



LARVICIDAL, ADULTICIDAL, AND REPELLENT ACTIVITY OF 'JATVAADI DHOOMAGAD' AGAINST AEDES AEGYPTI MOSQUITOES

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ABSTRACT

Mosquitoes are the most disturbing insects which are very hazardous to humanity. Mosquitoes are known to spread deadly vector-borne diseases. *Acharya Susrutaha* mentioned *Mashak* in *Kalpasthanana*. The *Keet-Visha* section also describes 5 different varieties of *Mashak*. *Agad Tantra* provides descriptions of a wide range of medications and formulas to prevent mosquito (Keet) harm. *Acharya Charaka* in this series described "*Jatvaadi Dhoomagad*" to prevent mosquito (Keet) harm. **Objective:** In this study, petroleum ether, chloroform, methanol, and aqueous extract of *Jatvaadi Dhoomagad* were utilised to test the larvicidal, adulticidal, and repellent properties. **Methods:** The Petroleum ether, Chloroform, Methanol, and Aqueous extract of *Jatvaadi Dhoomagad* were used against *aedes aegypti* in this study. **Results:** Statistically, In the chloroform extract of *Jatvaadi Dhoomagad*, the greatest rate of larval death against *Aedes aegypti* was observed in 24 hours. Accordingly, the petroleum ether extract of *Jatvaadi Dhoomagad* has shown better adulticidal action than other extract of *Jatvaadi Dhoomagad*. The knockdown times of the control group and petroleum ether extract were identical due to the poor mortality in other extracts of *Jatvaadi Dhoomagad*'s adulticidal action, hence there was no protection time recorded. **Conclusions:** These results suggest that the extracts of *Jatvaadi Dhoomagad* have no potential to be used as an ideal eco-

friendly approach for the control of aedes aegypti. This study provides a report on the larvicidal activity, adulticidal activity, and repellent effect of *JatvaadiDhoomagad* extracts against aedes aegypti mosquitoes.

Keywords: *Agad Tantra, Mashak*, larvicidal activity, adulticidal activity, repellent, *JatvaadiDhoomagad*.

INTRODUCTION

In *Agad Tantra*, bites of poisonous animals such as snakes, *Keet*, spiders, and rats, are described under *JangamVisha*. *Acharya Susrutahas* told about the origin of *Keet* and its 67 types. 5 types of *Mashak* are described under *KeetVisha*. *Acharya Susrutahas* mentioned *Mashakin Kalpasthana*. *Mashakis* very hazardous for human health. Which *ParvatiyaMashakis* most dangerous for human being^{1,2}. One of the most unsettling creatures that pose a serious threat to people is the mosquito. Deadly vector-borne diseases are known to be spread by mosquitoes. In India, there are more than 404 mosquito species and subspecies; the most prevalent ones are *Anopheles*, *Culex*, *Mansoniides*, and *Aedes*. The most hazardous kinds of mosquitoes among these are *Aedes*, which transmit diseases like dengue, chikungunya, zika, and yellow fever. The mosquito genus *Aedes* was first discovered in tropical and subtropical regions of the world. In India, the number of illnesses and fatalities brought on by *Aedes aegypti* mosquitoes is rising quickly every year. Over 17% of all infectious infections in recent years have been vector-borne illnesses. Over a million individuals pass away every year and up to 700 million people contract the disease³. In order to prevent mosquito (*Keet*) harm, a range of medications and formulas have been mentioned in the *Agad Tantra*. In the series, *Acharya Charaka* describes "*JatvaadiDhoomagad*" in chapter 23 of *Vish-Chikitsa*. *Laksha*, *Sevya (Khas)*, *Tejpatra*, *Guggulu*, *Bhalataka*, *Kakubh-pushp*, *Sarjrasa*, and *Shweta-Aparajita* are among its members. The effectiveness of these medications' Larvicidal, Adulticidal, and Repellent qualities could significantly reduce mosquito populations while potentially having no negative effects on people. We aimed to examine the larvicidal, adulticidal, and repellent actions of "*JatvaadiDhoomagad*" on *Aedes aegypti* because there is no specific treatment or vaccine available for mosquito-borne diseases, thus the focus must be on controlling them,

notably Dengue Fever and Chikungunya, considering the harmful effects of various types of chemical mosquito repellents, we tried to study larvicidal, adulticidal and repellent activities of – "*JatvaadiDhoomagad*" on *Aedes aegypti* mosquitoes.

AIMS AND OBJECTIVES- To evaluate the larvicidal, adulticidal, and repellent effect of *JatvaadiDhoomagad* extract against aedes aegypti mosquitoes.

MATERIAL AND METHODS-

Collection and Preparation of drug - Each of the 100gm of materials for *JatvaadiDhoomagad* were gathered in its purest state. Two components of *JatvaadiDhoomagad*, including *Kakubha-pushpa* and *Aparajita* pod, were procured at the Rishikul campus in Haridwar during the summer. We bought dried samples of *Laksha*, *Khas*, *Tezpatra*, *Guggulu*, *Bhallatak*, and *Shaal* from the Shriram Herbal Shop in Patel Nagar in Jaipur, Rajasthan. All the ingredients of *JatvaadiDhoomagad* were identified and verified by eminent experts of the *Dravyaguna* Dept. at Rishikul Campus, Haridwar Uttarakhand Ayurved University. The components for the *JatvaadiDhoomagad* were ground into a coarse powder at the neighbourhood-pisai Kendra in Haridwar. These components were all appropriately combined to create a powder. The obtained *JatvaadiDhoomagad* had a strong odour and brownish colour.

Preparation of the Extract - *Arjun* flower, *Aparajita* pod, and other dried samples of *Laksha*, *Khas*, *Tezpatra*, *Guggulu*, *Bhallatak*, and *Shaal* were mechanically ground into powder using an electrical stainless-steel blender. They were then extracted for 8 hours using petroleum ether (300 mL), chloroform (300 mL), methanol (300 ml), and water (300 ml) in a Soxhlet apparatus (boiling point range 60 The residue was collected and stored at 4° C after the extract was concentrated at 45°C under reduced pressure of 22–26 mmHg.

Preparation of different concentrations of each extract of *Jatvaadi Dhoomagad* for larvicidal activity^{5,6}.

The larvicidal activity of *Jatvaadi Dhoomagad* was tested at concentrations of 500 ppm, 750 ppm, and 1000 ppm. To prepare the 500 ppm solution for the experiment, 125 mg of extract was mixed with 0.5 ml of ethanol and then poured into a 500 ml plastic container containing 250 ml of water. For the preparation of the 750 ppm solution, 187.5 mg of extract was mixed with The steps were the same for the 1000ppm solution, and the control was set up using 0.5 ml of ethanol and dechlorinated tap water.

Mosquito Rearing - *Aedes* mosquito larvae were gathered from nesting locations in several parts of Haridwar. The immature stages from these places were gathered from various breeding sites, such as building sites, flower pots, buckets, coolers, storage tanks, iron vessels, etc. The gathered mosquito larvae were recognised at the Field Unit of the National Institute of Malaria Research B.H.E.L., Haridwar after the larvae were brought into the lab. Collected mosquito larvae were brought to the laboratory and kept in the laboratory at $26 \pm 20^\circ\text{C}$. The larvae were provided a mixture of dog biscuit and yeast powder in a 3:2 ratio as nutrients. The larvae were reared in the laboratory upto 3rd and 4th instars for testing of larvicidal activity. The larvae were reared up into adult mosquitoes. The adult mosquitoes were reared in a humidified cage and fed with a 10% glucose solution soaked into cotton. Female adult mosquitoes were separated from males and the adult female mosquitoes were used for testing of adulticidal and repellent activity.

preparation of filter paper for the adulticidal activity

0.2 gm of extract of *Jatvaadi Dhoomagad* with each solvent was diluted in 2.5 ml of ethanol and applied to Whatman filter paper of 180 cm^2 (size: $12 \times 15 \text{ cm}^2$) and dried. Control papers were treated with ethanol under similar conditions.

Larvicidal bioassay-

Bioassay tests for the determination of the larvicidal activity of particular extracts were performed on late 3rd or early 4th-stage larvae as per WHO protocol.

The plant fraction was dissolved in ethanol (0.5ml). Twenty larvae were introduced into a 500 ml container, containing 250 ml of distilled water. The larvae were exposed to different concentrations (500ppm,750ppm, and 1000ppm)of each extract. The test larvae were provided a mixture of dog biscuit and yeast powder. All the tests were carried out in 3 replicates along with the untreated control. Data were recorded and analyzed. Mortality of larvae was monitored after 24 h, 48 h, and 72 h.

Adulticidal bioassay-

The bioassay test for adulticidal activity was conducted using a standard protocol (WHO, 2006). Twenty female mosquitoes, 2-5 days old glucose, fed were collected and gently transferred into the plastic holding tubes. The mosquitoes were held in the holding tube for one hour and then exposed to test paper for one hour in the exposure tube and the knockdown of the mosquitoes was counted at end of one hour. At the end of one hour, the mosquitoes were transferred back to the holding tubes hour for a recovery period of 24 hours. Mortality of mosquitoes was recorded at the end of the 24-hour recovery period. Three replicates were carried out along with parallel control.

Repellent activity of petroleum ether extract -The petroleum ether extract of *Jatvaadi Dhoomagad* was evaluated for its repellent activity against *Aedes aegypti*. Repellency was tested against 3–6-day old blood starved, sucrose – fed (0.5 m solution) mosquitoes. 0.2 ml extract dissolved in 2.5ml ethanol and applied on the upper surface of the arm (wrist to fingertips) in the experimental and 2.5 ml coconut oil was applied on the upper surface of the arm in the control group. In an experimental test with repellents, only the upper surface of the arm (wrist to fingertips) was exposed, and the lower surface was covered. An untreated arm was first exposed for 5 min. in a cage containing 60 mosquitoes and the rate of mosquitoes, the landing was recorded. Then coconut oil was applied to the arm as a control. Then, it was exposed to other mosquito cages having 60 mosquitoes of the same batch and the number of landings was recorded for 5 min. The test arm was exposed for 5 min. until a confirmed landing was received. The test was over

after confirmation of the mosquito landing. The mosquito repellency was measured on the basis of the protection time.

RESULT-

LARVICIDAL ACTIVITY OF DIFFERENT EXTRACTS OF JATVAADI DHOMAGAD AGAINST AEADES LARVAE.

At 500ppm, results began to appear, and we increased the ppm to determine the efficacy of the *Jatvaadi Dhoomagad* compound in various solvents. In statistical analysis, it was observed that petroleum ether extract, methanol extract, and aqueous extract of *Jatvaadi Dhoomagad* had statistically no significant results and chloroform extract of *Jatvaadi Dhoomagad* had statistically significant larvicidal results in 24 hr. When we ran the data through a Probit model, we observed that petroleum ether extract of *Jatvaadi Dhoomagad* has LC 50 values of 1310, 375.237, and 221.535 in 24 hr, 48 hr, and 72 hr respectively, and LC 90 values of 12521.458, 10027.278 and 1714.026 in 24 hr, 48 hr and 72 hours respectively. And the p-value was $>.05$, indicating that the petroleum ether extract of *Jatvaadi Dhoomagad* had statistically no significant larvicidal activity.

□ In 24 hours of exposure, we observed that chloroform extract of *Jatvaadi Dhoomagad* has a significant outcome in the Probit model; its LC50 value was 1250.448, 2142.018 and 1237.900 in 24 hr, 48 hr, and 72 hr respectively LC90 value 4543.724, 21594.296 and 6713.113 in 24 hr, 48 hr, and 72 hr respectively, indicating that that chloroform extract of *Jatvaadi Dhoomagad* had statistically no significant larvicidal activity in 48 hr and 72 hr.

□ When we ran the data through a Probit model, we observed that the methanol extract of *Jatvaadi Dhoomagad* has LC 50 values of 1310.656, 829.450, and 249.887 in 24 hr, 48 hr, and 72 hr respectively and LC 90 values of 4549, 3972.682 and 2787.708 in 24 hr, 48 hr, and 72 hr respectively, and the p-value was $>.05$, indicating that the methanol extract of *Jatvaadi Dhoomagad* had statistically no significant larvicidal activity.

□ We observed that the aqueous extract of *Jatvaadi Dhoomagad* has LC 50 values of 1171.833, 867.725,

and 721.148 in 24 hr, 48 hr, and 72 hr respectively and LC 90 values of 4735.696, 4699.908 and 7008.294 in 24 hr, 48 hr, and 72 hr respectively, and the p-value was $>.05$, indicating that the aqueous extract of *Jatvaadi Dhoomagad* had statistically no significant larvicidal activity.

The adulticidal activity of extracts of *Jatvaadi Dhoomagad* against *Aedes aegypti* :

Adulticidal bioassay tests of petroleum ether, chloroform, methanol, and aqueous extracts of the *Jatvaadi Dhoomagad* compound on the field-collected *Aedes aegypti* against impregnated paper (WHO) were evaluated. 0.2gm of each extract of *Jatvaadi Dhoomagad* was dissolved in 2.5 mL ethanol and applied to Whatman filter paper of 180 cm² (size: 12x15) and dried. Control papers were treated with ethanol under similar conditions. Three replicates were carried out along with two controls. The knockdown time of *Aedes aegypti* at a sixty-minute interval was recorded. The first knockdown of *Ae. aegypti* was recorded at 1 hr. During the 24-hour recovery period, the maximum mortality of *Aedes aegypti* was 20 out of 60 exposed mosquitos in three replicates (20 mosquitos each). It was observed that the percent corrected mortality of *Ae. aegypti* was 33.3% (range: 30–40%) and the remaining 66.7% of mosquitoes were not affected by the *Jatvaadi Dhoomagad* extract (petroleum ether). Out of 60 mosquitoes exposed to the chloroform extract, nine died after a 24-hour recovery period in three replicates (20 mosquitoes each). *Ae. aegypti* was found to have a 15% adjusted mortality rate (range: 10–20%), while the remaining mosquitoes were unaffected by the *Jatvaadi Dhoomagad* extract. Out of 60 mosquitoes exposed to the methanol extract, 8 died after a 24-hour recovery period in three replicates (20 mosquitoes each). *Ae. aegypti* was found to have a 13.3% adjusted mortality rate (range: 10–20%), while the remaining mosquitoes were unaffected by the *Jatvaadi Dhoomagad* extract. Out of 60 mosquitoes exposed to the chloroform extract, nine died after a 24-hour recovery period in three replicates (20 mosquitoes each). *Ae. aegypti* was found to have a 15% adjusted mortality rate (range: 10–20%), while the remaining mosquitoes were unaffected by

the *JatvaadiDhoomagad* extract. The knockdown time of adult *Aedes* at one hour. The maximum mortality of *Ae. aegypti* at 24 hr recovery period was 0 out of 60 exposed mosquitoes in 3 replicates (20 mosquitoes each). It was found that the aqueous extract of *JatvaadiDhoomagad* had no effect on mosquitoes.

Repellency activity- The bioassay test for repellent activity was conducted using a standard protocol (WHO, 2006). On the recommendation of NIMR specialists, we further decided to conduct repellence in petroleum ether extract of *Jatvaadidhoomagad* due to low mortality in other extracts of *JatvaadiDhoomagad* in adulticidal action. Two replicates were carried out along with two control landing times of *aedes aegypti* at five min, 15 min, 30 min, and 45 min, which were recorded. The first landing of *aedes aegypti* was recorded at 5 min.

DISCUSSION

Mosquitos are described as Mashak in Ayurveda Literature. Mosquitoes are a nuisance to human health. They get attracted to humans through a sense of warm and humid convection arising from the human body and that of concentration of carbon dioxide. Generally, *Jatvaadidhoomagad yoga* contains aromatic plants as well as resins to bind them together. In *JatvaadiDhoomagad*, *Tamalpatra*, *Guggul*, *Sarja*, *Sevya*, etc are aromatic while *Laksha*, *Guggul*, and *Sarja* contain binding resins. The ingredient *Bhallatak* contains oil which is an irritant. *Sevya*, *Tamalpatra*, and *Aparajita* already showed mosquito repellent and larvicidal activity. Hence all these properties synergistically may act for obtaining the mosquito-repellent effect of *JatvaadiDhoomagad*. Most of the ingredients of *JatvaadiDhoomagad* are *Katu*, *Tikta*, *Kashayarasa*, and *Teekshnaguna* with *Ushnaveerya* hence can act as *vishagna* and *keetaghna*. Under the larvicidal study, we observed that petroleum ether extract of *JatvaadiDhoomagad* has LC 50 values of 1310, 375.237, and 221.535 in 24 hr, 48 hr, and 72 hr respectively and LC 90 values of 12521.458, 10027.278 and 1714.026 in 24 hr, 48 hr and 72 hours respectively.

And the p-value was $>.05$, indicating that the petroleum ether extract of *JatvaadiDhoomagad* had statistically no significant larvicidal effect. In 24 hours of exposure, we observed that chloroform extract of *JatvaadiDhoomagad* has a significant outcome in the Probit model; its LC50 value was 1250.448, 2142.018 and 1237.900 in 24 hr, 48 hr, and 72 hr respectively LC90 value 4543.724, 21594.296 and 6713.113 in 24 hr, 48 hr, and 72 hr respectively, indicating that the chloroform extract of *Jatvaadidhoomagad* had statistically no significant larvicidal effect in 48 hr and 72 hr. When we ran the data through a Probit model, we observed that the methanol extract of *JatvaadiDhoomagad* has LC 50 values of 1310.656, 829.450, and 249.887 in 24 hr, 48 hr, and 72 hr respectively and LC 90 values of 4549, 3972.682 and 2787.708 in 24 hr, 48 hr, and 72 hr respectively, and the p-value was $>.05$, indicating that the methanol extract of *JatvaadiDhoomagad* had statistically no significant larvicidal effect. We observed that the aqueous extract of *JatvaadiDhoomagad* has LC 50 values of 1171.833, 867.725, and 721.148 in 24 hr, 48 hr, and 72 hr respectively and LC 90 values of 4735.696, 4699.908 and 7008.294 in 24 hr, 48 hr, and 72 hr respectively, and the p-value was $>.05$, indicating that the aqueous extract of *JatvaadiDhoomagad* had statistically no significant larvicidal effect. **In adulticidal activity**, different extracts of *JatvaadiDhoomagad* against *Aedes* mosquitoes revealed that petroleum ether extract showed 33.3 % mortality in the range of 20.0-40.0 percent, chloroform extract showed 15 % mortality in the range of 10-20 %, methanol extract showed 13.3% mortality in the range of 10-20% and no mortality was observed in Aqueous extract. It means **chloroform extract** of *JatvaadiDhoomagad* has **better larvicidal activity** in 24 hours, while **petroleum ether extract** of *JatvaadiDhoomagad* has **better adulticidal activity** in comparison to other extract of *JatvaadiDhoomagad*. The mosquito repellency was measured on the basis of the protection time. In this experimental study, the knockdown time of the control group and petroleum ether extract was the

same so the repellency activity was found nil, and no protection time was recorded.

CONCLUSION

The significant increase in insecticide-based vector-mosquito control in the past decade resulted in their increasing resistance among vector-mosquito. Research has shown that *Sevya*, *Bhallatak*, and *patra*, have larvicidal and mosquito-repellent activity and *Aparajita* has larvicidal activity, but in the compound formulation of *Jatvaadi Dhoomagad*, it did not show such activity. This substance is a traditional formulation from the second century. The life cycle of *Aedes aegypti* is short and mutation for chemicals is very fast, so it might have developed resistance to our compound (*Jatvaadi Dhoomagad*) and thus showed no effect on this species of mosquitoes. No scientific study is reported on the larvicidal, adulticidal, and repellent actions of *Jatvaadi Dhoomagad*, therefore we took this formulation to investigate these actions. Larvicidal activity of the *Jatvaadi Dhoomagad* compound in different solvents extracts (petroleum ether, methanol, aqueous) had shown no significant effect, but in chloroform extract, there was a less significant result, which is scientifically not useful. In adulticidal activity, there was less than 80 % effect (according to WHO standards) of *Jatvaadi Dhoomagad* extract in different solvents, which did not show significant results. In repellent activity, there was no result shown in the petroleum ether extract of *Jatvaadi Dhoomagad*. Therefore, it is not acceptable according to the WHO standard. We found no significant effect of our compound *Jatvaadi Dhoomagad* in larvicidal activity.

FUTURE RECOMMENDATION

- Instead of using the extract form of *Jatvaadi Dhoomagad* in different concentrations, the

fumigation method can be used for the experiment.

- Oil extract of *Jatvaadi Dhoomagad* can be used for the larvicidal, adulticidal, and repellent activity.
- The study can be carried out on different species of mosquitoes like *Culex* or *Anopheles* for further research work.

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