Evaluation of Larvicidal Activities of *Parthenium Hysterophorus* L. Against *Anopheles Arabiensis* (Diptera: Culicidae), the Major Malaria Vector in Ethiopia

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Abstract

Background: Malaria is a leading public health problem in Ethiopia despite the implementation of effective indoor vector control strategies over several decades. Vector control operations in the country largely depend on the application of Indoor Residual Spraying (IRS) of synthetic insecticides and Long Lasting Insecticidal Nets (LLINs) which resulted in a significant reduction in the malaria burden. The spread of resistance to insecticides in these indoor vector control tools in vector species is a serious challenge. Alternatively, reports indicate that botanicals which are relatively safe possess toxic compounds with high larvicidal activities. However, there are limited evidences on the larvicidal effect of botanicals against the malaria vectors, *Anopheles arabiensis*. This study aimed to evaluate the larvicidal activities of the extracts of the roots, stems, and leaves of *Parthenium hysterophorus* against the 4th instar larvae of *An. arabiensis*.

Methods: Field collected leaves, stems and root parts of *P. hysterophorus* were dried and separately ground to powder and extracted in petroleum ether, hexane, acetone, and ethanol solvents. The extracts were concentrated using a Rota evaporator, and stock solutions were subject to serial dilutions for use as test concentrations. Laboratory reared 4th instar larvae of *An. arabiensis* were used for the larvicidal bioassays. Mortality data were subjected probit analysis to determine LC 50 and LC 90 of the extracts.

Results: The results showed that there were significant differences in percentage larval mortalities ($P < 0.05$) among different concentrations of the leaf, stem, and root solvent extracts, respectively. Hexane extracts of leaves and stem of *P. hysterophorus* at 480ppm caused the highest mortality of 4th larval instar of *An. arabiensis* (85% and 96.7%, respectively) compared to those of ethanolic, acetone, and petroleum ether extracts of these plant parts. Petroleum ether extracts of the root of *P. hysterophorus* were the most effective in causing very high larval mortality at 360ppm and 480ppm (98.3%). The LC 50 value of petroleum ether root extract (10.7ppm) and LC 90 value of petroleum ether root extract (105.5ppm) were significantly lower than those of ethanol, acetone, and hexane root extracts, respectively.

Conclusion: Petroleum ether root extract of *P. hysterophorus* has remarkably high larvicidal potential against 4th instar larvae of *An. arabiensis* which could be exploited for malaria vector control. Further studies on the larvicidal efficacy of the extracts under field conditions and identification of the bioactive components in the root parts of this weed plant are recommended.

Introduction

Mosquitoes are known to transmit human diseases including malaria, dengue fever, yellow fever, and filariasis [1, 2]. Globally, 212 million of malaria cases were reported in 2015 leading to 429,000 deaths, of which (92%) is reported from Africa [2]. Over 70% of the global malaria burden was estimated to have occurred in children less than 5 years old [2]. *Plasmodium falciparum* and *P. vivax* cause most of the malaria burden worldwide, particularly in sub-Saharan Africa [3–6]. Adult female mosquitoes of the genus *Anopheles* are known to transmit malaria. About 20 *Anopheles* species are known to transmit malaria in sub-Saharan Africa. In Ethiopia, *An. arabiensis*, a member of the *An. gambiae* complex, is the primary malaria vector, whereas *An. funestus*, *An. pharoensis*, and *An. nili* serve as secondary vectors [7].

A recent malaria indicator survey conducted in Ethiopia indicated that there is scaling up of the conventional malaria control strategies, mainly in the coverage of long-lasting insecticide treated bed nets (LLINs) and indoor residual spray (IRS). However, malaria is still a leading public health problem in the country [8]. Both these mosquito control strategies are based on the use of synthetic insecticides. However, the high cost of synthetic insecticides, and their adverse impacts on the environment and human health compromise the use of synthetic insecticides [9]. In Ethiopia, the development of insecticide resistance in the mosquito vectors and widespread multidrug resistant *Plasmodium* parasites are the challenges to the malaria control program. Studies indicate that *An. arabiensis* has developed resistance to a number of insecticides including DDT, permethrin, deltamethrin, and malathion [10, 11, 12]. The development of a behavioral resistance which led to an increased outdoor biting and resting proportion of *An. arabiensis* might play a significant role in the residual malaria transmission in Ethiopia. Furthermore, a shift in biting activities of *A. arabiensis* from late to early evening hours before people retire to bed has also been reported from Ethiopia [13]. Therefore, there is an urgent need to find sustainable alternatives or complements to vector management strategies.

Botanical larvicides are among such alternatives to synthetic insecticides which could be used against malaria vectors [14, 15]. Botanical larvicides are preferred over the conventional chemical insecticides because they are relatively ecologically safe, target-specific, and biodegradable properties [16].

P. hysterophorus (Asteraceae) is an extremely abundant invasive weed which causes severe economic loss, health problems, and habitat destruction [17, 18]. This species is reported to release allelochemicals that inhibit the germination and growth of field grasses and other plants. On the other hand, *P. hysterophorus* contain important bioactive compounds, such as sesquiterpene lactones, flavonoid glycosides and pinenes responsible for its ethnobotanical value [14, 19]. Previous studies reported that stem, leaf and root extracts of *P. hysterophorus* have larvicidal effects against *Culex quinquefasciatus* and *Aedes aegypti* [20, 21]. Thus, it is important to explore full profile larvicidal activities of *P. hysterophorus* against the larvae of mosquitoes for use in aquatic breeding sites.

To our knowledge, there are limited published reports on the larvicidal effects of root, stem and leaf extracts of *P. hysterophorus* against African malaria vectors, *An. arabiensis*. This study aimed to evaluate the larvicidal efficacy ethanolic, acetone, hexane and petroleum ether extracts *P. hysterophorus* root, stem and leaf parts against the 4th instar larvae of *An. arabiensis* under laboratory conditions.

Methods

Collection and processing plant parts

Parthenium hysterophorus (here after *P. hysterophorus*) whole plants were collected from the suburb areas of Akaki-Kaliti, Addis Ababa, Ethiopia. The plant specimens were identified as *P. hysterophorus* and voucher specimens have been deposited at the National Herbarium, Addis Ababa University. The fresh leaves, stem and root parts of *P. hysterophorus* were washed with tap water and dried under shade separately at room temperature (27 ± 2 °C). Finally, the dried leaf, stem and root parts were manually ground with mortar and pestle and sieved thoroughly to get a fine powder.

Plant extraction method

Ten gram of *Phyterophorus* leaf, root and stem powder were separately weighed and soaked in 100 ml of petroleum ether, hexane, acetone, and ethanol, using Erlenmeyer flasks, placed on the orbital shaker (VWR, USA) for 24 hours and were filtered through Whatman no. 1 filter paper (Whatman International Ltd., Maidstone, England). The crude extract filtrate were concentrated using a vacuum evaporator under low pressure. After complete evaporation of the solvent, 1 g of the concentrated extract was dissolved in 1000 ml of distilled water (1000 ppm) and stored as the stock solution in a refrigerator at 4°C. The stock solution was subject to serial dilution to prepare the desired concentrations of the extracts for the larvicidal bioassay.

Mosquito rearing

A colony of *An. arabiensis* was reared at 28 ± 1 °C and 70–80% RH; 12:12 h light: dark photoperiod in the insectary as recommended by WHO [12]. Newly emerged adults were fed on 10% sucrose solution soaked in cotton wicks. Female mosquitoes were provided with restrained rabbit as a blood source (1–2 h daily). Glass Petri dishes with wet filter paper placed inside the cage were used for oviposition and eggs were transferred to enamel trays with distilled water for egg hatching. The newly hatched larvae were reared on 30 mg/tray diet consisting of finely ground dog biscuits and yeast (3:2 w/w) in enamel trays (25 cm × 30 cm × 5 cm) containing de-chlorinated water. Pupae were collected daily and transferred to rearing cages for adult emergence.

Larvicidal bioassay

Fourth instar larvae of *An. arabiensis* were used for larvicidal bioassay performed at (28 ± 1) °C following the procedure described by WHO [22]. Test solvent extracts of root, stem and leaf parts of *P. hystrosporous* at concentrations of 40 ppm, 80 ppm, 120 ppm, 160 ppm, 240 ppm, 360 ppm, and 480 ppm were applied into separate glass beakers (200 ml) containing 20 4th instar *An. arabiensis* larvae in 100 ml of distilled water. Twenty 4th instar larvae in glass beaker containing 100 ml distilled water without any test extracts were used as controls. Dried yeast powder (20 mg) was added per glass beakers as a food of larvae for both treatment and control groups. The experiment was set up in a completely randomized design with three replications [23]. Larval mortality was recorded at the end of 24 h exposure time. The larvae were considered as dead when they failed to move after probing with a needle in the cervical region.

Phytochemical analysis of *P. hysterophorus* extracts

The solvent extracts of the leaf, stem and root parts of *P. hystrosporous* were analyzed to determine their phytochemical composition which could be responsible for larvicidal activities based on the procedures described in Harborne [24]. Accordingly, Mayer's test and Benedict's test were used to determine the presence of alkaloids and reducing sugars, respectively. Similarly, Foam test, Ferric chloride test (neutral 5%), Ferric chloride test (0.1%), and Ammonia test were used for detecting the presence of saponins, phenolic compounds, tannins, flavinoids, respectively. Salkowski test (for the presence of terpenoids) and Acid test (for the presence of phlobatannins) were also carried out for the plant extracts used in the larvicidal bioassays.

Data analysis

The larval mortality recorded after 24 h exposures were expressed as percentage of the number of larvae exposed to the treatments. If the observed mortality in the control is between 5–20%, mortality data was corrected using Abbot's formula [25]. When control mortalities are greater than 20%, experiments were discarded [22]. Data were analyzed using SPSS version 20 software (Armonk, NY: IBM Corp). A one-way ANOVA was used to test differences in percent larval mortality among concentrations of solvent extract. When difference are significant, means were separated using Tukey HSD test ($\alpha = 0.05$). Larval mortality data from bioassays with acetone, hexane and petroleum ether extracts of *P. hysterophorus* plant parts were subjected to a probit analysis to determine the LC_{50} and LC_{90} values for each solvent extract used in the bioassays.

Results

Larvicidal efficacy of *P. hysterophorus* leaf extracts

The data on larvicidal effects the leaf extracts of *P. hysterophorus* revealed that there were significant differences in percent larval mortality among different concentrations of hexane, acetone and petroleum ether leaf extracts, respectively ($P < 0.05$; Fig. 1). Hexane and acetone leaf extracts caused markedly high larval mortalities at 480 ppm concentrations (85% and 70% respectively) (Fig. 1B, 1C). Petroleum ether leaf extracts showed moderate larval toxicity at 360 ppm and 480 ppm concentrations (56.7% and 63.3%, respectively) (Fig. 1A). The LC_{50} of acetone, petroleum ether, and hexane extract of leaf of *P. hysterophorus* were 478 ppm, 393 ppm, 368 ppm respectively. (Table 1). Ethanolic leaf extracts of *P. hysterophorus* caused low larval mortalities (< 50%) and were not that effective against 4th instar *An. arabiensis* larvae ($P > 0.05$; Fig. 1D).

Table 1
Larvicidal activity of solvent extract of *P. hysterothorus* parts against 4th instar larvae of *An.arabiensis*.

Plant parts	Solvent	LC ₅₀ (ppm) (LCL-UCL)	LC ₉₀ (ppm) (LCL-UCL)	χ ² (df = 5)	Regression equation
Leafs	Hexane	368 (270.3-758.3)	809.2 (496.2-6053.2)	8.9	Y = 3.75x-9.6
	Acetone	478 (314.6-2672.4)	1358.2 (640.2-107680.2)	8.6	Y = 2.8x-7.6
	Petroleum ether	393.1 (300.3–619.0)	1352.6 (791.9-4227.7)	7.2	Y = 2.4x-6.2
Stem	Hexane	112.4 (61.4-167.6)	331.9 (211.9-1120.1)	10	Y = 2.7x-5.6
	Acetone	259.4 (185.1–442)	1814.6 (837.3-12354.6)	5.3	Y = 1.5x-3.7
	Petroleum ether	196.3 (98.9-572.5)	701.1 (332.4-68176.7)	16	Y = 2.3x-5.3
Root	Hexane	87.9 (32.9-136.7)	338.3 (203.2-1713.3)	9	Y = 2.2x-4.2
	Acetone	342.1 (208.7-2514.2)	769.7 (418.4-558486.5)	18.8	Y = 3.6x-9.2
	Petroleum ether	10.7 (0.02–31.1)	105.5 (45.7-238.1)	0.89	Y = 1.3x-1.3
Lower Confidence Limit; UCL: Upper Confidence Limit; χ ² : Chi-square; df: degree of freedom; ± SE: Standard Error. *Significant at P < 0.05 level.					

Larvicidal efficacy of *P. hysterothorus* stem extracts

There were significant differences in percent larval mortalities among different concentrations of petroleum ether, hexane, acetone and ethanol extracts of stem of *P. hysterothorus* ($P < 0.05$; Fig. 2). Petroleum ether and hexane stem extracts showed appreciably high percent larval mortalities at 360 ppm and 480 ppm (90% and 85%-97% respectively) (Fig. 2A; 2B). Hexane stem extracts also showed considerable larval mortality (70%-80%) at lower concentrations (120 ppm-240 ppm) (Fig. 2B). Hexane stem extracts (LC₅₀ = 112.4 ppm) and petroleum ether stem extracts (LC₅₀ = 196.3 ppm) showed moderate larvicidal activities (Table 1). Similarly, acetone stem extracts were effective resulting in 76% mortality but only at relatively higher concentrations (360 ppm and 480 ppm) (Fig. 2C), with LC₅₀ = 259.4 ppm and LC₉₀ = 1814.6 ppm (Table 1).

Ethanol extracts of stem of *P. hysterothorus* only cause relatively low mortalities (below 50%) at all the concentrations tested in the bioassays, and could not be considered for toxicological applications (Fig. 2D).

Larvicidal efficacy of *P. hysterothorus* root extracts

The data on larvicidal activities of extracts of *P. hysterothorus* revealed statistically significant differences in percent larval mortality among different concentrations of acetone, hexane and petroleum ether root extracts, respectively ($P < 0.05$) (Fig. 3).

Petroleum ether root extracts caused 93% -98% larval mortality of 4th instar *An. arabiensis* larvae at concentration ≥ 160 ppm, and lower concentrations (40 ppm and 120 ppm) also resulted in 80%- 82% larval mortalities (Fig. 3A). Petroleum ether root extracts were effective in a dose dependent manner with LC₅₀ = 10.7 ppm and LC₉₀ = 105.5 ppm (Table 1). Comparatively high larval mortalities (85.0% and 96.7%) were achieved with 360 ppm and 480 ppm hexane root extracts, respectively (Fig. 3B). Larval mortalities of 72%- 78.3% were recorded using 120 ppm-240 ppm hexane root extracts (Fig. 3B). Hexane root extracts also produced high larval mortality with LC₅₀ = 87.9 ppm and LC₉₀ = 338.3 ppm (Table 1).

Acetone root extracts were also effective in achieving 88.3% larval mortality at 480 ppm, but lower concentrations showed inferior larvicidal activities (Fig. 3C), with LC₅₀ = 342.1 ppm and LC₉₀ = 769.7 ppm (Table 1). Ethanol extracts of the root parts of *P. hysterothorus* showed relatively low larvicidal activities (with only 53% mortality at 480 ppm) (Fig. 3D).

Phytochemical analysis extracts of *P. hysterothorus*

Secondary plant metabolites including saponin, phenolic compounds, terpenoids, alkaloid and phlobatannins were detected from the phytochemical analysis of *P. hysterothorus* (Table 2). However, reducing sugars were not detected from all plant parts tested using Benedict's test.

Table 2
Phytochemical composition of *P. hysterothorus* leaf, stem and root

Tested components	Root Stem Leave											
	Hexane	PE	Ethanol	Acetone	Hexane	PE	Ethanol	Acetone	Hexane	PE	Ethanol	Acetone
Reducing sugars	-	-	-	-	-	-	-	-	-	-	-	-
Saponin	+	+	+	+	-	+	-	+	+	+	+	-
PC	-	-	-	-	-	-	-	-	-	-	+	+
Tannis	-	-	-	-	-	-	-	-	-	+	+	+
Flavonoid	-	+	-	+	+	-	+		+	-	-	-
Terpenoid	+	+	+	-	+	+	+	-	+	+	-	-
Phlobatannins	-	+	-	-	-	-	-	-	-	-	-	-
Alkaloids	-	+	-	-	-	-	-	-	-	-	+	-
+ = present and - = absent												

Discussion

This study evaluated the larvicidal activity of ethanol, acetone, hexane, and petroleum ether extracts of the leaf, stem, and root parts of *P. hysterothorus* against the 4th instar larvae of *An. arabiensis*. The toxic effect of *P. hysterothorus* against the 4th instar larvae of *An. arabiensis* depends on the solvent type, test concentration of the extract and plant parts. Petroleum ether and hexane extracts of the stem and root of *P. hysterothorus* resulted in relatively high larvicidal activity against 4th instar larvae of *An. arabiensis*. High larval mortality ($\geq 93\%$) was achieved with petroleum ether root extracts at ≥ 160 ppm concentrations. Lower concentrations of petroleum ether root extracts (40 ppm and 120 ppm) also caused appreciably high ($\geq 80\%$) larval mortalities (Fig. 3A) with dose dependent efficacy in toxicity. The LC_{90} value of Petroleum ether root extracts ($LC_{90} = 105.5$ ppm, Table 1) could be optimized to relatively low concentration formulations for application under field conditions. Hexane root extracts also produced promising efficacy (85.0% and 96.7%) but at relatively higher concentrations ($LC_{90} = 338.3$ ppm; Table 1). The high larvicidal activity of petroleum ether and hexane extracts of the root parts of this plant could be due to the presence of toxic phytochemicals such as saponins, phytosterols, phenols, flavonoids, and tannins, alkaloids and phenolic compounds detected in *P. hysterothorus* plant parts [9, 26–29].

The acetone ($LC_{50} = 478$ ppm, $LC_{90} = 1358.2$), hexane ($LC_{50} = 368$ ppm, $LC_{90} = 809.2$ ppm) and petroleum ether ($LC_{50} = 393.1$, $LC_{90} = 1352.6$ ppm) extracts of *P. hysterothorus* leaf part were moderately effective against 4th instar larval of *An. arabiensis* at higher concentrations and need further evaluation for use as mosquito larvicides under field conditions. In consistent with this result, acetone extracts of *Solanum trilobatum* leaf against 2nd instar larvae of *Culex quinquefasciatus* ($LC_{50} = 265.7$ ppm, $LC_{90} = 558.3$ ppm) and *Aedes aegypti* ($LC_{50} = 301.1$ ppm, $LC_{90} = 582.3$ ppm) were moderately effective [30]. The methanol extract of *Melaleuca cajuputi* leaf against *Ae. aegypti* ($LC_{50} = 183.4$ ppm and $LC_{90} = 1000$ ppm) and *Ae. albopictus* ($LC_{50} = 191.8$ ppm $LC_{90} = 1000$ ppm) [31], and methanol extract of the *Erythrina indica* leaf against the larvae of *An. stephensi* ($LC_{50} = 69.43$, $LC_{90} = 75.13$), *Ae. aegypti* ($LC_{50} = 91.4$ ppm, $LC_{90} = 125.5$), and *C. quinquefasciatus* ($LC_{50} = 134.3$, $LC_{90} = 167.14$) were moderately effective [32]. In addition, petroleum ether extract of *Cassia fistula* and *Nicotiana tabacum* leaf caused a relatively high mortality of 3rd instar larvae of *C. quinquefasciatus* with a respective LC_{50} and LC_{90} of (203.5 ppm, 542.8 ppm) and (223.9 ppm, 645.5 ppm) [33]. The Ethyl acetate and methanol extract of *Sterculia quinqueloba* leaf against late 3rd instar larvae of *An. gambiae* resulted in LC_{50} and LC_{90} of (178.9 $\mu\text{g/ml}$, 526.3 $\mu\text{g/ml}$) and (3762.4 $\mu\text{g/ml}$, 63762.4 $\mu\text{g/ml}$) respectively after 24 hours of exposure time [34]. In contradict with this study, Kumar *et al.*, [20] reported that acetone, hexane, petroleum ether and diethyl ether extract of *P. hysterothorus* leaf were not effective against 3rd and 4th instar larvae of *Ae. aegypti*. This variation could be attributed to the difference in the type of mosquito species involved in the experiment.

Petroleum ether, hexane and acetone extracts of *P. hysterothorus* stem showed a moderate larvicidal activity against the 4th larval instar of *An. arabiensis* with a respective LC_{50} and LC_{90} values of (196.3 ppm, 701.1 ppm), (112.4 ppm, 331.9 ppm) and (259.4 ppm, 1814.6) which also need a further field evaluation and screening. Similarly, hexane extract of *Achyranthes aspera*, *Cassia occidentalis*, *Catharanthus roseus*, *Lantana camara* and *Xanthium strumarium* stem exhibited a 100% mortality against 4th instar larvae of *Ae. aegypti* with LC_{50} and LC_{90} of (68.1 ppm, 115.1 ppm), (149.7 ppm, 206.3 ppm), (108.2 ppm, 184.2 ppm), (89.6 ppm, 125.9 ppm) and (460.9 ppm, 1074.0 ppm) respectively [35]. Ethyl acetate extracts of *Sterculia quinqueloba* stem resulted appreciable larvicidal activity against the 3rd instar larvae of *Ae. aegypti* ($LC_{50} = 227.3$ $\mu\text{g/ml}$, $LC_{90} = 642.03$ $\mu\text{g/ml}$) and *An. gambiae* s.s ($LC_{50} = 135.4$ $\mu\text{g/ml}$, $LC_{90} = 313.73$ $\mu\text{g/ml}$) [34]. Likewise, the respective (LC_{50} and LC_{90}) values of hexane and petroleum ether extract of *P. hysterothorus* stem were (379.8 mg/L, 1314.4 mg/L) and (438.5 mg/L, 870.6 mg/L) against 3rd larval instar of *Ae. aegypti* [20].

A number of studies indicated that root of different plant species have a larvicidal potential against different species of mosquitoes [36–38]. In this study, petroleum ether ($LC_{50} = 10.7$ ppm, $LC_{90} = 105.5$ ppm) and hexane ($LC_{50} = 87.9$ ppm, $LC_{90} = 338.3$ ppm) extract of *P. hysterothorus* root caused a significant mortality of 4th instar larvae of *An. arabiensis*; this makes petroleum ether root a most effective of all extracts tested. Likewise, methanol and ethanol extract of *Aristolochia saccata* root was effective against *Ae. albopictus* larvae with a respective LC_{50} and LC_{90} of (14.5 ppm, 42.7 ppm) and (17.3 ppm, 58.5 ppm) [39]. The same study also indicated that methanol ($LC_{50} = 31.9$ ppm, $LC_{90} = 81.1$ ppm) and ethanol ($LC_{50} = 19.8$ ppm, $LC_{90} = 60.4$ ppm) extract of *Aristolochia saccata* root caused a significant mortality of *Cx. quinquefasciatus* larvae starting from the lowest concentration. In addition, a significant larval mortality of *Cx. quinquefasciatus* was recorded because of petroleum ether extract of *Solanum xanthocarpum* root ($LC_{50} = 41.3$ ppm and $LC_{90} = 111.2$ ppm) [40]. Furthermore, a high (100% mortality at 200 mg/L within the 8th day of exposure time) larvicidal activity of aqueous-ethanoic extracts of *Syzgium guineense* root against *Ae. albopictus* was reported [41]. However, this result is by far superior to the larvicidal effect of petroleum ether and hexane extract of *P. hysterothorus* root against the 4th instar larvae of *Ae. aegypti* (LC_{50} and LC_{90} of (562.5 mg/L, 1232.1 mg/L) and (432.8 mg/L, 1118.5 mg/L) respectively) [20]. This could be due to the difference in mosquito species used for the experiment.

The phytochemical analysis of *P. hysterothorus* showed the presence of major secondary plant metabolites such as saponins, tannins, flavonoid, terpenoid and phlobatannins. Similarly, other studies revealed that *P. hysterothorus* is endowed with various chemical constituents such as, alkaloids, proteins, saponins, tannins, carbohydrate, glycosides, terpenoids, steroids, volatile oils, amino acids, amino sugars, lignans, phenolic compounds, flavonoids, metallic elements, organic acids, terpenoids [21, 26–28, 42].

Conclusion

Different solvent extracts of *P. hysterothorus* leaves, stems and roots have a larvicidal potential against the 4th instar larvae of *An. arabiensis* due to the presence of toxic secondary plant metabolites. Particularly, petroleum ether root, petroleum ether stem, hexane stem, and hexane root extract of *P. hysterothorus* possess a high larvicidal potential. The use of plant extracts in insect control offers a safer alternative to synthetic chemicals and can be obtained by the communities easily at a very low cost and used for protection against mosquito borne diseases. There is also a need for promoting the use of plant based larvicides through community based vector control programs. Further studies at larger scales, semi-field and field settings, are important to confirm their efficacies. In addition, screening of the active compounds of the plant which are responsible for its larvicidal activity is recommended.

Abbreviations

CL: Confidence Limit; DF: Degree of freedom; LC_{50} : Lethal concentration of the extract which kills 50% of the fourth instar larvae of *An. arabiensis* after 24 hours exposure; LC_{90} : Lethal concentration of the extract which kills 90% of the fourth instar larvae of *An. arabiensis* after 24 hours exposure time; χ^2 : chi square; PC: Phenolic compound; PE: Petroleum ether; ppm: parts per million; SE: Standard error

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data sets supporting the conclusions of this article are provided in the manuscript.

Competing Interest

The authors declare that there is no conflict of interest.

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Authors' contributions

MT, HT, YW and SD designed the study. HT, YW and SD supervised and MT conducted the experiments. MT conducted the statistical analyses. MT developed first draft, HT, YW and SD revised the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1
 Percentage mean mortality of 4th instar larvae of *An. arabiensis* with different concentrations of petroleum ether (A) hexane (B) acetone (C) and ethanol (D) extracts of *P. hysterophorus* leaves. The mean percentage larval mortalities with different letter designations are significantly different from one another with a Tukey HSD post hoc analysis at significance level ($P < 0.05$). Error bars represent the standard error of the mean.

Figure 2

Percentage mean mortality of fourth instar larvae of *An. arabiensis* with petroleum ether (A) hexane (B) acetone (C) and ethanol (D) extracts of *P. hysterophorus* stem. The mean percentage larval mortalities with different letter designations are significantly different from one another with a Tukey HSD post hoc analysis at significance level ($P < 0.05$). Error bars represent the standard error of the mean.

Figure 3

Percentage mean mortality of fourth instar larvae of *An. arabiensis* with petroleum ether (A) hexane (B) acetone (C) and ethanol (D) extracts of *P. hysterophorus* root. The mean percentage larval mortalities with different letter designations are significantly different from one another with a Tukey HSD post hoc analysis at significance level ($P < 0.05$). Error bars represent the standard error of the mean.