

**FORMULATION OF HERBAL MOSQUITO REPELLENT**

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Article Received on  
15 Nov. 2018,

Revised on 05 Dec. 2018,  
Accepted on 25 Dec. 2018,

DOI: 10.20959/wjpps20191-12939

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**ABSTRACT**

Plants exhibiting mosquito repellent activity have been used as personal protection measure against parasites. An attempt has been made to prepare herbal formulation of marigold along with other medicinal plants such as tulsi, aloe-vera and vanilla essence. The uses of plant extract which is Marigold flower can reduce the uses of chemical in mosquito repellent. The present study is attempted to get the marigold claimed to be used or associated with mosquito repellent activities. Thus a research will be conduct to study on formulation of natural mosquito repellent solution using extraction process for Marigold flower. Lutein has been extracted from Marigold using at various extraction conditions such as different temperature, extraction time and volume of solvent. The summary of this attempt on claiming

and efficacy of plant based repellents are suggested for further drug development programme. These plant dry petals may represent an alternative in formulating potent and affordable products in the control of mosquitoes.

**INTRODUCTION**

Human evolution has witnessed enormous application of natural products for medicine and health has been enormous. Since our earliest ancestors chewed on certain herbs to relieve pain, or wrapped leaves around wounds to improve healing, natural products have often been the sole means to treat diseases and injuries. During the past decades natural products have taken a secondary role in drug discovery and drug development, after the advent of molecular biology and combinatorial chemistry made possible the rational design of chemical compounds to target specific molecules. The past few years, however, have seen a renewed

interest in the use of natural compounds and, more importantly, their role as a basis for drug development. The modern tools of scientists to detail the exact nature of the biological effects of natural compounds on the human body, as well as to uncover possible synergy, which hold much promise for the development of new therapies against many devastating diseases, including dementia and cancer.

There has been a compelling curiosity to discover what compounds nature provides, but to obtain this information it is necessary to isolate compounds from their natural source and to determine their structures. This is seldom a difficult task, especially when the compound of interest is present at low concentrations such that enormous quantities of source material are required to extract even a few micrograms of the desired product. In this circumstance a high degree of skill and technology is required in both the isolation procedures and the subsequent investigations to establish the chemical structure. A second objective is the total synthesis of the compound from smaller molecules. Indeed, in the classical approach to structure determination, the assigned structure was not regarded as fully confirmed until the compound was synthesized and shown to be identical in all respects with the natural compound. This approach persists, although the enormous impact of modern methods of separation, spectroscopic and chromatographic analysis has made it possible to determine structure. The synthesis of natural products continues to be important.

N,N-Diethyl-meta-toluamide, also called DEET or diethyltoluamide, is the most common active ingredient in insect repellents. It is a slightly yellow oil intended to be applied to the skin or to clothing and provides protection against mosquitoes, ticks, fleas, chiggers, leeches and many biting insects.

DEET was developed in 1944 by Samuel Gertler of the United States Department of Agriculture for use by the United States Army, following its experience of jungle warfare during World War II. It was originally tested as a pesticide on farm fields, and entered military use in 1946 and civilian use in 1957.

The application solution for DEET was composed of 75% DEET and ethanol. DEET was historically believed to work by blocking insect olfactory receptors for 1-octen-3-ol, a volatile substance that is contained in human sweat and breath. The prevailing theory was that DEET effectively "blinds" the insect's senses so that the biting/feeding instinct is not triggered by humans or other animals which produce these chemicals. DEET does not appear to affect the insect's ability to smell carbon dioxide, as had been suspected earlier.

DEET is a synthetic compound which is tested and proven safe for the use on the skin, but in some cases like high concentration of the chemical, weakened Immunity of the person, allergy, children and infants it may cause problems. Side effects of DEET includes seizures, rashes, skin irritation, nausea, dizziness, slurred speech, numbness, headache and other serious side effects. The use of DEET has been restricted for children, pregnant women, and people with sensitive skin and so on to use it. The uses of plant extract can reduce the uses of chemical in mosquito repellent. To extract the plant to be uses in repellent, there are many ways to produce lutein from Marigold flowers and these types of extraction method have their own disadvantages due to long extraction time and poor stability of free lutein.

The current project was undertaken with the objective of developing aherbal formulation for application as mosquito repellent.

A mosquito repellent is a substance applied to skin, clothing, or other surfaces which discourages mosquitoes from landing on that surface. A mosquito is a very harmful insect which are carries disease causing viruses and parasites from person to person without catching the disease themselves. Mosquitoes are estimated to transmit disease to more than 700 million people annually in many countries. Due to this problem, many product of mosquito repellent are produced to use for personal protection and it is quite popular among citizens in many countries.

## **DESCRIPTION OF COMPONENT USED IN MOSQUITO REPELLENT**

### **1. Lutein extract**

#### **Description**

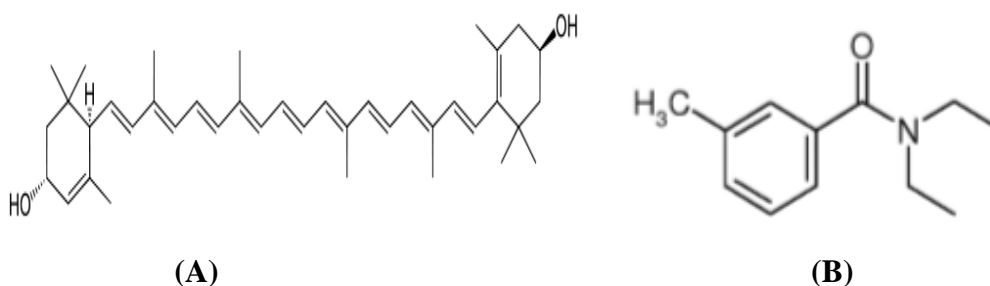
It flowers from July to September. The scented flowers are hermaphrodite (have both male and female organs). It is hardy to zone 6 and is frost tender. The lower leaves are broad and spatula shaped. Upper leaves may be oblong, are smooth at the edges, and are arranged alternately along the stem. Seeds are crescent to horseshoe shaped with the rough exterior. Its branching stem grows to the height of 30- 60 cm.

#### **Location**

Marigold is cultivated throughout India. It is widely grown in the Valley of Flowers, Ranthambore National Park in India.

### Medicinal uses

Marigold is used for stomach upset, ulcers, menstrual period problems, eye infections, inflammations, and for wound healing. It is antiseptic. If the Marigold flower is rubbed on the affected part, it brings relief in pain and swelling caused by a wasp or bee. A lotion made from the flowers is most useful for sprains and wounds and a water distilled from them is good for the sore eyes. The infusion of the freshly gathered flowers is beneficial in fever. Marigold flowers are mostly in demand for children ailments. Externally it is used in the treatment of alopecia. Internally it is used to treat bladder and kidney problems, blood in the urine, uterine bleeding and many more.



**Figure 1: Structures of A) lutein and B) DEET.**

### 2. Tulsi extract

Commonly known as tulsi, is an aromatic perennial plant in the family Lamiaceae. It is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. Tulsi is cultivated for religious and supposed traditional medicine purposes, and for its essential oil. It is widely used as an herbal tea, commonly used in Ayurveda. It has medicinal activities, including anti-stress, anti-lipidemic, antidiabetic and glycemic lowering properties.

### 3. Vanillin extract

Vanilla is a flavoring derived from orchids of the genus *Vanilla*, primarily from the Mexican species, flat-leaved vanilla (*V. planifolia*). Vanillin which has one less carbon and two less hydrogen atoms than ethyl vanillin, is 3-4 times less strong in its vanilla aroma. Vanillin is used as flavoring and fragrance.

### 4. Aloe Vera gel

The plant has triangular, fleshy leaves with serrated edges, yellow tubular flowers and fruits that contain numerous seeds. It shows healing properties, protection against UV and gamma radiation on skin, anti-inflammatory action, antiviral and antitumor activity, etc..

## MATERIAL AND METHODS

### 1. Lutein extract

- a. Dried petals of marigold which was grinded placed in amber coloured bottle with n – hexane.
- b. Kept in dark for approximately 2 days.
- c. Filtered extract of marigold using Whatmann filter paper no 41.
- d. Using vacuum rotary evaporator at 42°C solvent was evaporated.
- e. The obtained residue was sonicated to degas. (residue : oleoresin- dark reddish colour)
- f. Acetone added to residue to get solid precipitate.
- g. Filtered using Whatmann filter paper no 41 and residue was scrapped in petri dish. (solid : lutein, stored at 4°C)

### 2. Tulsi extract

- a. Dried leaves of tulsi which was grinded placed in amber colored bottle with ethanol.
- b. Kept in dark for approximately 2 days.
- c. Filtered using Whatmann filter paper no 41.
- d. Using vacuum rotary evaporator at 75°C solvent is evaporated.
- e. The obtained residue is sonicate to degas. (residue : tulsi extract dark green colour)
- f. Residue was kept in petri dish and stored at 4°C.

### 3. Vanillin extract

- a. Liquid-liquid extraction was done of vanilla extract using chloroform.(50ml / 150ml)
- b. Vanillin get separated in lower organic layer.
- c. This organic layer then transferred to round bottom flask for vacuum rotary evaporator at 65 °C.
- d. Stored in 10ml standard volumetric flask at 4°C.
- e. The TLC analysis of extracted vanillin is done using standard.
  - a) (mobile phase – 9:1 n-hexane : ethyl acetate
  - b) solvent to dissolve standard – chloroform
  - c) developing agent – heptanone : ethanol : sulfuric acid 4:5:1).

### 4. Aloe Vera gel

- a. The skin of leaf was peeled and the gel inside is scrapped out.
- b. The gel then grinded and excess water was removed to get smooth paste.

c. Stored in conical flask at 4°C for 24 hours.

### FORMULATION OF MOSQUITO REPELLENT

Usually the repellent exists in the form of lotions, aerosol spray, or cream, which displays the warning labels especially for the children. Such formulations are normally applied to the skin of humans to provide repellency for last a few hours. The compositions in the repellent should not cause any side effect or allergy to the human. Natural mosquito repellents consist of a combination of numerous ingredients that keep mosquitoes away. The formulations of repellent should be long lasting product and lesser of toxicity than chemical repellent.

#### Formulation concentration

Formulations were prepared in 2 ml eppendorf and each active ingredient was added according to the concentration using micropipette. As mentioned 5 different concentration was prepared namely A, B, C, D, E. This 5 different concentration was considered to be one batch. By repeating above procedure, 10 batches of 5 different concentration were prepared.

Extract Sample code	Lutein (ml)	Tulsi (ml)	Vanillin (ml)	Aloe-Vera (ml)
A	0.2	0.1	0.1	0.1
B	0.3	0.1	0.1	0.1
C	0.4	0.1	0.1	0.1
D	0.5	0.1	0.1	0.1
E	0.6	0.1	0.1	0.1

### DISCUSSION

Marigolds also have a distinct smell that repels mosquitoes. Most of the medicinal use of marigold are in its flower. Lutein ( $C_{40}H_{56}O_2$ ) is the major pigment present in the marigold flower. About 95% of lutein present in the flowers is in the form of esters out of which lutein palmitate is the major pigment (Gau et al. 1983). Dimyristate, myristate, palmitate, stearate and distearate are the other esters of lutein present in marigold flowers. Lutein ester concentrations in fresh marigold flowers vary from 4.0 mg/g in greenish yellow flowers to 800 mg/g in orange brown flower. Lutein was found to be present in a concentrated area of the macula, a small area of the retina responsible for central vision. The hypothesis for the natural concentration is that lutein helps protect from oxidative stress and high-energy light. Several studies show that an increase in macula pigmentation decreases the risk for eye diseases such as Age-related Macular Degeneration (AMD). Lutein (LOO-teen) is an oxygenated carotenoid found in vegetables and fruits. Lutein is found in the macula of the

eye, where it is believed to act as a yellow filter. Lutein acts as an antioxidant, protecting cells against the damaging effects of free radicals.

### **Vanillin as Stabilizer**

Vanillin is extracted from the vanilla pods. Vanillin shows good stabilizing property. It sometimes acts as catalyst. It also inhibits the reaction between two chemicals.

### **Stabilizer**

In chemistry a stabilizer is a chemical which tends to inhibit the reaction between two or more other chemicals. A stabilizer is a chemical that is used to prevent degradation.

### **Role of Stabilizer**

- a. The primary purposes of using stabilizers in any product is to produce smoothness in body and texture of the product.
- b. Stabilizer reduce the degradation rate of the product.
- c. It also prevents the degradation and spoilage of product.
- d. It increases of shelf life of the product.

### **Aloe vera as gel**

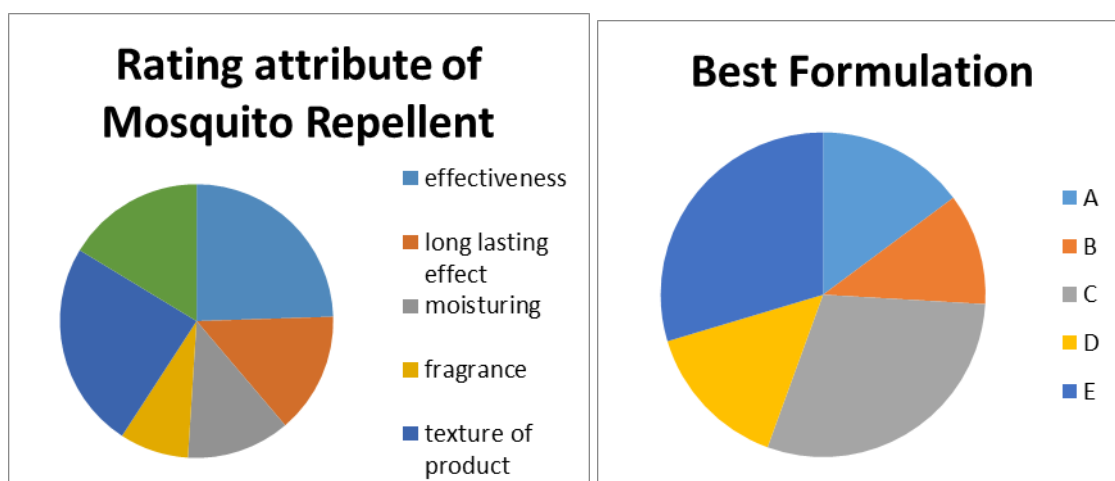
It gives smooth texture and consistency to the product and also particular galenical presentations.

## **RESULTS**

1. The lutein extract was in form of orange colour solid and from 1 g of marigold petals approximately 500 mg of lutein was obtained. The lutein extract shows almost similar property as of DEET and can be used as repellent. At the same time can lower the cost of mosquito repellent.
2. The tulsi was in form of dark green colour mist and from 1/2 g of tulsi leaves approximately 300 mg of mist was obtained. Tulsi enhances the antibacterial activity of product.
3. Vanillin was in form of colourless liquid and from 30ml of vanilla extract approximately 5ml of vanillin was obtained. From TLC analysis, extracted vanillin was similar to the standard. Vanillin gave pleasant and sweet smell to the product. Vanillin act as stabilizer. Vanillin extends the period of effectiveness in repellent thus resulting in an extension of the period effectiveness with relatively small quantities of actual active ingredient

(repellent), thus creating an effective and at the same time tolerable as mosquito repellent agent.

- Aloe vera gel was in form of homogenized gel (smooth paste). Gel act as base. Aloe vera gel help in moisturizing and gave smooth texture and also make available formulations that are pleasant and harmless to the skin, or particular galenical presentations can be offered, for example skin sprays, lotions, creams, or sticks.
- From the questionnaire, the natural compounds used in product were harmless. The set E was best formulation having maximum efficacy and when the lutein concentration was maximum in product the effectiveness was more were observed. This natural product can be used it is much safer.



- The concentration of the tulsi extract and vanillin extract was maintained at constant volume as tulsi have deterring smell and vanillin may be harmful to skin in more concentration.
- Essential oils are not used as they last long only for 2 hours.

## CONCLUSION

- Natural mosquito repellent of various formulation was developed using lutein extract from marigold, tulsi extract, vanillin essence and aloe vera gel. Lutein extract which is extracted from marigold petals act almost similar as the DEET. Tulsi is used as it has anti-microbial activity with its medicinal properties. Vanillin is used as stabilizer which increase the shelf- life of product. The smell of the vanillin also provides calmness to the body. Aloe vera also has many medicinal properties which is harmless when applied on skin and it is used as base of our product. This active ingredient which are used are natural and known to be harmless, it only consist some ethanol to increase the



effectiveness where as in other product DEET is used which harmful can cause many problems. This natural mosquito repellent can be used by the children as it is much safer as plant extract are base of mosquito repellent. This repellent is non-toxic and very safe for every age group. Thus, we conclude that lutein extract from marigold petals have a strong mosquito repellent activity and can be used in the natural mosquito repellent.

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