Evaluation of larvicidal activities of *Parthenium hysterophorus* L. against *Anopheles arabiensis* (Diptera: Culicidae), the major malaria vector in Ethiopia

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Abstract
Malaria is a leading public health problem in Ethiopia despite the implementation of effective indoor vector control strategies over several decades. In the country, the use of botanicals to prevent the transmission of malaria is limited. 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 *Anopheles arabiensis*. 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, 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. Probit analysis was used to determine LC₅₀ and LC₉₀ of the extracts. There were significant differences in percentage larval mortalities (P < 0.05) among different concentrations of the leaf, stem, and root solvent extracts, respectively. Petroleum ether extracts of *P. hysterophorus* root were the most effective in causing very high larval mortality at 360 ppm and 480 ppm (98.3%) when compared to other extract of the plant parts. The LC₅₀ and LC₉₀ value of petroleum ether root extract (10.7 ppm and 105.5 ppm respectively) were significantly lower than those of ethanol, acetone, and hexane root extracts. 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 plant are recommended.

Keywords: *Anopheles arabiensis* · Phytochemical · *Parthenium hysterophorus* · Larvicidal · Extract · Malaria

Introduction
Mosquitoes are known to transmit human diseases including malaria, dengue fever, yellow fever, and filariasis (Benelli et al. 2016; WHO 2018). Globally, 219 million of malaria cases were reported in 2017 leading to 435,000 deaths, of which 92% is reported from Africa and (WHO 2018). Over 61% of the global malaria death was estimated to have occurred in children less than 5 years old (WHO 2018). *Plasmodium falciparum* and *P. vivax* cause most of the malaria burden worldwide, particularly in sub-Saharan Africa (Gething et al. 2011, 2012; Howes et al. 2016). 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 (Gillies and Coetzee 1987).

A recent malaria indicator survey conducted in Ethiopia indicated that despite of the scaling up of the conventional malaria control strategies, mainly long-lasting insecticide treated bed nets (LLINs) and indoor residual spray (IRS), malaria is still a leading public health problem (FMoH 2017). 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 (Ghosh et al. 2012). 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 (WHO 1975; Yewhalaw et al. 2011; Balkew et al. 2013). 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 An. arabiensis from late to early evening hours before people retire to bed has also been reported from Ethiopia (Kibret and Wilson 2016). 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 (Maharaj et al. 2012; Shivakumar et al. 2013). Botanical larvicides are preferred over the conventional chemical insecticides because they are relatively ecologically safe, target-specific, and possess biodegradable properties (Dimetry 2012). P. hysterophorus (Asteraceae) is an extremely abundant invasive weed which causes severe economic loss, health problems, and habitat destruction (Maharjan et al. 2007; McConnachie et al. 2011). 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 pines responsible for its ethnobotanical value (Datta and Saxena 2001; Shivakumar et al. 2013). Previous studies reported that stem, leaf and root extracts of P. hysterophorus have larvicidal effects against Aedes aegypti (Kumar et al. 2012) and Culex quinquefasciatus (Bansode et al. 2016). 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 vector, An. arabiensis (Wachira et al. 2014). This study aimed to evaluate the larvicidal efficacy ethanol, 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

P. hysterophorus whole plants were collected from the suburb areas of Akaki-Kaliti, Addis Ababa, Ethiopia (8° 53’ 44.99” N, 38° 47’ 20.98” E). The plant specimens were confirmed as P. hysterophorus by botanists at the National Herbarium of Addis Ababa University and the voucher specimens have been deposited at the National Herbarium. The fresh leaf, 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 grams of P. hysterophorus 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 filtrates were concentrated using a vacuum evaporator under low pressure (400 mmHg). 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 subjected to serial dilution with distilled water to prepare the desired concentrations of the extracts for the larvicidal bioassay.

Mosquito rearing

Rearing of An. arabiensis was carried out at College of Natural Sciences, Zoological Science insectary (9° 03’ 35” N, 38° 76’ 36” E), Addis Ababa University. 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 (WHO 1975). 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 (WHO 2005). A 1 mL extracts of root, stem and leaf parts of P. hysterophorus 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 99 mL of distilled water. Twenty 4th instar larvae in glass beaker containing 100 mL distilled water without any test extracts were used as controls. A finely grounded dog biscuits and yeast (20 mg) was added in each 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 (Gomez et al. 1984). 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. hysterophorus* were analyzed to determine their phytochemical composition which could be responsible for larvicidal activities based on the procedures described by Harborne (Harborne 1998).

**Mayer’s test** A single drop of Mayer’s reagent was added to 2 mL of each extract along with the side of the test tube. Formation of a white precipitate indicated the presence of alkaloids.

**Benedict’s test** A 0.5 mL of Benedict’s reagent was added to 0.5 mL of each extract and heated for 2 min in a boiling water bath. Appearance of a red color precipitate indicated positive for carbohydrates.

**Foam test** A 2 mL extract was diluted with distilled water and made up to 20 mL. The suspension thus formed was shaken in a graduated cylinder for about 15 min. The development and persistent of about two-centimeter layer of foam at least for 2 minutes indicates the presence of saponins.

**Ferric chloride test** A 1 mL of the extract was diluted to 5 mL of distilled water to which a few drops of neutral 5% ferric chloride solution was added. The appearance dark green color indicates the presence of phenolic compounds.

**Foam test** About 0.5 mg of dried and powdered sample was boiled in 20 mL of water in test tubes and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration as the positive test for tannins.

**Ammonia test** A portion of the aqueous extract, as prepared in the previous test was added to 5 mL of the dilute ammonia solution. Subsequently, a few drops of concentrated sulphuric acid were added to this mixture of aqueous extract and ammonia solution. The appearance of yellow colorations in the solution indicated the presence of flavonoids.

**Salkowski test** A 5 mL of the extract was mixed with 2 mL of chloroform and concentrated sulphuric acid was added to the prepared solution along the sides of the tube in order to form a layer. A reddish brown color at the interface showed the presence of terpenoids.

**Acid test** If the boiling of aqueous extract of plant sample with 1% aqueous hydrochloric acid, forms red precipitate, it indicated the presence of phlobatannins.

**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 Abbott formula (Abbott 1925). When control mortalities are greater than 20%, experiments were discarded (WHO 2005). 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 ethanol, acetone, hexane and petroleum ether extract of *P. hysterophorus* parts was subjected to 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 of 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 and c). 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, and 368 ppm respectively (Table 1). Ethanol leave 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)
Larvicidal efficacy of *P. hysterophorus* 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. hysterophorus* (*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 and b). Hexane stem extracts also showed considerable larval mortality (70% - 80%) at lower concentrations (120 ppm - 240 ppm) (Fig. 2b). Hexane stem extracts (LC$_{50}$ = 112.4 ppm) and petroleum ether stem extracts (LC$_{50}$ = 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 LC$_{50}$ = 259.4 ppm and LC$_{90}$ = 1814.6 ppm (Table 1).

Ethanol extracts of stem of *P. hysterophorus* 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. hysterophorus* root extracts

The data on larvicidal activities of extracts of *P. hysterophorus* 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 LC$_{50}$ = 10.7 ppm and LC$_{90}$ = 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 LC 50 = 87.9 ppm and LC 90 = 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 LC 50 = 342.1 ppm and LC 90 = 769.7 ppm (Table 1). Ethanol extracts of the root parts of *P. hysterophorus* showed relatively low larvicidal activities (with only 53% mortality at 480 ppm) (Fig. 3d).

### Phytochemical analysis extracts of *P. hysterophorus*

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

### Discussion

This study evaluated the larvicidal activity of ethanol, acetone, hexane, and petroleum ether extracts of the leaf, stem, and root parts of *P. hysterophorus* against the 4th instar larvae of *An. arabiensis*. The toxic effect of *P. hysterophorus* against the 4th instar larvae of *An. arabiensis* depends on the solvent type, test concentration of the extract and plant parts. The insecticidal activity of plant extracts vary depending on plant species, mosquito species, geographical variation, extraction methodology and polarity of solvents used during extraction (Shaalan et al. 2005). Phytochemical analysis of *P. hysterophorus* indicated presence of various chemical constituents such as alkaloids, proteins, saponins, tannins, carbohydrates, glycosides, alkaloids, tannins, saponins, flavonoids, lignin and terpenes (Ghimire et al. 2014; Yu et al. 2014).

High larval mortality was achieved with 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. hysterophorus* root when compared to the other plant extract assayed in this study. This is in comparable with methanol and ethanol extract of *Aristolochia saccata* root against *Ae. albopictus* larvae with a respective LC 50 and LC 90 of (14.5 ppm, 42.7 ppm) and

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

| Plant parts | Solvent   | LC 50 (ppm) (LCL-UCL) | LC 90 (ppm) (LCL-UCL) | χ2(df = 5) | Regression equation |
|-------------|-----------|-----------------------|-----------------------|------------|---------------------|
| Leaves      | Hexane    | 368 (270.3–758.3)     | 809.2 (496.2–6053.2)  | 8.9        | Y = 3.75x − 9.6    |
|             | Ethanol   | 3080.6 (≥ 826.5)      | 24509.5 (≥ 2410.6)    | 5          | Y = 1.4x − 4.96    |
|             | 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    |
|             | Ethanol   | 498865.5 (≥ 498865.5) | 190,591,630 (≥ 190,591,630) | 5         | Y = 0.49x − 2.8   |
|             | 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    |
|             | Ethanol   | 793.4 (436.2-5248.9)  | 6627.8 (1800.2-670906.6) | 5       | Y = 1.4x − 4.0    |
|             | 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; χ2: Chi-square; df: degree of freedom
Das et al. (2007) indicated that methanol (LC50 = 31.9 ppm, LC90 = 81.1 ppm) and ethanol (LC50 = 19.8 ppm, LC90 = 60.4 ppm) extract of Aristolochia saccata root caused a significant mortality of C. quinquefasciatus larvae starting from the lowest concentration. Comparably, high larval mortality of C. quinquefasciatus was recorded because of petroleum ether extract of Solanum xanthocarpum root (LC50 = 41.3 ppm and LC90 = 111.2 ppm) (Mohan et al. 2006). However, this result is by far superior than the larvicidal effect of petroleum ether and hexane extract of P. hysterophorus root against the 4th instar larvae of Ae. aegypti (LC50 and LC90 of 562.5 mg/L, 1232.1 mg/L) and (432.8 mg/L, 1118.5 mg/L) respectively) (Kumar et al. 2012).

The phytochemical analysis of petroleum ether extract of P. hysterophorus root showed the presence of secondary plant metabolites such as saponin, flavonoids, terpenoids, phlobatannins and alkaloids. Similarly, hexane extract of the plant root was also found to incorporate saponin and terpenoids. Thus, the individual or synergistic effect of these secondary plant metabolites could be responsible for the larvicidal activity of petroleum ether and hexane extract of P. hysterophorus root. The highest larval mortality of An. arabiensis because of petroleum ether root extract of P. hysterophorus was recorded when compared with leaf and stem extracts. This might be associated with the presence of the highest toxic metabolites in the roots in terms of quality and/or quantity. Kishore et al. 2011, determined that the larvicidal activity of different plant extracts is associated with the presence of secondary metabolites such as such as, alkanes, alkenes, alkynes and simple aromatics, lactones, essential oils and fatty acids, terpenes, alkaloids, steroids, isoflavonoids, pterocarpans and lignans.

Petroleum ether and hexane extracts of P. hysterophorus stem showed a moderate larvicidal activity against the 4th larval instar of An.arabiensis with a respective LC50 and LC90 values of (196.3 ppm, 701.1 ppm), and (112.4 ppm, 331.9 ppm). Comparably, hexane extract of Achyranthes aspera, Cassia occidentalis, Catharanthus roseus, Lantana camara and Xanthium strumarium stem exhibited a moderate larvicidal activity against 4th instar larvae of Ae. aegypti with LC50 and LC90 of (68.1 ppm, 115.1 ppm), (149.7 ppm, 331.9 ppm).

Fig. 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.
206.3 ppm), (108.2 ppm, 184.2 ppm), (89.6 ppm, 125.9 ppm) and (460.9 ppm, 1074.0 ppm) respectively (Sharma et al. 2016). Ethyl acetate extracts of Sterculia quinqueloba stem resulted appreciable larvicidal activity against the 3rd instar larvae of Ae. aegypti (LC50 = 227.3 µg/ml, LC90 = 642.03 µg/ml) and An. gambiae s.s (LC50 = 135.4 µg/ml, LC90 = 313.73 µg/ml) (Wilson et al. 2014). Kumar et al. 2012, reported a higher LC 50 and LC 90 values of hexane (LC50 = 379.8 mg/L, LC90 = 1314.4 mg/L) and petroleum ether (LC50 = 438.5 mg/L, LC90 = 870.6 mg/L) extract of Fig. 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.

Table 2 Phytochemical composition of P. hysterophorus leaves, stem and root

| Tested components | Root | Stem | Leaves |
|-------------------|------|------|--------|
|                   | Hexane | PE | Ethanol | Acetone | Hexane | PE | Ethanol | Acetone | Hexane | PE | Ethanol | Acetone |
| Reducing sugars   | -     | -   | -       | -       | -       | -   | -       | -       | -       | -   | -       | -       |
| Saponin           | +     | +   | +       | +       | +       | +   | +       | +       | +       | +   | +       | +       |
| PC                | -     | -   | -       | -       | -       | -   | -       | -       | -       | -   | -       | -       |
| Tannins           | -     | -   | -       | -       | -       | -   | -       | -       | -       | -   | -       | -       |
| Flavonoids        | -     | +   | +       | +       | -       | -   | +       | +       | +       | +   | +       | +       |
| Terpenoids        | +     | +   | +       | -       | +       | +   | -       | -       | +       | +   | -       | -       |
| Phlobatannins     | -     | +   | -       | -       | -       | -   | -       | -       | -       | -   | -       | -       |
| Alkaloids         | -     | +   | -       | -       | -       | -   | -       | -       | -       | -   | +       | +       |

+ = present and - = absent
P. hysterophorus stem against 3rd larval instar of Ae. aegypti. This difference could also be associated with mosquito species, solvent polarity and concentration. Secondary plant metabolites such as flavonoids and terpenoid were isolated during the phytochemical analysis of hexane extract of P. hysterophorus stem. Saponin and terpenoids had been identified from petroleum ether extract of P. hysterophorus stem. The toxic effect of these plant metabolites could be the reason for the larvicidal activity of the plant extract (Detta and Saxena 2001).

The ethanol, acetone, hexane and petroleum ether extract of P. hysterophorus leaves were less effective against 4th instar larval of An. arabiensis when compared to other extracts used for this experiment. For instance, the respective LC values of acetone, hexane and petroleum ether leaves extract were (LC50 = 478 ppm, LC90 = 1358.2 ppm), (LC50 = 368 ppm, LC90 = 809.2 ppm) and (LC50 = 393.1 ppm, LC90 = 1352.6 ppm), which indicates they were effective only at higher concentrations. This finding is comparable with acetone extracts of Solanum trilobatum leaf against 2nd instar larvae of C. quinquefasciatus (LC50 = 265.7 ppm, LC90 = 558.3 ppm) and Ae. aegypti (LC50 = 301.1 ppm, LC90 = 582.3 ppm) (Lalitha and Thangapandiyan 2018). A relatively lesser LC values were recorded because of the methanol extract of Melaleuca cajuputi leaf against Ae. aegypti (LC50 = 183.4 ppm and LC90 = 1000 ppm) and Ae. albopictus (LC50 = 191.8 ppm LC90 = 1000 ppm ) (Bakar et al. 2018), and methanol extract of the Erythrina indica leaf against the larvae of An. stephensi (LC50 = 69.43 ppm, LC90 = 75.13 ppm), Ae. aegypti (LC50 = 91.4 ppm, LC90 = 125.5 ppm), and C. quinquefasciatus (LC50 = 134.3 ppm, LC90 = 167.14 ppm) (Govindarajan and Sivakumar 2014).

The preliminary screening crude extract of leaf of P. hysterophorus indicated the presence of secondary metabolites such as phenolic compounds, tannis, flavonoid, terpenoid, and alkaloids. However, the quantity (concentration) and quality effect of these secondary metabolites could determine the level of efficacy of the extract (Shaalan et al. 2005).

Conclusions

Different solvent extracts of P. hysterophorus 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. hysterophorus 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.

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Author 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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Data Availability

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

Compliance with ethical standards

Conflict of interest

The authors declare that there is no conflict of interest.

Ethics approval and consent to participate

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Abbreviations

CL, Confidence Limit; DF, Degree of freedom; LC50, 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; χ2, chi squire; PC, Phenolic compound; PE, Petroleum ether; ppm, parts per million; SE, Standard error

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