Research Journal of Environmental and Earth Sciences 4(7): 697-703, 2012
ISSN: 2041-0492
© Maxwell Scientific Organization, 2012
Submitted: May 04, 2012 Accepted: May 22, 2012 Published: July 25, 2012

Evaluation of Multi Potential Bioactive Endod, *Phytolacca dodecandra* (L' Herit) Berries Extracts Against Immature Filarial Vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

Nurie Misganaw, Shiferaw Moges, Muche Tadele, Mamaye Tesera,
Tigab Temesgen and Nagappan Raja
Department of Biology, Faculty of Natural and Computational Sciences,
Post 196, University of Gondar, Ethiopia

Abstract: Aim of the present study was to evaluate larvicidal and pupicidal properties of *Phytolacca dodecandra* plant extracts against immature filarial vector, *Culex quinquefasciatus*. The powdered berries were extracted with petroleum ether, acetone, benzene, methanol and water. The crude residue obtained from the extraction was used to prepare 62.5, 125, 250, 500 and 1000 ppm concentration, respectively. The experiment was conducted by using standard WHO protocol with modifications. The immature mosquitoes were exposed to selected concentration and the percentage mortality was observed continuously for 12, 24 and 48 h, respectively. Among the various solvent extracts tested, petroleum ether, acetone and benzene showed maximum mortality at 125 ppm concentration and above. At 1000 ppm concentration, all the solvent extracts tested showed 100% mortality. The III-instar larva was highly susceptible compared to IV-instar and pupa. The water and methanol extract was also proved to have larvicidal and pupicidal properties. This study showed *P. dodecandra* plant extract have bioactivity compound to kill the immature *Cx. quinquefasciatus*. These plants are growing naturally in Ethiopian highlands and proper utilization may prevent unwanted pollution to the environment.

Keywords: *Culex quinquefasciatus*, larvidical, *Phytolacca dodecandra*, pupicidal solvent extracts

INTRODUCTION

Soapberry, *Phytolacca dodecandra* (L. Herit) is belongs to the family Phytolaccaceae which is commonly called as Endod in Ethiopia. It is a perennial climbing plant growing rapidly in Ethiopian highlands (1600-3000 m above sea level) and produce fruits twice in a year from December to February and June to July (Lemma, 1970; Karunamoorthy *et al*., 2008). The powdered fruits/berries are commonly used in various parts of Ethiopia for washing cloth because when mixed with water it will produce foaming detergent solution (Lemma, 1970). The aerial part of the plant is possessing potential mosquito larvicidal active compound (Dahlman and Hibb, 1967). According to Spielman and Lemma (1973), butanol extract was highly toxic to 2nd and 3rd instar larvae of *Ae. aegypti*, *C. pipiens* and *An. quadrimaculatus*. The dissimilar level of LC50 and LC90 values was observed against *Ae. africanus*, *Ae. aegypti* and *Cx. quinquefasciatus* (Debella *et al*., 2007).

In Ethiopia, high mortality of snails was observed in natural water bodies where people using endod to wash their cloths. Subsequently, various parts of the plant were thoroughly investigated and confirmed molluscicidal activities from the berries (Lemma, 1970). The snail exposed to 19-25 ppm after 6 h and 6-7 ppm concentration after 24 h proved to shown 100% mortality (Baalawy, 1972). The type 44 Ethiopian *Phytolacca* species contained 25% of saponins in which Lemmatoxin, a potential molluscicide was isolated by using organic solvents (Lemma *et al*., 1972). The cercariae mortality was increased when exposed to aqueous extract of endod berries (type 44) with increased concentration and also exposure time (Birrie *et al*., 1998). The butanol extract of endod at less than 3 ppm was lethal to 50% of the fish and snails (Stobaeus *et al*., 1990). The crude berries extracts was reported to have strong toxicity effectiveness against aquatic macro invertebrates such as Baetidae and Hydropsychidide (Karunamoorthi *et al*., 2008). The *Haemonchus contortus* egg hatch inhibition was greater than 90% in ethanolic and dichloromethane extract of the related plant *P. icosandra* leaf extract at 0.90 mg/mL or higher concentration (Hermandez-Villegas *et al*., 2011).

Phytolacca dodecandra is native to sub-Saharan Africa and Madagascar (Schemelzer and Gurib-Fakim,

Corresponding Author: Nagappan Raja, Department of Biology, Faculty of Natural and Computational Sciences Post 196, University of Gondar, Ethiopia

2008) which is used for different medicinal purposes to treat various ailments in humans and also in animals (Ndamba *et al*., 1989; Nalule *et al*., 2011). The medicinal values of the plants are well documented in various parts of the World. In Ethiopia, people use leaf juice to treat Malaria (Mesfin *et al*., 2009). In addition, various medicinal uses are documented in worldwide with this plant species includes purgatives, antihelmintics, laxatives, emetics, diuretics, diarrhea, abdominal pains, edema and intestinal problems (Bizimana, 1994; Schemelzer and Gurib-Fakim, 2008; Nalule *et al*., 2011); wound treatment, skin diseases like ringworms, scabies, abortion induced by young leaves, dandruff, itching, headache, rheumatism, skin irritation, stomach pain and intestinal roundworms (Fonnegra and Jimenez, 2007; Schemelzer and Gurib-Fakim, 2008); treatment of emesis, otitis and pneumonia (EL-Kamali, 2009). The multi-potential bioactive plant *Phytolacca dodecandra* is growing naturally in Ethiopian highlands and also cultivated for use in the snail control program.

Objective: To determine larvidical and pupicidal activities of solvent extracts of immature berries against filarial vector, *Culex quinquefasiatus*.

MATERIALS AND METHODS

Larvicidal and pupicidal experiment was conducted at botany laboratory, Faculty of Natural and Computational Sciences, University of Gondar, Ethiopia from February 2012 to May 2012.

Collection and processing of plant materials: *Phytolacca dodecandra* immature berries were collected in and around Gondar in the month of February 2012. Plant species was identified by verifying the colour pictures followed by description and identification characters (Bekele-Tesemma, 2007). The immature berries were thoroughly washed with tap water to avoid dusts and other unwanted materials accumulated on the leaves from their natural environment. The dust free berries were allowed to dry under shade for 20 days or up to complete drying. The dried berries were powdered by using mortar and pestle, subsequently sieved to collect fine powder by using ASTM E11 Impact laboratory test sieve (no. 60 aperture 250 Mic, UK).

Extraction procedure: Twenty gram of powdered plant material was kept in 200 mL conical flask and added 100 mL of solvent such as petroleum ether, acetone, benzene, methanol and water individually. The mouth of the conical flask was covered with aluminum foil and kept in a Stuart reciprocating shaker for 24 h for continuous agitation at 150 Rev/min for thorough mixing and also complete elucidation of active materials to dissolve in the respective solvent. Then, extract was filtered by using muslin cloth followed by Whatman® filter paper (540 hardened ash less circles, 110 mm thickness, CAT No. 1540 110) and finally filtered by using vacuum and pressure pump (AP-9925 Auto Science). The solvent from the crude extract was removed by using rotary vacuum evaporator RE52 with the water bath temperature of 50°C except water extract. For water extract temperature was raised at 100°C. Finally, the residues were collected and used for the experiment.

Preparation of test concentrations: Stock solution of 10000 ppm concentration was prepared by adding 1 gm of plant residue with 1 mL of acetone and make up to 100 mL by adding tap water. From the stock solution 0.1% of soap powder was added for emulsification purpose. From the stock solution 1000, 500, 250, 125 and 62.5 ppm concentration was prepared by serial dilution method and tested against immature stages of *Cx. quinquefasciatus*.

Maintenance of immature mosquitoes: The immature stages of *Cx. quinquefasciatus* were collected from the stagnant water with rich organic load in and around Tewodros campus, University of Gondar and also pockets of stagnant water near the cattle shed around the basin of Kaka river, Gondar. Mosquito larval collection was done from the breeding site by using large kitchen strainer and transferred to large plastic container and transported to the laboratory. In the laboratory, homogenous immature stages were segregated and allowed to acclimatize in the laboratory for 24 h by using decholorinated tap water. The immature were provided with powdered dog biscuit with yeast powder (3:1 ratio) as a feed. After acclimatization of mosquito larvae in the laboratory, subsequent experiments were conducted.

Assessment of larvicidal potential of endod extracts: Larvicidal properties of various solvents extract of *P. dodecandra* was evaluated by using WHO method (WHO, 2005) with modifications. Twenty III, IV and pupal stage was released in to 250 mL of glass beaker individually. In each beaker, concentration of solvent extract was maintained at 1000, 500, 250, 125 and 62.5 ppm with the final water volume of 200 mL. In control, except plant materials remaining all added as mentioned in the concentration preparation. The mortality rates of immature mosquitoes were recorded at 12, 24 and 48 h exposure period, respectively. The dead larvae in five replicates were counted individually and converted in to percentage of mortality. Dead larvae were identified when they failed to move when the water was disturbed. The experiment was replicated five times and the percentage mortality was calculated. The corrected percentage mortality was calculated by using Abbott's formula (Abbott, 1925).

Corrected % mortality = [% mortality in test-% mortality in control]/[100-% mortality in control] X 100.

Assessment of pupicidal potential of endod extracts: Freshly emerged pupa was used for pupicidal activity. The concentration of plant extract and methods were followed as that of larvicidal activity. For each concentration 20 numbers of freshly emerged homogenous pupae were released individually and the percentage of mortality was recorded after 12, 24 and 48 h exposure period, respectively. The experiment was replicated five times and percentage mortality was calculated. The corrected percentage pupal mortality was calculated based on Abbott's formula as mentioned in larvicidal properties assessment.

Statistical analysis: The experimental data was subjected to descriptive statistical analysis to derive mean and standard deviation. The significant difference in concentration of the plant extract and various solvent extract of the plant was confirmed by two way Analysis of Variance (ANOVA). Further individual mean significant difference was calculated by using post hoc test LSD (Least Significant Difference test, $p<0.05$) by using SPSS software version 16.

RESULTS

Mean percentage mortality of IIIrd instar larvae of *Cx. quiquefasciatus* exposed to different concentration of *P. dodecandra* plant extract was mainly based on the concentration and type of solvent used for extraction (Table 1). Irrespective of tested concentration, 100% mortality was recorded in petroleum ether, benzene and methanol extract exposed to 12 h at 500 and 1000 ppm concentration. The mean percentage mortality rate at 500 and 1000 ppm concentration was not statistically significant ($p>0.05$; LSD). At 125 ppm concentration, except methanol and water extract remaining all percentage mortality was above 50%. The maximum mortality of 97 and 98% was recorded in acetone and benzene extract respectively exposed to 250 ppm concentration. The result of benzene and acetone extract was statistically not significant ($p>0.05$; LSD). However, compared to other tested solvent extracts the results was significantly different ($p<0.05$; LSD).

After 24 h exposure period in 500 and 1000 ppm concentration, except acetone (98%) extract remaining all the tested solvent extracts recorded 100% mortality (Table 2). The results was statistically not significant ($p>0.05$; LSD) within the solvent extract tested. At 250 ppm concentration, except methanol extract remaining all showed above 90% mortality. At 125 ppm concentration, maximum mortality rate of 93 and 83% was recorded in acetone and benzene extract, respectively. The mean percentage mortality of acetone and benzene extract was statistically significant compared to other tested solvent extracts ($p<0.05$; LSD).

After 48 h exposure period, there is no much significant difference at 62.5 ppm concentration. However, 500 and 1000 ppm concentration, 100% mortality was recorded in all the solvent extracts tested. At 250 ppm, benzene and water extract showed 100% mortality. The maximum larval mortality of 96% was recorded at 125 ppm concentration and the result was statistically significant ($p<0.05$; LSD) compared to other solvent extracts tested (Table 3).

Mean percentage mortality of homogenous stage of IVth instar larvae of *Cx. quinquefasciatus* exposed to *P. dodecandra* varied significantly after 12 h exposure period (Table 4 to 6). The larvae exposed to 1000 ppm concentration showed 100% mortality to all the solvent extracts tested. The percentage mortality in petroleum ether, acetone and benzene extract was not statistically significant ($p>0.05$; LSD) at 500 ppm concentration. However, compared to methanol and water extracts results was significantly different ($p<0.05$; LSD). At 250 ppm concentration, maximum mortality of 95 and 94% was recorded in acetone and benzene extract, respectively. Generally, lower concentration percentage mortality was decreased. Even though, benzene and acetone extracts showed above 50% larval mortality (Table 4).

Bio-potency of different solvent extracts of *P. dodecandra* showed fluctuation in irrespective of exposure period and solvents used for extraction after 24 h exposure period (Table 5). Results revealed that 100% mortality was recorded in 1000 ppm concentration. At 500 ppm concentration, 100% mortality rate was recorded only in benzene extract. The percentage mortality observed in petroleum ether, acetone and benzene extract was significantly different ($p<0.05$; LSD) compared to water and methanol extract. At 250 ppm concentration, percentage mortality was 97% in acetone and benzene extract and the remaining tested extract showed above 80% mortality.

After 48 h exposure period, percentage mortality rate was increased in increased concentration (Table 6). The larvae exposed to petroleum ether, acetone and benzene showed 100% mortality in 500 and 1000 ppm concentration. However, in methanol and water extract 98 and 94% mortality was observed at 500 ppm concentration. At 250 ppm concentration, all the tested solvent extracts showed above 90% mortality. The maximum percentage mortality of 89 and 82% was recorded in acetone and benzene extract, respectively. The mortality rate of all the solvent extracts tested in this concentration showed statistically significant difference ($p<0.05$; LSD).

The percentage mortality of homogenous stage of *Cx. quinquefascatus* pupa exposed to different solvent extracts of *P. dodecandra* berries varied significantly

Table 1: Mean percentage mortality of IIIrd instar larvae of *Cx. quinquefasciatus* after 12 h exposure period in *P. dodecandra* plant extracts

	Concentration in PPM				
Solvent extracts tested	62.5	125	250	500	1000
Petroleum ether	6±4.18^{e}	64±6.50^{d}	86±13.8^{a}	100±0.00^{a}	100±0.00^{a}
Acetone	38±5.70^{d}	93±5.70^{c}	98±2.73^{c}	98±2.73^{a}	100±0.00^{a}
Benzene	15±7.91^{c}	80±7.91^{b}	97±4.47^{c}	100±0.00^{a}	100±0.00^{a}
Methanol	29±9.61^{b}	44±8.21^{a}	64±9.61^{b}	100±0.00^{a}	100±0.00^{a}
Water	0±0.00^{a}	46±6.51^{a}	85±5.00^{a}	95±5.00^{a}	100±0.00^{a}

Values are mean±standard deviation of five replications; Within the column similar alphabets are statistically not significant (p>0.05) by LSD

Table 2: Mean percentage mortality of IIIrd instar larvae of *Cx. quinquefasciatus* after 24 h exposure period in *P. dodecandra* plant extracts

	Concentration in PPM				
Solvent extracts tested	62.5	125	250	500	1000
Petroleum ether	6±4.18^{a}	81±4.18^{d}	94±6.51^{a}	100±0.00^{a}	100±0.00^{a}
Acetone	38±5.70^{b}	93±5.70^{c}	98±2.73^{a}	98±2.73^{a}	100±0.00^{a}
Benzene	19±4.18^{c}	89±6.51^{c}	99±2.23^{a}	100±0.00^{a}	100±0.00^{a}
Methanol	36±7.41^{b}	54±9.61^{b}	88±5.70^{b}	100±0.00^{a}	100±0.00^{a}
Water	3±2.73^{a}	68±5.70^{a}	96±6.51^{a}	100±0.00^{a}	100±0.00^{a}

Values are mean±standard deviation of five replications; Within the column similar alphabets are statistically not significant (p>0.05) by LSD

Table 3: Mean percentage mortality of IIIrd instar larvae of *Cx. quinquefasciatus* after 48 h exposure period in *P. dodecandra* plant extracts

	Concentration in PPM				
Solvent extracts tested	62.5	125	250	500	1000
Petroleum ether	6±4.18^{a}	85±3.53^{d}	99±2.24^{a}	100±0.00^{a}	100±0.00^{a}
Acetone	41±5.47^{d}	96±4.18^{c}	98±2.73^{a}	100±0.00^{a}	100±0.00^{a}
Benzene	19±4.18^{c}	91±6.52^{b}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}
Methanol	36±7.41^{b}	62±6.70^{a}	95±5.00^{b}	100±0.00^{a}	100±0.00^{a}
Water	4±4.18^{a}	68±5.70^{a}	100±0.00^{a}	100±0.00^{a}	100±.0.00^{a}

Values are mean±standard deviation of five replications; Within the column similar alphabets are statistically not significant (p>0.05) by LSD

Table 4: Mean percentage mortality of IVth instar larvae of *Cx. quinquefasciatus* after 12 h exposure period in *P. dodecandra* plant extracts

	Concentration in PPM				
Solvent extracts tested	62.5	125	250	500	1000
Petroleum ether	0±0.00^{a}	31±4.18^{b}	77±10.37^{c}	96±4.18^{b}	100±0.00^{a}
Acetone	29±6.52^{d}	62±7.58^{d}	95±3.54^{b}	97±2.74^{b}	100±0.00^{a}
Benzene	11±6.52^{c}	53±8.36^{c}	94±6.52^{b}	100±0.00^{b}	100±0.00^{a}
Methanol	21±7.41^{b}	32±9.08^{b}	59±9.62^{a}	75±5.00^{a}	100±0.00^{a}
Water	0±0.00^{a}	43±7.58^{a}	63±5.70^{a}	73±7.58^{a}	100±0.00^{a}

Values are mean±standard deviation of five replications; Within the column similar alphabets are statistically not significant (p>0.05) by LSD

Table 5: Mean percentage mortality of IVth instar larvae of *Cx. quinquefasciatus* after 24 h exposure period in *P. dodecandra* plant extracts

	Concentration in PPM				
Solvent extracts tested	62.5	125	250	500	1000
Petroleum ether	3±3.94^{a}	32±5.70^{c}	84±9.62^{b}	97±4.47^{b}	100±0.00^{a}
Acetone	35±6.12^{d}	70±6.12^{b}	97±2.73^{c}	97±4.47^{b}	100±0.00^{a}
Benzene	12±6.70^{c}	76±7.41^{b}	97±4.47^{c}	100±0.00^{b}	100±0.00^{a}
Methanol	21±7.41^{b}	50±7.90^{a}	83±5.70^{b}	94±4.14^{a}	100±0.00^{a}
Water	0±0.00^{a}	48±4.47^{a}	89±7.41^{a}	91±6.51^{a}	100±0.00^{a}

Values are mean±standard deviation of five replications; Within the column similar alphabets are statistically not significant (p>0.05) by LSD

Table 6: Mean percentage mortality of IVth instar larvae of *Cx. quinquefasciatus* after 48 h exposure period in *P. dodecandra* plant extracts

	Concentration in PPM				
Solvent extracts tested	62.5	125	250	500	1000
Petroleum ether	3±2.73^{a}	44±8.22^{a}	95±5.00^{ab}	100±0.00^{b}	100±0.00^{a}
Acetone	38±5.70^{d}	89±4.18^{d}	98±2.74^{b}	100±0.00^{b}	100±0.00^{a}
Benzene	12±6.70^{c}	82±11.51^{c}	99±2.24^{b}	100±0.00^{b}	100±0.00^{a}
Methanol	21±7.41^{b}	57±7.58^{b}	91±6.51^{a}	98±2.73a^{b}	100±0.00^{a}
Water	2±2.73^{a}	49±4.18^{a}	91±8.94^{a}	94±6.51^{a}	100±0.00^{a}

Values are mean±standard deviation of five replications; Within the column similar alphabets are statistically not significant (p>0.05) by LSD

irrespective of tested concentration after 12 h exposure period (Table 7). The pupal mortality was recorded at the maximum of 100% except acetone extract (98%). At 500 ppm concentration, benzene extract showed 100% mortality. The percentage mortality rate of benzene and acetone extract was statistically not

Table 7: Mean percentage mortality of pupa of *Cx. quinquefasciatus* after 12 h exposure period in *P. dodecandra* plant extracts

	Concentration in PPM				
Solvent extracts tested	62.5	125	250	500	1000
Petroleum ether	3±2.73^{a}	56±4.18^{d}	72±5.70^{d}	85±5.00^{c}	100±0.00^{a}
Acetone	41±4.18^{d}	91±6.52^{c}	96±4.18^{c}	97±4.47^{b}	98±2.74^{a}
Benzene	9±6.51^{c}	62±5.70^{b}	87±5.70^{b}	100±0.00^{b}	100±0.00^{a}
Methanol	19±9.61^{b}	33±5.70^{a}	66±7.41^{a}	77±6.71^{a}	100±0.00^{a}
Water	0±0.00^{a}	31±8.21^{a}	67±5.70^{a}	78±9.08^{a}	100±0.00^{a}

Table 8: Mean percentage mortality of pupa of *Cx. quinquefasciatus* after 24 h exposure period in *P. dodecandra* plant extracts

	Concentration in PPM				
Solvent extracts tested	62.5	125	250	500	1000
Petroleum ether	4±2.23^{a}	70±7.90^{d}	84±4.18^{b}	95±5.00^{bc}	100±0.00^{a}
Acetone	41±6.52^{d}	91±8.94^{c}	97±2.73^{c}	99±2.23^{bc}	100±0.00^{a}
Benzene	11±4.18^{c}	65±6.12^{b}	95±5.00^{ac}	100±0.00^{b}	100±0.00^{a}
Methanol	24±7.41^{b}	47±7.58^{a}	84±6.51^{b}	96±4.18^{ab}	100±0.00^{a}
Water	0±0.00^{a}	44±4.18^{a}	92±5.70^{a}	92±9.08^{a}	100±0.00^{a}

Values are mean±standard deviation of five replications; Within the column similar alphabets are statistically not significant (p>0.05) by LSD

Table 9: Mean percentage mortality of pupa of *Cx. quinquefasciatus* after 48 h exposure period in *P. dodecandra* plant extracts

	Concentration in PPM				
Solvent extracts tested	62.5	125	250	500	1000
Petroleum ether	6±2.24^{e}	72±8.36^{e}	90±7.90^{bd}	100±0.00^{b}	100±0.00^{a}
Acetone	41±6.52^{d}	96±6.52^{d}	99±2.40^{c}	100±0.00^{b}	100±0.00^{a}
Benzene	11±4.18^{c}	65±6.12^{c}	96±6.52^{ac}	100±0.00^{b}	100±0.00^{a}
Methanol	24±7.41^{b}	49±7.41^{b}	88±7.58^{b}	97±2.73^{ab}	100±0.00^{a}
Water	0±0.00^{a}	44±4.18^{a}	92±2.73^{ad}	94±6.52^{a}	100±0.00^{a}

significant (p>0.05; LSD). However, compared to petroleum ether, methanol and water extract the results was statistically significant (p<0.05; LSD). The maximum percentage mortality of 96 and 91% was recorded in acetone extract at 250 and 125 ppm, respectively.

After 24hr exposure period, 100% mortality was recorded in all the plant extracts tested. At 500 ppm, benzene extract showed 100% mortality and remaining tested plant extracts showed above 90% mortality. At 250 ppm concentration, maximum percentage mortality of 97 and 95% was recorded in acetone and benzene extract, respectively. The result of benzene and acetone was not statistically significant (p>0.05; LSD). However, compared to petroleum ether, methanol and water extract the mortality difference was statistically significant (p<0.05: LSD). At 125 ppm concentration, maximum mortality of 91% was observed in acetone extract which was significantly different (p<0.05: LSD) from other tested plant extracts. At 62.5 ppm, maximum percentage mortality of 41% was recorded in acetone extract (Table 8).

After 48 h exposure period, all the tested solvent extracts showed 100% mortality at 1000 ppm concentration. At 500 ppm concentration, petroleum ether, acetone and benzene extracts showed 100% mortality; percentage mortality in methanol and water extract was 97 and 94%, respectively. At 250 ppm concentration, except methanol extract remaining all extracts showed above 90% mortality. The maximum percentage mortality of 96% was recorded in acetone extract at 125 ppm concentration (Table 9).

DISCUSSION

Natural product researches by using botanicals are progressively going on to identify novel bio-potency compounds to replace environmentally hazardous synthetic pesticides. The indiscriminate application of synthetic pesticides adversely affects the environment and human health. In addition, pesticide resistance has resulted in the resurgence of mosquito borne diseases (Becker *et al*., 2003). Recent report highlights botanical extracts not only having mosquitocidal properties and also have water purification properties (Arjunan *et al*., 2012). The mass breeding of *Cx. quinquefasicatus* was very common in highly polluted water with rich organic resources. Plant extracts possessing larvicidal and water purification properties are very much useful to maintain the aquatic environment free from pollution at the same time to control immature mosquitoes in their breeding sites.

Biopotency of *P. dodecandra* was based on the concentration, solvent used for extraction and also exposure period. Irrespective of the concentration, maximum percentage mortality of 100% was observed in all the tested solvent extracts at higher concentration (1000 ppm). The III-instar larva was highly susceptible compared to IV-instar larva and pupa. This will indicate comparatively late instar stages have high tolerance capacity under stress. The solvent used for the extraction process was one of the factors to determine the active ingredient. It was confirmed in the present finding that the mortality rate of immature varied widely in different solvent extracts. To confirm the

present findings Hermandez-Villegas *et al*. (2011) reported that *Haemonchus contortus* egg hatch inhibition was greater than 90% in ethanolic and dichloromethane extract compared to n-hexane extracts of similar plant species, *P. icosandra*. The percentage of larval mortality was based on the bioactive compounds present in the plants and dissolving nature to solvent used for extraction. Present findings also corroborate with the report of Hermandez-Villegas *et al*. (2011) they have reported that antihelmintic activity of similar plant species *P. icosandra* could be attributable to flavonoids, steroids, terpenoids, coumarin and or saponin presented in the ethanolic and dichloromethane extract. In addition, concentration of more than one compound may also responsible for observed antihelminthic activity against the life stages of *H. contortus.*

The percentage mortality of immature mosquitoes was significantly greater in petroleum ether, acetone and benzene extract at above 125 ppm; 100% mortality was observed at 1000 ppm in all the tested solvent extracts. It was observed that petroleum ether and benzene extract treated medium superficial oily layer was appeared which may block the oxygen supply to the immature mosquitoes; due to suffocation percentage mortality may increased. Similar findings was observed by Govindarajan *et al*. (2011a) they have confirmed significant larvicidal properties of crude benzene, acetone and methanol extracts of *C. fistula* leaf against *An. subpictus* and *Cx. tritaeniorthynchus*. The highest larval mortality was also found in the benzene extract of *E. coronaria* against the larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* (Govindarajan *et al*., 2011b).

In the present study, water and methanol extract was also proved to have larvicidal and pupicidal properties against immature *Cx. quinquefasciatus*. The berries of *Phytolacca* type 44 species in Ethiopia contained 25% by weight of saponin (Lemma *et al*., 1972). The percentage mortality of the present study may be due to the presence of saponin; it will produce foamy layer when mixed with water; that may also block the oxygen supply, leads to high mortality rate of immature mosquitoes. Kloos and McCullough (1984) also reported that immature berries of *P. dodecandra* have most potent molluscicides which are used to control schistosomiasis transmitting snail. Kovendan *et al*. (2012a) observed highest larval mortality in methanol extract of *Acalypa alnifolia* leaf against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The first to fourth instar larvae and pupae was highly susceptible to methanol extract of *Carica papaya* leaf extract (Kovendan *et al*., 2012b). The saponin from the *P. dodecandra* was the main active substance and responsible for bio-potency. The saponin was stable for 2 days and biodegrade (Molgaard *et al*., 2000). It is another advantage it may degrade quickly and may not pollute much on the aquatic environment. This study proved potential larvicidal and pupicidal activity of *P. dodecandra* against immature *Cx. quinquefasciatus*.

CONCLUSION

The results of the present study showed effectiveness of solvent extracts of *P. dodecandra* against immature *Cx. quinquefasciatus*. The biopotency was based on concentration, exposure period and solvent used for extraction. The water extract was also effective against immature mosquitoes and it can be suitable for controlling mosquitoes in small man made breeding places. However, large scale implementation needs to develop effective formulation. The application of these plant extract on mosquito breeding places surely prevent environmental pollution and also protect the earth from toxic chemical pollutants.

REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Arjunan, N., K. Murugan, P. Madhiyazhagan, K. Kovendan, K. Prasannakumar, S. Thangamani and D.R. Bernard, 2012. Mosquitocidal and water purification properties of *Cynodon dactylon*, *Aloe vera*, *Hemidesmus indicus* and *Coleus amboinicus* leaf extracts against the mosquito vectors. Parasitol. Res., 110: 1435-1443.

Baalawy, S.S., 1972. Laboratory evaluation of the molluscicidal potency of a butanol extract of *Phytolacca dodecandra* (endod) berries. Bull. World Hlth. Org., 47(3): 422-425.

Becker, N., D. Petric, M. Zgomba, C. Boase, J. Dahl and A. Kaiser, 2003. Mosquitoes and their Control. Kluwer Academic/Plenum, New York.

Bekele-Tesemma, A., 2007. Useful Trees of Ethiopia: Identification, Propagation and Management in 17 Agroecological Zones. Nairobi, RELMA in ICRAF Project, pp: 552.

Birrie, H., F. Balcha, B. Erko, A. Bezuneh and N. Gemeda, 1998. Investigation in to the cercariacidal and miracidiacidal properties of Endod (*Phytolacca dodecandra*) berries (type 44). East Afr. Med. J., 75: 311-314.

Bizimana, N., 1994. Traditional Veterinary Practice in Africa. Eschborn, Germany, ISBN: 3-88085-502-1, (GTZ).

Dahlman, D and E. Hibb, 1967. Plant extracts as larvicides. Ann. Entomol. Soc. Amer., 2: 60-73.

Debella, A., A. Taye, D. Abebe, K. Mudi, D. Melaku and G. Taye, 2007. Screening of some ethiopian medicinal plants for mosquito larvicidal effects and phytochemical constituents. Pharmacol. Online, 33: 231-243.

El-Kamali, H.H., 2009. Medicinal plants in east and central Africa: Challenges and constraints. Ethnobot. Leaflets, 13: 364-369.

Fonnegra, G.R. and R.S.L. Jimenez, 2007. Approved as medicinal plant in Colombia. 2nd Edn., Universidad de Antioquia, Medellin, Colombia, pp: 353, (In Spanish).

Govindarajan, M., M. Sivakumar and M. Rajeswari, 2011a. Larvicidal efficacy of *Cassia fistula* Linn. leaf extract against *Culex tritaeniorhynchus* Glies and *Anopheles subpictus* Grassi (Diptera: Culicidae). Asian Pacific J. Trop. Dis., 1(4): 295-298.

Govoindarajan, M., T. Mathivanan, K. Elumalai, K. Krishnappa and A. Anandan, 2011b. Mosquito larvicidal, ovicidal and repellent properties of botanical extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol. Res., 109: 353-367.

Hermandez-Villegas, M.M., R. Borges-Argaez, R.I. Rodriquez-Vivas, J.F. Torres-Acosta, M. Mendez-Gonzalez and M. Caceres-Farfan, 2011. Ovicidal and larvicidal activity of the crude extracts from *Phytolacca icosandra* against *Haemonchus contortus*. Vet. Parasitol., 179(1-3): 100-106.

Karunamoorthy, K., D. Bishaw and T. Mulat, 2008. Laboratory evaluation of Ethiopian local plant, *Phytolacca dedecandra* extract for its toxicity effectiveness against aquatic macroinvertebrates. Eur. Rev. Med. Pharmacol. Sci., 12: 381-386.

Kloos, H. and F.S. McCullough, 1984. Plant Molluscicides. In: Lemma, A., D. Heyneman and S.M. Silangwa (Eds.), *Pyhtolacca Dodecandra* (Endod) Dublin. Tycooly International Publishing Ltd., Ireland.

Kovendan, K., K. Murugan and S. Vincent, 2012a. Evaluation of larvicidal activity of *Acalypha alnifolia* Klein ex Willd: (Euphorbiaceae) leaf extracts against the malarial vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti* and Bancroftian filariasis vector, *Culex quinquefasciatus* (Diptera; Culicidae). Parasitol. Res., 110: 571-581.

Kovendan, K., K. Murugan, A. Naresh Kumar, S. Vincent and J.S. Hwang, 2012b. Bioefficacy of larvicidal and pupicidal properties of *Carica papaya* (Caricaceae) leaf extract and bacterial insecticide, spinosad, against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). Parasitol. Res., 110: 669-678.

Lemma, A., 1970. Laboratory and field evaluation of the molluscicidal properties of *Phytolacca dodecandra*. Bull. Wld. Hlth. Org., 42: 597-612.

Lemma, A., G. Brody, G.W. Newell, R.M, Parkhurst and W.A. Skinner, 1972. Endod (*Phytolacca dodecandra*), a natural product molluscicide: Increased potency with butanol extraction. J. Parasitol., 58: 104-107.

Mesfin, F., S. Demissew and T. Tekelehaymanot, 2009. An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. J. Ethnobiol. Ethnomed., 5: 28.

Molgaard, P., A. Chihaka, E. Lemmich, P. Furu, C. Windberg, F. Ingerslev and B. Halling-Sorensen, 2000. Biodegradability of the molluscicidal saponin of *Phytolacca dodecandra*. Regul. Toxicol. Pharmacol., 32: 248-255.

Nalule, A.S., J.M. Mbaria, D. Olila and J.W. Kimenju, 2011. Ethnopharmacological Practices in Management of Livestock Helmithes by Pastoral Communities in the Dry Lands of Uganda. Livestock Research for Rural Development, 23, Article 36, Retrieved from: http://www.lrrd.org/lrrd23/2/nalu23036.htm, (Accessed on: February 25, 2012).

Ndamba, J., S.K. Chandiwana and N. Makaza, 1989. The use of *Phytolacca dodecandra* berries in the control of trematode-transmitting snails in Zimbabwe. Acta Tropica., 46(5-6): 303-309.

Schemelzer, H.H. and A. Gurib-Fakim, 2008. Plant resources of Tropical Africa 11(1)-Medicinal Plants 1. Backhuys Publishers, Wageningen, Netherlands, ISBN: 978-90-5782-204-9.

Spielman, A. and A. Lemma, 1973. Endod extract, plant derived molluscicide: Toxic for mosquitoes. Amer. J. Trop. Med. Hyg., 22: 802-804.

Stobaeus, J.K., G.E. Heath, R.M. Parkhurst, W.O. Jones and J.E. Webster, 1990. A laboratory study of the toxicity of the butanol extract of endod (*Phytolacca dodecandra*) on two species of freshwater fish and two species of aquatic snails. Vet. Hum. Toxicol., 32: 212-216.

WHO, 2005. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCPP/2005.