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# Evaluation of larvicidal efficacy of *Diospyros montana* leaf extract on *Aedes albopictus*

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**Abstract**
In tropical and subtropical countries, mosquitoes play vital role as vectors of numerous dangerous diseases, such as dengue, yellow fever, filariasis, malaria, and zika, among others. *Aedes albopictus*, the Asian tiger mosquito can transmit the viruses of several life threatening diseases including, dengue, yellow fever, and zika. The present study aimed at assessment of the larvicidal activity of the crude extract of *Diospyros montana* leaves against *Ae. Albopictus*, and identification of the plant compound and functional groups responsible for larvicidal efficacy via FT-IR and different phytochemical analysis. Effect on the non-target organisms was also evaluated. However, more than 80% larval mortality is observed after 72 hours of exposure in all instars. After 72 hours of exposure, the $LC_{50}$ caused moderate mortality of 12% and 8% against *Chironomus circumdatus* and *Toxorhynchites splendens* respectively. Numerous secondary metabolites, including terpenoids, saponins, flavonoids, and alkaloids, coumarins were present in the crude extract of *D. Montana*. This is the first study to examine the efficacy of *D. Montana* leaf extract as a larvicidal agent against *Ae. albopictus* through determination of its secondary metabolites.

**Keywords:** *Diospyros Montana*, *Aedes albopictus*, larvicide, FTIR analysis, phytochemicals

## 1. Introduction
Mosquitoes play a crucial role as vectors of many harmful diseases, including dengue, yellow fever, filariasis, malaria, and zika, etc., mainly in tropical and subtropical countries. The Asian Tiger mosquito, *Aedes albopictus*, responsible for dengue, yellow fever and zika transmission, mainly breeds in coconut shells, flower tubs, buckets, tins, earthen pots, open tanks, unused shoes, ice-cream cups, discarded tires, etc.[1-2]. These diseases considerably worsen social mobility and poverty [3]. Since mosquitoes are the most ecologically significant insects in terms of their impact on public health, proper control programs are required. Up to a few years ago, only the adults were targeted; however, it is now widely accepted that focusing on the larvae will result in a greater reduction of mosquito populations. Regular treatment of chemically synthetic pesticides is typically used for larvae control. As the populations of mosquitoes continue to rise, the application of synthetic insecticides has increased day by day. Despite their effectiveness, their prolonged uses have negative impacts on human health, non-target animals, and the whole ecosystem and also developed resistance after a limited period. Eco-friendly control of mosquito larvae by some common exotic fishes, air-breathing fish, and dragonfly nymphs has been reported previously [4-6]. Changes in habitat composition may also reduce some mosquito species [7]. Alternatives of synthetic insecticides are needed everywhere in the world. From this perspective, botanical pesticides show promising results because they are efficient, eco-friendly, quickly biodegradable, and easily affordable [8-9]. The secondary chemicals from different plants are widely known for their overall insecticidal properties. Numerous substances from various plant extracts have been to have harmful effects on different mosquito species [10-11]. Efficacy of plant extracts as antimicrobial [12-13] and anthelmintic [14-15] agents have also been reported.
The genus *Diospyros* (family: Ebenaceae) consists of about 500 species that can be found in the tropical [16] and temperate regions of China, India, Indonesia, and the Malay Peninsula. There are 240 different species of *Diospyros*, 59 of which are scattered throughout India [17].

*Diospyros Montana* (Roxb.), common name Bombay ebony, has thin stems, a smooth bark, and young shoots that are glabrous or pubescent. The leaves are alternating, ovate-oblong, glabrescent, or gently pubescent, with a decurrent base and a simple petiole [18]. All parts of *Diospyros Montana* have significant therapeutic potential in traditional systems of medicine, making it one of the most important medicinal plants. Its roots act as an abortifacient, while the barks and gum are used to treat tuberculosis and jaundice, respectively [19]. *D. montana* has been reported to have anthelmintic, anticancer, anti-inflammatory, antileukemic, antimalarial, antiviral, prostaglandin synthesis inhibitor, and hypolipidemic properties and used in the treatment of dysuria, gravel, menor, biliousness, neuralgia, pleuracy, pneumonia, delirium, dysentery, cough, ulcer, anti-hypersensitive and snake bites [20-24].

The objective of the present study was to assess the larvicidal activity of the crude extract of *D. Montana* leaves against *Ae. albopictus*, the effect on non-targets like *Chironomus circumdatus* and *Toxorhynchites splendens* larvae, and identify the functional groups responsible for larvicidal efficacy through FT-IR analysis.

## 2. Materials and Methods

**2.1 Collection and rearing of mosquito larvae:** Population of *Ae. albopictus* was maintained in the Mosquito, Microbiology, and Nanotechnology Research Units, Department of Zoology, The University of Burdwan. The colony was kept free of all repellents, pathogens, and insecticides and kept at 27 ± 2° C temperature and 85% relative humidity under 14:10 cycles of light and dark in a day. A powdered mixture of dried Brewer's yeast powder, dog biscuits, and algae in a 3:1:1 ratio was supplied to the larvae as food.

**2.2 Collection and identification of the plant:** Mature green leaves of *Diospyros Montana* were gathered from The University of Burdwan campus in West Bengal, India (23°16'N, 87°54'E). A voucher specimen (GCRKM/2017 /S011) was submitted to the Department of Zoology, The University of Burdwan after Professor Ambarish Mukherjee, Department of Botany, identified the plant.

**2.3 Preparation of plant extract:** The obtained fresh, green leaves were first thoroughly rinsed with tap water, then in distilled water. After that, the leaves were soaked up on tissue paper. The juice from crushed mature leaves was filtered through Whatman No. 1 filter paper, and the filtrate was used as a stock solution. For future studies, the collected filtrate was kept in a refrigerator at 4°C. The necessary concentrations were made by adding distilled water with the stock solution.

**2.4 Larvicidal bioassay:** The larvicidal bioassays were carried out in accordance with the established WHO protocol. Five concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 percent) of crude extract were evaluated on four larval instars of *Ae. albopictus*. 25 larvae of each larval instar were put into a 250 ml sterilized glass beaker containing 100 ml of tap water. The larvicidal potentials of various crude extract concentrations were evaluated against each instar in a separate beaker. Beakers were left at room temperature (27 ± 2°C) and humidity (85 ± 2 %) for a total of 72 hours of observation. The mortality percentage was noted 24 hours, 48 hours, and 72 hours after exposure. Each experiment was triplicated. When the larvae did not move after being pricked with a needle in the siphon or cervical region, or when they could not reach the water surface, larvae were considered dead.

**2.5 Phytochemical analysis:** The standard methods of Trease and Evans [25], Sofowara [26] and Harborne [27] were used for the phytochemical examination of the crude extract of the leaves. The presence or absence of secondary metabolites such as tannins, saponins, steroids, flavonoids, and terpenes in the crude extract was checked.

**2.5.1 Tannins detection test:** According to the Ferric chloride test, 5–10 drops of $FeCl_3$ were mixed with 2 ml of crude extract. The appearance of bluish-black color indicates the presence of tannins. According to the Bromine water test, 1 ml of crude extract was added with a few drops of bromine solution. The decolorization of the bromine water indicates the presence of tannins. According to the alkaline reagent test, 1 ml of crude extract was mixed with 4–6 drops of 1N NaOH solution. Tannins can be detected by the rapid emergence of yellow to red precipitation.

**2.5.2 Terpenoids and steroids detection test:** 1N glacial acetic acid was used to acidify 1 ml crude extract, and 1 ml of concentrated 4N $H_2SO_4$ was added through the test tube wall in the ice chamber. Steroids are present when brown color develops, and terpenoids are present when green color develops [28].

**2.5.3 Flavonoids detection test:** According to the Zinc hydrochloride test, 1 ml of crude extract was mixed with 10 drops of 0.5N HCL, and then a small amount of zinc was added. The presence of Flavonoids is indicated by the precipitation of reddish-pink or pink color. According to the Shinoda test, a small amount of magnesium turnings was added to 1 ml of the crude extract, and then concentrated 2N HCL was gradually added. The appearance of pink, scarlet, or green hue denotes the presence of flavonoids. 4 ml of crude extract and 1.5 ml of 50% methanol solution were combined, and after that, the mixture was heated up, and 5-6 drops of strong 2N HCL and a few metal magnesium turnings were added. Red color formation shows the presence of Flavonoids [29-30].

**2.5.4 Saponins detection test:** Few drops of $NaHCO_3$ were added to 5 ml of crude extract and shaken vigorously. Then the sample was kept undisturbed for 3 mins. The formation of honeycomb-like stable foam indicates the presence of saponin.

**2.5.5 Alkaloids detection test:** The presence of alkaloids was examined using Mayer's and Wagner's tests. Mayer's reagent was prepared by adding 5 g of KI and 1.36 g of $HgCl_2$ in 100 ml of distilled water. Wagner's reagent was prepared by adding potassium iodide to an iodine solution. Glacial acetic acid was used to initially acidify the crude extract. Mayer's reagent was added to 1 ml of the acidified crude extract. According to this test, alkaloids are present when pale-yellow precipitate forms. Wagner's reagent was added in very small amounts to 1 ml of acidified crude extract. According to Wagner's test, reddish-brown precipitation determines the presence of alkaloids.

**2.5.6 Coumarins detection test:** 3 ml of 10% NaOH was added to 2 ml of crude extract. Instant conversion of the color of crude extract to yellow determines the presence of coumarin in the sample.

**2.5.7 Cardiac glycosides detection test:** 1 ml of glacial acetic acid was mixed with 2 ml of crude extract, then 2 ml of ferric chloride was added to it, and after that, 2 ml of concentrated $H_2SO_4$ was added. The appearance of brown color indicates the presence of cardiac glycosides.

**2.5.8 Anthocyanins detection test:** 1 ml of 2N HCL and 1 ml of $NH_3$ were added to 2 ml of crude extract. Immediate conversion of pink-red color to blue-violet indicates the presence of anthocyanin in the extract.

**2.6 Test on the non-target organisms:** The non-target organisms were chosen for testing, which share common habitats with the target species of mosquito larvae. The effect of *Diospyros montana* leaf crude extracts was studied on *Chironomus circumdatus* and *Toxorhynchites splendens* larvae. Non-target species were treated with $LC_{50}$ of crude extract (against 3rd instar *Ae. albopictus* after 24 hours) that is 0.2913%, and their mortality rates were recorded after 24 hours, 48 hours, and 72 hours of exposure [31].

**2.7 FT-IR analysis:** Fourier transformed infrared (FT-IR) spectroscopy was used to analyze the functional group present in the crude sample. The dried crude extract was combined with potassium bromide (KBR) using a mortar, and a pallet was then formed by applying hydraulic pressure to it. The pallet was then used in IR spectral analysis on a Jasco FT-IR Spectrometer (Model: FT/IR-4700) at a speed of 4 $cm^{-1}$; the scanning range was 450-4000 $cm^{-1}$.

**2.8 Statistical analyses:** Following Abbott's formula [32], the percentage of corrected mortality was determined. Experimental data were statistically analyzed to get the $LC_{50}$ and $LC_{90}$ values, regression equations (Y=mortality; X=concentrations), and regression coefficient values by using the software "STAT PLUS 2007 (Trial version)" and "MS Excel 2007". Three-way ANOVA analysis of the data was performed by using SPSS 11.0 software.

## 3. Results

Percent mortalities of *Ae. albopictus* larvae using the crude extracts of *D. montana* leaves are shown in Table 1. The results demonstrated that larval mortality gradually increased as extract concentration and exposure time increased. The outcomes of probit and regression analyses using the crude extracts for the *Ae. albopictus* mosquitoes have been tabulated in Table 2. The estimated marginal means of mortality against all instars of *Aedes albopictus* at 24h, 48h, and 72h of exposure against different concentrations of crude extract of *Diospyros montana* leaf are presented in Figure 1. Figure 1 showed that the mean mortality of all the instars elevated with increasing concentrations.

The outcome of three-way factorial ANOVA of mortality of *Ae. albopictus* using *D. montana* leaf extract revealed that different factors (time intervals, concentrations, and instars) significantly influenced larval mortality (Table 3).

The leaf extract had moderate impact on both the non-target organisms (Table 4). $LC_{50}$ of 3rd instar *Ae. albopictus* larvae showed maximum mortality of 12% against *Chironomus circumdatus* and 8% against *Toxorhynchites splendens* larvae after 72 h of exposure.

Phytochemical tests were performed to confirm the presence of plant compounds in the crude extract, which are shown in Table 5. The presence of various functional groups in the extract was determined by FT-IR analysis, and the characteristic peaks in the FT-IR spectrum indicated the presence of different functional groups, including alcohol, amide, amine, esters, alkanes and carboxylic acids. The FT-IR graph is presented in Fig 2, and the functional groups respective to the peak range are tabulated in Table 6.

**Table 1:** larval mortality of *Aedes albopictus* larvae in different concentrations of crude extracts of *Diospyros Montana* leaf

| Larval instars | Concentrations (%) | Mortality percentage (Mean± SE) | | |
|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours |
| 1st | 0.1 | 34.66±4.80 | 57.33±4.80 | 65.33±3.52 |
| | 0.2 | 42.66±2.66 | 64.00±2.30 | 72.00±2.30 |
| | 0.3 | 45.33±1.33 | 66.66±1.33 | 77.33±3.52 |
| | 0.4 | 56.00±2.30 | 78.66±1.33 | 92.00±2.30 |
| | 0.5 | 76.00±2.30 | 90.66±1.33 | 100.00±0.00 |
| | control | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 2nd | 0.1 | 33.33±3.52 | 54.66±3.52 | 61.33±4.80 |
| | 0.2 | 44.00±2.30 | 65.33±1.33 | 78.66±1.33 |
| | 0.3 | 48.00±2.30 | 74.66±1.33 | 84.00±2.30 |
| | 0.4 | 54.66±1.33 | 78.66±3.52 | 86.66±1.33 |
| | 0.5 | 70.66±2.66 | 85.33±1.33 | 100.00±0.00 |
| | control | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 3rd | 0.1 | 28.00±2.30 | 41.33±1.33 | 52.00±2.30 |
| | 0.2 | 40.00±2.30 | 57.33±1.33 | 65.33±3.52 |
| | 0.3 | 45.33±3.52 | 60.00±2.30 | 72.00±2.30 |
| | 0.4 | 61.33±1.33 | 74.66±1.33 | 82.66±1.33 |
| | 0.5 | 64.00±2.30 | 77.33±1.33 | 85.33±1.33 |
| | control | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 4th | 0.1 | 24.00±2.30 | 38.66±2.66 | 49.33±3.52 |
| | 0.2 | 38.66±3.52 | 50.66±1.33 | 66.66±1.33 |
| | 0.3 | 48.00±2.30 | 58.66±4.80 | 77.33±1.33 |
| | 0.4 | 54.66±1.33 | 66.66±3.52 | 78.66±3.52 |
| | 0.5 | 60.00±2.30 | 70.66±2.66 | 82.66±1.33 |
| | control | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |

**Table 2:** Log Probit and regression analysis of mortalities of *Aedes albopictus* larvae at different concentrations of crude extracts of *Diospyros Montana* leaf

| Larval Instars | Period of Exposure (h) | $LC_{50}$ | $LC_{90}$ | Regression equation | $R^2$ – value |
|---|---|---|---|---|---|
| First | 24 | 0.2542 | 2.3017 | y = 96.02x + 22.12 | 0.905 |
| | 48 | 0.0897 | 0.9490 | y = 81.32x + 47.06 | 0.946 |
| | 72 | 0.0771 | 0.3800 | y = 89.34x + 54.53 | 0.971 |
| Second | 24 | 0.2640 | 3.1599 | y = 85.32x + 24.53 | 0.949 |
| | 48 | 0.0895 | 0.9313 | y = 74.67x + 49.32 | 0.971 |
| | 72 | 0.0763 | 0.3653 | y = 85.34x + 56.52 | 0.924 |
| THIRD | 24 | 0.2913 | 2.4875 | y = 93.33x + 19.73 | 0.963 |
| | 48 | 0.1533 | 1.3334 | y = 89.33x + 35.33 | 0.940 |
| | 72 | 0.0988 | 0.8099 | y = 83.99x + 46.26 | 0.960 |
| FOURTH | 24 | 0.3308 | 2.8685 | y = 88.00x + 18.66 | 0.957 |
| | 48 | 0.1893 | 2.2337 | y = 80.00x + 33.06 | 0.970 |
| | 72 | 0.1005 | 0.8787 | y = 78.66x + 47.33 | 0.855 |

**Table 3:** Three-way ANOVA analysis of mortality of *Aedes albopictus* larva using exposure time, concentrations of crude leaf extract of *Diospyros Montana* and instars as three parameters

| Source of Variation | SS | D.F. | MS | F | p-level | F crit | Omega Sqr. |
|---|---|---|---|---|---|---|---|
| Instars | 3776.71 | 3 | 1258.90 | 61.04 | 0.00 | 5.78 | 0.06 |
| Concentration | 26467.02 | 4 | 6616.75 | 320.85 | 0.00 | 4.94 | 0.44 |
| Hour | 23880 | 2 | 11940 | 578.98 | 0.00 | 4.94 | 0.40 |
| Instar×Concentration | 1033.06 | 12 | 86.08 | 4.17 | 0.00 | 3.01 | 0.01 |
| Instars×Hour | 620.62 | 6 | 103.43 | 5.01 | 0.00 | 4.04 | 0.00 |
| Concentration×Hour | 188.44 | 8 | 23.55 | 1.14 | 0.34 | 3.55 | 0.00 |
| Instars×Concentration×Hour | 260.26 | 24 | 10.84 | 0.52 | 0.96 | 0.52 | 0.00 |
| Within Groups | 2474.66 | 120 | 20.62 | | | | |
| Total | 58700.80 | 179 | 327.93 | | | | |
| Omega squared for combined effect | 0.93 | | | | | | |

**Table 4:** Mortality of non-target organism in crude extract of *Diospyros Montana* leaves

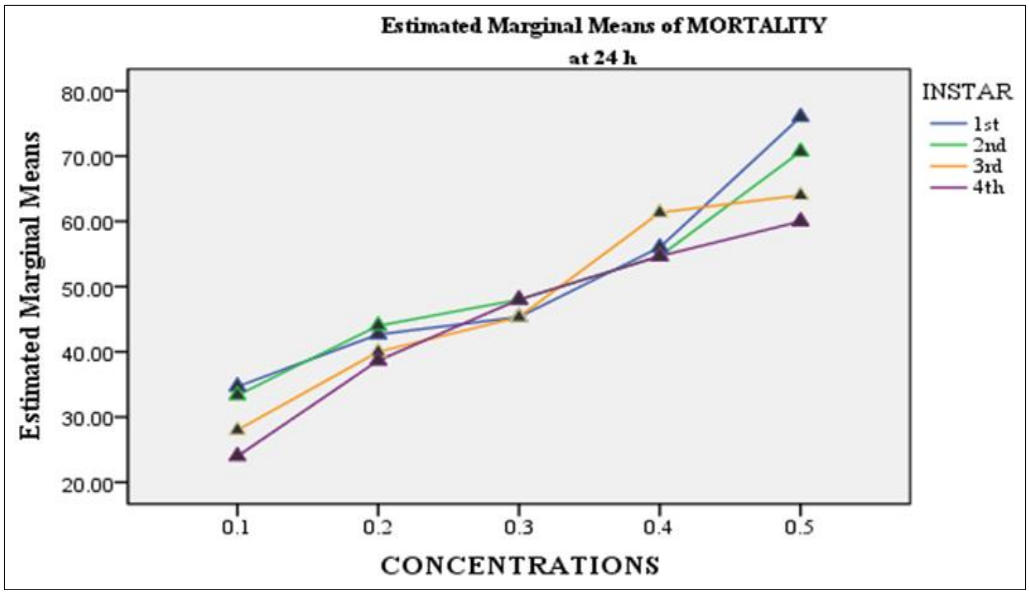
| Time of exposure | Mortality | |
|---|---|---|
| | ***Chironomus circumdatus*** | ***Toxorhynchites splendens*** |
| 24h | 1.33±1.33 | 1.33±1.33 |
| 48h | 6.66±1.33 | 5.33±1.33 |
| 72h | 12.00±2.30 | 8.00±2.30 |

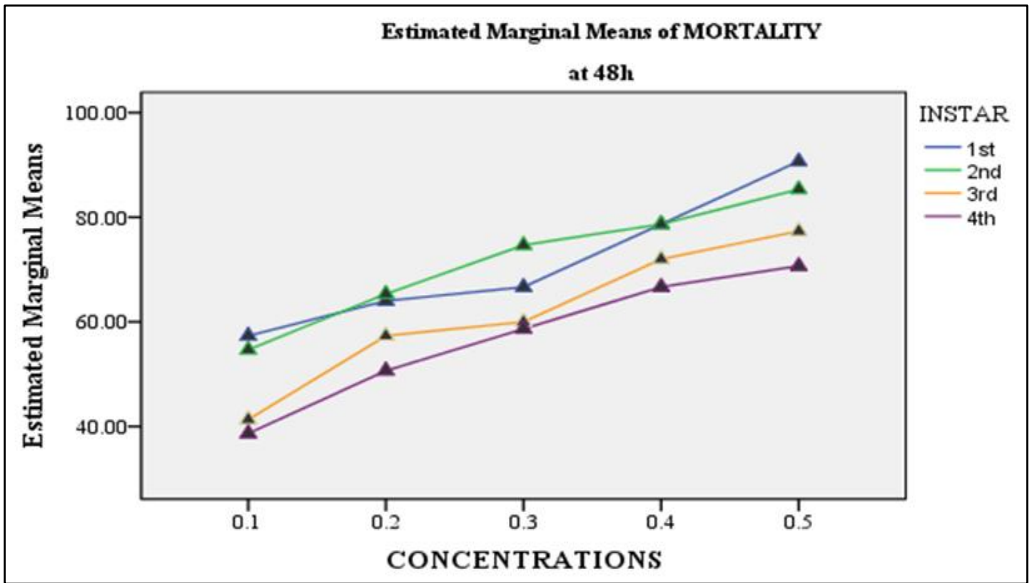
**Table 5:** Different Phytochemicals present in crude extract of *Diospyros Montana* leaf

| Present or absent | Name of the phytochemical | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Tannins | Terpenoids | Steroids | Flavonoids | Saponin | Alkaloid | Coumarin | Cardiac glycosides | Anthocyanin |
| | - | + | + | + | + | + | + | + | - |

**Table 6:** Functional groups identified in of crude extract of *Diospyros Montana* leaf, determined through FT-IR spectroscopy

| Absorption spectra peaks | Probable functional groups |
|---|---|
| 3274.54 $cm^{-1}$ | 1. C=O and N-H stretching of amide<br>2. N-H stretching of amine |
| 2919.70 $cm^{-1}$ | 1. CH stretching of alkanes<br>2. OH stretching of carboxylic acid |
| 1634.38 $cm^{-1}$ | 1. C=O stretching of ketones<br>2. C-C stretching of alkenes |
| 1321.00 $cm^{-1}$ | 1. C=O stretching of carboxylic acid<br>2. C-N stretching of amine |
| 1034.62 $cm^{-1}$ | 1. C-O stretching of alcohol<br>2. C-O-C stretching of esters |



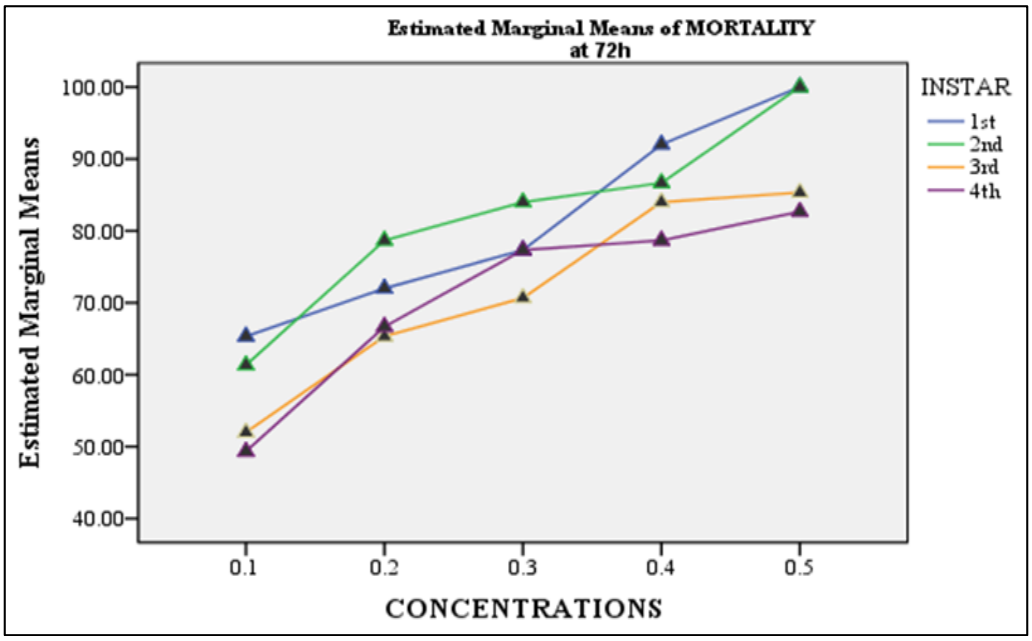




**Fig 1:** Estimated marginal means of mortality against all instar of *Aedes albopictus* at (a) 24h, (b) 48h, and (c) 72h of exposure against different concentrations of crude extract of *Diospyros Montana* leaf

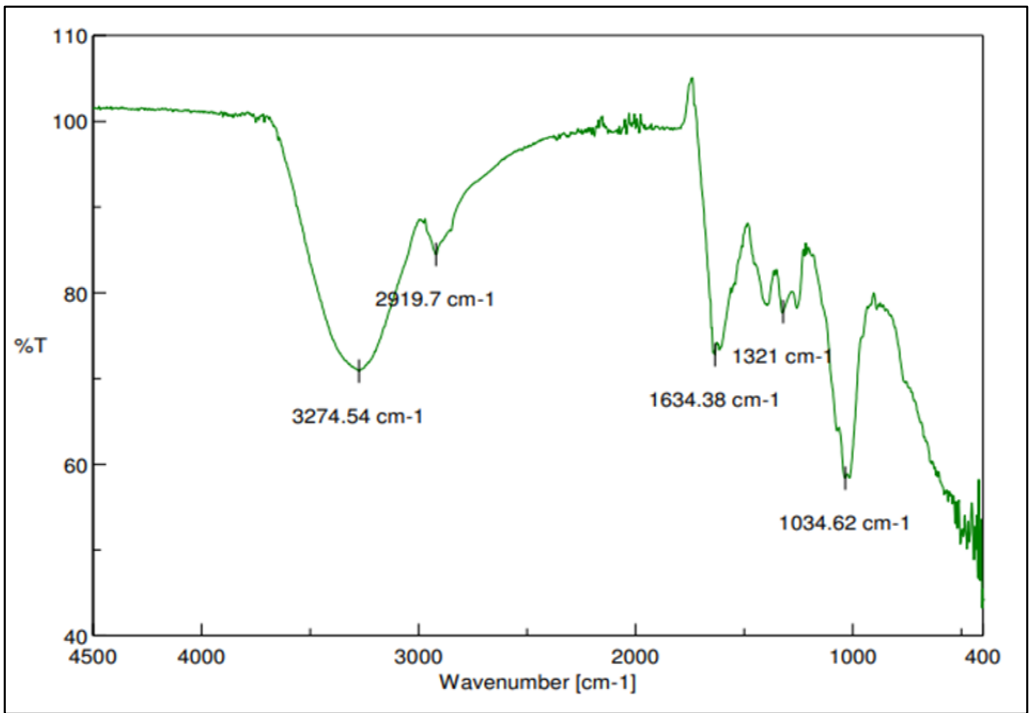



**Fig 2:** Fourier Transform Infrared (FT-IR) spectroscopy analysis of crude extract of *Diospyros montana* leaf

## 4. Discussion

Controlling mosquito vectors is the first and most important step in defending the human population against dangerous diseases carried by mosquitoes. Nowadays, several plant items are used as effective substitutes for synthetic insecticides to manage the mosquito vector population. Phytochemical have unique characteristics as they are readily available, easily biodegradable, target specific, inexpensive, and environment friendly [33]. Mosquito larva is the easiest target among all mosquito life stages because of its confinement to aquatic bodies. Secondary metabolites produced by plants, like alkaloids, terpenoids, steroids, tannins, saponins, coumarins, etc., have insecticidal capabilities that regulate the population of vectors on application. The effectiveness of these phytochemicals depends on several variables, including the type of plant, the components and extraction procedure, and the region and season from which and when the plant was harvested. Numerous studies have revealed a variety of herbs with larvicidal, pupicidal, adulticidal, phagodeterrence, oviposition deterrence, repellent, and smoke toxic effects against mosquitoes [34-36].

In the present study, cent percent larval mortality was found in 0.5 % of concentration of crude extract of leaves of *D. Montana* in 1st and 2nd instar larvae after 72 hours of exposure. However, over 80% larval mortality is observed in all instars on 72 hours post-exposure. The lowest $LC_{50}$ and $LC_{90}$ values, 0.07% and 0.36% were observed against 2nd instar larvae after 72 hours of exposure. The results of regression analyses revealed that the mortality rate was positively correlated with the concentration of crude extract, having a regression coefficient ($R^2$) value close to 1 in each case.

Plants' secondary compounds contain an abundant amount of biochemicals with diverse biological activities. Ghosh and Chandra also tested the impact of a saponin mixture derived from *Cestrum diurnum* as a larvicidal agent against the *Anopheles stephensi* mosquito [37]. Alkaloids extracted from the fruit of *Piper longum* and *Triphyophyllum pellatum* exhibited larvicidal activity against *Culex pipiens* and *Anopheles stephensi*, respectively [38]. Isoflavonoids and coumarins extracted from the tubers of *Neorautanenia mitis* were larvicidal against *Anopheles gambiae* and *Culex quinquefaciatus*, mosquitoes that transmit malaria and filariasis, respectively. [39].Crude extract of *D. Montana* contains various secondary metabolites like terpenoid, saponins, flavonoids, coumarins and alkaloids. These bioactive compounds singly or combinedly may be responsible for larvicidal efficacy.

Patil, *et al.* found the highest larval mortality in methanol extracts of *P. zeylanica* roots and *B. aegyptica* roots against *Ae. aegypti* with $LC_{50}$ 169.61 mg/lit and $LC_{90}$ 289.59 mg/lit [40]. Root of *Saussurea lappa* exhibited strong larvicidal activity against *Ae. albopictus* with $LC_{50}$ values of 3.26 µg/ml [41]. In this study, the $LC_{50}$ value is much lower compared to studies with other plants repoted earlier. Non-target organisms examined are found safer than target species *Ae. albopictus*.

## 5. Conclusion

This is the first study conducted regarding the effectiveness of leaf extract from *D. Montana* against *Ae. albopictus* as a larvicidal agent. In the near future, this extract may be used as a powerful source to produce larvicidal agent. However, more research is required to understand the chemical profile of the sample and its precise mode of action in the target species**.**

## 6. Acknowledgment

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**7. Conflict of interest**: We have no conflict of interest.

## 8. References


1. Chandra G. Mosquito. India: Sribhumi Publication Company; c2000. p. 1-102.
2. Malla RK, Mandal KK, Dutta M, Chandra G. An estimation of monthly propagation of dengue vector *Aedes aegypti* in rainwater filled tires. International Journal of Pest Management. DOI: 10.1080/09670874.2019.1616130
3. James AA. Mosquito molecular genetics: the hands that feed bite back. Science.1992;257(5066):37-38.
4. Chatterjee SN, Ghosh A, Chandra G. Eco-Friendly Control of Mosquito Larvae by *Brachytron pratense* Nymph. Journal of Environmental Health. 2007;69(8):44-49
5. Ghosh A, Mandal S, Bhattacharjee I, Chandra G. Biological control of vector mosquitoes by some common exotic fish predators. Turkish Journal of Biology. 2005;29(3):167-171.
6. Bhattacharjee I, Aditya G, Chandra G. Laboratory and field assessment of the potential of larvivorous, air-breathing fishes as predators of culicine mosquitoes. Biological Control. 2009;49(2):126-133.
7. Chandra G, Ghosh A, Biswas D, Chatterjee SN. Host Plant Preference of *Mansonia* Mosquitoes. Journal of Aquatic Plant Management. 2006;44:142-144.
8. Rawani A, Mallick Haldar K, Ghosh A, Chandra G. Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitology Research. 2009;105:1411-1417.
9. Chowdhury N, Chatterjee SK, Laskar S, Chandra G. Larvicidal activity of *Solanum villosum* Mill (Solanaceae: Solanales) leaves to *Anopheles subpictus* Grassi (Diptera: Culicidae) with effect on non-target *Chironomus circumdatus* Kieffer (Diptera: Chironomidae). Journal of Pest Science. 2009;82:13-18.
10. Singha S, Adhikari U, Ghosh A, Chandra G. Mosquito larvicidal potentiality of *Holoptelea integrifolia* leaf extract against Japanese Encephalitis Vector *Culex vishnui* Group. Journal of Mosquito Research. 2012;4(2):25-31.
11. Rawani A, Singh Ray A, Ghosh A, Sakar M, Chandra G. Larvicidal activity of phytosteroid compounds from leaf extract of *Solanum nigrum* against *Culex vishnui* group and *Anopheles subpictus*. BMC Research Notes. 2017;10:135.
12. Chatterjee SK, Bhattacharjee I, Chandra G. In vitro synergistic effect of doxycycline & ofloxacin in combination with ethanolic leaf extract of *Vangueria spinosa* against four pathogenic bacteria. Indian Journal of Medical Research. 2009;130(4):475-478.
13. Chandra G, Mukherjee D, SinghaRay A, Chatterjee S, Bhattacharjee I. Phytoextracts as Antibacterials: A Review. Current Drug Discovery Technologies. 2020;17(4):523-533.
14. Hossain E, Chandra G, Nandy AP, Mandal SC, Gupta JK. Anthelmintic effect of a methanol extract of leaves of *Dregea volubilis* on *Paramphistomum explanatum*. Parasitology Research. 2012;110:809-814.
15. Hossain E, Chandra G, Nandy AP, Mandal SC, Gupta JK. Anthelmintic effect of a methanol extract of *Bombax malabaricum* leaves on *Paramphistomum explanatum*. Parasitology Research. 2012;110:1097-1102.
16. Tanakaa T, Furusawa M, Ito T, Iliya I, Oyama M, Iinuma M, *et al*. Phenolic Constituents of Leaves of *Diospyros montana*. Natural Product Communication. 2007;2(1):55-59.
17. Puri VA. Microscopical Evaluation, Phytochemical Screening, and High-Performance Thin-Layer Chromatography Fingerprinting of Leaves of *Diospyros Montana* (Roxb.). International Journal of Green Pharmacy. 2018;12(3):205.
18. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Dehradun: International books distributers. 1995;3:1500-1.
19. Hazra B, Pal S, Banerjee A, Ray R, Bhattacharya DK. Pharmacological studies on the effect of the treatment of Swiss a Mice with diospyrin, a tumor-inhibitory plant product, and its synthetic derivatives. Phytotherapy Research. 1996;10:393-397.
20. Kokila K, Elavarasan N, Sujatha V. *Diospyros Montana* leaf extract-mediated synthesis of selenium nanoparticles and their biological applications. New Journal of Chemistry. 2017;41:7481-7490.
21. Sarma MD, Ghosh R, Patra A, Hazra B. Synthesis and antiproliferative activity of some novel derivatives of diospyrin, a plant-derived naphthoquinonoid. Bioorganic & Medicinal Chemistry. 2007;15:3672-3677.
22. Kottaimuthu R. Ethnobotany of the Valaiyans of Karandamalai, Dindigul District, Tamil Nadu, India. Ethnobotanical Leaflets. 2008;12(1):195-203.
23. Kadavul K, Dixit AK. Ethnomedicinal studies of the woody species of Kalrayan and Shervarayan hills, Eastern Ghats, Tamil Nadu. Indian Journal of Traditional Knowledge. 2009;8:592-597.
24. Rai K, Tiwari E. A poisonous forest tree Vishtendu (*Diospyros Montana* Roxb): Taxonomic evaluation. International Journal of Scientific Research. 2014;3:48-51.
25. Trease GE, Evans WC. Pharmacognosy Brailliar Tiridel can, 13th Edn, Macmillian Publishers; c1989.
26. Sofowara A. Medicinal plants and Traditional medicine in Africa, Spectrum Books Ltd, Ibadan, Nigeria; c1993. p. 289.
27. Harborne JB. Phytochemical methods, Chapman and Hall Ltd, London; c1973. p. 49-188.
28. Kantamreddi VSSN, Lakshmi YN, Kasapu VVVS. Preliminary phytochemical analysis of some important Indian plant species. International Journal of Pharma and Bio Sciences. 2010;1(4):351-357.
29. Singh A, Bhattacharya K, Chandra G. Efficacy of *Nicotiana plumbaginifolia* (solanaceae) leaf extracts as larvicide against malarial vector *Anopheles stephensi* Liston 1901. International Journal of Pharma and Bio Sciences. 2015;6(1):860-868.
30. Dutta S, Ray S. Evaluation of antioxidant potentials of leaf aqueous and methanolic extracts of *calophyllum inophyllum* in relation to total phenol and flavonoid contents. International Journal of Pharma and Bio Sciences. 2014;5:3.
31. Suwannee P, Amara N, Maleeya K, Usavadee T. Evaluation of larvicidal activity of medicinal plant extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on a non-target fish. Insect Science. 2006;13:179-188.
32. Abott WS, A method of computing the effectiveness of an insecticide. Journal of Economic Enotomology.

1925;18(2):265-267.
33. Rawani A, Halder KM, Ghosh A, Chandra G. Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitology Research. 2009;105:1411-1417.
34. Mallick S, Chandra G. Larvicidal, pupicidal, oviposition deterrent activity and smoke toxicity of mature leaf extracts of *Annona reticulata* Linn. Against filarial vector *Culex quinquefasciatus* Say. International Journal of Pharma and Bio Sciences. 2015;6:244-253.
35. Bhattacharya K, Chandra G. Phagodeterrence, larvicidal and oviposition deterrence activity of *Tragia involucrata* L. (Euphorbiaceae) root extractives against vector of lymphatic filariasis *Culex quinquefasciatus* (Diptera: Culicidae). Asian Pacific Journal of Tropical Disease. 2014;4:S226-S232.
36. Singha S, Adhikari U, Chandra G. Smoke repellency and mosquito larvicidal potentiality of *Mesua ferra* L. leaf extract against filarial vector *Culex quinquefasciatus* Say. Asian Pacific Journal of Tropical Biomedicine. 2011;1(1):S119-S123.
37. Ghosh A, Chandra G. Biocontrol efficacy of *Cestrum diurnum* (L.) (Solanales: Solanaceae) against the larval forms of *Anopheles stephensi*. Natural Product Research. 2006;20:371-379.
38. Lee SE: Mosquito larvicidal activity of pipernonaline, a piperidine alkaloid derived from long pepper, *Piper longum*. Journal of American Mosquito Control Association. 2000;16:245-247.
39. Joseph CC, Ndoile MM, Malima RC, Nkunya MH. Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpans from *Neorautanenia mitis*. Transaction of Royal Society Tropical Medicine and Hygiene. 2004;98:451-455.
40. Patil SV, Patil CD. Salunkhe RB, Salunke BK. Larvicidal activities of six plants extracts against two mosquito species, *Aedes aegypti* and *Anopheles stephensi*. Tropical Biomedicine. 2010:27(3):360-365.
41. Liu ZL, He Q, Chu SS, Wang CF, Du SS, Deng ZZ. Essential oil composition and larvicidal activity of *Saussurea lappa* roots against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitology Research. 2012;110:2125-2130.