

Annona glabra* Leaf Extract as a Dengue Mosquito Larvicide**S. R. Wickramarachchi¹ and L. D. Amarasinghe²¹Department of Chemistry, University of Kelaniya²Department of Zoology & Environmental Management, University of KelaniyaAnnona glabra***

A. glabra is a tropical fruit tree belonging to the family *Annonaceae* and related to several commercially grown *Annona* species, including *A. cherimola* (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;

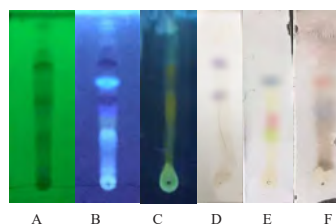
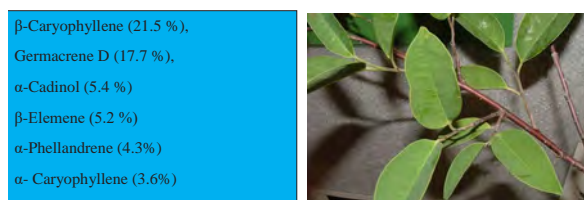
*Annona glabra*

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* (LC₅₀ = 2.51 mg/L and 2.43 mg/L respectively) (Figure 3 and Table 02).⁴

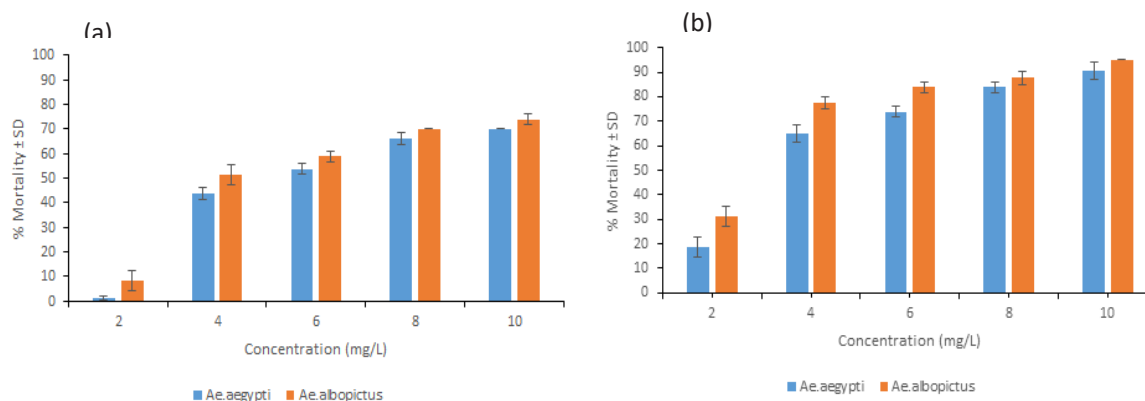


Figure 02: Mean mortality percentage of *A. glabra* leaf extract on *Ae. aegypti* and *Ae. albopictus* larvae at different concentrations; (a) after 24 h and (b) after 48h exposure

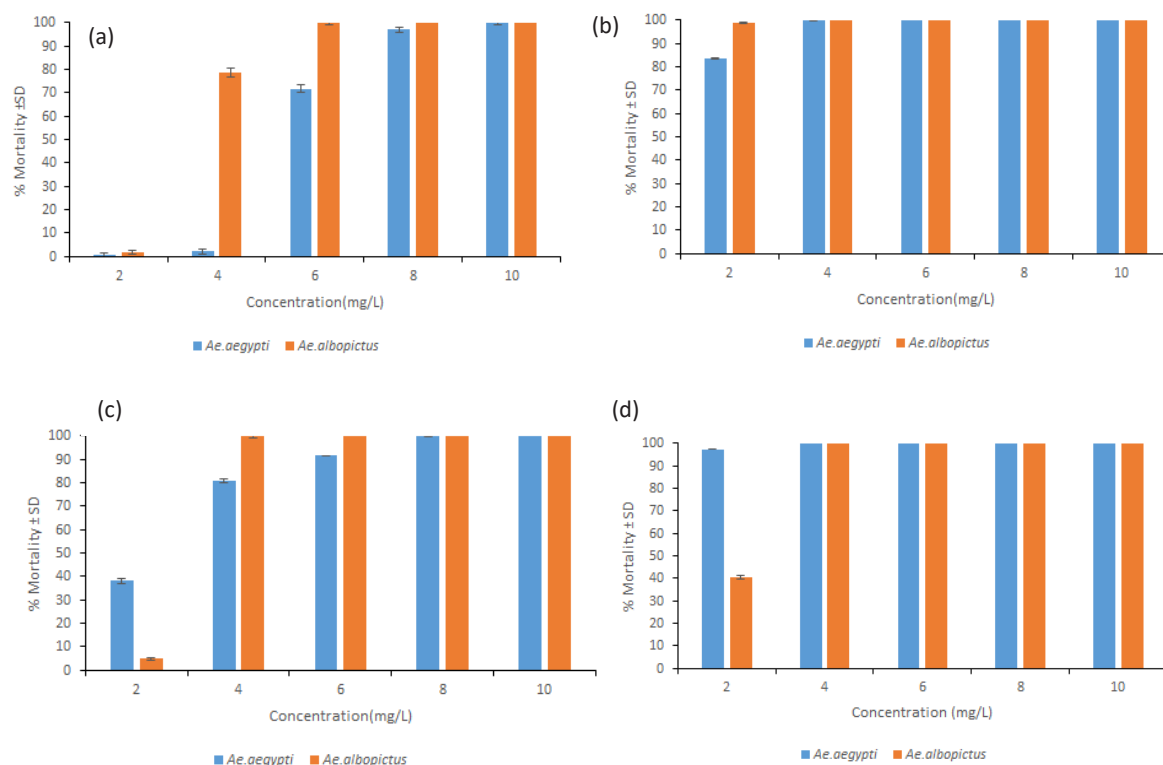


Figure 03. Percentage mortality \pm SD of *Ae. aegypti* and *Ae. albopictus* after exposing (a & c) 24 hours (b & d) 48 hours to different concentrations of *A. glabra* nano formulations of silver. (a & b) Plant extract : $AgNO_3$, 1:10 (c & d) Plant extract : $AgNO_3$, 2:10

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

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

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 : $AgNO_3$ 1: 10	<i>Ae. aegypti</i>	24	5.29	5.08	5.49
		48	1.51	1.34	1.65
	<i>Ae. albopictus</i>	24	3.02	2.86	3.17
		48	1.14	1.01	1.33
Plant extract: $AgNO_3$ 2:10	<i>Ae. aegypti</i>	24	2.43	2.19	2.45
		48	1.17	1.01	1.36
	<i>Ae. albopictus</i>	24	2.51	2.4	2.64
		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*.

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