

FORMULATION AND EVALUATION OF HERBAL MOSQUITO REPELLENT

By
LIZA CHOUDHARY

Under the Guidance
Of
Dr. NEERAJ KUMAR VERMA
(ASSOCIATE PROFESSOR)

Abstract

Mosquito-borne diseases such as dengue, malaria, and chikungunya continue to be major public health concerns, especially in tropical and subtropical regions. Synthetic mosquito repellents containing chemicals like DEET are effective but may cause skin irritation and other adverse effects upon prolonged use. Therefore, the present study was undertaken to develop a safe, effective, and eco-friendly herbal mosquito repellent using natural plant-based ingredients. In this study, various herbal ingredients such as neem oil (*Azadirachta indica*), citronella oil (*Cymbopogon citratus*), eucalyptus oil (*Eucalyptus globulus*), and turmeric extract (*Curcuma longa*) were selected based on their well-known insect-repellent, antimicrobial, and skin-friendly properties. The formulation was developed in the form of a cream/lotion/spray using suitable excipients to ensure stability, spreadability, and user acceptability. The prepared formulation was evaluated for its physicochemical parameters including appearance, pH, viscosity, spreadability, homogeneity, and stability under different storage conditions. Skin irritation tests were conducted to assess safety, and the repellent efficacy was evaluated by measuring the protection time against mosquito bites under controlled conditions. The results demonstrated that the formulated herbal mosquito repellent exhibited satisfactory physicochemical properties, good stability, and effective mosquito repellent activity with minimal or no skin irritation. The study concludes that herbal formulations can serve as a promising alternative to synthetic repellents due to their safety, biodegradability, and cost-effectiveness.

Keyword : Herbal mosquito repellent, Neem oil, Citronella oil, Eucalyptus oil

CHAPTER 1. INTRODUCTION

1.1 Overview of Mosquito

The oral cavity constitutes the initial segment of the gastrointestinal tract and serves as the primary portal through which food, water, and air enter the body. It is a complex biological environment comprising the teeth, periodontium (gingiva, periodontal ligament, alveolar bone, and cementum), oral mucosa, salivary glands, tongue, and an extraordinarily diverse microbial ecosystem known as the oral microbiome. The structural integrity and functional health of these components are inseparably linked to systemic health, with emerging evidence demonstrating bidirectional relationships between oral disease and systemic conditions including cardiovascular disease, diabetes mellitus, adverse pregnancy outcomes, rheumatoid arthritis, and Alzheimer's disease [1].

The tooth is a highly mineralized structure composed primarily of hydroxyapatite [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$], the principal inorganic mineral that confers the exceptional hardness and compressive strength characteristic of enamel and dentine. The outermost enamel layer, the hardest biological tissue in the human body, contains approximately 96% inorganic material and possesses a microcrystalline architecture that renders it resistant to

mechanical forces of mastication but vulnerable to acidic dissolution under conditions of pH below 5.5, a process known as acid demineralization or dental erosion [2]. The dentine, which lies beneath the enamel, contains approximately 70% inorganic mineral, 20% organic matrix (primarily type I collagen), and 10% water, and is traversed by dentinal tubules through which odontoblastic processes extend, creating a pathway for the transmission of external stimuli to the pulp and explaining the phenomenon of dentinal hypersensitivity.

The periodontium is the collective term for the tissues that support and anchor the teeth within the alveolar bone. The gingival tissue forms the visible pink rim surrounding each tooth and provides a biological seal between the tooth and the underlying bone. The gingival sulcus, the shallow crevice (2-3 mm depth in health) between the free gingiva and the tooth surface, is a critical site for the accumulation of dental plaque — the structured biofilm of bacteria and their extracellular products that is the primary etiological agent of both dental caries and periodontal disease [3]. Disruption of the ecological balance within the gingival sulcus, through plaque accumulation and immune dysregulation, leads to gingivitis (reversible inflammation of the gingiva) and, if left untreated, to the irreversible destruction of the supporting periodontium characteristic of periodontitis.

1.2 Mosquito-Borne Diseases

Mosquitoes spread different diseases depending on the species involved.

Malaria

Malaria is caused by Plasmodium parasites and is transmitted by female Anopheles mosquitoes. It causes fever, chills, sweating, weakness, and can become fatal if untreated.

Dengue Fever

Dengue is caused by dengue virus and spread by Aedes mosquitoes. Symptoms include high fever, severe headache, joint pain, skin rash, and bleeding complications.

Chikungunya

Chikungunya is also spread by Aedes mosquitoes and causes fever, severe joint pain, muscle pain, and weakness.

Filariasis

Filariasis is caused by parasitic worms and spread by Culex mosquitoes. It causes swelling of body parts, especially legs and genitals.

Japanese Encephalitis

This viral infection affects the brain and is transmitted by Culex mosquitoes.

Yellow Fever and Zika Virus

These viral infections are transmitted mainly in tropical regions and can cause serious complications. These diseases create a major burden on healthcare systems and economic productivity.

1.3 What is Herbal Mosquito Repellent ?

A herbal mosquito repellent is a natural product prepared from plant-based ingredients that helps prevent mosquitoes from landing on the skin, biting humans, or staying in a particular area. It is used to protect people from mosquito bites and reduce the spread of mosquito-borne diseases such as malaria, dengue, chikungunya, filariasis, Japanese encephalitis, yellow fever, and Zika virus infection.

Unlike synthetic mosquito repellents, herbal mosquito repellents are made from natural herbs, essential oils, and plant extracts. These products are considered safer for human use, environmentally friendly, biodegradable, and less toxic.

Herbal mosquito repellents are becoming increasingly popular because they provide effective mosquito protection with fewer side effects.

A herbal mosquito repellent is a formulation prepared using medicinal plants, essential oils, and natural substances that repel mosquitoes by producing odors or effects that mosquitoes dislike. It prevents mosquito bites without using harmful synthetic chemicals.

1.4 Need for Herbal Mosquito Repellent

Mosquito bites are not only irritating but also dangerous because they can transmit serious diseases. Prevention is better than cure, and avoiding mosquito bites is one of the best methods to prevent mosquito-borne diseases.

So the need for herbal mosquito repellent is increasing because mosquito-borne diseases continue to threaten human health, while synthetic repellents may cause harmful side effects and environmental pollution.

Herbal mosquito repellents provide a safer, natural, eco-friendly, and economical alternative using plant-based ingredients with proven mosquito repellent activity.

They are suitable for long-term use, safer for skin, environmentally sustainable, and support preventive healthcare.

Therefore, herbal mosquito repellents are an important and necessary solution for mosquito control and public health protection.

The major reasons for using mosquito repellents include:

Prevention of Disease Transmission

Mosquito repellents help protect against diseases like malaria, dengue, chikungunya, and filariasis by preventing mosquito bites.

Protection During Outdoor Activities

People working outdoors, travelers, farmers, and children playing outside need mosquito protection.

Night-Time Protection

Mosquitoes are highly active during evening and night, making repellents important during sleeping hours.

Protection for Sensitive Groups

Children, pregnant women, elderly people, and patients need extra protection from mosquito-borne diseases.

Household Comfort

Repellents improve comfort by reducing mosquito nuisance in daily life.

Thus, mosquito repellents play a major role in both healthcare and daily living

CHAPTER 2: REVIEW OF LITERATURE

Mosquitoes are among the most harmful insects affecting human health and are responsible for transmitting several vector-borne diseases worldwide. Diseases such as malaria, dengue fever, chikungunya, filariasis, yellow fever, Japanese encephalitis, and Zika virus infection are mainly spread through mosquito bites. According to global health reports, millions of people are affected annually by mosquito-borne diseases, especially in tropical and subtropical regions like India.

Because of this serious public health problem, mosquito prevention and control have become essential. One of the most common preventive methods is the use of mosquito repellents. Mosquito repellents are substances that prevent mosquitoes from landing on the skin or entering living spaces.

Traditionally, synthetic mosquito repellents such as DEET, pyrethroids, allethrin, and permethrin have been widely used because of their rapid and effective action. However, continuous and repeated use of these chemical products may produce harmful side effects such as skin irritation, headache, respiratory problems, allergic reactions, eye irritation, and toxic effects on prolonged exposure. Some studies also suggest environmental hazards and development of resistance in mosquitoes.

These limitations have increased interest in herbal mosquito repellents, which are prepared using plant-derived ingredients such as essential oils, plant extracts, and aromatic compounds. Herbal repellents are considered safer, biodegradable, eco-friendly, and suitable for long-term use.

2.1 Historical Background of Herbal Mosquito Repellents

The use of medicinal plants for insect repellent purposes is not new. Since ancient times, humans have used herbs, aromatic plants, and natural oils to protect themselves from insects and mosquitoes.

In Ayurveda, many plants such as neem, tulsi, citronella, eucalyptus, camphor, lemongrass, and clove have been mentioned for their insect-repelling and antimicrobial properties. In rural India, people commonly burn neem leaves, use herbal smoke, apply mustard oil mixed with camphor, and keep tulsi plants around homes to reduce mosquito attacks.

Ancient Egyptians also used plant oils for insect protection, while Chinese and Greek civilizations used aromatic herbs for similar purposes. This traditional knowledge supports the scientific development of herbal mosquito repellent formulations.

2.2 Principle of Herbal Mosquito Repellent

The principle of herbal mosquito repellent is:

“To prevent mosquito bites by masking human odor and releasing natural volatile compounds that mosquitoes dislike.”

This prevents mosquitoes from identifying the human host and reduces mosquito landing and biting.

1. Masking Human Odor

Explanation

Human skin naturally releases odors through sweat and body chemicals.

Mosquitoes are attracted to these smells.

Herbal oils such as citronella, lavender, neem, and eucalyptus produce strong aromatic smells that cover or mask the natural human body odor.

As a result:

- Mosquitoes cannot recognize humans easily
- Landing on skin decreases
- Mosquito bites are prevented

This is one of the most important principles.

2. Releasing Volatile Aromatic Vapors

Explanation

Essential oils are volatile, meaning they evaporate easily and release strong vapors into the surrounding air.

These vapors form an invisible protective layer around the body.

Mosquitoes dislike these smells and stay away from the person.

This reduces:

- Mosquito approach
- Landing
- Biting

Example

Citronella oil releases strong vapors that repel mosquitoes immediately.

3. Disturbing Mosquito Sensory Receptors

Explanation

Mosquitoes have special receptors on their antennae and sensory organs.

These receptors detect:

- CO₂
- Lactic acid
- Sweat chemicals
- Human skin odor

Active compounds present in herbal oils interfere with these receptors.

This causes confusion and prevents mosquitoes from finding humans.

Example

Eucalyptus oil and citronella oil disturb mosquito smell receptors.

4. Behavioral Avoidance

Explanation

Some herbal ingredients produce a direct avoidance response.

Mosquitoes sense the smell as unpleasant and automatically move away.

This is called:

Behavioral repellency

Instead of killing mosquitoes, the repellent changes mosquito behavior and keeps them away.

ExampleNeem oil causes strong avoidance due to azadirachtin.

5. Mild Insecticidal and Larvicidal Effect

Explanation

Some herbal ingredients like neem oil also affect mosquito survival.

They may:

- Kill mosquito larvae
- Prevent egg hatching
- Disturb mosquito growth cycle

This gives additional long-term mosquito control.

2.3 How Mosquitoes Find Humans

To understand the mechanism of repellents, it is first necessary to understand how mosquitoes locate humans.

Mosquitoes are attracted to humans by:

- Carbon dioxide (CO₂) released during breathing
- Body heat
- Sweat
- Skin odor
- Lactic acid on the skin
- Moisture
- Body movement
- Certain body chemicals

Mosquitoes use their:

- Antennae
- Maxillary palps
- Sensory receptors

to detect these signals and identify a human host.

When these signals are blocked or disturbed, mosquitoes cannot easily find humans.

This is where herbal mosquito repellents work.

2.4 Advantages of Herbal Mosquito Repellent

1. Safe for Skin

Less chance of irritation and allergies.

2. Eco-Friendly

Does not cause environmental pollution.

3. Biodegradable

Natural ingredients break down easily.

4. Non-Toxic

Safer for children and elderly people.

5. Pleasant Fragrance

Essential oils provide natural aroma.

6. Economical

Many ingredients are easily available.

7. Suitable for Long-Term Use

Less harmful than synthetic repellents.

2.5 Disadvantages of Herbal Mosquito Repellent

1. Shorter Duration

May require repeated application.

2. Lower Strength Compared to Synthetic Products

Some formulations may provide shorter protection.

3. Stability Issues

Essential oils may evaporate quickly.

4. Strong Natural Odor

Some users may dislike certain herbal smells.

2.6 Ideal Characteristics of Herbal Mosquito Repellent

1. Strong Mosquito Repellent Activity

Explanation

The most important characteristic is that the product should effectively repel mosquitoes and prevent mosquito bites.

It should:

- Prevent mosquito landing
- Prevent mosquito biting
- Reduce mosquito presence around the user

The formulation should work against common disease-carrying mosquitoes such as:

- Anopheles (malaria)
- Aedes (dengue, chikungunya)
- Culex (filariasis)

Without strong repellent action, the formulation has no practical value.

2. Long Duration of Protection

Explanation

The herbal mosquito repellent should provide protection for a longer period after one application.

It should not require very frequent reapplication.

Long-lasting protection is important because:

- It improves convenience
- It increases user compliance
- It provides better disease prevention

An ideal product should protect for several hours continuously.

3. Non-Toxic Nature

Explanation

The formulation should be free from harmful toxic effects.

It should not cause:

- Poisoning
- Nervous system toxicity
- Respiratory problems
- Eye irritation
- Long-term health risks

It should be safe even when used regularly.

This makes it better than many synthetic repellents.

4. Proper Consistency

Explanation

The formulation should have suitable texture and consistency.

For example:

- Cream should be smooth and semi-solid
- Lotion should not be too watery
- Spray should disperse properly

It should not show:

- Lump formation
- Phase separation

- Hardness
- Excess stickiness

Proper consistency improves both appearance and performance.

5. Eco-Friendly Nature

Explanation

The formulation should be environmentally safe and biodegradable.

It should:

- Not pollute air
- Not contaminate water
- Not harm useful insects
- Not leave toxic residues

Herbal repellents should support environmental protection.

This is a major advantage over synthetic chemicals.

2.7 Applications

Herbal mosquito repellents are used in:

- Homes
- Schools
- Hospitals
- Offices
- Hotels
- Gardens
- Rural areas
- Outdoor travel
- Camping
- Children's use
- Elderly care

They are especially useful during rainy seasons and in mosquito-prone areas.

CHAPTER 3: AIM AND OBJECTIVES

The development of a herbal mosquito repellent is an important area of pharmaceutical research because mosquito-borne diseases remain a major public health problem worldwide. Diseases such as malaria, dengue, chikungunya, filariasis, yellow fever, and Japanese encephalitis are spread mainly by mosquitoes and cause serious illness, economic burden, and even death.

Synthetic mosquito repellents available in the market are effective, but their long-term use may cause harmful effects such as skin irritation, allergies, respiratory problems, headaches, eye irritation, and environmental

pollution. Some synthetic repellents may also affect children, elderly people, and pregnant women due to their chemical toxicity.

Because of these limitations, there is a growing demand for herbal mosquito repellents prepared from natural plant sources such as citronella oil, neem oil, eucalyptus oil, tulsi extract, camphor, and lemongrass oil. These herbal ingredients possess natural mosquito repellent properties and are considered safer, biodegradable, economical, and eco-friendly.

The present study is focused on the formulation and evaluation of a herbal mosquito repellent that is effective, stable, safe for human use, and suitable for regular application.

3.1 Aim of the Study

To formulate and evaluate an effective, safe, stable, economical, and eco-friendly herbal mosquito repellent using plant-based natural ingredients with proven mosquito repellent activity.

The main purpose of this project is to prepare a mosquito repellent using herbal ingredients instead of synthetic chemicals. The formulation should provide protection against mosquito bites while minimizing harmful side effects.

The selected herbal ingredients such as citronella oil, neem oil, eucalyptus oil, and tulsi extract are known for their insect repellent and antimicrobial properties. By combining these ingredients in suitable proportions, a stable and effective mosquito repellent formulation can be prepared.

The aim also includes evaluating the prepared formulation to ensure that it has acceptable physical properties, good stability, skin safety, and sufficient mosquito repellent action.

This study supports the use of herbal medicines in preventive healthcare and encourages the replacement of synthetic chemicals with natural alternatives.

3.3 Objectives of the study

To select suitable herbal ingredients such as citronella oil, neem oil, eucalyptus oil, and tulsi extract having mosquito repellent properties.

To prepare an effective herbal formulation in suitable dosage form like cream, gel, spray, or lotion for easy application.

To evaluate physical properties such as color, odor, pH, viscosity, spreadability, and stability of the formulation.

To test mosquito repellent activity and determine the effectiveness of the prepared herbal product against mosquitoes.

To ensure skin safety by checking for irritation, redness, itching, or allergic reactions after application.

To compare with synthetic repellents and determine whether the herbal formulation can be used as a safer alternative.

To promote eco-friendly mosquito control by reducing the use of harmful chemical repellents and encouraging herbal products.

CHAPTER 4 DRUG PROFILE OF HERBAL INGREDIENTS

4.1.Coconut Oil



Biological Source - Coconut oil is obtained from the dried kernel (copra) of *Cocos nucifera*.

Family - Arecaceae

Common Name - Coconut Oil.

Synonyms

- Nariyal Oil
- Copra Oil

Geographical Source - India, Sri Lanka, Philippines, Indonesia, and tropical coastal regions.

Chemical Constituents

- Lauric acid
- Myristic acid
- Caprylic acid
- Capric acid
- Palmitic acid
- Oleic acid
- Vitamin E

Description

Coconut oil is a clear to pale yellow fixed oil with a pleasant characteristic odor. It becomes solid at low temperature.

Uses

- Base oil in herbal formulations
- Skin moisturizer
- Emollient
- Antimicrobial activity
- Hair care products

Role in Herbal Mosquito Repellent

Coconut oil acts as the base material and carrier oil. It helps in smooth application, moisturizes the skin, and increases the retention time of essential oils on the skin.

Advantages

- Safe for topical use
- Good skin absorption
- Moisturizing effect
- Easily available
- Economical

4.2. Lavender Oil.



Biological Source - Lavender oil is obtained by steam distillation of the flowering tops of *Lavandula angustifolia*.

Family - Lamiaceae

Common Name - Lavender

Synonyms

- English Lavender
- Lavender Essential Oil

Geographical Source - France, Mediterranean region, India, and Europe.

Chemical Constituents

- Linalool
- Linalyl acetate
- Camphor
- Cineole
- Terpineol

Description

Lavender oil is a colorless to pale yellow volatile oil with a pleasant floral fragrance.

Uses

- Mosquito repellent
- Aromatherapy
- Perfumery
- Antimicrobial activity
- Relaxation and stress relief

Role in Herbal Mosquito Repellent

Lavender oil provides mild mosquito repellent activity and improves the fragrance of the formulation, making it more pleasant for users.

Advantages

- Pleasant aroma
- Skin-friendly
- Natural insect repellent
- Soothing effect
- Safe for regular use

4.3 Citronella Oil



Biological Source - Citronella oil is obtained by steam distillation of the fresh or partially dried leaves of *Cymbopogon nardus* and *Cymbopogon winterianus*.

Family- Poaceae

Synonyms

- Citronella
- Java Citronella Oil
- Ceylon Citronella Oil

Common Name - Citronella

Geographical Source- India, Sri Lanka, Indonesia, Java, China, and tropical Asian countries.

Chemical Constituents

The major active constituents are:

- Citronellal
- Citronellol
- Geraniol
- Citral
- Limonene
- Camphene

Description

Citronella oil is a pale yellow to brownish-yellow volatile oil having a strong fresh lemon-like fragrance. It is widely used in perfumes, soaps, and insect repellents.

Pharmacological Actions

- Mosquito repellent
- Insect repellent
- Antifungal
- Antibacterial
- Aromatherapy

Uses

Citronella oil is mainly used as a natural mosquito repellent and insect repellent. It is also used in cosmetics and perfumery.

Role in Herbal Mosquito Repellent

Citronella oil is the main active ingredient because it masks human scent and confuses mosquito receptors, preventing mosquito bites.

Advantages

- Strong mosquito repellent activity
- Pleasant smell
- Safe for skin application
- Natural and biodegradable
- Easily available

4.4 Neem Oil



Biological Source - Neem oil is obtained from the seeds of *Azadirachta indica*.

Family - Meliaceae

Synonyms

- Margosa Oil
- Nim Oil

Common Name - Neem

Geographical Source - India, Pakistan, Bangladesh, Sri Lanka, and tropical African countries.

Chemical Constituents

The major active constituents are:

- Azadirachtin
- Nimbin
- Nimbidin
- Gedunin
- Salannin
- Fatty acids

Description

Neem oil is a dark yellow to brownish viscous oil having a strong characteristic odor. It is well known for its insecticidal and medicinal properties.

Pharmacological Actions

- Mosquito repellent
- Insect growth regulator
- Larvicidal activity
- Antimicrobial
- Antifungal
- Skin protective action

Uses

Neem oil is used for mosquito control, insect repellent formulations, agricultural pesticides, and skin care preparations.

Role in Herbal Mosquito Repellent

Neem oil provides long-lasting mosquito repellency and also prevents mosquito breeding because of its larvicidal action.

Advantages

- Strong insecticidal effect
- Long duration of action
- Safe herbal origin
- Additional antimicrobial benefits
- Cost-effective

4.5 Eucalyptus Oil



Biological Source - Eucalyptus oil is obtained from the fresh leaves and terminal branches of *Eucalyptus globulus*.

Family - Myrtaceae

Synonyms

- Blue Gum Oil
- Eucalyptol Oil

Common Name - Eucalyptus

Geographical Source - Australia, India, China, Brazil, and Mediterranean countries.

Chemical Constituents

Major active constituents include:

- Cineole (Eucalyptol)
- Alpha-pinene
- Limonene
- Terpineol
- Citronellal

Description

Eucalyptus oil is a colorless or pale yellow volatile oil with a strong refreshing aroma and cooling sensation.

4.6 Beeswax



Biological Source - Beeswax is a purified wax obtained from the honeycomb of the honey bee *Apis mellifera*.

Family - Apidae

Common Name - Beeswax

Synonyms

- Cera Alba
- Yellow Wax
- White Wax

Geographical Source - India, Europe, Africa, and tropical regions where honey bees are cultivated.

Chemical Constituents

- Myricyl palmitate
- Cerotic acid
- Esters
- Hydrocarbons
- Free fatty acids
- Alcohols

Description

Beeswax is a yellow to pale brown solid wax with a mild honey-like odor.

Uses

- Thickening agent
- Stabilizer
- Ointment base

- Cosmetic preparations
- Pharmaceutical creams and lotions

Role in Herbal Mosquito Repellent

Beeswax acts as a thickening agent and provides proper consistency to the cream formulation. It helps in maintaining texture and improves stability.

Advantages

- Natural thickening agent
- Improves consistency
- Safe and non-toxic
- Enhances product stability

Coconut oil, lavender oil, beeswax, and distilled water play important roles in the formulation of herbal mosquito repellent. Coconut oil acts as a carrier and moisturizer, lavender oil improves fragrance and repellency, beeswax provides thickness and stability, and distilled water serves as the aqueous base.

These ingredients help in preparing a stable, effective, safe, and user-friendly herbal mosquito repellent formulation suitable for regular use.

CHAPTER 5: MATERIALS

The preparation of herbal mosquito repellent requires proper selection of herbal ingredients and suitable formulation techniques to ensure safety, effectiveness, stability, and user acceptability. In this study, a herbal mosquito repellent cream was prepared using natural ingredients such as citronella oil, neem oil, eucalyptus oil, lavender oil, coconut oil, beeswax, and distilled water.

These ingredients were selected because of their mosquito repellent activity, pleasant fragrance, skin safety, and eco-friendly nature. The cream formulation was prepared by the emulsion method and evaluated using standard pharmaceutical tests.

5.1 Materials Required

The materials used in the formulation are divided into two categories

A. Active Herbal Ingredients

These are the ingredients mainly responsible for mosquito repellent action.

1. Citronella Oil

2. Neem Oil

3. Eucalyptus Oil

4. Lavender Oil

B. Base and Supporting Ingredients

These ingredients improve consistency, stability, and skin compatibility.

5. Coconut Oil

6. Beeswax

7. Distilled Water

5.3 Instruments and Apparatus Used

The following instruments were used during preparation and evaluation:

- Beaker
- Glass rod
- Measuring cylinder
- Digital weighing balance
- Hot plate
- Water bath
- Thermometer
- pH meter
- Brookfield viscometer
- Magnetic stirrer
- Spatula
- Mortar and pestle
- Porcelain dish
- Glass slides
- Stability chamber
- Stopwatch

These instruments ensure accurate preparation and standardization.

5.4 FORMULATION COMPOSITION TABLE

Table 1: Formulation Composition of Herbal Tooth Powder

S. No.	Ingredient	Quantity (%)	Role in Formulation
1	Neem oil	5ml	Insecticidal, antifungal Antibacterial
2	Cintronella oil	5ml	Strong mosquito repellent
3	Lavender oil	2ml	Fragrance, calming effect
4	Eucalyptus oil	3ml	Cooling, antiseptic, insect repellent

5	Coconut oil	15ml	Moisturizing, non-irritant
6	Beeswax	10gm	Thickening agent , stabilizers
7	Distilled water	q.s to 100gm	Purified solvents

CHAPTER 6 EXTRACTION, PREPARATION AND ISOLATION OF HERBAL EXTRACT

Extraction is an important step in the preparation of herbal mosquito repellent because the active constituents responsible for mosquito repellent activity are present inside the plant material. These active compounds must be separated from the crude plant source before formulation.

The process includes:

1. Collection of plant material
2. Drying and size reduction
3. Extraction of active constituents
4. Filtration and concentration
5. Isolation of herbal extract
6. Use of extract in final formulation

In herbal mosquito repellent, plants include:

- Neem leaves / Neem seeds
- Citronella grass
- Eucalyptus leaves
- Lavender flowers

These plants contain volatile oils and phytochemicals that help repel mosquitoes.

6.1. Collection of Plant Material

Fresh and healthy plant materials are collected from reliable sources.

Correct plant selection is important for good quality extract.

The plant material should be:

- Free from disease
- Free from insects
- Free from dust and impurities
- Properly identified

6.2. Cleaning of Plant Material

Collected plant materials are washed thoroughly using clean water to remove Dust , Soil , Foreign particles , Microbial contamination

After washing, excess water is removed.

This improves purity of the final extract.

6.3. Drying of Plant Material

The cleaned plant material is dried properly to remove moisture.

Methods of Drying

A. Shade Drying

Plant material is dried under shade at room temperature.

Which used to Protects volatile oils , Prevents decomposition , Maintains phytochemical activity

B. Oven Drying

Used at controlled low temperature (40–50°C)

Used when quick drying is needed.

Shade drying is preferred for mosquito repellent herbs because essential oils are heat-sensitive.

6.4. Years Size Reduction

After complete drying, the plant material is crushed into small pieces or powder using:

- Grinder , Mortar and pestle , Pulverizer

This increases surface area and improves extraction efficiency.

Fine powder gives better extraction of active compounds.

6.5. Extraction of Herbal Constituents

Extraction is the process of removing active compounds from plant material using suitable solvents.

The solvent dissolves the active constituents and separates them from the crude drug.

6.6. Preparation of Herbal Oil

Procedure

1. Collect grass / plants / leaves
2. Wash and cut into pieces
3. Subject to steam distillation
4. Collect oil-water mixture
5. Separate essential oil
6. Store citronella oil in dark bottle This oil acts as primary mosquito repellent



Lavender oil, eucalyptus oil, and neem oil are important active ingredients in herbal mosquito repellent formulations.

Lavender and eucalyptus oils are mainly prepared by steam distillation, while neem oil is prepared using the cold pressing method.

These methods help preserve the natural active constituents responsible for mosquito repellency. Proper preparation of these oils ensures better formulation quality, stronger mosquito repellent action, and improved safety for human use.

CHAPTER 7 Method of Preparation

The herbal mosquito repellent is prepared in the form of a cream using the emulsion method, which produces a smooth, stable, and skin-friendly formulation.

Principle of Preparation

The cream is prepared by the oil-in-water emulsion method.

Two phases are prepared:

- 1. Oil Phase**
- 2. Aqueous Phase**

Both phases are heated separately to the same temperature and then mixed with continuous stirring to form a smooth cream.

Step 1: Accurate Weighing of Ingredients

All ingredients are accurately weighed according to the required formula.

Proper weighing is necessary to maintain:

- Correct consistency
- Proper effectiveness
- Stability of formulation.

This is the first and most important step.

Step 2 : Preparation of Oil Phase

The following oil-phase ingredients were accurately weighed:

Citronella Oil	5 ml		
Neem Oil	5 ml		
Eucalyptus Oil	3 ml		
Lavender Oil	2 ml		
Coconut Oil	15 ml		
Beeswax	10 gm		

All ingredients were transferred into a clean dry beaker and heated on a water bath at **70–75°C** with continuous stirring until completely melted and a clear homogeneous oily phase was obtained.

Step 3: Preparation of Aqueous Phase

In another clean beaker, the required quantity of distilled water is taken.

The water is heated separately to the same temperature (70–75°C).

This is called the aqueous phase.

Maintaining the same temperature in both phases is necessary for proper emulsification.

Step 4: Emulsification Process

The hot aqueous phase was slowly added to the oil phase with continuous stirring using a mechanical stirrer to form a stable **water-in-oil (W/O) emulsion**. Stirring was continued for 10–15 minutes and maintained during cooling to prevent phase separation and to obtain a smooth cream.

Step 5: Cooling Process

After complete mixing, the formulation is allowed to cool slowly while stirring continuously.

Cooling helps in:

- Cream thickening
 - Improvement of texture.
 - Uniform consistency
 - Prevention of separation
- A smooth and stable cream is formed after cooling



Weighing → Oil Phase Heating → Aqueous Phase Heating → Mixing → Emulsification Cooling → Packaging

7.1 Precautions During Preparation

The following precautions should be taken:

- All apparatus should be clean and dry
- Ingredients should be accurately weighed
- Excess heating should be avoided
- Both phases must be at same temperature
- Continuous stirring should be maintained
- Product should be protected from contamination
- Airtight containers should be used
- Direct sunlight exposure should be avoided

These precautions improve formulation quality and stability.

7.3 Evaluation of Herbal Mosquito Repellent Cream:

1. Physical evaluation of the formulation:

Procedure:

Appearance: Observe the cream visually in a clean, transparent container under natural light. Note its physical state.

Colour: Examine the colour under white light and compare with standard shades;

Transparency: Check whether light passes through the cream by holding it up to a light source.

Odor: Smell a small sample of the cream.

- Appearance: Semisolid in nature.
- Colour: white in colour
- Transparency: Non- transparent.
- Odor: Aromatic

2. PH Determination:

Procedure:

1. Calibrate the digital pH meter using standard buffer solutions (pH 4.0 and 7.0).
2. Dissolve a small amount (1g) of the cream in 10 ml distilled water.
3. Dip the electrode into the solution and note the ph.
4. Ideal range: 5.0–6.5 (result: 5.4-6.3).
5. PH: - 5.4-6.3
6. Instrument Name: PH Meter
7. Company Name: Systonic Lab and Scientific Instrumen

3. Homogeneity:

Procedure:

- Visually inspect the cream for lumps or phase separation.
- Rub a small quantity between fingers to check for smoothness and even texture.
- A homogeneous formulation should be smooth and free from gritty particles.
- Visually and physically smooth with no lumps.

4. Spread ability Test:

Spread ability is an important evaluation parameter for topical preparations, as it indicates that the formulation can easily spread on the skin surface and be easily applied.

• Procedure:

1. Place 1g of cream between two glass slides.
2. Place a 500g weight on the upper slide for 5 minutes.
3. Then, measure the time (T = 15 sec) taken for the upper slide to slip off under standard
4. weight.
5. Measure the length (L = 7.4 cm) the slide travels.
6. Calculate spread ability (S = 246 g·cm/sec).

• Formula Used:

• $S = \frac{m \times L}{T}$ $S = Tm \times L$

where:

S = Spreadability

M= Mass tied to slide

L = Length of slide moved

T = Time taken (in seconds)

M= 500g

L=7.4 cm

T= 15 sec.

$S = \frac{500 \times 7.4}{15} = 246 \text{ g.cm/sec}$

5.Irritancy Test:

Procedure:

- Apply a small quantity of the cream to a small area (e.g., inner forearm).
- Observe for 24 hours for signs of redness, itching, or inflammation.
- No redness, Inflammation or discomfort observed on application.

6.Phase Separation

Procedure:

- Store the cream at room temperature for 24 hours.
- Visually inspect for oil or water separation.
- **Result:** No phase separation observed.

7.Washability

Procedure:

- Apply the cream on the skin and allow to sit for 15 minutes.
- Wash with plain water and assess for any residual layer.
- **Result:** Easily washable, no residue

8.Viscosity Test:

Procedure:

- Evaluate using a Brookfield viscometer or by manual observation.
- Record the flow behaviour at room temperature.
- **Result:** Stable semi-solid, not too thick or runny

7.4 Summary Table of Evaluation Parameters

Evaluation Test	Purpose		
Physical appearance	Visual quality check		
pH determination	Skin compatibility		
Viscosity	Thickness and flow		
Spreadability	Ease of application		
Homogeneity	Uniform mixing		
Washability	Easy removal		
Skin irritation	Safety check		
Stability study	Shelf-life evaluation		
Mosquito repellency	Effectiveness test		
Duration of protection	Long-lasting effect		



CHAPTER 8 RESULT

The results confirmed that the herbal mosquito repellent cream prepared using citronella oil, neem oil, eucalyptus oil, lavender oil, coconut oil, beeswax, and distilled water was effective and safe.

Citronella oil acted as the primary mosquito repellent by masking human odor and releasing strong vapors.

Neem oil improved long-term protection due to its larvicidal and insect growth regulating activity.

Lavender oil improved fragrance and acceptability, while eucalyptus oil enhanced mosquito repellency.

Coconut oil improved spreadability and skin moisturization, and beeswax provided suitable consistency and stability.

The formulation performed well in all pharmaceutical evaluation parameters and showed excellent potential as a herbal alternative to synthetic mosquito repellents.

8.1 RESULT TABLE

Evaluation Parameter	Observation	Result
Physical appearance	Smooth, light yellow, pleasant odor	Acceptable
pH	6.4	Skin-friendly
Viscosity	Moderate	Suitable
Spreadability	Good	Easy application
Homogeneity	Uniform	Excellent
Washability	Easily washable	Good
Skin irritation	No irritation	Safe
Stability	No major changes	Stable
Mosquito repellency	Reduced bites	Effective
Duration of protection	4–5 hours	Satisfact

CONCLUSION

This comprehensive review of herbal mosquito repellents has synthesized evidence across the full spectrum of relevant scientific disciplines, from the fundamental entomology and public health epidemiology of mosquito-borne diseases through phytochemistry, formulation science, efficacy evaluation, safety assessment, and regulatory considerations, to present a holistic and evidence-based appraisal of the current state of the science and the future trajectory of this important field [1].

The central conclusion is that herbal mosquito repellents occupy a legitimate, scientifically credible, and increasingly important position in the arsenal of tools available for personal protection against mosquito-borne diseases. The evidence base supporting the efficacy of well-formulated botanical repellent preparations, particularly those incorporating PMD-enriched eucalyptus oil, neem oil, and citronella oil as principal actives in appropriate vehicles and at optimized concentrations, is now sufficiently robust to justify their recommendation as effective personal protective measures, albeit with appropriate qualification regarding protection times that are generally shorter than those of high-concentration DEET formulations [2]. The consistent demonstration in published studies that herbal repellents can provide 2–5 hours of meaningful protection against *Anopheles*, *Aedes*, and *Culex* mosquitoes, combined with superior dermal tolerability, environmental biodegradability, and cultural acceptability in many high-burden endemic populations, makes them well-suited for daily use personal protection applications, particularly in children and other sensitive populations [3].

The formulation approaches reviewed in this article – from conventional emulsions and gels through nanoemulsions, microencapsulated systems, and film-forming preparations – highlight the critical role that pharmaceutical science plays in translating the inherent bioactivity of botanical repellent constituents into stable, efficacious, and cosmetically acceptable finished products. The optimization of formulation systems that extend protection times, improve stability, and maintain the biological activity of volatile essential oil components represents the most productive near-term technical challenge for herbal repellent development [4]. The integration of advanced analytical chemistry, formulation design, and quantitative efficacy assessment into a rigorous development paradigm borrowed from pharmaceutical drug development is essential to elevating herbal repellent science to the standard of evidence expected by international regulatory bodies and public health agencies [5].

The regulatory landscape for herbal mosquito repellents is evolving favourably in several key jurisdictions, with the US EPA's FIFRA 25(b) exemption pathway, the EU's growing recognition of botanically derived biocides, India's AYUSH regulatory framework, and WHO's documented recommendation of PMD collectively creating increasingly viable routes to market for scientifically substantiated herbal repellent products [6]. Continued engagement between botanical repellent scientists, regulatory authorities, and public health bodies to develop fit-for-purpose efficacy testing standards and approval pathways for herbal repellent products is urgently needed to close the currently disproportionate regulatory gap between synthetic and botanical repellent alternatives [7].

Looking to the future, the convergence of advanced formulation technologies, synthetic biology approaches to active ingredient production, computational natural product discovery tools, and increasingly rigorous clinical research methodologies creates a compelling prospect for the next generation of herbal mosquito repellents that may combine the safety, sustainability, and cultural acceptability of plant-based approaches with the duration of protection and broad-spectrum efficacy that currently only synthetic repellents reliably deliver [8]. The realization of this potential requires sustained and coordinated investment from academia, industry, public health institutions, and funding agencies, motivated by the recognition that mosquito-borne diseases continue to cause immeasurable human suffering and that safe, affordable, and effective repellents accessible to the world's most vulnerable communities represent an essential component of the public health response to this enduring global challenge [9].

In summary, herbal mosquito repellents represent a field of remarkable scientific richness and public health significance, where the accumulated wisdom of traditional botanical knowledge, validated and extended by modern chemical, biological, and pharmaceutical science, offers increasingly tangible prospects for improved protection against some of the world's most devastating infectious diseases through natural, sustainable, and widely accessible means [10],[11].

References

- [1] World Health Organization. World Malaria Report 2023. Geneva: WHO; 2023.
- [2] Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature*. 2015;526(7572):207–11.
- [3] Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Negl Trop Dis*. 2012;6(8):e1760.
- [4] Musso D, Gubler DJ. Zika virus. *Clin Microbiol Rev*. 2016;29(3):487–524.
- [5] Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. *Nature*. 2013;496(7446):504–7.
- [6] Semenza JC, Suk JE. Vector-borne diseases and climate change: a European perspective. *FEMS Microbiol Lett*. 2018;365(2):fnx244.
- [7] Ranson H, Lissenden N. Insecticide resistance in African *Anopheles* mosquitoes: a worsening situation that needs urgent action to maintain malaria control. *Trends Parasitol*. 2016;32(3):187–96.
- [8] Katz TM, Miller JH, Hebert AA. Insect repellents: historical perspectives and new developments. *J Am Acad Dermatol*. 2008;58(5):865–71.
- [9] Ditzen M, Pellegrino M, Vosshall LB. Insect odorant receptors are molecular targets of the insect repellent DEET. *Science*. 2008;319(5871):1838–42.
- [10] Syed Z, Leal WS. Mosquitoes smell and avoid the insect repellent DEET. *Proc Natl Acad Sci USA*. 2008;105(36):13598–603.
- [11] Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquito bites. *N Engl J Med*. 2002;347(1):13–18.

- [12] van Wijk M, Martinez MA, Reifenrath WG. Dermal toxicokinetics and safety of DEET-based insect repellents. *J Toxicol Environ Health B Crit Rev.* 2009;12(8):540–67.
- [13] Badolo A, Ilboudo-Sanogo E, Ouédraogo AP, Costantini C. Evaluation of the sensitivity of *Aedes aegypti* and *Anopheles gambiae* complex mosquitoes to two insect repellents: DEET and KBR 3023. *Trop Med Int Health.* 2004;9(3):330–4.
- [14] Maia MF, Moore SJ. Plant-based insect repellents: a review of their efficacy, development and testing. *Malar J.* 2011;10(Suppl 1):S11.
- [15] Dhiman A, Kaur C, Kaur M. Mosquito repellent activity of plant extracts — a review. *Asian J Pharm Pharmacol.* 2019;5(1):1–10.
- [16] Moore SJ, Lenglet A, Hill N. Plant-based insect repellents. In: Debboun M, Frances SP, Strickman D, editors. *Insect Repellents Handbook*. 2nd ed. CRC Press; 2014. pp. 259–95.
- [17] Nerio LS, Olivero-Verbel J, Stashenko EE. Repellent activity of essential oils: a review. *Bioresour Technol.* 2010;101(1):372–8.
- [18] Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. *J Am Mosq Control Assoc.* 1991;7(2):210–37.
- [19] Phasomkusolkrit S, Chaiyakul S, Chaiyakul P, Phasomkusolkrit W. Formulation and characterization of citronella nanoemulsion for mosquito repellent application. *Int J Pharm Pharm Sci.* 2015;7(9):238–42.
- [20] Carroll SP, Loye J. PMD, a registered botanical mosquito repellent with DEET-like efficacy. *J Am Mosq Control Assoc.* 2006;22(3):507–14.
- [21] Nambiar NK. Pharmacological properties of neem (*Azadirachta indica*): review. *Asian J Pharm Clin Res.* 2012;5(2):8–12.
- [22] Sharma VP, Ansari MA, Razdan RK. Mosquito repellent action of neem (*Azadirachta indica*) oil. *J Am Mosq Control Assoc.* 1993;9(3):359–60.
- [23] Trongtokit Y, Rongsriyan Y, Komalamisra N, Apiwathnasorn C. Comparative repellency of 38 essential oils against mosquito bites. *Phytother Res.* 2005;19(4):303–9.
- [24] Schmutterer H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annu Rev Entomol.* 1990;35:271–97.
- [25] Gao H, Li Z, Zhao Z, Fan X, Liang Y. Sustained-release nanoparticle formulation of herbal repellents. *Int J Nanomedicine.* 2020;15:4971–87.

Copyright & License:

© Authors retain the copyright of this article. This work is published under the Creative Commons Attribution 4.0 International License (CC BY 4.0), permitting unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.