

Repellent Activity of Catnip Extract (*Nepeta cataria L.*) Against *Aedes aegypti* Mosquito as Dengue Vector

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ABSTRACT

Introduction: Dengue infection has been a major concern for decades. Prevention from getting bitten by *Aedes aegypti* mosquito is considered better than cure as there is no specific antiviral treatment for dengue. Recently, catnip (*Nepeta cataria L.*) extract has been formulated and marketed as an alternative for protection against mosquitoes and act as a natural form of mosquito repellent.

Objectives: To understand the repelling properties of both DEET and *N. cataria L.* extract and to compare the repelling properties between DEET and *N. cataria L.* against *Ae. aegypti* mosquito.

Methods: The repelling properties was tested based on the number of mosquitoes that sits on the hand of the respondent after being applied to the gold standard of mosquito repellent, DEET, and compared to *N. cataria L.* extract in different concentrations in total of 15 minutes.

Results: Three different concentrations of catnip extract (10%, 30% and 50%) shows different efficacy in repelling *Ae. aegypti* mosquitoes at the range of 62.8%-80%, although the repellent activity is still lower than DEET that have 100% repellent activity.

Conclusion: *Catnip (N. cataria L.)* showed mosquitoes repellent properties at concentration 10-50% with repellent activity at the range 62.8%-80%.

Keywords: Dengue, *Aedes aegypti*, repellent, DEET (N,N- Diethyl-m-toluamide), catnip (*Nepeta cataria L.*) extract.

INTISARI

Pendahuluan: Infeksi Dengue merupakan masalah utama selama berpuluh tahun. Pencegahan dengan menghindari gigitan nyamuk *Aedes aegypti* dianggap lebih baik daripada mengobati karena tidak ada terapi antiviral yang spesifik untuk dengue. Akhir-akhir ini, ekstrak *catnip (Nepeta cataria L.)* telah diformulasi dan dipasarkan sebagai alternatif untuk perlindungan terhadap nyamuk dan berlaku sebagai bentuk alami dari repelen.

Tujuan: Untuk memahami agen penolakan dari N,N- Diethyl-m-toluamide (DEET) dan ekstrak *N. cataria L.* extract dan membandingkan agen penolakan antara DEET dan *N. cataria L.* terhadap nyamuk *Ae. aegypti*.

Metode: Agen penolakan diuji berdasarkan jumlah nyamuk yang mendarat di tangan responden setelah diolesi repelen nyamuk baku DEET, dan dibandingkan dengan ekstrak *N. cataria L.* pada berbagai konsentrasi selama total waktu 15 menit.

Hasil: Tiga konsentrasi ekstrak catnip (10%, 30% and 50%) menunjukkan daya penolakan nyamuk *Ae. aegypti* yang berbeda pada rentang 62.8%-80%, meskipun aktivitas penolakan nyamuknya masih lebih rendah dari DEET yang mempunyai aktivitas penolakan 100%.

Simpulan: Ekstrak *Catnip* (*N. cataria L*) menunjukkan daya penolakan nyamuk pada konsentrasi 10-50% dengan aktivitas repelant antara 62.8%-80%.

Kata kunci: Dengue, *Aedes aegypti*, repellent, DEET (N,N- Diethyl-m-toluamide), ekstrak catnip (*Nepeta cataria L.*).

INTRODUCTION

Dengue is a mosquito-borne infection which causes flu-like disease and may develop to the fatal dengue hemorrhagic fever (DHF). Dengue is most commonly found in tropical and subtropical climate areas in the world, mainly in the urban and suburban areas¹.

Dengue hemorrhagic fever is a topic constantly talked about in the mass media. This

is because DHF occurs almost throughout the year and does not have a season on its own. Asia is in the first place for the highest number of DHF patients every year. This may be due to the downpour in Asia and also the lack of sanitation, and more than 500,000 from 50 million cases of DHF require hospitalization².

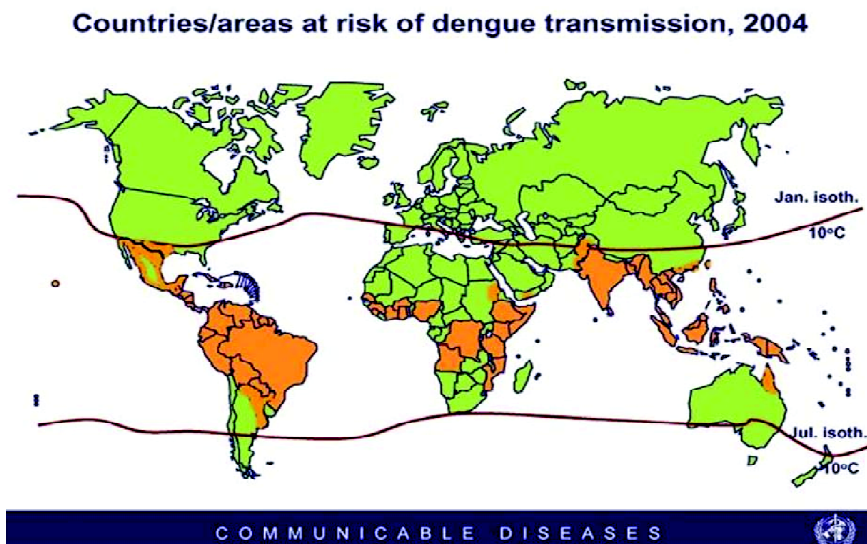


Figure 1: Countries or areas at risk of dengue transmission³.

Dengue in Indonesia was first found in Surabaya in the 1968, but the virologic confirmation was made only by 1972. Since then, dengue has spread to many regions⁴. Indonesia

is one of the top ten countries in South East Asia that has regular yearly reported incidents of dengue or DHF⁵.

Dengue is transmitted by the bite of an

Aedes mosquito which has already been infected with one of the four strains of dengue viruses. Dengue virus is from Flavivirus genus and has 4 serotypes which are, DEN-1, DEN-2, DEN-3, and DEN-4. Virus DEN-3 is the most common circulating serotype. Recovery from an infection strain provides a lifelong immunity against that virus but confers only partial and transient protection against subsequent infection by the other three. It is also said that sequential infection increases the risk of developing DHF⁶.

Dengue virus is single-stranded RNA virus. It is transmitted through the bite of an infected female *Aedes* mosquito. Once infected, the female mosquito can transmit the virus for the rest of its life during probing and feeding. Humans are the main carriers and multipliers of the virus as they can transmit the virus to another uninfected *Aedes* mosquito¹.

Up till now, the specific treatment against dengue virus such as antiviral therapy and vaccines are not available. So, prevention depends on control of and protection from the the mosquito's bites. Mass prevention could be done with cooperation of community and primary health center. The vector control is implemented through environmental and chemical methods. Community based programs such as educating people on the proper solid disposal, improved water storage practices which includes covering containers to prevent access by egg laying female *Aedes* mosquito has been done. Local authority also takes effort to do public spraying of insecticides or known as fogging and larva eradication¹.

However, these social campaigns do not produce satisfactory results due to the lack of social awareness and ignorance of the people.

Due to this, there is a need to develop a more natural based mosquito repellent that should not only be effective but safe to be used.

Nepeta cataria L. commonly known as catnip or catswort or catmint, is a species of genus *Nepeta* in the Lamiaceae family⁷. *Nepeta cataria* L. is a herbaceous perennial, growing 2–3 feet (61–91 cm) tall and wide. It resembles mint in appearance, erect, branched, square, grayish stems are clad with aromatic, opposite, coarsely-toothed, triangular to ovate, gray-green leaves (to 3" long). Leaves and stems are downy which gives them its gray-green appearance. Small, two-lipped, white flowers bloom in spike-like terminal clusters at the stem ends from late spring well into summer.

As a medicinal herb, catnip is used (fresh or dried) for making herbal tea that reportedly reduces anxiety, induces sleep, promote perspiration (fever or cold relief), it act as cough suppressant and comfort upset stomachs. As a garden plant, catnip acts as a repellent for certain insects.

Nepetalactone is the active ingredient in the catnip that affects cats, inducing a state of euphoria and stupor, which can be extracted by steam distillation from the stems and leaves⁷. To harvest catnip, stems are cut to the ground and hung upside down and cured in a warm place with good air circulation. When leaves are totally dry, then they can be used for variety of purposes.

N,N-diethyl-3-methylbenzamide (DEET), is the most effective, and best studied mosquito repellent currently in the market. This substance has a remarkable safety profile after 40 years of worldwide use, but toxic reaction still can occur if misused. DEET is an all purpose insect repellent which contains a minimum of 95% of the meta isomer, the most

effective form of diethyl toluamide, as a technical active ingredient⁸. N,N-diethyl-3-methylbenzamide (DEET) was first developed by scientists at the U. S. Department of Agriculture and patented by the U. S. Army in 1946. It was registered for use by the general public in 1957⁹.

N,N-diethyl-3-methylbenzamide (DEET) is a nearly colorless to faintly yellow liquid with an aromatic characteristic odor. It is relatively stable, highly hygroscopic and sensitive to light. Technical DEET is practically insoluble in water and glycerin but miscible with several organic solvents⁸. It is used primarily by dermal application to repel mosquitoes, flies, fleas, ticks, leeches. It is commercially available at concentration of 4% to 100% in formulations including lotions, creams, gels, aerosols, and pump sprays, usually with an ethyl or isopropyl alcohol base. N,N-diethyl-3-methylbenzamide (DEET) can enter the environment via its release into waste streams during its production and use. In the atmosphere, it exists in the vapor phase and is degraded by reaction with hydroxy radicals that produced photochemically. Its atmospheric half-life is approximately 15 hours. N,N-diethyl-3-methylbenzamide (DEET) has moderate mobility and is not expected to volatilize in moist or dry soil or to biodegrade under either aerobic or anaerobic conditions¹⁰.

Exposure to DEET can occur via ingestion, inhalation, or dermal contact. In humans, reported symptoms of overexposure include seizures, coma, hypotension, bradycardia, confusion, acute psychosis, abdominal pain, nausea and vomiting, skin irritation, and urticaria or contact rash. Veterans who used DEET-containing insect repellents showed signs of arthromyoneuropathy, a neurotoxic syndrome with symptoms including joint and muscle pain, fatigue after exertion, and tingling or numbing of the hands, arms, feet, and legs¹⁰.

In animals, DEET is absorbed rapidly from the skin and cleared rapidly from blood. It is metabolized in the liver and excreted rapidly in the urine; fecal elimination is minimal.

The aims of this study were to analyse the repelling properties of DEET and Catnip plant extract (*N. cataria L.*) against *Ae. aegypti* and to know the difference in the repelling properties between Catnip extract (*N. cataria L.*) and DEET against *Ae. aegypti*.

MATERIALS AND METHODS

This research was conducted through a simple experimental study in a laboratory setting, to compare the repellent efficacy of Catnip extract (*N. cataria L.*) in comparison to DEET against *Ae. aegypti* mosquito.

The subject used for this experiment is adult female *Ae. aegypti* aged 3 to 5 days old which were taken from Department of Parasitology, Universitas Gadjah Mada.

This research was conducted in Parasitology Laboratories of Faculty of Medicine, Universitas Gadjah Mada. The *N. cataria L.* leaf that was plucked from the plant was purchased from local market. The leaves were then washed, chopped and dried. Around 1.0kg of catnip leaves was obtained. Then, the leaves were heated in an oven at 50 to 60°C to ensure all the water inside the leaves has evaporated. The leaves were then grinded to obtain the powder form. The catnip leaves powder was then macerated using 50% ethanol solution at a ratio of 1:4 (powder: solvent). The mixture is then poured into an air-tight container and left to rest at room temperature for 48 hours. This mixture is then filtered to obtain the extract. The filtrate is then transferred to porcelain bowl and left to air dry for few days to enable the evaporation of

solvent. The substance left now in the porcelain bowl is the extract of catnip plant that will be used in the experiment. The final concentrated extract is kept in a screw-up vial and the mouth of the vial is covered with aluminium foil¹¹. The extract is then prepared in three different concentrations (10%, 30% and 50%) by Faculty of Pharmacy.

repeated about 3 times. Hands of respondent are applied with 1ml of both DEET and *N. cataria L.* extract up until the elbow. Each insectarium is filled with 30 mosquitoes and one hand is put inside of cage starting with *N. cataria L* extract 10% and then to 30% and 50% and lastly DEET 15%. Hand is observed for 15 minutes and the sampling is done three times which are on the

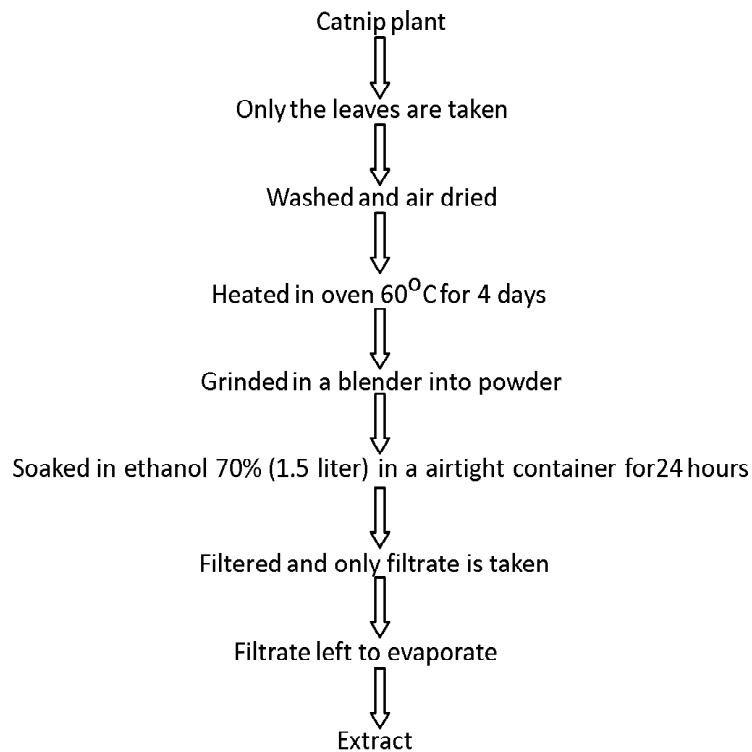


Figure 2. The steps of *N. cataria L.* leaves extraction

In this research, catnip extract (*N. cataria L.*) divided in 3 different concentration 10%, 30% and also 50% in comparison to DEET 15% against *Ae. aegypti* mosquito. The repelling properties were tested based on the number of mosquitoes that sits and bites on the hands of the respondent after being applied to both DEET 15% and *N. cataria L.* (10%, 30% and 50%) extract in total of 15 minutes. This action will be

5 minute, 10 minute and also 15 minute and this process is repeated again 3 times. After each experiment, the hand is washed properly with water and soap and rested for 30 minutes before being applied with the next repellent. In this experiment the both hands were used but switched after each experiment with different concentrations of catnip plant extract and also DEET. Before the hands are applied with *N.*

cataria L. extract and also DEET, one hand is put inside the cage to get the results for control.

To find out the repelling properties, the best parameter used is to calculate the number of *Ae. aegypti* mosquitoes sitting on the hand after the respondent has been applied with both DEET and also *N. cataria L.* extract. Analysis used in this research was the t-test. When the results are significant, then were proceed with the Anova test to check if the concentrations of catnip extract has different repelling properties.

RESULTS AND DISCUSSIONS

The results were obtained from the number of mosquitoes that have landed or bitten the hand of respondent before and after the application of catnip extract and DEET. The number of mosquitoes were then observed and counted at the 5th, 10th, and 15th minute. Three concentrations of catnip plant extract were used (10%, 30% and 50%) to compare which concentration has the highest repelling activity against *Ae. aegypti* mosquitoes.

Based on figure 1, it can be observed that there were an average of 7.6 mosquitoes per respondent sit on the hand of respondent applied with 10% of Catnip extract in 5th minutes observation and the mosquitoes approach increase to an average of 9.3 mosquitoes per respondent at the 15th minutes observation. These results indicated that 10% catnip extract repelled about 69.6% of 25 mosquitoes given in the experiment, however, the repellent activity reduced to 62.8% after 15 minutes exposure.

Higher concentration of Catnip extract 30% showed different pattern of repellent activity which was an average of 6 mosquitoes per respondent can be observed at 5 minutes

observation then mosquitoes number reduced to 5.3 mosquitoes at 10 minutes observation and to 5 mosquitoes at 15 minutes observation, respectively. This concentration contributed to repellent activity of 76% to 80% activity.

Catnip extract 50% showed similar activity to catnip extract 30% at 5 minutes observation, but its repellent activity reduced that indicated by increasing number of mosquitoes per respondent from 5 mosquitoes at 5 minutes to 7.3 mosquitoes at 10 minutes and 6.3 mosquitoes at 15 minutes. These results gave repellent activity of Catnip extract 50% ranging from 70.8% to 80%.

Catnip extract with 10% concentration still shows relatively high repelling properties against *Ae. aegypti* mosquito when compared to the control. However, comparing to the gold standard for mosquito repellent, DEET, the repellent activity of DEET was the highest efficacy in repelling mosquitoes. In the 15 minutes time period none of the female *Ae. aegypti* mosquitoes landed on the hand of the respondent in all three replications in DEET.

To know if there is a significant difference between the number of mosquitoes landed or bite on the hand of control and catnip extract is done by using Independent t-test, where the p value < 0.05. In the 5th minute the p-value between catnip 10%, 30% and 50% and control is lesser than <0.05. This means there is a significant difference between catnip extracts in different concentration and control in the 5th minute.

Meanwhile, ANOVA (p-value < 0.05) test is done to find out if there is significant difference in the time of observation in all three groups (control, catnip extract and DEET) on the scale

of 95% confidence interval. The ANOVA analysis shows that there is significant difference between the control, catnip extracts with different concentration and also DEET in 5th, 10th and also 15th minute. In the 5th minute, the F-

value was 131.886 with $p < 0.05$. In the 10th minute, F-value was 57.8765, $p < 0.05$ and in the 15th minute, F-value was 57.0632, $p < 0.05$. These results indicate significant difference between control, catnip extract and also DEET.

Table 1. The repelling activity of catnip extract (10%, 30% and 50%) and DEET 15% against *Ae. aegypti* mosquitoes.

Replication/ Time	Control			Catnip extract									DEET 15%		
	5	10	15	10%			30%			50%			5	10	15
Observation time (minute)															
1	25	25	25	9	12	12	7	5	3	6	7	5	0	0	0
2				8	7	9	3	5	9	5	10	9	0	0	0
3				6	4	7	8	6	3	4	5	5	0	0	0
Mean	25	25	25	7.6	7.6	9.3	6	5.3	5	5	7.3	6.3	0	0	0
SD ±	0	0	0	1.2	3.3	2.0	2.2	0.5	2.8	0.8	1.8	1.9	0	0	0

In the histogram below, there is a significant difference in number of mosquitoes that sit and bite in the first 5 minutes between the control and catnip extract in all the concentrations. This

shows that catnip extract in all concentrations provide some form of repelling properties against *Ae. aegypti* mosquito.

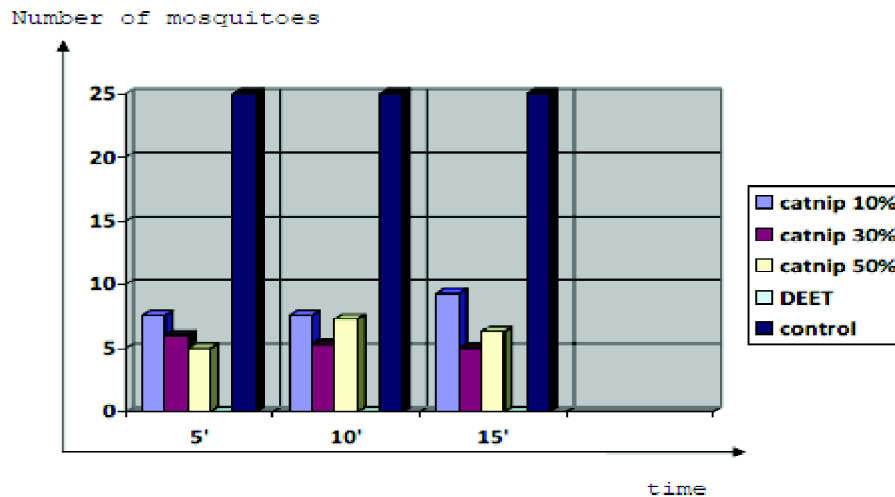


Figure 3. The repelling activities of catnip extract (10%, 30% and 50%) and DEET 15% against *Ae. aegypti* mosquito.

This research proves that catnip plant (*N. cataria L.*) extract does have repelling properties against *Ae. aegypti* mosquitoes. As there has been some mistakes done when the experiment was conducted, only the first 5 minutes of results are taken into consideration as the 10th minute and 15th minute is invalid. Based on the results obtained in the 5th minute, catnip extract shows repelling properties against *Ae. aegypti* mosquitoes.

During the conduction of this experiment, only the number of mosquitoes that sat and bite at that the point of 5, 10 and 15 minutes were counted. It supposed to be that even the numbers of mosquitoes that sat and flew were counted. The number of *Ae. aegypti* mosquito that sat and bite on the 10th and 15th minute were supposed to increase rather than decrease. This is because as the time increases, the efficacy of natural mosquito repellents should reduce and not vice versa. So in this experiment, the data collected is only valid if the 5th minute results were compared and the 10th and 15th minute results were disregarded.

This experiment also proves that the higher the concentration of catnip extract, the higher the repelling activity against *Ae. aegypti* mosquitoes. In 10% catnip extract shows significant repelling property compared to the results of the control. Although catnip 10% does show some form of repelling qualities, it is still not as effective as 30% concentration and also the 50% concentration of catnip extract. In 50% concentration of catnip extract possesses the highest repelling efficacy against *Ae. aegypti* mosquitoes.

However, catnip extract does not repel *Ae. aegypti* mosquito as efficiently as DEET. The

female *Ae. aegypti* mosquitoes did not land or bite the hand which was applied with DEET. According to Fradin and Day, an effective mosquito repellent is said to not allow any mosquito to land on that surface and it is also said to have a longer 'first bite' period. Meaning the repelling properties lasts for several hours¹².

Insects detect odors when volatile odors bind to receptors on the antennae and the maxillary pulps of the insects which are exposed called odorant receptor neurons. The DEET acts by blocking these receptors. Some plants produce some volatiles in order to deter herbivores when leaves are damaged¹³.

Many commercial repellents contain a few plant essential oils for fragrance or as repellents. But the combination of both DEET and essential oils of plants is said to not have better efficacy in repelling mosquitoes. Most essential oils are highly volatile, thus, contributes to the poor longevity as a mosquito repellent. Studies have shown that this problem can be overcome using fixatives and by careful formulations¹³.

Research says that catnip essential oil demonstrates repelling activity on mosquitoes. Meaning chasing the mosquito away and not by killing or irritate. Catnip also shows high recovery percentage which means that catnip is non toxic. Several studies shows that catnip oil is an effective repellent for up to 6 hours against *Ae. albopictus*¹⁴. In this research, the protection time against *Ae. aegypti* mosquitoes were not tested and evaluated. However, this research found that catnip is a strong irritant and has strong repellent on *Ae. aegypti* mosquitoes.

Researchers have said that the essential oil of catnip, nepetalactone is ten times more effective than DEET⁹. This experiment results does not agree with that statement, 50% catnip

extract seems to show highest repelling properties against *Ae. aegypti*. Higher concentrations of catnip extract show higher efficacy at early exposure.

The DEET 15% is said to repel mosquitoes up to an average of 7.6 hours and is said to be one of the most effective repellents¹⁵. The DEET at concentrations higher than 50% is said to cause dermal and ocular reactions¹⁶. American Academy of Pediatrics has recommended the proper use of DEET 10 to 30% were safe to be used on children and adults but not on infants below 2 months old. The higher the concentration of DEET has higher repelling properties but the graph comes to plateau from 50% concentration onwards. Most commercially available repellents use 40% DEET or lesser. Higher concentrations are used under intense biting circumstances. Lower concentration also means lower absorption into the skin and thus, lower toxicology reaction on the skin¹².

Based on the ANOVA results, show there was significant difference between the concentration of catnip extract and the repelling properties. This means higher concentration of catnip extracts provides better protection against *Ae. aegypti* mosquito. But natural repellents do not provide as much protection as the synthetic repellents and they also do not provide protection for a long period of time. The maximum number of hours tested on a natural repellent is said to be only 2 hours¹⁷.

Multiple factors play a part in determining the efficacy of a repellent. These factors includes, species of the biting organisms and density of organisms in the immediate surroundings, user's age, sex, level of activity, and biochemical attractiveness to biting arthropods, and ambient temperature, humidity, and wind speed. As a

result, a given repellent will not protect all users equally¹². It is also said that mosquitoes likes to bite the person who often consumes alcohol and mostly men compared to women and children¹⁸.

CONCLUSION

In conclusion, catnip extract with 10%, 30% and 50% concentrations show repelling properties by reducing the number of *Ae. aegypti* mosquitoes which land on the hand and bite the hands of respondent significantly. Higher the concentration of *N. cataria* L. have higher efficacy in repelling *Ae. aegypti* mosquitoes. DEET is still considered the gold standard repellent providing 100% protection against *Ae. aegypti* mosquitoes.

Further research is important to study on the efficacy of *N. cataria* L. as a mosquito repellent with different extract concentrations and under different circumstances for example in a bigger setting or a cooler place or a more humid place. It is also better to do more researches on the side effects on catnip plant and also DEET as a mosquito repellent lotion. Besides that, studies should be conducted in order to increase the longevity of natural based repellents as consumers are now demanding means of protections that are safe, pleasant to use and environmentally sustainable.

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