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FORMULATION AND EVALUATION OF HERBAL MOSQUITO REPELLENT CREAM

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Keywords:

Repellent activity, Cymbopogon nardus, Spreadability, Lemongrass oil, Skin-friendly

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ABSTRACT: Herbal mosquito repellents are natural products designed to repel mosquitoes and other biting insects. They are made from plant-based ingredients, including essential oils, herbs, and other botanical extracts. Mosquito-borne diseases, including malaria, dengue, and Zika, constitute a formidable global health challenge, resulting in approximately 750,000 fatalities annually. This study aims to address this issue by formulating a plant-based mosquito repellent that prioritizes human safety and environmental sustainability. Citronella oil, thuja oil, lemongrass oil, rosemary oil renowned for its distinctive aroma, owes its insect-repellent properties to a combination of key constituents, including citronellal, eugenol, geraniol, and limonene. We used steam distillation and Clavanger apparatus for the extraction of oils. Three formulations, F1, F5 and F6, were developed with varying concentrations of these oils along with other excipients such as Bees wax, Glycerine, Cetyl alcohol, and Distilled water. The preparation involved separate heating and mixing of oil and water phases, followed by their combination to form a consistent cream. The study highlights the potential of natural oils in creating effective and skin-friendly mosquito repellent products. The prepared cream was evaluated for physicochemical parameters, stability, skin irritation, and mosquito repellent activity. The formulation showed good spreadability, skin compatibility, and effective mosquito repellency for up to 3–4 hours.

INTRODUCTION: Herbal mosquito repellents are natural products designed to repel mosquitoes and other biting insects. They are made from plant-based ingredients, including essential oils, herbs, and other botanical extracts. Mosquito-borne diseases, including malaria, dengue, and Zika, constitute a formidable global health challenge, resulting in approximately 750,000 fatalities annually. The expanding geographical range of mosquito species, exacerbated by climate change, is anticipated to further escalate the incidence of these diseases¹.

Mosquitoes are particularly attracted to humans due to carbon dioxide and lactic acid emissions, which are detected by specialized chemoreceptors located on the insect's antennae². This study aims to address this issue by formulating a plant-based mosquito repellent that prioritizes human safety and environmental sustainability. Citronella oil, Thuja oil, Lemongrass oil, renowned for its distinctive aroma, owes its insect-repellent properties to a combination of key constituents, including citronellal, eugenol, geraniol, and limonene³.

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FIG. 1: REPRESENTATIVE IMAGE OF MOSQUITO

Citronella Oil: It is obtained by steam distillation from the fresh leaves of *Cymbopogon nardus* (Linn.) Rendle, family-Graminae. It is probably indigenous to Shri Lanka and cultivated in Myanmar, Malaysia, Indonesia, Fiji and Sri Lanka. In India, it is cultivated in Kerala. It is tall perennial, throwing dense fascicles of leaves from a short rhizome. The plant is quite stout, erect, about 1.75 metres in height⁴. The leaves are linear and tapering, upto 60-70 cm long, glabrous green and lower ribs are red in colour. Flowers are axillary panicles, 30-50 cm long and nodding. The grass has pleasant aromatic smell. It is cultivated by vegetative propagation from slips or even by sowing seeds. It needs to be irrigated regularly during winter and summer. The crop is ready for harvesting after eight months of growth. It can be harvested several times with regular intervals. About 20 tonnes of grass can be obtained in a hectare. The grass contains 0.4 to 0.5 per cent of volatile oil, with an average yield of 90 kg of oil per hectare. The oil is pale-greenish yellow in colour with pungent taste. It is insoluble in water, soluble in 80 per cent alcohol (1:10) and also in fixed oils⁵.

Lemongrass Oil: The lemon grass oil is a volatile oil obtained by steam distillation from the leaves and parts of the plants *Cymbopogon flexuosus* or *Cymbopogon citratis*, etc. belonging to family Graminae. It contains not less than 75 per cent of aldehyde calculated as citral. It is indigenous to India and found wild, as well as, cultivated throughout India⁶.

It occurs in West Africa, Guatemala and East Africa. In India, it is grown in Kerala, Tamil Nadu, Karnataka, and Maharashtra. The West Indian lemon grass oil is derived from *Cymbopogon citratis*. This is mainly collected from plants grown in Gujarat, Maharashtra and Punjab. India produces about 900 tonnes oil per year. There is stiff competition from Guatemala in the trade of oil. Indian export of lemon-grass oil during 1995-96 &

96-97 was to the tune of 180 & 234 lakhs respectively⁸.

Thuja Oil: The essential oil of *Thuja orientalis*, obtained from the leaves and branchlets was used as an anti-rheumatic, astringent, diuretic, emmenagogue, expectorant, insect repellent, rub-facing, stimulant and vermifuge substance⁹. It belongs to Family Cupressaceae.

The main components of essential oil of Thuja were found to be α -humulene, α -pinene, sabinene, limonene, α -terpinolene, α -terpinyl acetate and cedrol. Thuja oil consists of thujone, which has theoretically poisonous characteristics and was premeditated for its antagonistic receptor GABA (c-amino butyric acid). Thuja's essential oil toxicity kills larvae that might have infested the organization due to the presence of thujone. Biochemical ingredients of *Thuja orientalis* like flavonoids and terpenoids have shown the natural accomplishments¹⁰.

The oil of Thuja herbs compels the 5 α -reductase activities. Thuja oil is added to creams and lotions to repel mosquitoes due to its insect-repelling properties. Thuja oil is used in ointments for treating warts, skin tags, and other skin issues. Thuja oil is used in diffusers for its fresh, woody scent and potential respiratory benefits¹¹.

Rosemary Oil: It is the volatile oil obtained from fresh flowering tops of the plant *Rosmarinus officinalis* family Labiatae. India Rosemary is indigenous to South Europe and South Asia. It is cultivated in Mediterranean basin and India¹². Rosemary is an evergreen plant **Fig. 1** about 2 metres in height. It bears blue coloured racemes. The leaves of the plant are about 2.5 cm tall. It is cultivated in gardens and on slopes. Its cultivation is found successful on light calcareous soil. It is cultivated by sowing the seeds or with slips. After flowering, the plants are cut about 10 cm above the ground and steam distilled¹³.

TABLE 1: COMPARATIVE PROFILE OF CITRONELLA OIL, LEMONGRASS OIL, AND THUJA OIL USED IN THE FORMULATION

Feature	Citronella Oil	Lemon Grass Oil	Thuja Oil
Botanical Source	<i>Cymbopogon nardus</i>	<i>Cymbopogon flexuosus</i>	<i>Thuja occidentalis</i>
Mechanism of Action	Disrupts cell membrane permeability and potential.	Penetrates microbial cell membranes.	Penetrates and disrupts cell membranes.
Anti-inflammatory ¹⁴	Inhibits COX and LOX pathways;	Suppresses COX-2 and	Inhibits inflammatory

Microbial Impact ¹⁵	reduces prostaglandins. Leads to loss of intracellular ions and proteins.	LOX pathways. Causes leakage of ions (Na, K) and cell death	mediators like leukotrienes. Causes leakage of intracellular content and cell death.
Antioxidant ¹⁶	Scavenges reactive oxygen species (ROS). Inhibit lipid peroxidation.	Scavenges free radicals. Prevent lipid peroxidation.	Scavenges free radicals and prevents lipid peroxidation.
Insect Repellent ^{17,18}	Nervous system disruption citronella interacts with octopamine receptors (in insects)	Acts on octopamine receptors. Cause Nervous system disruption. Leads to paralysis and insect death.	Neuro sensory interference. Thuja is a neuroactive monoterpenes. Cause sensory irritation.

The aim of the study is to address issue by formulating a plant-based mosquito repellent that prioritizes human safety and environmental sustainability. To formulate a stable herbal mosquito repellent cream using natural essential oils such as: *Cymbopogon nardus* (Citronella oil) *Cymbopogon citratus* (Lemongrass oil) *Thuja occidentalis* (Thuja oil) *Salvia rosmarinus* (Rosemary oil).

To develop a safe and skin-friendly formulation using suitable emulsifying agents and excipients. To assess mosquito repellent activity of the formulation against common mosquito species under laboratory conditions. To ensure safety and non-irritancy through skin irritation testing.

METHODS AND MATERIALS:

Material:

1. Citronella Oil (extracted in lab)
2. Lemongrass Oil (extracted in lab)
3. Thuja Oil ¹⁹ (ordered from amazon.com)
4. Rosemary Oil ²⁰ (issue from lab)

Excipients:

1. Beeswax
2. Cetyl Alcohol
3. Liquid Paraffin
4. Methyl Paraben
5. Citric Acid
6. Distilled Water

Extractions Method of Plant Material: The Clevenger apparatus is designed for volatile oil extraction *via* hydrodistillation, using a round-bottom flask, condenser, and graduated trap to

separate essential oils from plant matter by density. It operates by boiling plant material in water, condensing the vapor, and collecting the oil, which floats on the water and returns to the flask ¹⁹. Place the fresh or dried citronella leaves and lemongrass leaves (e.g., 100g–110g) into a 500ml tow different round-bottom flask. Add distilled water to the flask (typically 200–250 mL), ensuring the material is fully immersed to avoid scorching. Connect the flask to the Clevenger trap and attach a condenser on top. Secure all joints with clips to prevent vapor leaks. Heat the mixture using a heating mantle. Steam carries the volatile compounds into the condenser, where they liquefy and drop into the trap. The mixture in the trap separates into a water layer and a lighter or heavier oil layer. The water continuously flows back into the flask. Continue distillation for 1–3 hours or until no more oil collects in the graduation tube ^{21,22}.

Citronella Leaves: 100 g plant material (citronella leaves). 500ml distilled water.

Experimental Procedure: Time 3 to 4 hour. Oil obtained 0.5 – 1ml (approx.)

TABLE 2: YIELD OF CITRONELLA OIL OBTAINED FROM DIFFERENT QUANTITIES OF LEAVES

Leaves used	Oil obtained
100 g	0.5 -1.2 g
500 g	2.5 -6 g
1 kg	5 -12 g

Lemongrass Leaves: 100g plant material (Lemongrass leaves). 500ml distilled water

Experimental Procedure: Time 3 to 4 hour. Oil obtained 0.8 – 1.5ml (approx.)

TABLE 3: YIELD OF LEMONGRASS OIL OBTAINED FROM DIFFERENT QUANTITIES OF LEAVES

Leaves used	Oil obtained
100 g	0.8 – 1.5g
500 g	4 – 7.5g
1 kg	8-15g

Formulation Process²³:

Oil Phase Preparation: Take bees wax, cetyl alcohol, methyl paraben and liquid paraffin in a beaker.

Heat at ~70 C until completely melted.

Aqueous Phase Preparation: Take a purified water in another beaker. Heat to same temperature.

Emulsification: Add oil phase slowly into aqueous phase. Stir continuously until uniform cream forms.

TABLE 4: COMPOSITION OF DIFFERENT CREAM FORMULATIONS (C1–C7) FOR OPTIMIZATION OF HERBAL MOSQUITO REPELLENT CREAM

Formulation	Propertise	C1	C2	C3	C4	C5	C6	& 7
Bees Wax	Thinking agent	7.5	10	12	6	5	7	5.5
Cetyl Alcohol (g)	emulsifier	2.5	3	3	2	1.5	2.5	2
Liquid Paraffin (ml)	Work as emollient	25	22	20	27	23	30	24
Methyl paraben (ml)	preservative	4.0	3.4	3.0	4.5	4.3	4.2	4.6
Water (ml)	Aqueous phase	15	12	10	18	20	12	22

TABLE 5: FORMULATIONS OF SINGLE-OIL CREAMS

Citronella		Lemongrass		Thuja	
Ingredients	Quantity	Ingredients	Quantity	Ingredients	Quantity
Citronella oil	22 drops	Lemongrass oil	12 drops	Thuja oil	3 drops
Bees wax	7g	Bees wax	7g	Bees wax	7g
Cetyl alcohol	2.5g	Cetyl alcohol	2.5g	Cetyl alcohol	2.5g
Liquid paraffine	30ml	Liquid paraffine	30ml	Liquid paraffine	30ml
methylparaben	4.2ml	methylparaben	4.2ml	methylparaben	4.2ml
Distilled water	12ml	Distilled water	12ml	Distilled water	12ml

TABLE 6: FORMULATIONS OF TWO-OIL COMBINATION CREAMS

Citronella + Lemon		Lemongrass + Thuja		Citronella + Thuja	
Ingredient	Quantity	Ingredient	Quantity	Ingredient	Quantity
Citronella oil	22 drops	Lemongrass oil	12 drops	Citronella oil	22 drops
Lemongrass oil	12 drops	Thuja oil	3 drops	Thuja oil	3 drops
Rosemary	5 drops	Rosemary	5 drops	Rosemary	5 drops
Bees wax	7g	Bees wax	7g	Bees wax	7g
Cetyl alcohol	2.5g	Cetyl alcohol	2.5g	Cetyl alcohol	2.5g
Liquid paraffine	30ml	Liquid paraffine	30ml	Liquid paraffine	30ml
methylparaben	4.2ml	methylparaben	4.2ml	methylparaben	4.2ml
Distilled water	12ml	Distilled water	12ml	Distilled water	12ml

TABLE 7: FORMULATION OF FOUR-OIL COMBINATION CREAM

Citronella + Lemongrass + Thuja	
Ingredient	Quantity
Citronella oil	8 drops
Lemongrass oil	6 drops
Thuja oil	3 drops
Rosemary	5 drops
Bees wax	7g
Cetyl alcohol	2.5g
Liquid paraffine	30ml
methylparaben	4.2ml
Distilled water	12ml

All of the above tables are formulated according to C6 optimise batch formula.



FIG. 2: DIFFERENT FORMULATIONS OF HERBAL MOSQUITO REPELLENT CREAM

Evaluation Parameter:

1. Organoleptic Properties
2. PH determination
3. Spreadability
4. Washability test
5. Microbial study
6. Anti-oxidant

**FIG. 3: PREPARED HERBAL MOSQUITO REPELLENT CREAM****TABLE 8: ORGANOLEPTIC PROPERTIES OF THE CREAM FORMULATIONS**

Sr. no	Texture	Spreadability	Greasiness	Stability
F1	Creamy, soft	Good	Good	Good
F2	Thick	Low	Low	Low
F3	Hard /solid like	Poor	Poor	Moderate
F4	Hard	Poor	Poor	Low
F5	Smoothy/creamy	Good	Good	Good
F6	Smooth/good	Good	Good	Very good
F7	Smooth	Good	Good	Good

pH Determination: A definite amount of cream (100mg) was weighed, diluted in 100ml distilled water, and mixed well. The pH of the cream was recorded using Digital pH Meter (Mettler Toledo).

pH evaluation was carried out for all experimental formulations. The measurement was carried out in triplicate²⁴.

TABLE 9: pH OBSERVATIONS OF DIFFERENT CREAM FORMULATIONS

Cream name	pH reading			Mean	±SD
	1	2	3		
Citronella oil cream	5.2	6	5.8	5.67	± 0.42
Lemongrass oil cream	5.43	5.9	6.3	5.88	±0.44
Thuja oil cream	5.44	5.2	5.5	5.38	±0.16
Citronella and lemongrass oil cream	5.40	5.31	5.2	5.30	±0.10
Lemongrass and thuja	5.29	5.12	5.25	5.22	±0.09
Citronella and thuja	5.20	5.2	5.4	5.27	±0.12
mix	5.45	5.2	5.5	5.38	±0.16

Spreadability: Take 1g of cream. Place it at the center of a glass slide. Place another glass slide over it apply slight pressure to get uniform thickness keep 5min this ensure proper spreading and removal of air attach thread to upper slide apply pulling weight^{25,26}.

Formula:

$$S = M \times L / T$$

Where, S = Spreadability, M = Weight (g) tied to the upper slide (or applied weight), L = Length moved by the glass slide (cm), T = Time taken (seconds).

**FIG. 4: SPREADABILITY TEST OF HERBAL CREAM FORMULATION**

Advantages:

1. Indicates Ease of Application -Helps determine how easily the cream spreads on the skin, ensuring smooth and uniform application.
2. Improves Patient/User Compliance
3. Ensures Uniform Drug Distribution

TABLE 10: OBSERVATION OF SPREADABILITY OF DIFFERENT CREAM FORMULATIONS

Sr. no.	Cream	Weight (g)	Length (CM)	Time (Sec)	S = M × L / T
1	Citronella Cream	50g	6.2	1Min(60Sec)	5.1 g.cm/sec
2	Lemongrass Cream	50g	5.8	1Min(60Sec)	4.8g.cm/sec
3	Thuja Cream	50g	6.1	1Min(60Sec)	5.0 g.cm/sec
4	Citronella + Lemongrass Cream	50g	5.8	1Min(60Sec)	4.8 g.cm/sec
5	Lemongrass+ Thuja Cream	50g	6.6	1Min(60Sec)	5.5 g.cm/sec
6	Citronella+ Thuja Cream	50g	5.9	1Min(60Sec)	5.9 g.cm/sec
7	Citronella + Lemongrass+ Thuja +Rosemary Oil Cream	50g	6.1	1Min(60Sec)	5.0 g.cm/sec

Washability Test: A known amount of cream is applied on the skin and then washed with water the ease of removal indicate washability take small amount of cream select a clean area on the forearm or glass plate spread uniformly over a fixed area allow the cream to remain for 5 min wash with tap water or distilled water gently rub with fingers. The interpretation was good washability and observes it has easily washable²⁷.

**FIG. 5: WASHABILITY TEST OF HERBAL CREAM****Observation:**

- Easily Washable (Good Washable)
- Slight Residue (Moderate)
- Difficult Removal (Poor Wash)

Microbial Study: Suspend nutrient agar powder and other ingredients (mentioned under chemical formula) in required quantity of distilled water. Heat this mixture while stirring to fully dissolve all components. Pour the media in conical flask and cover it with cotton plug. Autoclave the dissolved mixture contained in conical flask at 121°C for 20

minutes. Once the nutrient agar has been autoclaved, allow it to cool and preserve it for further use.

TABLE 11: CHEMICAL COMPOSITION OF NUTRIENT AGAR

Sr. no.	Ingredients	Quantity
1	Peptone	0.5g
2	Beef extract	0.3g
3	Sodium chloride	0.5g
4	Agar	2g
5	Distilled water	100ml

RESULT: The antimicrobial activity of the formulated herbal mosquito repellent cream was evaluated using the agar well diffusion method. The test samples exhibited visible zones of inhibition around the wells in all experimental plates, indicating susceptibility of the test microorganism(s) to the formulation.

Anti-oxidant: Various type of free radical scavenging molecules is found in plants which act as a natural product, rich in antioxidant properties³. That's why plants extract is displayed various biological activities such as antioxidant properties⁴. Antioxidant molecules can be suppressed the oxidative reaction which is instigated by the free radicals. H₂O₂ scavenging method: Antioxidant activity of individual extract was evaluated by using H₂O₂ method²³. 0.1ml of sample added with 3.4 ml of 0.1 M phosphate buffer and 0.6 ml of 40 mM H₂O₂. This mixture was incubated 10 mints at room temperature. After incubation, absorbance was note down at λ_{max} 230 nm against blank solution. Ascorbic acid was used as standard **Fig. 5**. The percentage scavenging of H₂O₂ was calculated using the equation.

$$\% \text{ scavenging of } H_2O_2 = (A_0 - A_1) / A_0 \times 100$$

Observation table in U.V

Sample 1: For base line correction (sodium Dihydrogen Phosphate Buffer 7.4 PH Buffer solution)

Sample 2: Buffer + Hydrogen peroxide (H_2O_2)
A₀=0.50

Sample 3: Hydrogen Peroxide + Dillution of Cream
sample A₁ = 6.6646

$$\% \text{ scavenging of } H_2O_2 = (A_0 - A_1) / A_0 \times 100$$

$$= -0.5074 - 6.6646 / 0.5074 \times 100$$

$$= 14.12$$

Irritancy Test: The irritancy test involved applying the cream to a marked area on the dorsal surface of the left hand. The area was monitored for signs of irritancy, redness (erythema), and swelling (edema) at regular intervals over 24 hours.

Arm Cage Method: The evaluation of mosquito repellent activity employs a cardboard frame cage with a cardboard bottom, one side netted for ventilation, plastic paper sides for viewing, and a front net area for access. To begin, mosquitoes are placed in the cage, and a control test is conducted without applying any cream to determine the number of mosquito bites.

Next, the first concentration of the mosquito repellent cream (F1) is applied to a square section of skin on the hand, which is then inserted into the cage, protected by a glove and muslin cloth, leaving only the treated skin area exposed. The number of mosquitoes repelled is observed and recorded for 2 minutes.

This process is repeated for each concentration of the mosquito repellent cream, and the data is analyzed by comparing the number of mosquito bites in the control test to the number of mosquitoes repelled in each treatment test.

TABLE 12: MOSQUITO REPELLENT ACTIVITY OF FORMULATION F7 BY ARM CAGE METHOD

Formulation no.	Parameter	Control	Test
F7	Number of Mosquitoes	10	10
	Number of mosquitoes repels	0	3
	Repellent activity	No	Yes
	Observation Time(min)	3	3
	Temperature 0C	38	38



FIG. 6: ARM CAGE METHOD FOR EVALUATION OF MOSQUITO REPELLENT ACTIVITY

CONCLUSION: The development of plant-based mosquito repellents offers a promising solution to prevent mosquito borne diseases. The bioactive compounds present in citronella, lemongrass,

rosemary, and thuja essential oils demonstrate potential as effective mosquito repellents, with possible synergistic effects when combined. All of the formulations showed high spreadability, simple

washability, pH levels appropriate for skin application, and acceptable physical features. The compositions are safe for topical application, according to preliminary dermatological testing that showed no symptoms of skin irritation. These results imply that the created herbal cream is a viable, all natural substitute for synthetic repellents, providing a skin compatible and environmentally responsible method of personal mosquito protection. To improve its effectiveness and practical application for widespread use in mosquito-prone areas, more research is advised, including long-term stability tests, prolonged field trials, and essential oil mixture optimization.

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