



MOSQUITO REPELLENT ACTIVITY OF NATURAL REPELLENT MADE OF NAVASĀRA AND LEMON JUICE ON ADULT ANOPHELES MOSQUITOES.

Adarsh G S*

Plakattu veedu, Anakottoor (P.O), Kottarakkara, Kollam. *Corresponding Author

Dr. R. Jyothi

Professor and HOD, Dept. of Swasthavritta, Govt. Ayurveda College, Tripunithura

Dr. Sajitha
BhadranAssociate professor, Dept. of Swasthavritta, Govt. Ayurveda College,
Thiruvananthapuram

ABSTRACT Mosquitoes are a serious threat to the public health as they are known vectors for all biological environments which result in many life threatening diseases like Malaria, Filariasis, Chikungunya, and Dengue fever etc. Hence for the prevention of mosquitoes bite the mosquito repellents on exposed skin area are strongly suggested. Most of the commercial mosquito repellents are prepared using non-biodegradable, chemicals which may lead to unacceptable health risks. With an increasing concern on public safety, a renewed interest on the use of natural products of natural origin is desired. Natural products are effective, environment friendly and biodegradable. In this study an attempt to find out the mosquito repellent action of a natural repellent made of *Navasāra* and Lemon juice explained in *Kriya koundudi* on Adult Anopheles mosquitoes.

KEYWORDS : Mosquito repellent, Navasāra, Anopheles mosquitoes

INTRODUCTION

Vector-borne diseases are a major public health concern, affecting around half of the world's population. Vectors are living organisms that can transmit infectious diseases like Malaria, Dengue fever, Chikungunya, viral encephalitis, yellow fever, Filariasis and Epidemic polyarthrititis to humans¹. Several vector control measures are taken to prevent the transmission. Insect repellents usually work by providing a vapour barrier preventing the arthropod from coming into contact with the skin surface. Most of the commercial mosquito repellents are prepared using non-biodegradable, chemicals like N, N-diethyl-3-methylbenzamide, dimethyl phthalate, and allethrin. It has been identified that chemical repellents are unsafe for public and should be used with caution because of their harmful impacts or toxic reactions like allergy, dermatitis, and cardiovascular and neurological side effects due to mishandling². With an increasing concern on public safety, a renewed interest on the use of natural products of natural origin is desired because natural products are effective, environment friendly, biodegradable, inexpensive, and readily available.

The Ayurvedic combination of Navasāra and Nimbuka is mentioned in *Kriya Kaumudi* of V M Kuttikrishna Menon is indicated for all types of *Mashaka visha*³. *Navasāra* (Ammonium chloride) is a specified *Ksāra* which is produced by burning the wood of *karīra* and *pīlu*, or else called *Chullikalavana*. While burning bricks in the brick furnace, a *pāndura* (yellowish white) coloured *lavana* is evolved, this is called *Navasāra* or *Chullikalavana*.

It ensures complete repellent action from mosquitoes. In this experimental study, a humble attempt is made to analyse the mosquito repellent activity of this herbo mineral combination.

MATERIALS AND METHODS

1. PREPARATION OF REPELLENT

INGREDIENTS	QUANTITY
Navasara(chullikalavana)	9g
Lemon juice	36ml
Caboxy Methyl Cellulose	2.55g
Glycerine	7.5 ml
SodiumBenzoate	0.20mg

a) *Navasāra* purification: - 150gm of impure *Navasāra* is taken in a clean stainless vessel added with 450 ml of clean water and stirred well. The liquid is filtered through a clean cloth. The filtrate is now taken in another clean vessel and boiled to evaporate all the liquid part. The white fine powder left at the base of the vessel were collected, dried and stored in a suitable airtight container as pure *Navasāra*⁴

b) Lemon juice - Squeeze the lemon juice from 5 lemon fruits into a glass and then filtered through a clean cloth and collected.

c) CMC, Glycerine, Sodium Benzoate are purchased from a laboratory supplies for manufacturing base of the gel.

2. RESEARCH TECHNIQUE

10 male volunteers were randomly selected with no allergic reaction to bites for this study trial. The age of the volunteers ranges between 20-30 years irrespective of gender. The test area of the volunteers forearm was thoroughly washed with unscented soap and rinsed with water. The hands of the volunteers were protected with surgical gloves from mosquitoes. During the time of experiment the volunteers were also asked to avoid rubbing, touching or wetting the repellent treated area as well as any activity that might lead to increase perspiration. The experiments were conducted following WHO guideline for efficacy testing of mosquito repellents for human skin.

4- 6 day old Female Anopheles mosquitoes without giving blood meal and fed with 10% glucose solution and are starved for 18 hours were taken for each trials. The mosquitoes were numbered and transferred from the rearing cages to 2 Glass Chambers with dimension 50cm × 50 cm × 50 cm⁵. Each Glass chamber consists of 150 adult mosquitoes. The glass chambers were marked Group A and Group B. Each of the Glass chambers was kept in a non-ventilated room of 8 feet dimension on all sides. The room is maintained at about 25°C and 75% relative humidity.

Natural gel prepared from *Navasāra* and Lemon juice was applied to the volunteer's right arm from the elbow to the wrist. The repellent cream was applied on an approximate 100 cm² area of one of the fore arm and the hand was covered with a glove. After application, the arms were left for 30 minutes to dry up. Base of the gel served as a control on the left arm. The untreated arm was inserted into Group A cage and the number of mosquito landings were recorded over 3 minutes. During testing the volunteer was requested to avoid any movements of the arm. Then the treated arm was inserted into the (Group A) same cage and the number of landings were recorded in the same way. Similarly in the next hour the untreated arm inserted into the Group B cage for 3 minutes and treated arm in Group B cage to avoid bias. This procedure is continued up to 6 hours and numbers of mosquito landing were recorded. Thus a total of 7 trials were done on each volunteers as Study group and as Control group.

RESULTS AND DISCUSSION

Table no: 1- Comparison of average of study group and control group

Time	0 minute	60 minutes	120 minutes	180 minutes	240 minutes	300 minutes	360 minutes
Study group	0	0	0	0	0	1	2
Control group	25	24	22	22	22	23	23

On comparison the average of mosquito landing in study group, no mosquito landing were observed till 240 minutes and only 1 mosquito landing were observed at 300th minute and 2 mosquitoes landing at 360th minute. In control group 25 mosquito landings were observed at 0 minutes, 24 mosquitoes at 60th minute, 22 mosquitoes at 120th minute,

22 mosquitoes at 180th minute, 22 mosquitoes at 240th minute, 23 mosquitoes at 300th minute and 23 mosquitoes at 360th minute.

Statistical analysis between the Study and Control group

In order to do paired t test between the study and control group, their baseline data analysis should be done. If the mean difference is not significant while comparing the baseline data analysis of 2 groups in all trials, paired t test can be done.

Table No: 2 - Effect of repellency during 0 minute to 360 minutes after application of repellent

Time	Mean difference	T – value	P – value
0 minute	-25.1	34.052	<0.001
60 minutes	-23.6	31.537	<0.001
120minutes	-21.8	32.865	<0.001
180minutes	-21.6	32.865	<0.001
240 minutes	-22.2	24.89	<0.001
300 minutes	-22	26.506	<0.001
360 minutes	-20.1	23.313	<0.001

Comparison of repellency in Study group by Tukey Kramer Multiple comparison test

Comparison group	significance	P value	Mean difference	Q
0 min vs 60 min	Ns	P>0.05	0	0
60 min vs 120 min	Ns	P>0.05	0	0
120 min vs 180 min	Ns	P>0.05	0	0
180 min vs 240 min	Ns	P>0.05	-0.1000	6.848
240 min vs 300 min	**	P>0.01	-0.9000	6.163
300 min vs 360 min	**	P>0.01	-0.8000	5.479

ns – non significant (P>0.05),

**Significant - P>0.01

Comparison of repellency in Control group by Tukey Kramer Multiple comparison test

Comparison group	significance	P value	Mean difference	Q
0 min vs 60 min	Ns	P>0.05	1.500	2.021
60 min vs 120 min	Ns	P>0.05	1.800	2.425
120 min vs 180 min	Ns	P>0.05	0.200	0.2695
180 min vs 240 min	Ns	P>0.05	0.700	0.9432
240 min vs 300 min	ns	P>0.05	0.700	0.9432
300 min vs 360 min	ns	P>0.05	1.100	1.482

Table: ns – non significant (P>0.05)

COMPLETE PROTECTION TIME ANALYSIS

The complete protection time (CPT) was defined as the time the first mosquito landed on or bit a treated arm.

REPELLENCY PERCENTAGE ANALYSIS

$$R(\%) = \left(\frac{C-T}{T} \right) \times 100$$

Where C is the number of mosquito bites on the control arm and T is the number of bites on the treated arm.

Table No: 3 - Repellency Percentages And Cpt Of Study Medicine Against Anopheles Mosquitoes

	R % in 0 min	R % in 120 min	R % in 240 min	R % in 300 min	R % in 360 min	CPT (min)
T1	100	100	100	100	92	360
T2	100	100	100	90.4	91.3	300
T3	100	100	100	96.1	89.2	300
T4	100	100	100	91.3	95.2	300
T5	100	100	100	94.7	95	300
T6	100	100	100	100	89.4	360
T7	100	100	100	95.4	94.4	300
T8	100	100	100	91.6	95.2	300
T9	100	100	100	95.6	91.6	240
T10	100	100	100	95.6	85	300

Repellency was calculated each hour and complete protection time (CPT) was determined by calculating the number of minutes from the time of repellent application to the first mosquito landing.

The observation of all the trials is analyzed by general observation,

graphical representations and appropriate statistical methods. While considering the general observations in the study group, mosquito repellent activity was noted at 0 min to 360 minutes. The rate of mosquito repellent activity is remains same up to 300 minutes. After that the repellent action is slightly reduced, but compared to the control group number of landing is relatively less. The study group showed significant mosquito repellent activity in all the trials than the control group. From these observations, we can assume that the mosquito repellent action remains in its peak level up to 240 minutes. After that the repellency is slightly reduced.

This repellent cream makes the person unattractive to the mosquitoes for biting. Mosquitoes are attracted by the odor of the skin, by applying mosquito repellent; you make the skin unattractive to mosquitoes. On the basis of results obtained it can be concluded that the repellent gel prepared by *Navasāra* and Lemon juice have no irritation and is able to repel mosquitoes. The ingredients of the formulation are easily available and cheap. But *Navasāra* should be use after purification. This natural repellent has got significant action similar to or more than the chemical repellents available today.

CONCLUSION

The topical application of this formulation on the participants have shown no side effects or irritation on the skin, thus it can be used on a wider population and have better utility than chemical repellents which cause several allergic reactions.

This study shows 100 percentage repellency rates up to 300 minutes. After that the repellency rate is reduced but not below to 90. which means even after 300 minutes the mosquito bite is very less in study group than control group. The t value is highly significant which shows that the study group produces far better results than the control group.

REFERENCES

- Aina SA, Banjo AD, Lawal OA, Jonathan K. Efficacy of some plant extracts on *Anopheles gambiae* mosquito larvae. Academic Journal of Entomology. 2009;2(1):31-<https://en.wikipedia.org/wiki/Anopheles>
- M.C.Gupta, B K Mahajan. Text book of preventive and social medicine, Third Edition ed. Ansari Road, Daryaganj, New Delhi: Jaypee Brothers Medical Publishers; 2003.)
- V.M.Kuttikrishna Menon. Kriya kaumudi. Kottayam, Kerala: Sahithya pravarthaka co-operative society, kottayam; 1986, page no 224.) <https://www.scientificamerican.com/article/is-it-true-that-the-deet/> [accessed on 28/5/2020]
- Dr. k. Rama Chandra Reddy, RASASASTRA, chaukambha Sanskrit bhavan Sanskrit, Ayurveda and indological publishers and distributors. Varanasi 221001, second edition 2010
- World Health Organization. *Guidelines for efficacy testing of mosquito repellents for human skin*. No. WHO/ HTM/ NTD/ WHOPES/ 2009. 4. World Health Organization, 2009.