

Development of Herbal Mosquito Repellent Spray

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ABSTRACT: Mosquitoes control and personal protection from mosquito's bites is one of the serious way the preventing of contagious disease proliferation. In recent years interest in plant based products has been revived because of the development of resistance, cross resistance and possible toxic hazards associated with synthetic insecticides. Presently most of the mosquito repellent available in market are based on chemical and they are toxic against the skin and nervous system like rashes, swelling, eye irritation and other health problems. Most of the people still dislike the smoke of the synthetic Pyrethroid containing mosquito coils. Hence herbal mosquito repellents were preferred than chemical based mosquito repellent. Essential oil belongs to various plant species and their mixtures have been seen to act as effective repellent against mosquitoes. The easy availability and less adverse environment impact have left to the increased interest in plant based repellents which are safe and biodegradable. The essential oil used mainly were *Ocimum sanctum* (Tulsi), *Eucalyptus citriodora* (Eucalyptus), *Curcuma longa* (Turmeric), *Citrus sinensis* (Sweet orange) peels, *Syzygium aromaticum* (Clove) buds, *Azadirachta indica* (Neem) seeds, Nika, Cinchona extract. The were formulation individually and in different combination with supportive ingredients. Such as Hexane, ethanol 95%, Tween 80 etc. Results of analysis showed that formulations containing cinchona quinine extract. *Azadirachta indica* and *Eucalyptus* these essential oil have the most promising activity exhibiting 80-90% repellency as compared to other repellent essential oils. Since it has no side effect on inhalation. Some formulation evaluated for texture, efficacy, and stability etc. The present study demonstrate the potential for using essential oil from medicinal plants in mosquito repellent formulation.

KEY WORDS: Mosquitoes control, Cinchona, Quinine, different essential oils, Smoke herbal mosquito.

INTRODUCTION

Mosquitoes are among the most disturbing blood insects afflicting human beings [1]. Mosquitoes belonging to genera *Anopheles*, *Culex* and *Aedes* are act as vector for pathogens of various diseases like Dengue, Malaria, Chikungunya, Yellow fever, Japanese Encephalitis and several other infections [2]. Mosquitoes transmit diseases to over 700 million people and over one million deaths are reported annually across the world malaria is transmitted by the female *Anopheles* mosquitoes [3, 4]. *Aedes aegypti* mosquitoes spreads Dengue fever.

Mosquitoes belong to family Culicidae. Its anatomy is divided into three basic parts namely head, thorax and abdomen. Head consists of sensors that helps mosquito find and feed on the prey they have two large compound eyes covered with tiny lenses that help them detect even slight movements. Mosquito antennae are long feathery organs which are positioned on their head and they contain sensitive receptors that detect Carbon dioxide in human breath from distance more than 100 feet. The odour of omental and other chemicals released by human sweat are detected by the maxillary palp between the antennae also between the antennae a long serrated mouth part is present which is used to suck blood and which is known as proboscis.

The thorax is part which is connected to the head and part of wings. The legs are also attached to thorax. The abdomen starts from thorax and serves as the mosquito's stomach and lungs [11]. Normally, male mosquitoes do not bite human beings, female mosquitoes bite human beings for protein from blood required for the maturation of their eggs after mating.

A mosquito repellent is used to avoid diseases caused by female mosquito's usually mosquito repellent masks human scent and has a scent which mosquitoes are avoided [14,15]. Topically on skin, cloths and other surfaces controlling mosquitoes is important due to increasing in number due to deforestation, industrialization and stagnant water. When synthetic products were not discovered organic mosquito repellents were prepared from Citronella grass, Nicotine from *Nicotiana glauca* (Tobacco) leaves, alkaloids are extracted from *Anabasis aphylla* (Russian weed) etc. Later, DEET was discovered i.e. - N, N diethyl -3- toluamide. It is mostly used mosquito repellent spray till date. It is safe yet some toxic effects has been observed such as encephalopathy in children, anaphylaxis, hypotension and decreased heart rate. Several others compound have been evaluated for repellent activity, but none has had the commercial success of DEET [16]. Nowadays many mosquito repellent sprays are available in market which contains higher concentration of DEET. Effect of spray lasts for several hours even in dense area. Creams or lotions are also available which are applied topically on the skin to prevent mosquito bite [17]. Moreover, mosquito coils are available which is burnt and smoke is created. However it can be harmful for human beings who were allergic to smoke. Liquidizers are also available mechanical methods such as electric mosquito zappers which work by using UV light are also available. There are also mosquito repellent products available based on sound productions, particularly ultrasound. Since the DDT is discovered. Mosquito control is done almost completely on synthetic organic insecticides. However, the use of synthetic insecticides has increased in last five decades which has resulting in environmental hazards and also in the development of physiological resistance in major vector species [2]. Even though, mosquito repellents based on chemicals have remarkable effects but has toxic effects on human skin and may cause rashes, swelling and irritation [15]. Therefore there is need for research and development of safe, biodegradable and low cost method for mosquito control using organic ingredients [2].

DRUG PROFILE:

CINCHONA OFFICIALIS (CINCHONA)

Kingdom: Plantae

Order: Gentianales

Family: Rubiaceae

Genus: Cinchona L. 1753

Species: C. officinalis

Synonyms: Cartagena bark, Calisaya

Cinchona officinalis is a shrub or tree with rugous bark and branchlets covered in minute hairs. Stipules lanceolate or oblong, acute or obtuse, glabrous. Leaves lanceolate to elliptic or ovate, usually about 10 centimetres (3.9 in) long and 3.5-4 centimeter's (1.4-1.6 in).wide; acute, acuminate, or obtuse tip; base rounded to attenuate; coriaceous, glabrous above and often lustrous; glabrous beneath or puberulent or short- pilose, especially on the veins. Inflorescences in terminal panicles, many –flowered; hypanthium with short coarse hairs; reddish calyx, glabrous or nearly so, with triangular lobes; pink or red corolla, sericeous, the lobes ovate, acute, the corolla tube being about 1 cm long. Fruit and oblong capsule, 1.5-2 cm. long, almost glabrous. *Cinchona officinalis* is a medicinal plant, one of several cinchona species used for the production of quinine, which is anti-fever agent. It is especially useful in the prevention and treatment of malaria. Other alkaloids that are extracted from these tree include cinchonine, cinchonidine and quinidine. Cinchona is also used for increasing appetite; promoting the release of digestive juices; and treating bloating, fullness, and other stomach problems. It is also used for blood vessel disorders including hemorrhoids, varicose veins, and leg cramps. Some people use cinchona for mild influenza, swine flu, the common cold, malaria and fever. Other use are for cancer, mouth and throat diseases, enlarged spleen and muscle cramps. Cinchona is used in eye lotions to numb pain, kill germs and as an astringent. Cinchona extract is also applied to the skin of hemorrhoids, ulcers, stimulating hair growth, and managing varicose veins. In foods, cinchona is used as a bitter flavoring in tonic water and alcoholic beverages.

Quinine is the most important alkaloid obtained from cinchona bark. Its molecular formula is

C₆H₅OH→21 (C₆H₅OH) 2 MATERIAL AND METHOD:

Material: Cinchona Bark Plant

Material:

The bark of cinchona was collected from cinchona tree. The bark was isolated and conserved for extraction.

METHOD:

1. Collection of plant material.
2. Extraction of cinchona bark.
3. Evaluation of mosquito repellent activity using in-vitro cage test method.
 - Extraction of the cinchona bark by using soxhlet apparatus:
 1. The bark is the first collected and air dried, then 500gm of the bark is powered.
 2. The sample is transfer to a 33mm X 100mm cellulose thimble and placed in the extraction chamber of a 200ml capacity soxhlet apparatus.
 3. The cellulose thimble is clogged with cotton in order to avoid transfer of sample powder particles to the distillation flask.
 4. The soxhlet apparatus is fixed in the condenser and is placed 500 ml distillation flask containing 300 ml of solvent boiling glass regulator.
 5. Sample is extracted under reflux with ethanol during 3 hours (5-6 cycles/hour) and quinine is extracted.

IDENTIFICATION TEST FOR QUININE:

1. Quinine when mixed with oxygenated acids such as sulphuric acid or acetic acid produces blue fluorescence.
2. Thalleoquin test: Quinine solution + 1 ml Br₂ water + 2ml NH₄OH=Green colour
(The green colour is obtained due to formation of compound called as Thalleoquin)

EVALUATION AND COMPARISON OF HERBAL MOSQUITO REPELLENT SPRAY:

1. Smoothness, relative density. Physical Examination (colour, odour, Test)
Formulated spray was evaluated for its colour. Visually color was checked. Odour was found by smelling the product .The smoothness was tested by rubbing the spray formulation on the skin.
2. pH determination test:
Take a 50 ml of given formulation, detect the pH of this formulation by using pH of this formulation by using pH meter.
3. Spray testing:

The nature of the spray determines whether a water soluble or oil soluble dye is applied.

TESTING THE MOSQUITO REPELLENT ACTIVITY OF EACH EXTRACT AND ESSENTIAL OIL USING ARM-IN-CAVE METHOD:

The mosquitoes used in this experiment were caught using a net while biting humans between 7 pm and 10 pm. Mosquitoes were starved for 24 hours and 20 mosquitoes were placed in the cage (45 X 15 X 30 cm).

Test timing was between 7 pm and since 10 pm since the mosquitoes typically bite at night. Host seeking behavior of the mosquitoes was tested prior to the experiment. This was done by placing a pre-cleaned hand in the cage and counting the number of mosquitoes that aligned within 10 seconds. If at least 5 mosquitoes aligned on the hand, the mosquitoes inside the cage were regarded as host-seeking and the repellency experiment was continued. Volunteers for arm which had been rubbed with 1 ml of the test solution was exposed to the cage and the number of mosquitoes that aligned or biting the arm was recorded in each minute for 5 minutes. Mosquitoes were given an over one hour interval and the above procedure was followed for each of the other extracts and essential oils. Each test was repeated thrice to get a mean value of mosquito repellent activity.

PREPARATION OF TEST SOLUTION:

1. 10% (V/V %) extract or essential oil containing ethanol solutions were prepared using each plant extract and essential oil.

- 0.3 ml of each plant extract or essential oil was mixed with 3 drops of Tween 80. Then ethanol was added until the volume was 3 ml 20% (V/V%) extract or essential oil containing ethanol solutions were separately prepared using Neem extract, Nika extract and Turmeric essential oil.
- 0.6 ml of each plant extract or essential oil was mixed with 3 drops of Tween 80. Then ethanol was added until the volume was 3 ml.
- In order to prepare the control test solution, ethanol was added to 3 drops of tween 80, until the volume was 3 ml.

CONSTITUENTS AND ITS QUANTITY USED FOR FORMULATION

Constituents	Quantity
Citronella essential oil	2.5 ml
Eucalyptus essential oil	2.5 ml
Tulsi essential oil	0.75 ml
Clove bud essential oil	1.75 ml
Sweet Orange peel essential oil	1.5 ml
Turmeric essential oil	1 ml
Nika extract	3 ml
Neem extract	3 ml
Hexane	1.5 ml
Ethanol	25 ml
Tween 80	7.5 ml
Distilled water	50 ml

RESULT:

Extract and Essential oil	Texture
Cinchona extract	Brown liquid
Eucalyptus oil	Colorless liquid
Tulsi essential oil	Light yellow liquid
Turmeric essential oil	Light yellow liquid
Sweet orange essential oil	Light yellow liquid
Clove bud essential oil	Red- brown liquid
Neem essential oil	Green- brown thick liquid
Nika essential oil	Green- dark brown thick liquid
Hexane	Colorless volatile liquid
Ethanol	Colorless volatile liquid

- Formulated Herbal Mosquito Repellent Spray was evaluated visually for its colour that is yellowish brown. Odour was found by smelling the product i.e Bitter orange.
- Essential oil show higher mosquito repellent activity compared to extract. The mosquito repellent activities were found to be in the order.

Eucalyptus essential oil (100%)> **Cinchona extract (98%)**> 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%).

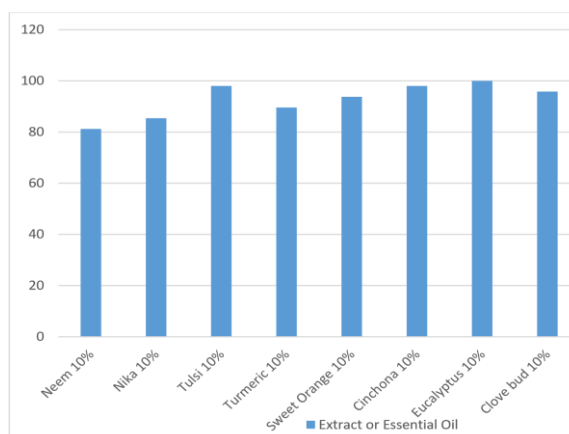


Fig: Graphical Representation of Mosquito Repellent (Percentage) of Extract and Essential oil.

CONCLUSION:

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.

Cinchona is especially useful in treatment and prevention of malaria. Eucalyptus and clove bud has characteristic odour. Tulsi essential oil has antibacterial, antiviral, and antifungal properties. Neem has medicinal properties that prove to be antifungal, antidiabetic and antibacterial.

The mosquito repellent spray which contained 10% (V/V %) total active ingredients each, showed 100% mosquito repellency for outdoor and indoor field trials which were carried out for three hours each day for two days.

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