

Larvicidal Efficacy of Five Medicinal Plant Extracts against *Aedes aegypti* L.: A Probit-Based Toxicity Assessment

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Abstract

Botanical products are widely investigated as alternatives to conventional mosquito larvicides, yet many candidate extracts are reported without quantitative dose-response metrics. Here we evaluated ethanolic extracts of five African medicinal plants against third-instar *Aedes aegypti* larvae using laboratory bioassays and probit analysis. Larvae were exposed for 24 h to 0.2% - 1.0% w/v extract concentrations, equivalent to 2,000 - 10,000 mg/L. *Carissa spinarum*, *Croton macrostachyus* and *Tylosema fassoglense* produced negligible mortality under the tested conditions. *Plectranthus barbatus* and *Albizia coriaria* produced concentration-dependent mortality, with LC₅₀ estimates of 0.79% and 0.91%, respectively. However, LC₉₀ estimates for both extracts exceeded the highest tested concentration, indicating that high mortality would require concentrations beyond the experimental range. These results suggest that the crude ethanolic extracts tested here are unlikely to be suitable for direct operational larval control. The observed activity in *P. barbatus* and *A. coriaria* is better interpreted as a basis for bioassay-guided fractionation, compound identification, formulation work, selectivity testing and environmental fate studies.

Keywords

Aedes aegypti, Botanical Larvicides, Ethanolic Extract, Medicinal Plants, Probit Analysis, LC₅₀, LC₉₀, Vector Control

1. Introduction

The mosquito *Aedes aegypti* L. is an important vector of dengue, Zika, chikungunya and yellow fever viruses and remains a major public-health challenge in many tropical and subtropical settings [1] [2]. Control of immature mosquito stages is

a central component of integrated vector management because aquatic breeding sites can be targeted before adult emergence [3]. Conventional larvicides, including organophosphates and other synthetic insecticides, have contributed to vector suppression, but their repeated use has raised concerns about resistance development, environmental persistence and impacts on non-target organisms [1] [2] [4].

Botanical larvicides remain attractive because they contain diverse secondary metabolites that may act through multiple biological pathways; however, their efficacy varies considerably with plant species, plant part, solvent system, extraction method and formulation [5]-[7].

Plant-derived products are often proposed as complementary or alternative larvicidal agents because they may contain multiple bioactive compounds and may degrade more readily in the environment than some conventional insecticides [8] [9]. Ethnobotanical knowledge provides a useful starting point for identifying candidate species. The African medicinal plants *Albizia coriaria*, *Plectranthus barbatus*, *Carissa spinarum*, *Croton macrostachyus* and *Tylosema fassoglense* have reported medicinal or bioactive properties and are therefore plausible candidates for preliminary screening [10]-[14].

However, the practical value of botanical larvicides cannot be inferred from mortality at a single high concentration. A candidate extract may show measurable laboratory activity yet remain unsuitable for field application if effective concentrations are too high, unstable, costly to produce or unsafe to non-target organisms. Quantitative dose-response measures, particularly LC_{50} and LC_{90} values with confidence limits, are therefore necessary to distinguish biological activity from operationally relevant potency [15] [16].

This study evaluated the larvicidal activity of ethanolic extracts from five African medicinal plants against third-instar *Ae. aegypti* larvae. The specific aims were to estimate dose-response parameters using probit analysis, identify extracts with concentration-dependent activity, and assess whether the crude extracts show sufficient potency to justify consideration as direct larvicidal products or, alternatively, as sources for further phytochemical investigation.

2. Materials and Methods

2.1. Plant Material and Extraction

Fresh materials of *A. coriaria* (stem), *P. barbatus* (aerial parts), *C. spinarum* (roots), *C. macrostachyus* (aerial parts) and *T. fassoglense* (roots) were collected from Uriri, Migori County, labelled and transported to the laboratory. Voucher specimens were collected *A. coriaria* (BOO 1027), *C. spinarum* (BOO 1051) *P. barbatus* (BOO 1291), *C. macrostachyus* (BOO 1266) and *T. fassoglense* (BOO 1156) authenticated and deposited at the University of Nairobi Herbarium (NAI). Plant materials were cleaned with distilled water and dried to constant weight. The dried material was ground into powder and stored in airtight containers until extraction.

For each species, 100 g of dried plant powder was extracted with ethanol using

Soxhlet extraction for 8 h. The extract was concentrated under reduced pressure at 40°C using a rotary evaporator, transferred into pre-weighed vials and dried to constant weight under vacuum. Extraction yield should be calculated as: dry extract mass divided by dry plant powder mass, multiplied by 100. Dried extracts were stored desiccated at 4°C until use. Before bioassay, each extract was reconstituted in distilled water containing less than 0.1% Polysorbate as an emulsifier.

2.2. Mosquito Larvae and Bioassay Conditions

Third-instar *Ae. aegypti* larvae were obtained from a standardized laboratory colony maintained at the University of Nairobi, School of Biological Sciences insectary. Larvae were held at 28°C, 45% relative humidity and a 12:12 h light/dark photoperiod. Larvicidal assays followed WHO laboratory guidelines for mosquito larvicide testing [16].

Five concentrations were prepared for each extract: 0.2%, 0.4%, 0.6%, 0.8% and 1.0% w/v, equivalent to 2,000, 4,000, 6,000, 8,000 and 10,000 mg/L, respectively. For each concentration, 20 larvae were placed in plastic containers with 40 mL de-ionized water and 1 mL of the appropriate extract solution. Each concentration was tested in triplicate, giving 60 larvae per concentration and 300 larvae per extract. The negative control contained de-ionized water and emulsifier only.

Larvae were not fed during the 24-h exposure period. Mortality was recorded after 24 h. Larvae were considered dead when they failed to move after gentle mechanical stimulation.

2.3. Statistical Analysis

Dose-response relationships were analyzed using probit regression. Mortality proportions were transformed to probit units and regressed against log₁₀-transformed extract concentrations. LC₅₀ and LC₉₀ values with 95% confidence limits were estimated only for extracts that generated sufficient concentration-dependent mortality as in [15]. Extracts with negligible mortality across the tested concentration range were not subjected to LC estimation because a reliable dose-response curve could not be fitted. Model fit should be reported using chi-square goodness-of-fit statistics, degrees of freedom and p-values. LC values estimated beyond the tested concentration range were interpreted cautiously as extrapolated estimates.

3. Results

Larval mortality varied substantially among the five plant extracts (Table 1). *A. coriaria* and *P. barbatus* produced concentration-dependent mortality, whereas *C. spinarum*, *C. macrostachyus* and *T. fassoglense* showed negligible activity across the tested concentration range. At 1.0% w/v, equivalent to 10,000 mg/L, *A. coriaria* caused 73.3% mortality and *P. barbatus* caused 56.2% mortality. The remaining extracts caused 0% - 6.7% mortality at the same concentration.

Table 1. Corrected 24 h mortality of third-instar *Ae. aegypti* larvae exposed to ethanolic plant extracts.

Plant species	0%	0.2% (2,000 mg/L)	0.4% (4,000 mg/L)	0.6% (6,000 mg/L)	0.8% (8,000 mg/L)	1.0% (10,000 mg/L)
<i>Albizia coriaria</i>	0	0	0	20.0	33.3	73.3
<i>Carissa spinarum</i>	0	0	0	0	0	0
<i>Croton macrostachyus</i>	0	0	0	0	0	0
<i>Plectranthus barbatus</i>	0	0	12.5	20.0	50.0	56.2
<i>Tylosema fassoglense</i>	0	0	0	0	0	6.7

Because only *A. coriaria* and *P. barbatus* generated sufficient concentration-dependent mortality, probit analysis was restricted to these two extracts. *P. barbatus* produced an LC₅₀ estimate of 0.79% w/v, equivalent to 7,900 mg/L, while *A. coriaria* produced an LC₅₀ estimate of 0.91% w/v, equivalent to 9,100 mg/L (**Table 2**). Estimated LC₉₀ values were 1.31% for *P. barbatus* and 1.18% for *A. coriaria*. Both LC₉₀ estimates exceeded the maximum tested concentration of 1.0% w/v and should therefore be interpreted as model-based extrapolations rather than directly observed effective concentrations.

Table 2. Probit-derived toxicity estimates for extracts with concentration-dependent larval mortality.

Plant species	LC50 % w/v	LC50 mg/L	95% CL	LC90 % w/v	LC90 mg/L	95% CL	Slope/fit
<i>Plectranthus barbatus</i>	0.79	7,900	0.68 - 0.93	1.31	13,100	1.12 - 1.58	Slope = 5.42; R ² = 0.952
<i>Albizia coriaria</i>	0.91	9,100	0.82 - 1.01	1.18	11,800	1.05 - 1.35	Slope = 6.89; R ² = 0.987

4. Discussion

The results indicate that crude ethanolic extracts from the five medicinal plants differed markedly in their larvicidal activity against *Ae. aegypti*. Only *P. barbatus* and *A. coriaria* produced concentration-dependent mortality, suggesting that these extracts may contain constituents with larvicidal activity. However, their LC₅₀ values were close to 1% w/v, and the LC₉₀ values were estimated beyond the highest tested concentration. This limits the immediate practical relevance of the crude extracts as direct larvicidal products.

The distinction between measurable activity and operational suitability is central to interpreting botanical larvicide studies. Extracts that achieve partial mortality at high laboratory concentrations may still be impractical for field use if large quantities of crude biomass are required. In this study, the active extracts required approximately 7,900 - 9,100 mg/L to achieve 50% mortality. Such concentrations may create logistical, economic and environmental constraints if translated directly into larval-source management. The findings therefore do not invalidate the ethnobotanical value of these plants, but they indicate that the crude ethanolic extracts should not be promoted as ready-to-use larvicides without further refine-

ment. The distinction between measurable biological activity and operational relevance is important.

Recent studies on essential oils and selected plant extracts have reported LC₅₀ values in the low to moderate mg/L range, whereas the active crude extracts in the present study required concentrations in the thousands of mg/L [17]–[19].

The weak activity observed for *C. spinarum*, *C. macrostachyus* and *T. fassoglense* may reflect the inherent variability of crude botanical preparations. Larvicidal performance can be influenced by plant part, harvesting season, geographical origin, extraction solvent, extraction method, storage conditions, mosquito species, larval stage and assay design. Differences between the present findings and previous reports should therefore be interpreted as evidence that botanical larvicidal activity is highly context-dependent rather than as simple contradictions. For example, activity reported against one mosquito species or using a different solvent system may not be replicated against *Ae. aegypti* using crude ethanolic extracts [11] [20].

Safety also requires careful consideration. The present study did not assess mammalian cytotoxicity, hemolytic activity, non-target aquatic toxicity or environmental persistence. Therefore, safety concerns should be treated as priorities for follow-up investigation rather than definitive conclusions from the current bioassay. Because some phytochemical groups commonly detected in medicinal plants, including saponins, alkaloids and polyphenols, can have broad biological effects, any future development should include selectivity testing against non-target organisms and mammalian cell models, as well as environmental degradation profiling [18] [21]–[24].

Because larvicidal activity does not necessarily imply target selectivity, candidate botanical larvicides should be evaluated for cytotoxicity, non-target aquatic toxicity and environmental persistence before field application is considered [18] [21]. Overall, the findings support a cautious development pathway. Crude extract screening remains useful for discovery, but the next stage should be bioassay-guided fractionation of *P. barbatus* and *A. coriaria*, followed by structural identification of active molecules, dose-response testing of purified fractions, formulation studies and safety assessment. A purified or enriched fraction may show higher potency than the crude extract; however, this must be demonstrated empirically before practical vector-control relevance can be claimed.

5. Conclusion

Under the tested laboratory conditions, ethanolic extracts of *C. spinarum*, *C. macrostachyus* and *T. fassoglense* showed negligible larvicidal activity against third-instar *Ae. aegypti* larvae. *P. barbatus* and *A. coriaria* produced concentration-dependent mortality, with LC₅₀ values of 0.79% and 0.91%, respectively. However, their LC₉₀ values were estimated above the maximum tested concentration, indicating that the crude extracts are unlikely to achieve high mortality at

practical direct-use concentrations. The two active extracts may still be valuable as starting materials for bioassay-guided fractionation and compound discovery, provided that potency, selectivity, formulation feasibility and environmental safety are rigorously evaluated.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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