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# Larvicidal properties of *Psychotria octosulcata* (W. A. Talbot.) (Rubiaceae) crude extracts on human vector mosquitoes *Aedes aegypti* (Linn.), *Culex quinquefasciatus* (Say.) and *Anopheles stephensi* Liston

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**Abstract**
*Psychotria octosulcata* plant extract potential in controlling mosquito larvae was investigated. The crude extracts from the plant leaves were obtained by soaking method using the solvents such as ethyl acetate, chloroform and hexane. The crude extracts were subjected to larvicidal activity of *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* of IV$^{th}$ instar larvae at 48hrs. *An. stephensi* showed maximum larvicidal activity at a minimum $LC_{50}$ value of 58.2ppm for ethyl acetate extract. Above 90% mortality was observed for ethyl acetate extract at $LC_{50}$ 62.2ppm and 65.7ppm for *C. quinquefasciatus* and *A. aegypti* respectively. The hexane also showed above 90% mortality for three species at 60-68ppm and chloroform showed comparatively lower mortality than other two solvents. The microscopic observations suggest that the extract has produced physical and physiological inconvenience for the survival of larvae. The results indicate that the plant extract has phytochemicals that are efficient to control mosquito larvae.

**Keywords:** *Psychotria octosulcata*, larvicidal activity, crude extracts, *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles stephensi*

## Introduction
Mosquitoes are one of the human vectors terrestrially display successful interactions with higher species. It has exploited the aquatic system for its abrupt development and has boosted its genetic print to cargo many dreadful diseases to humans. Hence it has become the most wanted species of insect control. Mosquitoes are popularly known to spread atrocious diseases like Dengue, Chikangunya, Malaria, Japanese encephalitis, Zika virus and other vector borne diseases. The fresh water mosquito Aedes disseminates dengue largely during the wet season in India. About 3.97 billion people around the world are at a risk of contracting Dengue [1]. Anopheles transmits malaria parasite and the incidence of malaria kills over 429, 000 people every year and children are easily affected [2]. *Culex* transmits Japanese encephalitis and Lymphatic filariasis. About six Culex species are involved in the transmission of Japanese encephalitis in India. Though death toll is reduced in recent years total elimination of the disease is targeted by 2020 [3].
However there is a high risk of the resurgence of the mosquito transmitted diseases among the susceptible and low socio economic population due to resistance developed by the mosquitoes and their disease carrier agent [4]. Mosquitoes develop special mechanisms, importantly diapauses [5] and pH [6] tolerance to synchronize their survival in every shifting environment and hence it influences the disease transmitting sequence. The fluctuations in the behaviour of the mosquito population and their infecting trends severely impinge on the mosquito control programmes. Therefore unseasonal vigil on the mosquito population and use of ecologically effective control measures incorporating appropriate control agent can consequently reduce vector population and re-entry of the disease. Though synthetic insecticides dominate the market for pest control; its prolonged use biomagnifies, hence affect non target organisms and induce resistant in target organisms.
Invariably plants with medicinal values are highly active against insects' species as they posses' complex of unique bioactive compounds which have individual or synergetic activity on the physiology and behaviour of the insects [7] and are easily biodegradable. There are

numerous medically valuable plants have been used as larvicides against mosquitoes like *Azadirachta indica* [8], *Carcuma aromatic* [9, 10], *Solanum villosum* [11], *Cassia nigricans* [12], *Riccinus communis* [13], *Abrus precatorius* [14], *Cynodon dactylon* [15], and *Musa paradisiaca* [16], *Syzygium aromaticum* [14, 15], *Phyllanthus emblica* [16], *Solanum nigrum* [17], *Areca catechu* [18], *Andrographis paniculata* [19] and, *Andrographis lineate* [19], *Tragia involucrate* [20] and many other plants were found to be effective against mosquito larvaes.

The plant Psychotria species of Rubiaceae family are traditionally used in various countries for different purposes. *Psychotria viridis* is used for inducing hallucinations [21], *Psychotria ipercacuanha* is used to treat *Entameoba hystolitica* infection [22], *Psychotria camptopus* with *Dissotis longisetosa* used to treat paralysis and activate nerves [23], *Psychotria henryiis* is used for revitalizing spleen and to reduce pain [24]. The psychotria species such as *Psychotria hoffmannseggiana*, *Psychotria capitata*, and *Psychotria goyazensis* has been reported to show insecticidal activities [25]. The earlier reports indicate that this species is medicinally important and also has insecticidal properties. The plant *Psychotria octosulcata* W. A. Talbot is a shrub endemic to south Western Ghats and also distributed in Eastern Ghats and evergreen forests in India. It is called malampaavattai in Tamil and the leaf paste is used by valaya tribes of Virudhunagar hills to cure muscle fracture [26]. It has vital use in ayurvedic pharmacological formulations and recent reports have shown anti-inflammatory [27] and anti-diabetic effect [28] of the plant extract. Based on our literature survey this is the first report on mosquito larvicidal activity using *P. octosulcata* plant.

## Materials and Methods

### Plant collection and Preparation of plant extracts

The fresh leaves of the plant were collected from Pullian Solai hills of Namakkal District, Tamil Nadu, and India. The plant was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Sytematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India. The herbarium (IPH-33) specimen of the plant was prepared and preserved in Entomology laboratory, Department of Zoology, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India.

To get a pure crude extract the plant leaves have to be devoid of dust and dirt's therefore the plants were washed with tap water thoroughly and it is dried under room temperature (25-27 ºC). The completely dried leaves were coarsely powdered using an electric vegetable blender [16].

The dried leaf powder of 400gm was extracted by soaking method [29] for 48 hrs at room temperature (25-27 ºC). The solvents used for soaking were Hexane, Chloroform, and Ethyl acetate in the ratio of 1:3(w/v). The leaf powder soaked in different solvents were filtered through Whatmann No 1 filter paper further the extract was concentrated using Rotary Vacuum Evaporator (Rotavap Model PBU-6). The concentrated extracts were stored in glass vials and refrigerated for further use.

### Mantainence of Insect culture

Larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* were collected from National Centre for Communicable Diseases control, Government of India, Ministry of health and family welfare field station, Mettupalayam, Coimbatore, Tamil Nadu, India. The collected larvae were cultured in tap water filled plastic trays protected by mosquito nets. Every day the larvae were fed with a mixture of yeast and dog biscuit (3:1 ratio). The matured IV instar larvae were transferred to cups and placed in oviposition cage. The emerged adults were fed with 10% sucrose solution. The female adult mosquitoes were allowed to feed blood meal from the hen. The filter paper lined water filled bowls were placed inside the cage to facilitate oviposition. The larvae from the F1 generation were used for the experiments. The whole setup was maintained at room temperature 25-27 ºC and 65-75% relative humidity [30].

### Larvicidal Bioassay and statistical analysis

Larvicidal assay for three species of mosquito larvae of IV$^{th}$ instar was used for the study. The concentration of 500, 250, 125, 62.5, 31.25ppm was prepared for each solvent extracts of Ethyl acetate, Chloroform, and Hexane. The bioassay was carried out according to the guidelines of WHO protocol [31] with slight modifications. The 500ppm concentration was prepared by dissolving 500mg of extract in 1000ml of dechlorinated tap water including 1ml of emulsifier (Tween 20 - Polyoxyethylene sorbitan mololaurate) and this preparation is followed for rest of their respective concentrations (250, 125, 62.5, and 31.25). Five replications were maintained and 200ml of the prepared mixture is used for each replication. Active and healthy larvae of 20 no's were introduced into each replicates. The control maintained was a mixture of 200ml of dechlorinated water including 1ml of emulsifier and introduced with 20 no's of healthy larvae. The experimental set up was observed closely until 48hrs. The mortality percentages were calculated according to Abbotts' formula [32] and $LC_{50}$ and $LC_{90}$ were determined by Probit analysis. The SPSS version 16.0 was used to perform One Way ANOVA followed by Tukey's test to determine variations among the larval mortality for different concentrations.

## Results and Discussion

The crude extracts of *P. octosulcata* using solvents such as ethyl acetate, chloroform and hexane were used for the bioassay. The mortality percentage of three mosquito species displayed variations between solvent extracts. The results of larvicidal activity for *Ae. aegypti*, *C. quinquefasciatus* and *An. stephensi* are given in the table 1, 2 and 3. About 0-10% mortality was obtained for the control mortality which is corrected by Abott's formula. The ethyl acetate extract showed highest mortality of *Ae. aegypti* at 500ppm of 96.1% at $LC_{50}$ of 65.7ppm followed by hexane and chloroform at 92.5% and 89.1% with $LC_{50}$ of 68.5ppm and 80.7ppm. Similarly at 500ppm *C. quinquefasciatus* showed 98.1% mortality at $LC_{50}$ of 62.2 ppm. The hexane and chloroform showed 96.5 and 91.3% mortality for $LC_{50}$ of 64.4 and 92.0ppm. *An. stephensi* also followed similar trend for 500ppm of maximum 98.1% mortality at $LC_{50}$ of 58.7ppm. The hexane leaf extract showed mortality of 97.3% at 60.9ppm and chloroform exhibited 95.1% at $LC_{50}$ 70.9ppm. All three crude solvent extracts have shown highest mortality at 500ppm for three mosquito larvae. The ethyl acetate extract was found to be more effective against three larval species followed by hexane and chloroform at higher concentration. Among three mosquitoes the ethyl acetate extract were more toxic to *An. stephensi* larvae at lower concentration 58.2ppm followed by *C. quinquefasciatus* and *Ae. aegypti*. The lowest mortality percentage was observed for 31.25ppm crude extract concentration for all the three larvaes. Overall the results indicate that 50% mortality can be achieved for ethyl acetate leaf extract between 58.2ppm to 65.7ppm for all the three species of mosquito larvae followed by hexane extract

between 60.9 to 68.5ppm. The chloroform extracts demonstrated 50% mortality at considerably higher concentration of 70.9, 80.7 and 92.0ppm for *An. stephensi*, *Ae. aegypti* and *C. quinquefasciatus*. Though all the solvents showed remarkable mortality the ethyl acetate extract dominated the other two.

The larvicidal activity characteristically differs according to the external environment and strength of larva. The larva continuously fights against the unpleasant environment and struggles to survive. In our experiment the larvae showed various activities on introducing in to the experimental set up. Initially fast movements of larvae were noticed further the movements cease and leads to crippling activity. Among three mosquitoes *An. stephensi* was found to be immediately affected by the extracts. The *C. quinquefasciatus* also showed earlier larvicidal effect than *Ae. aegypti.*

On observation the crippling activity of Ae. *aegypti* was much faster than the other two species. The larval crippling activity is performed by brushing mouth with the tail to get rid of the extract affecting the body. The extracts toxicity has affected the larval body as it caused elongation of the body (Fig 1. d, e, f and Fig 2. d, e, f), disintegration of the larval skin, bending of the larval body (Fig 1. d). Apart from its toxicity the extracts have specific adhesive properties that bind to larval tiny hairs thus preventing their free movements in the water (Fig 3. d, e, f). The Anopheles is the ones which are immediately affected by this physical barrier as they posses mouth brush, palmate hairs over their body and respire through air tubes near the tail as they do not posses siphon. It was observed that the plamate hairs of *An. stephensi* are largely adhere with the extract as seen in the figure (Fig 3. d, e, f). The extract affects both the internal and external part of the larvae lead to suffocation and death of larvae.

Several medicinal plant extracts have shown larvicidal, pupicidal and repellent activity against mosquitoes. The larvicidal activity of *Citrullus colocynthis* and *Curcurbita* maxima against *C. quinquefasciatus* was reported by Mullai and Jebanesan[33]. The ethyl acetate extract of these two plants have shown efficient larvicidal activity against the larvae at $LC_{50}$ value of 47.58ppm and 75.91ppm respectively. The plants like *Aloe turkanensis*, *Aloe ngongensis* and *Aloe fibrosa* of different solvent extracts showed larvicidal activity against *Anopheles gambiae*. The ethyl acetate extract of *A. turkanensis* showed 100% mortality of *An.gambiae* [34]. As ethyl acetate is a high polar solvent it extracts most of the bio active compounds from the leaf. Hence it produces a synergistic effect of bioactive compounds against the larvae and leads to subsequent mortality.

Alouni *et al* [35] experimented with Azadirachtin against *Culex pipiens*. It showed notable larvicidal activity. Kamaraj *et al*. [36] studied the larvicidal activity of three plants such as *A. squamosa*, *C. indicum* and *T. procumbens* against *An. subpictus* and *C. tritaeniorhyncus*. The ethyl acetate extract of *C. indicum* and acetone extract of *T. procumbens* showed 50% mortality at lower concentration of 39.98 and 51.57 mg/l against *An.subpictus*. The methanol extract of *C.indicum* and ethyl acetate extract of *T. procumbens* showed larvicidal activity at lower concentration against *C. tritaeniorhyncus*.

*Artemisia nilagirica* extracts were effective against *Anopheles stephensi* and *Aedes aegypti* larvae and showed efficient repellent activity [37]. Premalatha *et al*. [38] have studied the larvicidal activity of *Solanum trilobatum* leaf extracts against *Ae. aegypti*, *C. quinquefasciatus* and *An. Stephensi.* The methanol, chloroform and acetone extract showed above 90% mortality at 250ppm. Among three extracts the methanol showed 100% larvicidal activity at higher ppm. Kolli *et al*. [39] have reported larvicidal activity of *C. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* which was highest for methanol extract of *Pongamia pinnata*. The essential oil and hydrolat from aqueous, ethanol and methanol extracts of *Pseudocalymma alliaceum* has shown growth inhibitory activity of *C. quinquefasciatus* larvae. The delaying of larval and pupal duration is also demonstrated by this plant extract [40]. The different parts of the plant *Ipomoea cairica* was extracted with acetone and ethanol. Both the solvent extracts showed 100% mortality against *Ae. albopictus* and *Ae. aegypti* larvae[41]. The leaf extracts of *Plectranthus glandulosus* showed 100% mortality at 500ppm for Hexane extract against *Ae. aegypti* and *An. gambiae*. The *C. quinquefasciatus* showed 97% mortality at 1000 ppm [42]. *Delonix elata* leaf and seed extract was found to be effective against *Ae. aegypti* mosquito species[43]. These reports are in accordance with our results that high polar and the low polar solvent extracts have potentiality to exhibit larvicidal activity. The *Citrus grandis* peels from different places of Philipines were active against *Ae.aegypti.* The hexane extract of citrus peels showed larvicidal activity at lower $LC_{50}$ of 1.11 ppm, and 12.85 ppm for C*itrus grandis* peels collected from Davao, and Benguet respectively [44].

The oil extracted from the medicinal plants such as *Eucayptus globules*, *Azaditachta indica*, *Mentha piperita*, *Ocimum basilicum and Zingiber officinale* were tested against four larval stages of *Aedes albopictus*. It was found that *Z.officinale* was effective in producing 100% mortality at lower ppm followed by other plant extracts [45]. The study of larvicidal activity on *Ae.aegypti* using *Tephrosia purpurea* leaf extract against *Ae.aegypti*. The plant extract showed 61.4% mortality of IV instar larvae at 375 ppm and with the combination of *Bacillus sphaericus* it has showed above 80% mortality [46].

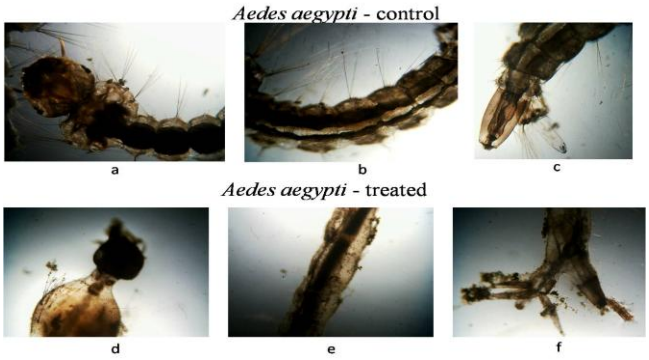


**Fig 1:** *Ae. aegypti* larvae observed under microscope. Control: a-head, b-abdomen, c-tail, Treated: d-head, e- abdomen, f- tail.

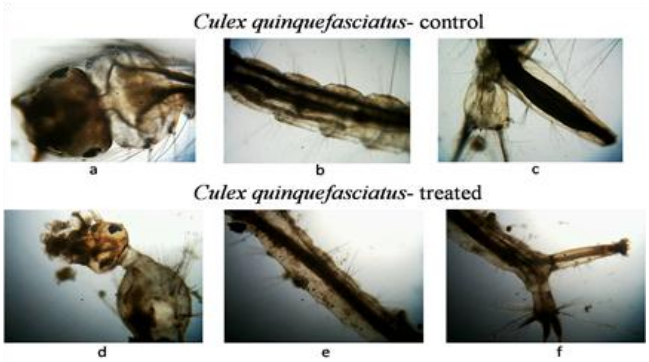


**Fig 2:** *C. quinquefasciatus* larvae observed under microscope. Control: a- head, b-abdomen, c-tail, Treated: d- head, e- abdomen, f-tail.

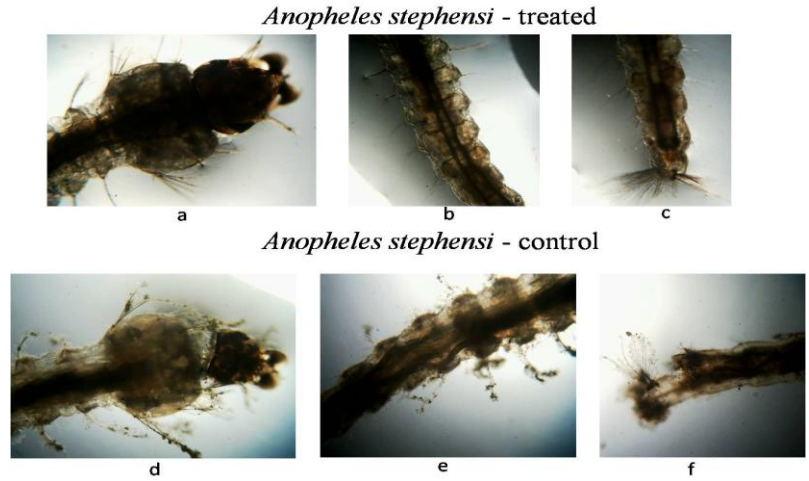


**Fig 3:** *An. stephensi* larvae observed under microscope. Control: a- head, b-abdomen, c-tail, Treated: d- head, e- abdomen, f- tail

**Table 1:** Larvicidal activity of crude extract of *Psychotria octosulcata* against *Aedes aegypti* at 48hrs

| Crude extract | Concentration (ppm) | Larval Mortality (%) | $LC_{50}$(ppm) (LCL-UCL) | $LC_{90}$(ppm) (LCL-UCL) | $X^2$ (df=3) |
|---|---|---|---|---|---|
| Hexane | 31.25 | 39.1±2.0[a] | 68.5 (23.7- 103.5) | 424.1 (363.2-518.6) | 2.6 |
| | 62.5 | 49.9±1.4[b] | | | |
| | 125 | 62.5±3.7[c] | | | |
| | 250 | 76.1±4.3[d] | | | |
| | 500 | 92.5±2.2[e] | | | |
| Chloroform | 31.25 | 38.5±2.0[a] | 80.7 (31.6-119.1) | 485.0 (411.8-602.1) | 2.4 |
| | 62.5 | 48.3±1.4[b] | | | |
| | 125 | 59.9±1.8[c] | | | |
| | 250 | 72.7±2.7[d] | | | |
| | 500 | 89.1±1.9[e] | | | |
| Ethyl acetate | 31.25 | 38.9±2.7[a] | 65.7 (28.4- 95.3) | 360.1 (310.8-434.4) | 2.1 |
| | 62.5 | 50.7±1.1[b] | | | |
| | 125 | 64.3±2.8[c] | | | |
| | 250 | 79.5±2.3[d] | | | |
| | 500 | 96.1±1.9[e] | | | |

Value represents mean ± S.D. of five replications. Different superscript alphabets in the column indicate statistical significant difference at $p<0.05$ levels by one way ANOVA followed by Tukey's test.

**Table 2:** Larvicidal activity of crude extract of *Psychotria octosulcata* against *Culex quinquefasciatus* at 48hrs

| Crude extract | Concentration (ppm) | Larval Mortality (%) | $LC_{50}$(ppm) (LCL-UCL) | $LC_{90}$(ppm) (LCL-UCL) | $X^2$ (df=3) |
|---|---|---|---|---|---|
| Hexane | 31.25 | 37.1±4.2[a] | 64.4 (28.5- 92.9) | 345.0 (298.2-415.3) | 4.0 |
| | 62.5 | 51.3±2.7[b] | | | |
| | 125 | 66.9±4.8[c] | | | |
| | 250 | 80.7±3.6[d] | | | |
| | 500 | 96.5±1.0[e] | | | |
| Chloroform | 31.25 | 34.9±2.0[a] | 92.0 (50.3- 126.2) | 455.4 (391.0-554.4) | 4.1 |
| | 62.5 | 46.5±2.3[b] | | | |
| | 125 | 59.7±2.3[c] | | | |
| | 250 | 74.7±4.1[d] | | | |
| | 500 | 91.3±1.6[e] | | | |
| Ethyl acetate | 31.25 | 37.9±4.4[a] | 62.2 (28.9- 88.6) | 318.6 (275.4-383.7) | 3.1 |
| | 62.5 | 52.3±1.8[b] | | | |
| | 125 | 67.7±4.3[c] | | | |
| | 250 | 81.3±1.6[d] | | | |
| | 500 | 98.1±1.1[e] | | | |

Value represents mean ± S.D. of five replications. Different superscript alphabets in the column indicate statistical significant difference at $p<0.05$ levels by one way ANOVA followed by Tukey's test.

**Table 3:** Larvicidal activity of crude extract of *Psychotria octosulcata* against *Anopheles stephensi* at 48hrs

| Crude extract | Concentration (ppm) | Larval Mortality (%) | $LC_{50}$ (ppm) (LCL-UCL) | $LC_{90}$ (ppm) (LCL-UCL) | $X^2$ (df=3) |
|---|---|---|---|---|---|
| Hexane | 31.25 | 39.3±3.3[a] | 60.9 (25.4- 89.0) | 334.14 (288.5-402.9) | 2.07 |
| | 62.5 | 52.1±1.4[b] | | | |
| | 125 | 65.7±2.3[c] | | | |
| | 250 | 81.3 ±2.4[d] | | | |
| | 500 | 97.3±0.8[e] | | | |
| Chloroform | 31.25 | 38.5±3.1[a] | 70.9 (32.7- 101.5) | 379.51 (327.5- 458.0) | 2.1 |
| | 62.5 | 49.3±1.0[b] | | | |
| | 125 | 63.1±1.9[c] | | | |
| | 250 | 78.3±3.0[d] | | | |
| | 500 | 95.1±1.3[e] | | | |
| Ethyl acetate | 31.25 | 39.3±3.0[a] | 58.2 (24.1- 84.8) | 314.7 (271.7- 379.9) | 2.02 |
| | 62.5 | 54.1±4.9[b] | | | |
| | 125 | 65.7±4.1[c] | | | |
| | 250 | 82.9±5.1[d] | | | |
| | 500 | 98.1±1.3[e] | | | |

Value represents mean ± S.D. of five replications. Different superscript alphabets in the column indicate statistical significant difference at $p<0.05$ levels by one way ANOVA followed by Tukey's test.

## Conclusion

In conclusion the results of our research work portrays that the leaf extract of *P. octosulcata* using different solvents were efficient in controlling the mosquito larvae. Therefore *P. octosulcata* can serve as a potential mosquito larvicide. Further investigations in this line are undertaken to unveil the potency of the plant extract for general utilization in mosquito control and other insecticidal properties.

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