e-ISSN 2320 –7876 www.ijfans.org
Vol.11, Iss.11, 2022
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DEVELOPMENT AND ANALYSIS OF VETIVERIA ZIZANIOIDES FOR EFFECTIVENESS AS A HERBAL MOSQUITO REPELLENT

[1]Suryajeet H Pawar, [2]Abhijit B Patil, [3]Prasad D Namewar
[1]Assistant Professor, [2]Professor, and [3]Associate Professor
Department of Agada Tantra, Bharati Vidyapeeth (Deemed to be University), College of Ayurved, Pune

Abstract

Numerous pharmacological effects of essential oils have been identified, one of which is their ability to deter insects and mosquitoes. The oil infused with Vetiveria zizanioides can reportedly treat insect bites and is antibacterial, astringent, and antifungal. Two different concentrations of mosquito repellent cream, 5% and 7.5%, were prepared and tested according to the American Society for Testing and Materials (ASTM) standard E951-83 by combining Vetiveria izanioides oil with two essential oils, clove oil and dill oil. A non-commercial mosquito repellent mixture has been tested on humans in a lab. All formulations were subjected to a sensory test to determine their consistency in terms of pH, clarity, homogeneity, gloss and spreadability, as well as colour, aroma, appearance and ease of use.

Keywords: Mosquito repellents, Vetiveria zizanioides infused oil**,** Skin sensitivity, Mosquito repellent activity, Plant extract, Essential oil

1. INTRODUCTION:

Mosquitoes are the most important vector for public health as they potentially carry the pathogen from one host to the other there by help in the dissemination of the pathogen thus spreading the disease. The various diseases transmitted by mosquitoes include, malaria, Japanese Encephalitis (JE), filariasis, chikungunya and Zika. Mosquito-borne diseases have been the important cause of severe mortality in the human populations, affecting around 700 million people from these diseases annually. Mosquito-borne diseases not only affect the life but have the socio economic impact, including losses in commercial and labor outputs, costs incurred in diagnosis, treatment, prevention and control particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases
Mosquitoes are among the most disturbing blood sucking insects afflicting human beings. [1]Several mosquito species belonging to genera *Anopheles*, *Culex* and *Aedes* are vectors for the pathogens of various diseases like Dengue fever, Malaria, Yellow fever, Japanese Encephalitis and several other infections. Mosquitoes alone transmit diseases to more than 700 million people and over one million deaths are reported annually across the globe. Therefore, the control of mosquitoes is an important public health concern around the world.
The substances which make surfaces unpleasant to mosquitoes are considered as mosquito repellents. It may be applied to skin or other surfaces which discourages mosquitoes from landing on those surfaces. [2] These substances typically contain active ingredients that repel mosquitoes, as well as secondary ingredients which aid in delivery of cosmetic appeal.

These repellents are available in many forms - creams to lotions to oils, but are most often sold as aerosol products. There are also repellents available based on sound productions, particularly ultrasound (inaudible high frequency sounds). Traditionally, various types of substances havebeen used to repel mosquitoes. These include smoke, plant extracts, oils, tars, and muds. Most of the repellents work by interfering with the mosquito's homing system. [3] This homing system, located on the antennae of the organism possesses a number of chemical receptors. Carbon dioxide, excretory products and lactic acid present in sweat in warm- blooded animals act as an attractive substance for female mosquitoes. The chemical receptors are activated by lactic acid, which naturally evaporates from the skin of warm-blooded animals.

Maharashtra having tropical climate is infested with several diseases such as malaria, dengue, etc. caused by mosquitoes. The mosquitoes act as a vector for the transmission of these deadly diseases. The need to protect ourselves from their bites seems more important. Much of the literature about mosquitoes provided by government agencies recommendsregular use of mosquito repellents most often containing DEET as the major chemical component. Many health problems such as headache, breathing difficulties, heart attack, etc. are the result of long term use of DEET either directly or indirectly.[4] Now-a-days, people are looking for mosquito repellents which are safer and preferably herbal based. There occur several plants around us containing certain essential oils, often found to be affective insect repellents. Many of these plants are herbs or shrubs often considered as weeds. Unfortunately, their use has been known only to some ethnic groups. [5] The use of herbs as mosquito or other insect repellents has been incorporated in many cultural rituals of these ethnic groups from time immemorial. Assam is bestowed with diversity of ethnic groups as well biodiversity with associated traditional knowledge. Taking traditional knowledge as clue certain plants have been selected processed and produced to prepare a mosquito repellent.

Vetiveria zizanioides(V. zizanioides) L. is a tall, tufted perennial, scented grass, with a straight stem, long narrow leaves and a lacework root system that is abundant, complex,[6] and extensive. It offers an inexpensive yet effective and eco-friendly tool to combat soil erosion. The roots have been used in Asia for centuries for their fragrance, and are woven into aromatic matting and screens. [7] The roots of some cultivars and ecotypes possess essential oil that has been utilized as fragrant material since ancient times. Water quality signifies the absence of contaminants, which are waste products, pollutants and nutrients. The plant extracts, e.g., vetiver extract, have many special characteristics that lend support for its uses in solving the water problem. [8] In the case of human health, when dealing with the contamination of water, prevention is better than cure. The plant also contains active ingredients used in traditional medicine and as a botanical pesticide.

Despite centuries of control efforts, insect -borne diseases are flourishing worldwide. [9] Witha disproportionate effect on children and adolescents, these conditions are responsible for substantial global morbidity and mortality. Insects (mosquitoes, lice, fleas, bed bugs) and ticks are able to transmit a number of diseases caused by infectious agents: viruses (Chikungunya virus, yellow fever, dengue fever, etc.), bacteria (Lyme disease, plague, etc.), parasites (malaria, sleeping sickness, leishmaniosis, filariasis, etc.). These diseases thrive mainly in tropical environments. [10]Mosquitoes cause more human suffering than any other

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organism and it was found that over one million people worldwide die from mosquito-borne diseases every year. In India, Mosquito borne diseases constitute a major public health problem in the list of communicable diseases.

An insect repellent is a substance applied to skin, clothing, or other surfaces which discourages insects (and arthropods in general) from landing or climbing on that surface. Insect repellents help prevent and control the outbreak of insect-borne diseases such as malaria, [11] Lyme disease, dengue fever, bubonic plague, and West Nile fever. Pest animals commonly serving as vectors for disease include the insects such as flea, fly, and mosquito; and the arachnid tick. Commercial insect repellents are recommended as the most effective form of bite-preventive treatment. The efficacy in providing long-lasting protection against a wide variety of mosquito species has been documented in several studies. Although they are effective against mosquitoes, there are concerns associated with its use. They are unsafe for children possibly causing encephalopathy.[12] Therefore, the development of alternative, environmentally friendly and sustainable approaches for mosquito control is with the use of natural products owning greater target specificity, lower bioaccumulation properties and reduction of malignancy in non-target animals.

Vetiver grass (Vetiveria zizanioides) also known as chrysopogom zizanioides (Poaceae family), is a graminaceous plant native to tropical and subtropical India. In western and northern India, itis popularly known as khus. Vetiver is the most versatile, multifarious grass with immense potential.[13] Vetiver is fibrous and its aromatic roots have been harvested for centuries and turned into perfumes, insect-repelling textiles, closet sachets, and even food flavorings. Vetiver root paste or its extract is used as a diaphoretic, stimulant, and refrigerant, flatulence and obstinate vomiting. It is a plant known for its ability to produce essential oil from the roots which is especially used in the perfume industry. Because of these activities khus has found vast applications making it a green treasure. There is increasing interest in the health and wellness benefits of herbs and botanicals, this is with good reason as they might offer a natural safeguard against the development of certain conditions and be a putative treatmentfor some diseases [14]. However, from ancient times, V. zizanioides have also been used as raw materials for cosmetics, pharmaceuticals, botanical pesticides, disinfectants, insect repellents, herbal teas etc. [15]. Amongst different elements, terpenes found a great amount of active repellent plant vital oil[16].

1.1 **Industrial Uses of Vetiveria Zizanioides**

There are many industrial uses of the Vetiveria Zizanioides as depicted in figure no. 1, such as, pharmaceutical and medical utilities, perfumery, Water and Soil phytoremediation, mushroom cultivation, botanical pesticides, Disruption of Biofilm and water and oil phytoremediation.

Possessing calming properties, vetiver oil has long been traditionally used in aromatherapy to relieve stress, anxiety, nervous tension and insomnia. Vetiver Oil is a complex blend of more than 150 sesquiterpenoid compounds.

Due to these unique features Vetiveria Zizanioides is a very useful aromatic medicinal plant

having many industrial applications.

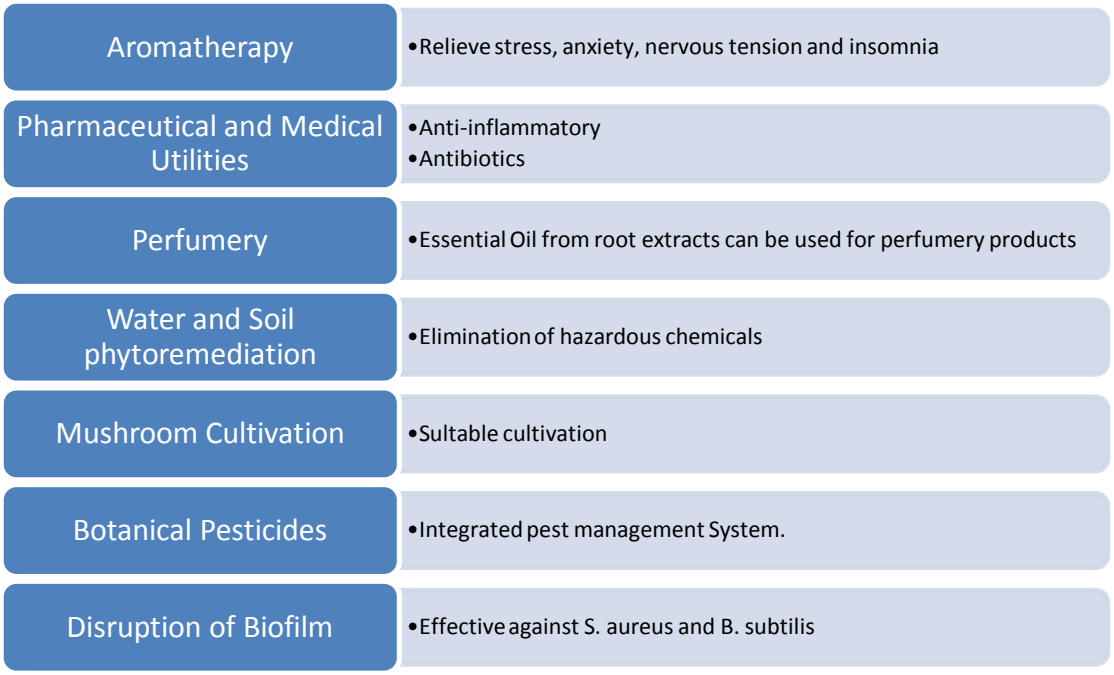

Figure No. 1: Industrial uses- Vetiveria Zizanioides

2. MATERIALS AND METHODOLOGY:

2.1. Preparation of materials used

The roots of Vetiveria zizanioides were collected from a nursery at Mulshi area (Pune). The plants were identified and authenticated at the Botany department of Pune University. All the chemicals were purchased from S.R. Traders, Ulhasnagar, and Mumbai. The mosquitoes were collected from R.L.Lab, Pune.

2.2. Extraction of Vetiveria zizanioides oil

The collected Vetiveria zizanioides roots were introduced into a glass jar. And the glassjar was then filled with castor oil. The Vetiveria zizanioides roots were kept for extraction. (Infusion) for about 3-4 days. After 3-4 days the Vetiveria zizanioides oil was filtered and collected into second jar which was incorporated in the formulation.

2.3. PREPARATION

Infusion of Vetiveria zizanioides oil was prepared first and was evaluated for repellent activity. The researchers found significant repellency activity in Vetiveria zizanioides oil which was compared with castor oil since the infusion was prepared in castor oil. With the data of Vetiveria zizanioides oil repellency activity it was decided to prepare a mosquito repellent cream of different concentrations i.e. 5% and 7.5%, a combination of Vetiveria zizanioides oil with two essential oils i.e. clove oil and dill oil.

2.4. Preparation of Mosquito repellent:

2.4.1. Aqueous Repellent Medicinal Cream: -

The medicinal cream (with oil in water base) made up of blending the essential oils in water base with a mixing wax. The formulation method for the same cream is as shown in

table no.1:

Table 1

Vital oils with Vetiveria zizanioides	7% or 10.5%
1-hexadecanol and palmityl alcohol	1%
Cream Base	1%
Liquid Paraffin	3%
Fatty Acid	10%
Glycerol	15%
Caustic Potash	1.5%
Water Q.S.	100%

2.5. EVALUATION OF ANTI-MOSQUITO ACTIVITY

The American Society for Testing and Materials (ASTM) standard E951-83 described testing applied and the anti-mosquito cream formulated assessed. As described same process applied for testing the cream. According to this process, 20 mosquitoes were transferred to the cage using a suction tube. The tested formulation was applied to the subject's forearm. A clasped hand was inserted into the cage and the number of bites was recorded. Every 30 min, the volunteer's hand was exposed to her cage for 3 min and the number of mosquito bites recorded. The test procedure was repeated by him three times. The same procedure was followed for controls (blank formulations), Vetiveria zizanioides oil and castor oil. The protection rate provided by repellent creams can be expressed by the following formula:

$$\text{Protection Rate} = \frac{\text{Bite with control} - \text{Treated Bites}}{\text{Bites on check}} \times 100$$

2.6 Primary skin irritation test:

Hermaphrodite guinea pigs (300-350 g) were used in the study. Animals were kept under standard housing conditions with a temperature of 27±20° C. and humidity of 60±5% RH. Animals were given an adequate diet and water ad libitum. Two animals were used for the study. The animals' backs were shaved prior to testing. Five patches of equal area (1 cm 2 each) were applied to the shaved back. The following formulations were used. Clean with 5% Vetiveria zizanioides oil as: i) Cream with 7.5% Vetiveria zizanioides oil ii)Cream with combination of volatile oil iii) Cream lacking active element/component iv)Controller Site

2.7 Procedure:

The quantity of 0.5 gm cream was applied on marked area, spread uniformly and covered cotton gauze, which was secured by hypo-allergic adhesive tape. The entire trunk was wrap with an impervious material for 24 hrs. period of exposure. This material aids in maintaining the test patch in position and retards evaporation of volatile substances. During the test period, guinea pigs were fed at regular interval. At the end of 24 hrs., the patches were removed and the skin was observed for any visible changes such as erythema or edema. Evaluation was done by using scale given by Draize. Observation was repeated after 72 hrs.

2.7.1. Draize Scale to assess skin reactions

Table 2

A. Erythema and Eschar formation		
a.	No Skin Irritations	0
b.	Very slight Skin Irritations (just traceable)	1
c.	Well define Skin Reactions	2
d.	Modest to Severe Skin Reactions	3
e.	Severe Skin Reactions with redness to Slight eschar development, damages in depth	4
	Total possible erythema reactions score	**4**

Table 3

Oedema(Fluid retention, dropsy, hydrops and swelling) formation		
a.	No oedema found	0
b.	Very slight oedema (just traceable)	1
c.	Slight oedema (edges of oedema well defined by definite raising)	2
d.	Moderate oedema (area elevated around 1 mm)	3
e.	Severe oedema (area elevated > 1 mm and growing behind the area of experience)	4
	Total possible oedema score	4
	Total possible score of primary skin irritation	**8**

The table no.2 and table no.3, lists Draize Scale assessment details of skin reactions ranging from no reaction to severe skin reactions. Where Total possible erythema reactions score is 4 and Total possible score of primary skin irritation 8.

2.7.2. pH Determination:

The pH of various gel formulations was determined using a digital pH meter.

Malleability:

Measured on a wood block and glass apparatus. A weight of approximately 20 g was placed in the dish and the time for the top slide (during movement) to completely separate from the stationary slide was recorded.

2.8 Therapeutic Potentials of Vetiveria zizanioides

Like the terrain of the jungle, vetiver has therapeutic potential, much of which remains untapped due to the complex nature of its essential oil, but the potential is enormous. Few other medicines, whether aromatic or not, exert the same depth of restorative action as vetiver, repairing the body's four core systems: the nervous system, the endocrine system, the gastrointestinal tract and the immune system. Vetiver oil has great potential for use as an insecticide. Vetiver oil extract is repellent and toxic to ants, mites and cockroaches. It was

previously thought that the tricyclic sesquiterpenoids dizanal and epizizanal isolated from vetiver oil were responsible for the repellent effect (Jain et al. 1982). Later, at least six compounds were observed: α- and β-vetivone, ximone, dizanal, epizizanal and (C)-(1S,10R)-1,10-dimethylbicyclo [,0]-dec-6 was done. - en-3-one has been proven to repel a wide variety of insects. Vetiver oil extracts have shown repellent and anti-oviposition properties when applied to Ceratitis capitata (Mishra 2000). Flavonoids isolated from aqueous extracts of vetiver oil exhibited 80% insecticidal activity against Lepidoptera stem borers of maize in humid tropical regions of West Africa at a concentration of 0.07 mg/ml.

2.8.1. Pesticide Activity

Vetiver oil has also been shown to be effective against stored grain pests. Adult Tribolium castaneum were shed by contact with feeding media treated with 2 and 5 g of vetiver oil dust per 10 g of diet (Vanden et al. 2000). Vetiver root extracts in petroleum ether, ethyl acetate, acetone, and methanol showed insecticidal activity against his XSM, SMC, SKS, and JTC strains of the mealworm T. castaneum. In larval bioassays, the highest petroleum ether toxicity (LD50 = 0.051 g/cm2) was recorded for strain XSM, and the lowest toxicity using methanol extract (LD50 = 11.351 g/cm2) for SMC strain (Sujatha 2010). Similarly, the non-polar petroleum ether fraction of vetiver oil, composed mainly of four discontinuous bis-tetrahydrofuran acetogenins, termed squamostatins B to E, showed insecticidal activity against Cytophilus oryzae parasitizing wheat seeds. I was. An acetone extract from fresh and preserved leaves of Callosobruchus maculates was found to be toxic to adults and an ethanol extract was found to be non-insecticidal. , exhibited insecticidal and repellent activity against Anopheles mosquito of the malaria vector, causing 85% mortality. The observed mortality suggests that the extract could be used as a biopesticide. LC50 values of 2nd, 3rd and the instar larvae of A. stephensi was 0.276, 0.285, and 0.305%, respectively.

2.8.2. Anti-tick Activity

Vetiver root extract was able to control the growth of larval and adult ticks, including the egg-laying period of Boophilus microplus bovine ticks. Ethanol extracts were most likely to control cattle ticks compared to other extracts (Korpraditkul et al. 1996). Chemical analysis showed that the sesquiterpene fraction dominated in the composition of vetiver essential oil, even though kushimol was the main active ingredient. It has been shown to have acaricidal activity against micro plus. These results indicate that C. zizanoides essential oil is a promising candidate as an acaricide. In this study, C. zizanioides essential oil decreased egg production and egg hatching by female ticks and reduced tick reproductive efficiency. Furthermore, the reduction in reproductive function observed in ticks treated with C. zizanioides essential oil was greater than that observed with the commercial reference products Natuneem (A. indica oil) and Butox P CE25 (deltamethrin). Concerning the effects of vetiver essential oils on the larval stage of the mite, the results obtained here indicate that these essential oils are effective against A. cajennense and R. Microplus larvae compared to the larvicidal activity of other botanical compounds.

2.8.3. Antibacterial Activity

Several studies have shown that vetiver oil has antibacterial activity against various strains of bacteria such as Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli and Corynebacterium ovis. Inhibition by pure oil was 60-70% greater than that by penicillin

(Gangrade et al. 1990). After hydrodistilling the essential oils of his two genotypes of V. zizanioides ('Gulabi' and 'KS-1'), hexane extracts of inflorescences, intact roots, and spent roots were added to the wild-type drug. Antibacterial activity against resistant strains was analyzed. of Mycobacterium smegmatis and E E. coli using disc diffusion and microbroth dilution methods. The extract showed antibacterial activity against both strains. The antibacterial activity of vetiver oil extract (10 mg/ml) in polar solvents (methanol, chloroform) and non-polar solvents (hexane) was tested against Staphylococcus aureus. Chloroform extracts showed antibacterial activity using the cup-drill method on agar solid media. Raw root extract of V. zizanoides (L.) Nash cultivar 'Surat Thani' showed antibacterial activity against four pathogens. The alkaloid vetiverine showed a minimum inhibitory concentration of 1.626 mg/mL. A polar ethanol extract showed better antibacterial activity against Staphylococcus aureus, E. Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis compared to aqueous extracts. Phytochemical analysis revealed the presence of flavonoids, glycosides, phenols, tannins, saponins and alkaloids. Vetiver oil is used for Acinetobacter baumannii, Aeromonas belonii, Candida albicans, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica, Serratia marcescens, S. The minimum inhibitory concentration for Staphylococcus aureus (Hammer et al. 1999) was found to be 0.008% (v/v). Root extracts contain two pathogenic bacteria, Escherichia coli (MTCC 3) and S. Staphylococcus aureus (MTCC 737). All these results confirm that vetiver extract is pharmacologically important and can be used for human disease.

2.8.4. Antifungal Activity

Vetiver oil has demonstrated broad-spectrum natural fungicidal activity against pathogens. The antifungal activity of sesquiterpenoid conversion products in vetiver oil has been demonstrated against two phytopathogenic fungi. H. Alternaria alternata (causing early blight in tomatoes and potatoes) and Fusarium oxysporium (causing tomato wilting) have been tested using the spore germination inhibition technique. Among the various compounds tested, xynodiol monobrosylate proved to be an effective antifungal agent against both fungi.Schiff base of sesquiterpenoids, i. H. N-(fusilidene)-p-methoxyaniline and N-(fusilidene)-p-bromoaniline were synthesized by reaction with p-methoxyaniline and p-bromoaniline. N-(Phuclidene)-p-methoxyaniline was found to inhibit the growth of A. alternata inhibited by 8 .7% and N-(khusiliden)-p-bromoaniline inhibited F. oxysporium growth by 7.5% at a concentration of 1 mg/ml..

2.8.5. Herbicidal Activity

It was previously hypothesized that certain substances secreted by vetiver plants may have allelopathic effects by inhibiting the growth of other plants. It has been shown to inhibit soybean seed germination and can be used for weed control (Techapanyawat 199). A Petri dish germination test was performed to test vetiver oil and his six common weed species of nootkatone, one of its minor constituents: red root foxwort, lamb quarters, ragweed, pitted morning glory, and tested its effect on figs. Vetiver oil not only inhibited germination of these weeds, but also inhibited seedling application of red root foxweed and common quarter

lamb, which acted as beneficial herbicides. Vetiver oil and its component nootkatone also inhibited the growth of Pisum sativum L plants. (Plant Height, Root Length, Dry Weight) Vetiveria zizanioides (L.) Nash: Magic Bullet Vetiver Oil and Two Non-edible Oil-producing Fence Plants Jatropha curcas L. and L. Allelopathic interactions with Ricinus communis L. were tested and vetiver plants were found to enhance the growth of Jatropha seedlings and enhance the growth of R. It suggests that vetiver jatropha is a suitable fence plant option for plant interactions.

2.8.6. Antioxidant Activity

Vetiver oil exhibits antioxidant and anti-inflammatory properties and can be used in medicines and perfumes (Chou et al. 2012). The antioxidant properties of vetiver oil have been evaluated using two different in vitro tests of his: the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging test and the Fe2 metal chelate test. The results showed that vetiver oil possessed potent free radical scavenging activity compared to standard antioxidants such as butylated hydroxytoluene (BHT) and α-tocopherol. Dissolved vetiver oil (0.01 mg/ml) showed 93% and 3
% Fe2 chelating activity at DPPH• in the metal chelation assay, respectively. In crude vetiver oil, β-vetivone, β-vetivone, and α-vetivone showed strong antioxidant activity.

3. RESULTS AND DISCUSSION

It must first provide too high a percentage of defense against mosquito bites and, second, be toxicologically harmless at the proposed application rate to get successful repellent. And after all, this repellent needs to be easy to put on and comfortable on the skin.

3.1. Formulation parameters

All formulations were sensory evaluated for color, fragrance, appearance, luster, and ease of use. All formulations had a fairly constant pH, were homogenous, soft, non-greasy, and easily removed after application. All formulations developed had pH values in the range of 6.7 to 7.

All developed formulations found having good clarity and uniformity without clumps or agglomerates. Spreadability is one of the key characteristics of all topical formulations as far as patient compliance is concerned. In addition, if the formula spreads easily, it will be more comfortable to apply to the skin.

3.2. Skin sensitivity study

Skin irritation studies are very important because many cosmetics have an inherent level of irritation. All formulations were safe for skin irritation and allergic sensitization, as the primary irritation index was found to be zero by the Draize patch technique, and there were no reports of edema or redness. It has shown skin acceptability of topical application.

3.3. Mosquito Repellency

Mosquitoes have highly olfactory chemoreceptors on their sensitive antennae that are stimulated by various odors, which can increase or decrease their attraction to the host. This behavior of reducing mosquito attraction to the host was observed with formulations containing a combination of essential oils and Vetiveria zizanioides oil.

The mosquito repellent effect of extracted Vetiveria zizanioides oil was tested against Aedes mosquitoes. Based on the results of Vetiveria zizanioides oil (61.24% protection) against mosquito bites, this indicated that the oil could be developed into a mosquito repellent

formulation. Therefore, the extracted Vetiveria zizanioides oil was incorporated into a cream base at various concentrations to assess its activity. A cream containing 7.5% vetiveria zizanioides oil provided a high level (57.01%) of protection against mosquito bites. A person treated with a cream containing 7.5% vetiveria zizanioides oil had only eight mosquito bites in 90 minutes. The average number of received bites for control volunteers was ± 19.62. Subjects treated with a cream containing 5% Vetiveria zizanioides oil received only 11.02 mosquito bites in 90 minutes. The average number of mosquitoes received by control volunteers was 20.01. It shows that it provided a high efficacy of cream containing 7.5.

Table 4 Values of evaluation parameters of developed formulations

Batch	Clarity	Homogeneity	pH	Spreadability g.cm/sec	Skin Problems
5 % VO Preparation	Clear	Good	6.8	6.8	Nil
7.5 % VO Preparation	Clear	Good	6.6	6.7	Nil
5 % VO + CO+DO Preparation	Clear	Good	6.7	7.0	Nil
VO: Vetiveria zizanioides oil; CO: Cinnamon oil; DO: Dill oil					

Table 5 Assessment of Anti-mosquito repellent Preprations

Batch	Mean Bites		% Mean protection
	Test	Control	
Vetiveria zizanioides oil	3.3±0.01*(**0.005789**)	8.62±2.09	61.24±10.21
5% VO Preparation	11.02±2.61* (**0.003494**)	20.01±1.60	45.10±16.01
7.5% VO Preparation	10±1.81*(**0.003591**)	19.62±3.3	57.01±7.05
5 % VO + CO+DO Preparation	2.89±2.65*(**0.000425**)	21±3	81.71±14.75
Values are represented as mean± S.D. of mosquito bites.*indicates the significantly($p<0.01$) different compared to respective control values. (Students unpair T-test) [VO: Vetiveria zizanioides oil; CO: Cinnamon oil; DO: Dill oil]			

A cream base containing combination of volatile oil with 5% Vetiveria zizanioides oil as an active ingredient had been also tested for mosquito repellent activity. The significance repellency effect exhibited by the formulation containing combination of volatile oils with % protection indicated excellent protection.

Based on protection rates, formulations containing a combination of volatile oils were found to be the best, followed by formulations containing Vetiveria zizanioides oil. For the Vetiveria zizanioides oil formulations, a dose-dependent response was observed as the

percentage of protection increased with increasing concentration of Vetiveria zizanioides oil.

4. FUTURE SCOPE FOR RESEARCH

Numerous Industrial applications and key anti-fugal and other medical uses of vetiveria zizanioides oil makes it important to focus this as traditional source of herbal and aromatic medicine. So, researchers are suggested to focus on all of its active ingredients as great source in other possible infections also and to further enhance extraction process and explore its medicinal uses faster and effective. The Research Studies such as drug-drug interactions and drug-food interactions and it's impact are another aspect of the future study that needs to be considered in the effective clinical use of the compounds. Researchers can also focus on advanced methods of fusion of vetiveria zizanioides oil in general skin cosmetics and medicines. Farmers also can be trained and motivated for this crop cultivation and production which can help farmers by two means of benefits as crop production and as remedy of water and soil phytoremediation therapy.

5. CONCLUSION

Active components are the focus and responsible element of all mosquito repellent preparations. A cream containing 7.5% vetiveria zizanioides oil provided a high level (57.01%) of protection against mosquito bites. For the Vetiveria zizanioides oil preparations, a dose-dependent response was observed as the percentage of protection increased with increasing concentration of Vetiveria zizanioides oil. Natural repellents, such as herbal essential oils, have been used as alternative compounds to repel mosquitoes and other insects. Mint, citronella, basil, thyme, neem, and lemongrass have been reported to repel insects. Although the history of other prominent repellents such as DEET and dimethyl phthalate is well established, it is correct to state that different essential oil combinations affect repellent performance. We have confirmed that natural formulations can be used as mosquito repellents.

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