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Original Article

Formulation and Evaluation of Polyherbal Mosquito Repellent Cream

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ABSTRACT

Nowadays majority of the disease or infection like malaria, dengue etc. caused by the different types of mosquitoes mainly female Anopheles mosquito to prevent these infection or diseases there are multiple synthetic pesticides are used in market. The various pesticides used as a mosquito repellent containing hazardous chemicals that causes the various health problems in humans and other living creatures, that pesticides also cause the environmental pollution by considering these facts we are here with mosquito repellent without hazardous chemicals known as Polyherbal Mosquito Repellent Cream as the herbs are easily accessible in our society, it is very beneficial to use these poly-herbs in mosquito repellent formulations. The herbs are the core substance in our formulations, there are different active constituents such as alkaloids, fixed oils, essential oil, resins, flavonoids, phenols, etc, which is present in specific parts of herbs as it is extracted by various methods like steam distillation, pressing techniques, solvent evaporation etc. Our study aims at investigating the herbs which has mosquito repellent activity but does not cause the health hazard, environmental hazard and pollution, those herbs which have mosquito repellent activity, which are used in specific proportion depending on their mosquito repellent activity in herbal mosquito repellent for formulations have been developed.

Keywords: Polyherbal formulation, Mosquito repellent cream, Essential oils, Cymbopogon nardus, Eucalyptus Plant-based repellent

INTRODUCTION

Mosquitoes such as Aedes, Anopheles, and Culex are a serious threat to the public health as they are known vectors for various protozoans, viruses, and bacteria which result in many life- threatening diseases like malaria, filariasis, yellow fever, Japanese encephalitis, chikungunya, and dengue. These vectors have been considered as a major obstacle to socioeconomic development of developing countries particularly in the tropical region. Despite considerable efforts in recent years to control vector-borne diseases, malaria alone produces 250 million cases per year and 800,000 deaths including 85% children under five years (WHO, 2010). Therefore, the prevention of mosquitoes could be better than the cure of vector-borne disease. Hence, use of the mosquito repellents on exposed skin area is strongly recommended. Insect repellents usually work by providing a vapor barrier deterring the arthropod from encountering the skin surface. Most of the commercial mosquito repellents are prepared using nonbiodegradable, synthetic chemicals like N, N-diethyl-3-methylbenzmide (DEET), dimethyl phthalate (DMP), and allethrin, which may lead to their higher exposure to the environment and, hence, the unacceptable health risks. With an increasing concern on public safety, a renewed interest on the use of natural products of plant origin is desired because natural products are effective, environment friendly, biodegradable, inexpensive, and readily available. Many plant extracts have been identified as having insect-repellent effects and there has been increasing research in the last decade proving plant-based mosquito repellents are just as, if not more effective than DEET like Citronella Oil, Neem Oil etc. Plant products are emerging as a potential source of mosquito control and among them essential oils have special interest due to their insecticidal or repellent properties. Essential oils are volatile, naturally occurring, complex compounds characterized by a strong odour and are formed by aromatic plants as

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secondary metabolites. They are liquid, volatile, rarely coloured, lipid soluble and soluble in organic solvents with a density generally lower than that of water. There are 17,500 aromatic plant species among higher plants and approximately 3,000 essential oils are known out of which 300 are commercially important for pharmaceuticals, cosmetics, and perfume industries apart from pesticide or repellent potential. Owing to the attraction for natural products like essential oils, despite their wide use and being familiar to us as fragrances, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture, and the environment. Some of them constitute effective alternatives or complements to synthetic compounds of the chemical industry without showing the same secondary effects. In nature essential oils play an important role in the protection of the plants as antibacterial, antiviral, antifungal, and insecticides. Hence the present study aims to develop an ecofriendly mosquito repellent, a substitute for chemical repellent by using different essential oils and to produce the natural repellent which is more cost effective, cheap and keeps the environment pleasant and health friendly. The aim of the present study is to formulate and evaluate a polyherbal mosquito repellent cream. The main objective of the study is to formulate a polyherbal mosquito repellent cream consisting of various herbal constituents such as Citronella oil, Eucalyptus oil, Tulsi oil, clove oil, neem oil, Sweet orange extract, Turmeric extract, etc. which exhibit mosquito repelling character.

Drug Profile

***Cymbopogon nardus* (Citronella)**

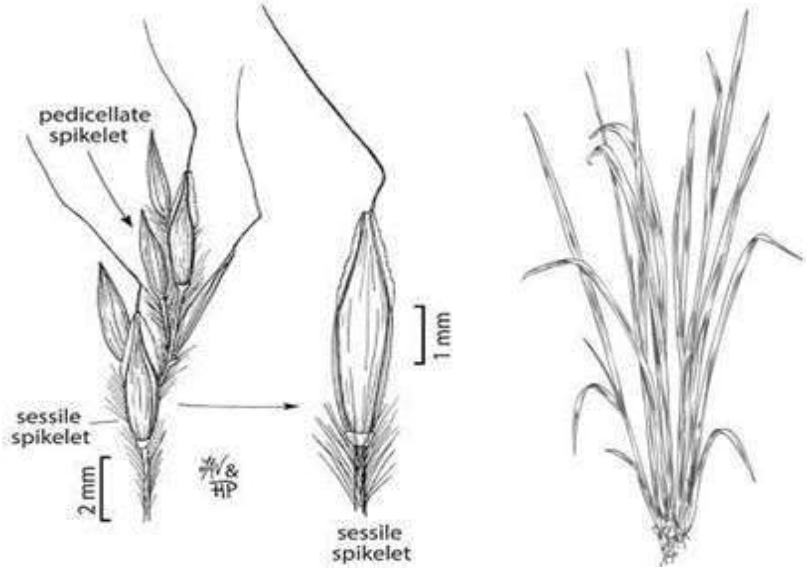

Figure 1: *Cymbopogon nardus.*

Kingdom: Plantae
Order: Poales
Family: Poaceae
Genus: Cymbopogon
Species: C. nardus

Cymbopogon nardus or Citronella grass (S. Pengiri) is a coarse and clump-forming tropical grass native to Sri Lanka. Citronella grass can grow up to 1.5-1.8 m tall and citronella stems are like canes. Citronella essential oil is extracted by steam-distillation of citronella leaves. Citronella essential oil is used as an insect repellent and it can be found in dozens of registered pesticide products such as sprays, lotions, and candles. Due to its antifungal properties, citronella oil is also used to treat insect bites. Additionally, citronella essential oil is one of the most common oils used in aromatherapy as it has an ability to treat and prevent fever and headache. Because of citronella oil's antiseptic properties, it is used in soaps, household cleaners and detergents. The main components of citronella essential oil are monoterpene hydrocarbons and alcohols such as geraniol (18-20%), citronellal (5-15%), citronellol (6.4- 8.4%), limonene (9-11%) and geranyl acetate (2%). Additionally, methyl eugenol, camphene, nerol, borneol, citronellic acid and citral are also present in citronella essential oil.

Eucalyptus globulus

Figure 2: *Eucalyptus globulus.*

Kingdom: Plantae
Order: Myrtales
Family: Myrtaceae
Genus: Eucalyptus
Species: E. globulus

Eucalyptus globulus (S. Eucalyptus) is named as "Blue Gum" and it is a medium to very tall forest tree which may reach 70 m in ideal conditions. However, Eucalyptus globulus is more commonly 15-25 m in height and it has a rough, greyish bark which is shed on the upper trunk and branches in long ribbons. Eucalyptus globulus leaves are glossy, dark green, thick, and leathery. The white flowers occur from winter to early summer and they are followed by hard, woody-capsules containing greyish fruits which are named as "Gum Nuts." Eucalyptus essential oil which is extracted by steam-distillation of eucalyptus leaves is taken orally for pain and inflammation of respiratory tract mucous membranes, coughs, asthma, and bronchitis. It is also used as an antiseptic, insect repellent and treatment option for wounds. Eucalyptus oil is also popularly used as a fragrance in perfumes and cosmetics and it is found in mouthwashes, toothpastes and cough drops as well. The main chemical constituent present in eucalyptus oil is 1,8-cineole (60-70%). In addition, α-pinene (9%), β-pinene (0.4%), limonene (0.04%), terpinen-4-ol (0.3%), aromadendrene (2.5%), epiglobulol (0.4%) and globulol (2.7%) are also present in eucalyptus oil.

***Curcuma longa* (Turmeric)**

Figure 3: Curcuma longa

Kingdom: Plantae
Order: Zingiberales
Family: Zingiberaceae
Genus: Curcuma
Species: C. longa

Curcuma longa (Turmeric) is an upright, relatively short and stout plant that rarely grows more than about 1 m in height. Its leaves are elongated, dark green, and pointed, often curling slightly along the margins. Each leaf arises on an individual stalk directly from the fleshy rhizome at its base. The rhizome appears scaly due to the remaining rings of previous leaves. Its outer skin is brownish but its flesh is deep orange-yellow inside. Rhizomes grow to about 5-8 cm x 1.5- 2.5 cm the flower stalk will appear among the leaves, also directly rising from the rootstock. The yellow-reddish flowers are arranged spirally along the cylindrical spike which may be partially protected by a leaf sheath. Turmeric is mainly used in culinary preparations and for medicinal purposes. For chemical purposes, it is used as an indicator and can identify a base. Turmeric turns red when it comes in contact with a base. Monoterpene hydrocarbons (46.9%) constitute the bulk of the Curcuma longa rhizome essential oil which is obtained by hydro-distillation and has antiseptic and anti- carcinogenic properties. Major constituents of the essential oil are β-bisabolene (13.9%), transocimene (9.8%), myrcene (7.6%), 1,8-cineole (6.9%), α-thujene (6.7%) and thymol (6.4%).

***Ocimum sanctum* (Tulsi)**

Figure 4: *Ocimum sanctum*

Kingdom: Plantae
Order: Lamiales
Family: Lamiaceae
Genus: Ocimum
Species: O. tenuiflorum

Ocimum sanctum or Tulsi is a branched sub-shrub which is 30-60 cm tall, with simple opposite green or purple leaves that are strongly scented. Tulsi leaves have petiole and are ovate, up to 5 cm long, usually slightly toothed. Flowers are purplish in elongate racemes in close whorls. Tulsi is native throughout the tropics and wide-spread as a cultivated plant. It is cultivated for religious and medicinal purposes and for its essential oil. Tulsi essential oil is used as a medicine for fever, headache, lung disorders, heart disorders and many other diseases. Tulsi essential oil has antibacterial, anti-viral and anti-fungal properties as well. Tulsi essential oil is also used in massage oils, perfumes, aromatherapy baths, soap making and candle making. Tulsi leaf essential oil contains methyl eugenol (46-68%), (E)- caryophyllene (17-27%) and β-elemene (16.3%) as the major constituents.

***Syzygium aromaticum* (Clove)**

Figure 5: *Syzygium aromaticum*

Kingdom: Plantae
Order: Myrtales
Family: Myrtaceae
Genus: Syzygium
Species: S. aromaticum

Syzygium aromaticum (Clove) is a triangular-shaped evergreen tree growing about 12 m tall with a smooth grey bark and about 12 cm long, glossy opposite leaves that resemble bay leaves. The attractive red and white bell-shaped flowers have four tiny petals surrounded by a long, four- parted calyx and numerous stamens. The clove buds are pink but calyx changes from yellow to deep red after the stamens fall. The fruit, called mother of cloves, is an edible purple berry about 2.5 cm long. The entire Syzygium aromaticum plant is extremely aromatic. Cloves are used in cooking, either in whole or in ground form as a spice throughout Europe and Asia. Clove essential oil is widely used and well known for its medicinal properties. Traditional uses of clove oil include use in dental care as an antiseptic and analgesic. The oil is active against oral bacteria associated with dental caries and periodontal diseases. Additionally, clove oil has anti-carcinogenic, anti-allergic, antioxidant and insecticidal properties. The major constituent in clove bud oils is eugenol (72.08-82.36%) and eugenyl acetate is the second major component (8.6-21.3%). In addition, β-caryophyllene (2.76-8.64%) and α-humulene (0.34- 1.04%) also see present in clove bud oil.

***Citrus sinensis* (Sweet Orange)**

Figure 6: *Citrus sinensis*

Kingdom: Plantae
Order: Sapindales
Family: Rutaceae
Genus: Citrus
Species: C. sinensis

Citrus sinensis or Sweet Orange is a small shallow-rooted evergreen tree which is about 6-13 m in height with enclosed conical top and spiny branches. Citrus sinensis leaves which are 5- 15 cm long and 2-8 cm wide are dark green in colour, glossy and oval shaped.

Greenish-white Citrus sinensis flower is small and it has five petals. Citrus sinensis fruits are reddish-green to yellowish-green in colour and consist of a leathery and tightly adhered peel which is 6 mm thick. Citrus sinensis peel protects the juicy inner pulp of the Sweet Orange fruit. Sweet Orange peels, leaves and flowers contain fine essences of oils that are used in the manufacture of cosmetic products and medicines. The main chemical components of Sweet Orange peel essential oil are α-pinene, sabinene, myrcene, limonene, linalool, citronellal, neral and geranial.

***Azadirachta indica* (Neem)**

Figure 7: *Azadirachta indica*

Kingdom: Plantae
Order: Sapindales
Family: Meliaceae
Genus: Azadirachta
Species: A. indica

Azadirachta indica is an evergreen and fast-growing tree that can reach a height of 15-20 m. The Neem branches are spread widely. The fairly dense crown is roundish or oval. The white and fragrant flowers arise from the junction of the stem and petiole. An individual flower is 5- 6 mm long and 8-11 mm wide. Neem fruit is a smooth olivelike drupe. The fruit skin is thin and turns yellow when ripe. The bitter-sweet pulp is yellowish-white. The white, hard inner shell of the fruit encloses one, rarely two or three, elongated seed having a brown seed coat. All parts of the tree such as seeds, leaves, flowers and bark, are used in the preparing of various different medical preparations. Neem products have medicinal properties that prove to be anti- fungal, anti-diabetic, antibacterial, antiviral and anti-fertility. Limonoid compounds contained in Neem seed extract seem to have the insecticide and pesticide properties. The main limonoid in Neem seed extract is azadirachtin. Additionally, azadiradione, fraxinellone, nimbin, salannin, salannol, vepinin and vilasinin are also present in Neem seed extract. Bitter property of the Neem oil is due to tannins, flavonoids and sesquiterpene derivatives.

***Vitex negundo* (Nika)**

Figure 8: *Vitex negundo*

Kingdom: Plantae
Order: Lamiales
Family: Verbenaceae
Genus: Vitex
Species: V. negundo

Vitex negundo or Nika is a branched, small and slender tree which grows up to 4.5-5.5 m in height, flourishing mainly in the Indian subcontinent. Vitex negundo leaf upper surface is green and the lower surface is silvery in colour. Flower is bluish purple and becomes black when ripe. All parts of the plant, from root to fruit, possess a multitude of phyto-chemical secondary metabolites which impart an unprecedented variety of medicinal uses to the plant. The hexane extract of Vitex negundo leaf contains viridiflorol (19.55%), β-caryophyllene (16.59%), sabinene (12.07%), 4- terpineol (9.65%), γ-terpinene (2.21%), caryophyllene (1.75%), 1- ocetene-3-ol (1.59%) and globulol (1.05%). Ethanol extract of Vitex negundo leaf contains flavonoids as the major constituents along with a few terpenoids.

MATERIAL AND METHODOLOGY

Collection

The ingredients; Citronella oil, Eucalyptus essential oil, Turmeric, Tulsi essential oil, clove oil, Sweet Orange peel essential oil, Neem oil, Nika extract used in the preparation of 50g polyherbal mosquito repellent cream were extracted using appropriate methods.

Ingredients and its Role

Table No.1: List of ingredients and its purpose.

Ingredients	Role of ingredient	Quantity
A] *Oil Phase*:		
Citronella oil	Mosquito repellent	1.5 ml
Eucalyptus oil	Mosquito repellent	1.5 ml
Tulsi oil	Mosquito repellent	0.5 ml
Clove oil	Mosquito repellent	0.2 ml
Sweet orange peel oil	Mosquito repellent	0.5 ml
Turmeric oil	Mosquito repellent	0.2 ml
Nika extract	Mosquito repellent	1 ml
Neem extract	Mosquito repellent	0.2 ml
Cetyl alcohol	Stiffening agent, Thickener	5 g
Stearic acid	Emulsifier	10 g
Beeswax	Emollient, Stabilizing agent	6.25 g
Liquid paraffin	Emollient, Lubricating agent	25 ml
B] Water Phase		
Glycerine	Humectant	2.5 ml
Potassium hydroxide	surfactant	3.75 g
Methyl paraben	Plasticizer, preservative	0.5 g
Water	Vehicle	12.5 g

Equipment Required

Table No.2: List of equipment

1.	Digital weighing balance
2.	Water bath
3.	pH meter
4.	Microscope
5.	Bunsen burner
6.	Glass rod or Stirrer
7.	Beakers
8.	Measuring Cylinders
9.	Thermometer

10.	Petri dishes.
11.	Container

Preparation of Herbal Mosquito Repellent Cream

1. Preparation of Oil Phase
   i. Beeswax, Paraffin oil, Cetyl alcohol, Stearic acid, and all the prepared essential oils are added in a beaker kept over the water bath which is set on the temperature of 70 degree Celsius.
2. Preparation of Water Phase
   i. Aqueous ingredients such as glycerine and water-soluble ingredients such as Potassium hydroxide and Methyl paraben are mixed and heated simultaneously at the same temperature.
3. Mixing Sequence
   i. Prepare aqueous phase and oil phase into separate containers before mixing.
   ii. To make W/O emulsion, keep the amount of oil phase higher than or equal to aqueous phases.
   iii. Add aqueous phase to oil phase through wall of the container to ensure little loss of two phases.
   iv. Stir emulsion at constant temperature (65 to 75 degree Celsius) for 45 – 60 min for proper emulsification.

EVALUATION PARAMETERS

The herbal mosquito repellent cream was evaluated for different parameters such as, appearance, viscosity, pH, irritancy test, thermal stability test, etc.

Organoleptic properties

i. Colour:

Off-White colour with slight shimmer

ii. Odour:

The odour of the cream was observed to be characteristic

iii. Presence of foreign particles:

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

iv. Dilution test:

1g of cream was taken in 2 test tubes; one is diluted with water and other with oily medium. Both the test tubes were shaken and then observed.

Stability studies

i. Phase separation:

Cream was kept in closed container away from sunlight. Then phase separation was observed every 24hrs for 30 days.

ii. Moisture absorption studies:

50mg of cream was taken on watch glass. A beaker was filled with water and kept in a desiccator without adsorbent, allowed to get saturated. Then watch glass with cream was introduced into desiccators and left it for 24hrs.

$$\text{Moisture absorbed (\%)} = \frac{\text{Final wt.} - \text{Initial wt.}}{\text{Initial wt.}} \times 100$$

Spreadability

The Spreadability was communicated as far as time in seconds taken by two slides to slip off from the cream, set in the middle of the slides, under a specific burden. Lesser the time is taken for partition of the two slides, better the Spread capacity. Two arrangements of glass slides of standard measurements were taken. The home-grown cream definition was set more than one of the slides. The other slide was set on the highest point of the definition, with the end goal that the cream was sandwiched between the two slides weight was put upon the upper slides with the goal that the cream between the two slides was squeezed consistently to shape a dainty layer. The weight was expelled. Also, the abundance of definition clinging to the slides was rejected off. The upper slide permitted slipping off openly by the power of weight attached to it. The time taken for the upper slide was noted.

Irritancy Test

Imprint a region (1sq.cm) on the left-hand dorsal surface. The cream was connected to the predetermined zone and time was noted. Irritancy, erythema, oedema, was checked if any for normal interims up to 24 hrs and detailed.

Thermal Stability test

The defined cream was kept in petri plates inside the hatchery at 45˚ ± 1° for 48 hrs. The example finished the test if on expulsion from the hatchery demonstrates no oil partition or some other stage detachment.

pH of the cream

The pH of cream was determined using Digital pH meter. 1gm of cream was weighed and dissolved in 100ml of distilled water and left for 2 hours. Till then pH meter was calibrated then for each formulation pH was measured in triplicate and average values were calculated.

Wash off property

Small amount of cream was applied on backside of palm and spread evenly. Then hand was washed under water tap, and it is observed whether it still feel greasy or not.

Testing the treated fabrics after washing

The treated fabric was washed with the recipe mentioned below in the launder-o-meter. After the fabric washed and dried, it was tested for mosquito repellent test to check whether the finish is durable or not.

Tensile strength test

Since the fabric strength tester was not functional the test was done on yarn form with the Following procedure. 10 warp and weft yarns each were taken out from the treated fabric. Similarly, 10 warp and weft yarns each were taken out from the untreated fabric samples. All The yarns were tested for tensile strength using SHIRLEY yarn strength tester.

Shrinkage test

This taste was done by plotting 10cm X 10cm rectangle on the fabric before treatment. Then This rectangle was measured again after treatment is given. The area difference was calculated and the shrinkage was expressed in percentage. 10 untreated fabric samples were prepared with the dimension 15cm X 15cm. 10 cm X 10cm box was plotted on each sample. The Mosquito repellent finish was applied on all the samples by pad-batch-dry method. Then the Previously plotted box dimension was measured again. Finally, the shrinkage was calculated by area difference in percentage.

Fabric stiffness test

This test measures the bending stiffness of a fabric by allowing a narrow strip of the fabric to Bend to a fixed angle under its own weight. Rectangular specimens of dimension 25 mm X 200 mm were cut from sample; three specimens were cut with the length parallel to the warp and three more with length parallel to weft. The specimens were placed on the platform with One end coincident with the front upper edge of the platform. The slide was placed on the Specimen so that the zero of the scale is in line with the notch. The slide was pushed forward at a uniform rate, carrying the specimen with it, until by looking in the mirror it is seen that the end edge of the specimen is in line with the two scribed lines at 41.5o to the horizontal. The procedure was repeated with the other side up and again at the other end of the Specimen.

Container testing

Prior to transferring the entire formulation to a container, different types of containers are chosen and a small, sample amount of formulation is kept in them for a day or two. Upon observing the quality of formulation in each container, the most appropriate and compatible container is chosen which protects the formulation and increases its shelf life.

RESULT

We have studied the Prepared Formulation and evaluated all its properties which has been found to be within limits.

- Herbal Ingredients has opened the way to formulate herbal formulations without harmful effects which imparts the required properties.
- The Preparation is an herbal cream and free from side effects therefore herbal products are high in demand.
- Expenses are less as compared to synthetic formulations.

DISCUSSION

Many researchers have found that Cymbopogon nardus, Eucalyptus globulus, Vitex negundo, Ocimum sanctum have better repellent efficacy rather than other plant materials. However, a single plant extract obtained from plants contributes to poor longevity as mosquito repellents. Therefore, three plant extracts of Cymbopogon nardus, Eucalyptus globulus, Vitex negundo, Ocimum sanctum are suitable to be used as a polyherbal mosquito repellent. Preliminary batches of formulated Mosquito repellent cream were characterized for their elegancy, emulsification, spreadability, stickiness and consistency based on sensorial assessment. All prepared formulations had nearly constant pH, homogeneous, emollient, non-greasy and were easily eliminated after the application. The pH values of all developed formulations were found to be in the range of 6.95 to 8. Under Stability Studies Colour, consistency, viscosity, texture profile, and pH of the prepared creams were found to be consistent, and no separation was observed throughout a 90-day study, which revealed the reproducibility of the physical and chemical parameters which ensures the consistent quality of the developed cream formulation. No skin irritations or rashes were detected on the arms of the test volunteers with extracts, essential oils and the prepared mosquito repellent formulations. All formulations were safe concerning skin irritation and allergic sensitization as the primary irritation index was found to be zero, and there was no report of any edema or redness. The mosquito repellent formulations from plants are exceptionally fruitful due to the prosperity of insecticidal ingredients found in plants as defences adjacent to insects. Repellents have an important place in protecting man from the bites of insect pests. An effective repellent will be useful in reducing man vector contact and in the interruption of disease transmission. The Cymbopogon nardus, Eucalyptus globulus, Vitex negundo, Ocimum sanctum not only has good mosquito repellent/mosquitocidal activity but also it has good odour characteristic and the effect was due to presence of various active ingredients in these plants.

CONCLUSION

During this study, mosquito repellent activity of ingredients of polyherbal showed that the product was very efficient and safe to use. Statistically, it showed that the formulated product is very good and safe for use. During this study, we found that the essential oils of as Citronella, Eucalyptus, Tulsi, clove, neem, Sweet orange, Turmeric extract showed higher repellent activity. No volunteers complain about allergic consequences. So, it is a safe product. The formulation was ecological, economical and pocket friendly. Further investigations are needed to elucidate the efficacy of the herbal mosquito repellent formulations against a wide range of mosquito species and also to identify active compounds responsible for mosquito repellent activity to utilize them, if necessary, in preparing a commercial product to be used as a mosquito repellent

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