



Larvicidal Activities of Soapberry (*Phytolacca Dodecandra*) and Chinaberry (*Melia Azedarach*) Powders, Separate and Combined Application against *Anopheles* Species (Diptera: Culicidae) in Ethiopia

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Abstract

*Malaria mosquito larvicides of plant origin have comparative advantages. They are easily available, accessible to end users, and environmentally friendly as compared to synthetic insecticides for controlling malaria vectors. This study was undertaken to assess larvicidal activities of Soapberry (*Phytolacca dodecandra*) and Chinaberry (*Melia azedarach*) seed powders, separately and in joint application, against wild *Anopheles* species in Western Ethiopia. Aqueous and methanol solvents were used for botanical extractions. The standard mosquito larvicidal bioassay procedures were employed using 1140 wild-collected 3rd instar larvae of *Anopheles* mosquitoes. Results revealed that methanol extracts outperformed aqueous extracts when used alone, and Chinaberry seed products displayed the highest mortality compared to Soapberry seed products. When paired with Soapberry, methanol extracts were the most poisonous crude extract with the lowest median fatal concentration values against the mosquito larvae (LC95, 3.368 mg/L; LC50, 1.009mg/L). On the other hand, aqueous extracts of Soapberry (LC95, 112.52 mg/L; LC50, 6.64 mg/L) had high fatal concentration values against the mosquito larvae. Larval mortality rate increased with concentration and the combination of the botanicals. The study recommends that joining Soapberry and Chinaberry seed extracts with higher concentrations of methanol is a promising control option against malaria mosquito larvae.*

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INTRODUCTION

Malaria is one of the most acute febrile mosquito-borne diseases worldwide (WHO, 2023). It demanded the lives of an estimated 619,000 people globally in 2021, down from 625,000 in 2020 (WHO, 2023). It has been a main public health concern and action throughout sub-Saharan Africa (SSA). In 2021, 96% of all malaria cases, as well as 95% of all deaths, were accounted for in SSA (WHO, 2023). The highest global load of malaria occurs in Africa, as the region experiences

95% of all cases (263 million cases) and 597,000 deaths in 2024. Around 75% of malaria deaths have been attributed to children below the age of five years (WHO, 2025).

Ethiopia is experiencing a lethal resurgence of malaria, marked by an unparalleled rise in the number of malaria cases, placing the country amongst the top high-burden countries in the WHO African Region (Platon & Ménard, 2026). Malaria is an endemic disease in areas with an elevation of less than 2000 m above sea level in

Ararsa et al.,

the country, and roughly 75% of the Ethiopian population lives in the malaria-endemic regions. However, now, there have been changes to the altitudinal limits for malaria infections, putting the vulnerable highland populations at risk of contracting the disease (Esayas et al., 2024). From western Ethiopia, the Oromia, Benishangul Gumuz, and Gambella regions remain malaria hotspots (Bonker et al., 2024). The primary malaria vector is *Anopheles arabiensis*, whereas *Plasmodium falciparum* and *Plasmodium vivax* are the dominant malaria parasites in the country.

Malaria control depends on vector control in Ethiopia. Indoor Residual House Spraying (IRS) and long-Lasting Insecticidal Nets (LLINs) are the major frontline malaria control interventions. Both IRS and LLINs have been highly effective at reducing malaria transmission (Alemu et al., 2012; Tadesse et al., 2025). Notwithstanding great progress in reducing malaria transmission, the feasibility and sustainability of the synthetic chemical insecticides have been challenging due to insecticide resistance development by vector mosquitoes (Balkew et al., 2012; Massebo et al., 2013; Gari et al., 2016), difficulties in attaining adequate population coverage due to logistic problems, high cost, and dependency on outside aid, and negative impact on non-target animals and the environment at large (Trudel & Bomblies, 2011). Therefore, botanical mosquito larvicides that might supplement IRS and LLINs are required for reducing the transmission of malaria.

Statement of the Problem

Botanicals are natural products and can be used as mosquito larvicides and replace environmentally hazardous synthetic insecticides (Getachew et al., 2016; Tadesse et al., 2025). Botanicals that possess larvicidal and water purification properties are important to maintain the aquatic environment free from pollution and also for controlling immature mosquitoes in their breeding sites (Zelege et al., 2017). Among the botanicals, Soapberry, *Phytolacca dodecandra* (L. Herit), and Chinaberry (*Melia azedarach*) have been evaluated to be effective larvicides against *An. arabiensis* (Trudel

Sci. Technol. Arts Res. J., April–June, 2026, 15(2), 01-10 and Momblies, 2011; Getachew et al., 2016; Zelege et al., 2017; Tadesse et al., 2025).

Soapberry (*Phytolaccaceae*) commonly grows in Ethiopian highlands and produces fruits that are traditionally used for washing clothes (Karunamoorthi et al., 2008). Likewise, Chinaberry (*Meliaceae*) is abundantly found in Ethiopia, particularly in malaria-endemic lowland areas. It is fast-growing and a beneficial source of shade in arid conditions. The Chinaberry seed powder extracts have been shown to be an effective larvicide against *Anopheles stephensi* and *An. arabiensis* (Trudel & Momblies, 2011) and *Aedes aegypti* (Wandscheer et al., 2004).

The feasibility and efficacy of Soapberry (Getachew et al., 2016; Zelege et al., 2017) and Chinaberry (Trudel & Bomblies, 2011) separate applications as locally available, low-cost mosquito larvicides have been evaluated. However, comparative studies of either a separate or combined application of the botanicals are lacking in Ethiopia and elsewhere in the world. The present study intended to assess the malaria mosquito larvicidal potency of Soapberry versus Chinaberry seed extracts, separately and in combined applications, in western Ethiopia.

Research Questions

1. What is the effect of Soapberry and Chinaberry seed extracts, single versus joint applications, against malaria mosquito larvae in western Ethiopia?
2. What is the efficacy of Soapberry and Chinaberry seed powder aqueous versus methanol extracts against *Anopheles* mosquito larvae?
3. What is the mosquito larvicidal effect of Soapberry as compared to Chinaberry seed extracts?

MATERIALS AND METHODS

Study Site

The experiment was carried out in Bako General Hospital, located in Bako Town, the capital of the Bako Tibe district (Figure 1). Bako is located in the Upper Gibe River Valley Basin. The main

Ararsa et al.,

highway connecting the Ethiopian capital, Addis Abeba, with Nekemte runs through Bako Town. The geographical location of the town is 9°15'30" N and 37°18'0" E. The Gibe River, which originates from the Orro highlands and drains to the Omo River and finally ends up in Lake Turkana of Kenya, is the ultimate source of malaria hydrology in the area that creates and maintains year-round malaria mosquito breeding sites. Bako Tibe is one of the malaria districts in Western Ethiopia. One general hospital and one health center in the town provide health care services. Both Soapberry and Chinaberry plants are commonly found in the area. Soapberry is found in protected and unprotected vegetation and

Sci. Technol. Arts Res. J., April–June, 2026, 15(2), 01-10

forests in the area, whereas the Chinaberry tree is planted for shade, ornaments, and fencing in home gardens and along streets in the town. The trend of malaria in the area is driven by bimodal rain. It peaks twice a year between May and June, following the short rainy season that occurs from March to May. And between September and October, following the major rain season that occurs from June to August. The study was conducted during the wet season, and the major mosquito breeding sites during these seasons are roadside pools, borrow pits, and tire tracks along footways and roadsides within villages. The mosquito larvae were collected from such larval habitats for the study.

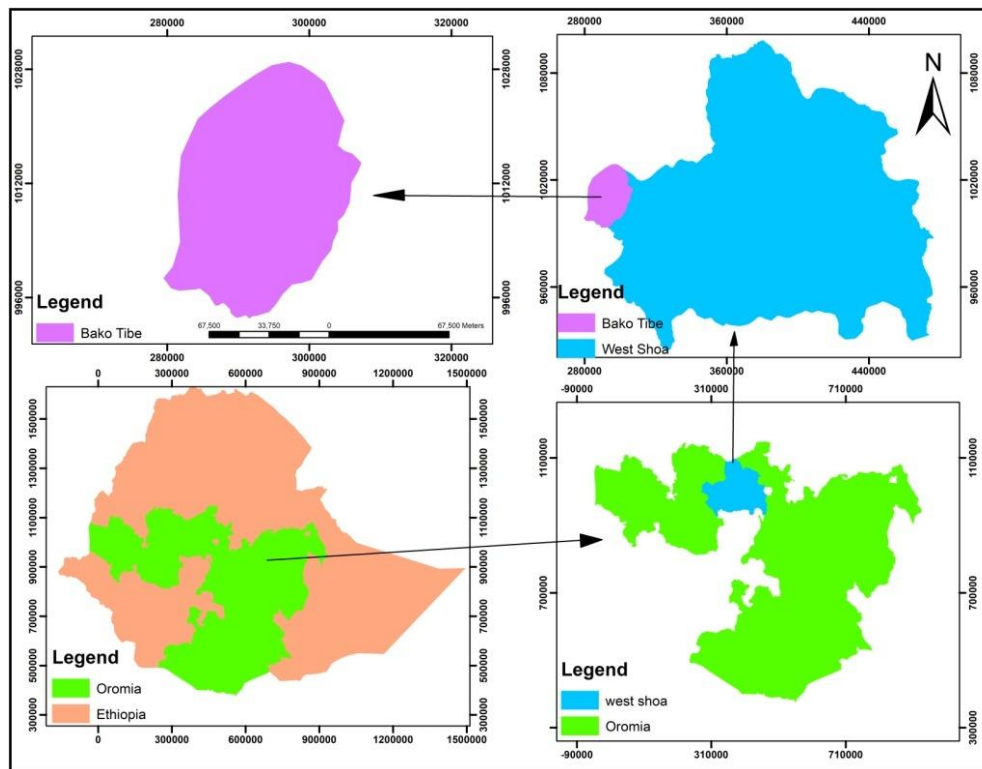


Figure 1. Geographical location of Bako Tibe in Ethiopia and Oromia.

Study design

An experimental study was conducted in Bako General Hospital from August to September 2023. A controlled experimental design (Kothari, 2004) was employed to investigate the efficacy of Soapberry and Chinaberry seed powder aqueous and methanol extracts against field-collected third-instar larvae of *Anopheles* mosquitoes.

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Soapberry and Chinaberry Seed Powder Preparation

The mature seeds of Soapberry and Chinaberry (Figure 2) were collected in plastic containers from Nekemte town in March 2023. Botanical identifications were done by plant taxonomists at Wollega University Herbarium. Procedurally, the

Ararsa et al.,

collected seeds were washed with distilled water, dried in the shade, and carefully ground with a mortar and pestle to reduce contamination. The

Sci. Technol. Arts Res. J., April–June, 2026, 15(2), 01-10
powder was stored for several days before use (WHO, 2005).



Figure 2. Chinaberry (left) and Soapberry (right)

Extraction methods

Aqueous extraction

Crude extracts of the botanicals (Soapberry and Chinaberry seed powders) were separately prepared by weighing 50 g of each plant powder in a beaker that contained 500 mL of water. It was sealed and shaken for 72 hours at room temperature. After 72 hours, double filtration was done with filter papers. The obtained solutions were evaporated using an oven at 40 °C to get a crude extract from each of those plants. Test concentrations were taken between 0.1 and 1.0 g from each crude extract of those plants (WHO, 2005). The test concentrations were set by adding 0.1 g, 0.3 g, and 0.5 g of each crude extract of Soapberry, Chinaberry, and their combined forms.

Chemical extraction

Methanol extractions were prepared by dissolving 50 g of Soapberry and 50 g of Chinaberry seed powders in 500 mL of methanol in separate beakers. It was sealed and stored for 72 hours at room temperature. The beakers were shaken well to dissolve and disperse the material in the solvent, due to many organic compounds being insoluble in water. To obtain the crude extracts, the samples were filtered through 200-mesh gauze. Finally, the filtrate samples were placed in an oven at 40 °C to concentrate the liquid and get the final concentration of the bioactive reagents. It was stored in a screw cap in a refrigerator at 4 °C for

the larvicidal bioassay test (WHO, 2005; Peng et al., 2021). Test concentrations were taken from crude extract between 0.1 and 1.0 g (WHO, 2005). The test concentrations were set using 0.1 g, 0.3 g, and 0.5 g of each crude extract of Soapberry, Chinaberry, and their combined forms, as was done for the aqueous extractions.

Mosquito larval collection

Mosquito larvae collection was conducted by using the standard dipping technique from mosquito-typical larval habitats such as roadside rain pool puddles and borrow pits within villages around Bako General Hospital (Imbahale et al., 2011). Procedurally, the larval habitats were observed visually for the occurrence of anopheline larvae, and then collections were done by dipping using 11.5 cm diameter and 350 ml capacity dippers, pipettes, and white plastic pans. The larval collections were carried out during 0900–1200 hrs in the morning or 1400–1700 hrs in the afternoon. Identification of *Anopheles larvae and culicines* was done based on their resting habits in water and the presence of a siphon. All the mosquito larvae were transported to Bako General Hospital, and the third-instar larvae were selected for the experiment.

Mosquito Larvicidal Bioassay

The standard mosquito larvicidal bioassay procedure (WHO, 2005) was followed to evaluate

Ararsa et al.,

the aqueous and methanol extracts of Soapberry and Chinaberry seed powders. Three replicated totals of 57 tests and 1140 larvae in aqueous and methanol extracts of Soapberry and Chinaberry, and their combination, were needed for the laboratory trial. 0.1, 0.3, and 0.5 g concentrations of each aqueous and methanol extract of Soapberry and Chinaberry, and their combined seed products, were added separately to different containers. The test contained 100 mL of distilled water and 20 mosquito larvae. The 20 larvae were added to each test container using a dropper from the larval tray. The control group was given distilled water only. Over the trial period (24 hours), the test was monitored, and the number of dead larvae was recorded. When the dead parts were found, they were taken out. The percentage mortalities were calculated using Abbott's formula as follows. Larval mortality percentage = Number of dead larvae/number of introduced larvae × 100

Data quality control

To ensure data quality, each bioassay test was replicated three times. The procedure for the

Table 1

Soapberry and Chinaberry aqueous extracts: separate and combined applications against Anopheles species larvae

Plant extract	Conc. (g/ml)	Number of Larvae	Larval mortality	Mortality (%)	X ± SD
Soapberry	0.1	20	3	15%	0.0 ± 0.0
	0.3	20	4	20%	3.0 ± 1.0
	0.5	20	7	35%	7.0 ± 0.0
	0.0	20	-	-	0.0 ± 0.0
Chinaberry	0.1	20	5	25%	5.0 ± 1.1
	0.3	20	6	30%	6.0 ± 1.0
	0.5	20	13	65%	13.0 ± 1.73
	0.0	20	-	-	0.0 ± 0.0
Soapberry and Chinaberry	0.1	20	8	40%	8.0 ± 1.0
	0.3	20	12	60%	12 ± 2.0
	0.5	20	17	85%	17.0 ± 2.0
	0.0	20	-	-	0.0 ± 0.0

Mean ± standard deviation of three replicates; Con. = concentration; g/ml = gram per milliliter.

However, the lowest larval mortality rates for the separate application of the aqueous extracts of the botanicals were recorded at 0.1 g. Likewise, the highest and lowest larval mortality rates for the

Sci. Technol. Arts Res. J., April–June, 2026, 15(2), 01-10 experiments was cleared, and larval containers were cleaned between each use. The key environmental variables, particularly temperature and humidity, were measured.

Data analysis

Data were edited, coded, and computed using the Statistical Package for the Social Sciences (SPSS) version 24.0. A one-way ANOVA was used to find the means and standard deviations of the tested organisms. Larval mortality among treatments and between treatments and controls was compared by using a chi-square test.

RESULTS AND DISCUSSION

Results

Soapberry and Chinaberry aqueous extracts against *Anopheles* species larvae

The highest mortality rate of Soapberry aqueous extract separate application against *Anopheles* species larvae was observed at 0.5 g, and so was the case for the Chinaberry (Table 1).

combined application of the aqueous extracts of the botanicals were recorded at 0.5 g and 0.1 g, respectively.

Table 2 presents separate and combined applications of Soapberry and Chinaberry methanol extracts against *Anopheles* species larvae. As can be seen from the table, the highest mortality rate of Soapberry methanol extract against *Anopheles* species larvae was observed at

Sci. Technol. Arts Res. J., April–June, 2026, 15(2), 01-10
0.5 g, and so was the case for the Chinaberry. However, the lowest larval mortality rates for the separate application of the methanol extracts of the botanicals were recorded at 0.1 g. Likewise, the highest and lowest larval mortality rates for the combined application of the methanol extracts of the botanicals were recorded at 0.5 g and 0.1 g, respectively.

Table 2

*Separate and combined application of methanol extracts of Soapberry and Chinaberry against *Anopheles* species larvae*

Plant extracts	Conc. (g/ml)	Number of Larvae	Larval mortality	Mortality (%)	X ± SD
Soapberry	0.1	20	5	25%	5.0 ± 1.1
	0.3	20	11	55%	11.0 ± 2.0
	0.5	20	13	65%	13.0 ± 2.46
	0.0	20	-	-	0.0 ± 0.0
Chinaberry	0.1	20	7	35%	7.0 ± 1.0
	0.3	20	9	45%	9.0 ± 1.0
	0.5	20	18	90%	18.0 ± 1.0
	0.0	20	-	-	0.0 ± 0.0
Soapberry and Chinaberry	0.1	20	11	55%	11.0 ± 2.0
	0.3	20	14	70%	14 ± 1.589
	0.5	20	20	100%	20.0 ± 0.0
	0.0	20	-	-	0.0 ± 0.0

Mean ± standard deviation of three replicates; Con. = concentration; g/ml = gram per milliliter.

Lethal concentrations (LC50 and LC95) of the botanicals

The lowest LC50 and LC95 values were found in the aqueous extract of *Phytolacca dodecandra*,

whereas the highest values were found in the methanol extract combined with *Phytolacca dodecandra* and *Melia azedarach* (Table 3).

Table 3

*Lethal concentrations (LC50 and LC95) of the separated and combined applications of the botanicals against *Anopheles* species larvae*

Plant extracts	LC50 (LCL-UCL) 95% CI	LC95 (LCL-UCL) 95% CI	P value
AE Soapb	6.64 (3.61 - 1049800)	112.52 (18.12-170536990)	.000
AE Chinab	2.46 (1.96 - 3.688)	16.925 (7.93-159.85)	.000
AE both	1.34 (1.015 - 1.608)	6.064 (4.14-355)	.000
ME Soapb	1.712 (1.19 - 2.309)	17.622 (7.408 - 460.468)	.001
ME Chinab	1.538 (1.274 - 1.793)	5.56 (4.012 - 10.547)	.000
ME both	1.004 (0.054 - 1.461)	3.368 (2.126 - 482.15)	.000

Keynote: AEsopab, aqueous extract of Soapberry; AEChinab, aqueous extract of Chinaberry; AEboth, aqueous extract of both; MESopab, methanol extract of Soapberry; MEChinab, methanol extract of Chinaberry; MEboth, a methanol extract of both, LC50, lethal concentration 50 that kills 50% of larvae; LC95, lethal concentration 95 that kills 95% of larvae; p value, probability value; LCL: Lower Confidence Limits; UCL: Upper Confidence Limits; 95% CI: 95%Confidence Limit.

There were statistically significant observations of mosquito mortality in aqueous and methanol

extracts of Soapberry and Chinaberry, and their combined $P \geq 0.005$ (Table 4).

Table 4

Comparisons of Soapberry, Chinaberry, and their combined effects in aqueous and methanol against Anopheles species larvae

Plant extracts	Plant extracts	X^2 (df=1)	P value	Significant(yes)and not significant (No)
AE Soapb	AEboth	8.340	0.004	Yes
	AEChinab	9.647	0.002	Yes
	MEChinab	10.366	0.001	Yes
	MEboth	9.712	0.002	Yes
AE Chinab	MEChinab	10.274	.001	Yes
	MESoapb	8.159	.004	Yes
	MEboth	9.273	.002	Yes
	AEboth	10.593	.001	Yes
ME Soapb	AEboth	9.426	.002	Yes
	MEChinab	8.596	.003	Yes
	MEboth	9.647	.002	Yes
AE both	MEboth	9.712	0.002	Yes

Note: AEsSoapb, aqueous extract of Soapberry; AEChinab, aqueous extract of Chinaberry; AEboth, aqueous extract of both; MESoapb, methanol extract of Soapberry; MEChinab, methanol extract of Chinaberry; MEboth, a methanol extract of both; P-value, probability value; X^2 , chi-square; Significant(yes)and not significant (No).

Discussion

Results indicate that *Phytolacca dodecandra* and *Melia azedarach* seed products had highly significant larvicidal effects on the malaria vector *Anopheles species* larvae when applied alone. These results are comparable to Zeleke et al. (2017) study on '*Phytolacca dodecandra* that shows an admirable larvicidal impact on *Anopheles arabiensis*. In addition, the larvicidal effect (larval mortality) significantly increased with an increase in the concentration of seed products from 0.1g/ml to 0.5 g/ml in both aqueous and methanol extracts. It was similar to Zeleke et al. (2017) studies on *Phytolacca dodecandra* against *Anopheles arabiensis*, which showed that the lowest and the highest mortality rates were observed at 5 and 50 mg/l of the crude seed extract of *P. dodecandra*. Therefore, the highest larval mortality rates were observed in the application of 0.5g of *Melia azedarach* and *Phytolacca dodecandra*. Results also indicate that aqueous extracts of *Melia azedarach* seed had the highest mortality rate compared to aqueous extracts of

Phytolacca dodecandra seed. It was supported by Trudel and Bombly's (2011) studies on the *Melia azedarach*, which observed larvicidal effects on *An. arabiensis* in Asendabo, Ethiopia. It is also supported by previous studies showing that different plants have different chemical compositions in nature (Hari & Nisha, 2018).

Phytolacca dodecandra and *Melia azedarach* combined were more effective than separate use in the case of aqueous seed extracts against the *Anopheles species* larvae. This was supported by (Hari & Nisha, 2018), who observed that combinations of plant products had more active ingredients than a single application and were more effective against mosquito larvae. In addition, the present results show that mortality rates increased as plant combinations increased, and 100% mortality rates were observed in the application of 0.5g combined of *Phytolacca dodecandra* with *Melia azedarach* methanol extracts. These results are expected because the combination of the botanicals might have synergic larvicidal effects. And the results are supported by Hari and Nisha (2018).

Ararsa et al.,

Results also show that the same plants with similar concentration levels had different mortality rates against mosquito larvae due to the solvent type. It was also supported by previous studies on Substance composition and the combined larvicidal impacts of chosen essential oils against *Anopheles mosquitoes*, which showed that combinations of different plants enhance larvicidal toxicity toward *Anopheles mosquitoes* (Intirach et al., 2012). Therefore, this result also indicates that *Phytolacca dodecandra* in aqueous solution with *Phytolacca dodecandra* in methanol ($x_2 = 8.34$, $p > 0.005$) and *Melia azedarach* in aqueous solution with *Melia azedarach* in methanol ($x_2 = 10.274$, $p > 0.005$) were statistically different. This would be because methanol can extract less weighty *phytochemicals* than aqueous extracts, and bioactive compounds of high molecular weights have low potency (Wandscheer et al., 2004).

The lethal dose enough to kill 50% (LC50) and 95% (LC95) of the larvae exposed were different with plant varieties and concentration levels. LC50 values were (6.64 mg/L, 2.46mg/L, and 1.34 mg/L), and LC95 values were (112.52 mg/L, 16.925 mg/L, and 6.064 mg/L) for the aqueous extract of *Phytolacca dodecandra*, *Melia azedarach*, and their combined extracts, respectively. Whereas, LC50 (1.712 mg/L, 1.538 mg/L, 1.004 mg/L) and LC95 (17.622 mg/L, 5.56 mg/L, 3.368 mg/L) for methanol extracts of *Phytolacca dodecandra*, *Melia azedarach*, and their combined extracts. It was supported by Nagappan's (2012) study on the evaluation of aqueous and ethanol extracts of bioactive medicinal plants. The outcome showed that the LC50 value of second instar hatchlings presented to ethanol extracts was 52.42 mg/L, and for watery concentrate, it was 62.66 mg/L. The current findings demonstrated that the most mortality effect with crude extract with minimum median lethal concentration values against *Anopheles species* larvae was methanol extracts combined with *Melia azedarach* and *Phytolacca dodecandra* (LC95, 3.368 mg/L) and (LC50, 1.009 mg/L). Whereas, less mortality effect with crude extracts with high lethal concentration values against

Sci. Technol. Arts Res. J., April–June, 2026, 15(2), 01-10
Anopheles species larvae were aqueous extracts of *Phytolacca dodecandra* (LC95, 112.52 mg/L) and *Phytolacca dodecandra* (LC50, 6.64 mg/L).

The main drawback of the study is that the effects of *Phytolacca dodecandra* and *Melia azedarach* were not assessed at all stages of the development of the *Anopheles species* larvae. Additionally, this research was unable to compare the laboratory-reared larvae with wild larvae because of resource-related constraints. Furthermore, the toxicity of those plants containing non-target organisms is not being investigated in this study. The phytochemicals responsible for the potential larvicidal activity were not identified. Thus, a precise phytochemical analysis warrants further studies. It also allows for more research on the larvicidal effectiveness of *Melia azedarach* and *Phytolacca dodecandra* seed extracts using other solvents.

CONCLUSION

The study is evident that the combined application of *Phytolacca dodecandra* and *Melia azedarach* has more larvicidal efficacy against the mosquito species. Methanol seed extracts of *Phytolacca dodecandra* and *Melia azedarach* were more efficacious than aqueous extracts in both separate and combined applications. *Melia azedarach* seed extracts were more efficacious than *Phytolacca dodecandra* in both methanol and aqueous extracts.

Recommendation

It is recommended that the joint application of Soapberry and Chinaberry seed extracts with higher concentrations of methanol is an excellent control option against malaria mosquito larvae.

CRedit Authorship Contribution Statement

Ararsa Mashu: Writing Original Draft, Data Curation, Resources, Project administration,
Oljira Kenea: Supervision, Writing - Review & Editing, Methodology, Funding acquisition, Validation,
Sisay Dugasa: Editing, Validation

Declaration of Competing Interest

There was no conflict of interest.

Ethical Approval

Not applicable

Data Availability Statement

The data for determining the results of this research is available on reasonable request to the corresponding author.

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