



Formulation And Evaluation Of Polyherbal Mosquito Repellent Gel

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ABSTRACT:

Mosquito-borne diseases remain a significant global health concern, necessitating effective and safe repellent solutions. This study explores the formulation of a gel-based mosquito repellent using extracts from *Tridax procumbens* (coat buttons), *Cymbopogon citratus* (lemongrass), and *Azadirachta indica* (neem). These plant species are known for their potent insect-repellent properties, attributed to compounds such as pyrethrins, citronella, and azadirachtin, respectively. The gel formulation was developed through a systematic extraction process followed by optimization of ingredient concentrations to enhance efficacy and stability. Physical and chemical characterization included viscosity measurements, pH determination, and stability testing under varying environmental conditions. In vitro testing against mosquito species known to transmit diseases such as malaria and dengue demonstrated significant repellent activity compared to control formulations. Furthermore, skin compatibility studies using human volunteers indicated minimal irritant potential, supporting its suitability for topical application. Overall, this mosquito repellent gel represents a promising alternative to synthetic repellents, harnessing the natural repellent properties of *Tridax procumbens*, *Cymbopogon citratus*, and *Azadirachta indica* while ensuring safety and efficacy for public health applications.

KEYWORDS: Mosquito Repellent, Polyherbal Gel, *Tridax Procumbens*, *Cymbopogon Citratus*, Neem

INTRODUCTION:

Globally, the tropical and sub-tropical regions are primarily affected by vector-borne diseases. Mosquito is the sole vector for the transmission of malaria, dengue, and chikungunya which transmits more than 750 million people every year. Nearly 3 million lost their life annually, of which nearly 90% of the mortality lies in infants. Currently, for the prevention of mosquito-borne diseases, the control of mosquito larval growth and personal protection from mosquito bites by the use of mosquito nets and mosquito repellent remained the chief methods. Scientific innovations include mosquito vaccines, but it is still at a nascent level and is not yet recommended for human use. For the complete elimination of adult mosquito and mosquito larva across the planet, dichloro diphenyl trichloroethane (DDT) was sprayed a few decades back, however mosquitoes developed rapid resistance and over the years, this became a rising problem for all individuals and there is an unmet need for the solution. Many commercial brands have flooded the market with N, N-diethyl- meta-toluamide (DEET) based mosquito repellent. For meeting the challenges, a large population across the globe started purchasing these DEET based mosquito repellents. Everything was going well until it was noticed that

the chemical component causes toxicity with hyperammonemia and encephalopathy in children after ingestions and applications. DEET was found to be comparatively harmful to the person suffering from urea cycle disorders such as ornithine transcarbamylase (OTC) deficiency and is therefore, contraindicated in individuals. These situations lead to the budding necessity of natural mosquito repellents which will have inexpensive, effectual, non-toxic, environment-friendly, and biodegradable attributes. Inspired from the upcoming global need, a carbopol 940 based mosquito repellent gel formulation was prepared from the essential oils of *Cymbopogon nardus*, *Murraya Koenigii*, *Cymbopogon citratus*, *Tridax procumbens*, *Eucalyptus globules*, and *Azadirachta indica*, and further evaluating them for their appearance, pH, viscosity, spreadability, extrudability, swelling index, and accelerated studies.[1-5]

TRIDEX PROCUMBENS :

Tridax procumbens, commonly known as coatbuttons or tridax daisy, is a perennial herbaceous plant belonging to the Asteraceae family. Native to tropical regions of Central and South America, it has spread widely to other parts of the world, particularly in tropical and subtropical climates. This invasive species is often found in disturbed areas such as roadsides, fields, and gardens. *Tridax procumbens* is characterized by its creeping stem, which can root at the nodes, and its distinctive small, yellow, daisy-like flowers with white petals. The leaves are ovate to lanceolate, serrated at the edges, and grow opposite each other on the stem. Despite being considered a weed in many areas, *Tridax procumbens* has some beneficial uses. It is known for its medicinal properties, including antimicrobial, anti-inflammatory, and wound healing effects and also act as mosquito repellent. Additionally, it is used in traditional medicine to treat ailments like dysentery, diarrhea, and liver disorders. The plant also has ecological benefits, providing food for pollinators and contributing to soil stabilization with its extensive root system. However, its aggressive growth can outcompete native vegetation, posing a threat to biodiversity.

Taxonomy Classification: [6]

Kingdom	Plantae
Division	Spermatophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	<i>Tridax</i>
Species	<i>Procumbens</i>

CYMBOPOGON CITRATUS :

Cymbopogon citratus, commonly known as lemongrass, is a tropical plant widely recognized for its culinary and medicinal uses. Native to regions of South Asia, Southeast Asia, and Australia, this tall, perennial grass thrives in warm, sunny climates and well-drained soils. Characterized by its long, slender leaves and a strong citrus aroma, lemongrass is a staple in many Asian cuisines, imparting a fresh, lemony flavor to dishes. Beyond its culinary applications, *Cymbopogon citratus* is valued in traditional medicine for its potential health benefits, including antimicrobial, antifungal, and anti-inflammatory properties. It is often used in teas, essential oils, and herbal remedies to alleviate digestive issues, reduce fever, and promote relaxation. In addition to its medicinal and culinary uses, lemongrass is also popular in aromatherapy and natural insect repellents due to its distinctive fragrance. Cultivating this versatile plant requires minimal maintenance, making it a favored choice for home gardens and commercial farming alike.

Taxonomy Classification: [7]

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Cyperales
Family	Poaceae
Genus	Cymbopogon
Species	C. citratus

AZADIRACHTA INDICA :

Neem (*Azadirachta indica*), a tree native to the Indian subcontinent, is renowned for its medicinal and environmental benefits. It belongs to the Meliaceae family and can grow up to 20 meters tall. Neem is often referred to as "Nature's Pharmacy" due to its wide array of bioactive compounds, including azadirachtin, nimbin, and quercetin, which are known for their antifungal, antibacterial, and antiviral properties. The leaves, bark, seeds, and oil extracted from neem are used in traditional Ayurvedic medicine to treat various ailments such as skin disorders, fever, and inflammation. Neem oil is a popular natural pesticide and insect repellent, effectively managing pests without

harming beneficial insects or the environment. Additionally, neem's ability to withstand arid conditions and improve soil fertility makes it a valuable resource for reforestation and combating desertification. The neem tree's versatility and sustainability highlight its importance in both ecological and health contexts.

Taxonomy Classification: [8]

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	Azadirachta
Species	A. indica

Aim:

- To formulate and evaluate a safe, effective, and long-lasting mosquito repellent gel using natural and/or synthetic ingredients that provide broad-spectrum protection against various species of mosquitoes.
- The gel should be easy to apply, non-greasy, skin-friendly, and suitable for use in diverse environmental conditions.
- The primary objective is to ensure the gel offers extended protection while minimizing potential side effects and environmental impact.

Objectives:

- Identify and select active ingredients with proven mosquito repellent properties.
- Ensure all ingredients are safe for human use and environmentally friendly.
- Develop a gel formulation that ensures the stable dispersion of active ingredients.
- Optimize the gel for consistency, skin absorption, and non-greasy application.
- Conduct laboratory and field tests to evaluate the effectiveness of the gel against different mosquito species.
- Compare the efficacy of the gel with existing commercial mosquito repellents.
- Perform dermatological tests to ensure the gel is non-irritating and safe for various skin types.

- Evaluate the potential toxicity of the gel to ensure it is safe for prolonged use.
- Conduct stability tests to ensure the gel maintains its efficacy and physical properties under different storage conditions.
- Assess user feedback on the gel's ease of application, scent, and overall feel on the skin.
- Make necessary adjustments based on feedback to improve user experience.
- Evaluate the environmental impact of the gel's ingredients and packaging.
- Ensure the formulation and packaging are environmentally sustainable.

PLAN OF WORK

PROPOSED METHODOLOGY

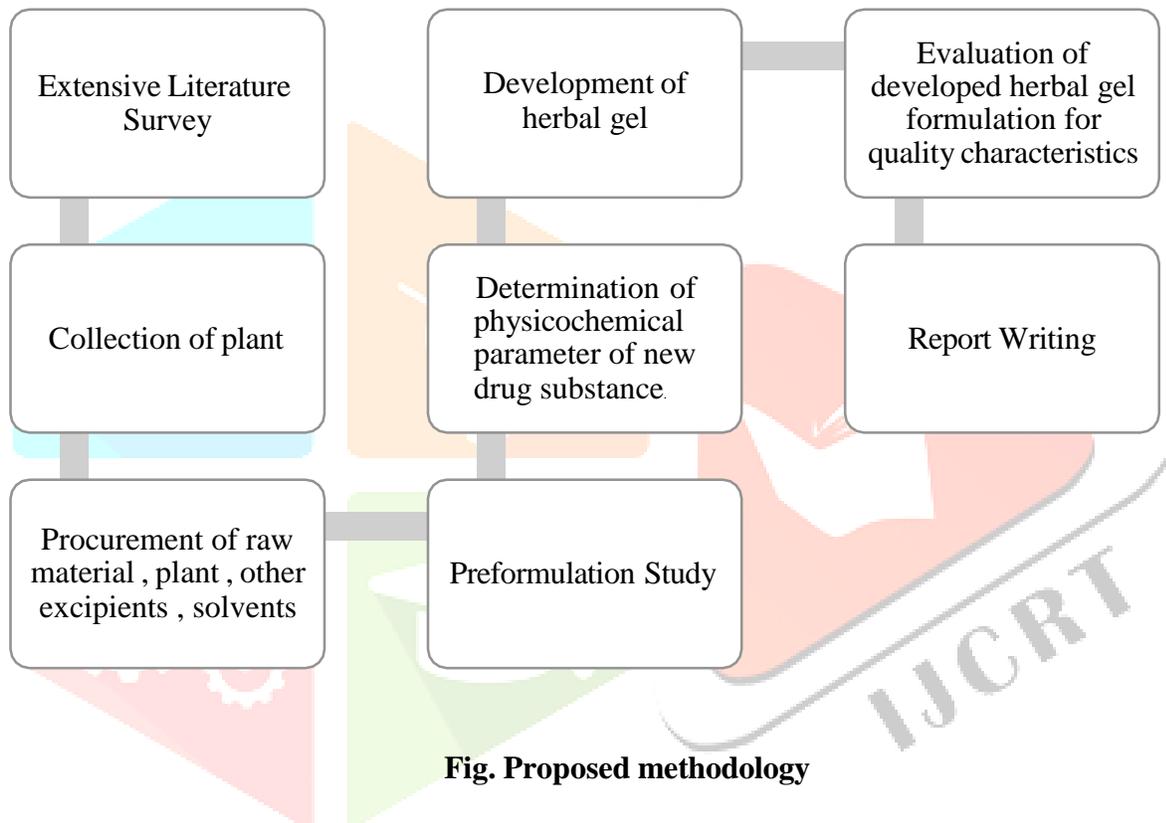


Fig. Proposed methodology

PLANT PROFILE:**TRIDAX PROCUMBENS:****Synonym:**

Amellus pedunculatus Ortega ex Willd., *Balbisia canescens* Rich. ex Pers., *Balbisia divaricata* Cass., *Balbisia elongata* Willd., *Balbisia pedunculata* Hoffmanns, *Chrysanthemum procumbens* (L.) Sessé & Moc.

Scientific Classification:

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Asterids
Order	Asterales
Family	Asteraceae
Genus	Tridax
Species	<i>T. procumbens</i>

Physical characteristics:**Height and structure:**

- Height: Generally grows up to 30-60 cm (12-24 inches) in height.
- Structure: It is a creeping, trailing plant with a spreading habit, often forming mats on the ground.

Leaves:

- Arrangement: Opposite or occasionally alternate.
- Shape: Leaves are ovate to lanceolate.
- Size: Typically 2-6 cm long and 1-3 cm wide.
- Margin: Coarsely toothed or serrated.
- Surface: Rough and hairy, with prominent veins.

Flowers:

- Type: Composite flowers, characteristic of the Asteraceae family.
- Structure: Consists of a central disc with small, yellow tubular florets, surrounded by white ray florets.
- Size: The flower head is about 1 cm in diameter.
- Blooming: Flowers year-round in favorable climates.

Fruit:

- Type: Achene, a small, dry, single-seeded fruit.
- Structure: Each achene is topped with a pappus of white bristles that aid in wind dispersal.
- Size: About 2-3 mm long.

Bark:

- Bark: As a herbaceous plant, it does not have a true bark like woody plants. The outer surface of the stems is green and hairy.

Wood:

- Wood: Being a herbaceous plant, *Tridax procumbens* does not have true wood. Its stems are soft, green, and herbaceous.

Stem:

- Structure: Thin, green, and covered with hairs.
- Growth: Prostrate or ascending, rooting at nodes that touch the ground, aiding in its spreading nature.

Root:

- Type: Fibrous root system.
- Structure: Roots are fine and spread widely in the soil, providing stability and nutrient uptake.

Chemical constituents:

Tridax Procumbens, Commonly Known As Coatbuttons, Has Been Studied For Its Diverse Range Of Chemical Constituents, Which Contribute To Its Medicinal Properties. The Plant Contains Flavonoids Such As Quercetin, Luteolin, And Apigenin, Which Are Known For Their Antioxidant And Anti-Inflammatory Effects. It Also Has Alkaloids, Including Beta-Sitosterol And Benzyl Alcohol, Which Exhibit Antimicrobial And Anti-Fungal Activities. Additionally, The Plant Contains Tannins, Saponins, And Carotenoids, Which Contribute To Its Pharmacological Activities. *Tridax Procumbens* Is Rich In Polysaccharides And Proteins That Have Been Shown To Have Immunomodulatory Effects. The Presence Of Essential Oils And Fatty Acids, Such As Palmitic And Linoleic Acids, Further Adds To Its Therapeutic Potential. These Chemical Constituents Collectively Make *Tridax Procumbens* A Valuable Plant In Traditional Medicine For Treating Various Ailments Such As Wounds, Infections, And Inflammation.

Pharmacological activities: [15,16]

- Anti-microbial activity
- Anti-inflammatory activity
- Anti-oxidant activity
- Wound healing activity
- Insect repellent activity

CYMBOPOGON CITRATUS :**Synonyms:**

Andropogon Cerifer Hack, Andropogon Ceriferus Hack, Andropogon Citratus Dc, Andropogon Citriodorus Desf. Nom. Inval, Andropogon Fragrans C.Cordem, Andropogon Roxburghii Nees Ex Steud.

Scientific Classification:

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Monocots
Clade	Commelinids
Order	Poales
Family	Poaceae
Genus	Cymbopogon
Species	C. Citratus

Physical Characteristics:

1. Height And Structure:

- Lemongrass Typically Grows In Dense Clumps That Can Reach A Height Of 2 To 4 Feet (60 To 120 Cm) Tall, Though In Ideal Conditions It Can Grow Taller.
- The Plant Has A Tufted Habit With Numerous Slender, Upright Stems Arising From A Central Base.

2 .Leaves:

- The Leaves Of Lemongrass Are Long, Narrow, And Lance-Shaped.
- They Are Typically About 1 Cm Wide And Can Grow Up To 1 Meter In Length.
- The Leaves Are Aromatic, Giving Off A Strong Lemon Scent When Crushed.

3. Flowers And Fruit:

- Lemongrass Produces Flower Clusters At The Tops Of Its Stems.
- The Flowers Are Small, Greenish-White, And Occur In Dense Spikes.
- However, Lemongrass Cultivated For Culinary And Ornamental Purposes Is Often Harvested Before Flowering, So Flowers And Fruits Are Not Commonly Seen.

4. Bark, Wood, And Stem:

- The Stems Of Lemongrass Are Smooth, Firm, And Cylindrical.
- They Are Fibrous And Contain A High Concentration Of Essential Oils That Contribute To The Plant's Fragrance And Flavor.
- The Outer Layer Of The Stems Can Have A Reddish Or Purple Tinge.

5. Roots:

- Lemongrass Has A Fibrous And Dense Root System.
- The Roots Grow Shallowly And Spread Out From The Base Of The Plant, Anchoring It Firmly In The Soil.

6. Bark And Wood:

- Lemongrass Is An Herbaceous Plant, Meaning It Does Not Have True Bark Or Woody Stems Like Trees Or Shrubs.
- The Stems Are Succulent And Green, Gradually Becoming Fibrous With Age.

Chemical Constituents:

Cymbopogon Citratus, Or Lemongrass, Contains A Variety Of Chemical Constituents That Contribute To Its Medicinal And Aromatic Properties. The Main Bioactive Compounds Found In Lemongrass Include Citral (A Mixture Of Geranial And Neral), Myrcene, Citronellal, Geraniol, And Limonene. Citral Is The Predominant Component Responsible For Lemongrass's Characteristic Lemony Scent And Flavor. These Compounds Are Concentrated Primarily In The Essential Oil Extracted From The Leaves And Stems Of The Plant. Lemongrass Essential Oil Is Valued In Traditional Medicine For Its Antimicrobial, Antioxidant, Anti-Inflammatory, And Antifungal Properties. It Is Widely Used In Herbal Teas, Culinary Dishes, And Aromatherapy. Studies Have Highlighted Its Potential Therapeutic Benefits, Such As Its Role In Treating Digestive Issues, Reducing Fever, And Even Possessing Anticancer Properties Due To Its Antioxidant Activity (Serafini Et Al., 2019; Bhalla Et Al., 2019). The Chemical Composition Of Lemongrass Underscores Its Significance Both In Traditional Medicine And Modern Scientific Research.

Pharmacological Activities:[17,18]

- Anti-bacterial activity
- Anti-fungal activity
- Anti-inflammatory activity
- Insect repellent activity

NEEM:**Synonyms:**

Antelaea azadirachta (L.) Adelb, *Antelaea canescens* Cels ex Heynh, *Antelaea javanica* Gaertn, *Azadirachta indica* subsp. *vartakii* Kothari, Londhe & N.P.Singh, *Melia azadirachta* L, *Melia fraxinifolia* Salisb, *Melia hasskarlii* K.Koch, *Melia indica* (A.Juss.) Brandis, *Melia japonica* Hassk, *Melia parviflora* Moon, *Melia pinnata* Stokes

Scientific Classification:

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Sapindales
Family	Meliaceae
Genus	<i>Azadirachta</i>
Species	<i>A. indica</i>

Physical Characteristics :

Height and Structure: Neem trees are typically medium to large-sized, reaching heights of 15 to 20 meters (about 50 to 65 feet) when fully grown. They often have a spreading canopy with a broad, rounded crown.

Leaves: The leaves of the neem tree are compound, consisting of multiple leaflets arranged alternately along the stem. Each leaflet is typically lance-shaped or elongated with serrated edges. The leaves have a vibrant green color and a somewhat coarse texture.

Flowers: Neem trees produce small, white, fragrant flowers arranged in dense clusters, typically appearing during the spring months. The flowers are often very aromatic and attract bees and other pollinators.

Fruits: Neem trees bear olive-sized fruits that are yellow or green when ripe. These fruits have a thin skin and contain a single seed encased in a fleshy pulp. The fruits are bitter to taste and have a somewhat pungent odor.

Bark: The bark of the neem tree is rough, furrowed, and dark gray to brown in color. It exudes a bitter taste and has a strong, pungent odor when crushed.

Wood: Neem wood is hard, durable, and termite-resistant, making it valuable for various purposes such as construction, furniture making, and agricultural implements.

Roots: Neem trees have deep and extensive root systems that enable them to withstand drought conditions and stabilize soil. The roots also play a role in nutrient absorption and water uptake.

Chemical Constituents:

Neem (*Azadirachta indica*) elaborates a vast array of biologically active compounds which are structurally complex and chemically diverse. Every part of this plant is used as herb. During the past two decades, the chemical constituents and biological activities of *Azadirachta indica* were intensively investigated in both developing and developed countries. Several published studies revealed a lot of biological compounds (Azadirachtin M and Azadirachtin N) and effects of these compounds on insects, and other inhibitory activities such as antitypanosomal and antiprotozoal. Extracted different constituents from the flowers of *Azadirachta indica* and analyzed their insecticidal activity, they found around 38 compounds in the flowers with insecticidal activity (such as: n-Hentriacontane, n-Nonacosane, n-Pentacosane, 2-Methoxy-5,40-dimethylbenzenebutanal, Methyl octadecanoate acid, etc). The group of tetranortriterpenoids, especially azadirachtin analogues is responsible for most of the active principles as it has many antiinfective and antimicrobial properties. The extracted chemical constituents of different parts of neem tree contained many biologically active compounds, including triterpenoids, alkaloids, phenolic compounds, flavonoids, carotenoids, ketones and steroid. The most biologically active compound is azadirachtin. This compound belongs to the C-seco limonoids which was classified as tetranortriterpenes.

Pharmacological Activities: [19,20]

- Anti-fungal activity
- Anti-bacterial activity
- Anti-oxidant activity
- Anti-inflammatory activity

Material and Instruments:**I. Instruments used for work:**

Sr.no.	Name of Instrument
1.	Soxhlet Apparatus
2.	Electronic weighing balance
3.	pH meter
4.	Brookfield viscometer
5.	Heating mantle
6.	Electronic waterbath

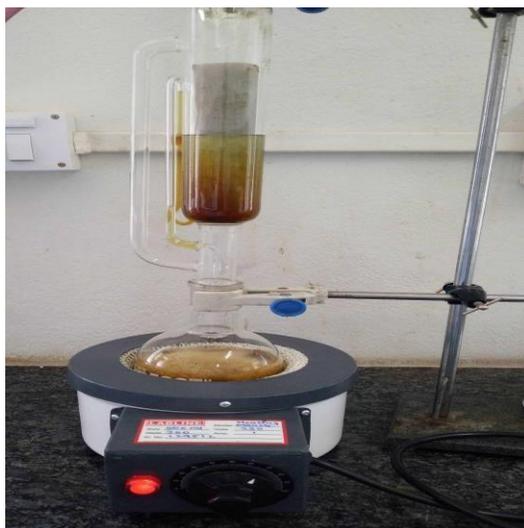
II. Chemicals used for work:

Sr. no.	Chemicals
1.	Ethanol
2.	Carbopol 940
3.	Propylene glycol
4.	Methyl paraben
5.	Triethanolamine
6.	Glycerine
7.	Distilled water

EXTRACTION

Tridax procumbens ethanolic extract preparation.

- The leaves were carefully chosen, cleansed to remove contaminants, and shade dried.
- The mechanical grinder ground the dried material into a fine powder.
- A 43-mesh sieve was used to filter the fine powder, which was then placed in an airtight container for later usage.
- Using the hot extraction method and the Soxhlet apparatus, about 40 gm of powdered material were extracted with ethanol as a solvent.
- The extraction was carried out until the solvent in the thimble turned clear, at which point a few drops were collected in the test tube during the cycle's conclusion and the solvent's chemical composition was tested.
- The extract was dried in a rotary vacuum evaporator following each extraction.
- Moreover, some part of the extract was preserved for preliminary Phytochemical screening.



Process of extraction using Soxhlet apparatus

PRELIMINARY PHYTOCHEMICAL INVESTIGATION:

The ethanolic extract was subjected to qualitative chemical investigation. The following procedures were adopted to test for the presence of various phytochemical constituents in the extract. Most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins, saponins and glycosides. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs. The following procedures were adopted to test for the presence of various chemical constituents in extract.

Test:

TEST FOR SAPONINS

- **Foam test:** A small amount of extract taken in a test tube with little quantity of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence of Saponin.

TEST FOR ALKALOIDS

- **Mayer's test:** 2-3 ml of filtrate with few drops of Mayer's reagent gives ppt.
- **Wagner's test:** 2-3 ml of filtrate with few drops of Wagner's reagent gives Reddish brown colour.

TEST FOR TANNINS

- **Ferric chloride test:** To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green colour indicates presence of Tannins.

TEST FOR STEROIDS

- **Liebermann's reaction:** Mix 3 ml extract with 3 ml acetic anhydride. Heat and cool. Add few drops of conc. H₂SO₄. Blue color appears.

TEST FOR FLAVANOIDS

- **Alkaline reagent test:** Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

TEST FOR TERPENOIDS

- **Salkowski reaction:** To 2 ml of extract, add 2 ml chloroform and 2 ml of conc. H₂SO₄. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

TEST FOR REDUCING SUGAR

- **Benedict's test:** Mix equal volume of Benedict's reagent and test extract in test tube. Heat in boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

TEST FOR PROTEINS

- **Biuret test:** Add 2ml of Biuret reagent to 2ml of extract. Shake well and warm it on water bath. Appearance of red or violet colour indicates presence of proteins. To 3 ml. extract add 4% NaOH and few drops of 1% CuSO₄ solution. Violate or pink colour appears.

Preformulation study

Preformulation studies are needed to ensure the development of a stable as well as effective and safe dosage form. It is a stage of development during which the pharmacist characterizes the physic-chemical properties of the drug substances and its interaction with various formulation components. Goals of Preformulation study:

- To determine the necessary physicochemical parameter of a new drug substance.
- To establish its incompatibility with excipients of formulation.

EXPERIMENTAL DESIGN

Formulation of mosquito repellent gel using natural volatile oils: Preparation of mosquito repellent gel:

1. Selection of excipients:

Cymbopogon Citratus oil and Azadirachta Indica oil is collected from ayurvedic shop from the kolhapur. The leaves of Tridax procumbens were collected from local farm from kolhapur. The raw materials and chemicals were taken from ashokrao mane institute of pharmacy, ambap, kolhapur.

All ingredients and excipients used are given in the table.

2. Method of preparation:

Different formulations of gels were formulated using gelling agents such as carbopol, HPMC, and HEC. Weighed all the actives and added one by one in the vortex in the beaker containing 3/4th ml of water and kept on a magnetic stirrer rotated at 300 rpm. Stir the mixture for 10 mins. Then add a weighed amount of carbopol in it. Continue the stirring for 20 mins until the mixture is free from carbopol lumps resulting in the gel. The mixture of triethanolamine (1 ml) and water (2.5 ml) was added dropwise to the gel to neutralize and thicken the gel.[21]

Formulation table:**Table no. 7: Formula for Herbal gel**

Sr.no.	Ingredients	Batches			Role of ingredient
		A1	A2	A3	
1	Ethanolic extract	1 gm	1 gm	1 gm	Therapeutic agent
2	Carbopol 940	2 gm	1.25 gm	1 gm	Thickner
3	Propylene glycol	5 ml	5 ml	5 ml	Humectant
4	Methyl paraben	0.3 gm	0.3 gm	0.3 gm	Preservative
5	Triethanolamine	qs	qs	qs	Nutralizer
6	Glycerin	1 ml	1 ml	1 ml	Vehicle
7	Distilled water	qs	qs	qs	Vehicle

Evaluation of gel:**Physical Evaluation:**

Physical parameters such as color and appearance were evaluated.

Washability:

The washability of formulations was examined by applying the gel on the skin and then evaluating the ease and the extent of washing it with distilled water and manually observing the effect.²²

Skin irritation test:

The formulated gel in the quantity of 0.5 g was applied to the normal hairless skin at an area of 6 cm² and then covered with a semi-occlusive bandage for the duration of 1 hr. After the application time, the bandage was removed, the applied gel was scrapped off completely, and the area was visually inspected for any rashes or similar symptoms. The test was done for a period of 7 days. The results were expressed in terms of grades.²³

Spreadability:

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. Weight of 1 kg was placed on the top of the slide for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 50 g. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 6.5 cm be noted. A shorter interval indicates better spreadability.

Spreadability was calculated using the following formula:

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

Where, S = Spreadability,

M = Weight in the pan (tied to the upper slide), L = Length moved by the glass slide and

T = Time (in sec.) taken to separate the slide completely each other.

Viscosity:

The viscosity of the formulation was determined by using the Digital Brookfield Viscometer using spindle no. 6 at 10 rpm and temperature of $25 \pm 1^\circ\text{C}$. A sufficient quantity of gel was filled in appropriate wide mouth container in such way that it should sufficiently allow to dipped the spindle and allowed to settle over 30 min before the measurements.²⁴

Swelling index:

The swelling index of the prepared dermal polyherbal gel was determined by taking 2 g of gel in a beaker containing 10 ml of distilled water. After 1 hr, the swelled formulation was removed from the beaker and was put on a petridish. The content was re-weighed and the swelling index was estimated from the formula:

$$\text{Swelling index (Si)} = W_t - W_o$$

Where, W_t = weight of swollen at t time; W_o = original weight of gel at zero time.

Mosquito repellent activity:

Initially, the forearm of volunteers was thoroughly washed with soap and completely dried. The left arm served as the control that was kept inside the mosquito cage. The frequency of the vector landed on the forearm in the duration of 30 seconds. If the mosquitoes landed >10 then the study was commenced. After 30 seconds of time duration, the arm was carefully removed from the mosquito cage. Afterward, the right arm smudged with mosquito repellent gel formulation was entered and analogously the study was performed at 30, 60, 120, 240, and 480 minutes. The number of mosquitoes that landed was determined and compared with the positive control (Odomos®). The study was performed in triplicate manner.[22-25]

Result and Discussion:**Collection and Authentication:**

The Leaves of *Tridax procumbens* were collected from the local farm from Kolhapur.

Extraction of Tridax Procumbens:**Extractive values of Tridax Procumbens**

Sample	Extraction method	Solvent used	Wt. of sample	Extraction value (%w/w)
Tridax procumbens leaves powder	Soxhlet extraction	Ethanol	30 gm	10% w/w

Physicochemical evaluation of Gel: 1] Physical Appearance**Physical appearance of gel**

Sr. no.	Batch	Color	Appearance
1	A1	Light Green	Green
2	A2	Green	Green
3	A3	Dark green	Green

All formulation batches were found to be homogeneous green gel preparations

3] washability and skin irritation:

Sr. No.	Batch	Washability	Irritability
1	A1	Good	No irritation
2	A2	Good	No irritation
3	A3	Good	No irritation

4] Spreadability

The time in seconds require to separate the two slides was taken as measure of spreadability.

pH and Spreadability of leaves extracts formulation.

Sr. No.	Batch	pH	Spreadability (gm.sm/sec)
1	A1	6.8 /±0.03	16.15/±0.005
2	A2	7.0 /± 0.03	15.40/±0.005
3	A3	7.1 /± 0.03	15.39/±0.005

5] Viscosity

Viscosity of gel was determined by using Brookfield rotational viscometer at 5, 10, 20, rpm. Each reading was taken after equilibrium of the sample at the end of two minutes. The samples were repeated three times.

Viscosity value of herbal gel

Sr. No.	rpm	Viscosity (Cps)
1	5	3615 ±0.11
2	10	3714 ±0.21
3	20	4137 ±0.43

6] Swelling index :

Sr .no.	Batch	Swelling index
1	A1	108
2	A2	115
3	A3	124

7] mosquito screening results of the formulated gel:

reatment stability	Repellency (%)			
	0	1hr	2hr	3hr
positive control	100±1.66	99.25±1.27	98.56±1.76	98.11±1.54
A1	84.29±1.86	82.38±1.13	81.55±1.21	79.43±1.33
A2	86.39±1.46	83.49±1.96	81.17±1.59	80.79±1.44
A3	87.37±1.15	86.13±1.55	85.94±1.88	85.16±1.99

Conclusion:

The mosquito repellent gel incorporating *Tridax procumbens*, *Cymbopogon citratus*, and *Azadirachta indica* plants offers a promising natural alternative to synthetic repellents. These plants have been traditionally recognized for their insect-repelling properties, validated by scientific studies demonstrating their efficacy against mosquitoes. *Tridax procumbens* provides a source of pyrethrins, *Cymbopogon citratus* contains citronella oil, and *Azadirachta indica* contributes azadirachtin, all of which act as effective repellents. The gel formulation ensures ease of application, potentially providing longer-lasting protection compared to traditional methods like sprays or lotions. Moreover, being plant-based, it is likely to be safer for human use and environmentally friendly compared to chemical alternatives. However, further research is necessary to optimize the formulation's efficacy, durability, and user-friendliness. Overall, the combination of these natural plant extracts in a gel form represents a promising direction in the development of effective and eco-friendly

mosquito repellents.

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