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# Screening of phytochemical compounds and mosquito larvicidal activity of *Allmania nodiflora* (L.) R.Br.ex Wight (Amaranthaceae)

**Bathrasamy R and A Arunprasath**

**Abstract**
In the present investigation phytochcmical analysis and larvicidal activity of *Allmania nodiflora.* were screened against *Aedes aegypti* in the phytochemical analysis of the petroleum ether and ethanol extract of *A. nodiflora* revealed the presence of alkaloids, flavanoids, saponins, glycosides, steroids. The phenol and tannin were absent in petroleum ether extract in ethanolic extract. The tannins were present and absent in petroleum ether extract. In GCMS analysis 9 bioactive phytochemical compounds were identified in the ethanolic extract of *A. nodiflora*. The extract were subjected for screening of larvicidal activity against *Aedes aegypti* at the concentration of (2%, 4%, 8%, 10%, 15%, 20%) were analyzed the highest mortality was observed after 96 hours in ethanolic extract. Lowest mortality was observed in petroleum ether extract 24hours.

**Keywords:** *Allmania nodiflora,* phytochemical, GCMS, parricidal activity, *Aedes aegypti*

## 1. Introduction
The mosquito is the principal vector of many of the vector-borne diseases affecting human beings and other animals. Mosquitoes constitute major public health problems vectors of serious human diseases [1]. Reported that *Culex pipiens* is the vector of West Nile Virus which causes encephalitis or meningitis which is known to affect the brain tissue, finally resulting in permanent neurological damage. Several mosquito species belonging to genera *Anopheles*, *Culex* and *Aedes* are vectors for the pathogens of various diseases like malaria, filariasis, apanese encephalitis, dengue fever, dengue hemorrhagic fever and yellow fever [2]. *Aedes aegypti* is the principal vector of dengue fever and dengue hemorrhagic fever and it is reported to infect more than hundred million people every year in more than 110 countries in the tropics [3]. One of the approaches for control of these mosquito Borne diseases is the interruption of disease transmission by either killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the breeding center soft the vectors. Conventional pesticides such Malathion, DDT and pyrethroides that are generally used for mosquito control are known to cause the problem of environmental pollution, residual effects and resistance by their in discriminate use. Development of resistance to malathion [4] and to deltamethrin [5] in adult *C. pipiens* has been reported.
The control of this vector is based on the destruction of breeding sites by using synthetic insecticides. However, the continued use of synthetic insecticides has resulted in resistance in mosquitoes [6]. In addition, synthetic insecticides are toxic and affect the environment by contaminating soil, water and air [7], then natural products may be an alternative to synthetic insecticides because they are effective, biodegradable, eco-friendly and safe to environment [8]. Natural products of plant origins present promising potentials for vector control. They are usually active against limited number of species, less expensive, readily biodegradable and less toxic to non-target organisms and suitable for use in both agricultural and public health purposes. Extracts from plants sources have been shown to possess insecticidal properties [9, 10, 11].
Current control is based on the use of commercial insecticides which have potential toxic effect on public health and the environment. Synthetic insecticides have been used during the past several decades to control varied dipteran pests [12]. Pesticides are indeed very effective in its use. But along with their useful effects, they also bring out serious harm to human health as well. Furthermore, these chemicals are expensive and are often toxic to both human and other animals and natural enemies [13]. The intensive use of chemical insecticides led to the development of resistant insect populations resulting in a reduced control and often to a negative impact on various non-target organisms and on the environment in general [14].

The present study is aimed to evaluate the phytochemical compounds and Larvicidal ability of *Allmania nodiflora* against *Aedes aegypti.*

## 2. Materials and methods

### 2.1. Collection of plant materials

The selected medicinal plant like *A. nodiflora* was collected in Othimalai (Annur), Coimbatore district, Tamil Nadu.

### 2.2. Preparation of plant extracts

30 g of powdered *A. nodiflora* leaf was successively extracted using 250 ml of ethanol and petroleum ether using the Soxhlet extractor for 8–10hrs. The extract was filtered through Whatman No. 1 filter paper to remove all undissolved matter including cellular materials and other constitutions that are insoluble in the extraction solvent.

### 2.3. Preliminary phytochemical studies

The methanol and petroleum ether extracts were subjected to preliminary phytochemical tests to determine the group of secondary metabolites present in the powdered *A. nodiflora*.

### 2.4. Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the ethanol extract of *A. nodiflora* leaf was performed using Shimadzu Japan GC QP2010 plus with a fused GC column coated with polymethylsilicon (0.25 nm × 50 m) and the conditions were as follows: Temperature programming from 80 to 200 °C held at 80 °C for 1 min, rate 5 °C/min, and at 200 °C for 20 min. Field ionization detector temperature of 300 °C, injection temperature of 220 °C, carrier gas nitrogen at a flow rate of 1 ml/min, and split ratio of 1:75 GC-MS were conducted using GCMS-QP 2010 plus Shimadzu Japan with an injector temperature of 220° and carrier gas presence of 116.9 kpa. The color length mentioned authors. Phenolic compounds were also detected in both solvents. They show a high degree of precipitation of phenolic compounds.

#### 2.4.1. GCMS analysis of *A. nodiflora*

The plant extract of *A. nodiflora* (ethnol extract) was analyzed by GC-MS. The presence of components was confirmed by comparing mass spectra of analyzed components with standard mass spectra of NIST and Willey library. In the The identified compounds represented with Retention Time, Chemical Formula, Molecular weight, Peak area %, Structure and Medicinal Uses.

### 2.5. Test for Larvicidal Activity

The laboratory colonies of *Aedes aegypti* were used for the larvicidal activity. The eggs of *A. aegypti* were collected from Centre for Research in Medical Entomology (ICMR) Madurai. The larvae were cultured and maintained in the laboratory at 27±1 °C and 85% of relative humidity.

### 2.6. Bioassay Experiment

Different concentration of extract between (0%, 2%, 4%, 8%, 10%, 15%, 20%) were prepared using distilled water. the mosquito larva were treated with extract using method of WHO. 20 larvae of *A. aegypti* were introduced in different test concentration of plant extract along with a set of control containing distilled water without any plant extract, after adding the larvae, glass dishes were kept in laboratory at room temperature. by counting the number of dead larvae at 24hrs/48hrs/72hrs/96hrs exposure, the mortality rate and the medium lethal concentration were obtained, three replication were maintained for each concentration. Dead larvae were removed as soon as possible in ordered to prevent decomposition, which may cause rapid death of the remaining larvae. The water used for the study analyzed by using the method of [15].

## 3. Results

### 3.1. Preliminary phytochemical analysis of *A. nodiflora*

Preliminary phytochemical analyses in *A. nodiflora* were carried to find out the presence of secondary metabolites. *A. nodiflora* is an erect herb distributed in throughout in India. The plants also screened for GCMS spectrum analysis and larvicidal activity against *A. agypti* in phytochemical evolution. Initially physical constants were evaluated for it is present as well as for its quality. (The alkaloids flavonoids, saponins and tannins were present in ethanol extract). The material was subjective to phytochemical analysis separately for observing the presence alkaloids, flavonoids, saponins, steroids and tannins (Table 1). All the result was observed in *A. nodiflora*. Flavonoids found in both hexane and ethanolic extract in the present study. The saponins, glycosides and steroids were present in both Petroleum Ether and ethanolic extract of *A. nodiflora*. In the presence study tannins were present only in ethanol extract and absent in Petroleum Ether extract.

### 3.2. GCMS-analysis of *A. nodiflora*

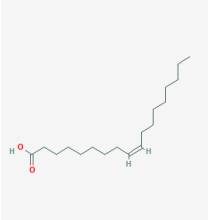
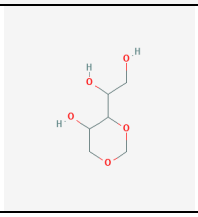
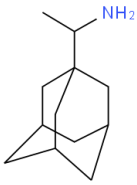
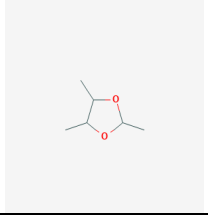
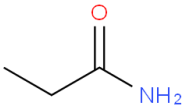
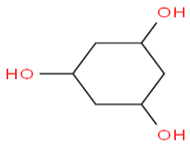
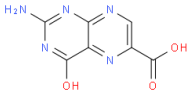
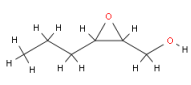
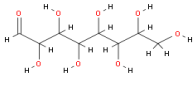
The plant extract of ethanol *Allmania nodiflora* were analysed by GCMS. The presence of components was comparing mass spectra of analysed components with mass spectra of NIST and willey library. In the GCMS analysis of *A. nodiflora*. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW), peak area (%), structure, medicinal uses in ethanolic extract of *Allmania nodiflora* were given in (Table 2).

**Table 1:** Preliminary Phytochemical Analysis of Petroleum ether And Ethanol Extract of *Allmania nodiflora*

| S. No | Name of The Secondary Metabolites | Petroleum Ether Extract | Ethanol Extract |
|---|---|---|---|
| 1 | Alkaloid | + | + |
| 2 | Flavanoid | + | + |
| 3 | Saponins | + | + |
| 4 | Glycosides | + | + |
| 5 | Steroids | + | + |
| 6 | Phenols | - | - |
| 7 | Tannins | - | + |

+ - Present
- - Absent

**Table 2:** GC-MS Analysis of Ethanolic extract of *A. nodiflora*

| S. No | RT | Compound | Formula | Molecular Weight (G/Mol) | Peak Area % | Structure | Medicinal Uses |
|---|---|---|---|---|---|---|---|
| 1 | 25.257 | Oleic Acid | $C_{18}H_{34}O_2$ | 282.468 g/mol | 5.553 |  | Food additive, Insecticide, Herbicide, Plant growth regulator(pubchem) |
| 2 | 25.803 | 1,3-Methylene-D-Arabitol | $C_6H_{12}O_5$ | 164.157 | 13.097 |  | No activity reported |
| 3 | 26.083 | 1-Adamantanemethylamine,. Alpha.-Methyl- | $C_{12}H_{21}$ | 179.302 | 18.252 |  | No activity reported |
| 4 | 26.268 | 1,3-Dioxolane, 2,4,5-Trimethylo | $C_6H_{12}O_2$ | 116.16 | 11.858 |  | Used as a flavor in alcoholic beverages(pubchem) |
| 5 | 26.618 | Propanamide | $C_3H_7NO$ | 73.052765 | 26.618 |  | antioxidant, cancer preventive, pesticide, Hypocholesterolemi, Dermatitigenic (Hesham Hussein Rassem *et al*., 2017) |
| 6 | 26.818 | Phloroglucitol | $C_6H_{12}O$ | 132.078644 | 13.369 |  | No activity reported |
| 7 | 26.968 | Pterin-6-Carboxylic acid | $C_7H_5N_5$ | 207.039246 | 26.968 |  | No activity reported |
| 8 | 27.173 | 2,3-Epoxyhexanol | $C_6H_{12}O$ | 116.083733 | 5.950 |  | No activity reported |
| 9 | 27.323 | L-Gala-L-Ido-octose | $C_8H_{16}O$ | 240.084518 | 5.909 |  | Used for memory Drugs production (Sirigiri Chandra Kala and Kandru Ammani 2017) |

### 3.3. Larvicidal activity of *A. nodiflora*

*A. nodiflora* crude extract was taken in six different concentrations. Including control it was ie., (0%, 2%, 4%, 8%, 10%, 15%, 20%) in different of the concentration of the plant extract 20 mosquito second stage live larvae were allowed to survive. The mortality rate was observed every 24hours.

Intervals till 96 hours (4days) without disturbing the set up. This observation made for ethanol extract treatment and petroleum ether treatment (Table 4-11). Since the discovery of DDT, control of disease-causing mosquito species has been almost completely based on synthetic organic insecticides. Following DDT, conventional pesticides such as malathion and pyrithroids are generally used for mosquito control. But extensive use of synthetic organic insecticides during the last 5½ decades has resulted in environmental hazards. Besides this also caused the development of physiological resistance in the major vector species. This has necessitated the need for search and development of environmentally friendly larvicidal substance from plant derivatives safe, biodegradable, low cost and indigenous methods for vector control, which can be used with minimum care by individual and communities in specific situation [16]. Either prohibiting the breeding cycle are killing or eliminating) the second stage of larvae through Larvicidal effect. The efficacy of the different concentration *A. nodiflora* was studied and results revalued petroleum ether extract quite promising extract which challenge the lethal does. 50% $LC_{50}$ in 24hrs (Table 4 & 5). It was noticed in the lower concentration of 4% petroleum ether extract within 48 hours (Table 6 & 7). Were as the same $LC_{50}$ was observed in ethanol extract 4% only after 72 hours (Table 8 & 9). 100% mortality was observed as the plant extract concentration increases by 8%, 10%, 15%,20% (Table 10 & 11). This directly proportionate to the increases trend or mortality rate even with in 96hours. It attends 100% death.

A survey of literature control of different species of mosquito revealed that assessment of efficacy of different phytochemicals obtained from various plants has been carried out by a number of researches on the field of vector control.[17] made an extensive review of plant derivatives in mosquito control. A large number of plant extracts have reported to have mosquitocidal or repellent activities against mosquito vectors, but very few plant products have shown practical utility for mosquito control [17].

**Table 3:** Mortality Rate of *Aedes agypti* in water (Control)

| S. No | Hours | Treatment % | No. of Mosquito Larvae | Rate of Death | Rate of Survival | Active | Inactive |
|---|---|---|---|---|---|---|---|
| 1. | 24 hours | 100ml water | 20 | 0 | 20 | 20 | 0 |
| 2. | 48 hours | 100ml water | 20 | 0 | 20 | 20 | 0 |
| 3. | 72 hours | 100ml water | 20 | 0 | 20 | 20 | 0 |
| 4. | 96 hours | 100ml water | 20 | 0 | 20 | 20 | 0 |

**Table 4:** Mortality Rate of *Aedes aegypti* in ethanolic extract of *Allmania nodiflora* (24hours)

| S. No | Treatment% | No. of Mosquito Larvae | Rate of Death | Rate of Survival | Active | Inactive |
|---|---|---|---|---|---|---|
| 1 | 2% | 20 | 3 | 17 | 13 | 4 |
| 2 | 4% | 20 | 7 | 13 | 10 | 3 |
| 3 | 8% | 20 | 20 | 0 | 0 | 0 |
| 4 | 10% | 20 | 20 | 0 | 0 | 0 |
| 5 | 15% | 20 | 20 | 0 | 0 | 0 |
| 6 | 20% | 20 | 20 | 0 | 0 | 0 |

**Table 5:** Mortality Rate of *Aedes aegypti* in Petroleum ether extract of *Allmania nodiflora* (24hours)

| S. No | Treatment% | No. of Mosquito Larvae | Rate of Death | Rate of Survival | Active | Inactive |
|---|---|---|---|---|---|---|
| 1 | 2% | 20 | 2 | 18 | 16 | 2 |
| 2 | 4% | 20 | 3 | 17 | 15 | 2 |
| 3 | 8% | 20 | 10 | 10 | 7 | 3 |
| 4 | 10% | 20 | 16 | 4 | 2 | 2 |
| 5 | 15% | 20 | 20 | 0 | 0 | 0 |
| 6 | 20% | 20 | 20 | 0 | 0 | 0 |

**Table 6:** Mortality Rate of *Aedes agypti* in ethanolic extract of *Allmania nodiflora* (48 hours)

| S. No | Treatment % | No. of Mosquito Larvae | Rate of Death | Rate of Survival | Active | Inactive |
|---|---|---|---|---|---|---|
| 1 | 2% | 20 | 7 | 13 | 9 | 4 |
| 2 | 4% | 20 | 12 | 8 | 6 | 2 |
| 3 | 8% | 20 | 20 | 0 | 0 | 0 |
| 4 | 10% | 20 | 20 | 0 | 0 | 0 |
| 5 | 15% | 20 | 20 | 0 | 0 | 0 |
| 6 | 20% | 20 | 20 | 0 | 0 | 0 |

**Table 7:** Mortality Rate of *Aedes aegypti* in Petroleum ether extract of *Allmania nodiflora* (48 hours)

| S. No | Treatment% | No. of Mosquito Larvae | Rate of Death | Rate of Survival | Active | Inactive |
|---|---|---|---|---|---|---|
| 1 | 2% | 20 | 8 | 12 | 7 | 5 |
| 2 | 4% | 20 | 10 | 10 | 7 | 3 |
| 3 | 8% | 20 | 15 | 5 | 4 | 1 |
| 4 | 10% | 20 | 20 | 0 | 0 | 0 |
| 5 | 15% | 20 | 20 | 0 | 0 | 0 |
| 6 | 20% | 20 | 20 | 0 | 0 | 0 |

**Table 8:** Mortality Rate of *Aedes aegypti* in Ethanolic extract of *Allmania nodiflora* (72 hours)

| S. No | Treatment% | No. of Mosquito Larvae | Rate of Death | Rate of Survival | Active | Inactive |
|---|---|---|---|---|---|---|
| 1 | 2% | 20 | 11 | 9 | 4 | 5 |
| 2 | 4% | 20 | 15 | 5 | 1 | 4 |
| 3 | 8% | 20 | 20 | 0 | 0 | 0 |
| 4 | 10% | 20 | 20 | 0 | 0 | 0 |
| 5 | 15% | 20 | 20 | 0 | 0 | 0 |
| 6 | 20% | 20 | 20 | 0 | 0 | 0 |

**Table 9:** Mortality Rate of *Aedes aegypti* in Petroleum ether extract of *Allmania nodiflora* (72 hours)

| S. No | Treatment % | No. of Mosquito Larvae | Rate of Death | Rate of Survival | Active | Inactive |
|---|---|---|---|---|---|---|
| 1 | 2% | 20 | 12 | 8 | 5 | 3 |
| 2 | 4% | 20 | 14 | 6 | 2 | 4 |
| 3 | 8% | 20 | 18 | 2 | 0 | 2 |
| 4 | 10% | 20 | 20 | 0 | 0 | 0 |
| 5 | 15% | 20 | 20 | 0 | 0 | 0 |
| 6 | 20% | 20 | 20 | 0 | 0 | 0 |

**Table 10:** Mortality Rate of *Aedes aegypti in* Ethanolic extract of *Allmania nodiflora* (96 hours)

| S. No | Treatment% | No. of Mosquito Larvae | Rate of Death | Rate of Survival | Active | Inactive |
|---|---|---|---|---|---|---|
| 1 | 2% | 20 | 16 | 4 | 1 | 3 |
| 3 | 4% | 20 | 18 | 2 | 0 | 2 |
| 3 | 8% | 20 | 20 | 0 | 0 | 0 |
| 4 | 10% | 20 | 20 | 0 | 0 | 0 |
| 5 | 15% | 20 | 20 | 0 | 0 | 0 |
| 6 | 20% | 20 | 20 | 0 | 0 | 0 |

**Table 11:** Mortality Rate of *Aedes aegypti* in Petroleum ether extract of *Allmania nodiflora (*96 hours)

| S. No | Treatment% | No. of Mosquito Larvae | Rate of Death | Rate of Survival | Active | Inactive |
|---|---|---|---|---|---|---|
| 1 | 2% | 20 | 15 | 5 | 2 | 3 |
| 2 | 4% | 20 | 17 | 3 | 1 | 2 |
| 3 | 8% | 20 | 20 | 0 | 0 | 0 |
| 4 | 10% | 20 | 20 | 0 | 0 | 0 |
| 5 | 15% | 20 | 20 | 0 | 0 | 0 |
| 6 | 20% | 20 | 20 | 0 | 0 | 0 |

## 4. Discussion

The phytochemical screening of flowers and flower buds are not been reported earlier although flower and flower buds of *A. nodiflora* also help in abortion and leucorrhea [18]. Alkaloids have been used as both antibacterial and antidiabetic properties and useful for such activities. Phenols and phenolic compounds have been extensively used in disinfection and remain the standard with which other bactericides are compared [19]. Phytochemical screening showed that the leaves and roots were rich in chemical constituents. Alkoloids, saponins, glycosides, phenolics, trepenoids and flavonoids has been documented in this study. These principles have known for many years to exhibit biological activity, such as effects on the central nervous system, antibacterial, antiturmour, and anthelmintic activity [20]. Many alkaloids known to have effect on the central nervous system and some act as the anti-parasitic (such morphine, a pain killer). For example, quinine was widely used against *Allmania nodiflora.* In this respect, it is found from the phytochemical screening that most plant traditionally used to treat malaria contain alkaloids among other things. Analgesia is anther property of many alkaloids containing plants used to in traditional medicine. Degenerative disorders, such as gouts and rheumatism, have also been traditionally treated with alkaloid-containing plants. Cochicine compounds are well known in treating gouts [21]. This present study has shown the larvicidal activity of extracts of *A. nodiflora* against *A. aegypti*. These findings are comparable to earlier reports of [22, 23, 24]. Plant extracts might have complex mixture of bioactive compounds, including phenolics, terpenoids, flavonoids and alkaloids which may jointly or independently contribute to mortality and delayed growth of larvae [25]. These factors might have accounted for the high mortality of the larvae recorded in this study.

The plant extracts exhibited a concentration dependent activity against mosquito larvae, since percentage mortality was observed to increase with increasing concentration and time of exposure. This observation agrees with the reports of [11, 22, 26, 27]. The ethanolic extract of the plant was more potent than the aqueous extract on the larvae of the mosquito species in this present study. This is consistent with the work of [17] who stated that the activity of extracts on target species varies with respect to the plant parts from which they are extracted and solvent of extraction among other factors. It is not unlikely that the difference in potency observed in ethanolic and petroleum ether extract of *A. nodiflora* in this study might be due to a for mentioned reasons. There was a drastic reduction in activity of larvae exposed to the different test solutions as evidenced by loss of wriggling and motility. These effects on behavior may be due to the presence of neuro toxic compounds in the plant extracts [28]. No behavioral changes were noticed in the control group, they were agile and wriggling throughout the duration of the study.

## 5. Conclusion

The extract of *Allmania nodiflora* has potent larvicidal activity against *Aedes agypti* Further studies on the screening, isolation and purification of bioactive compounds followed by

in depth and field bioassay are needed as to use *A. nodiflora* to control the vector mosquitoes.

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