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# Larvicidal activity of different plant parts of *Asparagus setaceus* against dengue vector *Aedes aegypti*

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**Abstract**
Plant-based natural products are preferred over synthetic insecticides because natural products are eco-friendly and much less prone to the development of resistance. This has necessitated research for the development of the eco-friendly, biodegradable indigenous method for the control of vectors. Mosquito larvicidal efficacy of crude and ethanol extracts of the leaf, bark, root, and fruit of *Asparagus setaceus* (Asparagaceae) against *Aedes aegypti* was evaluated in the present study. Phytochemical screening of all the plant parts was also done.
Crude and ethanol extracts of different parts of *A. setaceus* showed efficient larvicidal properties against *Ae. aegypti* in the following descending order fruit> root> bark> leaf. Fruit ethanolic extract exhibited the highest mortality (100%) against 1st and 2nd instars larvae at 24 hours of exposure. The result of the three-way factorial ANOVA of an ethanolic extract with different plant parts, different time intervals, and different instars revealed a significant difference in larval mortality ($P<0.05$). Phytochemical screening indicated the presence of alkaloids, saponin, tannin, and flavonoids. The isolated bioactive fraction possesses functional groups as aldehyde, alkane, alcohol, and keto.
*A. setaceus* indeed has remarkable mosquito larvicidal activity and can be used as eco-friendly insecticides in near future.

**Keywords:** *Asparagus setaceus*, *Aedes aegypti*, larvicidal activity, phytochemical

## 1. Introduction
Mosquitoes are vectors of various diseases and can transmit malaria, filariasis, dengue yellow fever, Japanese encephalitis, chikungunya, and other viral diseases [1, 2], which have a devastating effect on human beings. Among these mosquito-borne diseases dengue including dengue hemorrhagic fever, yellow fever and chikungunya are endemic in Southeast Asia and Africa [3], which are transmitted by *Aedes aegypti* (Linn.). The use of synthetic insecticides is the most common method for controlling mosquitoes. A recent study has proved that mosquitoes develop resistance to synthetic insecticides [4] and even to biopesticides such as *Bacillus thuringiensis* [5]. Also, synthetic insecticides adversely affect the environment by contaminating water and soil. There is an urgent need to find alternatives to synthetic insecticides which are potent, cost-effective and eco-friendly.
Botanicals do not have any hazardous effect on the ecosystem. A recent study has proved that phytochemical compounds, such as saponins [6], steroids [7, 8], flavonoids [9], alkaloids [10], aliphatic amides [11], and tannins [12] have potential as mosquito larvicides. Secondary metabolites of plants and their synthetic derivatives provide alternative sources in the control of mosquitoes [13, 14, 15]. Besides being anti-mosquito agents, plant products may act as antihelmintic [16, 17], antibacterials [18, 19] etc.
*Asparagus setaceus* (Asparagaceae) is a scrambling perennial herb growing up to 5 m. Roots are fibrous. Cladodes (flattened stems looking like leaves) are numerous per axils, 4 - 7 mm long, 0.5 mm wide. Occurring from spring to autumn, bell-shaped greenish-white flowers occur singly or in pairs, at the end of branches, hanging, 5 - 7 mm in diameter, flower stalks are 1 - 2.5 mm long. Sepals and petals are 3 - 4 mm long, 1 - 1.5 mm wide. Stamens are 2.5 - 3.5 mm long, filaments 2 - 3 mm long. Green berry is 4 - 5 mm in diameter which blackens

with maturity, fruit single, and 2.5 - 3.5 mm in diameter. It is native to Africa and is grown as a garden plant in parts of India. The objective of this study was to test larvicidal efficacy against *Ae. aegypti* and phytochemical screening of crude and ethanol extracts of leaf, bark, root, and fruit of *A. setaceus.*

## 2. Materials and Methods

### 2.1 Period and Location of the study

The study was carried out in Dec 2018. This work was conducted in the laboratories of the Department of Zoology, Kulti College, Kulti and Mosquito, Microbiology & Nanotechnology Research Units, Department of Zoology, The University of Burdwan, West Bengal, India.

### 2.2 Collection of plant materials and preparation of extracts

Fresh healthy leaf, root, bark, and green fruit were collected from the medicinal plant garden of Kulti College, Kulti, West Bengal, India and were brought to the laboratory for subsequent processing. After collection, different parts were separated and were initially washed with distilled water and dried on a paper towel. An amount of 15 g and 200 g of each leaf, root, bark, and fruit were weighed separately for crude and ethanolic extracts respectively. Different parts were crushed separately with a Jankel and Kunkel model A10 mill and the plant juice was filtered by Whatman No. 1 filter paper and the clear filtrate was used as a stock solution (100% concentration of crude extract) for bioassay experiments. Required concentrations (0.5%) were prepared by mixing up stock extract with the appropriate quantity of sterilized distilled water. For ethanolic extract samples were dried under shade for 4 weeks at room temperature. After grinding 50 g powder of leaf, root, bark, and fruit were shifted separately into filter paper thimble and were extracted for 72 hours with 500 ml of ethanol by using a set of four different soxhlet apparatus simultaneously [15]. A semisolid extract for each sample was obtained after the complete elimination of ethanol under reduced pressure. The extracts were stored in the refrigerator until use. Phytochemical analyses were carried out on the crude and ethanolic extracts using standard procedures to identify the chemical constituents like alkaloids, saponins, flavonoids, tannins, and steroidal glycosides [20, 21, 22].

### 2.3 Mosquito collection

Eggs of *Ae. aegypti* were collected from ovitraps set on the campus of Kulti College and were reared in trays containing tap water and maintained at 28 ± 2° C. When the eggs hatched into larvae, these were fed with a mixture of dog biscuits and yeast powder in the ratio of (3:1). Larvae were further reared to the adult stage and identified following the key provided by Christophers (1933) [23], Barraud (1934) [24], and Chandra (2000) [25]. The culture was kept free from exposure to pathogens, insecticides, or repellents.

### 2.4 Larvicidal Bioassay

The larvicidal bioassay was conducted according to the standard WHO larval susceptibility test methods [26] with slight modification. In the larvicidal bioassay, all the larval instars of *Ae. aegypti* were separately exposed to test concentrations of 0.5% and 100 ppm of crude and ethanol extract respectively of selected parts of *A. setaceus*. Twenty-five each of first, second, third, and fourth instars larvae were transferred gently to plastic bowls (contain 100 ml of water) separately, and simultaneously a control was maintained for crude and ethanol extracts. Larval mortalities in both treatment and control experiments were recorded after 24 h, 48 h, and 72 h of exposures. Moribund larvae were counted as dead larvae. This experiment was repeated three times with three sets of controls.

### 2.5 Effect on non-target organisms

Non-target organisms share common habitats with mosquito larvae and are not specifically targeted as a component by an interaction for which it was not the intended recipient. The effects of the ethanol extract obtained from different plant parts were tested against non-target organisms like *Chironomus circumdatus* larvae and *Daphnia* spp. These were exposed to 100 ppm concentration of the said extract to observe the mortality and other abnormalities such as sluggishness and reduced swimming activity up to 72 h of exposure [27].

### 2.6 Isolation and IR analysis of the active ingredient

The ethanolic stock extract of *A. setaceus* fruits was chromatogrammed and each of the spots was scrapped one by one from TLC plates according to their ratio of front [i.e. Retardation factor ($R_f$) - 9/14 or 0.642] value. From 45 chromatogrammed plates, each spot having same $R_f$ (0.642) value was assembled and dissolved in ethanol. The supernatant solution was taken in a beaker discarding the precipitate containing silica gel. After the desiccation of ethanol, an active ingredient containing solid fraction deposited at the bottom of the beaker was collected and different concentrations (20 ppm, 30 ppm, and 40 ppm) of bioactive fraction were made for bioassay experiment against 3rd instars larvae of *Ae. aegypti*. The spot ($R_f$ - 0.642) that showed a positive response in larval mortality of bioassay experiments was subjected to Infrared (IR) spectroscopic analysis using KBr plates (JASCO FT/IR Model-4700) with a scanning speed of 4 mm/s.

### 2.7 Statistical analysis

The percentage mortalities (% M) were corrected by using Abbott's formula [28]. For statistical justification, ANOVA analysis was done with the help of 'Stat plus 2009 professional' trial version software.

## 3. Results

The percentages mortality of four different larval instars of *Ae. aegypti* mosquito treated with four different plant parts of *A. setaceus* are presented in table 1. Fruit ethanolic extract showed the highest mortality rate of 100% against 3rd instars larvae in 48 hours (Table 2). The remaining parts like leaves, bark, and root were responsible for maximum mortality of 65.33% against 3rd instars larvae in 72 hours. No change in the swimming behaviour and survivability of non-target organisms were noted.

Results of the three-way factorial ANOVA of ethanolic extract of *A. setaceus* carried out with different plant parts, different time intervals and different instars revealed a significant difference ($P<0.05$) in larval mortality (Table 3).

In preliminary phytochemical analyses, secondary metabolites like alkaloids were detected qualitatively in all plant parts tested (Table 4). On the other hand, all the secondary

metabolites were present in the extracts of fruit and root of the tested plant.

Mortality of 3rd instar larvae treated with isolated compounds are recorded in table 5. The infrared spectrum of fruit ethanolic extract (Figure 1) revealed the presence of different functional groups 2918.73 cm-1 alkane, 2850.27 cm-1 aldehyde, 1738.51 cm-1 ketone, 1461.78 cm-1 alkane, 1168.65 cm-1 alkane, alcohol.

**Table 1:** Effect of crude extract (0.5%) of different plant parts of *Asparagus setaceus* against 3rd instars larvae of *Aedes aegypti*

| Larval instars | Plant parts used | Percent mortality (mean ± SE) | | |
|---|---|---|---|---|
| | | 24 hour | 48 hour | 72 hour |
| First | Leaf | 36.00 ± 0.54 | 42.67 ± 0.33 | 48.00 ± 0.33 |
| | Root | 52.00 ± 0.00 | 60.00 ± 0.33 | 65.33 ± 0.54 |
| | Bark | 49.33 ± 0.33 | 54.67 ± 0.67 | 61.33 ± 1.20 |
| | Fruit | 94.67 ± 0.67 | 98.67 ± 0.33 | 100.00 ± 0.00 |
| | Control | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Second | Leaf | 28.00 ± 0.33 | 34.67 ± 0.54 | 40.00 ± 0.54 |
| | Root | 48.00 ± 0.00 | 56.00 ± 0.88 | 58.67 ± 0.33 |
| | Bark | 44.00 ± 0.00 | 49.33 ± 0.33 | 54.67 ± 0.88 |
| | Fruit | 88.00 ± 0.54 | 93.33 ± 0.33 | 97.33 ± 0.33 |
| | Control | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Third | Leaf | 26.67 ± 0.54 | 32.00 ± 0.88 | 37.33 ± 0.33 |
| | Root | 46.67 ± 0.88 | 52.00 ± 0.33 | 57.33 ± 0.54 |
| | Bark | 41.33 ± 0.33 | 46.67 ± 0.33 | 52.00 ± 0.33 |
| | Fruit | 85.33 ± 0.33 | 90.67 ± 1.20 | 94.67 ± 0.67 |
| | Control | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Fourth | Leaf | 24.00 ± 0.00 | 25.33 ± 0.67 | 28.00 ± 0.00 |
| | Root | 36.00 ± 0.54 | 38.67 ± 0.00 | 40.00 ± 1.20 |
| | Bark | 33.33 ± 0.54 | 37.33 ± 0.54 | 38.67 ± 0.33 |
| | Fruit | 80.00 ± 0.00 | 81.33 ± 0.33 | 84.00 ± 0.00 |
| | Control | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

**Table 2:** Competence of ethanolic extract (100 ppm) of different plant parts of *Asparagus setaceus* against different larval instars of *Aedes aegypti*

| Larval instars | Plant parts used | Percent mortality (mean ± SE) | | |
|---|---|---|---|---|
| | | **24 hour** | **48 hour** | **72 hour** |
| First | Leaf | 41.33 ± 0.54 | 49.67 ± 0.33 | 52.00 ± 0.33 |
| | Root | 64.00 ± 0.00 | 69.33 ± 0.33 | 74.67 ± 0.54 |
| | Bark | 53.33 ± 0.33 | 60.00 ± 0.67 | 65.33 ± 1.20 |
| | Fruit | 100.00 ± 0.67 | 100.00 ± 0.33 | 100.00 ± 0.00 |
| | Control | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Second | Leaf | 32.00 ± 0.33 | 37.33 ± 0.54 | 44.00 ± 0.54 |
| | Root | 56.00 ± 0.00 | 62.67 ± 0.88 | 69.33 ± 0.33 |
| | Bark | 44.00 ± 0.88 | 49.33 ± 0.54 | 56.00 ± 0.88 |
| | Fruit | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| | Control | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Third | Leaf | 29.33 ± 0.33 | 36.00 ± 0.88 | 41.33 ± 0.33 |
| | Root | 53.33 ± 0.88 | 60.00 ± 0.33 | 65.33 ± 0.54 |
| | Bark | 40.33 ± 0.33 | 46.67 ± 0.33 | 52.00 ± 0.33 |
| | Fruit | 98.67 ± 0.33 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| | Control | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Fourth | Leaf | 22.67 ± 1.20 | 28.00 ± 0.67 | 30.67 ± 0.33 |
| | Root | 41.33 ± 0.54 | 45.33 ± 0.00 | 48.00 ± 1.20 |
| | Bark | 38.67 ± 0.54 | 32.00 ± 0.54 | 37.33 ± 0.33 |
| | Fruit | 84.00 ± 0.00 | 89.33 ± 0.33 | 92.00 ± 0.00 |
| | Control | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

**Table 3:** Completely randomized three way ANOVA analyses of the larvicidal activity using instars (I), hour (H) and Used Plant Parts (C) as three independent variables

| Source of variation | Sum of squares | Df | Mean sum of squares | F value | p-level |
|---|---|---|---|---|---|
| Instars(I) | 510.58 | 3 | 170.19 | 377.04 | 0.00 |
| Hours(H) | 117.26 | 2 | 58.63 | 129.89 | 0.00 |
| Plant part(C) | 4682.13 | 3 | 1560.71 | 3457.57 | 0.00 |
| Instars*Hours | 0.79 | 6 | 0.13 | 0.29 | 0.93 |
| Instars* plant part | 53.02 | 9 | 5.89 | 13.05 | 0.00 |
| Hour*plant part | 21.40 | 6 | 3.56 | 7.90 | 0.00 |
| Instars*Hour*plant part | 7.43 | 18 | 0.41 | 0.91 | 0.56 |
| Within groups | 43.33 | 96 | 0.45 | --- | |
| Total | 5435.97 | 143 | 38.01 | --- | |

**Table 4:** Preliminary qualitative analyses of crude and ethanol extracts of different parts of *Asparagus setaceus* for secondary metabolites

| Name of the Test | Phytochemical Constituents | Leaf | | Root | | Bark | | Fruit | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | E | A | E | A | E | A | E |
| Mayer's reagent | Alkaloid | + | + | + | + | + | + | + | + |
| $H_2So_4$ test | Saponin | + | + | + | - | + | + | + | - |
| Ammonia test | Flavonoids | - | - | + | + | - | - | + | + |
| Lead Acetate | Tanin | - | - | + | + | - | - | + | + |
| Liebermann's test | Steroidal glycosides | - | - | + | - | - | - | + | - |

A - Crude extract, E - Ethanol extract, (+) Positive, (-) Negative

**Table 5:** Percent mortality of *Asparagus setaceus* fruit ethanolic extract TLC fraction (with $R_f$ - 0.642 value) against third instars larvae of *Aedes aegypti*

| Larval Instars | Concentration (ppm) | Percent Mortality (Mean ± SE) | | |
|---|---|---|---|---|
| | | 24 hour | 48 hour | 72 hour |
| 3rd | 20 | 85.33 ± 0.88 | 92.00 ± 0.54 | 97.33 ± 0.33 |
| | 30 | 89.33 ± 0.33 | 96.00 ± 0.00 | 100.00 ± 0.00 |
| | 40 | 98.67 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |

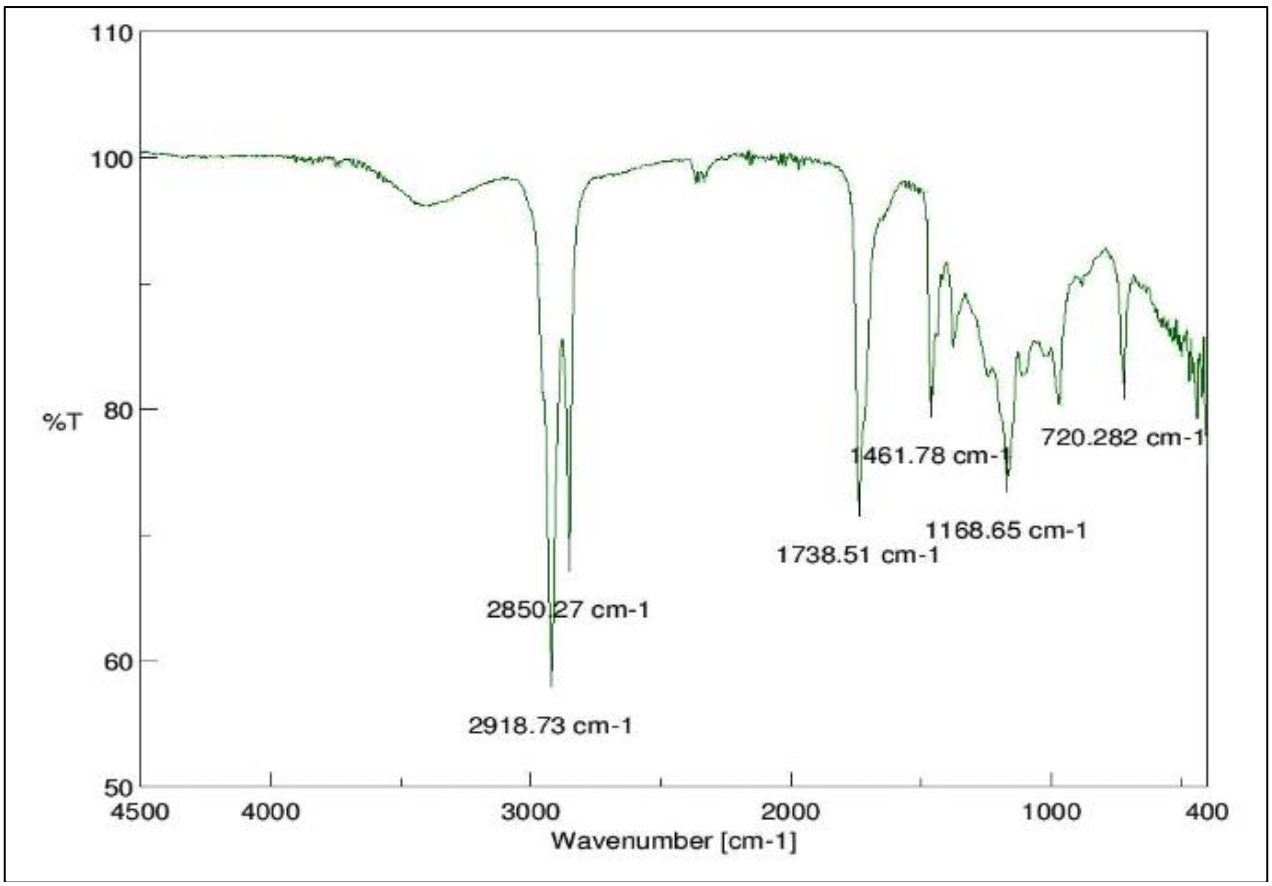


**Fig 1:** IR spectra of isolated bioactive fraction of fruit ethanolic extract of *Asparagus setaceus*

## 4. Discussion

Many of the defensive components of plants are biodegradable with non-residual effects on the biological environment. Botanicals can be an alternate source of bioactive chemicals being free from harmful effects on non-targets. The use of these botanical derivatives in mosquito control, instead of synthetic insecticides, can reduce the cost and environmental pollution [29].

The findings of the present investigation reveal that all plant parts of *A. setaceus* possess larvicidal activity against larval *Ae. aegypti* in the following descending order like fruit> root> bark> leaf. Moreover, fruit extract exhibits larvicidal activity in the following descending order i.e. isolated bioactive fraction> ethanolic extract> crude extract. The plant extracts are safe to tested non-target organisms that share the same habitat with *Ae. aegypti* larvae.

Alkaloids have a significant effect on the reproductive physiological, emergence, larvicidal, growth-regulating, and chemosterilant activities against mosquitoes. Reduced fecundity and fertility have been observed in adult female mosquitoes exposed to alkaloid-containing culture medium in their developmental stages [10]. Saponin acts as a potential mosquito larvicidal compound against *Ae. aegypti* and *Culex quinquefasciatus* [30]. Flavonoid compounds also have larvicidal, repellence and ovicidal and oviposition-deterrent activities [9]. Mosquito larvicidal activity reported in the present study can be due to any of the bioactive compounds or additively or synergistically by many compounds detected. In

our study, the isolated bioactive fraction ($R_f$ -0.642) possesses functional groups like aldehyde, alkane, alcohol, and keto. Many authors also have reported various functional groups from different solvent extractives of different plant parts. Chloroform extract of *Ocimum canum* leaf that shows larvicidal activity against *Ae. aegypti*, possess functional groups like alkenes, hydroxyl, phenol, alcohol etc. [31]. Ethyl acetate extract of leaves of *Glochidion lanceolarium*, which was shown to have significant larvicidal activity against *Cx. vishnui* group contains phenol as a functional group [32].

## 6. Conclusion

We can conclude from this study that in totality, *A. setaceus* indeed has larvicidal potential and can be used as a substitute for commercial insecticides. Further studies are necessary to find out the active ingredient/s specifically and to determine the mode of action.

## 7. Acknowledgement

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## 8. Conflict of interest

The authors have no conflict of interest.

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