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Full Length Research Paper

Larvicidal activity of extracts from different parts of Neem (*Azadirachta indica*) against *Aedes Aegypti* mosquitoes' larvae

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Dengue is one of the major health problem in many countries. The genus *Aedes* in which *Aedes aegypti (*Family: Culicidae) mosquito belongs to, is the major vector of dengue fever disease. Search for larvicidal active compound(s) is one of the several attempts to find effective and affordable ways to control this mosquito. The aim of this study is to investigate the toxic effect of different solvent (acetone, chloroform, ethanol) extracts from different parts (bark, leaf, root, and seed) of Neem, genus *Azadirachta* of *Azadirachta indica* (Family: *Meliaceae*) against *A. aegypti* larvae. For the larvicidal bioassay, four concentrations (50, 100, 500, and 1000 ppm) of plant crude extracts were prepared; 1 mL of dimethyl sulfoxide (DMSO) was used to solubilize the extracts in water. In each solution was inserted 10 larvae (second and third instar). A set of control using 2.0% DMSO and untreated sets of larvae in (tap) water were also run for comparison. Data were evaluated and the LC_{50} and LC_{90} values were read. The larvicidal activities of the crude extracts were varied and the LC_{50} and LC_{90} values ranges from 50 to 837.5 ppm and 94 to 950 ppm, respectively. Assays showed that leaf acetone and root chloroform extracts were more toxic against larvae and causes 100% mortality at concentration of 1000 ppm in 24 h. Whereas, the rest extracts achieved 100% mortality at 1000 ppm in 48 h. Bioactive phytochemical classes, such as alkaloids and sesquiterpene lactones were screened by TLC; and the results obtained were negative for alkaloids and positive for sesquiterpene lactones. Therefore, the results obtained in this study shows the potential of the crude extracts of *A. indica* against *A. aegypti* larvae; and this may warrant further research to determine the bioactive compound(s).

Key words: Neem (*Azadirachta indica*), crude extract, larvicidal activity, *Aedes aegypti*, larvae.

INTRODUCTION

Mosquitoes transmit serious human diseases, causing millions of deaths every year (Kamaraj et al., 2011). *Aedes aegypti (*Family: Culicidae) mosquito which comes from the genus *Aedes* is the major vector of dengue fever disease. Recently, high dengue alert has been issued nationwide as well as in Malaysia, due to the total deaths of more than 103 people between January and August 2010 (Cruez, 2010). The key to mosquito control is larval management because larvae occur in specific areas and can be controlled by modification of habitat with insecticides. The insectides weakens the cuticle defence system of the larvae causing penetration of pathogenic organisms, thus reducing the mosquito population (Batabyal et al., 2007; Dua et al., 2009).

Neem plant belongs to the genus *Azadirachta* of which *Azadirachta indica* (Family: Meliaceae) and its derived products have shown insecticidal property (Obomanu et

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al., 2006). Azadirachtin, a biologically active compound in *A. indica* is an eco-friendly insecticide than synthetic insecticides that contribute in high cost and health effects (Dua et al., 2009; Mondali et al., 2009; Shanmugasundram et al., 2008). Previously, studies have been conducted to test the larvicidal potential of the Neem (*A. indica*) extracts against fourth instar larvae of *Culex quinquefasciatus* and it reports positive (Alouani et al., 2009; Chakkaravarthy et al., 2011). *A. indica* products containing limonoids have been tested against different type of mosquito species and it is one of the best alternatives for mosquito control (Gunasekaran et al., 2009; Khalafalla et al., 2007; Nathan et al., 2005). Gurulingappa et al. (2009) reported on a limonoid, gedunin from *A. indica* exhibited 100% at 50 and 10 ppm, when evaluated for its toxic actions against fourth instar larvae of *A. aegypti* L. and *Culex quinquefasciatus* Say. Dua et al. (2009) reported that the *A. indica* oil formulation is effective and shows 85.2 to 98.1% reduction of *A. aegypti* larvae on day 1 of post application and 99.7 to 100% reduction up to 7 days. By this, the widespread of dengue can be controlled. Therefore, the purpose of this study was to determine the bioactivity of different solvent extracts from different parts of *A. indica* against dengue mosquito *A. aegypti* larvae.

MATERIALS AND METHODS

Plant material source

Neem (*A. indica*) plants were obtained from Teluk Intan, Perak, Malaysia, on 2nd February, 2011. The taxonomy identification of plant were done by a Botanist of the School of Environmental Sciences and Natural Resources, Natural University of Malaysia, Bangi, Selangor. The parts were separated into barks, leaves, roots and seeds, and placed on empty papers in an open area of a room and left to dry for about 1 month. The dried samples (barks, leaves and roots) were ground into coarse powder by using electrical blender. The seed kernels were removed from the seeds and crushed using mortar and pestle, then dried in oven at 109°C for 3 min.

Preparation of crude extracts

The crude extracts of ground parts: barks (30 g), leaves (50 g), and roots (40 g) were extracted by using acetone, chloroform, refluxed and maceration in ethanol. The macerated extracts were filtered after 5 days and concentrated by evaporating the solvents through rotary evaporator and further drying under open air. The fixed oil (seed oil) was extracted from seeds of Neem according to AOCS (1993) official method No. Aa 4-38 (Angers et al., 1996). Extraction was continued for 6 h in a soxhlet extractor used n-hexane as solvent.

Thin layer chromatography method

Precoated silica gel plates (1 mm thick, coated on glass of 20 × 20 cm) were used. After chromatography development, residual solvents were evaporated from the plates at room temperature. Separated spots were visualized under, UV Lamp and sprayed with Dragendroff and Mayer's reagents for alkaloids screening, and also vanillin-sulfuric acid reagent and UV light for sesquiterpene lactones.

Preparation of test concentrations

Four test concentrations (50, 100, 500, and 1000 ppm) were prepared through single dilution method and stored in labeled specimen bottles for larvicidal bioassay. DMSO was used to solubilize the extract in water.

Source of *A. aegypti* larvae

The *A. aegypti* larvae, were obtained from the Insectariums of Medical Entomology Unit of Institute of Medical Research, Kuala Lumpur, Malaysia. The supplied larvae (2nd and 3rd instars) were free from infection of virus and insecticides. The larvae were reared under the temperature of 20 ± 1°C and relative moisture of 80 ± 5%, and supplied with half cooked bovine liver during rearing process; during test period no food was given to the larvae.

Larvicidal bioassays

Larvicidal bioassay method, proposed by Dua et al. (2009) was conducted with slight modification. A number of 10 actively swimming larvae (2nd and 3rd instars) were released into 25-mL capacity beakers containing 16 mL of each larvicide extracts solution of different parts. A set of control using 2.0% dimethyl sulfoxide (DMSO) as Control 1 and untreated sets of larvae in (tap) water as Control 2, were run for comparison. The beakers were stored at room temperature at about 29°C ± 2°C and at 12L:12D. Mortality of larvae was recorded after 1-, 3-, 6-, 9-, 24-, and 48-h of exposure and moribund larvae were counted as dead. Toxicity and activity, were reported as LC_{50} and LC_{90}, representing the concentrations in ppm that killed 50 and 90%, respectively of larvae in 24 and 48 h.

Statistical analysis

Mortality data were observed and corrected mortality obtained by applying Abbott's formula (Abbott, 1925). LC_{50} and LC_{90} confidence intervals, were determined by the probit analysis method as described by Finney (1971).

RESULTS

Larvicidal properties of extracts from different part of Neem (*A. indica*)

The crude extracts from different parts of Neem (*A. indica*) were extracted using three solvents and each of them are respectively prepared into four different concentrations; and the mortality of the *A. aegypti* larvae was observed. All extracts showed considerable larvicidal activity when tested against *A.Aegypti* larvae.

Larvicidal activity of bark crude extracts

The effects of the plant bark crude extracts of different solvents, were shown in Figure 1 (a) to (d) as acetone,

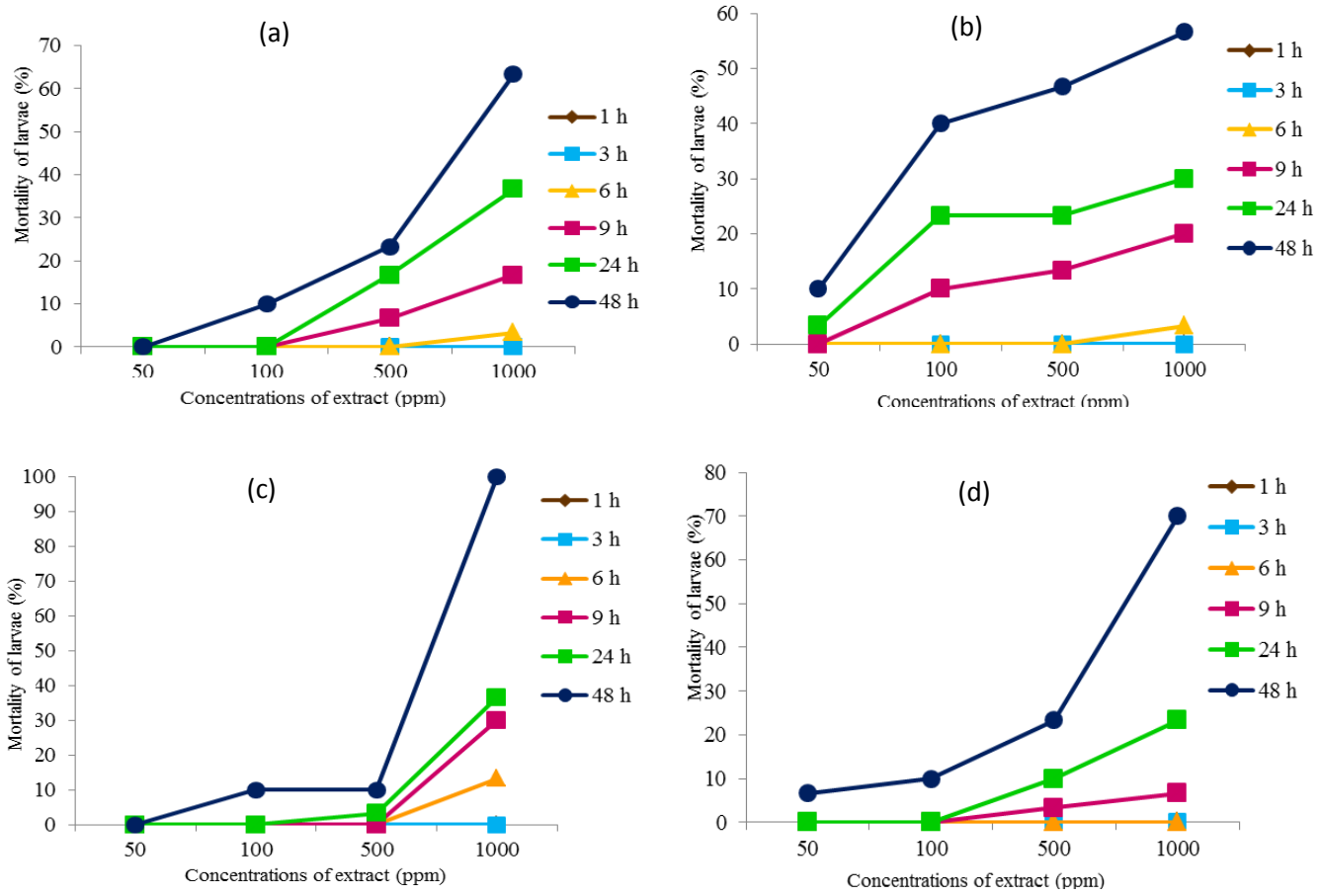

Figure 1. Larvicidal activity of the bark (a) acetone (b) chloroform (c) macerated in ethanol and (d) refluxed in ethanol extracts of Neem (*A. indica*) against *A. aegypti* mosquito larvae.

chloroform, macerated in ethanol, and refluxed in ethanol, respectively. At concentration of 1000 ppm after 48 h the macerated in ethanol extracts showed highest mortality (100%), followed by refluxed in ethanol extract (70%), acetone extract (63%) and chloroform extract (57%). The LC_{50} values were 725, 725, 787.5, and 837.5 ppm for the chloroform, refluxed in ethanol, macerated in ethanol and acetone extracts, respectively. On the other hand, the mortality of larvae in 24 h were, 46, 23, 23 and 10% for chloroform, acetone, refluxed in ethanol and macerated in ethanol extracts, respectively.

Larvicidal activity of leaf crude extracts

The larvicidal activity of different concentrations of leaf extracts were shown in Figure 2 (a) to (d) as acetone, chloroform, macerated in ethanol, and refluxed in ethanol, respectively. After 48 h and concentration of 1000 ppm all leaf crude extracts showed 100% mortality of larvae. Only leaf acetone achieved this mortality with 100 ppm; after 24 h the highest mortality was achieved by the acetone and chloroform extracts and reached 100% mortality at the test concentration of 1000 ppm; the mortality for both refluxed and macerated in ethanol was 80%. Both ethanol extracts (macerated and refluxed) could produce 100% mortality at the concentration of 500 ppm upon 48 h of exposure. The LC_{50} values after 48 h were 50, 70, and 72.5 ppm for the refluxed in ethanol, acetone and chloroform extracts, respectively. The LC_{90} values of 48 h were 94, 100, 320, and 400 ppm for the acetone, chloroform, macerated in ethanol and refluxed in ethanol extracts, respectively.

Larvicidal activity of root crude extracts

The rate of mortality of the root extracts were shown in Figure 3 (a) to (d) as acetone, chloroform, macerated in ethanol and refluxed in ethanol. After 24 h of larval exposure to the extracts, only root chloroform showed 100% mortality at the concentration level of 1000 ppm, followed by acetone (63%), refluxed in ethanol (26%) and macerated in ethanol (23%). After 48 h and at 1000 ppm

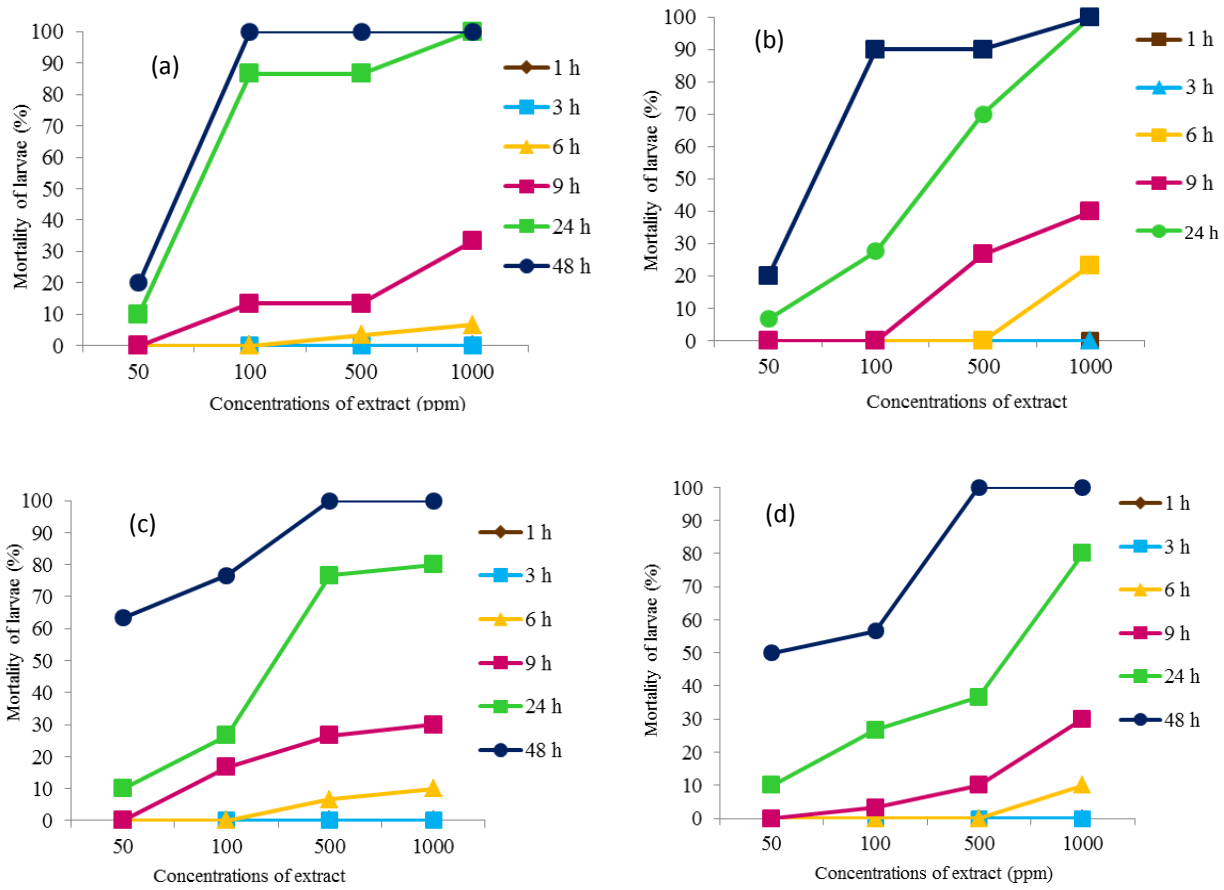

Figure 2. Larvicidal activity of leaf (a) acetone (b) chloroform (c) macerated in ethanol and (d) refluxed in ethanol extracts of Neem (*A. indica*) against *A. aegypti* mosquito larvae.

the mortality was 100, 93, 83, and 63% for chloroform, acetone, macerated in ethanol, and refluxed in ethanol respectively. The LC_{50} values of 48 h were 82.5, 375, 500, and 600 ppm for the acetone, chloroform, refluxed in ethanol, and macerated in ethanol respectively.

Larvicidal activity of seed oil

Figure 4 shows the larvicidal activity of fixed oil of *A. indica* against *A. aegypti* mosquito larvae. After 48 h of larval exposure to 500 and 1000 ppm of fixed oil, the mortality was 90 and 100% respectively. The LC_{50} and LC_{90} were 100 and 500 ppm, respectively. The highest mortality rate after 24 h larval exposure was 50 and 86% for 500 and 1000 ppm respectively.

Thin layer chromatography (TLC) screening for bioactive phytochemical classes

Thin layer chromatography (TLC) was performed for crude extracts of root, leaf and bark to screen for phytochemical classes such as alkaloids and sesquiterpene lactones. All tested crude extracts do not show any positive result towards alkaloids; whereas, the test for sesquiterpene lactones was positive.

DISCUSSION

The continuous use of various kinds of insecticides has increased mosquitoes' resistance. This action causes low insecticidal susceptibility in the mosquitoes and thus contributes to further development of their population. *Meliaceae* plant family of *A. indica* has been used as growth regulator against many insect pests. The crude extract of *A. indica* has been reported to be eco-friendly and non-toxic to vertebrates. The plant crude or partially-purified plant or botanicals extracts are less expensive and highly effective for the control of mosquitoes that contributes too many serious vector borne diseases rather than the purified compounds or extracts of the plant (Alouani et al., 2009; Khalafalla et al., 2007; Bagavan and Rahuman, 2011).

The experimental results obtained from this study

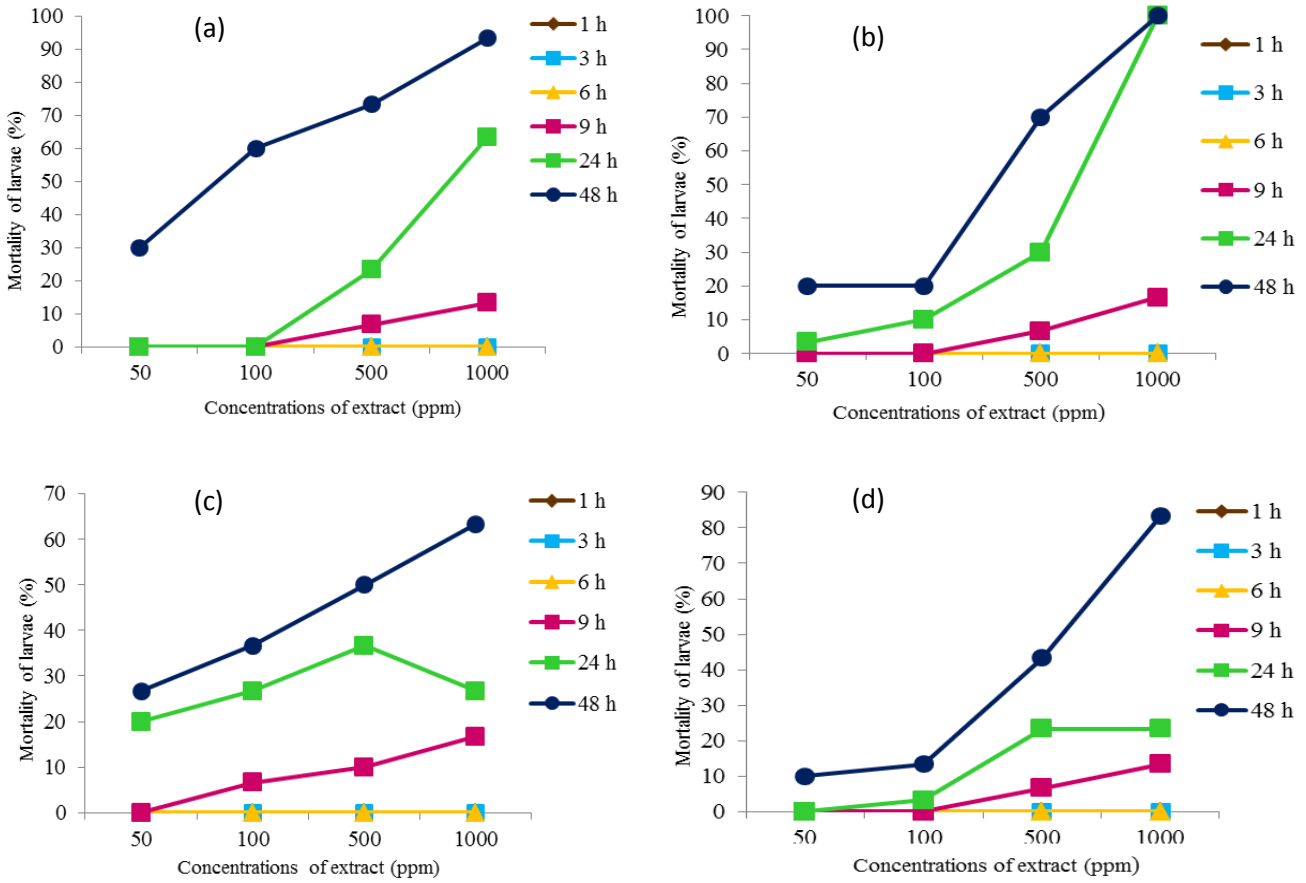

Figure 3. Larvicidal activity of root (a) acetone (b) chloroform (c) macerated in ethanol and (d) refluxed in ethanol extracts of Neem (*A. indica*) against *A. aegypti* mosquito larvae.

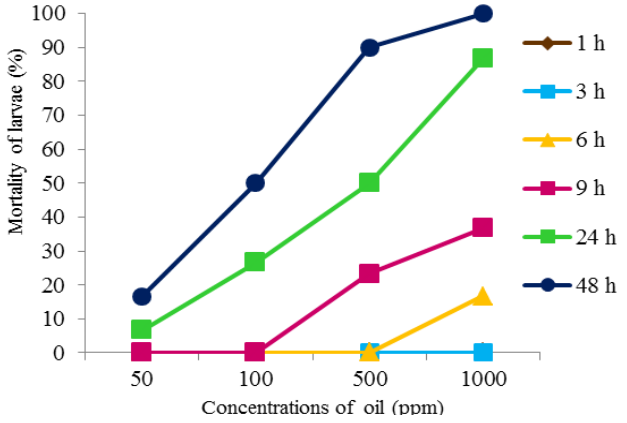

Figure 4. Larvicidal activity of fixed oil of Neem (*A. indica*) against *A. aegypti* mosquito larvae.

revealed that all *A. indica* extracts showed larvicidal activity against *A. aegypti* mosquito larvae. The larvicidal activity among extracts was extremely broad. The various biological activities of these plant extracts may be due to various phytochemical classes, existing in plant; these compounds may jointly or independently contribute to produce larvicidal activity.

Among extracts, leaf acetone and root chloroform extracts were more toxic and causes 100% mortality of larvae within 24 h at 1000 ppm (Figures 2a and 3b). Whereas, seed oil, bark macerated in ethanol, root acetone, leaf chloroform, leaf macerated and refluxed in ethanol extracts achieved 100% mortality of larvae after 48 h. Finally, in present study among the parts of *A. indica*, leaf extract was found to be most effective and thus have high larvicidal potency. The order of larvicidal potencies among plant parts were, leaf > root > seed > bark. Whereas, acetone was the best among the solvents that enables the extraction of larvicidal phytochemicals.

In previous study, the undiluted bark and leaf extract (crushed in a motor into watery paste) of *A. indica* produced 48 and 98% of mortality in 12 h (Gunasekaran et al., 2009). Authors reported that the chloroform extracts of *A. indica* leaf showed 12, 48.5, 56.5, 73 and 87% mortality at the concentration level of 62.5, 125, 250, 500 and 1000 ppm, when tested against the third instar larvae of *C. quinquefasciatus* (Say) (Diptera: *Culicidae*)

(El-Mahmood et al., 2010). Dua et al. (2009) showed that the Neem (*A. indica*) oil contributes towards reduction of *A. aegypti* larvae up to about 85.2 to 98.1% on day 1 of post application and 99.7 to 100% reduction of larvae up to 7 days at the dosage level of 0.5 to 5.0 ppm and the LC_{50} value for the oil formulation is 1.7 ppm, while the LC_{90} value was 3.8 ppm for 48 h. Aliero (2003) suggested that the 0.02% *A. indica* seed oil caused 100% mortality in *A. aegypti* and *C. quinquefasciatus*; whereas the chloroform extracts of *A. indica* showed larval mortality of 87% at 1000 ppm in *C. quinquefasciatus*.

Therefore, some of the obtained results differs from previous studies. This might be explained by the origin of the plants, types of solvents, method of extraction, formulation of the extracts and test material such as the larvae. Therefore, further study should be carried out to test the larvicidal potential against different type of mosquito species larvae with different formulation of extract and the dosage level.

Conclusion

This study demonstrates the potency of Neem (*A. indica*) in managing the larvae and thus contributes as an affordable way to control *A. aegypti* mosquito. Through this study the crude extracts from different parts of *A. indica* were extracted by different solvents. The extracts showed different larvicidal properties. This is explained by the different solvents properties, such as polarity that enables them to extract different type of compound(s), and variety of compounds that results in different larvicidal properties. Acetone was found to be the best among various tested solvents. The order of larvicidal potency among all the parts were leaf > root > seed > bark. Further study is needed for isolation and identification of bioactive compounds by different separation methods (such as column chromatography, TLC, and HPLC) and for identification methods using spectroscopy which includes UV, IR, MS and NMR.

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