repellent prepared against time duration. We have examined the stability for a time period of maximum of 20 days. This time period has been chosen on a trial basis. For possible commercial production, the time period need to be increased. The stability of the prepared formulations was examined visually based upon their phase separation, as the emulsifier 'okra mucilage' helps in stabilizing the lemon grass oil/water phase. At a very minimal concentration even (serial number 1 in the diagram) the emulsion shows stability for a period of 3 days indicating the efficacy of the okra based surfactant. A similar study was carried out in the formulation of a mosquito repellent cream, where the efficiency of an emulsifying wax was examined in forming a stable repellent, at varying concentration for optimization.¹⁸ An increasing trend of stability is observed with increase in okra concentration for all the formulations. However, the two typical concentrations (13.3 and 20% v/v, serial number 6 & 7 in the diagram) produce stability for a period of 20 days. Interestingly, with the okra/water extract concentration of 13.3 % v/v or serial number 6, the emulsion stability was achieved for 20 days, so this particular concentration is optimized in the present study.

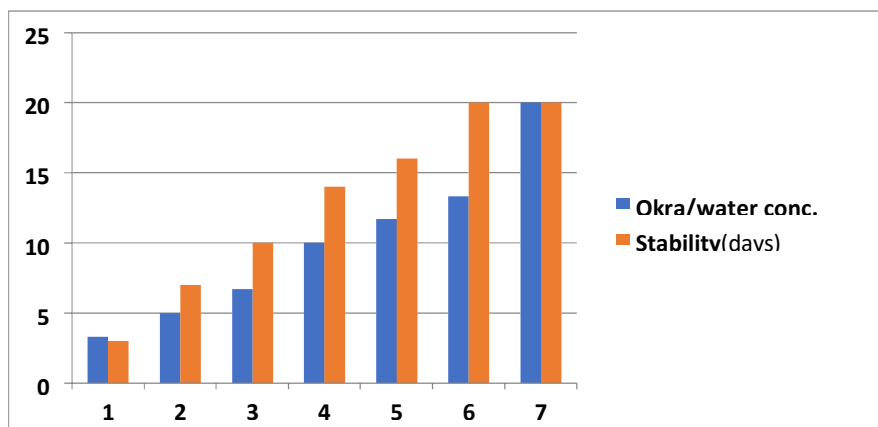


Figure 5 Stability (in days) vs. okra/water extract concentration (%v/v)

3.2 Optimization of the LO/water extract concentration for evaluating better stability of repellent:

For optimization of oil/water concentration, a total of four formulations were prepared in a total volume of 60 ml (figure 6). The okra mucilage/water extract concentration was kept fixed at 13.3% v/v in a total of 60 ml volume. The LO/water concentrations were varied from (20%, 27%, 33% and 53% v/v) in the four formulations (table 4). In section 3.1, the LO/water extract concentration was fixed at 17% v/v. Keeping it as a base, the LO/water concentrations were further varied in section 3.2. Rest volumes were adjusted with distilled water accordingly. Like earlier, the stability of the formulations was evaluated for a period of 20 days.

Table 4 Repellent formulations with varying LO/water concentration

Ingredients	S1	S2	S3	S4
LO/water	12ml	16ml	20ml	32ml
Okra/water	8ml	8ml	8ml	8ml
water	40ml	36ml	32ml	20ml



Figure 6 Repellent formulations with varying LO/water (S1 to S4)

The images in figure 6 clearly indicate the stability of the formulations at varying LO/water concentrations at a fixed okra based emulsifier concentration. However, a color change of the extracts was observed from the first day of preparation (left panel) up to the 20-25 days from preparation (right panel). This may be due to the possible structural changes of the ingredients that need further investigation. The optimization of the oil concentration can be achieved like section 3.1 by the stability comparison of the different phases (figure 7). The bar diagram is presented for optimization of the LO/water concentration in this study. The five formulations from (1 to 5) account for the stability at varying LO/water concentrations. Here all the five formulations exhibit stability from 20 to 25 days from the day of preparation. Serial number 1 & 2 results in comparatively higher stability with respect to the LO/water concentrations, which is very important and significant. At the same time, the rest three formulations (3 to 5) also results in stability for 22 to 25 days but at a comparatively higher LO/water concentration, which is not eco-friendly. The repellent was found to be stable for 20 days at the LO/water concentration 17 % (v/v) (Serial no. 1). At this concentration, stability is comparatively higher, so it is optimized for the selected range of concentrations in this present study. The highest stability of 25 days was also achieved but the corresponding LO/water concentrations were significantly higher than the stability, so not suitable for optimization. It was also

observed that the repellent was destabilized beyond 25 days (figure 8) probably due to the coagulation of the emulsion system resulting in precipitation. This needs further experimental investigation and hence confirmation. The 'Citral' present in LO/water extract interferes with the olfactory receptors, reducing their ability to locate human hosts.¹⁹ Additionally, the optimized LO/water concentration in the present case likely provide a more robust olfactory barrier, making it more difficult for the mosquitoes to penetrate and detect human skin. This is consistent with the theory that the intensity and duration of the repellent effect are directly proportional to the concentration of the active ingredient.¹⁵

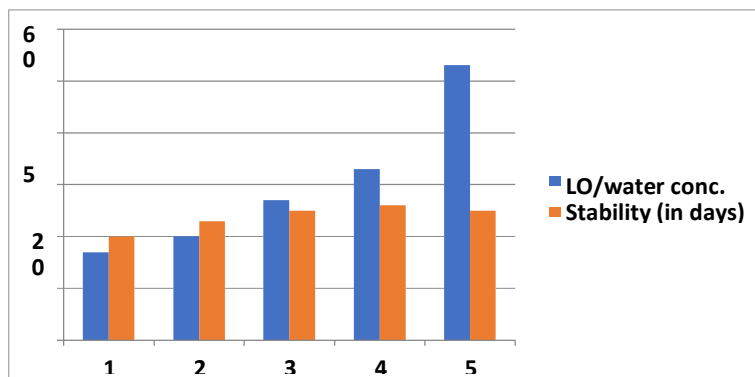


Figure 7 Stability (in days) vs. LO/water extracts concentration (%v/v)



Figure 8 Coagulation/precipitation of the repellent emulsion (after 25 days)

4. Conclusions and future scope

A stable herbal mosquito repellent was successfully formulated using lemongrass oil and okra water extract. The use of water as solvent and stirring techniques by magnetic stirrer ensured a homogeneous and stable mixture. This study highlights the potential of integrating natural ingredients into repellent formulations, paving the way for safer and more sustainable approaches to mosquito control. The formulation with okra/water extract at 13.3 % v/v and LO/water extract at 17 % (v/v) concentration was found to be stable for 20 days.

Beyond that the repellent was destabilized due to coagulation. There was no significant phase separation observed but involves a change in its color. For efficacy testing, proper scientific facility is required concerning the safety aspect. The pleasant lemon scent of lemongrass oil and the non- greasy texture achieved by incorporating okra mucilage is an attracting feature for users.



The determination of the average repellency duration via cage testing along with viscosity and citral concentration determination are future scope of the work.

Acknowledgment

The authors are thankful to Assam Don Bosco University, Assam for the facilities.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that are collected during experimentation are mentioned in the manuscript.

References:

- [1] Kaushik, M., Yadav, J., Singh, A., & Dubey, M. K., A systematic review of plant-based mosquito repellents and their activity, *Ind. J. Natural Prod. & Res.* **2023**, 14(3), 347-359.
- [2] Hazarika H, Krishnatreyya H, Tyagi V, Islam J and Gogoi N, The fabrication and assessment of mosquito repellent cream for outdoor protection, *Sci. Rep.* **2022**, 12(1), 2180.
- [3] Hazarika H, Boruah S, Islam J, Paul A and Zaman M K, Antimicrobial, mosquito larvicidal and repellent activity of essential oils isolated from three local species of ginger grown in upper Assam region, India, *Curr. Trends Pharm. Res.* **2020**, 7(1), 126-142.
- [4] Khote G.B, Marathe K.S, Dapke S.B, Morde P.F and Gite D.R, Formulation and evaluation of mosquito repellent paper from Lantana camara, *Int. J. Res. Public. & Rev.* **2025**, 6, 6166-6177.
- [5] Azad S and Chetia C, Analysis on the antimicrobial and repellent activities of *Cymbopogon martinii* essential oil, *Biosci. Biotech. Res. Comm.* **2021**, 14(2), 281-293.
- [6] Karthick R, Jeyabalan D and Mohanakrishnan H, Studies on mosquitocidal activity of Geranium oil (*Gelargonium graveolens*) against *Culex quinquefasciatus* say (Diptera: Culicidae), *Int. J. Emer. Tech. Innov. Res.* **2022**, 9(2), 487-494.
- [7] Baldacchino F, Tramut C, Salem A, Liénard E, Delétré E, Franc M, Martin T, Duvallet G and Jay-Robert P, The repellency of lemongrass oil against stable flies, tested using video tracking, *Parasite*, **2013**, 20, 21-27.
- [8] Oyedele A.O, Gbolade A.A., Sosan M. B., Adewoyin F.B., Soyelu O.L. and Orafidiya O.O., Formulation of an effective mosquito-repellent topical from lemongrass, *Phytomedicine*, **2002**, 9, 259-262.
- [9] Kalita B., Bora S., Sharma A. K. Plant Essential Oils as Mosquito Repellent- A Review, *Int. J. Res. & Dev. in Pharm. & Life Sci.*, **2013**, 3, 741-747.
- [10] Majewska E., Kozłowska M., Gruczynska-Sekowska E., Kowalska D., Tarnowska K., Lemongrass (*Cymbopogon citratus*) Essential Oil: Extraction, Composition, Bioactivity and Uses for Food Preservation, a Review, *Pol. J. Food Nutr. Sci.*, **2019**, 69, 327-341.
- [11] Lousinian S., Dimopoulou M., Panayiotou C. and Ritzoulis C., Self-assembly of a Food Hydro-colloid: the case of okra mucilage, *J. Biotechnol. & Biomat.*, **2017**, 66, 190-198.
- [12] Zaharuddin N.D., Noordin M.I. and Kadivar, A., The Use of *Hibiscus esculentus* (Okra) Gum in sustaining the Release of Propranolol Hydrochloride in a Solid Oral Dosage Form, *Bio-Med Res. Int.*, **2014**, Article ID 735891.
- [13] Lv, Y., Cai, X., Xv, T., Gao, H., Xv, Y., Gu, Y., Cheng, D., Yan, M. and Li, Y., Emulsions stabilized by okra polysaccharides: Physicochemical, interfacial, and emulsification properties, *Food Hydrocolloids*, **2024**, 155, 110338.

- [14] Dantas T. L., Alonso Buriti F. C. and Florentino E. R., Okra (*Abelmoschus esculentus* L.) as a Potential Functional Food Source of Mucilage and Bioactive Compounds with Technological Applications and Health Benefits, *Plants*, **2021**, 10, 1683.
- [15] Ashaq B., Rasool K., Habib S., Bashir I., Nisar N., Mustafa S., Ayaz Q., Nayik G. A., Uddin J., Ramniwas S., Mugabi R. and Wani S. Md., Insights into chemistry, extraction and industrial application of lemon grass essential oil -A review of recent advances, *Food Chemistry: X*, **2024**, 22, 101521.
- [16] Rajalakshmi M., Sangeetha S., Okra Mucilage Method of Extraction and a Novel Strategy for Pharmaceutical Drug Delivery System, *J. Pharmaceutical neg. results*, **2023**, 3, 542-550.
- [17] Zulfikar Z., Aditama W. and Sitepu F. Y., The effect of lemongrass (*Cymbopogon nardus*) extract as insecticide against *Aedes aegypti*, *Int. J. Mosquito Res.*, **2019**, 6, 101–103.
- [18] Agrawal S., Haldankar N. and Jadhav A., Formulation of Natural Mosquito Repellent. *Int. J. Adv. Res. Ideas & Innov. in Technol.* **2018**, 4, 11-17.
- [19] Kim M., Lee J., Park I., Kim J., Lee K., So J., Choi J.W., Jang J.E., Kwon H.J., Moon C., Choe H.K., Stable olfactory receptor activation across odor complexity, *i-science*, **2025**, 28, 113740.

