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Formulation and Evaluation of Mosquito Repellent Lotion of *Vasaka* Stem Extract

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Abstract
Medicinal plants contain numerous biologically active compounds which are helpful in improving the life and treatment of diseases and these are the primary source of synthetic and traditional herbal medicine. The presence of various life sustaining constituents in plants made scientists investigate these plants for their uses. The objective of the work was to formulate a mosquito repellent product containing *Adhatoda vasica* (Acanthaceace). *Adhatoda vasica* (Acanthaceace) is a well known medicinal plant from which certain alkaloids, phenolics, flavonoids, sterols and their glycoside derivatives have been isolated. Its diverse medicinal activities include cardiovascular protection, abortifacient, antitubercular, antimutagenic, antiulcer, antiasthmatic activities, hepatoprotective, antibacterial and antitussive activities. It is commonly used in indigenous and traditional folk medicine systems in South-East-Asia. The formulation of lotion was evaluated for various parameters like smooth texture and spreadability with a pH 7 which is a non irritant and suitable for the skin. The main evaluation is based on the cage test, cone test which determines the significant result for the activity. There is no phase separation during thermal stability. From the present work, it was concluded that 2ml and 6ml *Adhatoda vasica* containing lotion is safe, effective, usable for the skin and stable too and gives an effective result after the 4.0 and 4.5 intervals after application. The conclusion from the research, determines the potential to provide efficiency and can be used as a cheap, eco-friendly, safer for humans and the environment and also an efficient alternative to the chemical mosquito repellent.

Keywords: *Adhatoda vasica*, Acanthaceace, cone test, mosquito repellent, cage test.

Introduction
Adhatoda vasica belonging to the family Acanthaceae, commonly known as Adosa, is a small, evergreen shrub found in many regions of India and throughout the world, with a multitude of uses in traditional Ayurveda. Vasica is most well-known for its effectiveness in treating respiratory conditions. The leaves of Vasica are shows stimulant effect on the respiratory system.(1)

Vasica shows an antispasmodic and expectorant effect, and has been used for centuries with much success to treat asthma, chronic bronchitis, and other respiratory conditions. The powder of herb, boiled with sesame oil, is used to heal ear infections and arrest bleeding. Boiled leaves are used to treat rheumatic pain, and to relieve the pain of urinary tract infections.(1)

It is also believed to have abortifacient properties. It is used in some parts of India to stimulate uterine contractions, thus speeding childbirth. The substances which make surfaces unpleasant to mosquitoes are considered as mosquito repellents. It may be applied to skin or other surfaces which discourages

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mosquitoes from landing on those surfaces. These substances typically contain active ingredients that repel mosquitoes, as well as secondary ingredients which aid in delivery of cosmetic appeal.

these repellents are available in many forms - creams to lotions to oils, but are most often sold as aerosol products. There are also repellents available based on sound productions, particularly ultrasound (inaudible high frequency sounds) traditionally, various types of substances have been used to repel mosquitoes. These include smoke, plant extracts, oils, tars, and muds. Most of the repellents work by interfering with the mosquito's homing system.

This homing system, located on the antennae of the organism possesses a number of chemical receptors. Carbon dioxide, excretory products and lactic acid present in sweat in warm-blooded animals act as an attractive substance for female mosquitoes.(3)

The chemical receptors are activated by lactic acid, which naturally evaporates from the skin of warm-blooded animals. However, when a repellent ingredient containing the active repellent such as DEET (n-ndiethyl metatoluamide) is applied to the skin, it also evaporates. The repellents block the lactic acid receptors thus destroying upwind flight and as a result the mosquitoes lose its contact with the host.

This essentially "hides" the protected person from the mosquito. North East India, having a tropical climate is infested with several diseases such as malaria, dengue, etc. caused by mosquitoes. The mosquitoes act as a vector for the transmission of these deadly diseases. The need to protect ourselves from their bites seems more important. Much of the literature about mosquitoes provided by government agencies recommends regular use of mosquito repellents most often containing DEET as the major chemical component.(5)

Many health problems such as headache, breathing difficulties, heart attack, etc. are the result of long term use of DEET either directly or indirectly. Now-a-days, people are looking for mosquito repellents which are safer and preferably herbal based. There occur several plants around us containing certain essential oils, often found to be effective insect repellents.

Many of these plants are herbs or shrubs often considered as weeds. Unfortunately, their use has been known only to some ethnic groups. The use of herbs as mosquito or other insect repellents has been incorporated in many cultural rituals of these ethnic groups from time immemorial.

Material and Method

Material

Adhatoda vasica stem

Plant Material

The stem of *Adhatoda vasica* was collected from the Vasaka tree. The stems were then isolated and conserved for extraction.

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Method

- Collection of plant material
- Preparation of extract
- Evaluation of mosquito repellent activity using In-vitro cage test method.

Extraction of stem

Solvent extractions of stem were done by using different solvents like Ethyl alcohol, Diethyl ether and Petroleum-ether. Then one part of the extracts obtained was concentrated under reduced pressure in vacuum evaporator at (40 – 50) °C into a syrupy liquid. This syrupy liquid was again separated several times in separating funnel to get the desire oil. The constituents of the oil were determined. The constituents of the Petroleum-ether extract were also determined. The other part of the extracts was used for the preparation of mosquito repellent liquid. [12]

Preparation of herbal mosquito repellent from stem extract

The stem was first collected and air dried. Then 500 gm of stem were powdered and mixed. The extract obtained from Ethyl-alcohol, Diethyl ether and Petroleum-ether was kept separately and the extracts were concentrated by evaporating the solvent. Then each extract were mixed together in 1:1:1 ratio to form mixture.

Methods of evaluation of mosquito repellent

There are several methods to evaluate the treated textile with mosquito repellent. The most used techniques are cage test, cone test, and excito chamber. [12]

Cage test

The Cage test might assess the viability of repelling substance against mosquitoes for lotions, cream including impregnated material done fast and effective approach. It is designed to observe the mosquito landing on the untreated and treated fabric in the cage. The advantages of this method is it provides the real situation of the probing and biting of the mosquito to the human besides it can directly provide the observation of the mosquitoes behavior towards the treated materials[9].

Some studies reported in modification of the cage dimension; used a cage 18 × 18 × 18 cm dimension used cage 30 × 30 × 30 cm dimension, used 34 × 32 × 32 cm cage dimension, Used the 35 × 35 × 35 cm cage dimension. Two other studies reported the use of a bigger cage dimension, measuring 40 × 30 × 30 cm and 45 × 45 × 45 cm. The cage is concealed with transparent mosquito nets for easy observation and also to keep mosquitoes inside the cage. It has holes which are also covered with nets for arm accessing purpose. The cage needs to be filled with 200 mosquitoes that have been starved overnight and only were supplied with sucrose solution. Updated standards use a lesser number of mosquitoes in the cage (as low as 30 mosquitoes), as lower density provides more accuracy which better reflect the typical biting environment encountered during most indoor and outdoor activities and also give a comfortable condition to volunteer.

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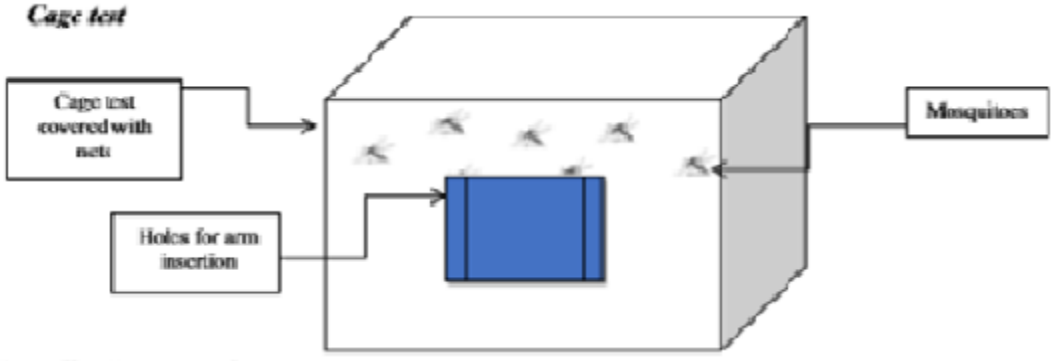
Volunteers should be preferable not to be tobacco users and should avoid using fragrance or repellent products for 12 h ad during testing. This factor may alter the person attractiveness to the mosquitoes and will affect to the outcome repellency assay. In preparation of the volunteer, their hand must be washed with unscented soap and rinsed with water and placed separated from each other by ≥20 m away. The arm covered with gloves or treated materials of the volunteers will be inserted into the cages. The left arm served as control while the right arm use as treated samples. Both forearms with untreated and treated materials will be exposed to the population of mosquitoes simultaneously for a period of 3 min. At least two mosquitoes landed or bite within the 3 min, the test will continue. If there is no mosquito landed within 3 min, the hand will withdraw from the cage. The number of mosquito landing will be counted independently using the digital camera for an accurate result. The exposition is done every 30 min up to 8 h or until the repellency fails. Each test samples done in triplicates at 28 ± 2 °C and 80 ± 5 % RH with 5 min waiting period between replicates. The time between applications of the treated Cage test covered with nets materials recorded as the protection time. The percentage of repellency or protection time was calculated using the formula.

Where U corresponds to the number of mosquitoes on untreated samples or control samples and T represents the number of mosquitoes on treated samples. This is the regularly utilized formulas as stated although the percentage of repellency was sometimes calculated with other formulas.



The Construction of cage test

Cone test

The cone test is formerly the custom to evaluate the toxicity of insecticide-treated bed nets against malaria, which also able investigate the toxicity of other impregnated (textile) surfaces. The fabric is treated, evaluated using the WHO cone test following the standard procedure described in the test procedures for insecticide resistance monitoring in malaria vectors, inefficacy and persistence of insecticides on treated surfaces. This test does not involve human participants as the bait to lure the mosquitoes comes to fabric and this is one of advantages of this method. Due to this factor, this method is less chosen by the researcher to conduct the mosquito repellency test on clothing.

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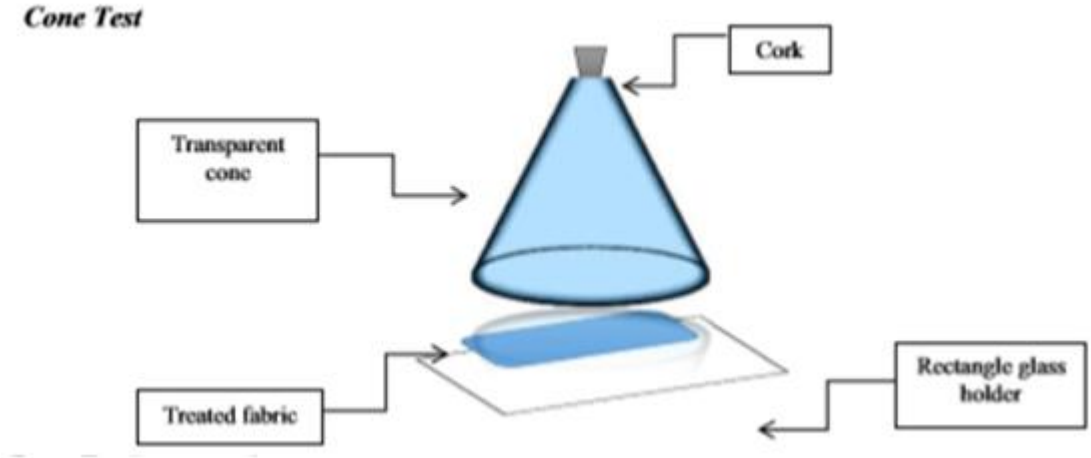
The use of artificial blood or animal blood as bait in order to attract host seeking mosquitoes, which in this cone test could help future studies to better assess the efficacy on the treated clothing. In the cone test, the mosquitoes might spend more time resting on the cone than on the treated surface during the 3 min exposition. The 3-min exposure test was carried out under the temperature of 27 °C. Five to ten female mosquitoes were blown into the cone using an aspirator and mosquitoes were exposed to the treated surface. The low density mosquito number used for this method made it easy to observe the mosquito behavior. The numbers of mosquitoes resting on the treated samples were counted within 3-min exposure.

% Mosquito mortality: (MR-MC)/ 100-c

MR represents the mosquito's mortality in test replicate

MC corresponds to the mosquito's mortality in control samples.



The construction of Cone Test

Excito chamber test

Excito chamber method is a modified custom method to observe the mosquito behavior change in the form of moving away from the treated to untreated fabric. This method and Cone test method does not involve the human subject to lure the mosquito. However, both methods can determine the behavior of the mosquitoes towards the treated materials. The box is made with one front and exit panel occupied with a single escape portal. It builds up with a screened inner chamber, glass holding frame and door cover. The mosquito was starved overnight or at least minimum 4 h before the test. The behavior of mosquitoes was observed in terms of the number of escaped mosquitoes to another space and remaining mosquitoes inside the chamber which filled with treated product. The observation is recorded after 10 and 30 min exposure. The test was conducted in daylight and repeated for four times. The percentage o f mosquito repellency was calculated using the formula:

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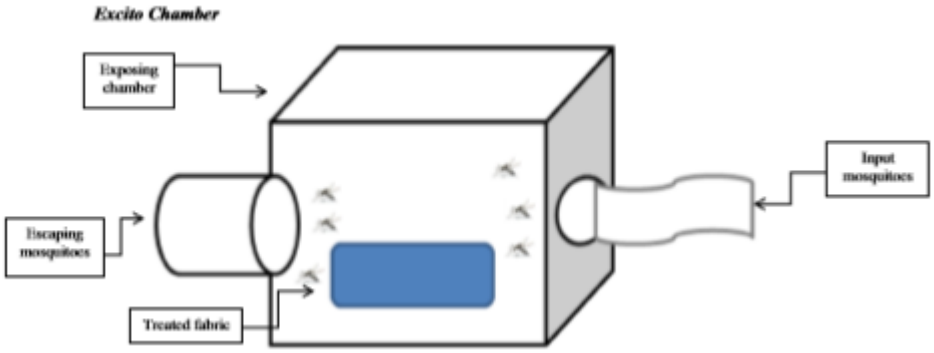
% Mosquito repellency: (NES + NDE)/ (NEX) × 100

Where, NES corresponds to the number of mosquitoes escaped while the NDE refer to the number of mosquitoes dead
NEX represents the number of mosquitoes exposed.
% Mosquito mortality: (MR − MC)/ (100 − C) × 100
% Mosquito repellency: (NES + NDE)/ (NEX) × 100



The construction of Excito Chamber

Result

Mosquitoes were observed to fly restlessly exhibiting frantic attempts to repel from applied lotion hand in contrast to non applied lotion hand. Time of immobility and no of dead mosquitoes was observed with time dependent and concentration dependent. The records are given below:

Time-dependent mosquito repellent by lotion of *Adhatoda vasica* on human volunteers.

Quantity of Lotion applied	**Number of Landing attempts/bites in time (Hours) (average values)**									
	0.5	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**	**Total**
2ml	0	0	0	0	0	1	0	2	5	8
6ml **0** **(control)**	0 3	0 3	0 2	0 4	0 2	0 2	0 2	0 1	1 2	1 21
Total	3	3	2	4	2	3	0	3	8	30

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Conclusion

Adhatoda vasica represents a class of herbal drug with a very strong conceptual or traditional base as well as strong experimental base for its use. Thus this plant has great potential to be developed as a drug by pharmaceutical industries. The essential oil as well as powder is having excellent mosquito repellent properties. Partial analyses of the constituents are done. Insect repellent properties of the stem are also proved.

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