# Repellent Activity of Plant Extracts, Essential Oils and their Combinations against Different Mosquito Species: A Review

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#### **ABSTRACT**

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Mosquitoes are majorly responsible for causing vector-borne diseases such as malaria, filariasis, chikungunya, dengue, zika, yellow fever, west nile etc. Aedes, Anopheles, Culex are the main genera among other mosquitoes' species, that are affecting human beings with such dreadful diseases worldwide. To control the transmission of diseases by mosquitoes generally mosquito nets, ITNs and LLINs, DDT, DEET, IR3535 are being used. Prolong use of these harmful chemicals not only cause development of resistance in mosquitoes but also have shown adverse effects on humans as well as on environment. So, there is a growing urge/need of finding a suitable mosquito repellent from natural origin, that have potential to prevent such vector-borne diseases with least side- effects on humans as well as on environment. In this review, we have enlisted various Essential oils (EO) that are having mosquito repellent properties and some of them such as Cymbopogon nardus, Eucalyptus maculata, Cymbopogon excavatus, Mentha piperita, Azadirachta indica are already used in various mosquito repellent formulations that are being marketed presently. EO of particular plant can be extracted from respective parts of it such as seeds, roots, rhizomes, stems, leaves, bracts, fruits, bark and inflorescences mainly by hydrodistillation and steam distillation. Gas chromatography coupled to mass spectrometry (GC-MS) is the identification technique used for identification of EO constituents. In addition, the combinations of potent EOs are also found to be the best effective way to be used as mosquito repellent so that repellent potential enhanced due to synergism as well as negates the harmful effects of individual oils. The efficiency of essential oils as well as its protection time can also be enhanced by using vanillin and formulation techniques like microencapsulation and nanoemulsion. Based on market coverage as well as economical cost, synthetic repellents are frequently marketed than essential oils but these EOs, extracts or natural products have the potential to provide efficient and safer repellents for humans as well as the environment.

**Keywords:** Herbal mosquito repellent, Essential oils combinations, Natural products, *Anopheles stephensi, Aedes aegypti,* Dengue, Malaria.



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### INTRODUCTION

According to World Health Organisation Report 2017, Seventy Lakh people died due to diseases caused by vectors. Mosquitoes are the prominent vectors for spreading of vector-borne diseases like malaria, filariasis, chikungunya, dengue, zika, yellow fever, west nile etc. The most dreadful genus is of Anopheles, Culex, and Aedes, which belong to Culicidae family.[1] Out of all the contagious diseases occurred worldwide, 17 % are caused by mosquitoes only. [2] Anopheles is a primary vector of malaria whereas Aedes albopictus act as secondary vector for diseases like dengue and chikungunia. These vectors require blood of host for production of their eggs and in process of sucking blood from infected host, they pass on the diseases to the healthy host and ultimately leads to dissemination of dreadful diseases. The chemical and microbial secretions from host's skin, the carbon dioxide from the exhaled air, urine, body heat, odour and moisture are the main factors which are responsible for proneness of host towards mosquito. [3] Bacillus cereus, Bacillus subtilis, Brevibacterium epidermidis, Corynebacterium minutissimum, Staphylococcus epidermidis are those bacterial species whose population is high in mosquito prone

individual as well as the degrading volatile products of the sweat by them also enhances attractiveness of mosquitoes towards the host. [4] L-Lactic acid, carboxylic acid mixtures, NH, and other secretions from the sweat act as an attractant for mosquitoes. [3] On the other hand, the carbon dioxide released in exhaled air shows synergistic effect with the body secretions that make body odour for attracting and orienting mosquitoes towards host.<sup>[5]</sup> To control the transmission of diseases by mosquitoes generally mosquito nets, ITNs and LLINs are used. But to overcome the restriction caused by mosquito nets, mosquito control by chemicals such as DDT, DEET, IR3535 in the form of IRS, Peridomestic space spraying is being done. After having so many advantages, DEET has many drawbacks just like disagreeable odour, numbness of lips as side effects as well as occurrence of resistance in mosquitoes against DEET and also causes damage to the surfaces or fabrics on which it is applied. Permethrin has efficacy against wider range of insects like mosquitoes, ticks, tsetse flies, fleas, lice etc. but longer use of higher concentrations of permethrin caused tremors, unorganised coordination, paralysis, rise in body temperature, skin and eye irritation as well as affects immune system also. Picaridin and DEPA must be avoided in breast-feeding or

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pregnancy. IR3535 must not be used in malaria endemic areas. [6] These chemicals develop resistance in various mosquitoes and causes harmful effects on humans as well as on environment too when longer used which emerges the need of mosquito repellent from natural origin so that it will become possible for prevention of diseases caused by mosquito without adversely affecting the environment. It is well established from the literature survey that various EOs have shown potential to be used as mosquito repellent and some of the EOs such as Cymbopogon nardus, Eucalyptus maculata, Cymbopogon excavatus, Mentha piperita, Azadirachta indica are used in commercial anti-mosquito formulations presently as well. There are problems associated with usage of individual EOs also because after having proven repellent potency against mosquitoes, some EO has pungent odour while other has skin and eye irritant property which make either of the EO unbearable if used in mosquito repellent formulations. And if individual EO does not have such unwanted properties which affect the humans, some require higher Repellent doses (RD50) to repel mosquitoes or some have capability to restrain only specific mosquito species. [7,8] The combinations of potent EOs are also found to new approach to be used as mosquito repellent so that repellent potential enhanced due to synergism as well as one EO is able to mask or negates the unwanted property of another EO used in combination. There is also benefit to use combination over individual oil because combination will be able to show repellency against all the selected species of mosquito not only against specific species as in case of some individual EOs. Apart from this, formulating these extracts, EOs or their combinations in various preparations like creams, sprays, ointments, polymer mixtures, or microcapsules provide controlled release and also enhances the effective repellenct time of respective EOs or extract.[9]

### Bioassays for Mosquito Repellent Activity

Various bioassays are enlisted in WHO 2013<sup>[10]</sup> and 2009<sup>[11]</sup> guidelines but still there is need for more appropriate bioassay for mosquito repellency. Researchers use various methods to assess the repellency of essential oils/ compounds against mosquitoes. The most used method for testing EOs, is the arm-in-cage assay. It was published in 1919 and it is likely the first well planned laboratory test of mosquito repellency.<sup>[12]</sup> Quantities of EO were previously dissolved in a fixed solvent volume and applied uniformly on the arm (from wrist to elbow) of a volunteer, whereas the other arm is treated with the solvent. The subject inserts his or her product-treated arm and the control in independent cages with a fixed number of blood unfed female mosquitoes. The time elapsed until the first bite in the treated arm is recorded and presented as PT, the number of mosquitoes biting in a determined time may also be recorded in order to calculate the percentage of repellency. Taking into account the quantity of EO applied and the area of the subject's arm, the dosage is calculated in  $\mu L/mm^2$  but difficulty in counting the number of mosquitos landing on the skin or bloodsucking accurately is the limitation of this assay.[13] The repellent activities are presented in values of RD50 and RD95 (calculated statistically), representing the dosage of EO capable to repel the 50% and 95%, respectively of the insect population. A similar method has been developed by using the depilated abdomen of a white mouse, instead of a human arm. [9,14]

Repellency was evaluated using Mosquito Repellency Bioassay. Aliquot (500 microliter) of the test solution of the oils or their combinations was dispensed separately over a cardboard sheet of size (22\*35 mm) and thickness (2.5 mm) equal to commercially available mosquito mats. Twenty adult mosquitoes were taken for the experiment and the observation of repellency studies was done by counting the migrated mosquitoes at an interval of 15 min for a total period of 1hr for assessment of true repellency and percentage repellency (PR) was calculated after

1hr experimental duration.<sup>[15]</sup> The Y-tube olfactometer bioassay has also been used for testing repellency, it consists on a glass Y-tube with a main arm (the stem) and two arms containing one repellent and the control in other one, where a low rate air movement is created by sucking the air in the two arms of the Y-tube with a pump connected to the stem. The essential oil sample and control are applied on a paper attached to the arms of the tube. Insects are introduced into the tube by a hole located at the centre (the joint point of the three tubes), after introduction the hole is closed with a rubber stopper and the pump is operated. After seconds of exposition, the number of insects on each of the two tubes (treated and control) are scored to assess the percentage repellency. [9,16] K&D bioassay system that determines the biting deterrence was developed for high throughput screening of compounds. In the room method, various body parts including feet, legs and arms of the test subject are treated with the desired chemical. The subject then walks into the mosquito-filled room or sits outside in case of open area testing. Number of mosquitoes landing on the treated parts of the body were collected with an aspirator at various time intervals after treatment to determine the repellency and protection time of various products. All the bioassay systems use positive control, commonly DEET and a negative control (solvent).[17] It is a common conclusion that the results of one bioassay don't compare with the other system at the same concentration of the material.

# Essential oils (EOs), plant extracts as Natural Mosquito Repellents

Several plant species indigenous to India had been evaluated for their mosquito repellent potential, are also being enlisted in Table 1. Govindarajan et al., (2016) reported that essential oil (EO) of leaves of Origanum scabrum had shown both larvicidal and repellent activity against four of the mosquito species and its maximum protection period against Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus, C. tritaeniorhynchus are 210 min, 180 min, 150 min, 120 min. Mathew, (2016) noted down mosquito repellent activity of 20% EO in ethanol of Plectranthus amboinicus leaves against Aedes aegypti with maximum protection period was six hours.<sup>[19]</sup> Champakaew et al., (2015) observed that Angelica sinensis hexane extract have shown maximum protection period of 7.5 hours against Aedes aegypti. [20] Nasir et al., (2015) reported repellent activities of Azadirachta indica leaves EO against Aedes aegypti and Aedes albopictus with protection period of 246 min and 256 min.<sup>[21]</sup> Peter and Mwangi, (2014) validated that Azadirachta indica EO had shown consistent repellency of 85.79% from concentration from 5% to 10%. [22] Govindarajan and Sivakumar, (2015) illustrated that methanolic extract of Erythrina indica leaves had shown best repellent activity against Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus with maximum protection period of 210 min, 210 min and 180 min.[23] Phukerd and Soonwera, (2014) noted that 10% concentration of Citrus hystrix peel EO had shown repellency against Aedes aegypti and Culex quinquefasciatus with protection period of 75 min and 11 min.[24] Tawatsin et al., (2001) reported the mosquito repellent activity of 25% EO of Citrus hystrix leaves with maximum protection periods of 3 hr, 1.5 hr, 2.5 hr (with 5% vanillin) and 1 hr, 0.5 hr, 0.5 hr (without vanillin) against Aedes aegypti, Anopheles dirus and Culex quinquefasciatus. [25] Govindarajan and Sivakumar, (2015) observed the repellent activity of Asparagus racemosus root extract against Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus with protection periods of 180 min, 180 min and 150 min. [23] Kumar et al., (2014) reported that Apium graveolens seed oil had both larvicidal and repellent activity against Aedes aegypti mosquito species with protection period of 150 min. [26] Kulkarni and Pawar, (2013) noted down that Lavandula gibsoni EO have shown repellent activity against Aedes aegypti with maximum protection period of 7 hr 25 min while its major constituents α-terpinolene and

thymol in a ratio of 2:1 gave synergistic effect for a period of 370 min which is higher than their individual protection periods against Aedes aegypti mosquito.[27] Conti et al., (2021) reported that EO of Hyptis suaveolens fresh leaves had both larvicidal and repellent activity against Aedes albopictus with maximum protection period of 134.8 min. [28] Govindarajan and Sivakumar, (2012) observed that methanolic extract of Andrographis paniculata had shown repellency with protection periods of 210 min and 180 min against Culex quinquefasciatus and Aedes aegypti. [29] Tawatsin et al., (2006) reported the repellent activity of EO of Hedychium coronarium against Aedes aegypti, Aedes albopictus, Anopheles dirus and Culex quinquefasciatus with protection periods of 0.9 hr, 8 hr, 8 hr and 7.5 hr in one minute exposure time. [30] Prajapati et al., (2005) illustrated that EO of Cinnamomum zeylanicum leaves had both larvicidal and repellent activity against Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus with respective repellent dose 50 (RD<sub>so</sub>) were 23 mg/mat, 28.4 mg/mat and 20.6 mg/mat in one hour. [30] Tawatsin et al., (2001) reported the mosquito repellent activity of 25% EOs of Cymbopogon winterianus and Ocimum americanum with maximum protection periods of each of the oil was 6.5 hr, 8 hr, 8 hr ( with 5% vanillin) and 3 hr, 4 hr, 8 hr (without vanillin) against Aedes aegypti, Anopheles dirus and Culex quinquefasciatus.[25]

Specifically focusing on the plant species that are found in North-Indian region narrows down screening to following plant species. Mathew, (2016) noted down the mosquito repellent activity of 20% EO in ethanol of Ocimum sanctum leaves against Aedes aegypti with maximum protection period was six hours. [19] Gokulakrishnan et al., (2013) reported that Pogostemon cablin leaves EO had shown repellency against Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus for 4.6 hr at a concentration of 2mg/cm<sup>2</sup> and patchouli alcohol, its major constituent have shown repellent activity for a period of 280 min. [32] Khandagle et al., (2011) noted down that Zingiber officinalis rhizomes EO had shown repellent activity against Aedes aegypti and Culex quinquefasciatus with protection period of 5 hr each at a concentration of 0.5mg/cm<sup>2,[33]</sup> Previously, Tawatsin et al., (2006) reported the repellent activity of EO of Zingiber officinalis rhizomes against Aedes aegypti, Aedes albopictus, Anopheles dirus and Culex quinquefasciatus with maximum protection periods of 3.4 hr, 7.2 hr, 8 hr and 7.7 hr in one minute exposure time when its 10% lotion in absolute alcohol plus excipients were used in experiment. [30] Kamaraj et al., (2011) reported that both methanolic and ethanolic extract of Piper nigrum seeds had mosquito repellent activity against Anopheles stephensi and Culex quinquefasciatus with maximum protection period of 150 min. [34] Tawatsin et al., (2006) illustrated the repellent activity of EO of Piper nigrum seeds against Aedes aegypti, Aedes albopictus, Anopheles dirus and Culex quinquefasciatus with maximum protection periods of 4.1 hr, 8 hr, 8 hr and 7.8 hr in one minute exposure time when its 10% lotion in absolute alcohol plus excipients were used in experiment. [30] Singh et al., (2009) reported that 10% hexane extract of Cyperus rotundus roots had shown repellency against Anopheles culicifacies, Anopheles stephensi and Culex quinquefasciatus with protection period of 4-6 hr, 6hr and 6 hr.[35] Tawatsin et al., (2006) reported the repellent activity of EO of Curcuma longa leaves against Aedes aegypti, Aedes albopictus, Anopheles dirus and Culex quinquefasciatus with protection periods of 3.6 hr, 8 hr, 8 hr and 8 hr in one minute exposure time. [30] Tawatsin et al., (2001) reported the mosquito repellent activity of 25% EO of Curcuma longa rhizomes with maximum protection periods of 4.5 hr, 8 hr, 8 hr ( with 5% vanillin) and 1 hr, 8 hr, 5 hr (without vanillin) against Aedes aegypti, Anopheles dirus and Culex quinquefasciatus.[25] Das et al., (2015) noted down that Curcuma longa rhizomes EO had maximum protection time of 96.2 min against Aedes albopictus at a concentration of 20%. [36] Tawatsin et al., (2006) reported the repellent activity of EO of Alpinia galanga rhizomes against Aedes aegypti, Aedes albopictus, Anopheles dirus and Culex

quinquefasciatus with protection periods of 2.2 hr, 7.8 hr, 8 hr and 7.2 hr in one minute exposure time. Similarly, author also described the repellent activity of Psidium guajava leaves EO against Aedes aegypti, Aedes albopictus, Anopheles dirus and Culex quinquefasciatus with protection periods of 4.4 hr, 7.4 hr, 8 hr and 7.7 hr in one minute exposure time. In continuation of the above plants, repellent activity of Piper betle leaves EO against Aedes aegypti, Aedes albopictus, Anopheles dirus and Culex quinquefasciatus with protection periods of 2.3 hr, 7.6 hr, 8 hr and 7.8 hr in one minute exposure time was also noted down. [30] Yang and Ma, (2019) reported the repellency of Citrus sinensis leaves EO against Aedes albopictus with maximum protection period of 4-8 hr. [37] Trongtokit et al., (2005) observed the repellent activity of 0.1 ml Cymbopogon nardus leaves EO against Aedes aegypti, Anopheles dirus, Culex quinquefasciatus and Anopheles stephensi with maximum protection periods of 2 hr, 1.16 hr, 1.6 hr and 8 hr. [38] Gillij et al., (2008) reported that 90% Rosmarinus officinalis EO have shown repellent activity for 90 min against Aedes aegypti.[13] Nasir et al., (2015) noted down the repellent activity of 10% Ocimum basilicum leaves EO against Aedes aegypti and Aedes albopictus with maximum protection periods of 64.34 min and 69.34 min.[21] Kamaraj et al., (2011) reported that methanolic extract of Trachyspermum ammi seeds had mosquito repellent activity against Anopheles stephensi and Culex quinquefasciatus with maximum protection period of 150 min.[34]

# Synergistic Combination of Essential Oils as a New Approach

As to prolong the protection time of mosquito repellents, combinations of essential oils are being built, tested and those showing synergistic effects may be further scaled up for commercial purposes also. For example, a 1:1:2 combination (20% concentration) of essential oils of Curcuma longa rhizomes, Zanthoxylum limonella and Pogostemon heyneanus leaves showed protection period of 329.4 min against Aedes albopictus mosquitoes, which is way more than their individual protection periods.[36] Combination of 50% clove: 50% thyme oil showed protection periods of 210 and 150 min against Aedes aegypti and Anopheles albimanus mosquito species. [60] Similarily, 0.075% concentration of combination of Litsea cubeba and Litsea salicifolia has proven to have significant synergistic effects against Aedes aegypti. [65] Combinations have been developed on the basis of the actual ratio of pure compounds present in the respective essential oils and tested for their repellent activity. Combination of perillaldehyde, perillyl alcohol, 1,8 cineole, limonene (29:4:10:7) of Conyza newii repelled 50% of mosquitoes at the lowest dose of  $12 \times 10-5$  mg/cm<sup>2</sup>. The blends of the main constituents of essential oil of Suregada zanzibariensis mixed in natural ratios showed higher repellency to A. gambiae s.s. than the parent oil. [66] Vanillin showed negligible repellency alone but enhances the protection period for about 2 hr when used in 5% concentration with most of the oils like cassia oil, rosemary oil, turmeric oil, and lemongrass oil etc. [67] Protection time of essential oil of Zanthoxylum piperitum and Kaempferia galanga increased from 60 to 190 min and 30 to 75 min against Aedes aegypti when mixed with vanillin, respectively.[40] Essential oil of O. americanum with vanillin provided 6.5-hr protection against Aedes aegypti, C. quinquefasciatus and Anopheles dirus in a caged experiment. Adding 5% vanillin with *C. winterinaius* oil provided 8-hr complete protection from C. quinquefasciatus, Anopheles dirus, and Aedes aegypti compared to 25% DEET.[25] A very few essential oil combinations have shown prominent synergistic effects so far. The high insect repellency and toxicity of the mixed oils might have resulted from the synergistic action of the main compounds in the oils. The mechanisms involved in how the interactions between the components of each essential oil result in the improvement of repellent activities, need further investigation.[9]

[46, 44]References [38] [40] [28] [41] [42] [30] [32] [45] Culex quinquefasciatus Culex quinquefasciatus Culex quinquefasciatus Culex quinquefasciatus **Targeted mosquito** Anopheles stephensi Aedes albopictus Aedes albopictus Aedes albopictus Anopheles dirus Aedes albopictus Anopheles dirus Aedes albopictus Aedes albopictus Aedes albopictus Aedes albopictus Aedes aegypti Aedes aegypti Aedes aegypti Aedes aegypti strong at low dose of min),30%(104.8 min) (0.08 µL/cm2 of skin) (43.8 min),20% (96.2 (0.4 µL/cm2 of skin) 5 hr, 8h (+ vanillin) 5% (22.4 min), 10% (0.7496 µg/cm<sup>2</sup> of  $4.6 \, hr \, (2 mg/cm^2)$ Max. Protection More than 1 hr 91.4 min(20%) 1-2.5 hr(+10% 1 hr, 4.5 hr (+ 1 hr (0.1 ml) 0.08 mg/cm<sup>2</sup> 8 hr (0.1 ml) vanillin) 134.8 min 92.4 min vanillin) 280 min\* (min/hr) 90 min  $(0.3 \, \text{ml})$ (0.1ml) Period 3.6 hr skin) 8 hr 8 hr 8hr Larvicidal Repellent Activity LC<sub>50</sub>-11.0% v/v LC<sub>50</sub>-115.6 ppm LC<sub>50</sub>-15.5% v/v 35.66 ppm 240.3 ppm 42.1 ppm 54.6 ppm  $LC_{50}$ LC<sub>50</sub>- $LC_{50}$ LC<sub>50</sub>/  $LC_{50}$ -۳ **Technique** GC-EIMS Analytical GC-EIMS GC-FID GC-MS GC-FID/ GC-MS GC/ GC/ GC/ MS MS MS Extraction distillation distillation distillation distillation distillation distillation distillation distillation distillation Method Steam Hydro-Hydro-Hydro-Hydro-Hydro-Hydro-Steam Patchouli alcohol (22.62%)\* Constituents with % phellandrene(5.04%), artumerone(23.12%, curlone (19.14%),α-Tumerone (41.11%, eucalyptol (3.92%) Aromadendrene 2-Nonanone imonene (25.70%) Linalool Sabinene (21.90%) Sabinene (83.6%) (37.99%) (37.4%)(21.8%)Aerial parts Aerial parts Dried Fruit Rhizomes Pericarp Leaves Used Fresh Leaves Leaves Fruit Zingiberacee Lamiaceae/ Lamiaceae Lamiaceae Lamiaceae Rutaceae Labiatae Rutaceae Rutaceae Apiaceae Family Korean pepper Japanese or Fringed rue Ma-khwaen Bushmint Peach sage Common Coriander Turmeric Monterey Patchouli Name cypress Hyptis suaveolens Salvia dorisiana Curcuma longa Zanthoxyl-um lumpiperitum Coriandrum Pogostemon Biological Zanthoxy Wild Ruta chalepensis macrocarpa Cupressus Source limonella sativum cablin s. è. 3 4; 5. 9 6

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Table 1: Biological Source, phytoconstituents and extraction methods with mosquito larvicidal and repellent activities of plant species.

quito References		sciatus [27]	iti [37,47] :tus	oti [17,37,48]	ynchus ti lingi	oti [24]	oti [24]	oti [24]
Targeted mosquito		Anopheles stephensi Culex quinquefasciatus Aedes aegypti	Aedes aegypti Aedes albopictus	Aedes aegypti Aedes albopictus	Culextritaeniorhynchus Aedes aegypti Anopheles darlingi	Aedes aegypti Culex quinquefasciatus	Aedes aegypti Culex quinquefasciatus	Aedes aegypti
Activity Larvicidal Repellent	Max. Protection Period (min/hr)	7 hr 25 min (2mg/cm²) 1. 310 min (0.4mg/cm²) 2. 250 min (0.2mg/cm²) 1+2. 370 min (0.6mg/cm²)	150 min 4 hr (7%)	2-3 hr 3 hr	1 hr (1,2.5,5mg/cm²) 1.47 hr (0.1 ml of 10% soln.) 2.5 hr (3 ml of 25% soln.)	16 min (10%) 80 min	30 min (10%) 260 min	16 min (10%)
<i>f</i> Larvici	LC <sub>50</sub> /	LC <sub>50</sub> - 62.79 ppm LC <sub>50</sub> - 54.72 ppm LC <sub>50</sub> - 48.3 ppm	LC <sub>50</sub> -111.9ppm	LC <sub>50</sub> -33.7% v/v 69 ppm		ı	1	ı
Analytical Technique		GC-FID	1		GC-FID	1	i .	
Extraction Method		Hydro-distillation	Hydro- distillation	Hydro- distillation	Steam distillation Steam distillation	Hydro- distillation	Hydro- distillation	Hydro- distillation
Major Constituents with %		1.a. terpinolene (22.22%) 2.Thymol (10.42%)			Geranial (41.30%)			ı
Parts Used		Aerial parts	Leaves	Aerial parts	Leaves	Leaves	Roots, Rhizomes	Rhizomes
Family		Lamiaceae	Lamiaceae	Poaceae		Zingiberacee	Zingiberacee	Zingiberacee
Common		Gibson`s Lavender, Nivli	Peppermint	Lemongrass			Finger root	Zedoary, White Turmeric
Biological Source		Lavandula gibsoni	Mentha piperita	Cymbopogon citratus		Атотит biftorum	Boesenbergia rotunda	Curcuma zedoaria
s. No.		ii	12.	13.		14.	15.	16.

Table 1: Con't									
Biological Common Family Source Name	Family	Parts Used	Major Constituents with %	Extraction Method	Analytical Technique	A Larvicid	Activity Larvicidal Repellent	Targeted mosquito	Refer- ences
						LC <sub>50</sub> /	Max. Protection Period (min/hr)		
Zingiber Common ginger Zingiberacee officinalis	Zingiberacee	Rhizomes	(E)-3,7-dimethyl-2,6-2,6- octadien al(24.72),eucalyptol(20.77),	Steam distillation		LC <sub>50</sub> -154 ppm LC <sub>50</sub> -197 ppm	5 hr (0.5mg/cm²) 5 hr	Aedes aegypti	[33]
			(Z)-3,7-dimethyl-2,6-octadienal(18.27), camphene (8.71)				3.4 hr (0.1 ml)	Culex quinquefasciatus Aedes aegypti	
							7.2 hr 8 h 7.7 hr	Aedes albopictus Anopheles dirus Culex quinquefasciatus	[30]
Zingiber Wild ginger Zingiberacee	Zingiberacee	Rhizomes		Hydro- distillation			17 min (10%) 185 min	Aedes aegypti Culex quinquefasciatus	[24]
Citrus Lime Rutaceae aurantifolia	Rutaceae	Peel		Hydro- distillation	O9		17 min (10%) 25 min	Aedes aegypti Culex quinquefasciatus Aedes aegypti	[24]
		Leaves					2 hr (15%)		[49]
Citrus hystrix Kaffir lime Rutaceae	Rutaceae	Peel		Hydro- distillation	1		11 min (10%)	Culex quinquefasciatus Aedes aegypti Aedes aegypti	[24]
		Leaves	,	Steam distillation	1	,	75 min 1 hr		[25]
							3 h (+ vanillin) 0.5 hr	Anopheles dirus	
							1.5 hr (+ vanillin) 0.5 hr 2.5 hr (+ vanillin)	Culex quinquefasciatus	
Citrus maxima Pomelo Rutaceae	Rutaceae	Peel		Hydro- distillation	gC	,	11 min (10%) 25 min	Aedes aegypti Culex quinquefasciatus	[24]
Fortunella Kumquat Rutaceae japonixa	Rutaceae	Peel		Hydro- distillation	OG		11 min (10%) 3 min	Aedes aegypti Culex quinquefasciatus	[24]
Litsea petiolata Litsea tree Lauraceae	Lauraceae	Leaves		Hydro- distillation	25		25 min (10%) 165 min	Aedes aegypti Culex quinquefasciatus	[24]

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s õ	Biological Source	Common	Family	Parts Used	Major Constituents with %	Extraction Method	Analytical Technique	A Larvicic	Activity Larvicidal Repellent	Targeted mosquito	Refer- ences
								LC <sub>50</sub> /	Max. Protection Period (min/hr)		
26.	Cymbopog-on winterianus	Citronella grass	Poaceae	Leaves		Steam distillation	1	1	3 h 6.5 hr (+5% vanillin)	Aedes aegypti	[25]
									4 hr 8 hr (+5% vanillin) 8 hr	Anopheles dirus	
										Culex quinquefasciatus	
27.	Ocimum americanum	Hairy basil	Lamiaceae	Leaves	E-Methyl Cinnamate (70.90%)	Steam distillation	1		3 hr 6.5 hr (+5% vanillin) 4 hr	Aedes aegypti	[25]
									8 hr (+5% vanillin) 8 hr	Anopheles dirus	
										Culex quinquefasciatus	
28.	Cymbopogon nardus	Citronella grass	Poaceae	Leaves	Citronellal, geraniol, citronellol, geranyl acetate	Steam distillation			2 hr 1.16 hr 1.6 hr 8 hr	Aedes aegypti Anopheles dirus Culex quinquefasciatus Anopheles stephensi	[38]
29.	Dianthus caryophyllum	Clove pink	Caryophyllaeae	Flowers	A-Pinene, β-Pinene, α-Terpineol, β-Citro nellol, Geraniol	Steam distillation	GC/ MS		4 hr	Aedes aegypti	[50]
30.	Citrus sinensis	Orange	Rutaceae	Leaves	Limonene(71,3%), Myrcene (1,28%), (Z) Linalool (5,4%)	Microwave- assisted Hydro- distillation	CC		4 - 8 hr	Aedes albopictus	[37]
31.	Mentha spicata	Spearmint	Lamiaceae	Leaves		1	r		7 hr	Aedes albopictus	[37]
32.	Eucalyptus globulus	Eucalyptus	Myrtaceae	Leaves		•	1	1	3 hr	Aedes albopictus	[37]
33.	Thymus vulgaris	Thyme	Lamiaceae		Thymol (61%)	Hydro- distillation	GC-MS		2 hr	Aedes albopictus	[51]
34.	Tribulus terrestres	Burra gokharu	Zygophyllaceae	Leaves				LC <sub>50</sub> - 64.6ppm	4 hr $(1.5 \text{ mg/cm}^2)$	Aedes aegypti	[52]
35.	Kaempferia galanga	Aromatic ginger	Zingiberaceae	Rhizomes		Steam distillation	GC/ MS	1	0.25 hr 1.25 hr (+10% vanillin)	Aedes aegypti	[40]

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ito References		[53]		[54]	[30]	ıtus	isi [34]	isi [34]	tsi [34]
Targeted mosquito		Aedes aegypti		Aedes aegypti	Aedes aegypti Aedes albopictus	Anopheles dirus Culex quinquefasciatus	Anopheles stephensi Culex quinquefasciatus	Anopheles stephensi Culex quinquefasciatus	Anopheles stephensi Culex quinquefasciatus
Activity Larvicidal Repellent	Max. Protection Period (min/hr)	0-0.5 hr (25%)	1-1.5 hr (25%)	Significant repellency (0.1%)	2.2 hr 7.8 hr	8 hr 7.2 hr	30 min 30 min (ethyl acetate extract)	150 min (500ppm) 150 min	150 min (500ppm) 150 min
β Larvici	LC <sub>so</sub> /	$LC_{50}$ - 36.30 ppm (volatile oil) $LC_{50}$ - 57.15	ppm (hexane extract)	1	ı		LC <sub>50</sub> -42 ppm LC <sub>50</sub> -31 ppm	LC <sub>50</sub> -34.76 Ppm LC <sub>50</sub> -37.49 ppm (methanol	extract)  LC <sub>so</sub> -37  ppm  LC <sub>so</sub> -48  ppm  (methanol
Analytical Technique		GC/ MS		GC-FID	GC.		1	GC-MS	GC-MS
Extraction Method		Steam		Hydro- distillation	Steam distillation		Hydro- distillation	Hydro- distillation	Hydro- distillation
Major Constituents with %		Xanthorrizol(35,08), Curcume ne(25,71%), 7-meth-anoazu lene(30,02%)		Eucalyptol(24,4%),Terpineno -4 - ol (26,0%),Sabinene(11,0 3%),Linalool(6,1%)	Eucalyptol(39,50%),1,3,3-trimethyl-2-oxabicyclo octan-6-ol acetate (28,61%),	4-allylphenyl acetate	β-caryophyllene, (32%), α-humulene (12%)		
Parts Used		Rhizomes		Flowers	Rhizomes		Leaves	Leaves	Leaves
Family		Zingiberaceae		Zingiberaceae	Zingiberaceae		Verbenaceae	Nelumbonaceae	Phyllanthaceae
Common Name		Wild turmeric		Shell ginger	Galangal, Kulanjan		Red sage, Putus	Lotus	Indian gooseberry
Biological Source		Curcuma aromatica		Alpinia zerumbet	Alpinia galanga		Lantana camara	Nelumbo nucifera	Phyllanthus amarus
S. No.		37.		38.	39.		40.	41. 1	42.

Targeted mosquito References		Aedes aegypti [30] Aedes albopictus Anopheles dirus Culex quinquefasciatus	Anopheles gambiae [55]		Aedes aegypti [49]	s s atus				
	otection iod v/hr)		Jo lm/						<u></u>	
Activity Larvicidal Repellent	,/ Max. Protection Period (min/hr)	2.3 hr 7.6 hr 8 hr 7.8 hr	120 min (3mg extract)	2 hr (15% conc.)		4.4 hr 7.4 hr 8 hr 7.7 hr	4.4 hr 7.4 hr 8 hr 7.7 hr 80.33 min (10%)	4.4 hr 7.4 hr 8 hr 7.7 hr 7.7 hr 60 min 81.67 min (10%)	4.4 hr 7.4 hr 8 hr 7.7 hr 7.7 hr 60 min 81.67 min (10% 95 min the 10 % oil) 256 min 256 min	
	LC <sub>50</sub> /		1	O			O	0		C
Extraction Analytical Method Technique		Steam GC distillation	Hydro	Hydro- GC distillation		Steam GC distillation				
			Hy	Hy						
Major Constituents with %		4-allyl-2-methoxy-phenol acctate (31.47), 3-allyl-6- methoxyphenol (25.96), 4-allyl-phenylacetate (5.21)	1			Caryophyllene oxide (21,97%), 4,4-di- methyltetracyclo(2,5%) tridecan-9-ol (14,49%),caryophyllene (11,76%), ±-(E)-nerolidol (9,39)	Caryophyllene oxid (21,97%), 4,4-di-methyltetracyclo(2,57 tridecan-9-ol (14,49%), caryophylle (11,76%), ±-(E)-nerol (9,39)  Eugenol (80,08%)	Caryophyllene (21,97%), 4,4 methyltetracycl tridecan-9 (14,49%),caryor (11,76%), ±-(E)-(9,39)  Eugenol (80,	Caryophyllene (21,97%), 4,4 methyltetracycl tridecan-9 (14,49%),caryor (11,76%), ±-(E)-(9,39)  Eugenol (80,	Caryophyllene (21,97%), 4,4 methyltetracycl tridecan-9 (14,49%),caryo (11,76%), ±-(E)-(9,39)  Eugenol (80,
Parts Used		Leaves	Leaves	Fruit		Leaves	Leaves	Leaves Flowers Leaves	Leaves Leaves Leaves	Leaves Leaves Leaves Leaves
Family		Piperaceae	Solanaceae	Rutaceae		Myrtaceae	Myrtaceae	Myrtaceae Myrtaceae Myrtaceae	Myrtaceae Myrtaceae Myrtaceae	Myrtaceae Myrtaceae Myrtaceae Malvaceae
Common		Betel nut	Thorn apple, Angel's trump-et	Pomelo		Guava	Guava	Guava Clove Nilgiri	Guava Clove Nilgiri	Guava Clove Nilgiri Neem Bala
Biological Source		Piper betle	Datura stramonium	Citrus grandis		Psidium guajava	Psidium guajava Syzygium aromaticum	Psidium guajava Syzygium aromaticum Eucalyptus globulus	Syzygium aromaticum arealyptus globulus Azadirachia indica	Syzygium aromaticum aromaticum globulus globulus Azadirachta indica
S. No.		44	45.	46.		47.	47.		49.	49.

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Refer-	ences	[58]	[34]	[30]	[19]	[19]	[59]	[21]
Targeted mosquito		Culex quinquefasciatus Aedes aegypti Anopheles stephensi	Anopheles stephensi Culex quinquefasciatus	Aedes aegypti Aedes albopictus Anopheles dirus Culex quinquefasciatus	Aedes aegypti	Aedes aegypti	Anopheles stephensi Aedes aegypti Culex quinquefasciatus C. Tritaeniorhynchus	Aedes aegypti Aedes albopictus
Activity	Larvicidal Repellent  / Max. Protection Period (min/hr)	120 min (5 mg/cm²) 150 min 180 min	150 min (500ppm) 150 min	2.5 hr 7.7 hr 8 hr 7.5 hr	6 hr (20%)	6 hr (20%)	210 min 180 min 150 min 120 min	64.34 min (10%)
<b>d</b> .	LC <sub>50</sub> /	LC <sub>50</sub> -96.15 ppm LC <sub>50</sub> -89.59 ppm LC <sub>50</sub> -79.08 ppm (Benzene extract)	LC <sub>50</sub> -53.94 ppm LC <sub>50</sub> -46.61 ppm (methanol extract)		1	1	LC <sub>50</sub> -61.65 ppm LC <sub>50</sub> -67.13 ppm LC <sub>50</sub> -72.45 Ppm LC <sub>50</sub> -78.87 Ppm	1
Analytical	Technique		GC-MS	29	GC-MS	GC-MS	GC-MS	ı
Extraction	Method	Steam distillation	Hydro-distillation	Steam	Hydro- distillation	Hydro- distillation	Hydro- distillation	Hydro- distillation
Major	Constituents with %			(E)-3,7-dimethyl, 2,6-octadienal (75,56%), 6-methyl- 5-hepten-2- one (7,92%)			Carvacrol (48,2%) etimol (16,6%), p – Cymene (3,9%), y-Terpinene (3,6%)	Estragol (58,26%), limonene + β-fellandrene(1 9,41%),fenchone(10.1%),α- Phellendrene (4, 37%)
Parts	Osed	Leaves	Leaves	Leaves	Leaves	Leaves	Leaves	Leaves
Family		Apocynaceae	Leguminosae	Lauraceae	Lamiaceae	Lamiaceae	Lamiaceae	Lamiaceae
Common	Name	Nanthiyavattai	Senna siamea	Mountain Pepper	Tulsi	Indian borage	Ornamental plant	Niazbow
S. Biological	Source	Ervatamia coronaria	Cassia siamea	Litsea cubeba	Ocimum sanctum	Plectranthus amboinicus	Origanum scabrum	Ocimum basilicum
S.	o Z	54.	55.	56.	57.	58.	59.	.09

	Refer-	والرق	[09]	[61]	[29]	[13]	[62]	[23]	[20]
	Targeted mosquito		Culex pipens	Aedes albopictus Aedes albopictus	Culex quinquefasciatus Aedes aegypti	Aedes aegypti	Culex quinquefasciatus Aedes aegypti	Anopheles stephensi Aedes aegypti Culex quinquefasciatus	Aedes aegypti
	Activity	Larvicidal Repellent  // Max. Protection Period (min/hr)	significant repellency at 0.005mg/cm² conc.	20 min 20 min	150 min (2.5 mg/cm²) 150 min (ethyl acetate extract)	90 min (90%)	120 min 120 min	180 min (5 mg/cm²) 180 min 150 min	7.5 hr (hexane extract)
		LC <sub>50</sub> /	1			1	LC <sub>50</sub> - 130ppm LC <sub>50</sub> - 140ppm		
	Analytical	Technique	GC/ MS	GC-FID GC/ MS	25	GC/ MS	25	1	GC/ MS
	Extraction	Method		Hydro- distillation	Hydro- distillation	Hydro- distillation	Steam	Hydro- distillation	Steam
	Major	Constituents with %	Sabinene (50,8%) $\beta$ -myrcene (13,2%), limonene (12,2%), cyclohexanol(6,9%)	Ent-kaur-16-ene 3- carene	·	Limonene(66,3%), α- Pinene(11, 8%) (E)- Ocimenone (19,1%)	3,5-di-te-rebutil-4-hidroxi benzaldeido (24,9%),benze no, 2-(1,1-dimetiletil)-1,4- dimetox i(22,54%).	· ·	3-N-Butylphthalide (25.81%), Butylidenephthalide (50.71%),β- espatulenol(2.52%), Ligustilide (1.92%)
	Parts	Used	Вепу	Leaf	Leaves		Flowers	Root	Rhizome and root
	Family		Cupressaceae	Cupressaceae	Compositse	Compositse	Compositse	Asparagaceae	Apiaceae
	Common	Name	Aaraar, Haubera	Japanese Cedar, ,Dhupi Sallaa	Bhringraj	Wild Marigold	East Indian globe thistle	Shatavari	Ginseng
Table 1: Con't	Biological	Source	Juniperus communis	Cryptomeria japonica	Eclipta alba	Tagetes minuta	Sphaeranthus indicus	Asparagus racemosus	Angelica sinensis
Table	v,	o Z	62.	63.	64.	65.		67.	

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Refer- ences		[26]	[64]	[29]	[13]	[13]	[33]	
Targeted mosquito		Aedes aegypti	Aedes aegypti Culex quinquefasciatus	Culex quinquefasciatus Aedes aegypti	Aedes aegypti	Aedes aegypti	Aedes aegypti	Culex quinquefasciatus
Activity Larvicidal Repellent	Max. Protection Period (min/hr)	150 min	88.7 min 126.7 min	210 min (5 mg/cm²) 180 min (methanol extract)	60 min (90%)	90 min (90%)	$\begin{array}{c} 1 \ h^1 \\ 2 \ h^2 \end{array}$	1 h <sup>1</sup> $2 h^{2}$ (0.5 mg/cm <sup>2</sup> )
Larvici	LC <sub>50</sub> /	LC <sub>50</sub> - 13.22 Ppm	1	1			LC <sub>50</sub> - 668ppm LC <sub>50</sub> - 761ppm	LC <sub>50</sub> - 355ppm LC <sub>50</sub> - 437ppm
Analytical Technique		1	25	25	GC- MS	GC- MS	1	
Extraction Method		Hydro- distillation	Hydro- distillation	Hydro- distillation	Hydro- distillation	Hydro- distillation	Steam	
Major Constituents with %		D -limoneno (80%)	Linalool (21,08%), Linalool acetate (16,14%), α-pine- ne(12,73%), Eugenol(8,86%)		α-ascaridol (99,4%) b-Curcumene (0,1%)	Camphor (33.6%), verbenone (24.9%), (E)-Caryophyllene (14.8%), borneol, bornyl acetate, α-terpineol, terpinen-4-ol		
Parts Used		Seed	1	1	1	Flowers	Leaf <sup>a</sup> Stem <sup>2</sup>	
Family		Apiaceae	Annonaceae	Acanthaceae	Amaranthaceae	Lamiaceae	Amaranthaceae	
Common		Root celery	Ylang-Ylang tree	Creat or green chiretta	Sugandha vastuka	Gulmehendi	Aghara	
S. Biological No. Source		Apium graveolens	Cananga odorata	Andrographis paniculata	Dysphania ambrosioid-es	Rosmarinus officinalis	Achyranthes aspera	
s. No.		70.	71.	72.	73.	74.	75.	

## CONCLUSION

Mosquitoes are majorly responsible for transmitting mosquito-borne diseases like malaria, dengue, chikungunya, zika, yellow fever and others. Prolong use of harmful chemical insecticide not only cause development of resistance in mosquitoes but also have shown adverse effects on humans as well as on environment. Plant extracts, essential oils and their chemical constituents also shown optimal repellent potential against various mosquito species. In addition, the synergistic combinations of EOs have also found to be effective against broad range of mosquitoes with least side-effects as well as combining these individual EOs with vanillin increases the total protection period against mosquitoes. Further research is required in the arena of EOs combination, to find most effective mosquito repellent combination against dengue and malaria causing mosquitoes and that can also have capability to be formulated as mosquito repellent preparations in future too.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## **ABBREVIATIONS**

ITNs: insecticide-treated nets; LLINs: Long-Lasting Insecticide-Treated Nets; DDT: Dichlorodiphenyltrichloroethane; DEET: N,N-diethyl-mtoluamide, IR3535: 3-(N-Butyl-N-acetyl)-amino propionic acid); EO: Essential oils; GC: Gas Chromatography; GC-MS: Gas Chromatography-Mass Spectroscopy; NH3: Ammonia; IRS: Indoor residual spraying; DEPA: Diethyl Phenyl Acetamide; LC<sub>50</sub>: Lethal Concentration; RD50, 95: Repellent doses; WHO: World Health Organization; PT: Percentage of Repellency; µL/cm2: Microlitre per centimeter square; µL/mm2: Microlitre per millimeter square; mm: Millimeter; PR: Percentage repellency; min: Minutes; %: Percentage; Hr: Hours; α: Alpha; Mg: Microgram; cm2: Centimeter square; mL: Millilitre; µL: Microliter; CSIR: Council of Scientific and Industrial Research; GC-EIMS: Gas Chromatography with Electron Impact Mass Spectrometry; FID: Flame Ionization Detector: Conc.: Concentration; %w/v: per cent weight by volume; Fig.: Figure; Gm: Gram; Mg: Milligram; PPM: Parts Per Millions; Sec: Seconds; %w/w: per cent weight by weight; mm: millimetre; v/v: volume by volume; DBT: Department of Biotechnology; JRF: Junior Research Fellow.

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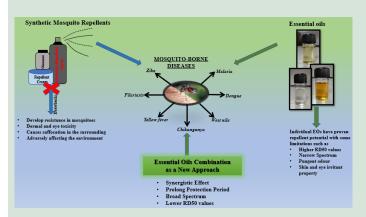
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#### **GRAPHICAL ABSTRACT**



#### **SUMMARY**

- Mosquitoes are responsible for spreading of vector-borne diseases.
- EO of particular plant is mostly extracted by hydro-distillation and steam distillation.
- GC/MS, GC-EIMS, GC-FID are the techniques used for identification of EO components.
- The EO combinations are also used as effective mosquito repellent.
- Due to provided synergism as well as negation of harmful effects associated with individual oils.
- Incorporation of vanillin also enhances the efficacy and maximize the protection period provided by EOs.

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