

A REVIEW ON MOSQUITO REPELLENT ACTIVITIES OF THUSI

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ABSTRACT

Mosquitoes are most important and abundant pest in urban, sub-urban and rural environment. Although, chemical control provides quick mortality, resistance of mosquito against the use of insecticide have been widely reported. Moreover, chemical mosquito repellent contains toxic synthetic pyrethroids as active ingredients whose exposure to food and water is hazardous to health. In this study an attempt has been made to develop an eco-friendly mosquito repellent sprayed with essential oil of Tulsi. This formulation is safe, eco-friendly, cheap, easy to use and has maximum repellence against mosquitoes. In addition, this, the mosquito repellent is less harmful to our health than the ones available in the market.

KEYWORDS: Mosquito repellent activity, Tulsi, Eugenol, *Aedes aegypti*.**INTRODUCTION**

The use of natural product as medicinal agents presumably predates the most basic recorded history. *Ocimum sanctum* L. is a plant which is used in several traditional medicinal systems to cure various diseases. The plant has been known possess antimicrobial activity, anti-anaphylactic activity, antidiabetic effect, antioxidant activity, anti-cancer property, wound healing effect, radio-protective effect, larvicidal property, anti-genotoxic effect, immunological effect and other miscellaneous activity.^[1]

Tulsi (Holi Basil)

Modern research has classified Tulsi as an adaptogenic herb. Adaptogens have been shown to support the body's healthy reactions to stress. Adaptogenic herb have been used in Ayurvedic tradition for thousands of years to promote and maintain wellness.^[1]

**Plant Profile**

Among the plant known for medicinal value, the plant of genus *Ocimum* belong to family *Labiatae* are very important for their therapeutic potential. *Ocimum sanctum* L. (*Labiatae*) is a strongly scented small annual herb, up to 18 inches tall, grows into a low bush and is commonly known as holi basil.^[2]

Scientific Classification

- Kingdom: Plantae
- (Unranked) Angiosperms
- (Unranked) Eudicots
- (Unranked) Asterids
- Order: Lamiales
- Family: *Lamiaceae*
- Genus: *Ocimum*
- Species: *O. tenuiflorum*
- Binomial name: *Ocimum tenuiflorum* or *Ocimum sanctum* L.^[3]

Characteristics of The Plant

Sacred basil or Holy basil, *Ocimum sanctum* Linn is a biennial or triennial shrub. The leaves of this plant on steam distillation yield a bright yellow colour volatile oil possessing a pleasant odour with an appreciable note of clove oil. The plant contains mainly phenols, aldehydes, tannins, saponin and fats. The essential oil major components are eugenol (71%), eugenol methyl ether (20%), carvacrol (3%) etc. The leaves are used as condiment in salads and other foods. *O. sanctum* is an erect, herbaceous, much-branched, softly hairy biennial or triennial, which grows to a height of 30-75 cm. Leaves

are entire, serrate, pubescent on both sides, flowers purplish or crimson, in racemes, fruits are sub-globose or broadly ellipsoid, slightly compressed, nearly smooth, pale brown or reddish with small black markings.^[4]

Cultivation Methods

• Soil Condition

Sacred basil thrives well on a wide range of soils. Rich loam, poor volcanic, saline and alkaline to moderately acidic soils are also well suited for its cultivation. Well drained soil helps in better vegetative growth. Water logged conditions can cause root-rot and results in stunted growth.

• Climate

It flourishes well under fairly high rainfall and humid conditions. Long days and high temperatures have been found favourable for plant growth and oil production. It can grow up to an altitude of 900 m. The plant is moderately tolerant to drought and frost. The plant can be grown under partially shaded conditions but with low oil contents.

• Propagation

Tulsi is propagated through seeds. Seeds will get deteriorated over generations, due to its high cross-pollination. Hence, for fresh plantings, the growers have to take fresh seeds from the pedigree stock.

• Planting Time

The nursery can be raised in the third week of February and transplanting is generally done in the middle of April.

• Harvesting

The crop is to be harvested at full bloom stage to obtain maximum essential oil yield and better quality oil. The first harvest is obtained at 90-95 days of planting. Thereafter, it may be harvested at every 65-75 days' interval. Harvesting should be done usually on bright sunny days for high and good quality oil. It is not desirable to harvest the crop if there was a rain in the previous day. The crop should be cut at 15-20 cm above the ground level.

• Processing

The harvested produce may be allowed to wilt in the field itself for 4-5 hours so as to reduce the moisture and also the bulkiness. However, oil quality and its yield do not diminish up to 6-8 hours after harvest, but further delay may cause considerable loss in yield and quality of oil. Steam distillation is found to be superior to hydro distillation and hydro cum steam distillation. Distillation unit should be clean, rust free and free of any other odour. The oil obtained is then decanted and filtered. The distilled oil is treated with anhydrous sodium sulphate or common salt at the rate of 20 g per litre to remove the moisture. The oil should be stored in sealed amber coloured glass bottles or containers made of stainless steel, galvanised tanks, aluminium containers and stored

in a cool and dry place. All processing activities should be recorded.^[5]

• Expected Yield

About 8 to 10 tons of fresh herbage per acre can be obtained by two to three harvests in a year. The oil yield varies with type, season and place of origin. Oil recovery ranging from 0.3–0.4%. Expenses is around Rs. 6,000/acre. Present market rate ranging from Rs.600 to Rs.800.

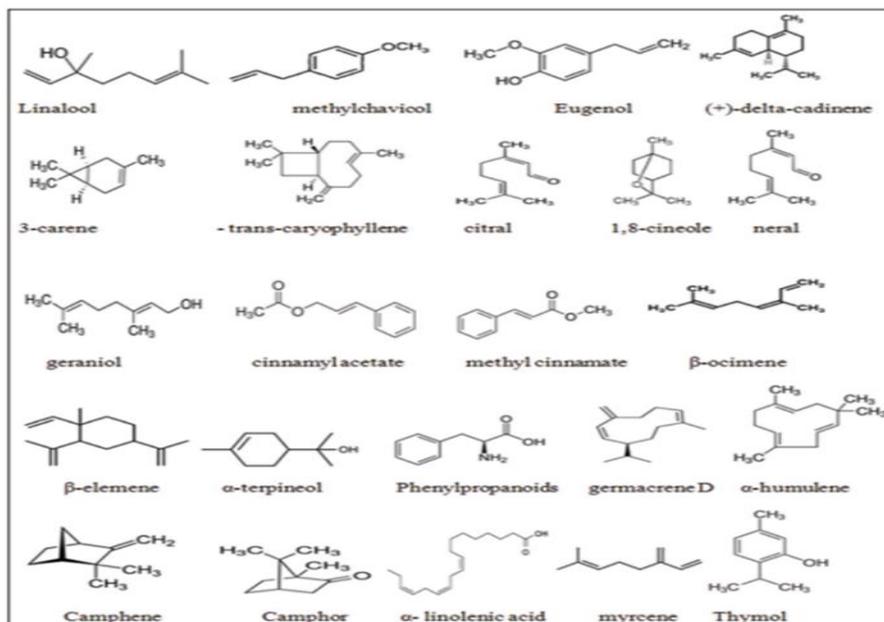
Nutritional Content

Ocimum sanctum L. contain vitamin C, A and minerals like calcium, zinc and iron as well as chlorophyll and many other phytonutrients. It enhances efficient digestion, absorption and use of nutrients from food and other herb. This plant contain protein: 4.2g, fat: 0.5g, carbohydrate: 2.3g, calcium: 25mg, phosphorus: 287 mg, iron: 15.1mg and edible portion 25mg; Vitamin C per 100 g.^[6]

Phytochemical Constituents

The chemical composition of *Ocimum sanctum* is highly complex, containing many nutrients and other biologically active compounds, the proportion of which may vary considerably between varieties and even among plants within the same field. Furthermore, the quantity of many of these constituents are significantly affected by differing growing, harvesting, processing and storage conditions that are not yet well understood.^[3]

The nutritional and pharmacological property of the whole herb in its natural form, as it has been traditionally used, results from synergistic interaction of many different active phytochemicals. Its leaf contains volatile oil eugenol, euginal (also called eugenic acid), urosolic acid, carvacrol, linalool, limatrol, caryophyllene, methyl carvicol (also called Estragol) while the seed volatile oil has fatty acid and sitosterol; in addition, seed mucilage contains some levels of sugars and anthocyanins are present in green leaves. The sugars are composed of xylose and polysaccharides.^[6]



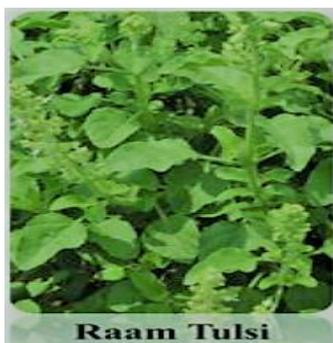
Different Varieties

Each variety has a slightly different look, taste and smell and when combined, create a perfectly balanced herbal infusion an array of health benefits. There are three varieties;^[7]

- Rama tulsi (*Ocimum sanctum*)
- Vana tulsi (*Ocimum gratissimum*)
- Krishna tulsi (*Ocimum sanctum*)

Ram Tulsi Type

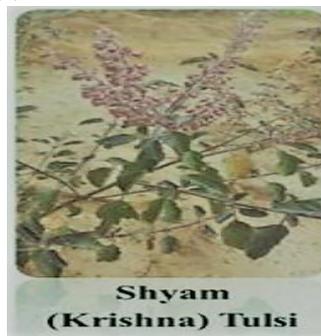
The most commonly occurring type is the green leafed tulsi (holi basil) known as Ram tulsi (*Ocimum tenuiflorum* or *Ocimum sanctum*). Ram tulsi type grows widely across China, Asia, India, Eastern Nepal and Brazil. The leaves, twigs, seed and even the root are aromatic with a characteristic fragrance. When crushed, the tulsi leaves release an aromatic oil. The plant yields oleanolic acid, eugenol, rosamarinic acid, ursolic acid, linalool, beta caryophyllen, beta elements and germacrene.



Shyam Tulsi Type

This is a variant of green leaf tulsi. Shyam or Krishna tulsi (holy basil) is characterized by purple fringed leaves and purple stems. The taste is rather pungent and strong.

Shyam tulsi type is not quite as widespread as the green leaf Ram tulsi.



Since it has higher amount of pungent essential oils, it proves more effective in treating coughs and colds, skin diseases, ear problems and problems of the respiratory tract.

VANA TULSI TYPE

Vana tulsi type grows wild, mainly in the Himalayan regions and the plains of India, Vana tulsi type (*ocimum gratissum*) is also cultivated in parts of country. Vana tulsi type include Maruvak tulsi and Babul tulsi, the chief characteristic of this type of tulsi is that it has the highest amount of eugenol.

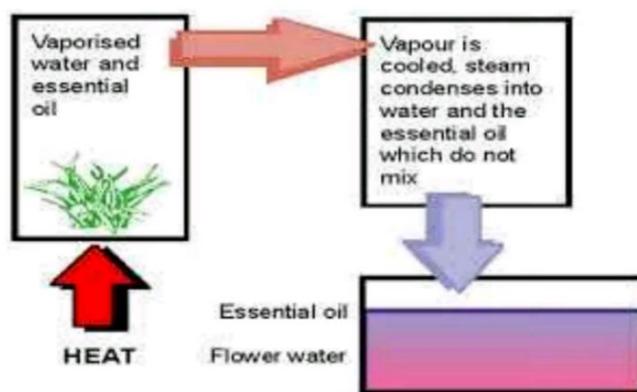


Vana tulsi due to its high amount of eugenol is effective in treating leukoderma and in removing poison from the body. Vana tulsi type is also effective in treating eye ailments. The Maruvak variety is used to treat bites and wounds, The Babul variety is used as a flavouring agent since its taste resembles that of clove. Vana tulsi type helps relax intestinal muscles and can be used to treat cramps and diarrhea. Vana tulsi type help to reduce blood glucose level in diabetics. Vana tulsi type also help to treat convulsion.

Apart from benefits when taken tulsi internally, all the above type also has beneficial effect on skin and hair. Tulsi leaf paste is applied to the hair helps control dandruff and fungal infection.^[8]

The Extraction of Essential Oil

The extraction of essential oil by steam distillation of *Ocimum basilicum* is a solid-liquid extraction process.^[9]



The process complete in

1. Collect the plant material and dry if it required
2. Fill this plant material in distillation vessel.
3. Add water or solvent (like ethanol, n-hexane) in distillation vessel.
4. Heat the mixture in well-equipped distillation vessel in control environment and controlled temperature, then
5. Diffusion of essential oil from inside the solid material of plant to its surface occurs, then
6. Transfer of mass from surface of plant solid material to surrounding liquid occurs, then
7. This liquid contains essential oil which is obtained by reverse cooler
8. Then the collection of above liquid obtained from reverse cooler is put to stand for some time then essential oil comes over water, then
9. We perform layer separation to separate out essential oil from water, then
10. Store this essential oil in dark, closed vessel for future use.^[8]

Essential Oil Steam Distiller

The experimental results prove that yield of essential oil from Tulsi (*Ocimum basilicum*) from only leaves of plant is slightly higher than from the mixture of leaves and stems (means plant crush).^[8,9]



The Science Behind Sacredness of Tulsi^[10]

- Mosquito-repellent/larvicidal properties
- Antimicrobial properties
- Hepatoprotective properties
- Anti-inflammatory properties
- Immunomodulatory effect
- Anti-carcinogenic properties
- Neuro-protective and cardio-protective properties
- Wound healing effect
- Radio-protective
- Antigenotoxic effect

Mosquito-Repellent Property



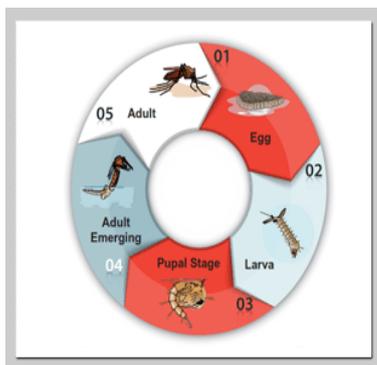
In search of safe natural products to repel/kill mosquitoes and plant pest's studies have been conducted on various plants including Tulsi. Mosquitocidal activity of Tulsi was investigated using its eugenol and triglycerid_e

(isolated from Tulsi's hexane extract) on fourth instars *Aedes aegypti* larvae.^[11] When seeds of Tulsi was placed in water, it exudes within one hour, a mucilaginous substance (polysaccharides) and larvae which came in contact with seeds became firmly attached to it and died due to drowning of larvae. When 100 larvae of *Culex fatigans* was spread over water containing 25, 50 and 75 Tulsi seeds/m² surface area of water for 48 h, 100% mortality was observed in 75 seeds/m² of water while 65% and 89% mortality observed in 25 and 50 seeds/m² of water respectively. Mosquitocidal efficacy of essential oil of *Ocimum sanctum* against adult mosquitoes of different species viz. *Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus* were investigated and 100% mortality observed in *A. stephensi*, *A. aegypti* at a dosage of 0.003 ml/43.0 cm². However, mortality of *C. quinquefasciatus* was observed at a higher dose (0.01 ml/43.0 cm²). Essential oil of Tulsi showed larvicidal efficacy against larvae of *A. stephensi*, *A. aegypti*, *C. quinquefasciatus*. The mosquito-larvicidal property of both leaf and flower extract of *Ocimum sanctum* against 4th instar larvae of *Aedes aegypti* and *Culex quinquefasciatus*. Compared to flower extract, leaf extracts were found to be more effective against both types of mosquitoes. In search of plant based insecticides, the anti-feedant and larvicidal properties of four plants including *Ocimum sanctum* against gram pod borer *Helicoverpa armigera*, cotton leaf roller *Sylepta derogata*, and mosquito *Anopheles stephensi* have been studied. Organic solvent extract of *Ocimum sanctum* were able to kill the larvae of tested pests and vector.^[12]

Larvicidal Activity

A larvicide (alternatively larvacide) is an insecticide that is specifically targeted against the larval life stage of an insect. Their most common use is against mosquitoes. Larvicides may be contact poisons, stomach poisons, growth regulators, or (increasingly) biological control agents.^[13]

The definition of larvicidal in the dictionary is of or relating to a chemical used for killing larvae.



Life Cycle of Mosquito

The Mosquito has 4 stages in its life cycle: Egg, Larva, Pupa and Adult.^[14]

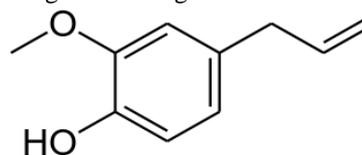
- The eggs of the mosquito are laid at the water surface.
- The name of the animal in the larva stage is the Wiggler. This is because it moves about by wriggling.
- The Larva lives in the water and breathes through a breathing tube at the bottom of its abdomen.
- The pupa stage is also known as the Tumbler because it tumbles about in the water. The Pupa stays close to the surface of the water to breathe with breathing tubes.
- During the Larva stage, the wriggler moults several times, getting larger each time.
- When it is ready to undergo metamorphosis, it moults one more time and turns into a Pupa.
- When it emerges as an adult mosquito, the male mosquito feeds on nectar of flowers and the female feeds on blood

Eugenol(1-hydroxy-2-methoxy-4-allylbenzene) the active constituent present in *ocimum scantum* L was found to be largely responsible for the therapeutic potential for tulsi. Mosquitocidal activity of Tulsi was investigated using its eugenol and triglyceride on fourth instars *Aedes aegypti* larvae. Eugenol kills the larve

Eugenol

Molecular Formula: C₁₀H₁₂O₂^[21]

Molecular Weight: 164.20 g/mol



Physical and Chemical Properties^[15]

Apparance: Clear colourless pale yellow or ambour coloured liquid.

Odour: Spicy pungent taste

Solubility: Insoluble in water

Boiling point: 255

Vapour pressure: 0.01

Density: 1.0652g/cm³

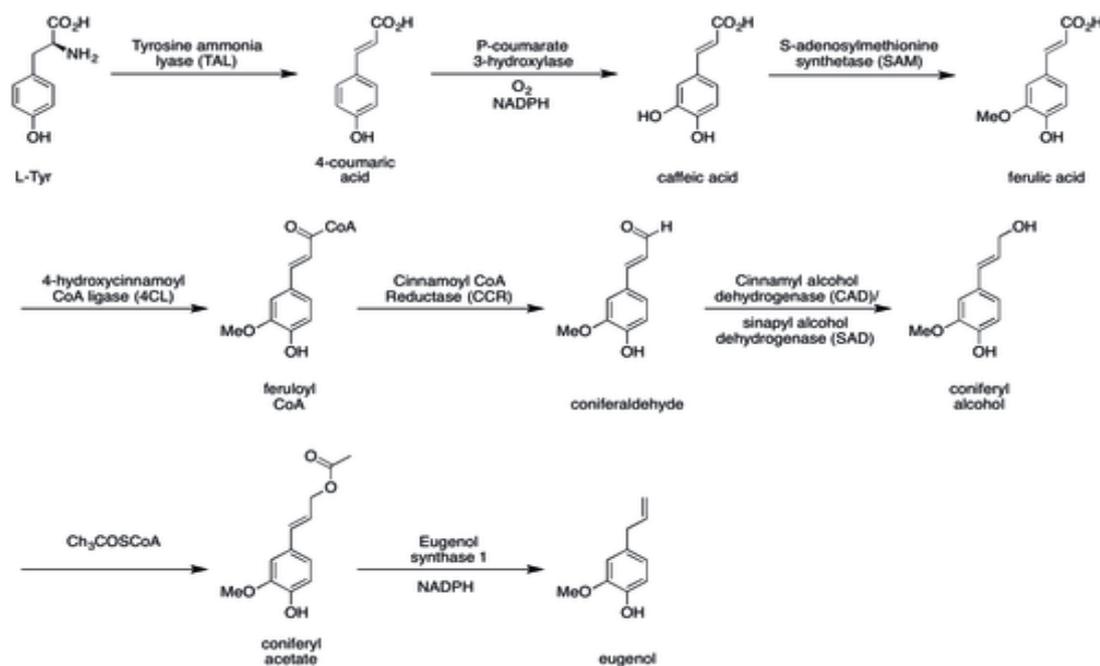
Refractive Index: 1.54

Partition Coefficient: 2.26

Biosynthesis of Eugenol

The biosynthesis of eugenol begins with the amino acid tyrosine. L-tyrosine is converted to p-coumaric acid by the enzyme *tyrosine ammonia lyase* (TAL). From here, p-coumaric acid is converted to caffeic acid by *p-coumarate 3-hydroxylase* using oxygen and NADPH. S-Adenosyl methionine (SAM) is then used to methylate caffeic acid, forming ferulic acid, which is in turn converted to feruloyl-CoA by the enzyme *4-hydroxycinnamoyl-CoA ligase* (4CL). Next, feruloyl-CoA is reduced to coniferaldehyde by *cinnamoyl-CoA reductase* (CCR). Coniferaldehyde is then further reduced to coniferyl alcohol by *cinnamyl-alcohol dehydrogenase* (CAD) or *sinapyl-alcohol dehydrogenase*

(SAD). Coniferyl alcohol is then converted to an ester in the presence of the substrate CH_3COSCoA , forming coniferyl acetate. Finally, coniferyl acetate is converted to eugenol via the enzyme *eugenol synthase 1* and the use of NADPH.^[14,15]



Analytical Method to Determine Eugenol Concentrations of Different Species of Ocimum

➤ Uv Analysis

By comparing the R_f value of standard eugenol from Thin layer chromatography, the band of eugenol obtained from extract of various species of ocimum was identified and scrub out. The scrub silica gel containing eugenol was thoroughly mixed with 5 ml of solvent and filtered. Then the solution was taken for UV Analysis.^[16]^[17]

➤ Hplc analysis

Development of method: After all trial and error, the following developed method having 50 parts of methanol, 25 parts of water and 75 parts of acetonitrile was fit strong for eugenol in an isocratic system and this method was developed for both standard eugenol and eugenol present in alcoholic extracts of different species of ocimum.^[18]

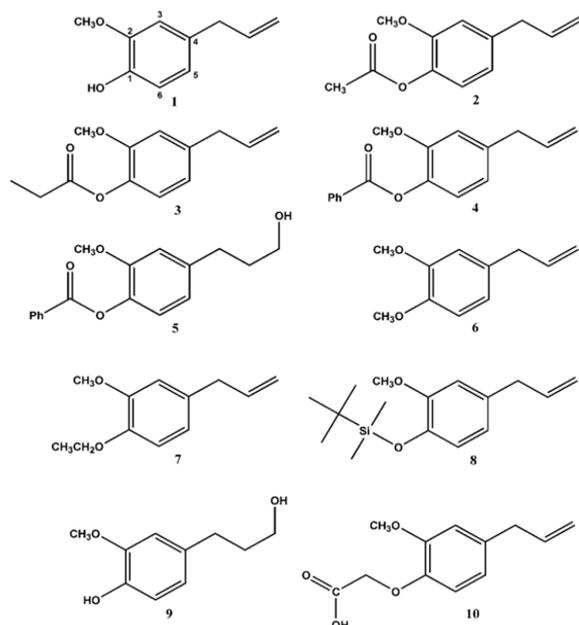
Calibration of eugenol by HPLC

1.01ml of 99% v/v eugenol when diluted up to 10 ml of solvent (MeOH: H₂O: ACN, 50:25:75) to give 100 µg / ml of eugenol. From this stock solution aliquot of 25, 50, 75, 100, 125 nano gram per ml of eugenol were prepared. After sufficient 30 min sonication and filtration through 0.45-micron filter paper those solutions were injected to HPLC for Calibration.

Structural–Activity Relationships of Eugenol

As eugenol exhibited larvicidal activity, it was of interest to synthesize nine derivatives either by protecting the

phenolic hydroxyl with a diverse set of esters and ethers or by region-selectively oxidizing the allylic double bond to a primary alcohol. The most active compounds in this group (compounds 1 (Eugenol) to 3 [1-propanoate-2-methoxy-4-(2-propen-1-yl) phenol] and 6 [1,2-Dimethoxy-4-(2-propen-1-yl) benzene] and 7 [1-ethoxy-2-methoxy-4-(2-propen-1-yl) benzene] have LC₅₀ values ranging from 67 to 113ppm (P < 0.05). Therefore, none of the derivatives tested significantly increased potency compared with the parent compound. The addition of bulky groups to the hydroxyl, for example in 4 [1-Benzoate-2-methoxy-4-(2-propen-1-yl)-phenol] and 8 [t-butyl dimethyl silyl ether] resulted in about an eight- and a threefold decrease in potency respectively. Similarly, the addition of an acidic group to the phenolic hydroxyl (compound 10 [2-(2-methoxy-4-(2-propen-1-yl) phenoxy) acetic acid] resulted in a three orders of magnitude decrease in potency. When the allyl side chain of eugenol was modified (compounds 5 [1-benzoate-2-methoxy-4-(3-hydroxy propyl)-phenol] and 9 [4-hydroxy-3-methoxy-benzopropanol]), larvicidal activity was significantly reduced, and compound 9 exhibited the lowest potency (LC₅₀ = 1415.1ppm).



A reasonable explanation for this result may be an increased number of hydroxyl groups preventing substance penetration in the larva cuticle from reaching its targets. Additionally, similar results were found by Belzile et al., who evaluated the synergistic activity of dillapiol derivatives against *Aedes atropalpus* Coq. Larvae.^[19] When the allyl side chain of dillapiol was modified, synergistic activity was either reduced or eliminated compared with dillapiol. The indiscriminate use of organophosphates, such as temephos, to control mosquito spreading has induced mosquito biological response to exhibit resistance to such compounds 4. As there are no significant differences between the LC₅₀ of temephos-resistant and temephos-sensitive strains, the present results indicate that there is no cross-resistance for the essential oil and evaluated compounds ($P < 0.05$). Using medicinal chemistry approaches, structural characteristics were identified that may contribute to the understanding of the larvicidal activity of phenyl propanoids. The present approach may help future work in the search for larvicidal compounds.^[20]



Marketed Preparations

- Tulsi herbal mosquito repellent liquid vaporizer refill
- Herbal strategi mosquito repellent-Mosrelief
- Repelz – Herbal mosquito repellent
- Texomos- Herbal mosquito repellent

- Herbal strategi just spray mosquito repellent-Mospray.

DISCUSSION

A. Mohamed Anees studied about the larvicidal activity of *Ocimum sanctum* against *Aedes aegypti* and *Culex quinquefasciatus*. The acetone, chloroform, ethyl acetate, hexane, and methanol leaf and flower extracts of *Ocimum sanctum* were studied against fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus*. The highest larval mortality was found in leaf extract of *O. sanctum* against the larvae of *A. aegypti* and *C. quinquefasciatus*. The LC₅₀ values of *O. sanctum* against the larvae of *A. aegypti* were 425.94, 150.40, 350.78, 575.26, and 175.67 and against the larvae of *C. quinquefasciatus* were 592.60, 93.92, 212.36, 76.61, and 82.12 ppm, respectively.

Vinod Krishan, Jyoti Uikey and R.C. Saxena conducted study on mosquito larvicidal activity of *Ocimum sanctum* on *Culex quinquefasciatus*. Mosquito borne diseases such as malaria, filaria, dengue, yellow fever and recently out broken chikunguniya are still continuing to be a major health problem in tropical and subtropical countries¹. Larval control during the past had been dependent mainly on the use of chemicals such as BHC and Paris green. Use of these chemical pesticides for a longer period posed number of health problems to the non-target organisms including human being. Moreover, mosquitoes have developed resistance to the pesticides. Therefore, it is necessary that alternate larvicidal compounds be evaluated for future use. In the present laboratory for the two decades more than twelve plant crude extracts have been evaluated against II and IV instar larvae of *Culex quinquefasciatus* as well as *Anopheles stephensi* which have shown anti-juvenile activity, loss in fecundity and fertility including some developmental defects. George and Vincent have reported the comparative efficacy of three plant extracts against *Culex quinquefasciatus*.^[21] Similarly, Madhumathy have evaluated the larvicidal potential of Capsicum against *Anopheles stephensi* and *Culex quinquefasciatus*.

Development of an analytical method to determine eugenol concentrations in alcoholic extracts of different species of ocimum's. Eugenol is used as a flavor in the food industry, has a variety of biological activity, and can serve as a biomarker. Because eugenol is present in the leaves of *Ocimum*, which are used as an herbal medicine, a sensitive and reliable quantitative Ultra-violet spectroscopy and high-performance liquid chromatographic method has been established for quantification of the compound in the leaves of the plant. A methanol extract of the powder of dried leaves of *Ocimum*, was spotted on the Merck aluminium plate pre-coated silica gel F254 with 0.2 mm thickness. MeOH: Chloroform (95:5) and MeOH: H₂O: ACN (50:25:75) used as mobile phase to isolate the eugenol and to prepare the sample for UV and HPLC analysis

respectively. The UV and HPLC method proposed for the quantitative monitoring of eugenol in *Ocimum* leaf powder is rapid, simple, and precise. Hence from that UV and HPLC analysis it was concluded that the *sanctum linn* contains higher amount of Eugenol.^[23,24]

Several studies reported that the essential obtained from Tulsi contain many nutrients and other biologically active compounds. Its leaf contains volatile oil eugenol, euginal (also called eugenic acid), urosolic and carvacrol, linalool, limatrol, caryophyllene. Eugenol (1-hydroxy-2-methoxy-4-allyl benzene) the active constituent present in *Ocimum sanctum L* has been found to be largely responsible for the therapeutic potential of Tulsi.

Extraction of essential oil from *Ocimum basillium* is a soild - liquid extraction. Mosquitocidal efficacy of essential oil of *Ocimum sanctum* against adult mosquitoes of different species viz. *Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus* were investigated. 100% mortality is observed in *A. stephensi*, *A. aegypti* at a dose of 0.003ml/43.0cm². However, mortality of *C. quinquefasciatus* was observed at a higher dose 0.01ml/43.0cm². The essential oil of Tulsi showed larvicidal efficacy against larvae of *A. stephensi*, *A. aegyptic*, and *C. quinquefasciatus*. Compared to flower extract, leaf extract was found to be more effective.

CONCLUSION

Plants are one of the most important source of medicine. Today large number of drug in use are derived from plants. The medicinal plants are rich in secondary metabolite (which are potential source of drug) and essential oils of therapeutic importance. Basil oil contains bioactive constituents they are insecticidal and repellents. The results of study of mosquito repellent activity were as follow *O. gratissimum* volatile oil exhibit the strongest activity. Volatile oil *O. gratissimum* showed complete repellence for a period of 2hr. The volatile oil of *O. bacillicum* and *O. tenuiflorum* also completely repelled the mosquito but their duration of action was shorter. The larvae were killed by eugenol which showed that mosquito larvae were killed when pure eugenol oil was spread on the water surface.

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