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Research Article

Formulation and Evaluation of Herbal Mosquito Repellent

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ABSTRACT

Herbal formulations involve combining herbs in specific quantities to offer therapeutic or cosmetic uses with minimal side effects compared to synthetic chemicals. Herbal Creams are semisolid emulsions, classified as oil-in-water (O/W) or water-in-oil (W/O) and are used for various purposes such as mosquito repellent and skin care. Mosquitoes, including species like Aedes and Culex are significant vectors for disease such as malaria and dengue, affecting millions of people globally. Effective mosquito control often involves repellents, which can be natural or synthetic. Natural options like those using Neem and Lemongrass extracts are being explored as safer, biodegradable alternatives to synthetic chemicals like DEET. Herbal mosquito repellent creams offer a promising solution to prevent mosquito bites and associated diseases, minimizing health risks compared to conventional repellents. The aim of the study was to develop an herbal mosquito repellent cream using Neem extract and essential oils from Clove and Lemongrass. The plant profiles include Neem (Azadirachta indica), known for its antimicrobial and mosquito-repellent properties; Lemongrass (Cymbopogon citratus), used for its insecticidal and antimicrobial benefits; and Clove (Eugenia caryophyllus), recognized for its insecticidal properties. The extraction methods involved maceration for Neem and Clevenger apparatus for the Essential oils. The cream was prepared using a water-in-oil emulsion method with ingredients including Beeswax, liquid paraffin, and coconut oil in the oil phase, and glycerin, borax, and rose water in the aqueous phase. Three formulations (F1, F2, and F3) were tested, with F1 showing the best stability, no phase separation, and no irritancy, making it suitable for further use. The cream demonstrated effective mosquito repellent properties in cage tests and exhibited favorable physical characteristics like smooth texture and good spreadability. The pH was found and no foreign particles were detected. The study highlights the potential of plant-based repellents in offering a safer alternative to synthetic products, with Neem's active compound, Azadirachtin playing a crucial role in repelling mosquitoes.

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INTRODUCTION

Before synthetic ointments came into use, plants and their derivatives were being used to kill the pests of agriculture, veterinary and public health importance. The use of plant-based embrocation is encouraged as they are comparatively less detrimental to the environment, biodegradable and have minimal or no use of a synthetic chemical which may result in causing a health hazard to the user. Moreover, many potent plants having biologically active constituents that can lead to the discovery of new insecticidal preparations. Therefore, plant materials have attracted significant interest in mosquito control programs in recent times.

Plant derivatives such as creams, essential oils, coils, incense, mist based products having plant-based derivatives have shown up in markets with high demand as compared to synthetic products. This has provided researchers with new scopes in the field to come up with more plants possessing mosquito repellent properties. Many polyherbal formulations having added qualities have also become the central element for recent research in this sector.

A mosquito repellent is a substance applied to skin, clothing or other surfaces that rejects insects (and arthropods in general) from infecting that surface. As most of the mosquito repellent products and devices are either of synthetic origin or have an evident effect on human health leading to several complications, the need for a repellent that is more effective and less harmful has arisen.

Herbal formulations are dosage forms that contain one or more raw or processed herbs in predetermined amounts to offer targeted nutritional or cosmetic advantages for the diagnosis, treatment, or mitigation of human or

animal illness. Herbal preparations are said to be more effective and to have fewer negative effects than any other synthetic chemical preparations since they are made by extracting the active ingredients from the biological sources of herbal plants.[8] Numerous illnesses spread by mosquitoes have been documented in our nation. Each year, millions of people worldwide are afflicted by mosquito-borne illnesses, which are mostly spread by these arthropod vectors.

Aedes, Culex, and Anopheles mosquitoes pose a severe threat to public health since they are known to carry a variety of viruses, bacteria, and protozoa that can cause life-threatening illnesses including dengue, chikungunya, malaria, yellow fever, and Japanese encephalitis.[2] Because mosquito borne illnesses are becoming more common, more people are using repellents against mosquitoes every day. In the nation, repellents such as vaporizing mats, sprays, mosquito coils, and liquid vaporizers are often employed.

Nowadays majority of serious diseases or infections are caused by mosquitoes mainly female anopheles' mosquito like Malaria, Dengue, Chikungunya, Lymphatic Filariasis, Zika, etc.

MALARIA: Malaria is an arthropod borne disease caused by plasmodium parasite (P.vivax, P.falciparum, P.malariae, P.ovale) transmitted by the infected mosquito. Major Vector: Anopheles stephensi, A. culicifacies.

DENGUE: Dengue (break-bone-fever) is a viral infection caused by flavi virus (DEN-1, DEN-2, DEN-3, DEN-4) serotypes. Major Vector: Aedesegypti, Aedesalbopictus.

CHIKUNGUNYA: Chikungunya is an illness caused by a virus called chikungunya. Transmitted to people through the bite of infected mosquito Major Vector: Aedesalbopictus, A.aegypti.



LYMPHATIC FILARIASIS: This disease is caused by filariadea type round worm parasite (Wucheresiabancrofti). Major Vector: Anopheles culex ,Aedes and Monsonia.

ZIKA VIRUS: Zika virus is a mosquito-brone virus associated with a birth defect called microcephaly.

Major Vector: Aedesaegypti , Aedesalbopictus. Dizziness Nausea and Vomiting Breathless Itching and Rash to prevent this disease there are multiple conventional Synthetic Mosquito Repellent in Market. This synthetic formulations consist of various hazardous chemicals that causes various acute and chronic health problem in humans and may lead to environmental pollution.

Numerous plant extracts have been shown to be highly effective in keeping mosquitoes away and to have mosquitocidal properties against mosquito vectors. Neem extract can effectively suppress mosquito populations in place of synthetic insecticides by killing Aedes pupae at very low doses.[2] The most popular natural repellents in the world are made by Cymbopogon.[13] Additionally, clove oil has insecticidal and repellent properties against a wide variety of pest species.[12] As a result, two distinct plant leaves— lemongrass, and neem leaves—have been chosen for use in this study as the best environmentally friendly method of controlling mosquitoes.

A chemical that is applied to skin, clothes, or other surfaces to deter mosquitoes from landing there is known as a mosquito repellent. Their active component serves as the sole means of keeping mosquitoes away by obstructing their ability to detect lactic acid and carbon dioxide, which are generated when a person perspires.[7] The aim of present study was to formulate a herbal cream using Neem extract, Essential oils of Clove &

Lemongrass as active ingredients for a potent mosquito repellent property.

MATERIALS AND METHODS

COLLECTION OF PLANT MATRIALS

1. NEEM

Fresh leaves of neem were collected and the leaves were cleaned and dried in room temperature at 25-27oC for 2 weeks. All dried leaves were subjected for grinding by using grinder mixer and after that screening of powder was done passing through sieve no: 60 to get very fine powder. Then the powdered material were preserved in an air tight container [6, 17, 2]

2. LEMONGRASS

Plants were cut about 10 -20 cm above the ground level. The first harvested was taken 6-9months after planting. The grasses were harvested in morning during dry periods. The leaves were separated from its stalks and air-dried at room temperature after which it was cut into small pieces. [3, 13]

3. CLOVE

From the age of six to seventy-nine, the drug may be gathered annually. As clove buds develop, their color changes. After being gathered, the drugs are removed from their stalks and spread out on coconut mats to dry in the sun. When the buds dry, they lose almost 70% of their weight and turn a dark reddish brown in color. [14]

EXTRACTION

EXTRACTION OF LEMONGRASS, NEEM AND CLOVE BY CLEVANGER APPARATUS



Weighed quantity of materials (lemongrass: 30g, clove & tulsi: 50g) was taken in a sample flask and required quantity of water was added (lemongrass & tulsi: 300ml, clove; 250ml). Connected the flask with Clevenger apparatus and opened the tap water for running the flow water in a condenser. Started heating on a heating mantle and controlled the heat so that oil with water vapours comes into the graduated distillate receiving tube and excess water goes back into the flask. Continued heating for 8 hours, cooled the assembly and removed the water from the distillate receiving tube, and the oil was taken into the rotary evaporator for removing the traces of water and cooled the flask in a desiccator. After cooling, it was weighed accurately. [3]

FORMULATION CONSIDERATION OF CREAM

WATER

This is one of the most important materials used in the cream formulation. It is the cheapest and easily available and are used as vehicle in the formulation. It is used in preparation of cream to dissolve other ingredients. Water can also form emulsion which can be water-in –oil or oil-in – water emulsion depending on the quantity of water used. [1]

MINERAL OIL

Mineral oil is widely used in preparation of cosmetics rarely causes allergic reactions and do not clog pores of the skin. It is also used as moisturize the body as it reduces water loss from the body. Some examples of mineral oil used in cream formulation are light liquid paraffin, heavy liquid paraffin, liquid petroleum.[1]

WAXES

Waxes are used in preparation of cream include beeswax, carnauba wax, spermaceti etc. Wax is used in cosmetics because it prevents the separation of liquid and oil components in an emulsion. These waxes also increase the thickness of the liquid portion and sticks on the surface of skin. [1]

GLYCERIDE OIL

Glyceride oil is mostly vegetable oils. Examples of glyceride oils are almond oil, arachis oil, castor oil, coconut oil etc. [9]

VEGETABLE OILS

It forms a barrier on the surface of skin and slow down the loss of water helping to maintain plumpness of skin. Vegetable oil may also be used to increase the thickness of the liquid or oil portion of cream. Examples almond oil, sunflower oil. [9]

EMOLLIENTS

Emollients, also commonly referred as moisturizers, are product that helps to soften skin or to treat skin that has become dry. They work by increasing the ability of skin to hold water, providing the skin with layer of oil to prevent water loss and lubricating the skin. They are moisturizing treatments applied directly to the skin to smooth and hydrate it. [9]

HUMECTANTS

These are important multifunctional ingredients found in most skin care formulations. Humectants are hygroscopic organic compound. These are the substances with the ability to absorb and hold moisture. These has many benefits such as moisturization, exfoliation etc. Examples of humectants are glycerin, sodium PCA. [9]

PRESERVATIVES



Preservatives are the essential part of cosmetics to prevent the growth and alterations caused by microorganisms. Contamination during formulation, shipment, storage and consumer use can be prevented using preservatives. Antioxidant is also used to protect from alteration caused by exposure to oxygen. The most commonly used synthetic preservatives are effective to prevent the growth of microorganisms in the product even at low concentrations. [1]

FORMULATION OF HERBAL MOSQUITO REPELLENT CREAM

Preparation of Oil Phase

1. Weigh 2.8g of beeswax and place it in a china dish.
2. Add liquid paraffin and other oil-phase ingredients like coconut oil to the china dish.
3. Heat the mixture in a water bath at 70°C until completely melted.
4. Just before mixing with the aqueous phase, add 0.5g Neem extract and essential oils

INGREDIENTS	Quantity (25g) F1	Quantity (25g) F2	Quantity (25g) F3
Beeswax	3.8g	2.9g	3.7g
Liquid paraffin	12ml	9ml	9ml
Clove oil	1ml	1ml	1ml
Lemongrass oil	0.5ml	1ml	1ml
Glycerine	2ml	3ml	--
Coconut oil	2ml	2.5ml	4ml
Borax	0.2g	0.5ml	0.6g
Neem oil	0.5g	0.5g	0.1g
Rose water	2.5ml	3.6ml	4.6ml

(Clove oil, Lemongrass oil, Tulsi oil) to the oil phase.

Preparation of Aqueous Phase

1. Add borax to the aqueous phase, which consists of glycerin and rose water.
2. Heat the aqueous phase in a water bath at 70°C, ensuring uniform heating.

Emulsification Process

1. Slowly add the aqueous phase to the oil phase with rapid stirring to create a uniform emulsion.
2. Stir the mixture continuously to ensure proper blending of the two phases.

Cooling & Packaging

1. Allow the emulsion to cool down gradually.
2. Once the temperature drops to 30°C, transfer the cream into a suitable container for storage.

EVALUATION

PHYSICAL PROPERTIES

The color, odor, and appearance of the cream were examined.

DILUTION TEST

A small amount of cream was taken in two test tubes one of the test tubes is diluted with water and other with oily medium. Both of these test tubes were shaken for few minutes and then observed to determine the type of emulsion.

PRESENCE OF FOREIGN PARTICLES



A little amount of herbal cream is taken and spread on a glass slide and which is then observed against light.

CAGE TEST

The effectiveness of mosquito repellent cream was tested commonly by the cage test method. By performing the experiment, the effectiveness of mosquito against the repellent cream can be found out. While conducting the experiment mosquitoes are placed inside the cage. Mosquitoes needed for the experiment are caught using net while biting the humans. Repellent formulation was applied and rubbed evenly. Then arm is inserted into the cage and exposed to mosquitoes. The number of mosquitoes that land and probe is observed and recorded.

STABILITY STUDIES

PHASE SEPARATION:

The cream to be tested was placed in a closed container away from sunlight and the phase separation was observed every 24 hours for 30 days.[8]

pH DETERMINATION:

The pH meter was calibrated. The pH measurement of the cream was carried out by dissolving 0.5g of cream in 50ml of distilled water and the measurement was done using a digital pH meter.[8]

IRRITANCY TEST:

The cream was applied to the specific area on the dorsal surface of the left hand and the time was noted. Irritancy, edema, erythema is tested at regular interval for 24 hours.

HOMOGENEITY:

Homogeneity of the formulated cream was evaluated by visual appearance and touch. The consistency is determined by examining its softness, greasiness, and stickiness. [11]

SPREADABILITY:

A small sample of cream was taken in a glass slide and it was compressed between two glass slides to uniform thickness. The time in which the upper glass slide moved over the lower slide is calculated as measure of spreadability. [11]

$$S = d^2 \times \pi/4$$

Where, S = spreading area depending on mass (mm²) d = spreading area diameter (mm)

RESULT AND DISCUSSION

1. ORGANOLEPTIC EVALUATIONS

The physical properties and all formulated cream were evaluated by its color, odor, and texture. The results are tabulated below:

Sr. No	Formulation	Colour	Odor	Texture
1.	F1	Olive Green	Characteristic Odor	Smooth
2.	F2	Olive Green	Characteristic Odor	Smooth
3.	F3	Olive Green	Characteristic Odor	Hard

2. PRESENCE OF FOREIGN PARTICLE

All the prepared formulations are spread on glass slide and which is tested against light and no foreign particle was observed.





3. TYPE OF EMULSION

Tests were performed to determine the type of emulsion and were found to be water in oil emulsion.

STABILITY STUDIES

1. PHASE SEPARATION ANALYSIS OF PREPARED FORMULATION

Sr. No	Formulation	Observation
1	F1	No phase separation
2	F2	Phase separation
3	F3	Phase separation

From this it was concluded that F1 shows no phase separation while F2 and F3 formulation is unstable. Therefore, F1 is used for the further studies.

2. IRRITANCY TEST

Sr. No	Formulation	Observation
1	F1	No irritancy

3. HOMOGENEITY

Homogeneity of formulated herbal cream was evaluated by visual appearance and touch. The visual appearance and touch were good

4. SPREADABILITY

The prepared herbal cream formulation was tested for spreadability and the area covered by the fixed amount of cream was found to be 9.62mm².

5. pH DETERMINATION

The pH of the formulated herbal cream was found to be 5.

CONCLUSION

Mosquitoes are vector for humanities most deadly illnesses and was most disturbing blood sucking insects affecting the health of the individuals. Plant derived repellents usually do not show hazards of toxicity to humans and are easily biodegraded. By promoting more use of natural repellents, one can reduce the noxious effect of synthetic mosquito repellent on human health and environment. Neem extract contain active compound azadirachtin, which has the ability to act as natural insecticide. Herbal formulations are growing demand in the world market. The formulated cream was evaluated for parameters like spreadability, stability, pH, and irritancy. The appearance of the cream was good and has a smooth texture. Irritancy test was conducted and show no sign of redness and itching which indicate that the cream was safe for topical application. The present study has shown that leaves of Neem and oil of Lemongrass, Clove has potent mosquito repellent activity.

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