DEVELOPMENT OF NATURAL MOSQUITO REPELLENT FINISH USING *PLUCHEA WALLICHIANA* PLANT EXTRACT ON COTTON FABRIC

April, 2023

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B.E. (Textile Processing)

DEVELOPMENT OF NATURAL MOSQUITO REPELLENT FINISH USING *PLUCHEA WALLICHIANA* PLANT EXTRACT ON COTTON FABRIC

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Family and Community Sciences

By

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April, 2023

CERTIFICATE

This is to certify that the research work presented in this dissertation entitled "**Development of Natural Mosquito Repellent Finish Using *Pluchea wallichiana* Plant Extract on Cotton Fabric**" in pursuit of a Masters' Degree in **The Department of Clothing and Textiles** is her original bonafide work.

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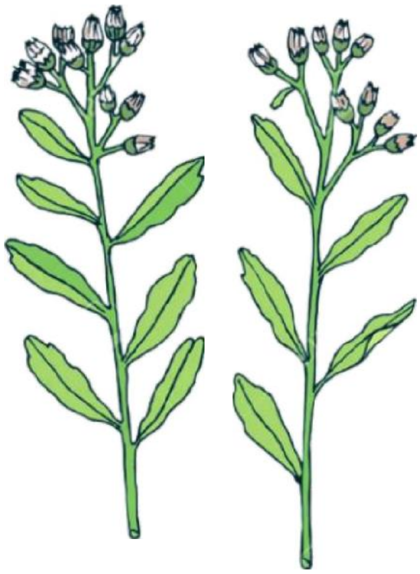

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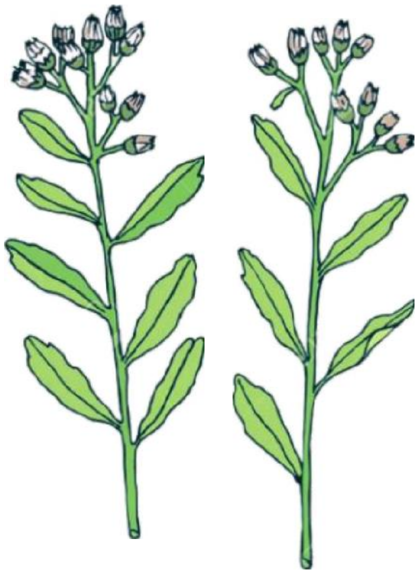

ABSTRACT

ABSTRACT

In India, the use of mosquito repellent textiles has gained popularity in recent years due to the increasing awareness about mosquito-borne diseases such as malaria, dengue, and chikungunya. Mosquito repellent textiles are specially designed fabrics that are treated with chemicals or natural extracts to repel mosquitoes. There are several types of mosquito repellent textiles available in India, such as mosquito repellent bed sheets, nets, curtains, clothing, and outdoor gear. These products are widely available in the market.

There are various synthetic mosquito repellent products available in the market, each with its own advantages and disadvantages. Here are some of the most common synthetic mosquito repellents, DEET (N,N-diethyl-meta-toluamide) is one of the most widely used mosquito repellents. DEET-based products are effective in repelling mosquitoes and other biting insects, but high concentrations of DEET can cause skin irritation and may not be safe.

Natural mosquito repellents are often made from ingredients that are considered safe and non-toxic. Synthetic mosquito repellents can be harmful to the environment, particularly if they are not disposed of properly. Natural mosquito repellents, on the other hand, are often made from biodegradable ingredients and are less likely to have a negative impact on the environment. Natural mosquito repellents have been in use for centuries, with many traditional remedies relying on plant-based ingredients to repel mosquitoes. However, in recent years, there has been increased interest in the development of natural mosquito repellents that are both effective and safe for use. One approach to developing natural mosquito repellents involves identifying the active compounds in plant extracts that are effective at repelling mosquitoes.

The use of natural plant extracts as mosquito repellents is becoming more popular in India, especially among those who prefer eco-friendly and non-toxic alternatives. There are several natural plant extracts and essential oils that are known to repel mosquitoes, including citronella, eucalyptus, neem, lavender, and lemongrass. These extracts are usually applied to the textile using different techniques such as impregnation, coating, or infusion. With the growing demand for eco-friendly and non-toxic alternatives, the use of natural plant extracts as mosquito repellents is likely to increase in the future.

Natural mosquito repellent textiles are fabrics that are treated with natural plant extracts or essential oils to repel mosquitoes. These textiles are designed to provide a safe and non-toxic alternative to synthetic insecticides that may have harmful effects on human health and the environment. *Pluchea wallichiana* leaves extract is a rich source of various phytochemical compounds which may possess good mosquito repellent properties. Hence, this study focuses on the development of a natural mosquito repellent finish using *Pluchea wallichiana* plant extract on cotton fabric.

The research aimed to investigate the effectiveness of the leaf extract as a mosquito repellent and its potential as an eco-friendly alternative to synthetic repellents.

In the present study, researcher experiments with *Pluchea wallichiana* leaves to use it as mosquito repellent finish. To estimate the effectiveness of leaf extract as mosquito repellent finish. Crude extract is obtained from leaves using Soxhlet extraction and rota evaporator was used to separate extract and solvent. Phytochemical analysis was conducted for knowing active mosquito repellent compounds. The extracted finish was applied through a pad-dry-cure method involving two techniques, i) Direct technique and ii) Resin cross-linking technique. The effectiveness of the finished fabric in repelling mosquitoes was evaluated using a standard laboratory test method.

The results showed that the *Pluchea wallichiana* leaf extracted using ethanol as a solvent had a 4.6 percent yield. A phytochemical analysis of the crude extract was also done to ascertain the compounds present in the extract. Flavonoids, Alkaloids, Phenols and glycosides are the compounds present which generally contribute to mosquito repellent property. Extract was applied on cotton fabric through pad-dry-cure method using different concentration of extract (10 and 20 gpl) and two different techniques,i.e. Direct and resin cross-linking and tested the fabric to evaluate efficacy of mosquito repellency through mosquito behaviour test and an extract was effective in repelling mosquitoes. The experiments were conducted using two species of mosquitoes for testing which are known as *Aedes* and *Anopheles stephensi*. In the direct technique, the highest mosquito mortality rate was up to 75 percent for directly tested samples, 60 percent after wash fastness test and 75 percent after light fastness test of treated sample using 20 gpl extract. The finished fabric exhibited poor wash fastness and poor light fastness. In resin cross-linking technique, a highest mortality up to 90 percent for directly tested samples was achieved, 65 percent MMR after wash fastness test and 75 percent after light fastness test of treated sample using 20 gpl extract. The finished fabric exhibited poor wash fastness and poor light fastness. Overall, the 20 gpl treated samples were more effective than 10 gpl treated samples. The treated samples after washing had a poor repellency compared to light fastness. The samples were tested using the standard WHO cone test method. A hundred percent repellency rate was obtained during the efficacy testing of the crude extract using the cage test. This study forms a baseline research in the development of a mosquito repellent fabric, using natural plant based crude extract, which can be a potential environmentally and healthy natural resource based repellent that protects the human beings from the mosquito bites and thereby reducing the risk of mosquito borne diseases.

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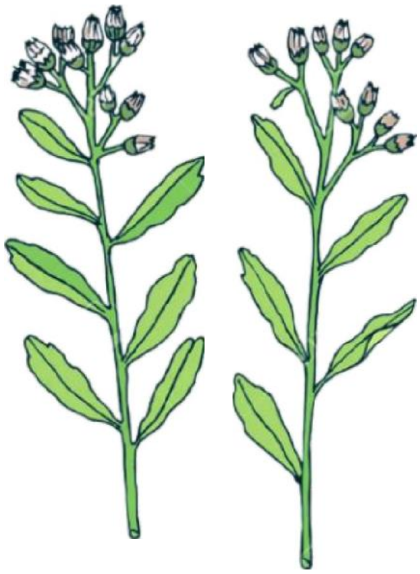


INTRODUCTION

CHAPTER I

INTRODUCTION

Mankind's quest for protection has led to a number of developments in every field with regards to textile. After fabricating the mansions of fashion and comfort, the field textiles has progressed towards the high tech era of performance, which has brought up diversification and expansion of technologies, in textiles for protection, performance and comfort.

Today, textiles continue to be an important part of our daily lives, with a wide range of applications including clothing, household items, and industrial products. The textile industry remains a major global industry, with many countries producing and exporting textiles to meet the demands of consumers around the world.

Textile finishes refer to the treatments applied to textiles to alter their appearance, performance, or durability. These treatments can include dyeing, printing, coating, and laminating, among others. Textile finishes can also be used to add functional properties such as water repellency, fire resistance, or antimicrobial properties.

The development of new textile finishes is an important area of research and innovation in the textile industry, as it allows for the creation of new products with enhanced properties and functionality. The use of sustainable and eco-friendly textile finishes is also an increasingly important consideration in the industry, as consumers become more aware of the environmental impact of textile production and seek out more sustainable options.

Overall, textiles and textile finishes are an integral part of our daily lives, with applications in clothing, home furnishings, and industrial products. As the textile industry continues to evolve, new materials and finishes will emerge, creating new opportunities and challenges for textile designers and manufacturers.

In the modern era "SMART TEXTILE" has become a buzzword. Protective textiles are among one such smart application of smart technology in textiles. Protective textiles refer to those textile products which have a functionality of giving protection from something in some or the other sense. With regard to textiles, the protective textile field of smart textiles has to fulfil this requirement. By using this part human beings solve many problems and mosquito repellent textile is one of them. By using this anyone can keep themselves far away from mosquitoes (G. S. Siva Kumar, 2015).

Mosquitoes are one of the most harmful vectors which transmit parasites in the human body, spreading deadly ailments such as malaria, dengue, chikungunya fever, yellow fever, etc. And every year a lot of people die affected by these diseases.

Mosquito repellent is a type of product that is designed to keep mosquitoes away from people, animals, or areas where they are not wanted. Mosquito repellents are products that are designed to prevent mosquitoes from biting humans by using chemicals or natural substances to repel these insects.

Mosquito repellent products are widely available in the market, and there are a variety of options to choose from. Some of the most common mosquito repellent products include: Insect repellent sprays, Insect repellent lotions, Mosquito repellent wristbands, Mosquito nets, Insecticide-treated clothing, Electric mosquito repellent devices, etc.

The development of textiles with mosquito-repellent properties involves the use of specialized finishes and treatments applied to the fabric. These finishes can include synthetic repellents such as DEET (N,N-diethyl-meta-toluamide), as well as natural repellents such as citronella, eucalyptus, and lemongrass. The use of textiles to repel mosquitoes and other insects has become an increasingly important area of research and innovation in the textile industry (Mynul Islam Sajib, et.al, 2020).

The effectiveness of mosquito-repellent textiles is determined by factors such as the type and concentration of repellent used the durability of the finish, and the breathability and comfort of the fabric. The use of sustainable and eco-friendly repellents is also an important consideration in the development of these textiles.

The potential applications of mosquito-repellent textiles are wide-ranging, including clothing, bedding, and outdoor gear such as tents and mosquito nets. These textiles have the potential to significantly reduce the transmission of mosquito-borne diseases, particularly in regions where these diseases are endemic.

Natural mosquito repellents and chemical mosquito repellents both offer effective ways to protect against mosquito bites and the diseases they can carry. However, there are some key differences between the two.

Natural mosquito repellents are typically made from plant-based ingredients, such as essential oils, that are known to repel mosquitoes. These ingredients include citronella, lemongrass, peppermint, and eucalyptus, among others. Natural mosquito repellents are generally considered safe and non-toxic, and may be a good choice for individuals who are sensitive to chemicals or who prefer to use natural products.

One advantage of natural mosquito repellents is that they tend to have a milder scent than chemical repellents, which can be important for individuals who are sensitive to strong smells. However, natural repellents may also need to be reapplied more frequently than chemical repellents, as they tend to have a shorter duration of effectiveness.

Tribal communities around the world have a long history of using natural mosquito repellents to protect themselves from mosquito-borne diseases. Traditional knowledge passed down through generations has helped these communities identify plants and other natural substances that are effective at repelling mosquitoes.

Plants have been used for centuries as natural mosquito repellents. Many plants contain essential oils that have insecticidal and repellent properties, making them effective at deterring mosquitoes. Plants can be used in various forms, including essential oils, dried or fresh leaves, and even live plants, to provide natural mosquito protection.

The use of medicinal plants as a source of relief from illness can be traced back to over five million years in the early civilization of China, India and North East, which is as old as mankind. It has been estimated that in developed countries such as the US, plant based drugs contribute about 25% of the total drugs, while in fast developing countries such as India and China, the contribution is about 80%. Thus the economic importance of medicinal plants is much more to developing countries than the rest of the world (Maheshwari, V. and Ramya, K., 2014).

In India, the use of neem as a natural mosquito repellent has been documented for centuries. The neem tree produces bitter oil that is effective at repelling mosquitoes, and the leaves and bark of the tree are commonly used in traditional medicines to treat mosquito-borne diseases.

In recent years, there has been an increasing demand for natural mosquito repellent products in the modern market. Natural mosquito repellent finished fabrics are becoming increasingly popular in the modern market as a way to protect against mosquito bites.

The use of natural mosquito repellent finished fabrics in the textile industry is becoming more common due to the growing demand for eco-friendly and sustainable products. These fabrics are often made from natural fibres such as cotton, bamboo, or hemp, which are environmentally friendly and biodegradable.

Overall, the use of natural mosquito repellent finished fabrics in the textile market is a great option for those looking for a safe and effective way to protect against mosquito bites without the use of harsh chemicals. The present study focuses on developing an eco-friendly natural mosquito repellent finished fabric treated using the plant extracts of *Pluchea wallichiana*.

Pluchea wallichiana is commonly known as wallich camphor-weed “RASANA” is an erect, glutinous, much branched shrub, with stems 1-2 m tall, branches round, striped, glandular velvet-hairy, young ones densely velvet-hairy. Leaves are alternate, broadly elliptic or ovate-oblong, half stem-clasping, blunt or somewhat pointed, often apiculate, margins obscurely toothed or almost entire, 3-6 x 1.5-3 cm, somewhat leathery, velvet-hairy, veins prominent on both surfaces. Wallich Camphor-Weed is found in Punjab, Rajasthan, Gujarat and South India (i).

Pluchea wallichiana has a variety of traditional medicinal uses in different cultures. In traditional Ayurvedic medicine, the plant is used to treat a range of ailments, including fever, cough, bronchitis, and rheumatism. It is also used as an insecticide and insect repellent due to its natural repellent properties against mosquitoes and other insects.

Pluchea wallichiana has been used as a natural mosquito repellent in some regions, with its leaves and stems crushed and applied to the skin or used as incense to repel mosquitoes. In recent years, research has focused on developing natural mosquito repellent products from *Pluchea wallichiana* extract, including repellent sprays, lotions, and fabric finishes.

Literature in Ayurveda suggests that Pluchea *wallichiana* is a valuable plant species with potential uses in medicine and as a natural mosquito repellent. Cotton is the most commonly used fibre hence the use of cotton fabric will be explored for the benefit of the end consumers.

Textiles for personal use or for home use can be treated with natural or synthetic mosquito repellent finishes to create a protective barrier against mosquitoes. A mosquito repellent finish applied to textile will be potentially safe to use as the application is not directly on the skin and does not cause any irritation or allergic reactions. In this research, objective analysis of mosquito repellent finish applied on cotton fabric was done. The results of the repellent activity were based on the cage test for evaluating the effectiveness of mosquito repellent finish.

1.1 PURPOSE OF THE STUDY

Untiring efforts are made by national and international healthcare bodies, in order to control the menace of vector borne diseases and great success has been achieved so far. However, there is still scope for use of natural mosquito repellent that works against the nuisance of biting insects or mosquitoes that transmit tropical diseases such as malaria, dengue, chikungunya, yellow fever, etc. The natural repellent is safer than synthetic chemicals. With the surge in consumers becoming more conscious about organic and plant based sources, naturally extracted mosquito repellent oil products will be the next buzz word due to their environment friendly nature and concerning human health. So, from the non-toxicity and availability point of view, traditional use of repellent products should be promoted in society. The purpose of applying natural mosquito repellent finish on cotton fabric is to create a natural protective barrier against mosquitoes.

The major tool in mosquito control operation has been the application of synthetic insecticides. But this has not been very successful because it has harmful effects on human health i.e. unpleasant smell, oily feeling to some users and potential toxicity. Using natural plant products is a simple and sustainable method of mosquito control.

When cotton fabric is treated with a natural mosquito repellent finish, such as that made from *Pluchea wallichiana* leaves, the repellent creates a barrier on the fabric's surface that prevents mosquitoes from landing and biting.

In addition to its potential for public health benefits, a natural mosquito repellent finish using *Pluchea wallichiana* plant extract could also have environmental and economic advantages. Unlike synthetic insecticides, plant-based repellents are typically biodegradable and have low toxicity, making them safer for the environment and for human health. Additionally, using plant extracts as a source of insect repellent could support local agriculture and provide economic benefits to communities where the plants are grown. Hence there appears to be great scope to undertake, this study as a promising area research contribution to public health, through functional finish application.

1.2 OBJECTIVES OF THE STUDY

1.2.1 To develop an Eco friendly natural mosquito repellent finish using plant extract.

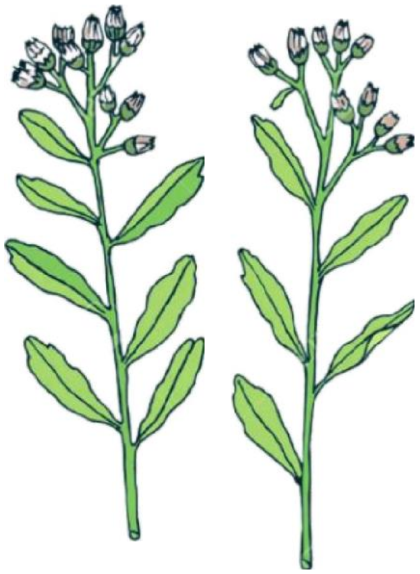
1.2.2 To study the application of plant extract as a finish on cotton fabric.

1.2.3 To study the effect of a mosquito repellent finish on cotton fabric.

1.2.4 To evaluate wash and light durability of finished fabric.

1.3 DELIMITATIONS OF THE STUDY

1.3.1 The study is limited to using only one solvent (i.e. Ethanol).



REVIEW
OF
LITERATURE

CHAPTER II
REVIEW OF LITERATURE

The review of literature for the present study was gathered from various secondary sources such as libraries, journals, articles, handbooks and from various websites. The review of literature related to the study has been broadly classified into two major sections and further in subsections:

2.1 Theoretical Review

2.1.1 Extraction techniques of medical plants

2.1.2 Phytochemical analysis

2.1.3 Finishing of the fabric using extract

2.1.4 Mosquito repellency test

2.1.5 Mosquito behaviour test

2.1.6 Soxhlet extraction method

2.1.7 *Pluchea wallichiana* plant

2.1.8 Fastness tests repellency

2.2 Related Research Review

2.2.1 Research related to extraction process

2.2.2 Research related to preliminary phytochemical test

2.2.3 Research related to eco-friendly mosquito repellent finished fabric

2.2.4 Research related to mosquito test

2.2.5 Research related to application of mosquito repellent finish

2.2.6 Research related to pad-dry-cure method

2.2.7 Research related to *Pluchea* family

2.1 THEORETICAL REVIEW

2.1.1 Extraction techniques of medical plants

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. (Handa S. et al., 2008)

These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts and powdered extracts. Such preparations popularly have been called galenicals, named after Galen, the second century Greek physician. The purposes of standardized extraction procedures for crude drugs are to attain the therapeutically desired portion and to eliminate the inert material by treatment with a selective solvent known as *menstruum*.

Methods of extraction:

i) Decoction

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat-stable constituents. This process is typically used in preparation of Ayurvedic extracts called “quath” or “kawath”. The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further.

ii) Infusion

Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.

iii) Maceration

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.

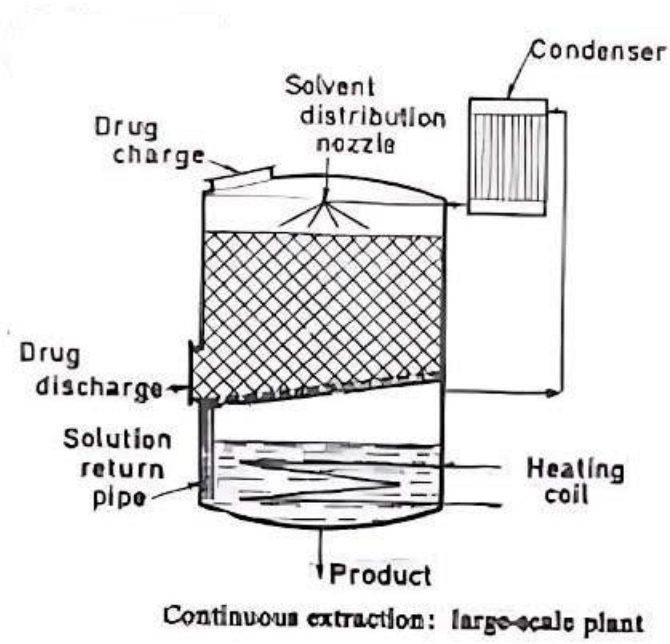


Figure 2.1: Circulatory extraction
Image source: http://surl.li/gfgcb

iv) Digestion

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the *menstruum* is thereby increased.

v) Percolation

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified *menstruum* (A liquid that dissolves a solid; a solvent, especially one used to extract a drug from a plant) and allowed to stand for approximately 4 hr in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional *menstruum* is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 hr. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional *menstruum* is added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient *menstruum* is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting.

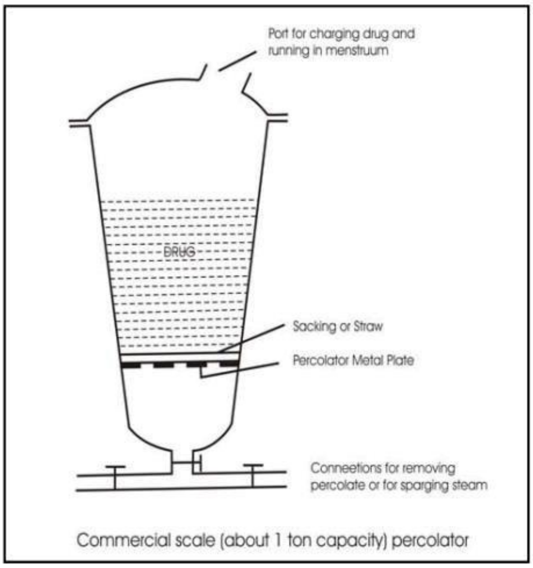


Figure 2.2: Percolation extraction
Image source: http://surl.li/gfggt

vi) Hot Continuous Extraction (Soxhlet)

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in the chamber of the Soxhlet apparatus (Figure 2.2). The extracting solvent in the flask is heated, and its vapours condense in the condenser. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in the chamber rises to the top of the siphon tube, the liquid contents of the chamber siphon into a flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This affects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

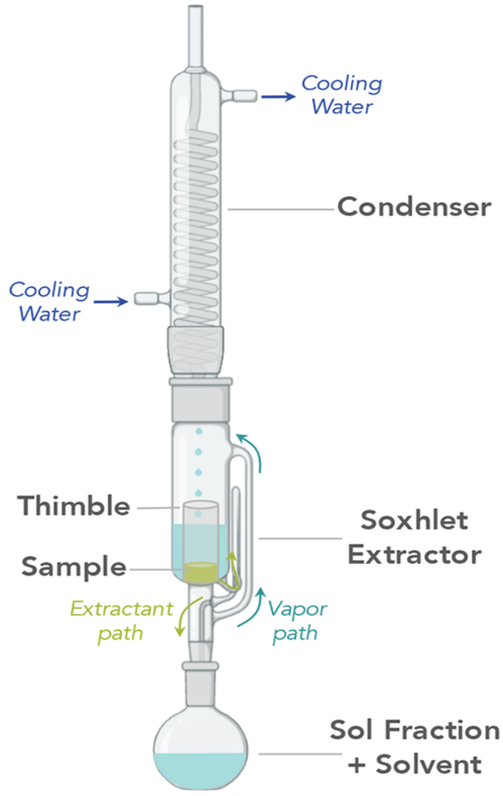


Figure 2.3: Soxhlet extraction

Image source: https://www.researchgate.net/profile/Shu-Wang-24/publication/

vii) Ultrasound Extraction (Sonication)

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of *Rauwolfia* (Sarpagandha) root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules.

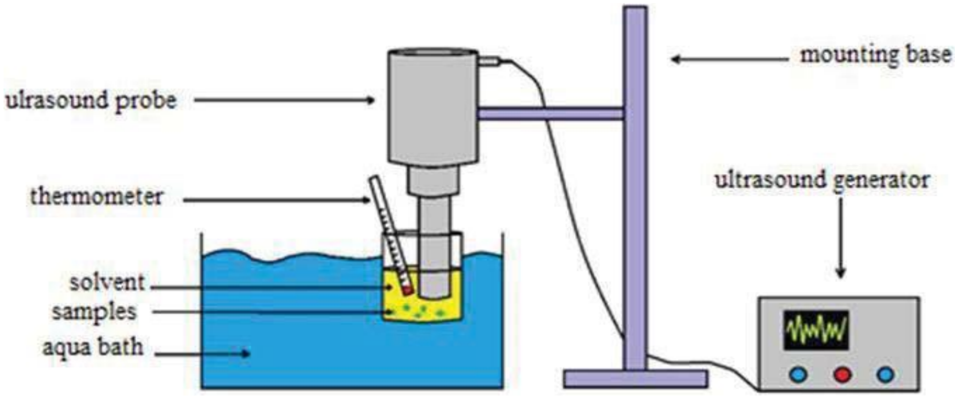


Figure 2.4: Ultrasonic extraction

Image source: https://onlinelibrary.wiley.com/cms/asset/

viii) Hydrodistillation

In order to isolate essential oils by hydrodistillation, the aromatic plant material is packed in a still and a sufficient quantity of water is added and brought to a boil; alternatively, live steam is injected into the plant charge. Due to the influence of hot water and steam, the essential oil is freed from the oil glands in the plant tissue. The vapour mixture of water and oil is condensed by indirect cooling with water. From the condenser, distillate flows into a separator, where oil separates automatically from the distillate water.

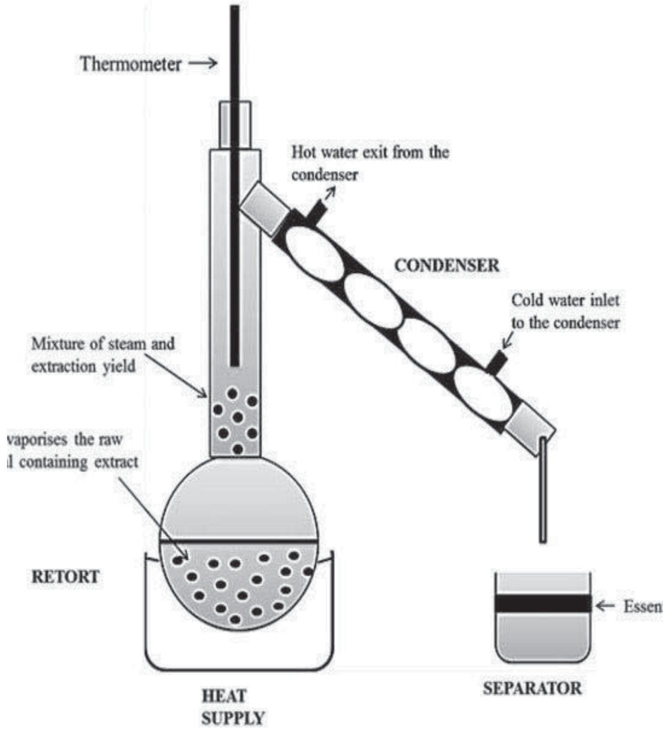


Figure 2.5: Hydro-distillation extraction

Image source: https://www.researchgate.net/profile/Siti-Nuurul-Huda-Mohammad-Azmin/

ix) Steam Distillation:

Steam distillation is a type of distillation (a separation or extraction process) for a temperature-sensitive plant such as natural aromatic compounds. It once was a popular laboratory method for purification of organic compounds but has become obsolete by vacuity distillation. Steam distillation is still important in certain industrial sectors. Steam distillation is one of ancient and officially approved methods for isolation of essential oils from plant materials. The plant materials charged in the alembic are subjected to the steam without maceration in water. The injected steam passes through the plants from the base of the alembic to the top. Steam distillation is a method where steam flows through the material as shown in Figure 6. This steam functions as agents that break up the pores of the raw material and release the essential oil from it. The system yields a mixture of a vapour and desired essential oil. This vapour is then condensed further and the essential oil is collected. The principle of this technique is that the combined vapour pressure equals the ambient pressure at about 100°C so that the volatile components with the boiling points ranging from 150 to 300°C can be evaporated at a temperature close to that of water. Furthermore, this technique can be also carried out under pressure depending on the essential oils extraction difficulty.

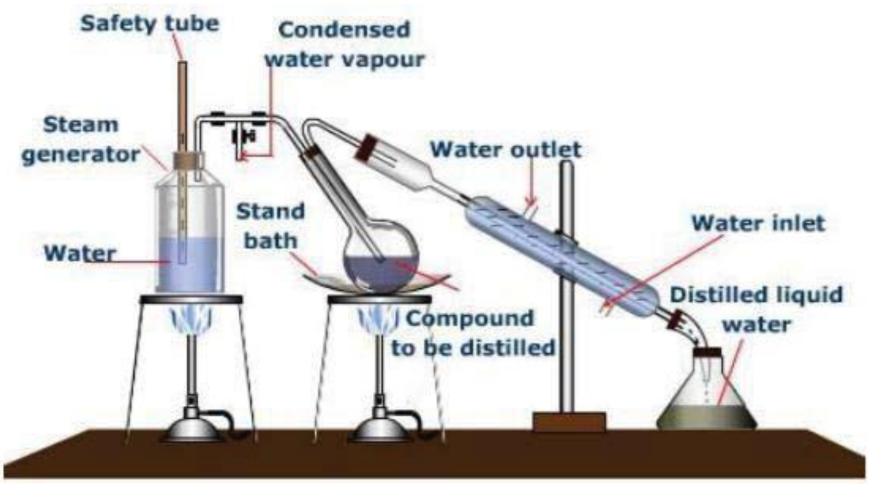


Figure 2.6: Steam distillation extraction

Image source: https://encrypted-tbn0.gstatic.com/

2.1.2 Phytochemical analysis (Harborne J. et al., 1998)

The different qualitative chemical tests can be performed for establishing the chemical composition profile of the extract. The following tests may be performed on extracts to detect various phyto-constituents present in them.

i) Detection of Alkaloids (Evans, 1997)

Solvent free extract, 50 mg is stirred with a 3 ml of dilute hydrochloric acid and filtered. The filtrate is tested carefully with various alkaloidal reagents as follows:

- Mayer's test (Evans, 1997)

To a 3 ml of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate indicates the test as positive.

Mayer's Reagent

Mercuric chloride (1.358 g) is dissolved in 60 ml of water and potassium iodide (5.0 g) is dissolved in 10 ml of water. The two solutions are mixed and made up to 100 ml. with water.

- Wagner's test (Wagner, 1993)

To a few ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube. A reddish-brown precipitate confirms the test as positive.

Wagner's reagent

Iodine (1.27 g) and potassium iodide (2 g) is dissolved in 5 ml of water and made up to 100 ml. with distilled water.

- Hager's test (Wagner et al., 1996)

To a few ml of filtrate, 1 or 2 ml. of Hager's reagent (saturated aqueous solution of picric acid) are added. A prominent yellow precipitate indicates the test as positive.

- Dragendorff's test (Waldi, 1965)

To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent are added. A prominent yellow precipitate indicates the test as positive.

Dragendorff's reagent

Stock solution: Bismuth carbonate (5.2 g) and sodium iodide (4 g) are boiled for a few min with 50 ml glacial acetic acid. After 12 h, the precipitated sodium acetate crystals are filtered off using a sintered glass funnel. Clear, red-brown filtrate, 40 ml is mixed with 160 ml ethyl acetate and 1 ml. water and stored in amber-coloured bottle.

Working solution: Ten mL of stock solution is mixed with 20 mL of acetic acid and made up to 100 ml. with water.

ii) Detection of Carbohydrates and Glycosides (Ramakrishnan et al., 1994)

The extract (100 mg) is dissolved in 5 ml of water and filtered. The filtrate is subjected to the following tests.

- Molish's test

To 2 ml of filtrate, two drops of alcoholic solution of a-napthol are added, the mixture is shaken well and ml. of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

- Fehling's test

One ml of filtrate is boiled in a water bath with 1 ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar. Fehling's Solution A: Copper sulphate (34.66 g) is dissolved in distilled water and made up to 500 ml using distilled water. Fehling's Solution B: Potassium sodium tartrate (173 g) and sodium hydroxide (50 g) is dissolved in water and made up to 500 ml.

- Barfoed's test

To 1 ml of filtrate, 1 ml of Barfoed's reagent is added and heated in a boiling water bath for 2 min. Red precipitate indicates the presence of sugar.

Barfoed's reagent

Copper acetate, 30.5 g is dissolved in 1.8 ml of glacial acetic acid.

- Benedict's test

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent is added. The mixture is heated in a boiling water bath for 2 min. A characteristic coloured precipitate indicates the presence of sugar.

Benedict's reagent

Sodium citrate (173 g) and sodium carbonate (100 g) are dissolved in 800 ml of distilled water and boiled to make it clear. Copper sulphate (17.3 g) dissolved in 100 ml distilled water is added to it.

For detection of glycosides, 50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hr in a water bath, filtered and the hydrolysate is subjected to the following tests.

- Borntrager's test (Evans, 1997)

To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates the presence of glycosides.

- Legal's test

Fifty mg of the extract is dissolved in pyridine; sodium nitro-prusside solution is added and made alkaline using 10% sodium hydroxide. Presence of glycoside is indicated by the pink colour.

iii) Detection of Saponins (Kokate, 1999)

The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 min. A two cm layer of foam indicates the presence of saponins.

iv) Detection of Proteins and Amino acids (Fisher, 1968; Ruthmann, 1970)

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate is subjected to tests for proteins and amino acids.

- Millon's test (Rasch and Swift, 1960)

To 2 ml. of filtrate, 3-5 drops of Millon's reagent are added. A white precipitate indicates the presence of proteins.

Millon's reagent

Mercury (1 g) is dissolved in 9 ml of fuming nitric acid. When the reaction is completed, equal volume of distilled water is added.

- Biuret test (Gahan, 1984)

An aliquot of 2 ml of filtrate is treated with one drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicates the presence of proteins.

- Ninhydrin test (Yasuma and Ichikawa, 1953)

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to two mL of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

v) Detection of Phytosterols (Finar, 1986)

- Libermann-Burchard's test

The extract (50 mg) is dissolved in 2 mL acetic anhydride. To this, one or two drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array of colour changes shows the presence of phytosterols.

vi) Detection of Fixed Oils and Fats (Kokate, 1999)

- Spot test

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

- Saponification test

A 3-4 drops of 0.5 N alcoholic potassium hydroxide solutions are added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated in a water bath for 2 hr. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

vii) Detection of Phenolic compounds and Tannins

- Ferric chloride test (Mace, 1963)

The extract (50 mg) is dissolved in 5 ml. of distilled water. To this, 2-3 drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds.

- Gelatine test (Evans, 1997)

The extract (50 mg) is dissolved in 5 ml of distilled water and 2 ml. of 1% solution of gelatine containing 10% sodium chloride is added to it. White precipitate indicates the presence of phenolic compounds.

- Lead acetate test

The extract (50 mg) is dissolved in distilled water and to this; 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

- Alkaline reagent test

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

- Magnesium and hydrochloric acid reduction (Harborne, 1998)

The extract (50 mg) is dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (drop wise) are added. If any pink to crimson colour develops, presence of flavanol glycosides is inferred.

viii) Detection of Gum and Mucilages (Whistler and BeMiller, 1993)

The extract (100 mg) is dissolved in 10 ml of distilled water and to this; 25 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilages.

ix) Detection of Volatile Oil (James et al., 1996)

In a volatile oil estimation apparatus, 50 g of powdered material (crude drug) is taken and subjected to hydro-distillation. The distillate is collected in a graduated tube of the assembly, wherein the aqueous portion automatically separates out from the volatile oil.

2.1.3. Finishing of the fabric using extract

i) Microencapsulation (Simon B., 2006)

Many methods for eco-friendly textile processing have been developed by science, including enzymatic textile finishing, plasma technology, natural product finishing, and microencapsulation.

Microencapsulation has become a difficult way to develop novel biotechnological materials. Microencapsulation is the process of forming small “packaging” known as micro particles, microspheres, or microcapsules, which are made up of structures that contain one or more bioactive compounds or are immobilized by one or more polymers.

Encapsulating liquid droplets, solid particles, or gas molecules in an encapsulating agent/matrix/ wall material is known as microencapsulation. These chemicals are completely encapsulated in a covering material or integrated into a homogeneous or heterogeneous matrix to generate tiny capsules with a variety of characteristics. The core and the shell/wall of microcapsules are split into two portions.

The microencapsulation technique can protect active chemicals from potentially harmful conditions such as oxidation, heat, acidity, alkalinity, moisture, or evaporation.

Morphology of microcapsules

Microcapsules have two parts: the core and the shell. The core (intrinsic component) contains an active substance (for example, a hardener), whereas the shell (extrinsic part) shields the core from the external environment permanently or temporarily (Figure 2.7).

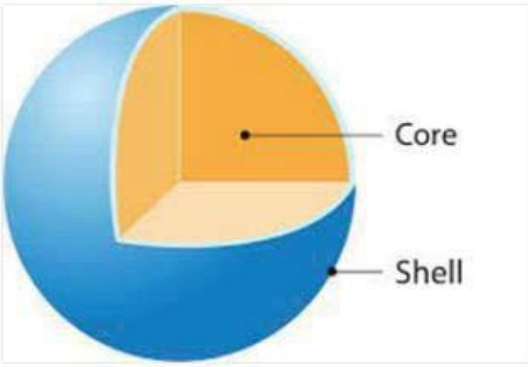


Figure 2.7: Morphology of microcapsules

Image source: https://www.google.co.in/url?sa=i&url=https%3A%2F%2Fjtcps.journals.

Core material

The core material, which is defined as the exact substance to be coated, might be solid, liquid, or gaseous. The major forms of the core materials are solution, dispersion, and emulsion. Because the liquid core might contain scattered and/or dissolved material, the core material composition can vary. Active ingredients, stabilizers, diluents, excipients, and release-rate retardants or accelerators can all be found in the solid core. The capacity to change the composition of the core materials gives significant flexibility and utilizing this feature frequently enables for effective design and development of desired microcapsule features.

Shell material

Diffusion, permeability, and controlled release applications all benefit from shell type and shape. The physical and chemical qualities of the resulting microcapsules/microspheres are determined by the shell material selected.

Classification of microcapsules:

Microcapsules can be classified based on their size or morphology.

Micro/Nanocapsules

Microcapsules range in size from a thousandth of a millimeter to a few millimeters. Nano capsules are microcapsules with a diameter in the nanometer range to emphasize their microscopic size.

Aroma/Fragrant Textiles

Fragrance/Scent finishing is one such growing sector that adds value and utility to textile products by incorporating aroma into them. The perfumes produced from plant sources, i.e. essential oils not only have a nice smell but have also been used as antiseptics, anti-inflammatory, antimicrobials, and emotional soothing. Aromatherapy textiles include essential oils generated from plant-based raw materials, which can help to improve the body’s mental and physical state. Microcapsules burst as a consequence of mechanical pressure on the capsules during wear, releasing the active component (Figure 2.8). To create scent textiles, several research studies have been conducted.

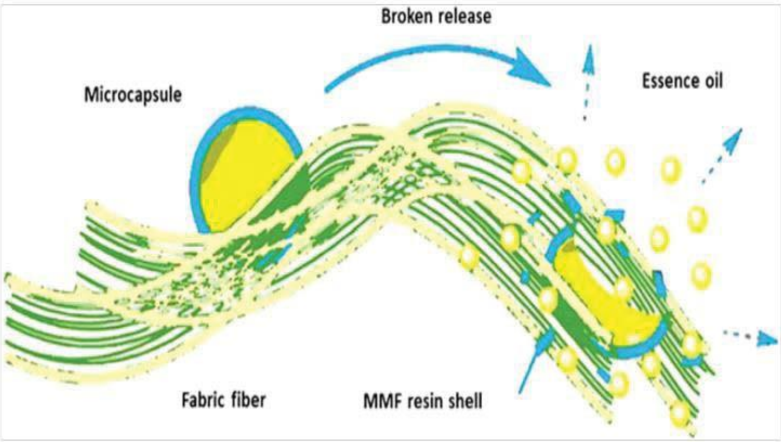


Figure 2.8: Release of aroma microcapsules under external force

Image source: https://encrypted-tbn0.gstatic.com/images

ii) Pad-dry-cure method

The most common application method for easy-care and durable press finishes is a pad–dry–cure procedure. In this process, the crosslinking reactant, catalyst, softener, and other components are dried on the fabric prior to the crosslinking reaction that takes place during the curing step. If the finish is cured immediately after drying, while the fabric is still in an open width configuration, the finish is referred to as a ‘pre-cure’ finish; the finish is cured *prior* to garment manufacture. Textiles with a pre-cure finish are most suited for fabrics and apparel that require wrinkle resistance, such as sheeting, shirting and casual trousers.

The traditional pad–dry–cure method is a ‘dry’ curing process, i.e. all of the water has been removed from the fabric prior to the actual crosslinking reaction. It is also possible to crosslink cellulose in a ‘wet’ process. Fabric padded at ~80 % wet pickup with the finishing chemicals is wrapped in plastic film and batched at room temperature for about 24 hours before washing and drying.

The water content of the cellulose fibres during the crosslinking step greatly affects the final fabric properties as is seen in Table 2.1.

Table 2.1: Effects of different curing conditions on fabric properties

‘Dry’ curing, short times at high Temperatures	‘Wet’ curing, long times at low temperatures
Loss of tear strength and abrasion resistance	Good tear strength and abrasion resistance
High dry crease recovery angle	Lower dry crease recovery angle
Lower wet crease recovery angle	High wet crease recovery angle

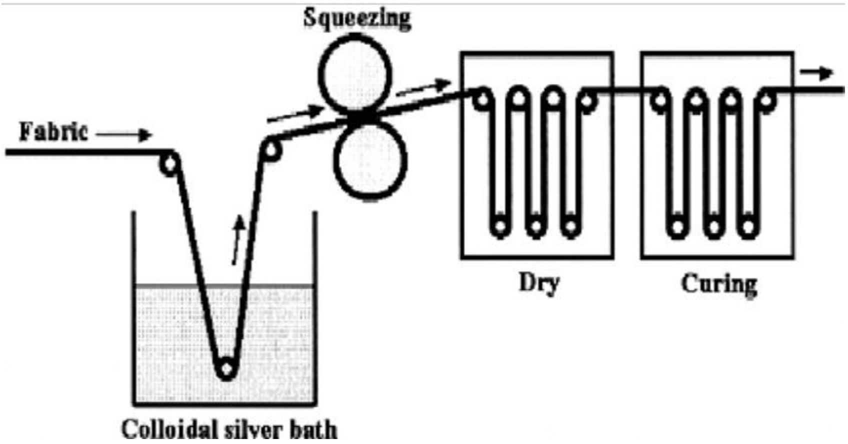


Figure 2.9: Pad-Dry-Cure Method

Image source: https://www.researchgate.net/profile/Mohammed-Ramadan-5/publication/pad-dry-cure

2.1.4 **Mosquito repellency test** (Debboun M., 2015)

Repellents are currently used by millions of people worldwide to prevent nuisance bites from blood-feeding insects. Recently, there was limited scientific evidence on the efficacy of repellents to reduce disease. Although the inhabitants of tropical countries with low per capita incomes may still use smoke and plant materials to keep biting arthropods at bay, the majority of research into the highly effective mosquito repellents that are available today has been carried out by scientists employed by or funded by the military to protect troops stationed in high-disease-risk areas. Some of the world's most important programs involved in the understanding and prevention of arthropod-borne diseases have risen as a result of conflicts in tropical regions that lead to massive loss of life from diseases such as yellow fever, louse-borne typhus, and malaria. Two of these discoveries, N,N-diethyl-3- methylbenzamide (DEET), which is a topical repellent and long-lasting permethrin-treated clothing other repellent is p-menthane-3,8-diol (PMD).

Topical repellents are oils or lotions applied to the exposed skin or clothes of the consumer, with the most safe and effective. Natural mosquito repellents can be effective in preventing mosquito bites to some extent. However, their effectiveness may vary depending on various factors such as the type of repellent, the concentration of the active ingredient, the duration of exposure, and the level of mosquito activity in the area.

Mosquito repellency refers to the ability of a substance or product to prevent mosquitoes from landing on and biting a person or animal. Mosquitoes are attracted to humans by the carbon dioxide, heat, and moisture as well as by certain chemicals in human skin. Mosquito repellents can be either synthetic or natural and come in various forms, such as sprays, lotions, and candles.

There are several methods for testing the mosquito repellency of different products. Here are some common methods used for testing the mosquito repellency of different products:

- Arm-in-Cage Method: This method is used for testing the efficacy of mosquito repellents, such as lotions and sprays. It involves applying the product to a volunteer's forearm and placing it in a cage containing mosquitoes. The number of mosquito bites on the treated and untreated areas of the skin is recorded over a set period of time.

- Petri Dish Test: This method is used for testing the efficacy of mosquito repellent-treated fabrics, such as clothing and bed nets. In this test, a petri dish is divided into two sections, one treated with the product and the other left untreated. Mosquitoes are then released into the dish and the number of mosquitoes in each section is counted over a set period of time.

- Y-Tube Test: This method is used for testing the efficacy of mosquito repellent-treated surfaces, such as walls and floors. It involves placing mosquitoes in a Y-shaped tube and exposing them to two different odors, one of which is the treated surface. The number of mosquitoes that are attracted to each odour is recorded, and the percentage of mosquitoes repelled by the treated surface can be calculated.

- Field Test: This method is used for testing the efficacy of mosquito repellents in a natural setting, such as a forest or a residential area. Mosquitoes are collected before and after treatment with the product, and the reduction in the number of mosquitoes is calculated.

It is important to use established protocols and methods for testing to ensure accuracy and consistency of results. Testing should be conducted in a controlled environment, and appropriate safety measures should be taken to protect volunteers and researchers from mosquito-borne diseases.

2.1.5 Mosquito behaviour test

There are several methods to evaluate the treated textile with mosquito repellent. The most used techniques are cage test, cone test, and excito chamber, which have been discussed here.

i) Cage test

The cage test assesses the viability of repelling substances against mosquitoes for use in lotions, cream including impregnated material. It is a test that can be executed in a quick and effective manner. The test was designed to observe the mosquito landing on the untreated and treated fabric in the cage. The advantages of this method was that it provided the real situation of the probing and biting of the mosquito to the human besides it can directly provide the observation of the mosquitoes behaviour towards the treated materials. The drawbacks of the cage test involve human participation, it takes a lot of preparation either in terms of paper works such as needed to apply ethical approval, the human and mosquito preparation. In terms of human participation, the consent form and incentives must be prepared as an appreciation to the volunteer. The mosquitoes used in the test need to be free from pathogen as the human subject involved in the test must have the assurance that the test will not harm them. The cage measurement is according to WHO guideline for efficacy testing of mosquito repellents for human skin the range of 35–40 cm per side. Some studies reported in modification of the cage dimension used a cage 18 × 18 × 18 cm dimension and used cage 30 × 30 × 30 cm dimension.

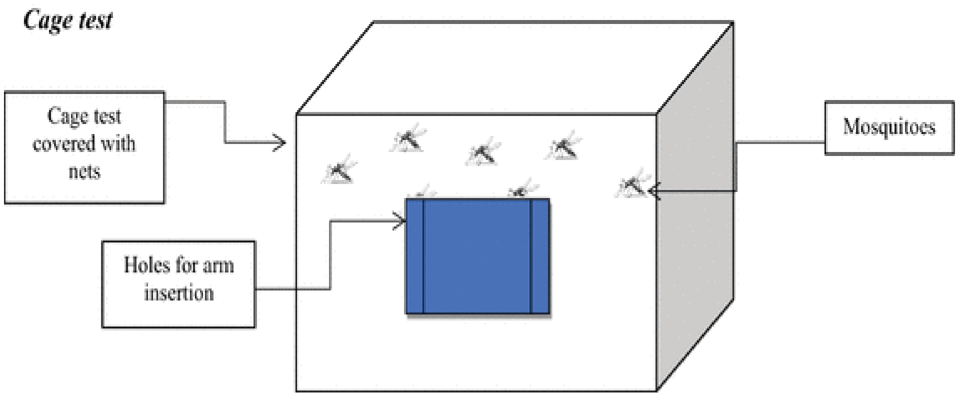


Figure 2.10: Cage test

Image source: https://media.springernature.com/lw685/springer-static/image/

ii) Cone test

The cone test was formerly the custom to evaluate the toxicity of insecticide-treated bed nets against malaria, which was also able to investigate the toxicity of other impregnated (textile) surfaces. The fabric treated, evaluated using the WHO cone test following the standard procedure described in the WHO 1998: test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. This test does not involve human participants as the bait to lure the mosquitoes comes to fabric and this is one of advantages of this method. Due to this factor, this method is less chosen by the researcher to conduct the mosquito repellency test on clothing. The use of artificial blood or animal blood as bait in order to attract host seeking mosquitoes, which in this cone test could help future studies to better assess the efficacy of the treated clothing. In the cone test, the mosquitoes might spend more time resting on the cone than on the treated surface during the 3 min exposition. The 3-min exposure test was carried out under the temperature of 27 °C.

The standard WHO plastic cone was placed on top of the treated surface of the sample and secured using a masking tape. Five to ten female mosquitoes were blown into the cone using an aspirator and mosquitoes were exposed to the treated surface. The low density mosquito number used for this method made it easy observation for the mosquito behaviour. The numbers of mosquitoes resting on the treated samples were counted within 3-min exposure. At the end of the exposition, the mosquitoes were transferred to the plastic cones for further observation. The plastic cup kept in an insecticide-free air and supplied with 10 % sucrose solution. The number of immobilized, knocked down test mosquitoes was determined 1 h after the exposition and the mortality rate was determined after 24 hr. The percent mosquito repellency was calculated using the following formula:

$$\% \text{ Mosquito mortality: } (MR - MC)/(100 - C) \times 100\%$$

Where, MR represents the mosquito's mortality in test replicate while the MC corresponds to the mosquito's mortality in control samples. The natural mortality rate is determined with an untreated fabric.

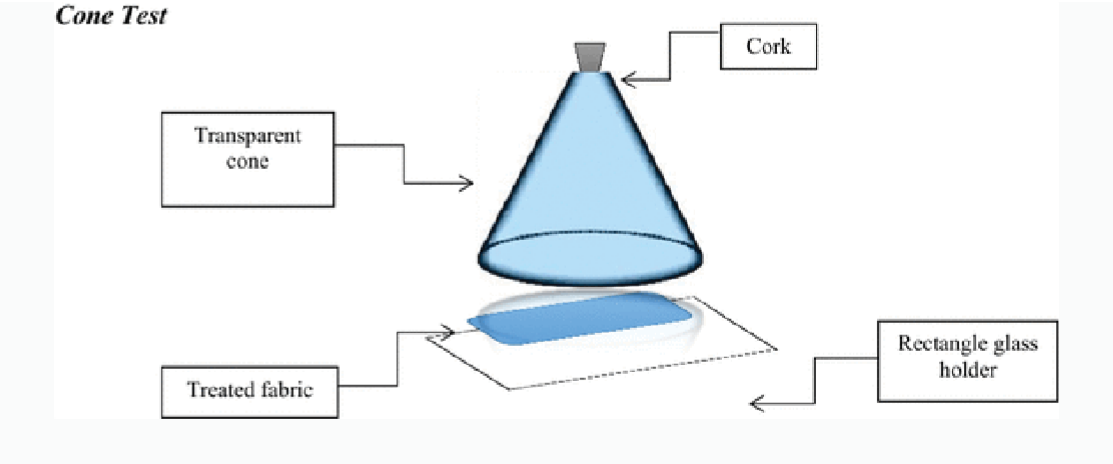


Figure 2.11: Cone test

Image source: https://media.springernature.com/lw685/springer-static/image/

iii) Excito chamber test

The Excito chamber method was a modified custom method to observe the mosquito behaviour change in the form of moving away from the treated to untreated fabric. This method and Cone test method does not involve the human subject to lure the mosquito. However, both methods can determine the behaviour of the mosquitoes towards the treated materials. The box is made with one front and exit panel occupied with single escape portal. It builds up with screened inner chamber, glass holding frame and door cover. The mosquito was starved overnight or at least a minimum 4 hours before the test. The behaviour of mosquito was observed in term of number of escaped mosquitoes to another space and remains mosquitoes inside the chamber which filled with treated product. The observation is recorded after 10 and 30 min exposure. The test was conducted in daylight and repeated for four times. The percentage of mosquito repellency was calculated using the formula:

$$\% \text{ Mosquito repellency: } (NES+NDE)/(NEX)\times 100\%$$

Where, NES corresponds to the number of mosquitoes escaped, while the NDE refer to the number of mosquitoes dead and last is NEX represents the number of mosquitoes exposed.

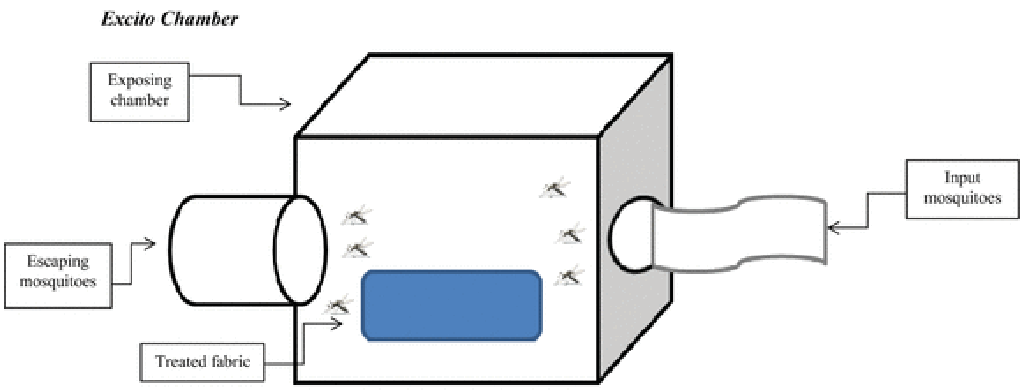


Figure 2.12: Excito Chamber

Image source: https://media.springernature.com/lw685/springerstatic/image/

2.1.6 Soxhlet extraction method

The original Soxhlet extractor was developed by Franz Von Soxhlet, a German agricultural chemist, in the early part of this century. In Soxhlet extractor, within an enclosed flask there is an inverted condenser pointing down into the flask from the top. Just below that condenser will be suspended either what's called a Soxhlet basket or a recovery vessel depending on whether you are extracting or recovering solvent. The condenser will have cold liquid circulating through it to keep the condenser cold. In the bottom of the main flask, solvent is placed. To do an extraction, the powdered plant material is placed in the Soxhlet basket which is a vessel with perforated sides and bottom so that liquid can fall through it. When gentle heat is applied to the main flask, the solvent begins to evaporate and the solvent vapours reach the cold condenser at the top of the flask and begin to liquefy on the sides of the condenser (much the same way that a cold glass of water becomes wet on the outside of itself on a hot day). The re-condensed solvent on the sides of the condenser begins flowing down the sides of the condenser and begins dripping off of drip points on the end of the condenser. This solvent drips into the top of the Soxhlet basket where it saturates the herb being extracted. The solvent flows through the basket and out of the holes in the bottom of the basket carrying the extract with it into the bottom of the flask. The extract laden solvent falling from the Soxhlet basket is dark in colour and as it becomes clearer, one can know that the plant material is leached out and the process is finished.

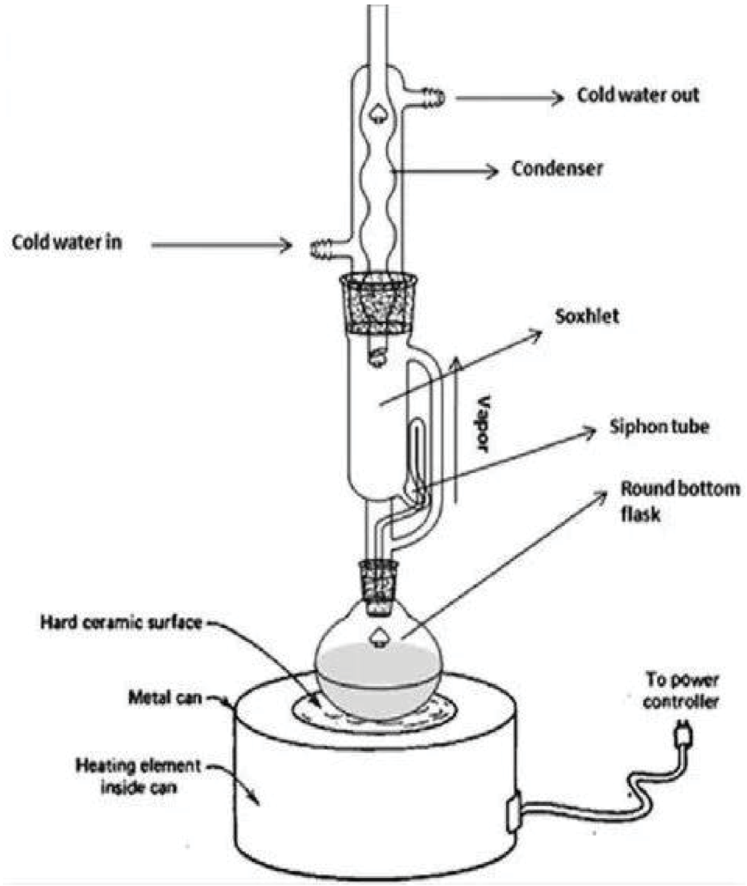


Figure 2.13: Soxhlet Instrument

Image source: https://5.imimg.com/data5/CM/HP/LT/SELLER-1742194/extraction-apparatus-500x500.jpg

The recovery vessel is simply a cup which is suspended below the condenser. As solvent vapours re-condense and fall off the tip of the condenser, they fall into the cup and are thus separated from the extract itself.

At this point one can do one of 3 things:

1. Stop the operation and pour the extract infused solvent out of the main flask.
2. Hook up the recovery vessel and remove the solvent from your extract which generally leaves a paste behind.
3. Dump and squeeze out the spent plant material in the Soxhlet basket, then start a fresh basket of herb in the extractor using the same solvent which continually re-distils and extracts regardless of how much extract is infused into it in the bottom of the main flask.

2.1.7 *Pluchea wallichiana* (Rasana)

The genus *Pluchea* belongs to the family *Compositae* and is the name- giving genus of the tribe *Plucheeae*. The tribe comprises 28 genera and the genus *Pluchea* forms the largest group with 40 species. Although *Pluchea* was first described as early as 1817 no revisionary or monographic works have been prepared since then *Pluchea* species are shrubs and subshrubs of tropical and subtropical regions. They are aromatic plants and most of them have a pleasant odour. Various species of *Pluchea* are used medicinally in Africa, Arabia and India and most frequently the aromatic leaves and stems are used.

The genus *Pluchea* (Compositae, Plucheeae) comprises 45 species distributed worldwide in tropical and subtropical regions. This revision is focused on the species in the Old World. 29 species are accepted, which are distributed in Africa, the Middle East, South Asia, South East Asia and Australia. And *Pluchea wallichiana* is one of them.

Pluchea wallichiana, known from Pakistan and India, are the only endemic *pluchea* species from the Indian Subcontinent. It is also mentioned that it has test epidermis cells dorsally thickened in *Pluchea wallichiana* which covers the leaves, flowers, roots and stems of plant. It forms a boundary between the plant and external environment. Leaves 2 to 6 cm long, 1.5 to 2.5 cm wide, lanceolate, oblanceolate or oblong. Distributed mainly in the states Gujarat, Rajasthan, Punjab and also in South

India in the state Tamil Nadu. It has been found in the dangs district in Gujarat. *Pluchea wallichiana* is known to be rare in Jodhpur (Rajasthan, India).

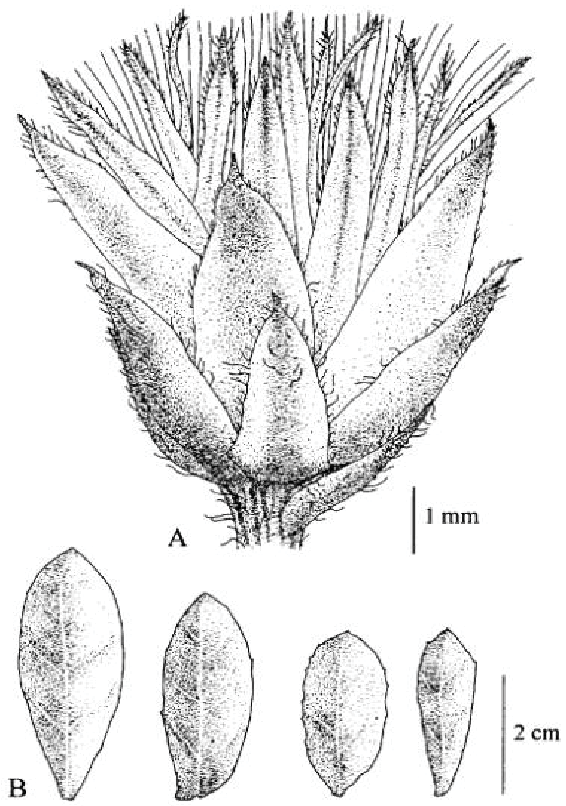


Figure 2.14: *Pluchea wallichiana* - A: Capitulum, from Wallich (G), B: Leaf shapes.
Image source: https://www.jstor.org/stable/3776750?origin=crossref

2.1.8 **Fastness tests** (Rastogi D. et al., 2017)

Fastness of coloured or finished fabrics is of utmost importance for their efficient end use. The ability of a fabric to retain its original effect is one of the most important parameters which can affect consumer's satisfaction. Fastness properties of textiles were evaluated to a variety of agents such as washing, light, rubbing, perspiration, bleaches, dry cleaning, heat, etc. A coloured or finished textile generally assessed for specific fastness parameters which depends on its intended use. For instance, fastness to washing is important for clothes which are washed frequently, while fastness to light is important for curtains. Similarly, fastness to bleach is important for swimwear, as water in swimming pools contain chlorine used as disinfectant, while fastness to perspiration may be more relevant to test for socks, shirts, and other such apparel.

The various parameters that affect fastness are fibres used, dyes and chemicals used and the method of application. Standard tests that are regularly updated and published are available from various organisations such as AATCC (American Association of Textile Chemists and Colourists), BIS (Bureau of Indian Standards), ISO (International Organizations for Standardization). Any one of these standard tests can be used to determine the fastness properties of textile materials.

i) Wash fastness

Wash fastness is a measure of how well a fabric can withstand repeated washing without losing its colour or appearance. It is an important property to consider when selecting or designing textiles for applications where frequent washing is expected, such as in clothing or household linens.

Various factors can affect the wash fastness of a fabric, including the type of fibre, dye or printing method used, and the washing conditions. To determine the wash fastness of a fabric, it is tested by washing it under controlled conditions, such as using a standardized detergent and washing machine, and then evaluating any changes in the fabric's appearance or colour.

A fabric that has good wash fastness will maintain its appearance and colour even after several washes, while a fabric with poor wash fastness may fade, bleed, or lose its colour intensity or brightness. Various treatments can be used to improve the wash fastness of a fabric, such as using colourfast dyes, finishing treatments, or adding additional layers of protection to the fabric.

A launder-o-meter is used to test wash fastness of dyed or finished fabric. It is a machine consisting of stainless steel canisters which rotate in a thermostatically controlled water bath. Conditions of temperature, detergent solution and abrasive action similar to those at home and commercial laundering are created by selecting suitable standard test methods so as to obtain appropriate results. The change in colour or staining in the launder-o-meter can be due to the abrasive action against canister walls and steel balls. Assessment of fastness involves a visual determination or another testing.

Figure 2.15: Launder-o-meter

Image source: https://www.rycobel.com/assets/uploads/site/products/launder-ometer/_1600xAUTO_crop_center-center_none/big-container.jpg

ii) Light Fastness

Light fastness refers to a fabric's ability to resist fading or discoloration when exposed to light, especially sunlight. It is an important property to consider when selecting or designing textiles for applications where the fabric will be exposed to natural or artificial light, such as in outdoor furniture, curtains, or upholstery.

Various factors can affect the light fastness of a fabric, including the type of fibre, dye or printing method used, and the intensity and duration of exposure to light. To determine the light fastness of a fabric, it is tested by exposing it to light under controlled conditions, such as using a standardized light source and measuring any changes in the fabric's appearance or colour.

A fabric that has good light fastness will maintain its appearance and colour even after prolonged exposure to light, while a fabric with poor light fastness may fade, change colour, or develop unsightly stains or discolorations. Various treatments can be used to improve the light fastness of a fabric, such as using UV-resistant dyes, finishing treatments, or adding additional layers of protection to the fabric.

The instrument used for light fastness of textile material is known as fade-o-meter. The light exposure conditions are controlled in terms of temperature, humidity, and intensity to simulate the effects of natural sunlight. The fabric sample is periodically removed and evaluated for any changes in its appearance or colour using standard colour evaluation methods.

Figure 2.16: Fade-o-meter

Image source: https://www.biophlox.com/image/cache/Products/Globe-Tex-Industries/digital-light-fastness-tester-800x800.jpg

The Fade-o-meter is used to test the light fastness of textiles, especially those intended for outdoor use, such as upholstery, curtains, and outdoor apparel. The test results can be used to determine the fabric's suitability for outdoor use and to evaluate the effectiveness of different dyeing or finishing treatments that may improve the fabric's light fastness.

2.2 RELATED RESEARCH REVIEW

2.2.1 Research related to extraction process

Jahan N. and Nahar S., (2022) reported Antimicrobial activity and mosquito repellency of dyed mulberry filament silk swatches with neem leaf extracts were investigated. FTIR analysis showed that dyed samples with bioactive components and neem-treated silk fabric did not cause an allergic reaction on human skin. The aqueous extraction method was employed for extracting colouring matter from the leaves of the neem plant. The extracted solutions were prepared in powder form in shade dried with different material liquor ratios of 1:4, 1:6, 1:8 and 1:10. The pH meter was calibrated with pH 4, pH 7, and pH 10 buffer solutions before measuring pH. Every 8-h shift, the pH meter was calibrated in the laboratory. Due to differences in pH and liquor ratios, the colour of the extracted solutions varied from dark brown to light brown.

Singh N. and Sheikh J., (2020) mentioned that functionalities can be imparted to textiles by the application of a variety of encapsulated materials, including both natural and synthetic ones. The use of sustainable natural materials for functionalization of textiles is the growing interest and rising demand from modern consumers. In this case, the use of essential oils as an encapsulated material can serve the requirements of sustainable chemical finishing of textiles. Essential oils have a broad spectrum of antimicrobial and biological activities because of their complex chemical composition, resulting in the presence of several functional properties like antiviral, antifungal, insect and mosquito repellents, antibacterial and pest control.

Rassem H. et al., (2016) studied on techniques for extraction of essential oils from plant and mentioned that essential oils are natural products which consist of many volatile molecules. They have been used for several applications in pharmaceutical, cosmetic, agricultural and bioactivity example flowers. Extraction of essential oils could be carried out by various techniques. Have Innovative methods avoid shortcomings of content optional techniques to reduced chemical risk, extraction time and high energy input and obtain yield quality of essential oils. Despite their numerous applications, except if essential oils are very sensitive to environmental factors used as such.

Tesfaye B. et al., (2017) reported on extraction of essential oil from neem Seed by using soxhlet extraction methods and it was intended to investigate the influence of different factors such as Particle sizes, solvent type, temperature, and time on the quantity of neem oil. There are different methods for essential oil extraction from neem seed. In this study, soxhlet extraction method was selected. In this extraction method the maximum oil yield obtained was 43.71% by using ethanol-hexane 60:40% solvent for three hours extraction time. The experimental quantitative difference in the quantity of the oil was due to particle size and extraction time variability. Volume proportions of ethanol-hexane mixtures (60:40 and 40:60% respectively) served as efficient solvent alternatives to the use of hexane in essential neem oil extraction.

Abah S. et al., (2011) mentioned that the solubility of extract, amount of a particular phytochemicals and the total amount of extract varies with the extraction method used in this research. Cold method of extraction produced wider zones of inhibition and more activity, indicating that cold method could be better than soxhlet method in antimicrobial assay of *Anchomanes dtfformis* (Blume).

2.2.2 Research related to preliminary phytochemical test

Vishnu B. et al., (2019) conducted a study on ‘phytochemical analysis’. Plants are an important source of phytochemicals which are an important source of drug and medicine. These phytochemicals have extraordinary properties like antibacterial, antifungal, anti-cancerous, antioxidant, anti-inflammatory, anti-diabetic activities etc. The identification of this compound relies on the tools of phytochemical analysis and hence the knowledge about these techniques is important. This article was helpful in the collection, identification, extraction and analysis of phytochemicals that are extracted from the plants. The methods followed for this analysis should be standard and following non-standard protocols could lead to the false results that are not reproducible.

Ammar A. et al., (2017), plant extracts showed strong antioxidant capacity, and the extracts can be considered a good source of natural antioxidants and antimicrobials. Polyphenol extraction from plants using fast and appropriate techniques is a low-cost method due to the reduction in the amount of solvent used, in addition to avoiding the need for longer extraction times compared to the conventional extraction method. Moreover, natural bioactive compounds have been found to interfere with and prevent all kinds of cancer. Flavonoids have been shown to work as anti-tumour (benign, melanoma) agents involving a free radicals quenching mechanism (i.e., OH, ROO). In fact, many studies have shown that flavonoids play significant multiple roles including mutagenic, cell damage, and carcinogenic, due to their acceleration of different aging factors.

Venkitachalapathi K. et al., (2016) reported that the results of leaf analysis gave a contribution to the world of traditional medicines. The different leaf extracts of petroleum ether, chloroform and distilled water of Mimusops elengi L. were subjected to qualitative analysis for the identification of various primary and secondary metabolites. Powdered dried leaf sample was successively extracted with petroleum ether, chloroform and distilled water by using soxhlet apparatus until the decolourisation of the solvents. The presence of alkaloids, flavonoids, terpenoids, steroids, tannins, saponins, phenols, carbohydrates, proteins and aminoacid were confirmed in various extracts of leaf. Fixed oils and fat were present in both chloroform and aqueous extracts, whereas tannins were present only in petroleum ether extract.

Nandagoapalan V. et al., (2016) studied the Phytochemical Analysis of Some Traditional Medicinal Plants. Natural products especially from plant sources, including species have been investigated for their characteristics and health effects. The preliminary qualitative phytochemical screening of the crude powder of 25 plants was done to assess the presence of bioactive components. The presence of alkaloids, flavonoids, tannins, phenols, steroids, glycosides, terpenoids and saponins was determined. Among these compounds alkaloids, phenols, flavonoids, saponins and tannins are important secondary metabolites and are responsible principles for medicinal values of the respective plant. Terpenoids are found in 12 medicinal plants out of 25 plants selected. Terpenoids and tannins are attributed for analgesic and anti-inflammatory activities. Apart from this tannins contribute property of astringency. Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plants serve as defense mechanism against predation by many microorganism, insects and herbivores. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids etc. The major chemical substances of interest in the earlier reports have been the alkaloids and steroidal sapogenins (saponins) however; other diverse groups of naturally occurring phytochemicals such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils etc. also have been reported.

Dinesh K. et al., (2015) found that pluchea lanceolata is a traditional medicine containing important bioactive compounds having antibacterial properties against isolated new multidrug resistant strains of Vibrio cholera. Phytochemical study would help to establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin against Vibrio cholera. Pluchea lanceolata is also used to treat various human diseases like anti-inflammatory, and analgesic activity. The alkaloids and flavonoids of the plant possesses various applications in the field of medicine and greatly used in rheumatoid arthritis and neurological diseases. The plant is still used in their native places due to its medicinal properties.

Yusuf A. Z. et al., (2014) worked on 'Phytochemical analysis of the methanol leaves extract of Paullinia pinnata linn'. The presence of the constituents was also found to be similar to those reported for most medicinal plants. The phytochemial constituents of the leaf of P. pinnata were investigated. The leaf was found to constitute steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids.

Kumara swamy M. et al., (2011) attempt to study the Phytochemical and antimicrobial studies of leaf extract of Euphorbia neriifolia. The chloroform and ethanol extracts have shown relatively greater activity than that of any other extracts. The preliminary phytochemical screening of E. neriifolia leaf extracts has revealed the presence of secondary metabolites of therapeutical importance. The major phytochemicals found were phlobatannins, saponins, flavonoids, tannins, phenols, terpenoids and cardenoloids. However, all extracts tested showed the absence of sterols, anthraquinones and cardiac glycosides.

RNS Yadav and Munin Agarwala (2011) worked on phytochemical analysis of some medicinal plants. The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extracts from medicinal plants could be seen as a good source for useful drugs.

Anjoo K. et al., (2008) studied on phytochemical and pharmacological profile on ageratum conyzoides L. A wide range of chemical compounds including alkaloids, cumarins, fl avonoids, chromenes, benzofurans, sterols and terpenoids had isolated from this species. Extracts and metabolites from this plant had found to possess pharmacological and insecticidal activities.

2.2.3 Research related to eco-friendly mosquito repellent finished fabric

Salunke M. et al., (2022) reported on herbal mosquito repellent, the findings of this study revealed that essential oils and extracts from certain plants have strong repellent action against Anopheles spp. mosquitoes. In the last two decades, researchers have been looking for novel natural repellents, and while certain plants have shown to have repellent properties, few natural products have been developed. Entomologists and individuals working in the field of mosquito-borne diseases should read this review to learn more about the usefulness and possible role of plant-derived repellents in disease control.

Ghada A. and Ahmed G., (2022) mentioned that the he textile industry has become well advanced in technology with value-added textile and clothing products. Insect repellent textiles assist in the prevention of vector-borne diseases such as malaria and dengue fever, which insects spread. Insect-resistant finishes can be added to textiles and garments in various ways, using chemicals or natural materials. The wet cleaning method can be used on curtains, upholstery materials, shoes, and carpet cloth, and other things. The efficacy of insect repellent finished fabric to provide protection can be measured and tested using standard methods. Future insect repellent finishes will focus on achieving efficient and long-lasting insect repellent properties while being environmentally sustainable.

Gupta R. et al., (2022) studied on novel formulation and evaluation of poly herbal mosquito repellent and mentioned that natural based mosquito repellent was successfully developed in this research study. The drug has been found to be very effective and safe to use. Owing to its beneficial use in the human body, the prepared eight separate essential oil-based mosquito repellent gel and candle formulations have proven to be an effective tool in preventing mosquito-borne diseases. In many developed countries, where most residents have no access to mosquito net, high-cost mosquito repellent creams, and miscellaneous physical methods, this formulation of gel and candle can be an efficient, affordable, and easily accessible way to deter mosquito-borne diseases such as malaria, dengue, etc. in the lower parts of society.

Maia M. et al., (2011) worked on plant based insect repellents, their efficacy, development and testing. The field of plant-based repellents is moving forward as consumers demand means of protection from arthropod bites that are safe, pleasant to use and environmentally sustainable. Perhaps the most important consideration is improving the longevity of those repellents that are effective but volatile such as citronella. New developments have also been seen in understanding the function of plant-based repellents in insects. Several studies have investigated the behavioural mode of action of repellents through structure-activity studies of contact versus spatial repellency. The field of plant-based repellent evaluation and development had become far more rigorous in recent years and developments in methods of dispensing plantbased volatiles means that extension in the duration of repellency and consequent efficacy of plant-based repellents will be possible in future.

2.2.4 Research related to mosquito test

Mynul Islam S. et al. (2020) from Bangladesh worked on the mosquito test on textile fabrics by using different medicinal natural plants. The research work has given a new idea in finishing of cotton fabric with medicinal plants for mosquito repellent activity.

The treated fabric was found to be very hygienic but not the same for all types of finishes. The result was checked after washing the fabric. The finished fabric showed a great result until the 9th wash as the extract only coated a surface without any bonding on the fabric which was removed by repeated washing. After washing 9 times we have found that the repellency behaviour of fabric was decreasing gradually. After 9^{th} wash the leaves extracted chemicals treated fabric lost its repellency effect but other fabrics had few repellency behavior.

Golam Emamul M. et al. (2018) found through the mosquito repellency behavior test that plant extraction of Kalamegh (Andrographis paniculata) is more efficient at driving away mosquitos as mosquito repellant than inorganic compound DEET. As well as it is eco-friendly and economic. So the use of mosquito repellants from natural sources like Kalamegh will be more efficient. The volume of mosquito repellents to be applied on fabric will increase the more repellency percentages. Authors have found that microencapsulated application of mosquito repellant has a longer and higher repellency percentage than the direct application. Though authors have found knitted fabric is slightly more capable for mosquito repellency than woven fabric, both of these fabrics will be very efficient as mosquito repellent fabric in different uses of it. The use of mosquito repellant finished fabric can be used in clothing and home textile which will help to skip hazardous processes for driving away mosquitoes. Authors are hoping that this analysis of comparison between different mosquito repellent with different volume and its different application process on different types of fabric will help for future development of making mosquito repellent finished fabric industrially.

Aufa A. and Nurain Y. (2016), from Malaysia mentioned that Vector borne disease from mosquitoes are one of the major problem arises. In order to avoid the transmission of diseases to other human, fabrics can act as a physical barrier between human skin and the blood sucking mosquito. This review outlines that most commonly used textile materials to impart the mosquito repellent comes from cotton, polyester and blended fabrics. By using these fabrics, an efficient textile material to treat with the mosquito repellent agent has been proven to demonstrate the good properties. It also showed that there are various techniques of imparting the repellent into the textile substrate which most used methods is a pad dry cure method with microencapsulation of repellents. In this review, it summarized the type of mosquito repellency assessment to conduct the efficacy of the impregnated textile. Differences in the findings of the included studies could be attributed to many factors such as type of textile materials used or type of technique used to impart repellent. Therefore, it suggested that the fabrics with a suitable repellent agent with proper methods of mosquito repellency test are hoped to develop a new knowledge to other future.

Idris M. et al., (2014) worked on Phytochemical Screening and Mosquito Repellent Activity of the Stem Bark Extracts of Euphorbia Balsamifera (Ait) and concluded that the repellent activity of the chloroform fraction from the bark of Euphorbia balsamifera is an important discovery in our struggle to find a lasting solution to the menace of mosquitoes in particular, and insects in general.

Marta M. and Sarah M. (2011) are studied on Plant-based insect repellents: a review of their efficacy, development and testing and found that the field of repellent development from plants is extremely fertile due to wealth of insecticidal compounds found in plants as defences against insects. The modern pyrethroids that are the mainstay of the current malaria elimination program that is making excellent progress, are synthetic analogues based on the chemical structure of pyrethrins, discovered in the pyrethrum daisy, Tanacetum cinerariifolium from the Dalmation region and Tanacetum coccineum of Persian origin. The insecticidal component comprising six esters (pyrethrins) is found in tiny oil-containing glands on the surface of the seed case in the flower head to protect the seed from insect attack.

2.2.5 Research related to application of mosquito repellent finish

Krishna Raj G. et al., (2022) worked on developing a fabric with mosquito repellent finish and Coating cotton fabric with different natural material finish. To consolidate the repellence properties of Neem, Tulasi and Betel into the fabric. By using this process, to prevent mosquito related diseases like dengue, malaria, etc., since use natural materials to apply finish it is harmless to human skins. No need to use external chemical repellent creams or coils and produced at a low cost. By implementing this process they didn't need any kind of external mosquito repellent agents.

Laura N. et al., (2020) studied on Efficacy of Plant-based Repellents against Anopheles Mosquitoes. The results of this show that some plants essential oils and extracts have significant repellent activity against Anopheles spp. mosquitoes. The studies conducted in the last two decades have focused on the search for new natural repellents and some plants displayed good repellent properties. However, few natural products have been developed so far. This review underscores the need to understand the potential role of plant-derived repellents in disease control.

Mamta R. et al., (2017) reported the research study on development of mosquito repellent finish. Enzymatic desizing, scouring and bleaching were done to prepare the fabric for mosquito repellent finish. Extraction was done in methanol to get the extract of plant material. The fabric was treated with plant extract using two application techniques i.e. Direct and Resin cross-linking techniques. For direct technique, the concentration of citric acid was 5 percent and for resin cross-linking technique, 40 g/l Fixapret F-eco with 8 g/l catalyst proportion along with 10 g/l extract, MLR 1:20 and treatment time 20 minutes. The optimum temperature for drying were 80°C for 5 minutes and curing temperature were 120°C for 60 seconds. Highest efficacy was observed in samples using direct technique followed by resin cross-linking.

Ranasinghe M. et al., (2016) studied on Development of Herbal Mosquito Repellent Formulations and find that plant essential oils showed higher mosquito repellent activities compared to plant extracts. When 10 % (V/V %) extracts and essential oils were compared, the mosquito repellent activities occurred in the following order: Citronella essential oil and Eucalyptus essential oil (100 %) > Tulsi essential oil (97.94 %) > Clove bud essential oil (95.81 %) > Sweet Orange essential oil (93.75 %) > Turmeric essential oil (89.56 %) > Nika extract (85.44 %) > Neem extract (81.25 %). When 20 % (V/V %) extracts and essential oils were compared, the mosquito repellent activity was found to be in the following order: Turmeric essential oil (100 %) > Nika extract and Neem extract (97.94 %). The mosquito repellent gel and the mosquito repellent spray which contained 16 % (V/V %) total active ingredients each, showed 100 % mosquito repellency for out-door and indoor field trials which were carried out for six hours each day for two days.

Ramya K. and Maheshwari V. (2014) mentioned that Andrographis paniculata plant extracts treated fabrics has found to have good mosquito repellent property, by both direct application method and microencapsulation method. The wash durability of the encapsulated samples showed better efficiency than by directly applied samples and the microencapsulated samples which have a high retention of the repellent activity. This form of natural extraction of the mosquito repellent finishes is very safe and eco-friendly and protect the body from mosquitoes. The sample treated with Andrographis paniculata extract is effective, economical and eco-friendly.

2.2.6 Research related to pad-dry-cure method

Dr. Archana D. and Rimpi P. (2020) reported on Natural dyeing of marigold leaves extract against insects and found that Cotton fabric finished with marigold leaves protects the human beings from the bite of insect and there by promising safety from insect vector diseases and it is eco-friendly, biodegradable, non- toxic, non-irritant to the skin and low cost for vector control and can be used with minimum care. It can be successfully utilized in apparel, insect net, window curtain and other home furnishings. It shows good repellent property when applied on cotton fabric.

Dr. Prathusha K. et al., (2020) worked on natural finishes on textiles to combat the mosquitoes and they reported that in today's world, the growing number of mosquito-borne diseases necessitates the discovery of new forms of mosquito repellents, in both synthetic and natural forms. In recent years, the trend is shifting towards the development of mosquito repellent finished textiles that provides a much-necessitated feature of repelling mosquitoes through use of natural active agents derived from plant extracts. The developed fabrics were assessed for mosquito repellent efficacy. From an extensive list of sources prepared based on ethno botanical review, thirteen sources were selected and tested for mosquito repellent efficacy using modified cage test method. This study proved that mosquito repellent finishes can be given to the textile materials in order to provide external protection in the form of door curtains, bed sheets, table and sofa covers, to prevent the mosquito bites.

Gupta A. and Dr. Singh A. (2017) reported that Mint leaves extracts treated fabrics have found to have good mosquito repellent property by direct application method.

Vector borne diseases are one of the major problems in developing countries. To avoid such sort of disease transmission to humans can be avoided using mosquito repellent fabrics. These forms of natural extraction of the mosquito repellent finishes are very safe and eco-friendly and protect the body from mosquitoes. The sample treated with mint leaves extract is effective, economical and eco-friendly.

Granch B. T. (2016) found that Sample treated with binder and oil was effective on mosquito repellency property. Thus, treatment should be given by Pad-Batch-Dry (PBD) method. The wash fastness was poor so it is recommended not to wash the treated fabric or retreat it after washing by spraying. The strength of the treated fabric was better than the untreated on therefore the finish doesn't degrade the fabric. The finish results slight shrinkage on the fabric. But this is the most noticeable effect on many other finishes and chemical treatments of cotton. The stiffness property of the fabric was not also increased much. Finally it is concluded that the finish applied on the fabric was effective without washing and it has no remarkable problem on the property of the fabric rather than increasing the strength of the fabric. The future work may potentially link the gap by improving the wash fastness using some chemicals.

2.2.7 Research related to *pluchea* family

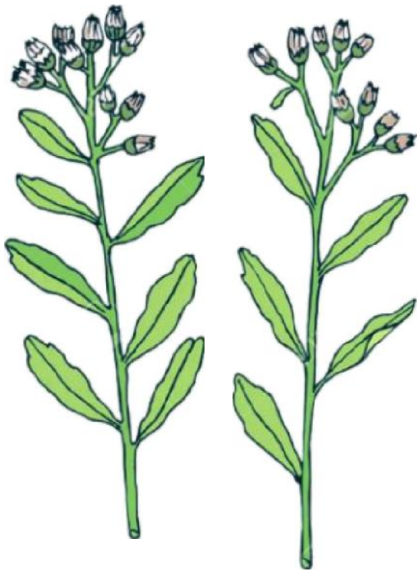
Sachan A. et al., (2019) studied on the extract of *Pluchea Lanceolata*, *Alhagi pseudalhagi*, *Caesalpinia bonduc*. Conventionally, many herbal formulations are using as single herb or in combinations of several different herbs. It believed that poly herbs show synergistic effect. The herbal formulation includes either plant raw material or plant extracts. Here, all selected plants are collected from the Chambal Valley of India to investigate the antidiabetic properties. This study provides the evidence that 50% v/v ethanolic extracts of all three plants *P. lanceolata*, *A. pseudalhagi* and *C. bonduc* are having potent enzyme inhibitory actions which are responsible for hyperglycemia. However, more efforts are needed for the isolation and characterization of bioactive compounds and further evaluation of biological properties.

Yuliani and Rahayu Y., (2018) mentioned that *Pluchea Indica* leaf extract effect on larva mortality of *Spodoptera litura*. Concentration of 12% gives an optimal mortality effect of 81.90%. *Pluchea Indica* leaf extract effect on seed germination (percentage and rate of germination) *Amaranthus spinosus*. The higher the concentration (1%), the germination inhibition is greater. *Pluchea Indica* leaf extract can be developed as bio-herbicide and insecticide.

Agni Febrina P. et al., (2016) worked on Antibacterial effects of *Pluchea indica* Less. leaf extract on Enterococcus faecalis and Fusobacterium nucleatum (in vitro). This study aimed to determine antibacterial activity of *Pluchea indica* Less. leaves extract against *E. faecalis* and *F. nucleatum* bacteria. Dilution method was conducted first to show Minimum Inhibitory Concentration (MIC) of the extract against *E. faecalis* and *F. nucleatum*. The antibacterial activity test on *Pluchea indica* Less. leaves extract was performed on *E. faecalis* and *F. nucleatum* bacteria using agar diffusion method. The *Pluchea indica* Less. leaves extract used for antibacterial activity test was at a concentrations of 100%, 50%, 25%, 12.5%, and 6.25%. Thirty-five petridiscs were used and divided into five groups based on the extract concentration. The results showed strong and moderate antibacterial effects of the *Pluchea indica* Less. leaves extract on *E. faecalis* at the concentrations of 100% and 50%, while on *F. nucleatum* only at the concentration of 100% with moderate effect. *Pluchea indica* Less. leaves extract has antibacterial activity against *E. faecalis* and *F. nucleatum* bacteria with strong-moderate effect.

Pooja S. and Karuna S. (2012) tried to find the chemical and biological potential of Rasayana herb used in traditional system of medicine and mentioned that the traditional usage in Indian system of medicine and ethno pharmacological point of view, pluchea lancelolata would be worthwhile to investigate the biochemical and physiological mechanisms involved in the different biological properties particularly.

Ching-I Peng et al., (1998) mentioned that four species of the *Pluchea* (Asteraceae) are recognized from Taiwan. e. A taxonomic treatment, line drawings, and a distribution map of *Pluchea* species in Taiwan are provided. Shrubs or herbs with alternate, simple leaves, more or less aromatic. Capitula many-flowered, disciform, usually numerous, in panicled, elongated to terminal flat-topped inflorescences. Involucral bracts imbricated. Outer florets numerous, in several peripheral rows, filiform, pistillate and fertile; achenes 4-5 angled, longitudinally grooved.



METHODOLOGY

CHAPTER III

METHODOLOGY

The study reported, takes an experimental approach towards the development of natural mosquito repellent finish using *Pluchea wallichiana* plant extract on cotton fabric. The study attempts to explore the possibility of developing a repellent finish through natural sources to protect people from diseases caused by mosquitoes keeping in mind a sustainable approach that will not cause any harm to people's skin or the environment. Present study was also planned to extract the *Pluchea wallichiana* leaves and make a natural mosquito repellent finish for selected fabric. Finally to test the selected fabric treated with a natural mosquito repellent finish for their performance against the mosquitoes. This chapter deals with the materials and methods followed for fulfilling the objective of this study.

The experimental procedure of the study undertaken has been subdivided into the following subsections:

3.1. Research design

3.2. Market Survey

3.3. Selection and composition of the material used

- 3.3.1. Selection of the fabric
- 3.3.2. Selection of the plant source for Mosquito Repellent finish

3.4. Preliminary data of the fabric

- 3.4.1. Determination of fibre content of the fabric
- 3.4.2. Determination of thread count of fabric
- 3.4.3. Determination of fabric weight per unit area
- 3.4.4. Determination of thickness of the fabric

3.5. Experimental study for selection of application method

3.6. Preparation of the finish

- 3.6.1. Extraction
- 3.6.2. Filtration

3.7. Evaluation of components of the extract

- 3.7.1. Preliminary phytochemical analysis of *Pluchea wallichiana* leaves extract

3.8. Procedure for the application of the finish

3.8.1. Scouring of the sample

3.8.2. Application of Natural Mosquito Repellent finish

3.9. Testing of untreated and treated fabrics using relevant standard method

3.9.1. Mosquito collection

3.9.2. Assessment of efficacy of finish

3.9.3. Test method of Mosquito Repellency on treated fabric

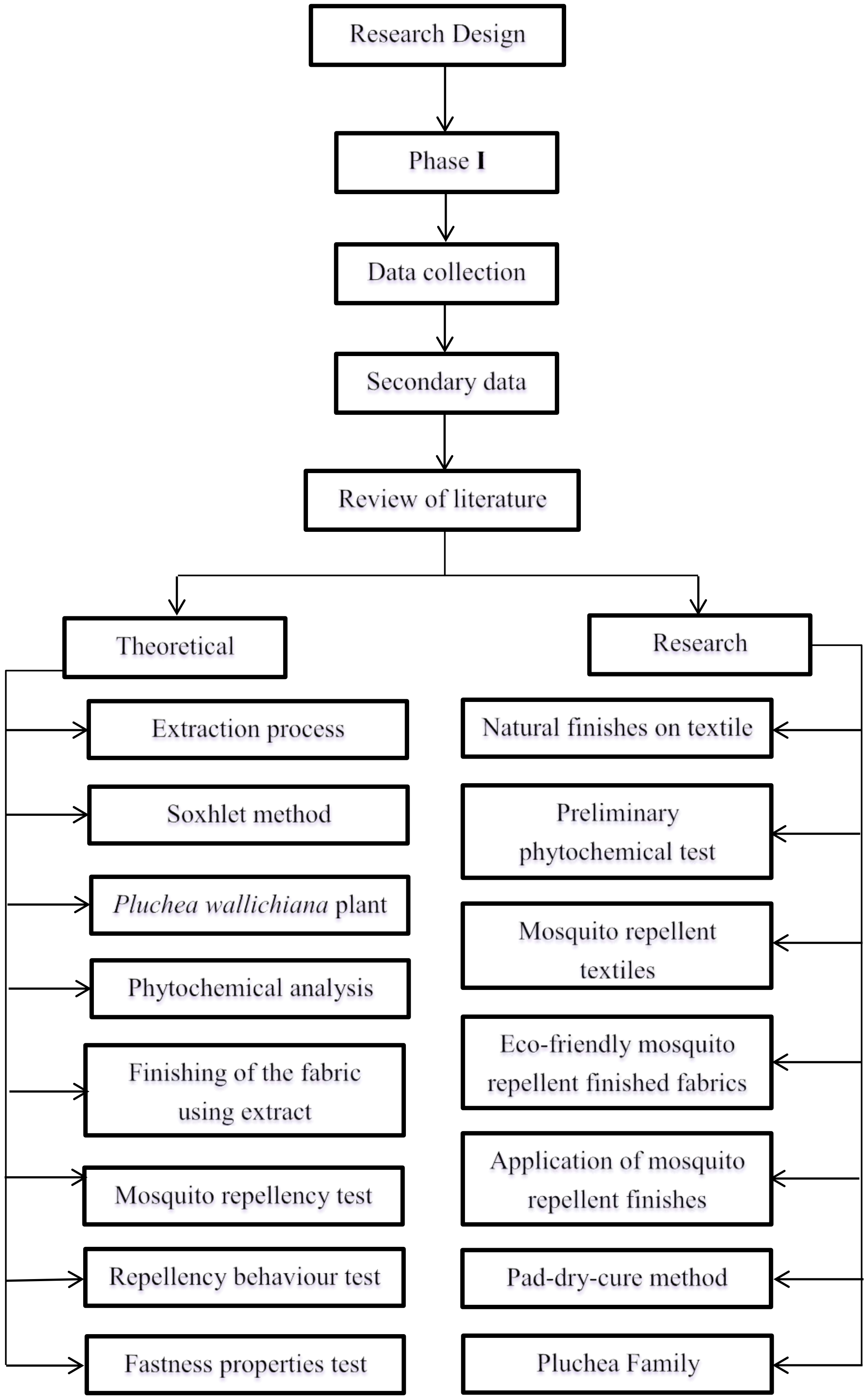
3.9.4. Calculation of Repellency behaviour on treated fabric

3.10. Evaluation of fastness properties of treated fabric

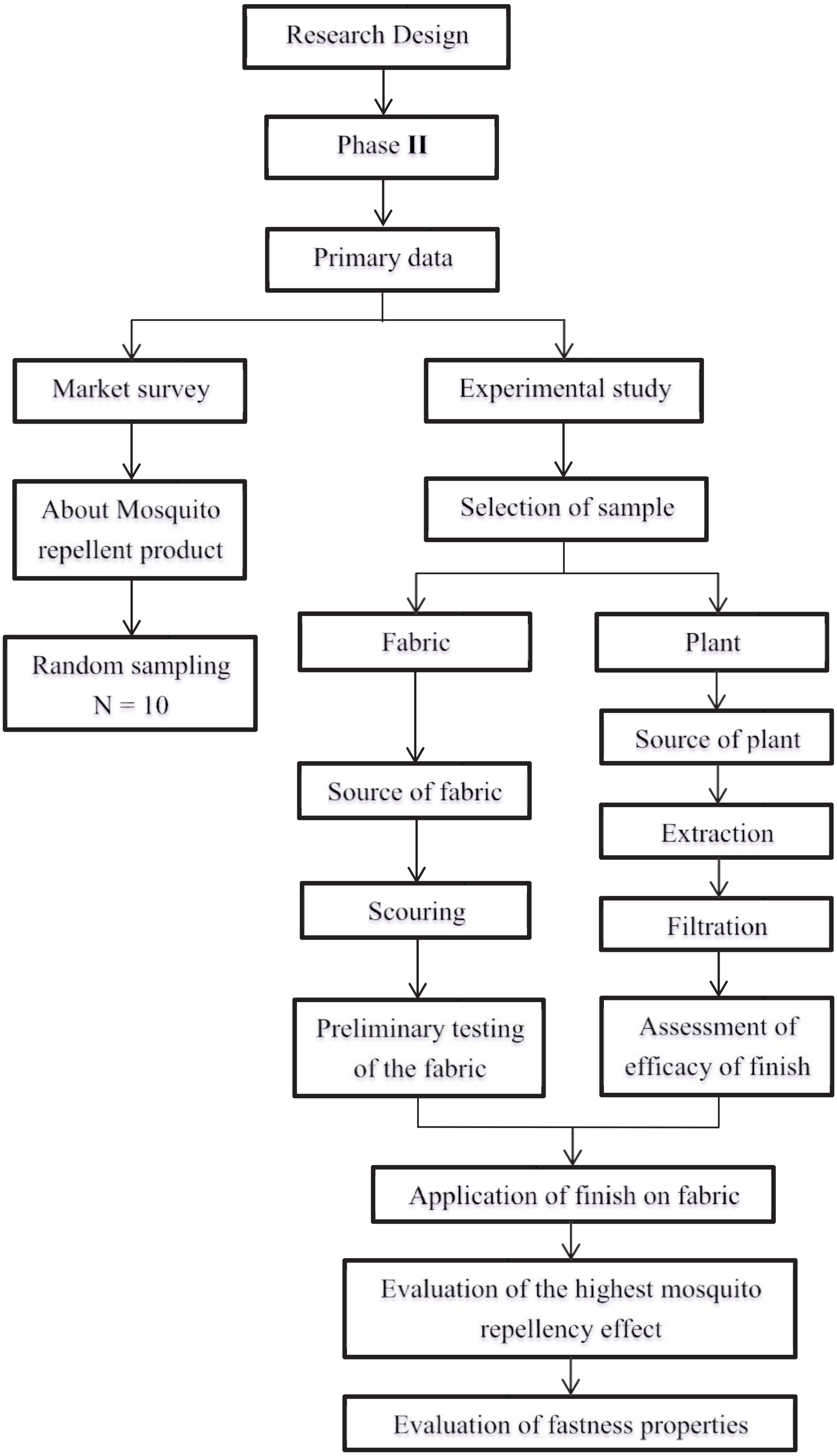
3.10.1. Assessment of durability of finish to wash

3.10.2. Assessment of durability of finish to light

3.1. (a) Research design for phase I



(b) Research design for phase II



3.2. Market Survey

The market in India for insect repellents is significantly large, due to the all-pervading breeding of mosquitoes and the serious diseases that mosquitoes inflict on the people. Applying mosquito repellents either on their skin, house, even on their clothing or upholsteries may help in protecting humans against mosquito bites. Repellents that applied to skin or clothing produce a vapour layer that has an offensive smell or taste and makes a person unattractive for feeding and therefore repels the mosquito several studies showed that most repellents was developed in the form of lotions, cream, essential oils, spray, or solution where in most cases require direct application to the human skin.

To take the study forward, the first step was market survey. This survey was done on all the local shops near the residential area where mosquito repellent products are sold. This was done for which types of mosquito repellent products are available in the market and which product is sold the most. It is also to know which mosquito repellent product people use the most.

Also want to know from this survey that in this research study which textile will be suitable for applying mosquito repellent finish. As mosquito repellent sprays are sold more so we can focus on clothing area, if there is a vaporizer then try to focus on home textiles, also there are lotions or creams then can be focus on medical textiles, if use of essential oil that is also used in sustainable textiles, etc.

List of the all synthetic mosquito repellent products:

- Liquid vaporizer
- Fabric roll-on
- Spray
- Fast card
- Cream or lotion
- Coil
- Mosquito repellent patches

Plate 3.1: Synthetic mosquito repellent products

3.3. Selection and composition of the material used

3.3.1. Selection of the fabric

The research aimed to develop natural mosquito repellent finish, for its application to home textiles. Hence, a market survey was done in the city of Baroda, and fabric for application was selected from amongst the samples collected through the survey. Care was taken that the fabric widths surveyed were of cotton and available in higher widths for home application, so that they can be used for curtain or bed-sheet material. Since large width fabrics were not available in retail a plain cotton bed-sheet was chosen as the fabric for test. Selection of the fabric was based on their utility and suitability as per the climatic condition of the locale for the indoor uses like curtains, pillow covers, bed sheets, etc.

Cotton has been the most commonly used fibre in the Indian home textile hence the researcher used cotton fabric for the study so that the end consumers are also able to get the benefits of this project.

In home textile, cotton the prime choice of fabric to make curtains, bed sheets, pillow covers that are not only soft, comfortable, durable but also hypoallergenic and environment-friendly.

100% Cotton fabric was chosen for the application of the natural mosquito repellent finish against the mosquito bites and protection from the diseases like dengue, malaria, chikungunya fever, etc. Also this can be used to protect the Classrooms of School, Houses, Office rooms, Laboratories, Hospitals, etc.

Pure cotton fabric was sourced from a home décor shop in Vadodara. The fabric was made by Vision India- Grapes The Linen based from Panipat, Haryana. Vision India, a name synonymous with above par quality, has been bringing inspiration to homes since 2011. Their brand "Grapes The Linen" offers inspired living through high-quality products and exclusive designs. Each product is crafted in a sustainable environment and carries its own unique identity and stands with international quality standards. Cotton fabric is one of them products because it is a one of the popular natural mosquito repellent finished fabrics in the modern market.

3.3.2. Selection of the plant source for Mosquito repellent finish

The major tool in mosquito control operation was the application of synthetic insecticides. But this has not been very successful because it has harmful effects on human health i.e. unpleasant smell; oily feeling to some users and potential toxicity.

Using natural plant products is a simple and sustainable method of mosquito control. Repellents of plant origin do not pose hazards of toxicity to humans. Enhancing the health and hygiene qualities of consumer products, make it necessary to find new ways of applications on textiles with medicinal plants.

The plant was selected based on their mosquito repellent property and use as a finish on the textile fabric. Firstly, after analysing from different sources, make a list by sorting all the natural mosquito repellent plants available. After that availability of the plant and their mosquito repellent properties, we choose the *Pluchea wallichiana* (Wallich Camphorweed) plant for this study.

Fresh leaves of *Pluchea wallichiana* were collected from **Arboretum Cum Medicinal Plant Garden**, Faculty of Science, The Maharaja Sayajirao University of

Baroda, Vadodara. *Pluchea wallichiana* (leaves) extracted using ethanol was used for mosquito repellent finish on cotton fabric.

Botanical name: *Pluchea wallichiana*
Common name: Rasana
Family: Asteraceae
Part used: Leaves
Reason: Mosquito repellent finish
Origin: Punjab, Rajasthan and Gujarat
Climate and soil: Open waste lands, sandy-saline lands in low rainfall areas

Plate 3.2: *Pluchea wallichiana* Plant (Rasana)

3.4. Preliminary data of the fabric used.

Pre-treatments of the fabric were conducted for assuring the quality and properties. Preliminary data of the fabrics was determined as per standard procedures given below:

3.4.1. Determination of fibre content of the fabric

Confirmation of the type of fibre in the fabric was done through various tests:

i) Burning test
ii) Chemical solubility test
iii) Microscopic analysis

3.4.2. Determination of thread count of fabric (ASTM D3775-17e1)

Thread count refers to the number of threads or strands per square inch of fabric, counting horizontal and vertical threads. Thread count which is the number of yarns/cm^2 helps to describe the tightness of the weave. Fabric count was determined using pick glass.

The fabric count was determined by counting the number of threads in one square centimetre in the warp and in the weft directions. Determine the number of warp and weft threads per centimetre or inch from at least in 3 different places. And put down the readings of warp and weft threads per inch or centimetre. Identify weave under pick glass or a microscope.

3.4.3. Determination of fabric weight per unit area (ASTM D3776-20)

The weight of a fabric can be described in two ways, either as the 'weight per unit area or the weight per unit length. The circular GSM cutter was used to determine the weight per unit area which is defined in grams per square meter.

The fabric was cut through the GSM cutter. After cutting these samples were then kept in the desiccators for 24 hours for conditioning and weighed. After 24 hours the samples were taken out from desiccators and weighed.

Calculate the average weight of the cut samples in grams and multiply by 100 to give the weight per square meter in grams.

3.4.4. Determination of thickness of the fabric (ASTM D1777-96)

Fabric thickness tester was used to determine the thickness of the fabric. Determination of thickness of fabric samples in the laboratory is usually carried out with the help of a precision thickness gauge. The fabric whose thickness is to be determined is kept on a flat anvil. The circular pressure foot is pressed gently onto it from the top under a standard fixed load, the contact faces of the anvil and the pressure foot being ground flat and set in planes parallel to each other. The dial indicator directly gives the thickness in mm. This procedure is repeated to obtain the values of thickness at least at 5 different locations. The mean value of all the readings of thickness determined and the result is the average thickness of the sample under test.

3.5. Experimental study for selection of application method

The present study was undertaken to develop natural mosquito repellent cotton fabric with extraction of *Pluchea wallichiana* (Rasana) leaves using ethanol as a solvent. Soxhlet apparatus was used for this extraction process. And extract applied on fabric using two different types of techniques: Direct and Resin cross-linking application techniques by pad-dry-cure method. After that all the samples were tested under standard mosquito repellency tests.

For testing, three different tested fabrics were prepared:

i) Direct tested after application

ii) Fabric tested to evaluate durability of finish to wash

iii) Fabric tested to evaluate durability of finish to light

3.6. Preparation of the finish

3.6.1. Extraction

Fresh leaves of *Pluchea wallichiana* plant used for the study were obtained from **Arboretum Cum Medicinal Plant Garden**, Vadodara. Prior to use, the *Pluchea* leaves were surface cleaned with running tap water to remove dirt and other impurities material. Fresh *Pluchea wallichiana* leaves were cut into small pieces and prepared for the extraction process.

Pluchea wallichiana leaves were packed in Whatman's Circular ashless filter paper and placed in the Soxhlet chamber. Selected solvent was placed in a round bottom flask and assembled for Soxhlet extractor then the distillation process was begun. Here, the material liquor ratio was 1:40 of sample and 98% ethanol solvent. Which means, each 3 gm of fresh leaves was taken and 120 ml of 98% ethanol. This process was conducted at 70°C for 3 hours. After that extract was heated in open flask for evaporate the preheated solvent at 50°C for 10 min. This extract was filtered through whatman no. 42 filter paper into conical flask. After completed the extraction process, take the extract out of the airtight flask and fill it in a black bottle and stored at 4°C for further uses.

Plate 3.3: Fresh leaves

Plates 3.4: Fine chopped leaves

Plate 3.5: Soxhlet apparatus for extraction

Plate 3.6: Chopped leaf after extraction

Plate 3.7: Leaf extract

3.6.2. Filtration

A rota evaporator is an instrument in chemical laboratories used for the effective separation of solvents from given samples via distillation. The rotary evaporator working principle is that the boiling point of liquids lowers on decreasing their pressure. This allows the solvent to vaporize at lower temperatures than when boiled in a normal atmosphere. For this process, extract was filled in the conical flask which is on the heating mangle, temperature was 78℃ (temperature based on the boiling point of the solvent) and the flask started rotating 70 to 80 rpm. This one cycle takes up to 1 hour. During this process solvent was separated and stored in another conical flask.

Then extracted *Pluchea wallichiana* was weighed by using the following equation:

$$\% \text{ yield} = \frac{W2}{W1} \times 100$$

Where: W_1 = Sample weight initially placed in the conical flask.

W_2 = Sample weight after the extraction and evaporate the solvent.

Plate 3.8: Rota evaporator

3.7. Evaluation of certain components of the extract

3.7.1. Preliminary phytochemical analysis of *Pluchea wallichiana* leaves extract

Phytochemicals are chemical compounds that occur naturally in plants (Phyto means “Plant” in Greek). Proper selection and identification is important for any phytochemical analysis, any defect in this could severely affect the research and may reduce the value of the study.

The chemicals that are produced by plants are called phytochemicals. These are produced by the plant’s primary and secondary metabolism. Phytochemicals are known to have a role in the protection of human health. These are majorly classified as primary and secondary metabolites.

Phytochemical analysis of *Pluchea wallichiana* leaves extract was tested for the presence of bioactive compounds by using following standard methods:

Qualitative analysis of primary metabolites:
The primary metabolites are responsible for the basic development of the plant.

- Test for carbohydrates

Molisch's test: To about 2 ml of the sample, 2 drops of alcoholic solution of α-naphthol was added to the mixture after being shaken well. 3-4 drops of conc. H_2SO_4 were added along the sides of the test tube. A violet ring indicates the presence of sugars.

- Test for proteins

Biuret test: 2 ml of filtrate was taken to which 1 drop of 2% copper sulphate solution was added; 1 ml of 95% ethanol was added. Then it was followed by excess addition of KOH. The appearance of pink colour indicates the presence of protein.

- Test for amino acids

To 2 ml of extract few drops of nitric acid were added along the sides of the tube the appearance of yellow colour indicates the presence of protein and free amino acids.

- Test for fatty acids

1 ml of the extract was mixed with 5 ml of ether. Their extracts were allowed to evaporate on a filter paper and the filter paper was dried. The appearance of transparency indicates the presence of fatty oils.

Miscellaneous compounds:

- Test for fixed oils and fats

Spot test: small quantity of the extract was taken and pressed between 2 filter papers. The appearance of spots indicates the presence of oils.

- Test for gums

To 1 ml of extract, distilled water, 2 ml of absolute ethanol was added with constant stirring white or cloudy precipitate indicates the presence of gums or mucilage.

Qualitative analysis of secondary metabolites:

The secondary metabolites are those which are needed for the survival of the plants in a harsh environment.

- Test for anthraquinones

To 5 ml of extract, few ml of conc. H_2SO_4 was added and 1 ml of diluted ammonia was added to it. The appearance of rose pink confirms the presence of anthraquinones.

- Test for quinones

To 1 ml of extract, alcoholic KOH is added the presence of red to blue colour indicates the presence of quinones.

- Test for alkaloids

Mayer's test: To a few ml of filtrate, 2 drops Mayer's reagent was added a creamy or white precipitate shows a positive result for alkaloids

Mayer's Reagent: Mercuric chloride (1.358 g) is dissolved in 60 ml of water and potassium iodide (5.0 g) is dissolved in 10 ml of water. The two solutions are mixed and made up to 100 ml with water.

- Test for glycosides

Borntrager's test: To 2 ml of filtrate, 3 ml of chloroform is added and shaken. The chloroform layer is separated and 10% ammonia solution was added. The pink colour indicates the presence of glycosides.

- Test for cardiac glycosides

5 ml of solvent extract was mixed with 2 ml of glacial acetic acid and a drop of ferric chloride solution was added followed by the addition of 1 ml of conc. H_2SO_4. A brown ring in the interface indicates the presence of deoxy sugars of cardenoloides. A violet ring may appear beneath the brown ring while acetic acid layer a green ring may also form just gradually towards the layer.

- Test for phenol

To 1 ml of extract add in 1 ml of sodium nitrite and few drops of dilute sulphuric acid then 2 ml of dilute NaOH was added and mixed gently. Deep red or green or blue colour develops show the presence of phenol.

- Test for tannins

To 5 ml of extract a few drops of neutral 5% ferric chloride solution was added, the production of dark green colour indicates the presence of tannins.

- Test for flavonoids

1 ml extract was dried over a water bath and 5-10 drops of concentrated HCl was added followed by Zn powder. A pink, reddish pink or brown colour develops and it indicates the presence of flavonoids.

- Test for saponins

0.5 mg of extract was vigorously shaken with a few ml of distilled water. The formation of frothing is positive for saponins. The froth from the above reaction is taken and a few drops of olive oil is added and shaken vigorously and observed for the formation of emulsion.

- Test for terpenoids

Salkowski test: 3 ml of the extract was taken and 1 ml of chloroform and 1.5 ml of concentrated H_2SO_4 are added along the sides of the tube. The reddish brown colour in the interface is considered positive for the presence of terpenoids.

- Test for steroids

2 ml of extract with 2 ml of chloroform and 2 ml of concentrated H_2SO_4 are added, the appearance of red colour and yellowish green fluorescence indicates the presence of steroids.

3.8. Procedure for the application of the finish

3.8.1. Scouring of the sample

The fabric was first scoured to remove any impurities in form of grease, dust, dirt, residue (“suint”), vegetable matter or foreign matter before application of any finish.

Cotton fabric was scoured with 2 g/l of commercial detergent and 2 g/l soda ash in a material liquor ratio of 1:40. The fabrics were thoroughly washed and then air dried.

3.8.2. Application of Natural Mosquito repellent finish

Pluchea wallichiana (leaves) ethanol extract was used for mosquito repellent finishing on cotton. Fabric was scoured. For mosquito repellent finishing on fabric two application techniques i.e. direct and resin cross-linking were used through pad-dry-cure method.

i) Direct technique:

The direct method in pad-dry-cure is used in textile finishing processes to add functional properties to fabrics. In this method, a solution of the desired finishing agent is prepared, and the fabric is passed through the solution using a padding machine. The fabric is then dried at high temperatures to fix the finishing agent onto the fabric.

In the procedure, 10 g/l *Pluchea wallichiana* (leaves) extract, 5% citric acid was mixed in 1:20 MLR and the fabric immersed for 20 minutes. The treated fabric samples were padded by laboratory padding mangle machine at pressure maintaining 2 bar pressure followed by 2 nip 2 dips and dried in a preheated oven at 80°C for 15 minutes and cured at 120°C for 3 min.

Also prepare 20 g/l *Pluchea wallichiana* (leaves) extract, 5% citric acid was mixed in 1:20 MLR and the fabric immersed for 20 minutes. The treated fabric samples were padded by laboratory padding mangle machine at pressure maintaining 2 bar pressure followed by 2 nip 2 dips and dried in a preheated oven at 80°C for 15 minutes and cured at 120°C for 3 min.

ii) Resin cross-linking technique:

Resin cross-linking is another method used in the pad-dry-cure finishing process in the textile industry. This method involves the use of a cross-linking agent, which creates chemical bonds between the fibres of the fabric, resulting in improved fabric properties.

In the resin cross-linking method, the fabric is first padded with a resin solution containing a cross-linking agent, followed by drying at high temperatures to cure the resin and create the cross linking bonds. The process is typically carried out using a padding machine, which applies the resin solution to the fabric uniformly.

The choice of resin and cross-linking agent depends on the desired properties and the type of fabric being treated. Resin cross-linking is a widely used method in textile finishing, particularly for cotton fabrics.

40 g/l DMDHEU (dimethylol dihydroxy ethylene urea) and 8 g/l magnesium chloride mixed along with 10 g/l *Pluchea wallichiana* (leaves) extract in 1:20 MLR and fabric immersed for 20 minutes. Padding, drying and curing parameters were the same as per direct technique.

Also make 40 g/l DMDHEU (dimethylol dihydroxy ethylene urea) and 8 g/l magnesium chloride mixed along with 20 g/l *Pluchea wallichiana* (leaves) extract in 1:20 MLR and fabric immersed for 20 minutes. Padding, drying and curing parameters were the same as per direct technique.

Plate 3.9: Padding mangle for pad-dry-cure method

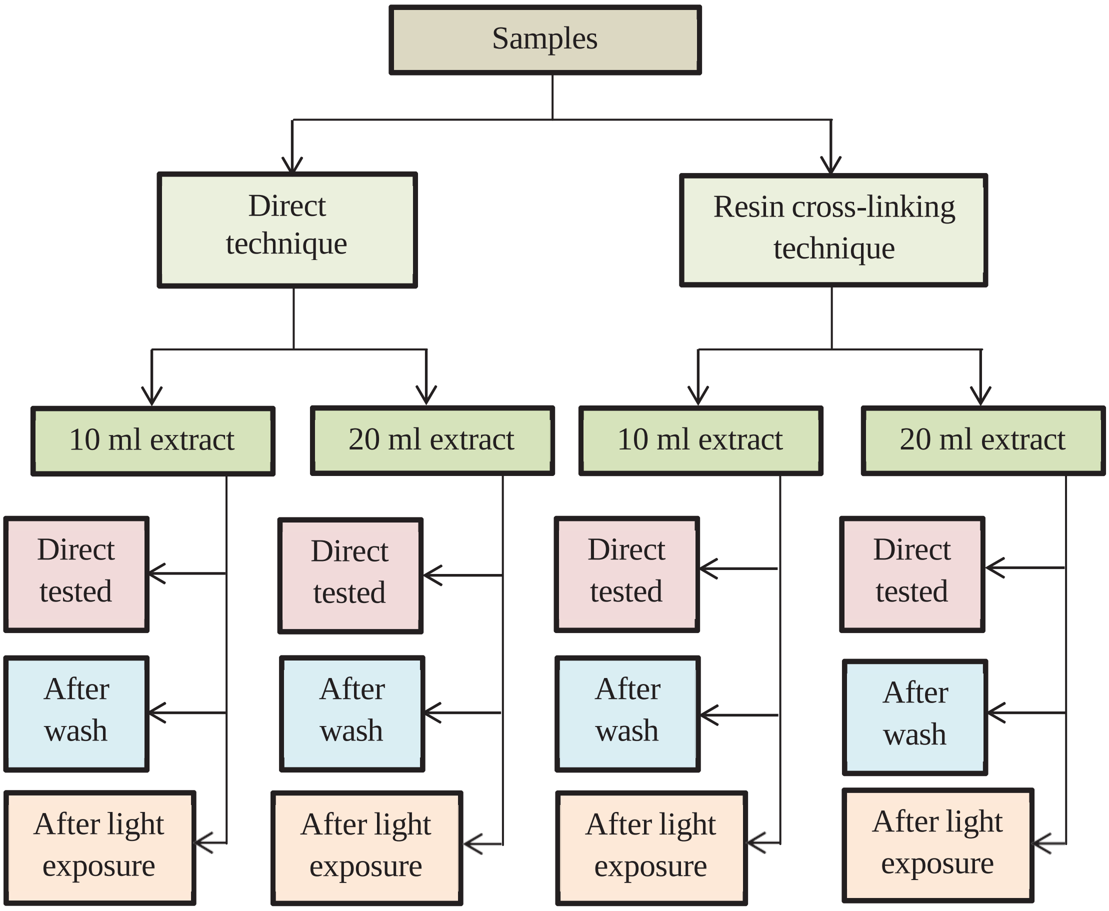


Figure 3.1: Flowchart of the treated samples

Hence, a total of 12 samples and one control sample were treated then studied for mosquito repellency.

In textile finishing, pick up refers to the amount of finishing solution or chemical that is absorbed by the fabric during the finishing process. The pick-up and add-on levels affect the final properties of the finished fabric, such as its hand, appearance, and performance characteristics.

i) pick-up (%):

This is the amount of liquid retained by the fabric after it has been passed through a finishing bath. It is typically expressed as a percentage of the weight of the wet fabric.

$$\text{Pick-up (\%)} = \frac{w2-w1}{w1} \times 100$$

Where, w_1=Weight of dry sample

w_2=Weight of wet sample

ii) Add-on (%).

This refers to the amount of solids deposited on the fabric after the liquid has been evaporated. Add-on is expressed as a percentage of the weight of the dry fabric.

$$\text{Add-on (\%)} = \frac{w2 - w1}{w1} \times 100$$

Where, w_1=Weight of untreated sample

w_2=Weight of treated sample after dried

3.9. Testing of untreated and treated fabrics using relevant mosquito testing standard method

The samples are tested from **NIMR** (National Institute of Malaria Research), **Department of Health Research, Ministry of Health and Family Welfare, Government of India**. Field station: Nadiad, Civil Hospital, 387001- Gujarat.

3.9.1. Mosquito collection

Two different species of mosquitoes were used for testing the fabrics. At first some *Anopheles stephensi* mosquitoes which are a primary mosquito vector of malaria were collected from Kanjarti village, Kheda district, Gujarat and *Aedes* mosquitoes which are a mosquito vector of dengue were collected from Nadiad village, Nadiad, Gujarat and were collected during the evening hours with the help of suction tube. They were stored in a container which had the facility to enter air and oxygen. All mosquitoes were starved of blood and sugar for 4 hours before the tests. These mosquitoes are also growing in the laboratory for the research work.

Plate 3.10: Mosquito collection

3.9.2. Assessment of efficacy of finish

It is important to carefully evaluate the performance of a mosquito repellent finish before selecting it for use. Assessing the efficacy of a mosquito repellent finish can be done through several methods. And in these methods, one method was selected for the assessment which was the laboratory testing.

In the laboratory, the extract was tested in a cage with different concentrations of extract (5 gpl, 10 gpl and 20 gpl) applied on a glucose cotton pad. And evaluate the mosquito repellency and take readings after 1 hr and determination of % repellency after 24hr. Compare the results then choose the two concentrations of extract which are most effective compared to the other one. After that the finish was applied on the samples which tested mosquito repellency.

The cage measurement is according to WHO guidelines for efficacy testing of mosquito repellents. Some studies reported in modification of the cage dimension used a cage 18 × 18 × 18 cm dimension.

Plate 3.11: Extract applied on glucose cotton pad

Plate 3.12: Cage test of glucose cotton pad to evaluate efficacy of extract

3.9.3. Test method of Mosquito Repellency on treated fabric

The mosquito repellent of the *Pluchea wallichiana* extracts treated fabrics was tested using a Mosquito Repellency Behavioural Test.

There are several methods to evaluate the treated textile with mosquito repellent. The most used techniques are cage test, cone test, and excito chamber.

Cone test was preferred for testing all the treated samples to evaluate mosquito repellency following the standard procedure.

- Cone test:

The cone test is formerly the custom to evaluate the toxicity of insecticide-treated bed nets against malaria, which can also investigate the toxicity of other impregnated (textile) surfaces. The fabric treated, evaluated using the WHO cone test following the standard procedure described in the WHO 1998: test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. This test does not involve human participants as the bait to lure the mosquitoes to fabric and this is one of advantages of this method. The use of artificial blood or animal blood as bait in order to attract host seeking mosquitoes which in this cone test could help future studies to better assess the efficacy of the treated clothing.

In the cone test, the mosquitoes might spend more time resting on the cone than on the treated surface during the 3 min exposition. The 3-min exposure test was carried out under the temperature of 28±2°C with 60 to 70% RH. The standard WHO plastic cone was placed on top of the treated surface of the sample and secured using a masking tape or clips. Twenty female mosquitoes were blown into the cone using a suction tube and mosquitoes were exposed to the treated surface. The low density mosquito number used for this method made it easy observation for the mosquito behaviour.

Plate 3.13: Mosquitoes inlet using suction tube

Plate 3.14: Cone test of all finished fabric to evaluate mosquito repellency

3.9.4. Calculation of Repellency behaviour on treated fabric

Cone test method was employed for testing mosquito repellency of treated samples. 20 mosquitoes were released into the cone and allowed to settle for 3 minutes. Mosquitoes were deprived of all the nutrition and water for a minimum of 4 hours before exposure. At the end of the exposition, the mosquitoes were transferred to the plastic cones for further observation. The number of immobilized, knocked down test mosquitoes was determined 1 hr (at 15, 30, 45 and 60 minutes intervals) after the exposition and the mortality rate was determined after 24 hr. The percentage of mosquito repellency was calculated using the following formula:

$$\% \text{ Mosquito mortality: } (MR - MC) / (100 - C) \times 100\%$$

Where, MR represents the mosquito’s mortality in test replicate while the MC corresponds to the mosquito’s mortality in control samples. The natural mortality rate is determined with an untreated fabric.

Laboratory tests were performed during daylight hours only. The anti-mosquito effectiveness was noted by counting the number of mosquitoes that rested on the untreated and treated samples. Mosquito repellent percentages were analysed after each test.

Plate 3.15: Dead mosquitoes in plastic cone

3.10. Evaluation of fastness properties of treated fabric

The samples are evaluated under fastness testing to identify the effectiveness of the mosquito repellency. The all samples were assessed for the following parameters:

- Wash fastness
- Light fastness

3.10.1. Assessment of durability of finish to wash

The laundering test was done to evaluate wash fastness of the samples carried out with launder-o-meter as per the A.A.T.C.C. standard test method IA 6-1962. Principle of launder-o-meter is that the specimens are laundered under appropriate conditions of temperature, alkalinity and abrasive action such that the desired loss of the colour or effect is obtained in a conveniently short time.

Soap solution was made by dissolving 2 g/l of non-ionic detergent. The material liquor ratio was maintained at 1:40. The samples were placed in jars of 800 ml capacity at room temperature. The test method consisted of one level of laundry for 30 minutes. The specimen was then removed, rinsed and squeezed. Specimen was opened out and dried in the air. Evaluate the effectiveness of the mosquito repellency on treated fabrics.

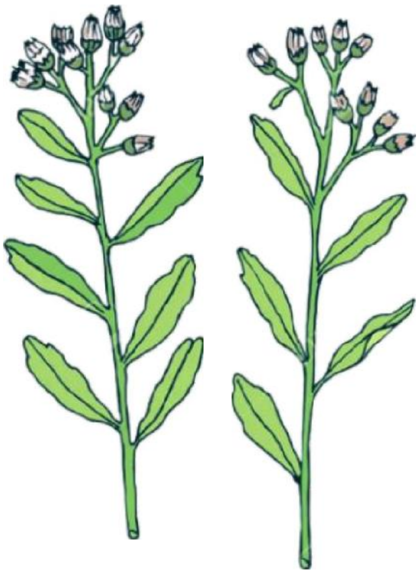
Plate 3.16: Launder-o-meter

3.10.2. Assessment of durability of finish to light

To test the light fastness of samples, Digital light fastness tester was used as per the A.A.T.C.C standard test method 16A-1963. The specimens from the textile to be tested and an effect of applied finish are exposed simultaneously to a specified calibrated carbon-arc light under specified conditions for the standard fading hours.

The test specimens were then exposed to light for 5, 10 and 15 standard fading hours. And here the samples are exposed to light for 5 hours. Evaluate the effectiveness of the mosquito repellency on treated fabrics.

Plate 3.17: Fade-o-meter



RESULT AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

The present study deals with the development of a natural mosquito repellent finish using *Pluchea wallichiana* plant extract on cotton fabric an innovative application of traditional plant medicinal application with potential benefits for public health and sustainable textile protection application. The use of *Pluchea wallichiana*; a plant species known for its medicinal properties has been explored through this study. In this study *Pluchea wallichiana* leaves has been explored for its potential use as a natural mosquito repellent finish.

To develop a natural mosquito repellent finish, the first step was to extract the active compounds from the plant material using a suitable solvent, such as ethanol or water. The extracted compounds were then tested for their mosquito repellent properties using standard laboratory methods i.e. efficacy test and mosquito behavioural test.

To ensure the safety and effectiveness of the natural mosquito repellent finish, it was important to ascertain the finish's repellent efficacy against mosquitoes and its durability on the fabric. Hence, tests for repellency behaviour and fastness to wash and light were performed.

The results of the study have been discussed under the following subsections:

4.1. Market survey

4.2. Preliminary data of the fabric

4.2.1. Determination of fibre content of the fabric

4.2.2. Determination of thread count of fabric

4.2.3. Determination of fabric weight per unit area

4.2.4. Determination of thickness of the fabric

4.3. To develop an Eco-friendly natural mosquito repellent finish using plant extract.

4.3.1. Extraction of fresh *Pluchea wallichiana* Leaves

4.3.2. Filtration of extract *Pluchea wallichiana* Leaves

4.4. Preliminary phytochemicals analysis

4.5. Assessment of efficacy of finish

4.6. To study the application of plant extract as a finish on cotton fabric

4.6.1. Direct application method

4.6.2. Resin cross linking method

4.7. To study mosquito repellency of the finish applied to cotton fabric

4.7.1. Assessment of mosquito repellency property

4.7.2. Effect of mosquito repellent efficiency of cotton fabric

4.7.3. Evaluation of the highest mosquito repellency effect

4.8. Evaluation of fastness properties of treated fabric

4.8.1. Assessment of durability of finish to wash

4.8.2. Assessment of durability of finish to light

4.1. Market survey

Synthetic mosquito repellent products are widely available in many different types, including sprays, lotions, liquid vaporizers, patches, roll-on and wristbands. They typically contain chemical compounds such as DEET, Picaridin, or IR3535, which are effective at repelling mosquitoes and other biting insects. In terms of pricing, synthetic mosquito repellent products can range from affordable to relatively expensive, depending on the brand, the product type, and the concentration of the active ingredients.

Survey was done in 10 different shops where synthetic mosquito repellent products were to be found near Gorwa area, Vadodara, Gujarat. The most selling and consumer choice mosquito repellent products are liquid vaporizers, lotions and fabric roll-on. On the basis of this survey, finally to conclude that liquid vaporizers are the most widely used product and sometimes these products are harmful to human health, causing allergic sneezing and breathing difficulty in some citizens. Hence, the present study explored the use of natural mosquito repellent finished fabric, for its application to home textiles products like bed sheets, pillow covers, curtains, etc., which are alternative solutions of liquid vaporizers and coils.

4.2. Preliminary data of the fabric

Preliminary data testing for fabric was done to determine the fabric's properties and characteristics. This testing helped to determine whether the fabric was suitable for a particular application or use.

The cotton fabric was chosen for the study. For its preliminary data following parameters: fibre content, fabric count, weight per unit area and thickness of the fabric were studied.

4.2.1. Determination of fibre content of the fabric

The selected fabric was scoured and then after drying kept in a desiccator. To confirm the fibre content three tests were conducted i.e. microscopic analysis, chemical solubility test, burning test as shown in Table 4.1.

Table 4.1: Fibre Identification of the Selected Fabric

Sr. No.	**Microscopic Analysis**	**Chemical Solubility Test**	**Burning Test**	**Fibre confirmed**
1	A flattened collapsed spirally twisted tube with a rough surface and frequent convolutions that change direction.	Dissolved in 70% H_2SO_4	Burns quickly without melting, leaving small fluffy grey ash or no ash and produced a smell of burning paper.	Cotton

After the microscopic analysis, chemical solubility test and burning test it was confirmed that the fabric used for the study was 100% cotton.

4.2.2. Determination of thread count of fabric

Thread count is a measurement of the number of threads woven into one square inch of fabric. For cotton fabric, a thread count of 100 to 800 is considered a good range for bedding and clothing. (Robinson. K., 2021)

This 100 % Cotton fabric thread count was 195 ends per inch and 122 picks per inch.

4.2.3. Determination of fabric weight per unit area

The grams per unit area (GSM) of cotton fabric can vary depending on the specific type of cotton fabric and its intended use. Generally, the GSM of cotton fabric ranges from 100 to 200, with higher GSM indicating a denser and heavier fabric. 100% Cotton fabric has 116.73 gms/m^2.

4.2.4. Determination of thickness of the fabric

The thickness of the cotton fabric used by the thickness tester can vary depending on the specific type of cotton fabric. The thickness of the cotton fabric was 31.4 mm.

Table 4.2: Preliminary data of the selected fabric

Sr. No.	Fibre Content	Fabric Count		Weight/ Unit Area (Gms/m^2)	Fabric Thickness (mm)	Weave
		End per inch	Picks per inch			
1	100% cotton	195	122	116.73	31.4	Satin

The results showed that the selected satin woven cotton fabric was an unbalanced fabric with count of 195 ends per inch and 122 picks per inch. The weight per unit area of cotton fabric was 116.73 gms/m^2. Thickness of the cotton was 31.4 mm.

4.3. To develop an Eco-friendly natural mosquito repellent finish using plant extract.

4.3.1. Extraction of fresh *Pluchea wallichiana* Leaves

Extraction of the leaves typically refers to the process of extracting bioactive compounds or other valuable materials from plant leaves. This involves using a solvent such as ethanol to dissolve the bioactive compounds in the leaves. The resulting solution is then filtered and concentrated to obtain the desired extract.

Pluchea wallichiana leaves were collected, cleaned, cut into small pieces and wrapped in filter paper for extraction using Soxhlet apparatus. The solvent used for extraction was 98% ethanol. The flowchart present below explains the extraction process and yield from *Pluchea wallichiana* leaves.

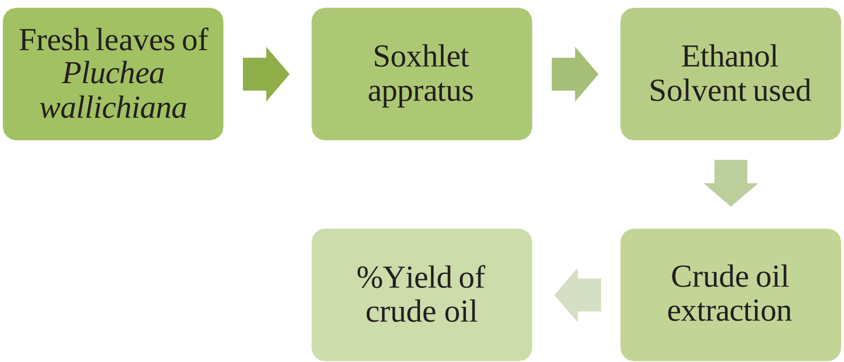


Figure 4.1: Flowchart of the extraction process

A total of 141 gms of leaves were subjected to extraction using ethanol solvent. Material to liquor ratio was 1:40. A total 1830 ml extract was collected using Soxhlet extraction method.

4.3.2. Filtration of extract *Pluchea wallichiana* Leaves

Filtration of the leaves refers to the process of separating solid particles or impurities from a liquid extract obtained from plant leaves. This is typically done to obtain a clean and clear extract that is free from any unwanted materials.

Rotary evaporation is a commonly used method for concentrating plant extracts, which involves heating the extract under vacuum to remove the solvent and leave behind a concentrated extract.

Rota-evaporator separated the solvent and extract. There was a total 1830 ml extract in this instrument and separate the ethanol and crude oil extract. There is 85 ml of crude oil extract collected after separation of the solvent. Calculated the % yield of extract using this formula given below:

$$\% \text{ Yield} = \frac{W2}{W1} \times 100$$

Where, W1 = Weight of total extract

W2 = Weight of extract after separated the solvent

$$\% \text{ Yield} = \frac{85}{1830} \times 100$$

= 4.6 % yield of crude oil

Total % of yield was 4.6 from *Pluchea wallichiana* leaf extract. This extract was used for further procedure to develop natural mosquito repellent finished cotton fabric.

Table 4.3: Some physical properties of leaf extracts of *Pluchea wallichiana*

Sr. No.	**Solvent**	**Weight(g)**	**% Yield**	**State**	**Colour**
1	98% Ethanol	127.5	4.6	Liquid	Dark green

4.4. Preliminary phytochemicals analysis

Phytochemicals are compounds which are produced by plants ("Phyto" means "Plant" in Greek). They are found in fruits, vegetables, grains, beans and other plants and herbs. Phytochemical analysis of *Pluchea wallichiana* leaves were carried out using standard phytochemical procedure. The leaves of this plant contain various phytochemicals, which are biologically active compounds that have potential health benefits. Some of the phytochemicals identified in *Pluchea wallichiana* leaves include:

Table 4.4: Phytochemical Analysis of *Pluchea wallichiana* leaves

Sr. No.	Phyto-compounds	Result	Confirmation
1	Carbohydrates	-	Violet ring
2	Proteins	-	Pink colour
3	Amino acids	+	Yellow colour
4	Fatty acids	-	Appearance of transparency
5	Fixed oils and fats	+	Appearance of spots
6	Gums	-	White or cloudy precipitate
7	Anthraquinones	-	Rose pink
8	Quinones	-	Red to blue colour
9	Alkaloids	+	Creamy or white precipitate
10	Glycosides	+	Pink colour
11	Cardiac glycosides	+	Violet, brown and green ring
12	Phenols	+	Deep red or green or blue colour
13	Tannins	+	Dark green colour
14	Flavonoids	+	Pink, reddish pink or brown colour
15	Saponins	-	Formation of frothing
16	Terpenoids	+	Reddish brown colour
17	Steroids	+	Red colour and yellowish green fluorescence

Key = "+" Active compound present, "-" Active compound absent

Phytochemical analysis as given in Table 4.4 shows the presence of phytochemical present in *Pluchea wallichiana* leaves.

Preliminary phytochemical studies are helpful in finding out chemical constituents in the plant material that may well lead to their quantitative estimation. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different compositions which occur as secondary metabolites (Kalaiselvi V. et al., 2016). For the preliminary phytochemical analysis, leaf extracts (Ethanol) of *Pluchea wallichiana* were taken. In this study, the results revealed the presence of medically active compounds in the plant studied. From the table, it could be seen that, amino acids, fixed oils and fats, glycosides, cardiac glycosides, phenols, alkaloids, tannins, flavonoids, terpenoids and steroids were present in the *Pluchea wallichiana* leaves.

Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plants serve as defence mechanisms against predation by many plants, microorganisms, insects, and herbivores.

Amino acids: Amino acids derived phytochemical compounds have a wide range of medicinal properties, and may be beneficial for a variety of conditions. Several amino acids have been shown to possess anti-inflammatory properties. These amino acids can help reduce inflammation in the body, which may be beneficial for a variety of conditions, including arthritis, asthma, and inflammatory bowel disease. Amino acids also have antioxidant properties, which can help protect cells from oxidative stress and damage. Some amino acids have hydrophilic properties; which means they have an affinity for water. This can be useful in textiles for their ability to absorb and retain moisture, which can help keep the fabric feeling soft and comfortable (Wadaskar. S. et al., 2022).

Fixed oils and fats: Some plant-derived fats and oils have been shown to have anti-microbial properties. These oils can be used in textile production to help prevent the growth of bacteria, fungi, and other microorganisms that can cause odour and infection. Many plant-derived fats and oils have moisturizing properties. These oils can be used in textile production to help create soft and comfortable fabrics that are gentle on the skin. Some plant-derived fats and oils contain compounds that possess anti-inflammatory properties. These compounds can be used in textile production to create fabrics that may be beneficial for people with conditions such as arthritis or other inflammatory skin conditions.

Glycosides: Some non-toxic glycosides can be hydrolysed to phenolic compounds that can repel insects (Evans et al., 1986). It can therefore be assumed that the repellent activity in *Pluchea wallichiana* may be due to the presence of these metabolites (glycosides) (Idris M. et al., 2014). Glycosides are known to lower the blood pressure. There has been reported presence of bioactive compound that offers antisecretory and antiulcer effects (Jadon and Dixit, 2014).

Cardiac glycosides: Cardiac glycosides have been used for over two centuries as stimulants in case of cardiac failure (Trease and Evans, 1998; Olayinki et al., 1992). Cardiac glycosides can help to lower blood pressure, which can improve overall cardiovascular health. Some studies have suggested that cardiac glycosides may have anti-inflammatory properties, which could potentially be useful in the treatment of various inflammatory conditions. Some cardiac glycosides have been shown to have antitumor properties and are being investigated as potential anticancer agents.

Alkaloids: Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity which means how toxic a substance is to cells (Nobori T. et al., 1994). Several workers have reported the analgesic, antispasmodic and antibacterial properties of alkaloids (Okwu, D. E. et al., 2004). Alkaloids belong to the beta-carboline group which possess antimicrobial, anti HIV and antiparasitic activities (Patel et al., 2012).

Phenols: Phenols are potent antioxidants that can protect the body from damage caused by free radicals, which are unstable molecules that can damage cells and contribute to aging and disease (Tungmunnithum D. et al., 2018). Phenols have anti-inflammatory properties that can help to reduce inflammation in the body, which can be useful in the treatment of various inflammatory conditions. Some phenols have antimicrobial properties and can be used to treat infections caused by bacteria, fungi, and viruses. They can have a positive effect on cardiovascular health by reducing the risk of heart disease, improving blood circulation, and lowering blood pressure.

Tannins: Tannins are attributed for analgesic and anti-inflammatory activities. Apart from these tannins contribute properties of astringency i.e. faster the healing of wounds and inflamed mucous membrane (Okwu and Josiah, 2006). Tannins have antibacterial activity. They are thought to be responsible for antidiarrheal activity (Enzo, 2007). Also believed to have antimicrobials and antioxidants activities even at lower concentrations it can prevent bacterial growth and at higher concentration it behaves as antifungal agent (Sumathy et al., 2011).

Flavonoids: The biological functions of flavonoids apart from its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumours (Doss A. et al., 2016). Flavonoids are thought to be responsible for antidiarrheal activity. Flavonoids were found in all the extracts and are potent water-soluble antioxidants which prevent oxidative cell damage suggesting antiseptic, anticancer, anti-inflammatory effects and mild hypersensitive properties (Kumaraswamy M. et al., 2019). They also are effective antioxidants and show strong anticancer activities. They are potent water-soluble antioxidants and free radicals scavenger, which prevents the cell from oxidative cell damage (Yadav et al., 2014).

Terpenoids: Some terpenoids act as natural insecticides or repellents, helping to protect plants. Many terpenoids have been shown to have medicinal properties, such as anti-inflammatory, antimicrobial, and antitumor activities. Terpenoids are attributed for analgesic and anti-inflammatory activities (Doss A. et al., 2016).

Steroids: Steroids have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones (Idris M. et al., 2014). Steroids are potent anti-inflammatory agents that can reduce inflammation and swelling in various parts of the body.

In phytochemical analysis of *Pluchea wallichiana* leaves were extracted for use as a mosquito repellent finish, and *Pluchea wallichiana* is known to have natural mosquito repellent properties. Several Phytochemical compounds present in the leaves of plants are responsible for this activity. Flavonoids, Alkaloids, Phenols and glycosides are the present compounds which are responsible for mosquito repellent property.

1. Alkaloids: Alkaloids are a class of nitrogen-containing compounds that are present in many plants, including *Pluchea wallichiana*. They have been shown to have insecticidal properties against mosquitoes.
2. Flavonoids: Flavonoids are a class of plant pigments that are known to have a range of biological activities, including insecticidal and repellent properties against mosquitoes.
3. Terpenoids: Terpenoids are a class of compounds that are found in many plants, including *Pluchea wallichiana*. They have been shown to have insecticidal and repellent properties against mosquitoes.
4. Phenols: Phenolic compounds are a class of compounds that are present in many plants, including *Pluchea wallichiana*. They have been shown to have insecticidal and repellent properties against mosquitoes.

The purpose of these compounds in *Pluchea wallichiana* leaves is to protect the plant from being attacked by insects, including mosquitoes. These compounds act as natural insecticides and repellents for the plant, through the experimentation under this study, these active compounds are made available through extraction, as mosquito repellent.

4.5. Assessment of efficacy of finish

The efficacy of a mosquito repellent finish can be assessed by evaluating the ability of the finished fabric to repel mosquitoes. This can be done through laboratory testing or field trials.

Laboratory test known as the ‘Glucose pad test’ involves exposing the glucose and repellent applied to cotton pad for the hungry mosquitoes to feed upon and evaluating the number of mosquito landings on the cotton pad for feeding. The results of the testing can be used to determine the effectiveness of the mosquito repellent finish.

Different amounts of extract (5 ml, 10 ml and 20 ml) were applied on a glucose cotton pad to find the efficacy of the crude extract. Glucose cotton pad was put in the cage for mosquitoes, where it is expected that mosquitoes fed glucose from cotton pad but due to the presence of the crude repellent extract the mosquitoes kept away from the glucose cotton pad and they cannot feed the glucose because of the effect of the extract, all mosquitoes were observed to be attempting to get out of the cage.

After 24 hrs mosquitoes were not dead but they were not able to feed from that cotton pad and also they could not land on the cotton pad. Hence it was inferred that the efficacy of the extract as a mosquito repellent finish was 100 percent. The anti-mosquito effectiveness was noted by counting the number of mosquitoes that escaped on the glucose cotton pad at 15, 30, 45 and 60-minutes intervals as mentioned in table 4.5 below.

Table 4.5: Efficacy of mosquito repellent finish n=20

Sr. No.	Time of observation (min)	Extract (ml)	No. of mosquito exposed	No. of mosquito escaped	% Efficacy
1	15	5	20	1	5
2	30		20	3	15
3	45		20	5	25
4	60		20	7	35
5	15	10	20	4	20
6	30		20	6	30
7	45		20	9	45
8	60		20	13	65
9	15	20	20	4	20
10	30		20	7	35
11	45		20	13	65
12	60		20	18	90

It was observed that 5 ml extract was less effective compared to 10 ml and 20 ml of extract. After 15 min interval the observation of the number of mosquitoes that escaped was 1 in 5 ml of extract which was less effective and 4 mosquitoes in 20 ml of extract which was more effective compared to 5 and 10 ml of extract. Also, after 30 min, 5 ml applied extract cotton pad had 3 mosquitoes escape, 10 ml applied extract cotton pad was 6 mosquitoes escaped and 20 ml applied extract cotton pad had 7 mosquitoes escaped. After 45 min, 5 ml applied extract cotton pad had 5 mosquitoes escaped, 10 ml applied extract cotton pad had 9 mosquitoes escaped and 20 ml applied extract cotton pad had 13 mosquitoes escaped. And last 60 min, 5 ml applied extract cotton pad had 7 mosquitoes escaped, 10 ml applied extract cotton pad had 13 mosquitoes escaped and 20 ml applied extract cotton pad had 18 mosquitoes escaped.

The highest efficacy of 90 percent was observed in 20 ml extract applied on cotton pad after 60 min and the lowest efficacy of 35 percent was observed in 5 ml extract applied on cotton pad after 60 min. Average efficacy was determined in 10 ml applied extract on the cotton pad which was 65 percent

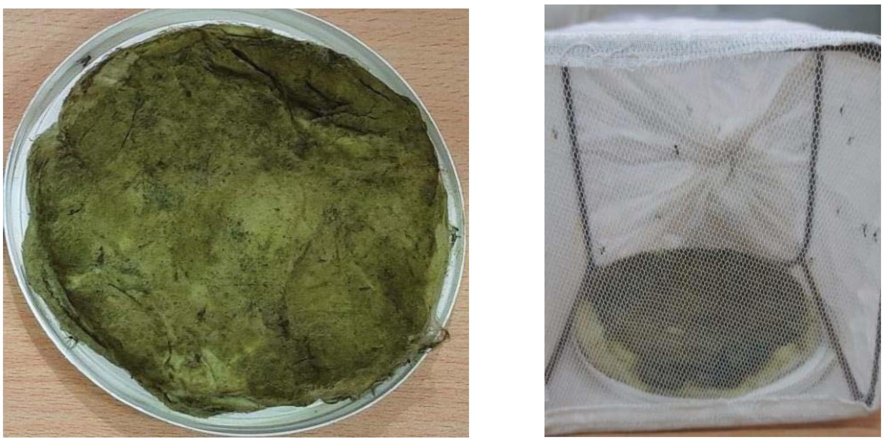


Plate 4.1: Extract applied on glucose cotton pad and Cage test for efficacy

4.6. To study the application of plant extract as a finish on cotton fabric

The natural mosquito repellent finish which was extracted from *Pluchea wallichiana* leaf was applied on 100% cotton fabric using Pad-dry-cure method on padding mangle. Finish was applied using two different techniques: i) Direct technique and ii) Resin cross-linking technique.

4.6.1. Direct application method

The direct method of finishing involves applying finishing chemicals directly onto the fabric using padding. The pick-up and add-on level in the direct method of finishing depended on the type of finishing chemical used and the method of application.

Cotton fabric was treated by direct application method using two different amounts of extract (10 and 20 gpl) with 5% citric acid and common salt. After the application of finish, pick up and add-on of the fabric has been mentioned in Table 4.6.

Table 4.6: Per cent pick-up and add-on of treated fabric using direct application method

Sr. No.	Extract	Pick-up (%)	Add-on (%)
1	10 gpl	56.95	4.48
2	20 gpl	65.47	7.62

The pick-up of treated fabric using 10 gpl extract had 56.95 Per cent and Add-on was 4.48 percent. And using 20 gpl extract had a 65.47 percent pick-up and 7.62 percent add-on in direct application method.

4.6.2. Resin cross linking method

In textile finishing, the resin cross-linking method involves applying a resin-based finishing chemical to the fabric and then curing it to create a cross-linked network within the fabric. The pick-up and add-on level in the resin cross-linking method depended on the type of resin used and the method of application.

The pick-up and add-on level in the resin cross-linking method was typically higher than in other finishing methods due to the need for enough resin to be absorbed into the fabric to create the cross-linked network.

Cotton fabric was treated by resin cross-linking application method using two different amounts of extract (10 and 20 gpl) with DMDHEU (Dimethyl dihydroxy ethylene urea) and Magnesium chloride ($MgCl_2$). After the application of finish, percent pick up and add-on of the fabric was mentioned in Table 4.7.

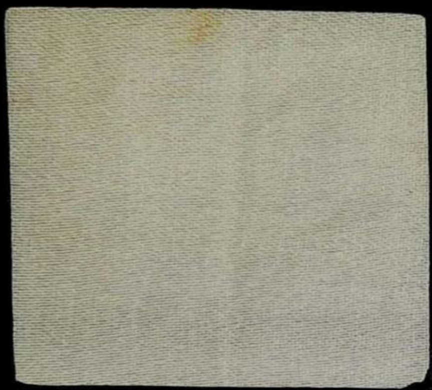
Table 4.7: Per cent pick-up and add-on of treated fabric using Resin cross-linking application method

Sr. No.	Extract	Pick-up (%)	Add-on (%)
1	10 gpl	68.61	5.38
2	20 gpl	75.34	8.52

The percentage wet pick-up of treated fabric using 10 gpl extract was 68.61 percent and add on was 5.38 percent And using 20 gpl extract have 75.34 percent wet pick-up and 8.52 percent add on in resin cross-linking application method.

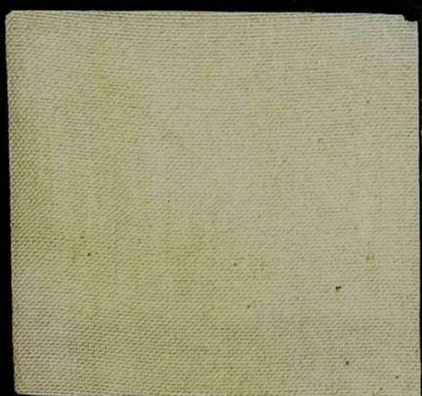
Plate 4.2: Repellency test of applied finish

i) Control Sample



ii) Direct technique (using 10 gpl extract)

iii) Direct technique (using 20 gpl extract)

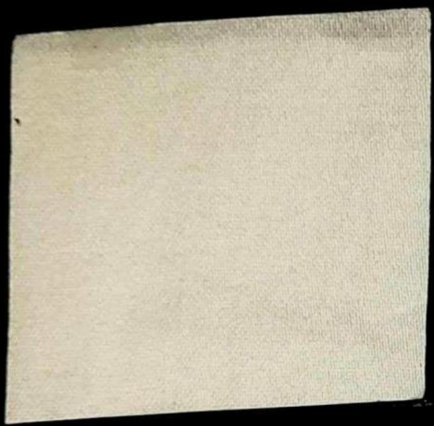


iv) Resin cross-linking technique (using 10 gpl extract)

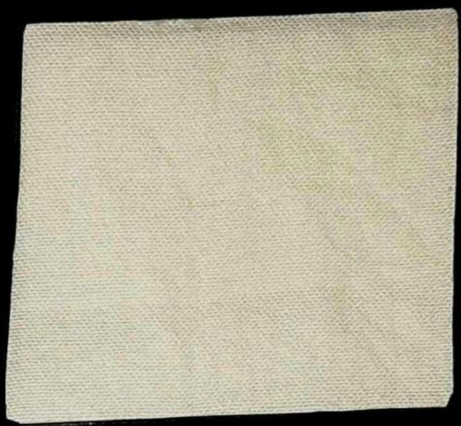
v) Resin cross-linking technique (using 20 gpl extract)

Plate 4.3: Repellency test of applied finish - Post wash

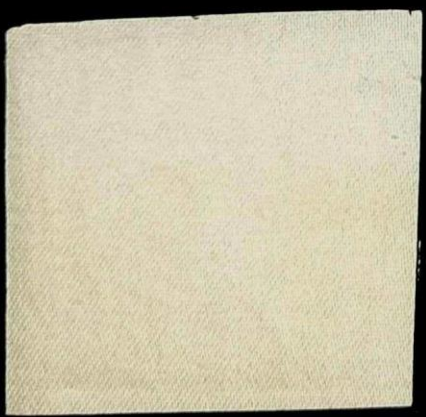
i) Control Sample



ii) Direct technique (using 10 gpl extract)



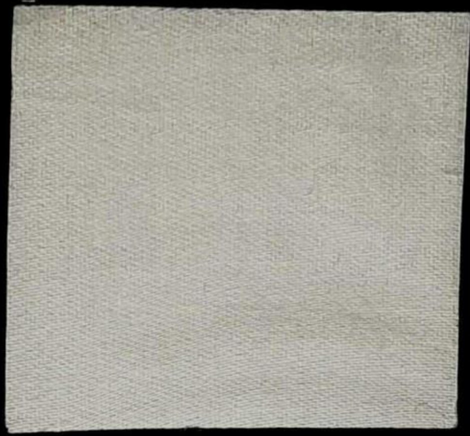
iii) Direct technique (using 20 gpl extract)



iv) Resin cross-linking technique (using 10 gpl extract)

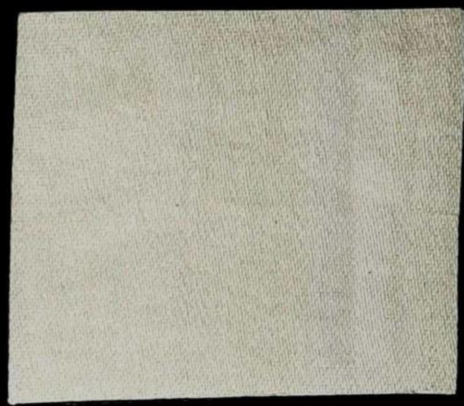
v) Resin cross-linking technique (using 20 gpl extract)

Plate 4.4: Repellency test of applied finish - Post light exposure

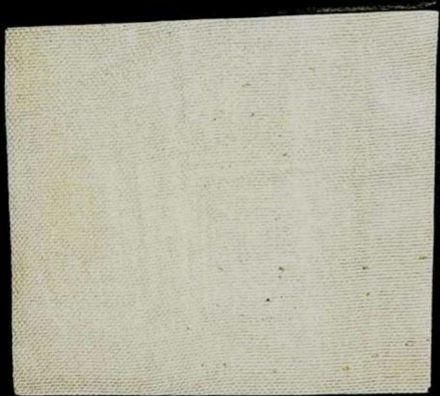


i) Control Sample

ii) Direct technique (using 10 gpl extract)



iii) Direct technique (using 20 gpl extract)



iv) Resin cross-linking technique (using 10 gpl extract)

v) Resin cross-linking technique (using 20 gpl extract)

4.7. To study mosquito repellency of the finish applied to cotton fabric

For mosquito repellent test, control (untreated) and treated cotton fabrics were tested and evaluated for mosquito repellent property and effectiveness was studied through per cent repellency and mosquito mortality percentage was also computed. The mosquito mortality of all treated fabric using different application methods were also compared.

The treated fabrics were tested for mosquito repellent efficacy against untreated fabrics using modified WHO cone test method, while exposing 20 mosquitoes in cone. The mosquitoes were allowed to settle for two minutes. After 2 minutes, the numbers of mosquitoes resting on the treated and untreated fabrics were counted and noted at 15, 30, 45 and 60 minutes intervals. The number of immobilized, knocked down test mosquitoes was determined after 1 hour of exposure and the mortality rate was determined after 24 hours. The percentage of mosquito repellency was calculated using the following formula:

$$\% \text{ Mosquito mortality: } (MR - MC) / (100 - C) \times 100\%$$

Where, MR represents the mosquito mortality in test replicate while the MC corresponds to the mosquito mortality in control samples.

4.7.1. Assessment of mosquito repellency property

The cone test is a commonly used method for assessing the mosquito repellency properties of various products, such as lotions, sprays, and fabrics. The test involves placing a plastic cone over a treated area of fabric and then releasing a controlled number of mosquitoes into the cone. The number of mosquitoes that land on the treated area versus the untreated area is then counted and recorded.

The tables below show the results of the cone test conducted at different concentrations of the mosquito repellent finish applied to the cotton fabric. The effectiveness of the mosquito repellent was indicated as a percentage of mosquitoes that did not land on the treated fabric compared to the untreated fabric. It was generally observed that as the concentration of the mosquito repellent agent increases, the repellency effectiveness also increases.

Plate 4.5: Cone test for evaluate mosquito repellency of treated fabric

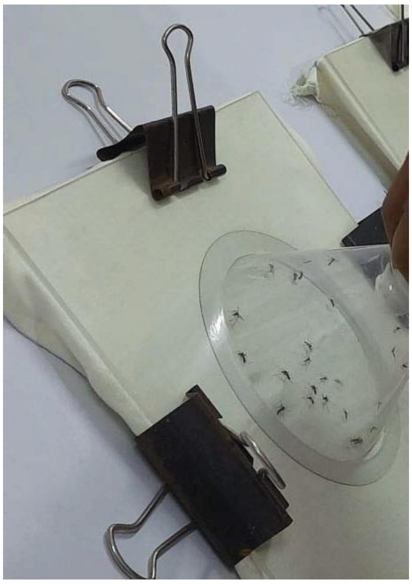


Plate 4.6: Mosquitoes that land on the treated area and the untreated area

i) Direct application method

A total of six treated samples using direct application method with different amount of extract were tested. The result of mosquito repellency has been reported in the tables below:

Table 4.8: Mosquito repellency of sample DT10D

Sr. No.	Test day	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	3	15
2		30	20	4	20
3		45	20	7	35
4		60	20	9	45
5	3	15	20	2	10
6		30	20	4	20
7		45	20	5	25
8		60	20	6	30
9	5	15	20	3	15
10		30	20	4	20
11		45	20	4	20
12		60	20	5	25
13	7	15	20	3	15
14		30	20	4	20
15		45	20	4	20
16		60	20	3	15
17	9	15	20	2	10
18		30	20	2	10
19		45	20	3	15
20		60	20	2	10

Key: DT10D – Direct technique using 10 gpl extract direct tested

The samples were treated with 10 gpl extract using direct technique for evaluation of mosquito repellency of direct tested fabrics. As shown in table 4.8 the samples were observed for the no. of mosquitoes escaping the cone for 1 hr with 15 min intervals and then observed at the end of 24 hours. There was 15 % repellency observed after 15 min and 45% repellency observed after 60 min on the 1st day of testing. On the 9th day of testing, a direct tested sample was observed at a lowest repellency which was 10 % after 1 hr. When after applying the finish, the effect we get after doing the first test, then its effect starts decreasing.

Table 4.9: Mosquito repellency of sample DT20D

Sr. No.	Test day	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	4	20
2		30	20	6	30
3		45	20	9	45
4		60	20	10	50
5	3	15	20	3	15
6		30	20	4	20
7		45	20	6	30
8		60	20	7	35
9	5	15	20	3	15
10		30	20	4	20
11		45	20	4	20
12		60	20	5	25
13	7	15	20	2	10
14		30	20	4	20
15		45	20	4	20
16		60	20	4	20
17	9	15	20	1	05
18		30	20	1	05
19		45	20	2	10
20		60	20	3	15

Key: DT20D - Direct technique using 20 gpl extract direct tested

As shown in the table 4.9 samples were observed after one day intervals. There was 20 % repellency observed after 15 min and 50 % repellency observed after 60 min on the 1st day of testing. On the 9th day of testing, direct tested sample showed a lowest repellency which was 15 % after 1 hr. This fabric had better repellency compared to 10 gpl extract applied sample in direct technique. The results of this test showed that as the concentration of the mosquito repellent agent increased, the effectiveness of the repellent increased as well.

Table 4.10: Mosquito repellency of sample DT10W

Sr. No.	Wash cycle	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	1	05
2		30	20	3	15
3		45	20	4	20
4		60	20	5	25
5	2	15	20	1	05
6		30	20	2	10
7		45	20	3	15
8		60	20	4	20
9	3	15	20	1	05
10		30	20	2	10
11		45	20	2	10
12		60	20	3	35
13	4	15	20	1	05
14		30	20	1	05
15		45	20	1	05
16		60	20	2	10
17	5	15	20	0	00
18		30	20	0	00
19		45	20	1	05
20		60	20	1	05

Key: DT10W - Direct technique using 10 gpl extract tested after wash

These samples were applied 10 gpl extract using direct technique to evaluate mosquito repellency which was tested after washing. As shown in the table 4.10 samples were treated after every wash. There was 5 % repellency observed after 15 min and 25 % repellency observed after 60 min on 1st wash. On the 9th day of testing (5th wash), a direct tested sample observed a lowest repellency which was 5 % after 1 hr. When after applying the finish, the effect we get after doing the first wash, then its effect decreases up to 20 % compared to directly tested fabric (10 gpl extract) and 5% less effective compared to sample after light fastness testing (10 gpl extract).

Table 4.11: Mosquito repellency of sample DT20W

Sr. No.	Wash cycle	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	2	10
2		30	20	3	15
3		45	20	4	20
4		60	20	6	30
5	2	15	20	2	10
6		30	20	2	10
7		45	20	3	15
8		60	20	4	20
9	3	15	20	2	10
10		30	20	2	10
11		45	20	2	10
12		60	20	3	15
13	4	15	20	1	05
14		30	20	1	05
15		45	20	1	05
16		60	20	2	10
17	5	15	20	0	00
18		30	20	1	05
19		45	20	0	00
20		60	20	2	10

Key: DT20W - Direct technique using 20 gpl extract tested after wash

These samples were applied 20 gpl extract using direct technique to evaluate mosquito repellency which was tested after washing. As shown in the table 4.11 samples were treated after every wash. The observation of no. of mosquitoes escaped evaluated after 1 hr with 15 min intervals. There was 10 % repellency observed after 15 min and 30 % repellency observed after 60 min on 1st wash. On the 9th day of testing (5th wash), the direct tested sample observed a lowest repellency which was 10 % after 1 hr. When after applying the finish, the effect we get after doing the first wash, then its effect decreases up to 20 % compared to directly tested fabric (20 gpl extract) and 15% less effective compared to sample after light fastness testing (20 gpl extract).

Table 4.12: Mosquito repellency of sample DT10L

Sr. No.	Test day	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	2	10
2		30	20	3	15
3		45	20	4	20
4		60	20	6	30
5	3	15	20	2	10
6		30	20	2	10
7		45	20	3	15
8		60	20	5	25
9	5	15	20	3	15
10		30	20	2	10
11		45	20	2	10
12		60	20	4	20
13	7	15	20	1	05
14		30	20	2	10
15		45	20	3	15
16		60	20	2	10
17	9	15	20	1	05
18		30	20	2	10
19		45	20	2	10
20		60	20	2	10

Key: DT10L - Direct technique using 10 gpl extract tested after light exposure

These samples were applied 10 gpl extract using direct technique for evaluated mosquito repellency which tested after light fastness. As shown in the table 4.12 samples were treated after light fastness test. There was 10 % repellency observed after 15 min and 30 % repellency observed after 60 min on the 1st day of testing. On the 9th day of testing, the direct tested sample observed a lowest repellency which was 10 % after 1 hr. When after applying the finish, the effect we get after doing the first light fastness test, then its effect decreased up to 15 % compared to directly tested fabric (10 gpl extract) and 5% more effective compared to samples after washing. (10 gpl extract).

Table 4.13: Mosquito repellency of sample DT20L

Sr. No.	Test day	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	2	10
2		30	20	4	20
3		45	20	6	30
4		60	20	9	45
5	3	15	20	3	15
6		30	20	3	15
7		45	20	5	25
8		60	20	7	35
9	5	15	20	2	10
10		30	20	2	10
11		45	20	3	15
12		60	20	5	25
13	7	15	20	3	15
14		30	20	3	15
15		45	20	4	20
16		60	20	4	20
17	9	15	20	1	05
18		30	20	2	10
19		45	20	2	10
20		60	20	2	10

Key: DT20L - Direct technique using 20 gpl extract tested after light exposure

These samples were applied 20 gpl extract using direct technique for evaluated mosquito repellency which tested after light fastness. As shown in the table 4.13 samples were treated after light fastness test. There was 10 % repellency observed after 15 min and 45 % repellency observed after 60 min on the 1st day and the 9th day of testing, the direct tested sample observed a lowest repellency which was 10 % after

1 hr. When after applying the finish, the effect we get after doing the first light fastness test, then its effect decreased up to 5 % compared to directly tested fabric and 15% more effective compared to samples after washing (20 gpl extract).

ii) Resin cross linking method

There were total 6 samples that were treated using resin cross-linking application method the results of mosquito repellency have been reported in the tables below:

Table 4.14: Mosquito repellency of sample RCL10D

Sr. No.	Test day	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	5	25
2		30	20	7	35
3		45	20	8	40
4		60	20	11	55
5	3	15	20	4	20
6		30	20	6	30
7		45	20	6	30
8		60	20	7	35
9	5	15	20	3	15
10		30	20	5	25
11		45	20	4	20
12		60	20	6	30
13	7	15	20	3	15
14		30	20	3	15
15		45	20	4	20
16		60	20	5	25
17	9	15	20	2	10
18		30	20	2	10
19		45	20	3	15
20		60	20	3	15

Key: RCL10D - Resin cross linking technique using 10 gpl extract direct tested

These samples were applied 10 gpl extract which directly tested using resin cross-linking technique to evaluate mosquito repellency. As shown in the table 4.14 samples were treated after one day intervals. There was 25 % repellency observed after 15 min and 55 % repellency observed after 60 min on the 1st day of testing. On the 9th day of testing, the direct tested sample observed the lowest repellency which was 15 % after 1 hr.

Table 4.15: Mosquito repellency of sample RCL20D

Sr. No.	Test day	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	6	30
2		30	20	8	40
3		45	20	10	50
4		60	20	14	70
5	3	15	20	4	20
6		30	20	7	35
7		45	20	8	40
8		60	20	10	50
9	5	15	20	3	15
10		30	20	6	30
11		45	20	6	30
12		60	20	7	35
13	7	15	20	4	20
14		30	20	4	20
15		45	20	4	20
16		60	20	5	25
17	9	15	20	1	05
18		30	20	2	10
19		45	20	2	10
20		60	20	4	20

Key: RCL20D - Resin cross linking technique using 20 gpl extract direct tested

These samples were applied 20 gpl extract which direct tested using resin cross-linking technique for evaluate mosquito repellency. As shown in the table 4.15

samples were treated after one day intervals. There was 30 % repellency observed after 15 min and 70 % repellency observed after 60 min on 1st day of testing. And 9th day of testing, direct tested sample was observed a lowest repellency which was 20 % after 1 hr.

Table 4.16: Mosquito repellency of sample RCL10W

Sr. No.	Wash cycle	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	1	05
2		30	20	3	15
3		45	20	5	25
4		60	20	4	20
5	2	15	20	2	10
6		30	20	3	15
7		45	20	4	20
8		60	20	3	15
9	3	15	20	1	05
10		30	20	2	10
11		45	20	3	15
12		60	20	3	15
13	4	15	20	2	10
14		30	20	1	05
15		45	20	2	10
16		60	20	2	10
17	5	15	20	0	00
18		30	20	1	05
19		45	20	1	05
20		60	20	1	05

Key: RCL10W – Resin cross-linking technique using 10 gpl extract tested after wash

These samples were applied 10 gpl extract using resin cross-linking technique to evaluate mosquito repellency which was tested after washing. As shown in the table 4.16 samples were treated after every wash. The observation of no. of mosquitoes escaped evaluated after 1 hr with 15 min intervals. There was 5 % repellency observed after 15 min and 20 % repellency observed after 60 min on 1st wash. On the 9th day of testing (5th wash), the direct tested sample observed the lowest repellency which was 5 % after 1 hr. When after applying the finish, the effect we get after doing the first wash, then its effect decreases up to 35 % compared to directly tested fabric (10 gpl extract) and 15 % less effective compared to sample after light fastness testing (10 gpl extract).

Table 4.17: Mosquito repellency of sample RCL20W

Sr. No.	Test day	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	3	15
2		30	20	5	25
3		45	20	6	30
4		60	20	8	40
5	2	15	20	3	15
6		30	20	3	15
7		45	20	5	25
8		60	20	6	30
9	3	15	20	2	10
10		30	20	4	20
11		45	20	4	20
12		60	20	5	25
13	4	15	20	2	10
14		30	20	2	10
15		45	20	2	10
16		60	20	2	10
17	5	15	20	1	05
18		30	20	1	05
19		45	20	1	05
20		60	20	2	10

Key: RCL20W – Resin cross-linking technique using 20 gpl extract tested after wash

These samples were applied 20 gpl extract using resin cross-linking technique to evaluate mosquito repellency which was tested after washing. As shown in the table

4.17 samples were treated after every wash. There was 15 % repellency observed after 15 min and 40 % repellency observed after 60 min on 1st wash. On the 9th day of testing (5th wash), the direct tested sample observed the lowest repellency which was 5 % after 1 hr. When after applying the finish, the effect we get after doing the first wash, then its effect decreases up to 20 % compared to direct tested fabric (20 gpl extract) and 5 % less effective compared to sample after light fastness testing (20 gpl extract).

Table 4.18: Mosquito repellency of sample RCL10L

Sr. No.	Test day	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	3	15
2		30	20	4	20
3		45	20	5	25
4		60	20	7	35
5	2	15	20	3	15
6		30	20	3	15
7		45	20	3	15
8		60	20	5	25
9	3	15	20	2	10
10		30	20	2	10
11		45	20	3	15
12		60	20	4	20
13	4	15	20	2	10
14		30	20	3	15
15		45	20	2	10
16		60	20	2	10
17	5	15	20	1	05
18		30	20	1	05
19		45	20	2	10
20		60	20	2	10

Key: RCL10L – Resin cross-linking technique using 10 gpl extract tested after light exposure

These samples were applied 10 gpl extract using resin cross-linking technique to evaluate mosquito repellency which tested after light fastness. There was 15 % repellency observed after 15 min and 35 % repellency observed after 60 min on the 1st day and the 9th day of testing, the direct tested sample observed a lowest repellency which was 10 % after 1 hr. When after applying the finish, the effect we get after the first light fastness test, then its effect decreases up to 10 % compared to fabric which directly tested using resin cross linking technique (10 gpl extract) and 15% more effective compared to samples after washing (10 gpl extract). (Table 4.18).

Table 4.19: Mosquito repellency of sample RCL20L

Sr. No.	Test day	Time of observation (min)	No. of mosquito exposed	No. of mosquito escaped	% Repellency
1	1	15	20	3	15
2		30	20	4	20
3		45	20	7	35
4		60	20	9	45
5	2	15	20	3	15
6		30	20	3	15
7		45	20	5	25
8		60	20	7	35
9	3	15	20	4	20
10		30	20	5	25
11		45	20	5	25
12		60	20	5	25
13	4	15	20	2	10
14		30	20	2	10
15		45	20	3	15
16		60	20	3	15
17	5	15	20	2	10
18		30	20	1	05
19		45	20	1	05
20		60	20	2	10

Key: RCL20L – Resin cross-linking technique using 20 gpl extract tested after light exposure

These samples were applied to 20 gpl extract using resin cross-linking technique to evaluate mosquito repellency which was tested after light fastness. As shown in the table 4.19 samples were treated after light fastness test. There was 15 % repellency observed after 15 min and 45 % repellency observed after 60 min on the 1st day of testing. On the 9th day of testing, the direct tested sample observed a lowest repellency which was 10 % after 1 hr.

When after applying the finish, the effect we get after the first light exposure, then its effect decreased up to 25 % compared to fabric which directly tested using resin cross linking technique (20 gpl extract) and 5% more effective compared to samples after washing. (20 gpl extract).

4.7.2. Effect of mosquito repellent efficiency of cotton fabric

Cotton fabric was treated with natural mosquito repellent to improve its efficiency in repelling mosquitoes. The effectiveness of the mosquito repellent on cotton fabric was depended on concentration of the repellent, the method of application, and the duration of the treatment.

The treated fabric was exposed to mosquitoes and the number of mosquito bites is counted over a certain period of time.

Cotton fabric treated with an effective natural mosquito repellent was provided good protection against mosquitoes. However, the effectiveness of the repellent may decrease over time due to washing, exposure to light, and other factors.

The results of the cone test showed that as the concentration of the mosquito repellent agent increased, the effectiveness of the repellent increased as well. The control sample showed no repellency effect, with all mosquitoes landing on the treated fabric. The results are shown in the tables below:

Table 4.20: Mosquitoes mortality of all samples tested direct after the application

Sr. No.	Test day	Sample	No. of mosquito exposed	No. of mosquito dead	% Mortality
1	1	Control	20	0	00
2		DT10D	20	13	65
3		DT20D	20	15	75
4		RCL10D	20	16	80
5		RCL20D	20	18	90
6	3	Control	20	0	00
7		DT10D	20	11	55
8		DT20D	20	13	65
9		RCL10D	20	14	70
10		RCL20D	20	14	70
11	5	Control	20	0	00
12		DT10D	20	8	40
13		DT20D	20	9	45
14		RCL10D	20	9	45
15		RCL20D	20	11	55
16	7	Control	20	0	00
17		DT10D	20	7	35
17		DT20D	20	8	40
18		RCL10D	20	8	40
19		RCL20D	20	10	50
20	9	Control	20	0	00
21		DT10D	20	6	30
22		DT20D	20	6	30
23		RCL10D	20	7	35
24		RCL20D	20	9	45

Key: DT10D – Direct technique using 10 gpl extract direct tested, DT20D - Direct technique using 20 gpl extract direct tested, RCL10D - Resin cross linking technique using 10 gpl extract direct tested, RCL20D - Resin cross linking technique using 20 gpl extract direct tested

There are 4 samples which were directly tested with different two application techniques which were direct and resin cross linking and evaluate % mortality of the treated and untreated samples after 24hr. After the application, (Table 4.20) the first test gave a highest percent of mortality compared to other days.

DT10D treated sample had 65 percent mortality, DT20D treated samples had 75 percent mortality, RCL10D treated sample had 80 percent mortality and RCL20D treated sample had 90 percent mortality. And 9th day of testing after application of finish gave less results compare to 1st day of testing which were respectively DT10D treated sample had 30 percent mortality, DT20D treated sample have 30 percent mortality, RCL10D treated sample had 35 percent mortality and RCL20D treated sample had 40 percent of mortality which up to 45 percent less result given than first day tested. The percent mosquito mortality of direct tested fabrics were more effective than other treated fabrics.

Control sample was also tested to evaluated % mosquito mortality of the untreated fabric which obtained no % mortality compared to treated fabrics with direct and resin cross-linking techniques.

There are 4 samples which were tested after washing with two application techniques which were direct and resin cross linking and evaluate % mosquito mortality after 24hr. After the application, (Table 4.21) the first test gave a highest percent mortality compared to other days. DT10W treated sample had 50 percent mortality, DT20W treated sample had 60 percent mortality, RCL10W treated sample had 50 percent mortality and RCL20W treated sample had 65 percent of mortality. And 9th day (5th wash) of testing after application of finish gave less results compare to 1st wash of testing which were respectively DT10W treated sample had 20 percent mortality, DT20W treated sample had 25 percent mortality, RCL10W treated sample had 20 percent mortality and RCL20W treated sample had 30 percent of mortality which up to 30 percent less result given than 1st wash of treated fabric.

The results of mosquito mortality of treated samples after wash were mentioned in table 4.21 below:

Table 4.21: Mosquitoes mortality of all samples tested after washing fastness testing

Sr. No.	Wash cycle	Sample	No. of mosquito exposed	No. of mosquito dead	% Mortality
1		Control	20	0	00
2		DT10W	20	10	50
3	1	DT20W	20	12	60
4		RCL10W	20	10	50
5		RCL20W	20	13	65
6		Control	20	0	00
7		DT10W	20	6	30
8	2	DT20W	20	9	45
9		RCL10W	20	5	25
10		RCL20W	20	10	50
11		Control	20	0	00
12		DT10W	20	5	25
13	3	DT20W	20	7	35
14		RCL10W	20	5	25
15		RCL20W	20	7	35
16		Control	20	0	00
17		DT10W	20	5	25
17	4	DT20W	20	5	25
18		RCL10W	20	4	20
19		RCL20W	20	5	25
20		Control	20	0	00
21		DT10W	20	4	20
22	5	DT20W	20	5	25
23		RCL10W	20	4	20
24		RCL20W	20	6	30

Key: DT10W - Direct technique using 10 gpl extract tested after washing fastness testing, DT20W - Direct technique using 20 gpl extract tested after washing fastness testing, RCL10W – Resin cross-linking technique using 10 gpl extract tested after washing fastness testing, RCL20W – Resin cross-linking technique using 20 gpl extract tested after washing fastness testing

Table 4.22: Mosquitoes mortality of all samples tested after light fastness testing

Sr. No.	Test day	Sample	No. of mosquito exposed	No. of mosquito dead	% Mortality
1	1	Control	20	0	00
2		DT10L	20	13	65
3		DT20L	20	15	75
4		RCL10L	20	11	55
5		RCL20L	20	15	75
6	2	Control	20	0	00
7		DT10L	20	9	45
8		DT20L	20	11	55
9		RCL10L	20	7	35
10		RCL20L	20	12	60
11	3	Control	20	0	00
12		DT10L	20	8	40
13		DT20L	20	9	45
14		RCL10L	20	6	30
15		RCL20L	20	9	45
16	4	Control	20	0	00
17		DT10L	20	6	30
17		DT20L	20	7	35
18		RCL10L	20	5	25
19		RCL20L	20	7	35
20	5	Control	20	0	00
21		DT10L	20	4	20
22		DT20L	20	7	35
23		RCL10L	20	6	30
24		RCL20L	20	6	30

Key: DT10L - Direct technique using 10 gpl extract tested after light fastness testing, DT20L - Direct technique using 20 gpl extract tested after light fastness testing, RCL10L – Resin cross-linking technique using 10 gpl extract tested after light fastness testing, RCL20L – Resin cross-linking technique using 20 gpl extract tested after light fastness testing

There are 4 samples which were tested after washing with different two application techniques which were direct and resin cross linking and evaluate percent mosquito mortality of the treated and untreated samples after 24hr. After the application, (Table 4.22) the first test gave a highest percent mosquito mortality compared to other days. DT10L treated sample had 65 percent mortality, DT20L treated sample had 75 percent mortality, RCL10L treated sample had 55 percent mortality and RCL20L treated sample had 75 percent mortality. And 9th day of testing after application of finish gave less results compare to 1st day of testing which were respectively DT10L treated sample have 20 percent mortality, DT20L treated sample have 35 percent mortality, RCL10L treated sample have 30 percent mortality and RCL20L treated sample have 30 percent of mortality which up to 35 percent less result given than 1st day of testing of treated fabric.

All treated fabrics have different % of mortality with their effectiveness and it depends on the concentration of the extract and application of the techniques. Direct treated fabric with 20 gpl using resin cross-linking technique was more effective and treated fabric after washing with 10 gpl using direct technique was less effective and low percent of mortality.

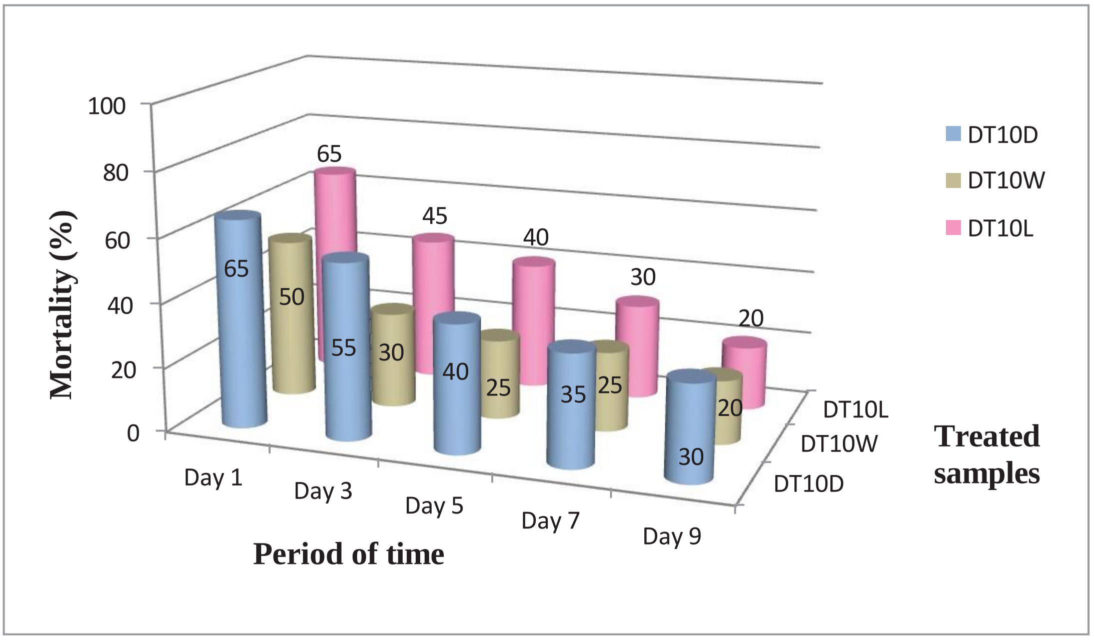
4.7.3. Evaluation of the highest mosquito repellency effect

The results were reported as a graph showing the percentage of mosquitoes that died on the treated fabric compared to the untreated fabric for each concentration and type of treated fabric. The observation helped in determining which type of treated fabric provided the highest level of protection against mosquitoes.

i) Direct technique

All the treated samples which were respectively directly tested, after washing and after light fastness testing were treated under direct technique using pad-dry-cure method of application were compared for their values of mosquito mortality and effectiveness of treated samples were kept under observation until the repellency effect diminished as per standards for repellent chemicals.

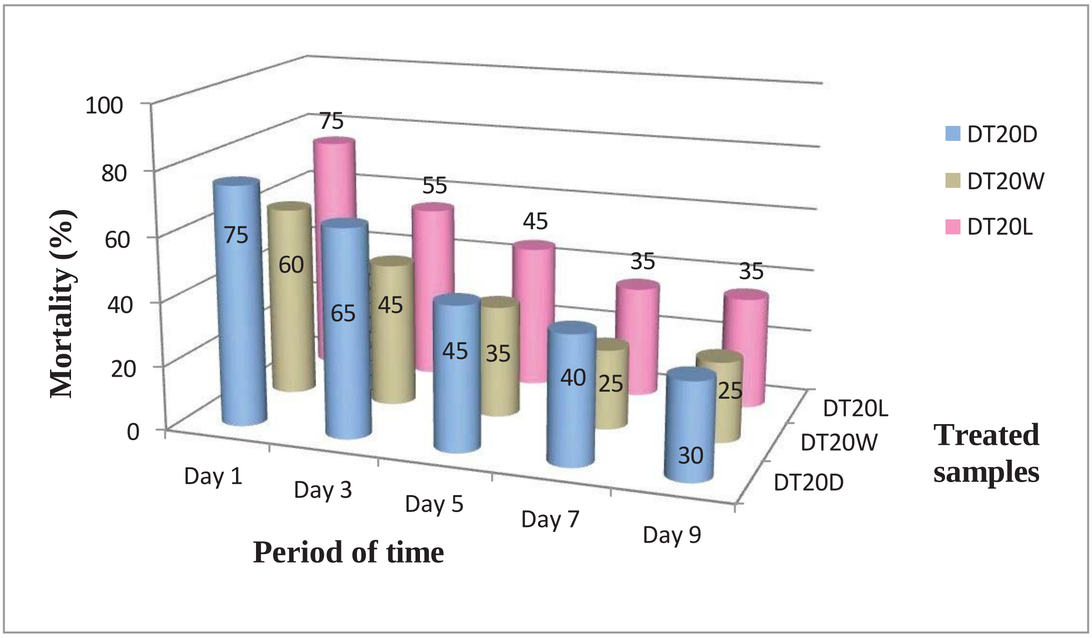
The graphs below represent the day wise results of testing of all treated fabrics and the value of the highest percentage of mortality of different types of tested fabric.



Graph 4.1: Comparison of the treated fabric using direct technique with 10 gpl extract

Key: DT10D – Direct technique using 10 gpl extract direct tested, DT10W - Direct technique using 10 gpl extract tested after wash fastness testing, DT10L - Direct technique using 10 gpl extract tested after light fastness testing

From the time the finish was applied on the fabric using direct technique until the effect of the applied finish becomes less till then all the tests of the mosquito mortality are mentioned in the graph 4.1. Three samples were tested in which one sample was direct tested, one after washing and one after light exposure. As per the graph, the highest percent of mortality was shown on 1st test in sample DT10D and DT10L had 65 percent. The lowest percent of the mortality was shown in samples after washing and light exposure. Washed sample had a poor repellency compared to other tested fabric. Here, also evaluated that mosquito mortality of the tested fabric DT10W had 50 percent of the mosquito mortality which was less effective compared to DT10D and DT10L samples. The three samples have poor repellency compared to other tested fabrics.



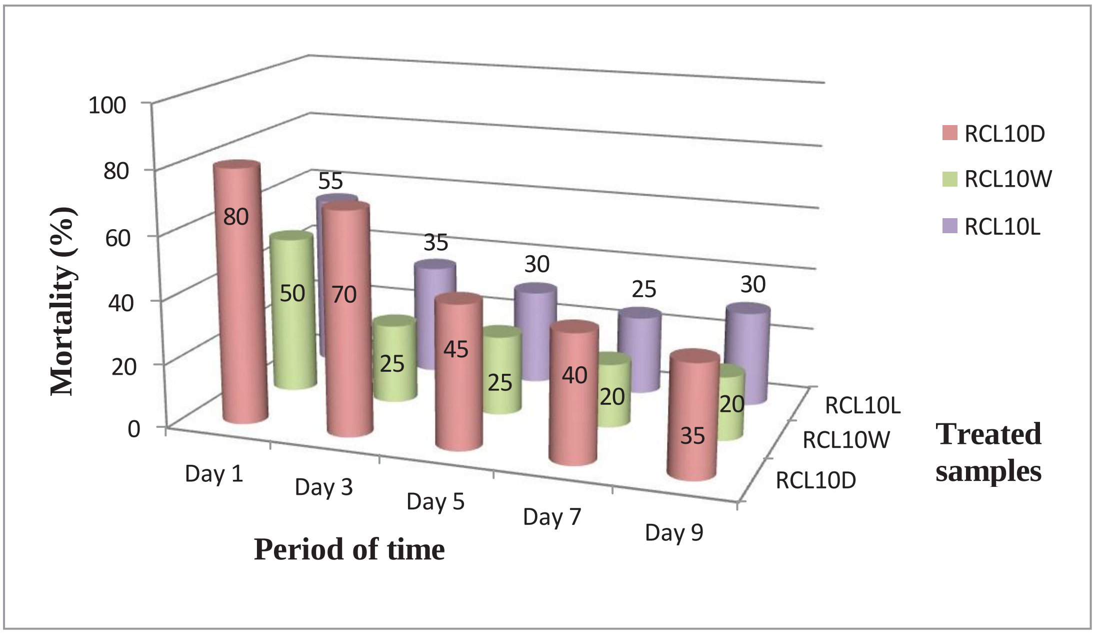
Graph 4.2: Comparison of the treated fabric using direct technique with 20 gpl extract

Key: DT20D – Direct technique using 20 gpl extract direct tested, DT20W - Direct technique using 20 gpl extract tested after wash fastness testing, DT20L - Direct technique using 20 gpl extract tested after light fastness testing

In the graph 4.2, three samples were tested in which one sample was directly tested, one after washing and one after light exposure. As per the graph, the highest percent of mortality was shown on 1st test in sample DT20D and DT20L had 75 percent of mortality. The lowest percent of the mortality was shown in samples after washing. Washed sample had a poor repellency compared to other tested fabric. Here, also evaluated that mosquito mortality of the tested fabric DT20W had 60 percent of the mosquito mortality which was less effective compared to DT20D and DT20L samples. These all three samples have average repellency compared to other tested fabrics. These three tested samples were more effective compared to the samples DT10D, DT10W and DT10L.

ii) Resin cross-linking technique

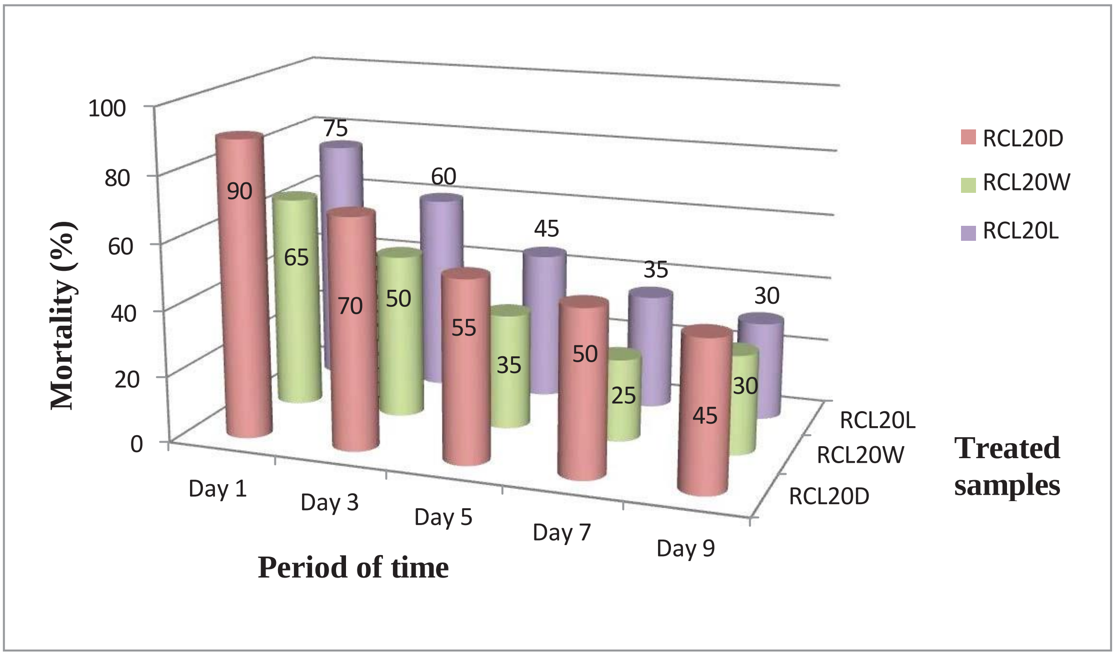
The all treated samples which were respectively directly tested, after washing and after light fastness testing were treated under resin cross-linking technique using pad-dry-cure which compared with their value of mortality and effectiveness of treated samples day by day.



Graph 4.3: Comparison of the treated fabric using resin cross-linking technique with 10 gpl extract

Key: RCL10D – Resin cross-linking technique using 10 gpl extract direct tested, RCL10W – Resin cross-linking technique using 10 gpl extract tested after wash fastness testing, RCL10L – Resin cross-linking technique using 10 gpl extract tested after light fastness testing

From the time the finish was applied on the fabric using resin cross-linking technique until the effect of the applied finish becomes less till then all the tests of the mosquito mortality are mentioned in the graph 4.3. Three samples were tested in which one sample was direct tested, one after washing and one after light exposure. As per the graph, the highest percent of mortality were shown on the 1st test in sample RCL10D. The lowest percent of the mortality was shown in samples after washing had 20 percent. Washed sample had a poor repellency compared to other tested fabric. Here, also evaluated that mosquito mortality of the tested fabric RCL10W had 50 percent of the mosquito mortality which was less effective compared to RCL10D and DT10L samples. These all three samples have average repellency compared to other tested fabrics. These treated samples were more effective compared to the fabrics treated using direct technique. RCL10D sample was more effective compared to RCL10W and RCL10L samples in every testing.



Graph 4.4: Comparison of the treated fabric using resin cross-linking technique with 20 gpl extract

Key: RCL20D – Resin cross-linking technique using 20 gpl extract direct tested , RCL20W – Resin cross-linking technique using 20 gpl extract tested after wash fastness testing, RCL20L – Resin cross-linking technique using 20 gpl extract tested after light fastness testing

Observations were taken from time the finish was applied on the fabric using resin cross-linking technique until the effect of the applied finish becomes less till then all the tests of the mosquito mortality are mentioned in the graph 4.4. It was observed that the highest mosquito mortality compared other tested fabric. Three samples were tested in which one sample was direct tested, one after washing and one after light exposure. A per the graph, the highest percent of mortality was shown on 1st test in sample RCL20D had 90 percent of mosquito mortality. The lowest percent of the mortality was shown in samples after washing and after light exposure had 30 percent. Washed sample had an average repellency after compared to other tested fabric. Here, also evaluated that mosquito mortality of the tested fabric RCL20W had 50 percent of the mosquito mortality decreased after the 3rd wash cycle. These all three samples have good to excellent repellency compared to other tested fabrics. These samples had more effective for mosquito repellency compare to all the other tested fabrics.

4.8. To evaluate wash durability of finished fabric

Assessment of light fastness and wash fastness tests are commonly performed on treated fabrics in textile finishing to evaluate the durability and effectiveness of the treatment. The results of these tests are usually reported in a standard format to provide a clear understanding of the performance of the treated fabric.

4.8.1. Assessment of durability of finish to wash

Assessing the durability of finish to wash for mosquito repellent fabric is crucial to ensure mosquito mortality and longevity of the finish. Here are the table 4.23 and 4.24 shows data for the durability of finish to wash for mosquito repellent fabric:

Table 4.23: Durability of finish after washings, n=20 (using extract -10 gpl)

Sr. No.	Application technique	Wash cycle	% mortality	Remark
1	Direct	0	65	Average repellency
2	Resin cross-linking		80	good repellency
3	Direct	1	50	Poor repellency
4	Resin cross-linking		50	Poor repellency
5	Direct	2	30	Poor repellency
6	Resin cross-linking		25	Poor repellency
7	Direct	3	25	Poor repellency
8	Resin cross-linking		25	Poor repellency
9	Direct	4	25	Poor repellency
10	Resin cross-linking		20	Poor repellency
11	Direct	5	20	Poor repellency
12	Resin cross linking		20	Poor repellency

0= treated control sample (without washing), one wash cycle - 30 minutes
n= no. of mosquitoes exposed in each cone

In table 4.23, without washing the mosquito repellency of treated samples (10 gpl extract) using direct and resin cross-linking techniques were respectively 65 percent with average repellency and 80 percent with good repellency. After the 1st wash cycle, the percent mortality of the treated fabric using 10 gpl extract decreased up to 15 percent and 30 percent with poor repellency. After 2nd wash the treated samples have less than 50 percent mortality with poor repellency property. After 5th wash (9th day), tested samples have 20 percent mosquito repellency which indicated poor effectiveness. With the increase in washing cycles the effectiveness of treated fabric decreased.

Table 4.24: Durability of finish after washings, n=20 (using extract -20 gpl)

Sr. No.	Application technique	Wash cycle	Mortality %	Remark
1	Direct	0	75	Good repellency
2	Resin cross-linking		90	Excellent repellency
3	Direct	1	60	Average repellency
4	Resin cross-linking		65	Average repellency
5	Direct	2	45	Poor repellency
6	Resin cross-linking		50	Poor repellency
7	Direct	3	35	Poor repellency
8	Resin cross-linking		35	Poor repellency
9	Direct	4	25	Poor repellency
10	Resin cross-linking		25	Poor repellency
11	Direct	5	25	Poor repellency
12	Resin cross linking		30	Poor repellency

0= treated control sample (without washing), one wash cycle - 30 minutes
n= no. of mosquitoes exposed in each cone

In table 4.24, without washing the mosquito repellency of treated samples (20 gpl extract) using direct and resin cross-linking techniques were respectively 75 percent with good repellency and 90 percent with excellent repellency. After the 1st wash cycle, the percent mosquito mortality of the treated fabric using 20 gpl extract decreased up to 15 and 25 percent with average repellency. After 3nd wash the treated samples have less than 50 percent mortality with poor repellency property. After 5th wash (9th day), tested samples have 25 to 30 percent mosquito repellency which is poor effectiveness. When the washing cycle was increased the effectiveness of treated fabric was decreased. The result showed us the treatment had poor washing fastness as its repellency decreases proportionally as the number of wash increases.

4.8.2. Assessment of durability of finish to light

Assessing the durability of finish to light for mosquito repellent fabric is essential to ensure the longevity of the finish and its efficacy. Here are the table 4.25 and 4.26 which assessed the durability of finish to light for mosquito repellent fabric:

Table 4.25: Durability of finish after light fastness, n=20 (using extract -10 gpl)

Sr. No.	Application technique	Test day	% mortality	Remark
1	Direct	0	65	Average repellency
2	Resin cross-linking		80	Very good repellency
3	Direct	1	65	Average repellency
4	Resin cross-linking		55	Average repellency
5	Direct	3	45	Poor repellency
6	Resin cross-linking		35	Poor repellency
7	Direct	5	40	Poor repellency
8	Resin cross-linking		30	Poor repellency
9	Direct	7	30	Poor repellency
10	Resin cross-linking		25	Poor repellency
11	Direct	9	20	Poor repellency
12	Resin cross linking		30	Poor repellency

0= treated control sample
n= no. of mosquitoes exposed in each cone

In table 4.25, without light fastness testing the mosquito repellency of treated samples (10 gpl extract) using direct and resin cross-linking techniques were respectively 65 percent with average repellency and 80 percent with very good repellency. After 1st light fastness testing, the percent mosquito mortality if the treated fabric using 10 gpl extract was decrease up to 25 percent with average repellency. After 3nd light fastness test, the treated samples have less than 50 percent mortality with poor repellent properties. After 5th test (9th day), tested samples have 20 to 30 percent mosquito repellency which is poor effectiveness. When light fastness testing was increased the effectiveness of treated fabric was decreased.

Table 4.26: Durability of finish after light fastness, n=20 (using extract -20 gpl)

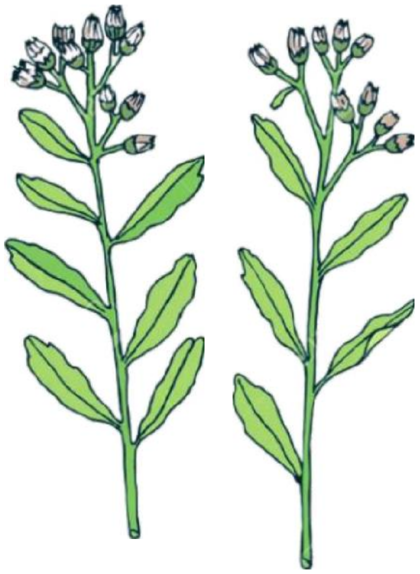
Sr. No.	Application technique	Test day	% mortality	Remark
1	Direct	0	75	Good repellency
2	Resin cross-linking		90	Excellent repellency
3	Direct	1	75	Good repellency
4	Resin cross-linking		75	Good repellency
5	Direct	3	55	Average repellency
6	Resin cross-linking		60	Average repellency
7	Direct	5	45	Poor repellency
8	Resin cross-linking		45	Poor repellency
9	Direct	7	35	Poor repellency
10	Resin cross-linking		35	Poor repellency
11	Direct	9	35	Poor repellency
12	Resin cross linking		30	Poor repellency

0= treated control sample
n= no. of mosquitoes exposed in each cone

In table 4.26, without light fastness testing the mosquito repellency of treated samples (20 gpl extract) using direct and resin cross-linking techniques were respectively 75 percent with good repellency and 90 percent with excellent repellency. After 1st light fastness testing, the percent mosquito mortality if the treated fabric using 20 gpl extract decreased up to 15 percent with average repellency. After 3nd light fastness test, the treated samples have less than 50 percent mortality with poor repellency properties. After 5th test (9th day), tested samples have 30 to 35 percent mosquito repellency which is poor effectiveness. When light fastness testing was increased the effectiveness of treated fabric was decreased. Comparatively, washing samples were less effective than light fastness tested samples. After 9 days, washing samples have 20 to 25 percent effectiveness when light fastness tested samples have 20 to 35 percent of effectiveness.

Results showed that the fabric was highly effective in repelling mosquitoes under direct tested using resin cross-linking technique with 20 gpl extract. In the direct test, the fabric repelled less compared to fabric treated by resin cross-linking technique.

After washing, the fabric gradually lost its repellent properties, which was indicated by decrease in effectiveness to repel the mosquitoes. Similarly, after light exposure, the fabric was averagely effective in repelling mosquitoes. Hence, it could be averred that the developed mosquito repellent finish can be effectively applied to home textiles to maximise protection against vector born diseases. The finish is effective for 9-10 days in its direct application method, hence can be effectively used for application to bead spreads and curtains, as it has reasonable fastness to light.



SUMMARY AND CONCLUSIONS

CHAPTER V

SUMMARY AND CONCLUSION

Pluchea wallichiana is a plant species that belongs to the Asteraceae family. It is native to the Himalayan region and can be found growing in various parts of Asia, including India, Nepal, Bhutan, and China. The plant was traditionally used for medicinal purposes in Ayurveda and other traditional medicine systems. The leaves of the plant contain several bioactive compounds, including sesquiterpene lactones, flavonoids, and phenolic acids, which have been shown to exhibit various pharmacological activities such as anti-inflammatory, analgesic, and antioxidant properties. In recent years, there has been a growing interest in the use of natural plant based extracts for their potential applications in the textile industry.

Several studies have investigated the use of *Pluchea wallichiana* leaf extracts as a natural mosquito repellent and have reported promising results. These extracts have been shown to be effective against different species of mosquitoes, including *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. The use of *Pluchea wallichiana* leaves as a natural mosquito repellent has the potential to provide a safer and more environmentally friendly alternative to conventional chemical-based repellents.

A natural mosquito repellent finish using *Pluchea wallichiana* plant extract on cotton fabric has been developed as an alternative to chemical-based repellents. The development of this finish involved the extraction of the active ingredient from the plant and its application onto the cotton fabric through a pad-dry-cure method. The effectiveness of the finish was evaluated through standard mosquito repellency tests, which showed that the treated fabric exhibited significant mosquito repellent properties compared to untreated fabric.

The present study was executed by a sample selection of woven fabric, through market survey of home textiles fabrics; cotton satin which being the most widely available and suitable material for home textile application was treated with *Pluchea wallichiana* leaf extract to explore the natural mosquito repellent property.

To achieve the purpose of the study, the specific objectives thus framed were as follows:

5.1. Objectives of the Study

5.1.1. To develop an Eco friendly natural mosquito repellent finish using plant extract.
5.1.2. To study the application of plant extract as a finish on cotton fabric.
5.1.3. To study the effect of a mosquito repellent finish on cotton fabric.
5.1.4. To evaluate wash durability of finished fabric.

5.2. Delimitation of the Study

5.2.1. The study is limited to cotton fabric.
5.2.2. The study is limited to using only one solvent (i.e. Ethanol).
5.2.3. The study is limited to one plant source.

5.3. Material and methods

The methodology was divided into two phases. Review of literature formed the Phase-I of the study. In a Phase-II experimental study was conducted to assess the effect of mosquito repellency and mortality of the treated fabric. Samples were treated using pad-dry-cure methods. Tests for the fastness to wash and light were conducted as per standard procedures. All the finished samples have been analysed according to the test needed to perform as per the standard methods. For mosquito repellent property, Cage test as well as cone test, as per the WHO standard testing method were conducted.

For the present work, an experimental approach was taken in which the variables considered were two different application techniques, condition time, concentration, temperature, pressure, humidity, percent pick-up and testing of samples treated with pluchea extract was done.

An experimental procedure was undertaken to explore the potential of *Pluchea wallichiana* fresh leaf extract applied on cotton fabric using different amounts of extract which respectively 5, 10 and 20 gpl to find out the efficacy of the mosquito repellent. Here, considering the effectiveness of the crude extract 10 and 20 gpl extract were selected for the final application on fabric. Finishing process was carried out on padding mangle using pad-dry-cure method with two different techniques, i.e. Direct and resin cross-linking technique at room temperature and pressure was 2 bars with 2 dips 2 nips. After padding, treated fabric was dried in a preheated oven at 80°C for 15 minutes and cured at 120°C for 3 minutes using the oven.

The treated samples were assessed for mosquito repellent property using WHO standard cone test. And then the evaluations of the samples were done. The fastness properties of treated samples in terms of wash fastness and light fastness were also done.

Durability of the repellency effect of the *Pluchea* extract treated sample was achieved up to five wash cycles. The samples were subjected to five washes using a launder-o-meter at room temperature. The evaluation after first to five washes was done to ascertain the repellency and percent mosquito mortality of the treated fabric.

Stability of the finish for exposure to light was carried out through light fastness test in the standard Xenon arch lamp fadometer. The samples were subjected to light using fade-o-meter as per the specified time limit in the test method (5 hours) and the evaluation was done by evaluating the repellency and percent mosquito mortality of the treated fabric.

5.4. Results and discussion

The results and discussion of the study have been discussed under the following headings:

5.4.1. Preliminary data of the fabric

The results of preliminary data of fabric revealed that the fabric selected was 100 % cotton with fabric count 195 x 122 ends per pick per inch, GSM was 116.73 gms/m^2, thickness 31.4 mm and had a satin weave.

5.4.2. Preliminary data of the *Pluchea wallichiana* extract

Pluchea wallichiana extract was a form of liquid extracted from the fresh leaf. It was obtained after subjecting the leaves of the Pluchea *wallichiana* plant to an ethanolic extraction method. It is an organic and volatile compound. Extraction was done on Soxhlet apparatus. During the time of extraction the crude extract liquid had a very dark green colour. Fresh leaves were used for ethanolic extraction.

5.4.3. Filtration of *Pluchea wallichiana* extract

The extract obtained through the Soxhlet method was further filtered and separated by a rota evaporator which helped to separate ethanol from the extract and gave a crude extract. In this procedure, total per cent of yield was 4.6 from the *Pluchea wallichiana* leaf extract which was used further to develop natural mosquito repellent finish. The extract was first subjected to phytochemical screening, to establish the presence of phytochemicals responsible for mosquito repellency.

5.4.4. Phytochemical analysis of *Pluchea wallichiana* extract

The phytochemical properties of *Pluchea wallichiana* extract have been determined. The phytochemical analysis of the extract reveals that amino acids, fixed oils and fats, alkaloids, glycosides, cardiac glycosides, phenols, tannins, flavonoids, Terpenoids, and Steroids were present. A number of studies reported that alkaloids, flavonoids, terpenoids and phenols were responsible for mosquito repellent properties.

5.4.5. Assessment of efficacy of extract using as a finish

The research proposed to assess the efficacy of the *Pluchea wallichiana* extract. Three different amounts of extract were applied to the glucose cotton pad which is 5 ml, 10 ml and 20 ml of extract respectively. It was found that among the three different amounts of extract, 20 ml extract applied on a glucose cotton pad showed the best repellency results compared to 5 and 10 ml of extract. All there gave a good to excellent repellency and 100% mortality which indicated that the crude extract had a great potential as a mosquito repellent.

5.4.6. Application of extract as a finish on cotton fabric

Treated fabrics were tested for mosquito repellency. A total of 12 samples were prepared with 10 gpl of extract of *Pluchea wallichiana* and another set of samples were 20 gpl of extract. Crude extract applied on cotton fabric using pad-dry-cure method with direct and resin cross-linking techniques. Using direct technique, the highest wet pick-up value of the treated samples was 65.47 % and the dry pick-up value of the treated sample was 75.34 %. As well as fabric treated under resin cross-linking techniques, the highest wet pick-up value of treated sample was, and dry pick-up 7.62 % of treated sample was 8.52 %.

5.4.7. Effect of a mosquito repellent finish on cotton fabric

Treated fabric was tested for 10 days with types of the testing whereas directly tested sample, treated sample after washing and treated sample after light fastness testing. The highest effect of a mosquito repellent finish was shown on the directly treated fabric and the lowest effect of a mosquito repellent finish was shown on the treated fabric after washing. Also observed that the treated fabrics with 20 gpl extract were more effective than the treated fabrics with 10 gpl extract.

5.4.8. Evaluation of the mosquito repellent properties

They were evaluating 3 types of samples that were directly tested, after washing and after light fastness. The higher mosquito repellency was shown in the direct treated samples. The fastness to laundering and light fastness test was carried out for the treated samples. The repellency for all the treated fabric on 1th day after washing was average repellency and the treated fabric after light fastness testing was good to average repellency when the direct tested fabric was excellent repellency. The repellency for all the treated fabric on 9th day after washing was poor repellency and the treated fabric after light fastness testing was average to poor repellency when the directly tested fabric was good repellency.

5.5. Conclusion

The research was aimed to develop a natural mosquito repellent finish using *Pluchea wallichiana* plant extract on cotton fabric. The present study was an attempt to scientifically investigate the development of natural mosquito repellent extracted from *Pluchea wallichiana* leaf extract.

From the present study following conclusions were derived:

1) A natural mosquito repellent finish using *Pluchea wallichiana* plant extract on cotton fabric was effective in repelling mosquitoes.
2) The fresh leaves of the Pluchea *wallichiana* plant had a strong effect of mosquito repellent property, hence the extract had excellent efficacy.
3) Efficacy of the extract was 100% because the strong effect of extracting mosquitoes was not able to feed or land on the glucose cotton pad. There was no mortality value and no knock down mosquitoes but the effect of the extract affected the mosquito to get out of the cage.
4) The best result was obtained in directly treated fabric with resin cross-linking technique using 20 gpl extract that had excellent repellency.
5) The result was obtained in treated fabric after wash using 10 gpl extract that had poor repellency.
6) It was observed that all the treated samples after washing had poor repellency compared to direct treated and light fastness testing.
7) It was also observed that all the treated samples after light fastness testing had average repellency compared to direct treated and wash fastness testing.
8) The finished sample for washing treatment, after the 3^{rd} wash they lose less than 50% of their mortality.
9) The finished sample for light fastness test, after the 4^{rd} light fastness testing they lose less than 50% of their mortality.
10) The directly treated fabric was effective for up to 7 days. After the 7th day, their mortality was also less than 50%.
11) Application of the finish using resin cross-linking technique was more effective than the direct technique.

12) Concentration of extract was increased; the effect of mosquito repellent property was also increased.

13) The finish was providing a more eco-friendly and sustainable alternative to conventional chemical-based mosquito repellents.

14) This study highlights the potential of natural plant extract as a viable option for mosquito repellent finish in the textile industry.

15) Re-application of the extract on the treated fabric had different criteria:

i) For direct treated fabric - After 9 days

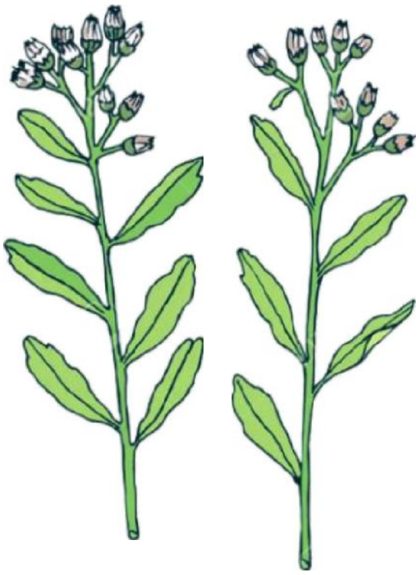
ii) For treated fabric after durability of wash - After 5th wash cycle

iii) For treated fabric after durability of light – After 6 days

RECOMMENDATIONS FOR FURTHER STUDIES:

The present study was a foundation study; exploring and experimenting with natural plant sources to develop an alternative mosquito repellent. This research can be further explored in the area of:

- Reducing the green colour of the extract, for application to very light or white fabrics.
- Exploring combinations of other plant based natural repellents.
- Developing products to extend its use as an ‘off shelf’ consumer product.
- Investigate different extraction and finishing techniques to optimize the concentration and distribution of Pluchea wallichiana plant extract on cotton fabric. This could include investigating the effects of different solvents and application methods on the repellent properties of the finished fabric.
- Conduct field trials to determine the efficacy of the finished cotton fabric in real-world situations. This could involve testing the fabric in areas with high mosquito populations and comparing the results to control groups.
- Evaluate the commercialization potential of the finished cotton fabric as a natural mosquito repellent. This could involve investigating the market demand for natural mosquito repellents, determining the cost of production, and identifying potential partners for commercialization.



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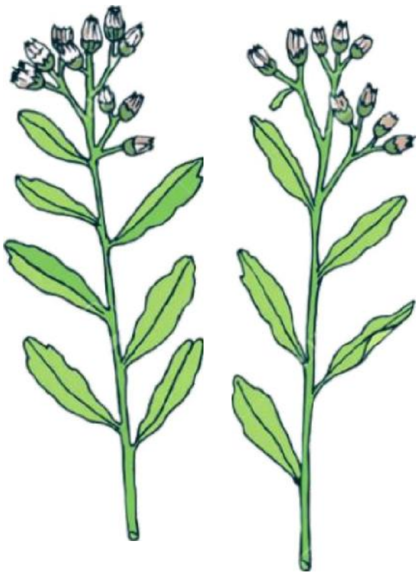

ANNEXURE

Annexure – I

Consent Form for procurement of raw material and its appropriate utilization

To, Respected Sir/ Ma'am,

This is to inform you that I Ms. Janvi Dabhi is doing M.Sc. in Clothing and Textiles. The study is being carried out by a researcher on "Development of Natural Mosquito Repellent Finish Using Pluchea Wallichiana Plant Extract on Cotton Fabric." as partial fulfilment of M.Sc. Dissertation under the guidance of Dr. Falguni Patel, Department of Clothing and Textiles.

In tropical countries mosquito threat is one of the greatest problems faced by the people in their everyday lives. Mosquitoes cause more human suffering than any other organism. Mosquitoes are attracted to carbon dioxide as well as the warmth and humidity people give off, so differences in body chemistry between people make some people more prone to getting bitten by mosquitoes than others. A variety of mosquito repellent products in the form of lotions, cream, coils, spray, liquidators are available in markets and they are limited in their use due to various reasons. But these types of synthetic chemical contained products cause health hazards to human kinds. So, to overcome these problems, mosquito repellent fabrics are used for safety purposes. With regard to textiles, the protective textile field of smart textiles has to fulfil this requirement. By using this part human beings solve many problems and mosquito repellent textile is one of them. By using this anyone can keep themselves far away from mosquitoes. Natural repellents are identified by researchers to control the Mosquitoes. Market for fragrant clothing has also been expanded and due to an increase in awareness about health and hygiene, people increasingly want their clothing to be hygienically fresh. Natural mosquito repellent finish textiles are one of the revolutionary ways to advance the textile field by providing the much-needed features of driving away mosquitoes, especially in the tropical areas in an eco-friendly way.

The present study focuses on developing an eco-friendly natural mosquito repellent finished fabric treated using the plant extracts of *Pluchea wallichiana.*

The properties of the plant will be tested and explored for the determination of their utilization in the field of technical textiles. For the same, I require consent from you for the procurement of the raw materials which includes the leaves of Pluchea Wallichiana. The procured raw material will be utilized appropriately for the extraction and testing of the extracted essential oil.

We hope that the findings of this study will contribute to the development of newer materials with unique functional properties in the field of sustainable technical textiles. Due acknowledgement will be given to you in the dissertation.

Please sign on the consent letter in the space below that you are willing to accept the due consent for the procurement of the raw material and supply us with the required quantity for the research.

Thanking you,

Yours sincerely

Guide,	Investigator,
Dr. Falguni Patel	Janvi Dabhi
patel.falguni-ct@msubaroda.ac.in	janvidabhi3446@gmail.com

Signature of the Provider

ANNEXURE – II

Department of Clothing and Textiles
Faculty of Family and Community Sciences
The Maharaja Sayajirao University of Baroda
Prof.C.C.Mehta Road, Vadodra-390002
Ph: (+91-0265)2795523 Extension 13

सत्यं शिवं सुन्दरम्
Estd. 1949
Accredited Grade "A+" by NAAC

Date: 28/03/23

To,
Dr. Rajendra Kumar Baharia
Officer In charge & Scientist C
Assistant Professor, Acedemy of Scientific
And Innovative Research (AcSIR)
ICMR-National Insitute of Malaria Research,
Field Station: Nadiad, Civil Hospital
Gujarat-387001

Subject: Requesting testing for mosquito repellency for Master's Dissertation Research by Ms. Janvi Dabhi

Respected Sir,
The student Ms. Janvi Dabhi, is pursuing a masters dissertation titled 'Development of Natural Mosquito Repellent finish for cotton fabric'. She is a regular student of the Department of Clothing and Textiles, Faculty of Family and Community Sciences.

The Department follows all norms for 'Ethics in Research' and her work has an ethical clearance passed by the Faculty Ethics Committee, please find attached here with the ethical clearance letter from our institution.

In the initial stages of her research Ms. Janvi had identified your esteemed institute as a testing facility for the product developed under her research. I hereby request you to allow her the testing of effectiveness of the fabric sample developed with natural repellent finish, in repelling mosquitoes at your research lab (Nadiad-Civil hospital). The testing results will be used for her research purpose only. Her work has reached the application and testing stage and she is due for her dissertation submission on the 15th of April 2023. Kindly allow her for testing at the earliest.

Shall be highly obliged if she gets a quick permission.

Thanking you in anticipation.

Sincere regards,

Approved by Director NIMR

Dr. Falguni Patel
Assistant Professor
Department of Clothing and Textiles,
Faculty of Family and Community Sciences,
The Maharaja Sayajirao University of Baroda

ANNEXURE - III

Department of Clothing and Textiles
Faculty of Family and Community Sciences
The Maharaja Sayajirao University of Baroda
Prof.C.C.Mehta Road Vadodara-390002
Ph: (+91-0265)2795523 Extension 13

Date:05/09/2022

To,
The Dean,
Faculty of Science,
The Maharaja Sayajirao University of Baroda,

Subject: Requesting '*Pluchea wallichiana*' leaves for Master's Dissertation research work.

Respected Sir,
The student Ms. Janvi Dabhi, is pursuing a masters dissertation titled 'Development of Natural Mosquito Repellent finish for cotton fabric'. She is a regular student of the Department of Clothing and Textiles, Faculty of Family and Community Sciences.

I hereby request you to please allow her to procure '*Pluchea wallichiana*' leaves for her research work. She will be experimenting on aqueous as well as essential oil extracts. Hence, she shall require approximately 3-5 kg leaves, distributed in two to three batches.

Thanking you in anticipation.

Sincere regards,

Dr. Falguni Patel
Assistant Professor
Department of Clothing and Textiles,
Faculty of Family and Community Sciences,
The Maharaja Sayajirao university of Baroda

Offg. Dean
Faculty of Science
The Maharaja Sayajirao University of Baroda
Vadodara - 390 002.

Through,

Prof. Anjali Karolia
I/c Head and Dean,
Department of Clothing and Textiles.

ANNEXURE - IV

Department of Clothing and Textiles
Faculty of Family and Community Sciences
The Maharaja Sayajirao University of Baroda
Prof.C.C.Mehta Road Vadodara-390002
Ph: (+91-0265)2795522

Ref No. : FFCSc/CT/

Date : 06/3/2023

To, Dr. Padamnabhi S. Nagar
Department of Botany,
Faculty of Science,
The Maharaja Sayajirao University.

REF:PERMISSION TO

Dear ~~Madam~~/Sir,

This is to request you to kindly permit Ms. Janvi Dabhi.

~~B.Sc~~./M.Sc./~~Ph.D~~ student of Clothing & Textile Department to make use of facilities of your ~~Library~~ / Laboratory / Visit to R & D Lab ./~~visit to Mill~~ etc. Kindly allow her to use 'Rota evaporator'.

Thanking You,

Yours Sincerely.
Falguni patel.
Dr. Falguni Patel.

I/c Head
Clothing & Textile Department