Guest Articles

Annona glabra Leaf Extract as a Dengue Mosquito Larvicide

S. R. Wickramarachchi¹ and L. D. Amarasinghe² ¹Department of Chemistry, University of Kelaniya ²Department of Zoology & Environmental Management, University of Kelaniya

Annona glabra

A. glabra is a tropical fruit tree belonging to the family Annonaceae and related to several commercially grown Annona species, including A. cheromola (cherimoya), A. muricata (soursop), A. reticulata (custard apple or bullock's heart) and Annona squamosa (sugar apple). It is commonly known as pond apple, alligator apple swamp apple, corkwood, bobwood, and monkey apple.¹. A. glabra is native for North, South and Central America and West Africa. It is regarded as an invasive weed in Sri Lanka and Australia where it grows in estuaries and chokes mangrove swamps. The tree grows up to 12 m in dense thicket. The trunk is narrow and gray in colour and the leaves are ovate to oblong. The upper surface of the leaf is light to dark green. Leaves have a distinct smell, similar to green apples, which makes it distinguishable from mangroves.

Uses

The fruit is edible and can be made into jam. It is a popular ingredient in fresh fruit drinks in Maldives. The crushed seed cooked in coconut oil was applied to get rid of head lice in older days. *A. glabra* is used in traditional medicine against several human ailments and disorders such as constipation, fever, ulcers and tumor including cancer.

Chemical composition of leaf

The main classes of phytochemicals present in an aqueous leaf extract are flavonoids, glycolipids, alkaloids, aromatic hydrocarbons, phenols, sugars, steroids and terpenes (Fig 01). The terpenes identified in oil samples are mainly mono and sesquiterpenoids. The composition of oil is as follows¹;



Annona glabra



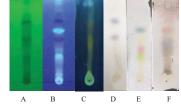


Figure 01: Thin layer chromatogram of crude aqueous leaf extract of *A. glabra* observed under UV light at (A) 254 nm (B) 360 nm (C) 360 nm, showing flavonoids (D) showing glycolipids (E) alkaloids and aromatic hydrocarbons (F) Phenols, sugars, terpenes, steroids.² C, D, E and F are treated with spray reagents.

Dengue Fever

Dengue is a mosquito borne tropical disease. According to statistics 390 million infections per year are reported worldwide. Common symptoms of dengue are high fever, headache, vomiting, muscle and joint pains and a characteristic skin rash. It is an arboviral disease of humans that has four distinct serotypes of dengue virus (DEN 1–4). The mosquito, *Aedes aegypti*, is the main vector responsible for virtually all dengue virus serotypes causing dengue fever, dengue hemorrhagic fever and dengue shock syndrome. *Aedes albopictus* has been considered as a vector in which the DENV is maintained but does not contribute to the transmission rate in epidemics. Both species of *Aedes* mosquitoes are primarily container breeders and they thrive in both clean and organically rich water in both natural and artificial containers.





Aedes aegypti (female)³

Aedes albopictus (female)³

Control of Dengue Fever

No vaccine is currently available for any of the Dengue viral fever types. The disease prevention is mainly achieved by controlling the mosquito population. Traditional methods use chemical pesticides targeting both adults and larvae. DDT and malathion were widely used to control all mosquito vectors in the past.

Mosquito Larvicides

Larvicides are among the main tools in recent mosquito control programs. The most widely used larvicides are organophosphates such as Temephos, Methoprene and biological control by *Bacillus thuringiensis israelensis* (Bti). Since the larvicides are applied to either natural or artificial bodies of water, they must be harmless to beneficial and other nontarget organisms, including humans. Effect on non-target populations, high toxicity to mammals, bioaccumulation in non-target organisms, non-biodegradable nature, ecological imbalance, the emergence of refractory vector behaviour and environmental pollution are some of the drawbacks of synthetic chemical insecticides. Hence, there is a high demand for botanical based natural insecticides.

Annona Extracts as a Larvicide

It is reported that the genus *Annona* shows strong insecticidal properties. According to reports *A. crassiflora* shows larvicidal activity against *Ae. Aegypti. A. squamosa* have a larvicidal activity against *Ae. albopictus* and *Culex quinquefasciatus.* Seed extract of *A. muricata* shows larvicidal activity against *Ae. aegypti.*⁴

Larvicidal Efficacy of A. glabra

Ethanol stem bark extract of *A. glabra* is larvicidal to *Ae. aegypti.*⁵ *A. glabra* aqueous leaf extract shows larvicidal properties on *Ae. aegypti* and *Ae. Albopictus* (5.94 mg/L and 5.00 mg/L respectively) (Figure 2 & Table 01).⁴

A. glabra nano formulations of silver has shown enhanced larvicidal efficacy on Ae. aegypti and Ae. Albopictus ($LC_{50} = 2.51 \text{ mg/L}$ and 2.43 mg/L respectively) (Figure 3 and Table 02).⁴

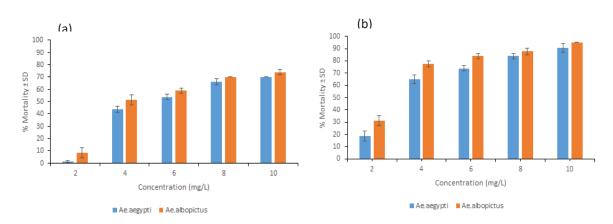


Figure 02: Mean mortality percentage of *A. glabra* leaf extract on *Ae. aegypti* and *Ae. albopictus* larvae at different concentrations; (a) after 24 h and (b) after 48h exposure



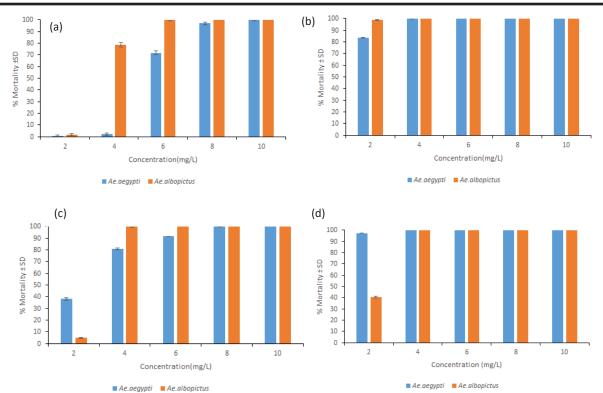


Figure 03. Percentage mortality \pm SD of *Ae. aegypti* and *Ae. albopictus* after exposing (a & c) 24 hours (b & d) 48 hours to different concentrations of *A. glabra* nano formulations of silver. (a & b) Plant extract : AgNO₃, 1:10 (c & d) Plant extract : AgNO₃, 2:10

| Mosquito species | Exposure period (hours) | LC ₅₀ (mg/L) | 95 % confidence interval for LC ₅₀ | | |
|------------------|----------------------------|-------------------------|---|------------|--|
| | | | LCL (mg/L) | UCL (mg/L) | |
| Ae. aegypti | 24 | 5.94555 | 5.33000 | 6.55850 | |
| | 48 | 3.54850 | 3.06765 | 3.99527 | |
| Ae. albopictus | 24 | 5.00402 | 4.43068 | 5.55518 | |
| | 48 | 2.73467 | 2.31874 | 3.11563 | |

Table 01: Larvicidal activity of A. glabra crude leaf extract against Ae. aegypti and Ae. albopictus

Table 02: Larvicidal activity of A. glabra nano formulations of silver against Ae. aegypti and Ae. albopictus

| Product | Mosquito species | Exposure period (hours) | LC ₅₀ (mg/L) | 95 % confidence interval for LC ₅₀ | |
|-------------------|-------------------------------|----------------------------|----------------------------|---|------------|
| | | | | LCL (mg/L) | UCL (mg/L) |
| Plant extract : | Ae. aegypti Ae. albopictus | 24 | 5.29 | 5.08 | 5.49 |
| A ~NO | | 48 | 1.51 | 1.34 | 1.65 |
| AgNO ₃ | | 24 | 3.02 | 2.86 | 3.17 |
| 1:10 | | 48 | 1.14 | 1.01 | 1.33 |
| Plant extract: | A | 24 | 2.43 | 2.19 | 2.45 |
| AaNO | Ae. aegypti | 48 | 1.17 | 1.01 | 1.36 |
| AgNO ₃ | Ae. albopictus | 24 | 2.51 | 2.4 | 2.64 |
| 2:10 | | 48 | 2.10 | 2.01 | 2.18 |

Summary: Dengue is a widely spread arboviral disease of humans. It is mainly transmitted by mosquito vectors, *Aedes aegypti* and *Aedes albopictus*. *A. glabra* is an invasive weed in Sri Lanka. *Annona* sp. are known to possess insecticidal properties. *A. glabra* is larvicidal to *A. aegypti* and *A. albopictus*.

References:

- Thang, T. D.; Dai, D. N.; Hoi, T. M.; Ogunwande, I. A. Study on the volatile oil contents of Annona glabraL, Annona squamosaL, Annona muricataL, and Annona reticulataL, from Vietnam, *Natural Product Research.*, 2013, 27(13), 1232–1236
- Paragodaarachchi, Y. L., Wickramarachchi, P. A. S R., De Silva, C. R., Amarasinghe, L. D. (2020), Toxicity analysis of green sybthesized silver nanoparticles using leaf extract of Anonna glabra against Daphnia magna, *In the proceedings of International Conference* on Frontiers in Chemical Technology 2020, Colombo, Sri Lanka, July 20th -22nd, pg 67.

- 3. Kindly provided by Ms Rasika Dalpadado, PhD candidate (2020), University of Kelaniya
- L.D. Amarasinghe, P.A.S.R. Wickramarachchi, A.A.A.U. Aberathna, W.S. Sithara, C.R. De Silva, Comparative study on larvicidal activity of green synthesized silver nanoparticles and Annona glabra (Annonaceae) aqueous extract to control Aedes aegypti and Aedes albopictus (Diptera: Culicidae), Heliyon 6 (2020) e04322.
- De Omena, M.C., Navarro, D.M., de Paula, J.E., Luna, J.S., Ferreira de Lima, M.R., Sant'Ana, A.E., Larvicidal activities against Aedes aegypti of some Brazilian medicinal plants. *Bioresour. Technol.* 2007, 98 (13), 2549–2556, 2007.

Dr S. R. Wickramarachchi obtained the BSc from the University of Kelaniya, Sri Lanka and PhD from Sheffield Hallam, UK. She is currently serving as a Senior Lecturer at the Department of Chemistry, University of Kelaniya, Sri Lanka.

Professor L D Amarasinghe obtained the BSc from the University of Kelaniya, Sri Lanka, and PhD from University of London. She is a Professor at the Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya, Sri Lanka.