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Research Article

Evaluation of Insect Repellent Finish using "*Leucas aspera*" Leaves Extract

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Abstract

This study examines using *Leucas aspera* leaves to create an insect-repellent finish on textiles via the padding mangle process. The extract was evenly applied to cotton fabric, then dried and cured. The results show that *Leucas aspera* provides an effective, natural alternative to synthetic insect repellents for textiles.

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1. INTRODUCTION

The growing concerns over mosquito-borne diseases such as malaria, dengue, and chikungunya have led to increased interest in the development of effective insect repellents. Traditional insect repellents often contain synthetic chemicals, which can pose risks to human health and the environment. As a result, researchers are turning to natural sources for safer, eco-friendly alternatives. One such plant, *Leucas aspera*, commonly known as "Thumbai," has been recognized in traditional medicine for its insect repellent properties. In this study, we explore the potential of *Leucas aspera* leaves as a natural insect repellent finish on textiles using the padding mangle method. This method is widely used in the textile industry to apply finishes to fabrics, ensuring an even distribution of the repellent on the surface of the material. The application of such natural repellents to fabrics could provide a safe and sustainable solution for protecting individuals from insect bites, particularly in regions where mosquito-borne illnesses are prevalent. The primary objective of this research is to evaluate the efficacy of *Leucas aspera* as a bio-based insect repellent finish when applied using the padding mangle technique.

2. REVIEW OF LITERATURE

Natural insect repellent agents are substances derived from plants or other natural sources that repel insects without the use of synthetic chemicals. These natural repellents offer a safer and more environmentally friendly alternative to conventional insect repellents containing synthetic compounds like DEET. Here are some commonly used natural insect repellent agents:

- **Citronella Oil:** Citronella oil is derived from the leaves and stems of citronella grass (*Cymbopogon nardus* or *Cymbopogon winterianus*). It is known for its strong insect-repelling properties and is commonly used in candles, sprays, and lotions.
- **Lemon Eucalyptus Oil:** Lemon eucalyptus oil is extracted from the leaves of the lemon eucalyptus tree (*Corymbia citriodora*). It contains a compound called PMD (para-menthane-3,8-diol), which has been shown to effectively repel mosquitoes.
- **Lavender Oil:** Lavender oil, derived from the flowers of the lavender plant (*Lavandula angustifolia*), has a pleasant aroma and is known for its calming properties. It also has insect-repelling properties and can be used in sprays, lotions, and sachets.
- **Peppermint Oil:** Peppermint oil is extracted from the leaves of the peppermint plant (*Mentha × piperita*). It has a strong scent that repels insects such as mosquitoes, ants, and spiders. Peppermint oil can be used in sprays, diffusers, or as a natural deterrent around the home.
- **Neem Oil:** Neem oil is derived from the seeds of the neem tree (*Azadirachta indica*). It contains compounds that repel insects and has been used for centuries in traditional medicine and agriculture. Neem oil can be applied topically or used as a natural insecticide in gardens.
- **Cedarwood Oil:** Cedarwood oil is extracted from the wood of cedar trees (*Cedrus* spp.). It has a woody aroma and contains compounds that repel insects such as mosquitoes, moths, and ticks. Cedarwood oil can be used in sprays, diffusers, or as a natural insect repellent for clothing and linens.
- **Catnip Oil:** Catnip oil is derived from the leaves of the catnip plant (*Nepeta cataria*). It contains a compound called nepetalactone, which is highly effective at repelling mosquitoes. Catnip oil can be used in sprays, lotions, or as a natural insect repellent for pets (Carroll, 2018).

Methods of Insect Repellent finish

Insect repellent finishes are applied to fabrics to provide protection against insect bites and pests. These finishes utilize various methods to incorporate insect-repelling properties into the fabric. Here are several methods commonly used for insect repellent finishes:

- **Topical Application**: In this method, the insect repellent solution is directly applied to the surface of the fabric. The solution typically contains natural or synthetic insect-repelling agents such as permethrin, DEET, or essential oils. Fabrics are soaked or sprayed with the solution and then allowed to dry thoroughly before use.
- **Encapsulation**: Encapsulation involves trapping insect repellent agents within microscopic capsules and then applying them to the fabric surface. When the fabric is worn or rubbed, the capsules rupture, releasing the repellent and providing long-lasting protection against insects.
- **Microencapsulation:** Similar to encapsulation, microencapsulation involves enclosing insect repellent agents within tiny particles or microcapsules. These microcapsules are then bound to the fabric fibers using a binding agent or adhesive. As with encapsulation, the repellent is gradually released when the fabric is worn or rubbed.
- **Impregnation**: In this method, the fabric is treated with a solution containing insect repellent agents. The solution penetrates the fabric fibers, effectively impregnating them with the repellent. Impregnated fabrics provide durable and long-lasting insect protection, even after repeated washings.
- **Coating:** Coating involves applying a layer of insect repellent material directly onto the fabric surface. This can be done using spraying, brushing, or roller coating techniques. The coating forms a protective barrier that repels insects upon contact, providing immediate and continuous protection.
- **Embedding**: In embedding, insect repellent agents are incorporated into the fabric during the manufacturing process. This can be achieved by mixing the repellent agents with the fabric fibers before weaving or by incorporating them into the fabric coating or treatment.
- **Fibre Modification**: In some cases, insect repellent properties can be imparted to the fabric fibers themselves through chemical modification or treatment. This can involve introducing insect-repelling compounds into the

fiber structure during synthesis or processing (Gupta., 2011)

Evaluation of Insect repellent

There are various testing methods for evaluation of mosquito-repellent textiles which are given below:

The field test

It is the most meaningful evidence for the efficacy of treated textile. The field test is especially performed in locations where numerous of floodwater mosquitoes are present. In this method solution of 0.5ml of repellent is applied on forearms and forearms are exposed to mosquitoes. No. of mosquito bite is recorded (Hossan et al., 2011).

WHO cone test

In WHO cone test [29] a standard WHO plastic cone is attached to the treated test surface. Afterwards, 5mosquitoes are transferred into the cone with an aspirator and exposed to the treated surface for 3 minutes. At the end of the exposition, test mosquitoes are removed from the cones, placed in small cages for further observation and kept in insecticide-free air. The number of immobilized, knocked-down test mosquitoes is documented one hour after the exposition, the mortality rate is determined using the following formula. The natural mortality rate is determined with an untreated textile. It is found that mosquito might spend more time resting on the cone than on the treated surface (Srinivasan *et al.,* 2011).

Mortality % = (No of Dead specimen/ Total No. of exposed specimen) X 100

Mosquito Cage Test

American Society for Testing and Materials (ASTM) standard E951-83 [31]

This method is for non-commercial mosquito repellent formulation on the skin in this method mosquito-repellency activity is assessed using the test cage as shown in figure 6. The flexor regions of the forearms of volunteers are outlined with five circular 29mm diameter test areas. 0.025 ml of dilutions of the essential oils in ethanol and 0.025 ml of the diluents is applied to the marked areas of circles. Ethanol 95% was applied at the middle, which was the third circle as the control test. The test cages are positioned securely on the arms of each volunteer with Velcro tapes to ensure that only the test areas are exposed for mosquito bites.

Fifteen female mosquitoes, between four and seven days old, are introduced into each cage and the numbers of bites are recorded at the end of 120 seconds.

Insect repellent finishes can be categorized into two main types: topical treatments and fabric impregnation. Topical treatments involve applying repellents directly to the fabric's surface, providing temporary protection. Fabric impregnation, on the other hand, involves incorporating insect repellents into the fabric during manufacturing, offering longer-lasting effectiveness.

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3. MATERIAL AND METHODS

MATERIALS

Scoured and desized 100% Cotton fabric was used for the application of Insect Repellant Finish. The leaves of *Leucas aspera* were used for the Insect Repellant finish.

METHODS

Extraction process

The collected *Leucas aspera* leaves were shadow dried to remove the moisture content, proper drying is carried out otherwise the compounds may get contamined. After drying, the griding was carried out to the break the leaves into small units. 50g of Leucas *aspera* was soaked in 500ml of methanol for 24 hours and then filtered.

Padding Mangle Technique

The padding mangle process for applying an insect repellent finish using *Leucas aspera* extract involves immersing the selected fabric, such as cotton, in the prepared herbal extract solution. Once the fabric is thoroughly saturated, it is passed through the padding mangle, a machine equipped with a series of rollers that squeeze the fabric to ensure even distribution of the extract while removing excess liquid. This controlled padding step ensures the uniform application of the insect-repellent finish across the fabric surface. Following padding, the treated fabric is typically dried at a moderate temperature to remove any remaining moisture and then cured at a specific temperature to fix the herbal finish onto the fibers, enhancing its durability and effectiveness.

Human Ethical Clearance

To survey the evaluation of insect repellent finishes using *Leucas aspera,* submit a research proposal and consent form to an ethics committee for approval. The proposal should outline study objectives, methods, and participant rights.

4. RESULT AND DISCUSSION

Phytochemical Test Analysis

The Phytochemical analysis was carried out to identify the functional group present in *Leucas aspera* leaves. The functional groups are found using the graph show in figure I and presented in table 1.

Table 1: Phytochemical Analysis

S. No	Metabolite	Test performed	Observation	Result	Figure No
1	Alkaloids	+Mayer'sreagent	PresenceofCreamcoloured precipitate	+	1
		+Dragendorff's reagent	Presenceofreddishbrownprecipitate	+	2
2	Flavonoids	+H_2SO_4	Presenceofreddish Orangecolour	+	3
		+leadacetate	Presenceofwhite precipitate	+	4
3	Sterols (Libermanntest)	+$CHCl_3$+Acetic anhydride+ Conc.H_2SO_4	Presenceofreddish brown ring	+	5
7	Proteins	+conc. HNO_3	PresenceofYellow colouration	+	6
10	Carbohydrates	Molisch'stest	PresenceofVioletring	+	7
		Fehling'stest	PresenceofRed precipitate	+	8
13	Cardiacglycosides	Brominewatertest	Presenceofyellowprecipitate	+	9
		Keller-killanitest	Presenceofbrownring	+	10
15	Lignin	+Gallic acid	Presenceofolive-greencolour	+	11
17	volatileoils	fluorescencetest	Pinkish fluorenscence	+	12

Phytochemical analysis in *Leucas aspera* extracts showed the presence of alkaloids, flavonoids, sterols, proteins, carbohydrates, cardiac glycosides, lignin, and volatile oil.

FT-IR Spectroscopic Test Analysis

The FT-IR analysis was carried out to identify the functional group present in *Leucas aspera* leaves. The functional groups are found using the graph show in figure I and presented in Table 2.

Table 2:

Sample	Wavelength (cm-1)	Functional Group	Compound class
Leucas aspera **(LA)**	3356.14	OH	Alcohol
	29339.52	CH and CH_2	Aliphatic group
	1689.64	C=O	Quinone compound
	1450.47	CH	Aliphatic bending group
	1111.00	S=O	Sulfone compound
	102613	SiO_2	Silica
	686.66	C-Br	Halo compunds

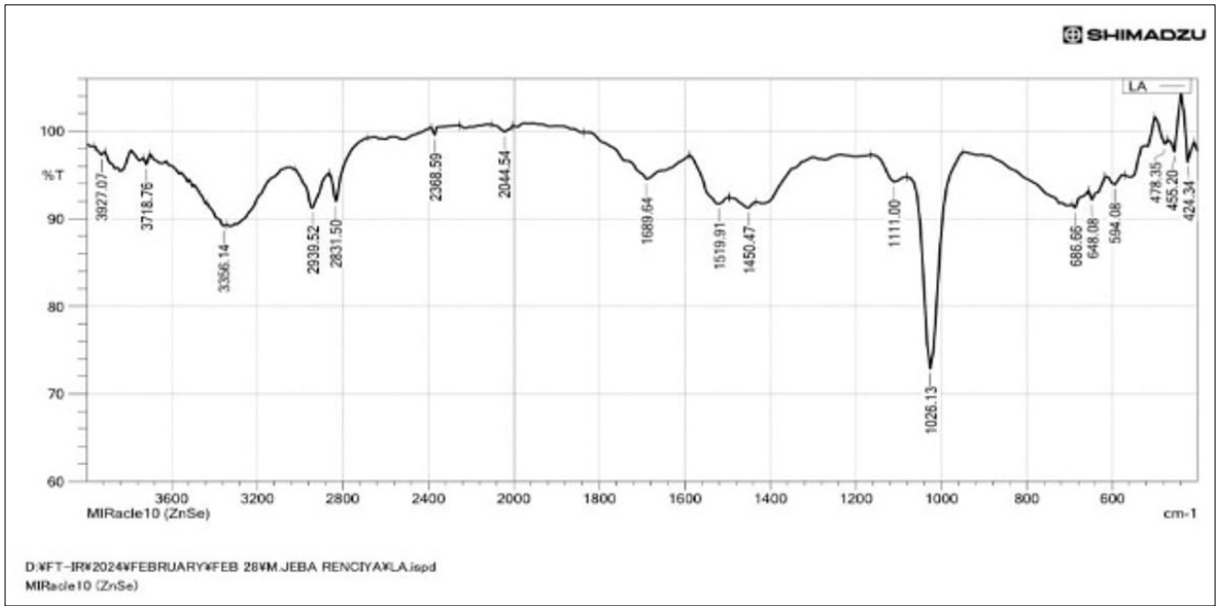

Fig: 1

Aliphatic compounds often have distinct odors, some of which insects may find repellent. For example, the odor of certain aldehydes, such as citronellal found in citronella oil, is known to repel mosquitoes. Quinone compounds, which are aromatic compounds containing two carbonyl groups. Researchers may synthesize quinone derivatives with specific structural modifications to enhance their insect-repellent properties. These modifications could include adding functional groups or altering the quinone ring structure to optimize repellence

Cytotoxicity Test

Table: 3

Samples	Concentrations	OD values(triplicate)-24hrs					
		1	2	3	Average	% of viability	% of cytotoxicity
Controlcells (without treatment)		1.65 4	1.658	1.647	1.653	100%	No cytotoxicity
Sample: LA	25µg	0.11 4	0.117	0.108	0.113	93.16	6.84
	50µg	0.14 5	0.148	0.146	0.146	91.17	8.83
	75µg	0.20 2	0.208	0.218	0.209	87.36	12.64
	125µg	0.25 4	0.265	0.268	0.262	84.15	15.85
	150µg	0.28 7	0.298	0.265	0.283	82.88	17.12

In the table 3 proves that the extract is treated with different concentration level of acid propanol to determine its viability and toxicity 25µg concentration shows 93.16% viability and the highest concentration level 150µg shows 82.88%. The test analysis shows that the extract has excellent cell viability and cytotoxicity level.

Anti-bacterial Test-AATCC97

Antibacterial testing evaluates the ability of substances to inhibit or kill bacterial growth. It involves exposing bacterial cultures to the substance and assessing its impact using various methods such as agar diffusion or broth dilution assays.

Table 4: Anti-bacterial Test

Samples	Zone of Inhibition (mm)	
	Staphylococcus aureus	***Escherichia coli***
LA uncoated fabric	0 mm	0*
LA Coated fabric	0*	18 mm

Fig 2: Anti-bacterial Test

It is observed from Table 4 and Figure 2 the antibacterial analysis, *Escherichia coli were* used to evaluate the zone of incubation in coated fabric. In the coated fabric, an 18-mm zone of incubation is formed, and there is no bacterial growth beneath the original fabric treated against *Escherichia coli.*

Repellent Specialization Test

Cage Test (Insect Repellent)

This test is conducted by placing mosquitoes in a cage equipped with a treated surface, such as insecticide-treated nets or walls, or by introducing substances like repellents or attractants. Researchers then observe mosquito behavior, including feeding, resting, and mortality rates, to gauge the effectiveness of the intervention. The mosquito cage test provides valuable insights into the potential impact of control measures on mosquito populations and helps inform strategies for combating mosquito-borne diseases like malaria, dengue fever, and Zika virus (Raja et al., 2015).

Mosquito Repellency activity Before Washing

The mosquito repellency Test before washing of original and treated cotton fabric (Mosquito)

Table : 5

S. No	Samples	Batched or unbatched	No of Mosquitoes (Death)
1	CO	Batched	Nil
2	LAT	Batched	46
S. No	**Samples**	**Batched or unbatched**	**No of larvae (Death)**
1	CO	Batched	Nil
2	LAT	Batched	23

The Mosquito Repellency Test after washing of original and Treated Cotton Fabric

Table 6: Cage Test after Wash

S. No	Samples	No of Mosquitos	Remark
1	CO	19	Poor Repellency
2	LAT	27	20

It is observed from Table VII that the treated fabric has excellent repellency compared to the untreated fabric. Mosquito repellency activity is observed by doing the cage test. The fabrics were observed before and after washing to evaluate the efficiency of the repellency in the fabric. The result reveals that after washing, it is less than before washing.

5. CONCLUSION

Leucas aspera leaves extract can serve as an effective insect repellent. By applying the extract on skin or clothing, it may deter insects, providing protection against bites and potential vector-borne diseases. However, it's crucial to consider factors such as concentration, frequency of application, and individual sensitivity. Additionally, incorporating other preventive measures, such as wearing protective clothing and using mosquito nets, can enhance the overall effectiveness of using *Leucas aspera* as an insect repellent. Overall, judicious and informed usage of *Leucas aspera* leaves extract can offer a natural and potentially sustainable solution for repelling insect.

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