# Repellency Effect of *Artemisia vulgaris* Essential Oil against *Aedes aegypti* Mosquitoes in Laboratory Condition

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The mosquito species *Aedes aegypti* L. is a major vector of dengue fever (DF), dengue haemorrhagic fever (DHF), Chikungunya, yellow fever and Zika fever. Dengue virus transmission (serotypes 1-4) by *Aedes* mosquitoes is a public health problem that principally affects tropical countries. Myanmar is one of the DF and DHF endemic areas in Southeast Asia Region. In mosquito control programs, botanical origin insecticides (pyrethrum, rotenone, sabadilla etc.) have the potential to be used as ovicidal, larvicidal and adulticidal of eggs, larvae and adult mosquitoes. *Artemisia vulgaris* (derived from Taunggyi Township, Shan State) dried leaves powder was subjected to hydro-distillation in a Clevenger-type distilling apparatus. The resulting oil was dried over anhydrous sodium sulphate and resulting 8 gm of essential oils were obtained from 100 gm of dried leaves powder. Mosquito repellency assay was done to test the efficacy of the essential oil against *Aedes aegypti* on human volunteers. *Artemisia vulgaris* essential oil applied on average 342 $cm^2$ area of arm for 100% protection against *Aedes aegypti* mosquitoes landing to probe the skin was found with the dose 0.08 g/ml or 0.0002 $g/cm^2$. Repellency activity of complete protection time of *Artemisia vulgaris* oil dose 0.0002 $g/cm^2$ provided 97.62% protection for 30 minutes, 92.86% protection for 60 minutes and 85.71% protection for 120 minutes against *Aedes aegypti*. *Artemisia vulgaris* leaves essential oil did not cause dermal irritation when applied to human skin. No adverse effects on human volunteers were observed after application. Essential oil of *Artemisia vulgaris* leaves was found to have very effective protection from biting of *Aedes aegypti*.

*Key words*: Repellency, Dilution, Protection, *Artemisia vulgaris*, Essential oil

## INTRODUCTION

Each year, an estimated 50 to 100 million new cases of dengue occur around the world, of these, 500,000 cases correspond to dengue haemorrhagic fever with mortality of 5% (25000 cases).[1, 2] The WHO initiative for vaccine Research (IVR), in collaboration with a wide range of partners, aims to facilitate the development and future introduction of safe, effective and affordable dengue vaccines.[3] The mosquito species *Aedes aegypti* L. is a major vector of dengue fever (DF), dengue haemorrhagic fever (DHF), Chikungunya, Yellow fever and Zika fever. Dengue virus transmission (serotypes 1-4) by *Aedes* mosquitoes is a public health problem that principally affects tropical countries. Dengue haemorrhagic fever outbreaks were recognized as new diseases first in Myanmar in 1970 caused by dengue type one.[4] Dengue fever and dengue haemorrhagic fever are increasingly becoming serious public health

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problems especially among the 5-10 and 11-15 years old age groups and now noted 15 years above, a vast majority of the cases occur in 5-8 years old age group.[5, 6] Almost half of the global population lives in high-risk areas and currently more than 100 countries experience dengue fever and dengue haemorrhagic fever.[7]

Rapid, poorly planned urbanization in association with weak regulatory policies for discharge of solid waste has resulted in the accumulation of discarded containers in most developing countries. These accumulations have favored the establishment and geographic spread of this mosquito. The strategies to control dengue transmission used by the public vector control programs have not been adequate in most countries. The emergence of insecticide resistance, the difficulty of eliminating larval population through environmental sanitation and lack of efficacy of ultra-low volume insecticide spraying to control adults are factors which have limited the effectiveness of vector control programmes.[8] The indiscriminate use of synthetic insecticides is creating multifarious problems like environmental pollution, insecticide resistance, and toxic hazards to humans.

Globally, there have been conscientious efforts to overcome these problems, and great emphasis has been placed recently on environment friendly and economically viable methodologies for pest control. Phytochemicals obtained from the huge diversity of plant species are important source for safe and biodegradable chemicals, which can be screened for mosquito repellent, larvicidal and insecticidal activities. Large numbers of plant products have been reported to have mosquito larvicidal and repellent activity against adult mosquitoes.[9] In recent years essential oils have received much attention as potent bioactive compounds against various mosquitoes species.[10] *Artemisia vulgaris* L. is a member of the Asteraceae family. It is a tall (1-1.5 meter) aromatic, threatened perennial herb distributed throughout the northern temperature regions of Africa, Asia, Europe, India, Myanmar and North America. In Myanmar *Artemisia vulgaris* L. plants are abundantly present in Chin, Kachin and Shan State. In traditional medicine, this plant is widely used for the treatment of diabetes and extract of the whole plant is used for epilepsy and in combination for psychoneurosis, depression, irritability, insomnia and anxiety states.[11]

It is necessary to find out more potential natural product in plants pest. Synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air.[12] Therefore, there is a need to find out the alternative ways for environmental safety, biodegradable, cost effective and indigenous methods to prevent mosquito bites. Recently increased interest is developing plant origin repellency effects as an alternative to chemical repellency. The study was undertaken to assess the repellent potential of essential oil of *Artemisia vulgaris* L. against *Aedes aegypti* mosquitoes.

## MATERIALS AND METHODS

*Aedes aegypti* mosquito larvae and adult *Aedes* mosquitoes emerged from pupae were collected from Hlinethaya Township were reared in laboratory of Medical Entomology Research Division, Department of Medical Research. Larvae were fed on DMR larva food. Adults were provided with 10% sucrose solution and 8 weeks old mouse for blood meal. Mosquitoes were held at 26±2ºC, 65-75% relative humidity with a photo period of 12-hour light and 12-hour dark. Mosquitoes were reared continuously in laboratory for repellency test. Laboratory-reared 3-5 days old mosquitoes were used for repellency of essential oil of *Artemisia vulgaris.*

### *Mosquitoes species identification*

Larvae and adult mosquitoes emerged from larva survey were identified by morphological methods.[13]

*Collection and preparation of extraction of Artemisia vulgaris leaves essential oil*

*Artemisia vulgaris (*Myanmar name: Shanmanaing leaves) were collected from Taunggyi Township, Shan State. A total of 10 kilograms of *Artemisia vulgaris* leaves were cleaned and dried in shed place at room temperature for 30 days. Dried leaves were ground by grinding machine to make powder and finely ground 100 grams of dried leaves powder were mixed with 1000 ml distilled water and subjected to hydro-distillation in a Clevenger-type distilling apparatus for two hours. The resulting oil was dried over anhydrous sodium sulphate and resulting 8 gm of essential oils was obtained from 100 gm of dried leaves powder. The essential oil was stored in airtight fuscous glassware in a refrigerator at 4ºC until use. The extraction was done in Department of Botany, Yangon University.

*Repellent activity testing*

The repellent study was done according to the method of World Health Organization.[14] Three to five days old blood starved 50 numbers of female *Aedes aegypti* mosquitoes were kept in a steel net cage (59 x 59 x 59 cm). The volunteer had no contact with lotions, perfumes, or perfumed soaps on the day of the assay. Using a pipette, 1 ml of ethyl alcohol (95%) diluent used in the preparation of the test repellent was applied evenly to average 342 $cm^2$ of forearm skin between the wrist and elbow of the volunteer and allowed to dry 1 minute. Before insertion of arm into the cage containing 50 *Aedes* female mosquitoes, the hands were protected by plastic gloves to protect mosquitoes bite. This procedure was done according to WHO.[14]

The first step, ethyl alcohol applied forearm was inserted into the cage and the number of mosquitoes that landed on the skin during a 30-second period was counted. The control forearm was carefully withdrawn and this arm was then treated with 1 ml of 0.1 g/10 ml of *Artemisia vulgaris* essential oil solution and allowed to dry. The treated arm was placed in the cage for another 30 seconds period and observed for mosquito landing. This procedure was repeated for each additional incremental of *Artemisia vulgaris* oil dose. The tests were carried out one after the other without delay and *Artemisia vulgaris* oil dose at each test was calculated as the sum of the doses applied to arrive at the cumulative dose for each test. Test was proceeded until the mosquito landing rate on the exposed forearm was less than 10 females in 30 seconds. Two trained technicians recorded the number of landings. At the conclusion of the dose response experiment, 1 ml of ethyl alcohol was applied on the other forearm and allowed to dry. This forearm was inserted in the cage for 30 seconds to verify that the number of landings was more than 10 per 30 seconds as was observed at the beginning of the experiment.

*Estimation of complete protection time*

The complete protection time of *Artemisia vulgaris* oil extract was determined when 99 to 100% protection dose (0.08 g/ml) was used on 342 $cm^2$ area of forearm skin between the wrist and elbow. Four mosquito cages (size 59 x 59 x 59 cm) each containing 50 non blood fed 5 days old *Aedes aegypti* female mosquitoes were normally used. Two cages were used for testing two female volunteers and another two cages were used for testing two male volunteers. Same procedure was followed as above. In the first step, ethyl alcohol applied forearm was inserted into the cage and the number of mosquitoes that land on the skin during a 3-minute period was counted. The control forearm was carefully withdrawn from the cage. Then, 0.08 g of *Artemisia vulgaris* oil extract, prepared in 1 ml of ethyl alcohol solution was applied evenly on 380 $cm^2$ of another forearm skin between the wrist and elbow. The treated arm was placed in and mosquito landing was observed.

After 30 minutes, the 3-minute period *Artemisia vulgaris* oil repellent treated arm was inserted again into the cage and exposed for 3 minutes to determine landing activity. This procedure was repeated at

30 minutes intervals for 180 minutes and the procedure was used consistently throughout the experiment. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood. Complete protection time was estimated after experiment. Same repellency test procedure was followed on other three volunteers. As *Aedes aegypti* is a day time biter, test was done between 08:00 hours and 16:00 hours. Tests were done in 15 x 10 x 10 ft room, at 25-27ºC and relative humidity of 60-80%.

*Data analysis*

Data entry and processing was made using Microsoft Excel software. The average repellency data were subjected to calculating percentage protection of mosquito bite.

P=1-(T/C)=(C-T)/C

Protection (P) was expressed as a proportion of the number of mosquitoes landing on treated arm, (T) in relation to the number of landings on the control arm, (C) of the same individual. C was the average landing on two untreated arms.

## RESULTS

Table 1. Experiment of successive *Artemisia vulgaris* oil doses applied to arrive at a cumulative dose for *Aedes aegypti*

| A | B | C | D | E |
|---|---|---|---|---|
| Left arm control average area 342 $cm^2$ | 1 ml ethyl alcohol | 0 gm | 18±1.83 | Average control (Left+Right) 17.5 (C) |
| Left arm dose 1 | 0.01 | 0.01 | 4±0.82 | 77.14 |
| Left arm dose 2 | 0.01 | 0.02 | 3±0.82 | 82.86 |
| Left arm dose 3 | 0.02 | 0.04 | 2±0.82 | 88.57 |
| Left arm dose 4 | 0.04 | 0.08 | 0 | 100 |
| Right arm control | 1 ml ethyl alcohol only | 0 | 17±1.41 | |

A=Application sequence, 4 replicates
B=Repellent solution concentration to be applied in 1 ml (g/ml), frequently added
C=Cumulative amount of repellent (g/342 $cm^2$ area) (Total amount)
D=Average mosquito landing to probe the skin
E=% protection [P=1-(T/C)=(C-T)/C]

Successive cumulative dose of *Artemisia vulgaris* essential oil applied on 342 $cm^2$ area of arm for 100% protection of *Aedes aegypti* mosquito landing to probe the skin was found to be dose of 0.08 g/ml or 0.0002 g/$cm^2$ as shown in Table 1.

Table 2. Repellency test of *Artemisia vulgaris* oil 0.08 g/ml on 4 volunteers' arms in laboratory

| Replicates (Repellent applied areas) | Control* | Duration of repellency test (*Artemisia vulgaris* oil 0.08 gm in 1 ml of ethylalcohol) & No. of mosquito bite | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F |
| Male 1 (362 $cm^2$) | 16 | 1 | 2 | 3 | 5 | 7 | 9 |
| Male 2 (359 cm) | 17 | 0 | 1 | 2 | 2 | 4 | 7 |
| Female 1 (325 $cm^2$) | 22 | 1 | 2 | 2 | 3 | 6 | 7 |
| Female 2 (321 $cm^2$) | 29 | 0 | 1 | 2 | 2 | 4 | 7 |
| Total bite (Average 342 $cm^2$) | 84 ±5.94 | 2 ± 0.58 | 6 ±0.58 | 10 ±0.5 | 12 ±1.14 | 19 ±1.5 | 30 ±1 |

A=30 minutes (No. of mosquito bite)
B=60 minutes (No. of mosquito bite)
C=90 minutes (No. of mosquito bite)
D=120 minutes (No. of mosquito bite)
E=150 minutes (No. of mosquito bite)
F=180 minutes (No. of mosquito bite)
*=3 minutes (No. of mosquito bite)

Table 3. Estimation of complete protection time of *Artemisia vulgaris* essential oil repellent against *Aedes aegypti*

| Dose 0.0002 g/$cm^2$ | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| Control* | 84 | - | - | - | - | - | - |
| Percentage** | 0 | 2 (97.62) | 6 (92.86) | 10 (88.10) | 12 (85.71) | 19 (17.38) | 30 (64.29) |

A=0 minute (No. of mosquito bite)
B=30 minutes (No. of mosquito bite)
C=60 minutes (No. of mosquito bite)
D=90 minutes (No. of mosquito bite)
E=120 minutes (No. of mosquito bite)
F=150 minutes (No. of mosquito bite)
G=180 minutes (No. of mosquito bite)
*=Total number of bite (4 replicates)
**=Percent protection of mosquito bites (4 replicates)

Repellency activity of complete protection time of *Artemisia vulgaris* oil dose 0.0002 gm/$cm^2$ provided 85.71% protection for 120 minutes against *Aedes aegypti* and over 90% protection was found for 60 minutes, i.e. 97.62% protection for 30 minutes and 92.86% protection for 60 minutes (Table 2 & 3). The essential oil of *Artemisia vulgaris* leaves was found to have very effective protection from biting of *Ae. aegypti* mosquitoes.

## DISCUSSION

Insecticide residues in the environment as a result of chemical insecticide usage have turned the researcher's attention towards natural products. In the past years, the plant kingdoms synthesize a variety of secondary metabolite which plays a vital role in defense of plants against insects/ mosquitoes. Mosquito repellents may be one of the most effective tools for protecting human from vector-borne diseases and nuisance caused by mosquitoes. Natural products are safe for humans when compared to that of synthetic compounds. Other researchers revealed that the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health.[15] Different parts of the plants contain a complex of chemicals with unique biological activity[16, 17] which is thought to be due to toxins and secondary metabolites which act as mosquitocidal agent.[18] Smoke is still, the most widely used common methods of repelling biting insects that is used throughout the world.[19]

Most households in the developing world rely on personal protection measures of limited effectiveness, such as burning mosquito coils or leaves. The bundles of dried *Artemisia vulgaris* L. were burned to repel biting insects since they contain insect repellents that can be released by combustion. The larvicidal, pupicidal, adulticidal, and repellent activities of *Artemisia nilagirica* against the mosquitoes, *Anopheles stephensi* and *Aedesa egypti*, were suggested that the leaf extract was used as a potent larvicidal agent.[20] *Artemisia* extracts contain secondary metabolites mainly monoterpenoids such as vulgarole, spathulenol, vulgarin, triterpenoide: α-amyrin, α-amryin acetate and fernenol.[21]

The insecticidal properties of *Artemisia vulgaris* and other plants of the genus *Artemisia* have been attributed to the presence of these secondary metabolites. The compounds isolated from *Artemisia vulgaris* were mainly monoterpenoids such as linalool, camphor, isoborneol, borneol, terpinen-4-ol, isobornyl, nonanone-3, (α+β)-thujone, bornyl acetate, β-pinene, myrcene, α-terpinene, limonene, and cineote. These compounds were reported as effective repellent compounds against *Aedes aegypti*.[22]

Repellency activity of complete protection time of *Artemisia vulgaris* oil possessed significant repellent activity against *Aedes aegypti*. The dose 0.0002 g/cm$^2$ provided 85.71% protection for 120 minutes against *Aedes aegypti* and over 90% protection was found for 60 minutes i.e. 92.86% protection for 30 minutes and 97.62% protection for 60 minutes, respectively. Although it exerted an effective biting protection time against *Aedes aegypti,* it is lower than the protection time of currently used synthetic compounds such as DEET, A13-37220, A35765 and CIC-4.[23, 24]

These chemical compounds provide better and longer protection against many biting insects (ED50 and ED95 level=0.37-25.37 µg/cm$^2$, 3-8 hours). Repellent protection time in laboratory may change depending on the biological characteristics of the mosquito test population. Different in species and body size, sugar water availability, adult density in test cages, and mosquito age can affect test results.[25-27]

Mixture of 5% vanillin and essential oil of *Curcuma longa* found effective repellent activity for 8 hours against *Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus*.[28] Other researchers also observed that the repellent activity of methanol extract of *Ferronia elephantum* leaves against *Aedes aegypti* activity at 1.0 and 2.5 mg/cm$^2$ concentrations gave 100% protection up to 2.14±0.16 hours and 4.00± 0.2.4 hours, respectively and the total percentage protection was 45.8% at 1 mg/cm$^2$ and 59.0% at 2.5 mg/cm$^2$ concentrations for 10 hours.[29] These results revealed that essential oil of *Artemisia vulgaris* plant

extracts was very effective to prevent *Aedes aegypti* mosquitoes bite i.e. 85.71% protection for 120 minutes and over 90% protection for 60 minutes in day time.

*Conclusion*

From these results, the study concluded that the essential oil of *Artemisia vulgaris* leaves exhibits repellent activities against dengue vector mosquitoes. More studies are needed to elucidate the ovicidal activity against a wide range of mosquito species. The active compound responsible for repellent activity should be identified, which could be used to control different mosquito species in the future. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

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