Development and Optimization of Herbal Mosquito Repellent Vaporizer Using Essential Oils and its Evaluation

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ABSTRACT

Mosquito-borne diseases are a major global health hazard, and each year they affect millions of people around the world. Even though synthetic chemicals such as DEET provide protection against mosquitoes, long-term usage of these products poses a risk for both environmental and human health. In this study, a sustainable and safe herbal vaporizer mosquito repellent containing essential oils has been designed and optimized. The ingredients used in the formulation were clove oil, lemongrass oil, and peppermint oil together with ethanol, propylene glycol, and Tween 20 as solvents and emulsifiers. Optimizations have been done by a 2^2 factorial design where the effect of ethanol and propylene glycol amounts on evaporation rate has been investigated. A total of twelve formulations have been prepared and studied. ATR-FTIR spectroscopic analysis revealed the compatibility between essential oils. The optimized formulation (F3) showed the minimal evaporation rate (0.20 g/hr). The results of mosquito repellency tests performed by cage test method revealed better repellent effect than commercial products, with no landing being recorded in the first 10 minutes. Physical and chemical stability of the formulated mosquito repellent was established by carrying out studies at 2°C, 25°C, and 40°C for 30 days with no phase separation. This herbal formulation of mosquito repellent is equally effective to that of the market but with more safety and environmental friendly qualities.

Keywords: Herbal mosquito repellent, essential oils, clove oil, lemongrass oil, peppermint oil, optimization, evaporation rate, stability, etc.

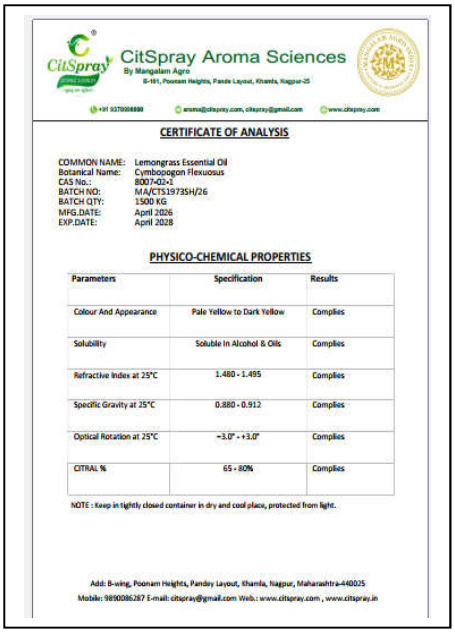
INTRODUCTION

Mosquitoes are known to be the most lethal carriers of various human infections, transmitting malaria, dengue, Zika, chikungunya and many more infectious diseases.[1,2] According to the World Health Organization, malaria alone results in an estimated mortality of 14 million and 2.3 billion cases per year.[25] Although the traditional approach to mosquito control includes environmental management, biological control, and chemical methods using insecticides like deltamethrin and cypermethrin, none of them can be effective individually.[5] Also, vector control programs on a larger scale may not always prove sustainable. Hence, personal protection against mosquito bite through mosquito repellents has become highly essential. Of the repellent agents available in the market, such as cream, spray, and roll-on, mosquito repellent vaporizer is found to be more efficient and economic, because it gives continuous protection and no need for repeated application; it is a practical approach for populations that lack access to health care.[3,9] DEET (N, N-diethyl-3-methyl toluamide) is the most commonly used artificial mosquito repellent agent.[6] However, it has been observed that the prolonged use of this chemical causes skin irritation, nausea, seizure, muscular disorders, memory loss, and loss of sensory and motor activity. In addition, DEET is ineffective in giving complete long-term protection against malaria-causing mosquitoes.[7] To counteract this problem, an increased effort is being made to develop natural agents from natural botanical resources.[4,10] Essential oils are those fragrant essences that are obtained from plants in their purest form. Essential oils are used in various fields such as flavor and fragrance, pharmaceuticals, and aromatherapy. The strong peppery odor of clove oil, which is made up of eugenol, is one of the odors that are very distasteful to mosquitoes.[12] Tests have shown that clove oil can provide 100% protection against Aedes aegypti mosquitoes for at least two hours. The oil of lemongrass contains citral and geraniol that interfere with the sense of smell of mosquitoes to make it difficult for them to locate their hosts.[8,13] The oil of peppermint contains menthol and menthone that confuses their sensory perception to make them unable to detect food and danger.[14] The use of essential oil in making a liquid vaporizer of herbal mosquito repellent can be explored further.[11,21]

MATERIALS AND METHODS

Materials

Materials utilized were sourced from authorized vendors for natural substances. Clove oil, lemongrass oil, and peppermint oil were sourced from Cit Spray Aroma Science Nagpur. Propylene glycol and ethanol were acquired from authorized chemical vendors. Tween 20 (Polysorbate 20), a non-ionic surfactant, was used as the emulsifier.[22]



CitSpray Aroma Sciences
By Mangalam Agro
B-191, Poonam Heights, Pande Layout, Khamla, Nagpur-25

CERTIFICATE OF ANALYSIS

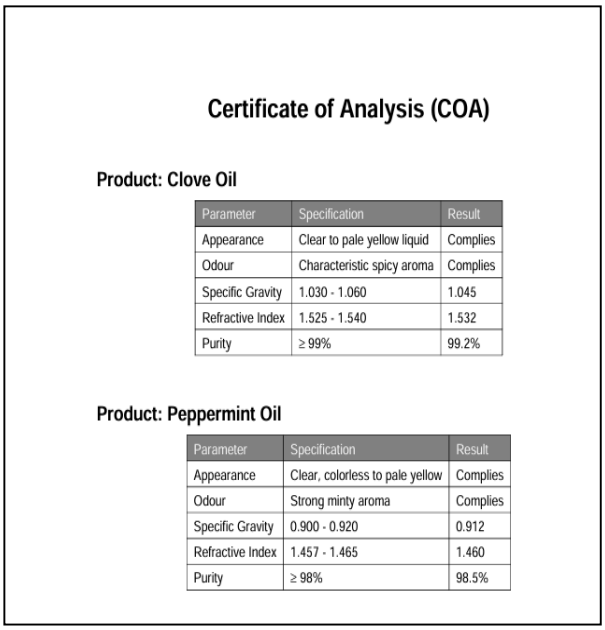
COMMON NAME: Lemongrass Essential Oil
Botanical Name: Cymbopogon Flexuosus
CAS No.: 8007-01-1
BATCH NO: MA/CTS1973SH/26
BATCH QTY: 1500 KG
MFG.DATE: April 2026
EXP.DATE: April 2028

PHYSICO-CHEMICAL PROPERTIES

Parameters	Specification	Results
Colour And Appearance	Pale Yellow to Dark Yellow	Complies
Solubility	Soluble in Alcohol & Oils	Complies
Refractive Index at 25°C	1.480 - 1.495	Complies
Specific Gravity at 25°C	0.880 - 0.912	Complies
Optical Rotation at 25°C	-3.0° - +3.0°	Complies
CITRAL %	65 - 80%	Complies

NOTE : Keep in tightly closed container in dry and cool place, protected from light.

Add: B-wing, Poonam Heights, Pandey Layout, Khamla, Nagpur, Maharashtra-440025
Mobile: 9890086287 E-mail: citspray@gmail.com Web.: www.citspray.com , www.citspray.in



Certificate of Analysis (COA)

Product: Clove Oil

Parameter	Specification	Result
Appearance	Clear to pale yellow liquid	Complies
Odour	Characteristic spicy aroma	Complies
Specific Gravity	1.030 - 1.060	1.045
Refractive Index	1.525 - 1.540	1.532
Purity	≥ 99%	99.2%

Product: Peppermint Oil

Parameter	Specification	Result
Appearance	Clear, colorless to pale yellow	Complies
Odour	Strong minty aroma	Complies
Specific Gravity	0.900 - 0.920	0.912
Refractive Index	1.457 - 1.465	1.460
Purity	≥ 98%	98.5%

Formulation Development

Methodology of preparation of herbal vaporizer formulation for mosquitoes was done through a systematic process. The oils used in making the formulation included clove oil (6ml), lemongrass oil (8ml) and peppermint oil (4ml). These were mixed in a clean and dry beaker to make the oil phase. Propylene glycol and tween 20 were added as co solvent and emulsifier, respectively. This solution was then stirred gently at 50-100 rpm for 5-10 minutes until formation of a clear phase was realized. Ethanol was thereafter added gradually while stirring to prevent phase separation.

Optimization Using Factorial Design

The optimization experiment was conducted by 2^2 factorial design method to examine the effects of two factors, ethanol concentration (Factor A) and propylene glycol concentration (Factor B), on the response factor (evaporation rate), using three replications.[16] Twelve formulations (F1-F12) were prepared based on the factorial design scheme.

Pre-formulation Studies

The identification of essential oils was carried out by using ATR-FTIR technique. The spectra were recorded using Alpha-T FTIR Model (Shimadzu, Japan) equipped with ATR sample holder that uses one-bounce diamond crystal.[15,17] The spectra were obtained in the range of 4000 cm^{-1} to 400 cm^{-1} by accumulating 20 scans at spectral resolution of 4 cm^{-1}. A small drop of the essential oil was placed on the surface of the diamond ATR crystal.

Evaluation Parameters

Rate of Evaporation: Evaporation rate was measured by weighing the mass loss of the liquid vaporizer at definite time intervals during the normal operation using an electronic balance.[23] This process was done by taking the mass of the formulation before and after the operation for

three hours. The evaporation rate was determined using the following equation: Evaporation rate (g/hr) = (Initial mass – Final mass) / Time interval.

Mosquito Repellency Testing: Tests were done in the laboratory for repellency using cage tests for various mosquito types.[24] The trials were done inside a chamber with known volume (30 m^3) at controlled temperatures (27±2°C) and relative humidity (RH: 75±5%), and with the vaporizer being used in its standard plug-in position.

Mosquitoes and allocation of cages: Female non-blood fed mosquitoes of the Aedes aegypti species about 5–7 days old (about 20–25 per cage) were placed in three screened cages located equally distant from the device: Cage 1 (negative control: attractant only, device turned off), Cage 2 (test: herbal vaporizer), Cage 3 (positive control: commercial synthetic vaporizer). Positions of the cages were switched for each replicate to minimize effects of room positions; minimum of 3 replicates were done per formulation.

Exposure and documentation: While the apparatus was working, mosquito landings and probing of the cage mesh was documented in 5-minute intervals for 30 minutes. The knockdown (loss of flight/stance coordination) was also noted using the same time frame; mosquitoes that survived were kept alive using 10% sucrose solution for 24 hours for post-exposure mortality assessment.

Response variables: Landing rate = total number of landings / observation time. Percentage repellency = [(C-T)/C] x 100 (C and T = landing rates for control and treatment cages). Knockdown (%), Mortality (24 hours, %).

Stability Testing: The stability tests were carried out in accordance with International Conference on Harmonisation (ICH) [18,19] criteria at three different temperatures namely: Room Temperature (25°C ± 2°C/60% RH ± 5% RH), Refrigerator Temperature (2°C/60% RH ± 5%) and Elevated Temperature (40°C ± 2°C/75% RH ± 5% RH). The samples were tested at 0, 10, 20, and 30 days in respect of phase separation, evaporation rate, solubility, pH and odor properties. In stability testing, the samples were kept in tightly sealed containers and were weighed intermittently to measure the loss of weight.

RESULTS AND DISCUSSION

Optimization Study

Table 1: 2^2 Factorial Design for Optimization with 3 replicates

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Clove oil	6	6	6	6	6	6	6	6	6	6	6	6
Lemongrass oil	8	8	8	8	8	8	8	8	8	8	8	8
Peppermint oil	4	4	4	4	4	4	4	4	4	4	4	4
Tween 20	2	2	2	2	2	2	2	2	2	2	2	2
Propylene glycol	5	20	20	5	5	20	5	20	5	20	20	5
Ethanol	15	2	2	15	2	15	2	15	2	2	15	15
Total (ml)	40	42	42	40	27	55	27	55	27	42	55	40

Optimization was carried out based on the rate of evaporation for the various formulations (F1-F12) for a 3-hour time interval. There were marked variations in evaporation rates due to the presence of variations in ethanol and propylene glycol concentrations. Formulations having higher ethanol concentration had higher evaporation rates. However, formulations with higher propylene glycol concentration had lower evaporation rates owing to the humectant property of propylene glycol.

Formulation batches with the least evaporation rate were F3 (0.20 g/hr), F9 (0.23 g/hr) and F12 (0.22 g/hr). On the other hand, formulations having the maximum evaporation rate were F4 (1.42 g/hr), F1 (1.39 g/hr) and F10 (1.35 g/hr). It is clear that F4 and F1 were not very stable formulations since evaporation from these formulations was quite fast. Based on the above results, formulation F3 could be considered as the optimized batches.

ANOVA for selected factorial model

Table 2: ANOVA Table

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.09	2	1.05	348.36	< 0.0001	significant
A-ethanol	1.32	1	1.32	440.01	< 0.0001	
B-pg	0.7701	1	0.7701	256.71	< 0.0001	
Residual	0.0270	9	0.0030			
Lack of Fit	0.0192	1	0.0192	19.69	0.0022	significant
Pure Error	0.0078	8	0.0010			
Cor Total	2.12	11				

Response 1: Rate of evaporation

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**

The Model F-value of 348.36 suggests that the model is significant. The probability of obtaining an F-value as large as this from noise alone is only 0.01%.
A p-value of less than 0.0500 indicates the significance of model terms. In this situation, A and B are significant model terms. A p-value greater than 0.1000 suggests that the model terms are not significant. Should there be several insignificant model terms, excluding those necessary to satisfy hierarchy, model simplification might enhance the model.
Lack of Fit F-value of 19.69 suggests that the Lack of Fit is significant. The probability of obtaining a Lack of Fit F-value as large as this from noise alone is only 0.22%. It is bad if there is a significant lack of fit since it means that the model does not fit.

Table 3: Fit Statistic and Actual Equation

Std. Dev.	0.0548		**R^2**	0.9872
Mean	0.7617		**Adjusted R^2**	0.9844
C.V. %	7.19		**Predicted R^2**	0.9773
			Adeq Precision	42.7224

Fit Statistics
The **Predicted R^2** of 0.9773 is in reasonable agreement with the **Adjusted R^2** of 0.9844; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 42.722 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Actual Factors

This equation expressed in terms of the real factors can help us in making predictions for the responses corresponding to the specific values of the factors. The specific values of the factors must be provided in their original units. This equation should not be used for the comparison of the effects of each factor on the response since the factors have been scaled based on their units.

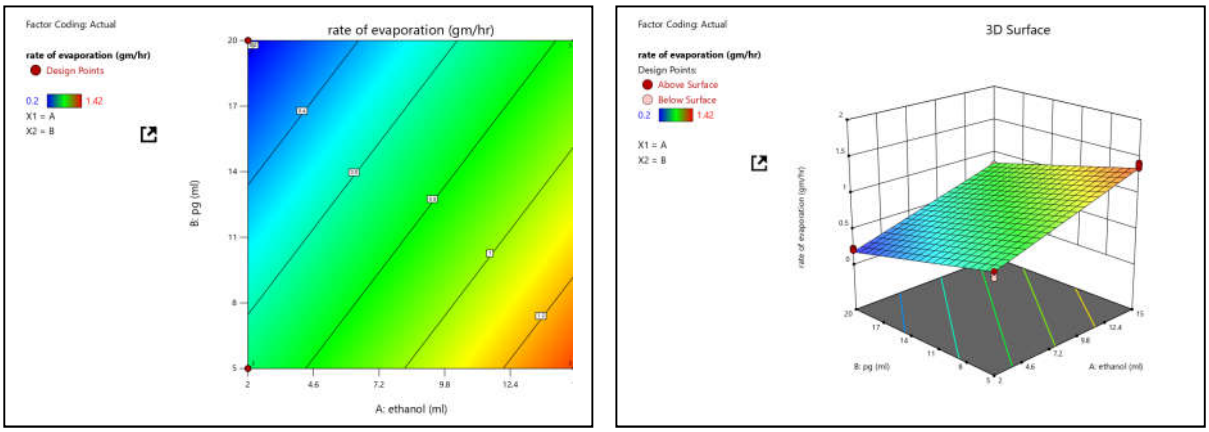


Figure 1: 3D surface plot and counter plot

Table 4: Final Optimized Batch

Sr.no	Content	Quantity
1	Lemongrass Oil	8ml
2	Clove Oil	6ml
3	Peppermint Oil	4 ml
4	Tween 20	2 ml
5	Propylene Glycol	20ml
6	Ethanol	2ml

The optimized batch formulation (F3) contained ethanol 2 mL, propylene glycol 20 ml, lemongrass oil 8 ml, clove oil 6 ml, peppermint oil 4 ml, and Tween 20 2 ml, for a total volume of 42 ml.

Pre-formulation Studies

ATR-FTIR Characterization

ATR-FTIR spectroscopy confirmed the presence of functional groups characteristic of the essential oils.

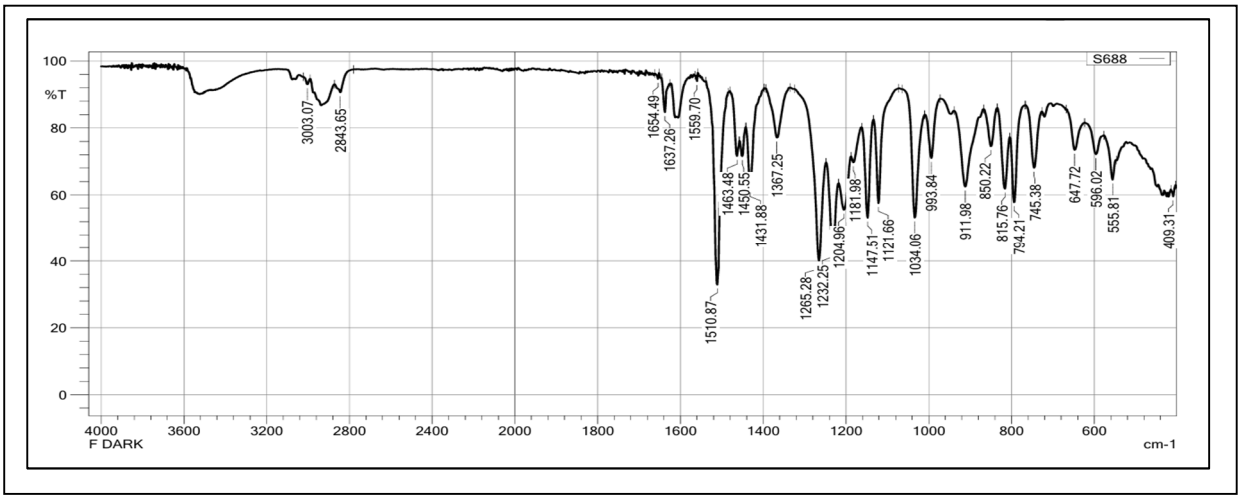
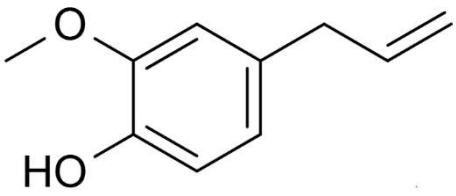


Figure 2: ATR-FTIR spectra for clove essential oil

Table 5: Stretching of spectra of clove oil

Wavenumbers (cm-1)	Assignments	Functional Group/ vibration
3003, 2843	C-H stretching	Aromatic and aliphatic C-H
1637, 1590,1510	C=C stretching	Aromatic ring vibration
1458, 1431	C-H bending	CH2 and CH3 groups
1265-1222	C-O stretching	Phenolic and ether groups
1147-1034	C-O stretching	Alcohols and ethers
910-810	C-H out of plane bending	Aromatic substitution
651-555	Out of plane bending	Aromatic substitution skeletal

The infrared spectra of clove oil revealed high absorption peaks at 3400 cm^{-1} (stretching vibration of O-H group of phenolic compounds), 1500-1600 cm^{-1} (structure of aromatic rings), and 1200-1000 cm^{-1} (stretching vibration of C-O group of ether.[10,18]



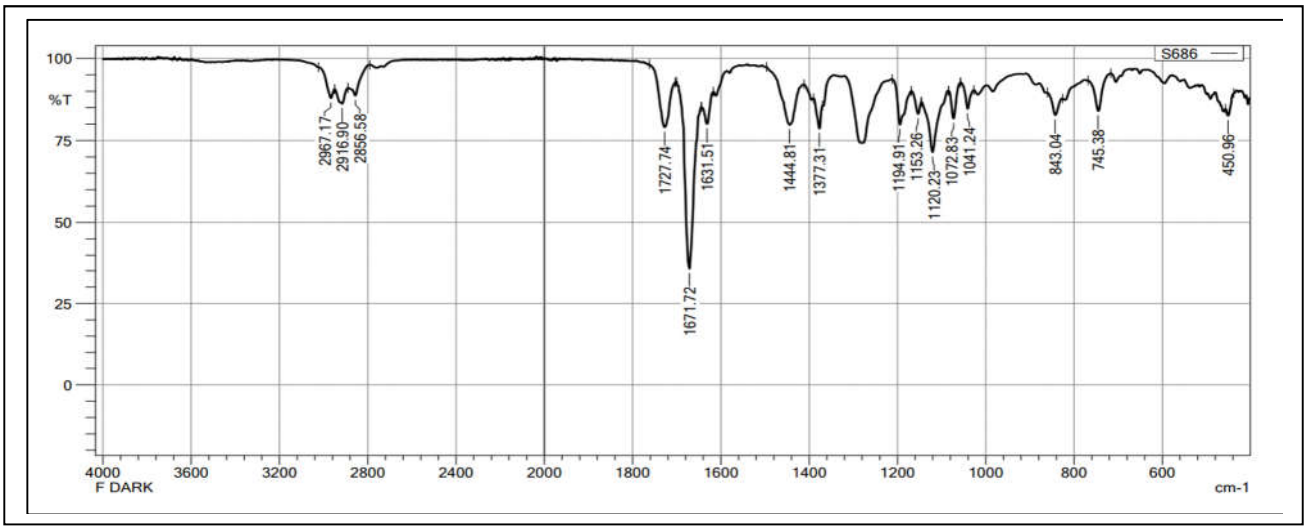
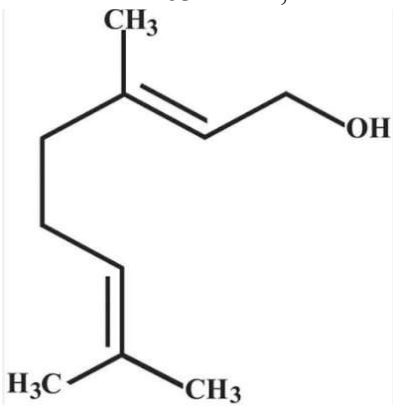


Figure 3:ATR-FTIR spectra for Lemongrass essential oil

Table 6: Stretching spectra of lemongrass oil

Wavenumber (cm-1)	**Assignment**	**Functional Group**
2967, 2916, 2856	C-H stretching	Aliphatic CH2, -CH3 groups
1727	C=O stretching	Aldehyde/ esters groups
1671	C=C stretching	Alkenes (conjugated)
1631	C=C stretching/ skeletal	Conjugated double bonds
1445, 1377	C=H bending	-CH2, -CH3 groups
843, 745	C-H out of plane bending	Alkenes
450	Fingerprint region	Skeletal vibration

There were prominent absorption bands corresponding to the carbonyl group at 1727 cm^{-1} (indicative of aldehyde and ester groups), C-H bonds in lemongrass oil at 2900 cm^{-1}, and conjugated double bonds at 1671 cm^{-1} and 1631 cm^{-1}, indicating the presence of citral.



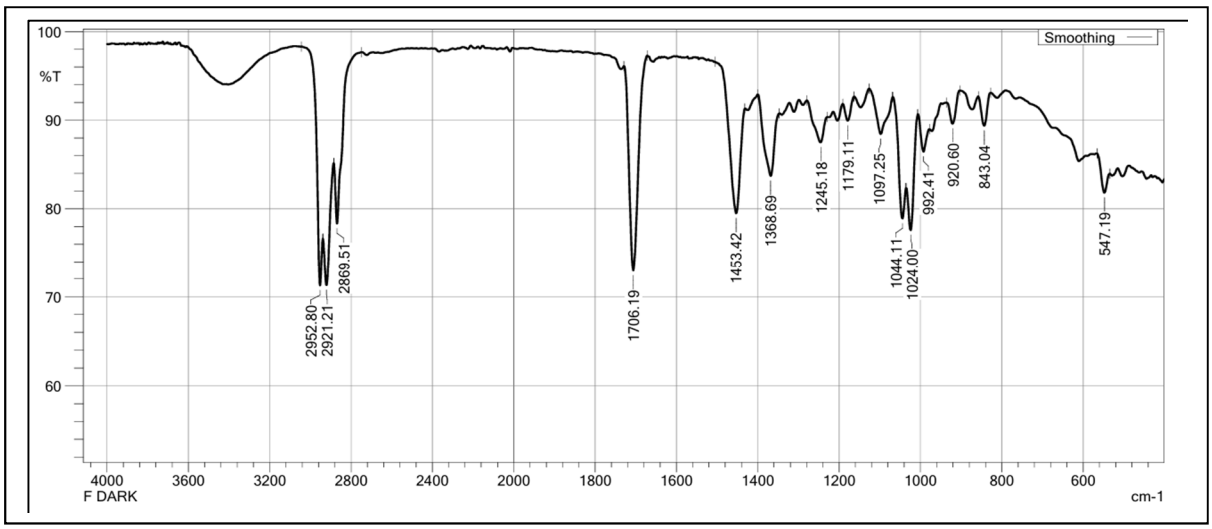
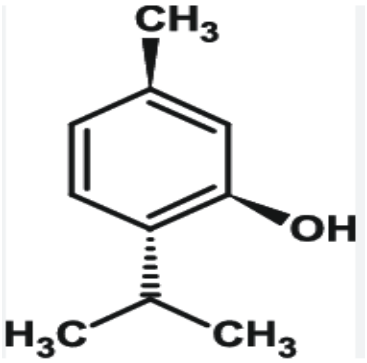


Figure 4: ATR-FTIR spectra for peppermint essential oil

Table 7: Stretching spectra of peppermint oil

Wavenumber (cm-1)	**Assignment**	**Functional Groups/ vibrations**
2952.80, 2921.21	C-H stretching (CH3, CH2 groups)	Menthol, Menthone, Limone
1706.19	C=O stretching (carbonyl group)	Menthone, Menthyl acetate
1453.42, 1368.69	C-H bending (CH2, CH3)	Hydrocarbon (terpenes,menthol)
1245.18, 1179.11, 1097.25, 1044.11	C-O stretching (alcohols, esters, ethers)	Menthol(alcohol)Menthyl acetate (ester)
992.41, 920.60	=C-H bending (alkenes)	Limonene, other monoterpenes
843.04	=C-H bending (alkenes)	Monoterpenes

In the spectra of peppermint oil, the presence of carbonyl group was seen with absorption peaks of 1706 cm^{-1} (menthone), C-H stretching vibration at 2900 cm^{-1}, and absorption peaks of C-O stretching vibrations were observed at 1245, 1179,



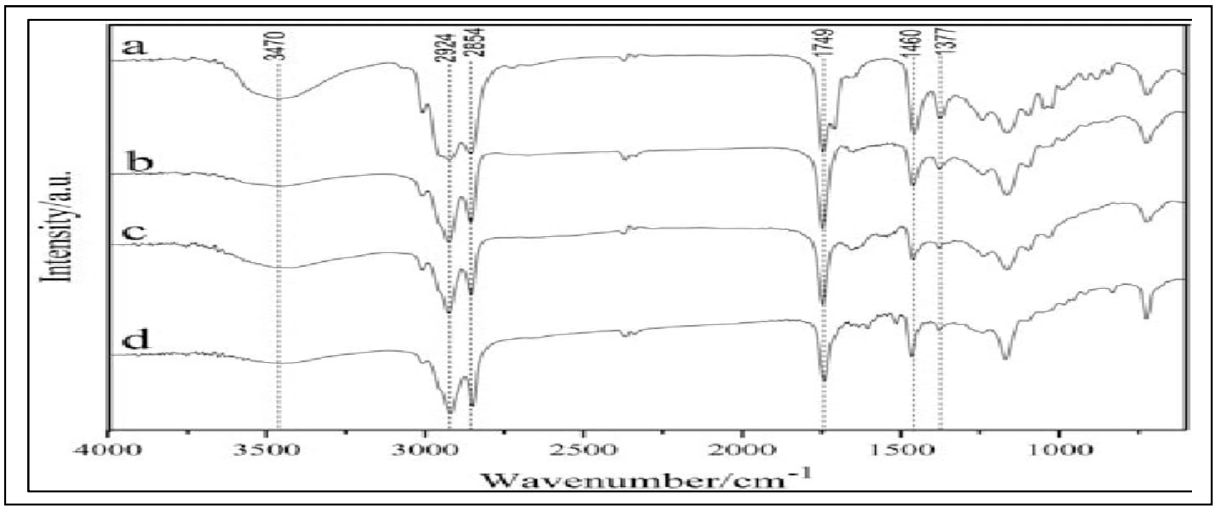


Figure 5: ATR-FTIR Spectra of mixture of Essential oil

Table 8: Stretching spectra of mixture of essential oil

Wavenumber (cm-1)	Assignments	Functional Group/vibration
~3400 - 3350	Broad O-H stretching	Phenolic / alcoholic group
~2920 - 2850	C-H stretching	Aliphatic chain from terpens
~1740 - 1710	C=O stretching	Aldehyde / ester groups
~1630 - 1600	C=C stretching	Aromatic ring and alkenes
~1260 - 1050	C-O stretching	Alcohols and ethers
~880 - 750	C-H bending	Aromatic compoud

In the FTIR spectroscopy test conducted on the mixed essential oil sample, all the expected absorption peaks belonging to each individual oil were visible, and no new peaks appeared or any previous peaks disappeared, signifying that there was no reaction between the components.

Evaporation Rate Evaluation

Table 9: Evaporation rate of Factorial batches

Batches	Weight after 3hour time interval		Weight loss after 3 hour	Weight loss after 1 hour
	Initial	**Final**		
F1	58.53gm	54.36gm	4.17gm	1.39gm
F2	58.53gm	56.61gm	1.92gm	0.64gm
F3	58.53gm	57.93gm	0.60gm	0.20gm
F4	58.53gm	54.27gm	4.26gm	1.42gm
F5	58.53gm	56.07gm	2.46gm	0.82gm

F6	58.53gn	56.55gm	1.98gm	0.66gm
F7	58.53gm	56.19gm	2.34gm	0.78gm
F8	58.53gm	56.46gm	2.07gm	0.69gm
F9	58.53gm	57.84gm	0.69gm	0.23gm
F10	58.53gm	54.48gm	4.05gm	1.35gm
F11	58.53gm	56.13gm	2.40gm	0.80gm
F12	58.53gm	57.87gm	0.66gm	0.22gm

Table 10: Evaporation rate of Synthetic and marketed herbal

Components	**Weight of 3hour time interval**		**Weight loss after 3 hour**	**Weight loss after 1 hour**
	initial	**final**		
Marketed Synthetic Mosquito Repellent	58.53gm	58.08gm	0.45gm	0.15gm
Marketed Herbal Mosquito Repellent	58.53gm	58.13gm	0.40gm	0.13gm

Optimized formulation (F3) showed a mean evaporation rate of 0.20 g/hr, while the commercial herbal and synthetic repellents had evaporation rates of 0.13 g/hr and 0.15 g/hr, respectively. Even though the developed formulation showed a slightly higher evaporation rate than the commercial products, it was still one of the lowest evaporation rates among all the developed formulations, which means the active ingredients are released consistently and controlled. A controlled evaporation rate is important because it ensures controlled release of the active ingredient, hence making the vaporization effective in repelling mosquitoes.

Table 11: Mosquito Landing Counts and Landing Rates at Different Time Intervals Following Application of the Marketed Mosquito Repellent

Total No. of Mosquitoes [N]	**Time(T)**	**Landing (L) Marketed Repellent**	**Landing Rate=** $\frac{L}{T}$
20	5	0	0±0
20	10	2	0.2±0.01
20	15	5	0.33±0.006
20	20	9	0.45±0.01
20	25	10	0.4±0.01
20	30	12	0.4±0.01

Mosquito Repellency Assessment

It was evident from the cage testing method that all three formulations had some degree of mosquito repellent properties; however, their efficiencies depended on time. It was apparent that the optimized herbal mosquito repellent had superior mosquito repellency performance since there were no mosquitoes landing on the formulation within the first 10 minutes.

Table 12: Cage test method for herbal repellent

Total No. of Mosquitoes [N]	**Time(T)**	**Landing (L) Herbal Repellent**	**Landing Rate=$\frac{L}{T}$**
20	5	0	0±0
20	10	0	0±0
20	15	1	0.0066±5.773
20	20	2	0.1±0.005
20	25	5	0.2±0.01
20	30	8	0.266±0.001

Table 13: Cage test method for marketed herbal repellent

Total No. of Mosquitoes [N]	**Time(T)**	**Landing (L) Herbal Marketed Repellent**	**Landing Rate=$\frac{L}{T}$**
20	5	0	0±0
20	10	1	0.10±0.005
20	15	3	0.20±0.005
20	20	6	0.30±0.005
20	25	8	0.32±0.01
20	30	10	0.33±.0.01

At 5 minutes, there were no landings on the herbal repellent. After 30 minutes, the landing rate for the herbal repellent was 0.266, while that of the synthetic repellent was 0.40 and that of the herbal repellent was 0.33.

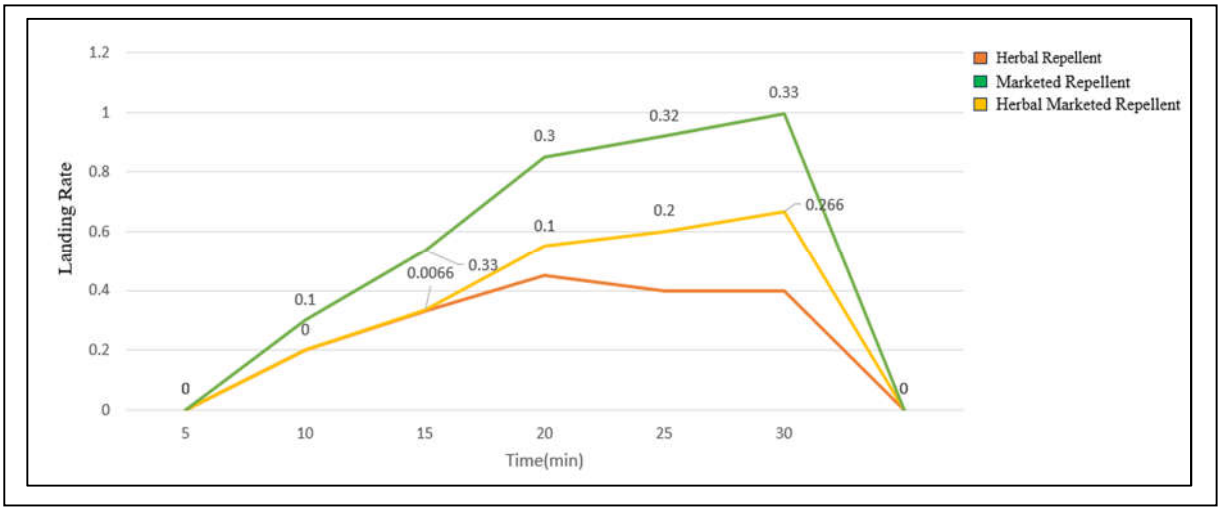


Figure 6: Landing rate vs time

The high repellent property of the formulation is due to the synergistic action of the three essential oils along with their optimum concentration in the formulation and the controlled release system obtained by solvent optimization.

Stability Studies

The stability of the formulation was examined under three thermal conditions and found to be highly physically and chemically stable for the duration of 30 days. There was no phase separation or change in color, odor, and solubility in any of the test conditions.

Table 11: The stability study at room temperature (25°C)

TIME	PHASE SEPERATION	RATE OF EVAPORATION	SOLUBILTY	COLOUR/ ODOUR
INITIAL (0)	Single phase	0.00	Soluble	Pale yellow/strong medicinal aroma
10 DAYS	No change	0.003	No change	No change
20 DAYS	No change	0.001	No change	No change
30 DAYS	No change	0.000	No change	No change

Table 12: The stability study at high temperature (40°C)

TIME	PHASE SEPARATION	RATE OF EVAPORATION	SOLUBILITY	COLOUR/ ODOUR
INITIAL (0)	Single phase	0.00	Soluble	Pale yellow/strong medicinal aroma
10^{TH} DAY	No change	0.002	No change	No change
20^{TH} DAY	No change	0.000	No change	No change
30^{TH} DAY	No change	0.001	No change	No change

Table 13: The stability study at low temperature (2°C)

TIME	PHASE SEPARATION	RATE OF EVAPORATION	SOLUBILITY	COLOUR/ ODOUR
INITIAL (0)	Single phase	0.00	Soluble	Pale yellow/strong medicinal aroma
10^{TH} DAY	No change	0.003	No change	No change
20^{TH} DAY	No change	0.001	No change	No change
30^{TH} DAY	No change	0.003	No change	No change

In ambient temperature (25°C), the formulation retained very good stability with little evaporation (0.000-0.003 g/hr). In refrigeration temperature (2°C), the formulation retained its stability with evaporation rate of 0.000-0.002 g/hr. In high temperature (40°C), slightly more evaporation took place (0.001-0.003)

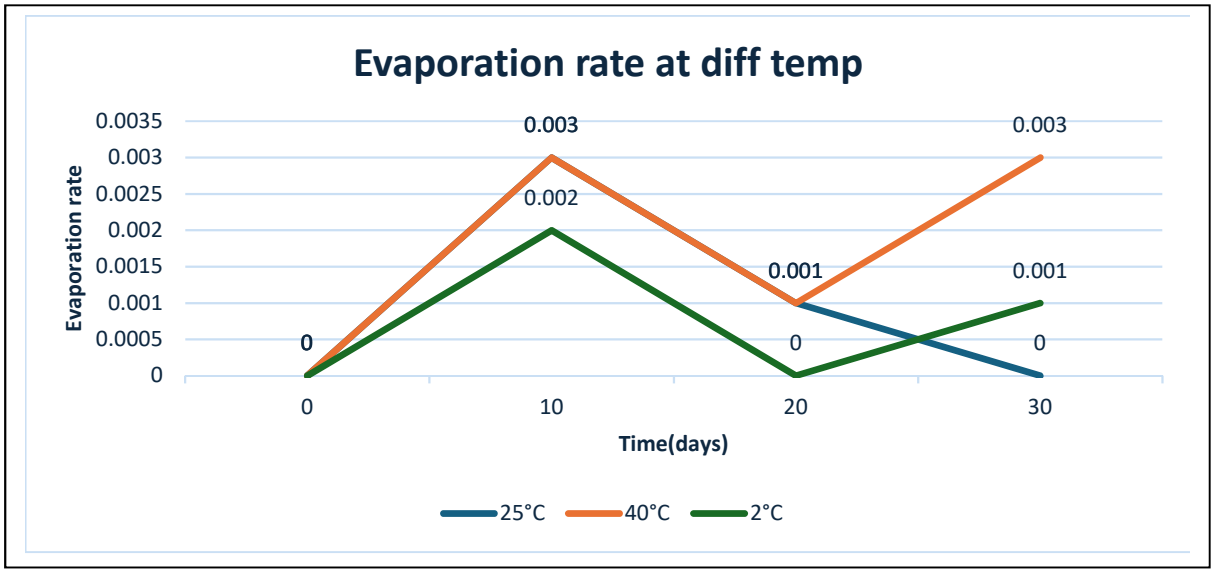


Figure 7: Evaporation rate at different temp

The results indicate that the formulation maintains excellent stability across standard storage conditions, making it suitable for commercial distribution and consumer use.

CONCLUSION

This present study successfully formulated and optimized herbal mosquito repellent vaporizer formulation with the incorporation of clove, lemongrass, and peppermint essential oils. Based on 2^2 factorial designs with three replicates (12 formulations; F1 to F12), F3 formulation was found to be the optimal formulation which showed evaporation rate of 0.20 g/h and suitable formulation stability.

ATR-FTIR analysis showed the presence of functional groups characteristic of the essential oils which include O-H stretching vibrations (~3400 cm^{-1}), C-H stretching vibrations (2920-2850 cm^{-1}), and C=O stretching vibrations (~1740 cm^{-1}) and thus it can be seen that the major bioactive components have not undergone any chemical alteration.

It is evident that the formulated spray exhibited evaporation properties similar to those of other commercially available mosquito repellants and high repellency efficiency without any mosquito landing within 10 minutes of exposure and for the entire 30-minute evaluation period. The stability test results revealed that the formulation retained physical and chemical stability irrespective of whether it was stored at room temperature, refrigeration or accelerated conditions without any changes in physical properties such as appearance, odour, phase separation, and solubility.

On the whole, it can be said that the herbal mosquito repellent vaporizer is an eco-friendly and efficient alternative to the existing mosquito repellents with great potential of being applied for commercial purposes.

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