Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors

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ARTICLE INFO

Article history:
Received 19 October 2010
Received in revised form 27 October 2010
Accepted 15 December 2010
Available online 20 January 2011

Keywords:
Anopheles vagus
Armigeres subalbatus
Culex vishnui
Plant extracts
Larvicidal activity

ABSTRACT

Objective: To evaluate the mosquito larvicidal activity of plant extracts. Methods: The hexane, chloroform, ethyl acetate, acetone, and methanol leaf, flower and seed extracts of Abrus precatorius (A. precatorius), Croton bonplandianum (C. bonplandianum), Cynodon dactylon (C. dactylon), Musa paradisiaca (M. paradisiaca) and Syzygium aromaticum (S. aromaticum) were tested against fourth instar larvae of Anopheles vagus (An. vagus), Armigeres subalbatus (Ar. subalbatus) and Culex vishnui (Cx. vishnui). Results: The highest larval mortality was found in seed ethyl acetate extracts of A. precatorius and leaf extracts of C. bonplandianum, flower chloroform and methanol extracts of M. paradisiaca, and flower bud hexane extract of S. aromaticum against An. vagus with LC50 values of 19.31, 39.96, 35.18, 79.90 and 85.90 μg/mL; leaf ethyl acetate and methanol extracts of C. dactylon, flower methanol extract of M. paradisiaca, flower bud methanol extract of S. aromaticum against Ar. subalbatus with LC50 values of 21.67, 32.62, 48.90 and 78.28 μg/mL, and seed methanol of A. precatorius, flower methanol extract of M. paradisiaca, flower bud hexane extract of S. aromaticum against Cx. vishnui with LC50 values of 136.84, 103.36 and 149.56 μg/mL, respectively. Conclusions: These results suggest that the effective plant crude extracts have the potential to be used as an ideal ecofriendly approach for the control of disease vectors. This study provides the first report on the larvicidal activity of crude solvent extracts of different mosquitoes.

1. Introduction

Mosquitoes are responsible for the spread of more diseases than any other group of arthropods. Mosquito-borne diseases still remain a major health problem in both human and veterinary sectors. Diseases transmitted by mosquitoes include malaria, dengue hemorrhagic fever, Japanese encephalitis, yellow fever and filariasis. One to two million deaths are reported annually due to malaria worldwide. Lymphatic filariasis affects at least 120 million people in 73 countries in Africa, India, Southeast Asia, and Pacific Islands. These diseases not only cause high levels of morbidity and mortality but also inflict great economic loss and social disruption on developing countries such as India, China, etc. India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about 720 crores[1]. The annual incidence and mortality estimates for Japanese encephalitis (JE) are 30 000–50 000 and 10000, respectively[2]. The use of conventional pesticides in the water sources, however, introduces many risks to people and/or the environment. Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors[3]. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. Many researchers have reported on the effectiveness of plant extracts against mosquito larvae[4,5].

Anopheles vagus (An. vagus) is a zoophilic species, which was more often collected in human–landing captures during the rainy season. The rainy season may be important in malaria transmission due to high biting populations[6,7]. A vector species that employs a unique, robust immune response against an invading pathogen is the Armigeres subalbatus (Ar. subalbatus), a natural vector of the nematode parasites that cause lymphatic filariasis. It also serves as a
of considerable medicinal importance \[13, 14\]. The bioassay extracts of \emph{Croton bonplandianum} (C. bonplandianum) have been conducted around the world. The larvicidal efficacy of the extracts from the seed of \emph{Croton bonplandianum} against three medically important species of \emph{JE} virus activity in endemic areas of Tamil Nadu, southern India \[12\].

Many studies on plant extracts against mosquito larvae have been conducted around the world. \emph{Abras precatorius} (A. precatorius) locally known as “Kundu mani,” is a slender woody vine plant.

\emph{Croton bonplandianum} (C. bonplandianum) is a plant of considerable medicinal importance \[13, 14\]. The bioassay results showed that the essential oil of \emph{Croton regelianus} (C. regelianus) and the isolated compound strongly effective against \emph{Aedes aegypti} (A. aegypti) \[15\], and the essential oil obtained from stalks and leaves of \emph{Croton arborescens} (C. arborescens), \emph{Croton nepetaefolius} (C. nepetaefolius), \emph{Croton sonderianus} (C. sonderianus) and \emph{Croton zehntneri} (C. zehntneri) were evaluated against \emph{Aedes aegypti} (A. aegypti) larvae \[16\]. \emph{Cydonia dactylon} (C. dactylon) infusions was effective attractant to ovipositing female mosquitoes of \emph{Culex quinquefasciatus} (Cx. quinquefasciatus), \emph{Culex nigripalpus} (Cx. nigripalpus), and \emph{Culex erraticus} (Cx. Erraticus) \[17\].

\emph{Musa paradisiaca} (M. paradisiaca) (banana) is a perennial tree like herb widely distributed in moist tropics. To enriched food value and versatile medicinal value, banana is one of the most important fruits and the vegetable crops of India. The leaves methanol and 95% ethanol extracts of \emph{M. paradisiaca} were tested against the III instar larvae of \emph{Anopheles stephensi} (An. stephensi) and L4 larvae of \emph{Ae. aegypti}, respectively \[18,19\]. \emph{Syzygium aromaticum} (S. aromaticum) (clove) undiluted oils was the most effective and provided 2 h of complete repellency against \emph{Cx. quinquefasciatus} and \emph{Anopheles dirus} (An. dirus) \[20\] and the methanol and ether extracts showed complete inhibition of adult emergence at 200 and 600 ppm, respectively against \emph{Culex pipiens} (Cx. pipiens) \[21\].

In the light of earlier literature, it is known that larvicides play a vital role in controlling mosquitoes in their breeding sites, but still vectors resistance to them remains unanswered. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess in the present communication, an attempt has been made to evaluate the larvicidal efficacy of the extracts from the medicinal plant against three medically important species of \emph{Anopheles vagus} (An. vagus), \emph{Armigeres subalbatus} (Ar. subalbatus) and \emph{Cx. vishnui}.

2. Materials and methods

2.1. Collection of plant materials

The seed of \emph{A. precatorius} L. (Fabaceae), leaf of \emph{C. bonplandianum} Baill. (Euphorbiaceae), \emph{C. dactylon} L. (Poaceae), flower of \emph{M. paradisiaca} L. (Musaceae), \emph{S. aromaticum} L. Merr. et Perry (Myrtaceae) were collected from Javadhu Hills, Tiruvannamalai district and Nilgiri Mountain, Nilgiri district, Tamil Nadu, South India in February 2010. The plants were authenticated by Dr. C. Hema, Department of Botany, Arignar Anna Govt. Arts College for Women, Vellore, India. Voucher specimens have been deposited in the laboratory of Zoology, C. Abdul Hakeem College, Melvisharam.

2.2. Preparation of plant extracts

The dried (7–15 days in the shade at the environmental temperatures, 27–37 °C day time) leaf (500 g), flower (400 g) and seed (550 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with hexane (1500 mL, Fine), chloroform (2 000 mL, Fine), ethyl acetate (2 800 mL, Qualigens), acetone (1 800 mL, Qualigens) and methanol (4 000 mL, Qualigens) in a soxhlet apparatus (boiling point range 60–80 °C) for 8 h. The extract was concentrated under reduced pressure of 22–26 mmHg at 45 °C and the residue obtained was stored at 4 °C. One gram of crude extract was first dissolved in 100 mL of acetone (stock solution). From the stock solution, 1 000 – 3.125 μg/mL were prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05 % in the final test solution. The control was set up with acetone, dechlorinated tap water and polysorbate 80.

2.3. Mosquito culture

\emph{An. vagus} larvae were collected from the rice fields, open muddy pools and ditches, \emph{Ar. subalbatus} breeding in open and uncovered septic tanks and \emph{Cx. vishnui} larvae were collected from rice fields stagnant water area of Melvisharam and identified Dr. V. Rajagopal, Senior Entomologist, Zonal Entomological Research Centre, Vellore, Tamil Nadu. To start the colony, the larvae were kept in plastic and enamel trays containing tap water. They were maintained at (27 ±2) °C and 75%–85% relative humidity under 14:10 light and dark cycles. Larvae were fed a diet of Brewers yeast, dog biscuits and algae collected from ponds in a ratio of 3:1:1, respectively. Pupae were transferred from the trays to a cup containing tap water and were maintained in our insectary [(45×45×40) cm] where adults emerged. Adults were maintained in glass cages and continuously provided with 10% sucrose solution in a jar with a cotton wick. On day five, the adults were given a blood meal from a pigeon. Glass petridishes with 50 mL of tap water lined with filter paper were kept inside the cage for oviposition \[22\].

2.4. Larvicidal bioassay

During screening with the laboratory trial, the fourth instar larvae of \emph{An. vagus}, \emph{Ar. subalbatus} and \emph{Cx. vishnui} were collected from the insect rearing cage and identified in Zonal Entomological Research Centre, Vellore. One gram of crude extract was first dissolved in 100 mL of acetone (stock solution). From the stock solution, 1 000 μg/mL was prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. The larvicidal activity was assessed by the procedure of WHO \[23\] with some
From the stock solution, different concentrations ranging from 3.125 to 1 000 μg/mL were prepared. Based on the screening results, crude different solvent leaf, flower and seed extracts of *A. precatorius*, *C. bonplandianum*, *C. dactylon*, *M. paradisiaca*, and *S. aromaticum* were subjected to dose response bioassay for larvicidal activity against the larvae of *An. vagus*, *Ar. subalbatus* and *Cx. vishnui*. The numbers of dead larvae were counted after 24 h of exposure and the per cent mortality was reported from the average of five replicates. However, at the end of 24 h the selected test samples turned out to be equal in their toxic potential.

2.5. Dose – response bioassay

From the stock solution, different concentrations ranging from 3.125 to 1 000 μg/mL were prepared. Based on the screening results, crude different solvent leaf, flower and seed extracts of *A. precatorius*, *C. bonplandianum*, *C. dactylon*, *M. paradisiaca*, and *S. aromaticum* were subjected to dose response bioassay for larvicidal activity against the larvae of *An. vagus*, *Ar. subalbatus* and *Cx. vishnui*. The numbers of dead larvae were counted after 24 h of exposure and the per cent mortality was reported from the average of five replicates. However, at the end of 24 h the selected test samples turned out to be equal in their toxic potential.

### 3. Results

The preliminary screening is a good mean of evaluation of the potential larvicidal activity of plants popularly used for this purpose. The effect of the flower and seed hexane, chloroform, ethyl acetate, acetone and methanol extracts of *A. precatorius*, *C. bonplandianum*, *C. dactylon*, *M. paradisiaca*, and *S. aromaticum* were tested at 1 000 μg/mL and showed activity against the fourth instar larvae of *An. vagus*, *Ar. subalbatus* and *Cx. vishnui* (Table 1). All plant extracts showed moderate larvicidal effects after 24 h; however, the highest larval mortality was found in seed ethyl acetate, acetone and methanol extracts of *A. precatorius*, leaf ethyl acetate extract of *C. bonplandianum*, leaf ethyl acetate and methanol extracts of *C. dactylon*, flower chloroform, acetone and methanol of *M. paradisiaca* and flower bud hexane extracts of *S. aromaticum* against the fourth instar larvae of *An. vagus* (LC50 = 19.31, 191.58, 90.69, 39.96, 68.12, 41.13, 35.18, 119.33, 79.90 and 85.90 μg/mL; LC90 = 71.71, 843.10, 372.15, 218.14, 241.46, 183.83, 311.55, 748.50, 381.93 and 408.89 μg/mL), seed ethyl acetate, acetone and methanol of *A. precatorius*, leaf ethyl acetate and methanol of *C. dactylon*, flower chloroform, acetone and methanol of *M. paradisiaca*, flower bud hexane, ethyl acetate and methanol extracts of *S. aromaticum* against the fourth instar larvae of *Ar. subalbatus* (LC50 = 93.94, 99.19, 88.72, 21.67, 32.62, 89.42, 167.19, 48.90, 151.53, 48.90 and 78.28 μg/mL; LC90 = 413.27, 486.35, 662.61, 98.34, 267.21, 327.30, 1071.41, 187.82, 519.71, 187.82 and 641.32 μg/mL), seed methanol of *A. precatorius*, flower methanol of *M. paradisiaca*, flower bud hexane extract of *S. aromaticum* against the fourth instar larvae of *Cx. vishnui* (LC50 = 136.84, 103.36 and 149.56 μg/mL; LC90 = 1248.23, 340.44 and 995.56 μg/mL), respectively. Chi–square value was significant at P < 0.05 level (Table 2).

### 4. Discussion

The vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either counter measures or development of newer insecticides[25]. It is evident from our results that a rise in the concentration of plant extracts was the main cause of mortality in *An. vagus*, *Ar. subalbatus* and *Cx. vishnui* larvae. Similar study was conducted by Mathew et al.[26] and reported that the seed methanol extract of *Clitoria ternatea* (C. ternatea) was effective against the larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* with LC50 values of 65.2, 154.5, and 54.4 ppm, respectively. The leaf extract of *Cassia obtusifolia* (C. obtusifolia) had significant larvicidal effect with LC50 and LC90 values were

### Table 1

| Botanical name/ Family/ Vernacular names | Parts used | Species | Hexane | Chloroform | Ethyl acetate | Acetone | Methanol |
|----------------------------------------|------------|---------|--------|------------|--------------|---------|----------|
| *A. precatorius* L. / Fabaceae / Seed  |            | *An. vagus* | 71.000 ± 3.115 | 72.000 ± 3.912 | 100.000 ± 0.000 | 100.000 ± 0.000 | 100.000 ± 0.000 |
| *Kunda mani*                           |            | *Ar. subalbatus* | 87.000 ± 1.140 | 78.000 ± 2.325 | 100.000 ± 0.000 | 100.000 ± 0.000 | 100.000 ± 0.000 |
| *C. bonplandianum* Baill. / Leaf       |            | *Cv. vishnui* | 82.000 ± 2.191 | 85.000 ± 1.581 | 81.000 ± 1.923 | 89.000 ± 1.302 | 100.000 ± 0.000 |
| *Euphorbiaceae / Reilpoondu*           |            | *Ar. subalbatus* | 62.000 ± 2.170 | 84.000 ± 1.483 | 100.000 ± 0.000 | 53.000 ± 3.240 | 41.000 ± 2.588 |
| *C. dactylon* L. / Poaceae / Leaf      |            | *Cv. vishnui* | 39.000 ± 1.164 | 59.000 ± 1.788 | 61.000 ± 2.735 | 68.000 ± 1.140 | 61.000 ± 1.788 |
| *Arugampallu*                          |            | *Ar. subalbatus* | 79.000 ± 2.407 | 86.000 ± 1.643 | 90.000 ± 1.100 | 89.000 ± 1.095 | 85.000 ± 1.581 |
| *M. paradisiaca* L. / Musaceae / Flower | Vazhai    | *An. vagus* | 73.000 ± 2.408 | 75.000 ± 2.549 | 100.000 ± 0.000 | 86.000 ± 1.923 | 100.000 ± 0.000 |
|                                        |            | *Ar. subalbatus* | 84.000 ± 1.788 | 80.000 ± 2.236 | 100.000 ± 0.000 | 89.000 ± 1.302 | 100.000 ± 0.000 |
| *S. aromaticum* L. Merr. et Perry / Myrtaceae / Lavangam / Bud |            | *Cv. vishnui* | 85.000 ± 2.435 | 71.000 ± 1.924 | 73.000 ± 2.966 | 75.000 ± 1.581 | 92.000 ± 1.140 |

Control – Nil mortality, * Mean value of five replicates.
The larvicidal activities of different crude solvent extracts of acetone, chloroform, ethyl acetate and methanol extracts of Annona squamosa (A. squamosa), methanol extract of Centella asiatica (C. asiatica), acetone and methanol extracts of Gloriosa superba (G. superba) against the larvae of A. stephensi showed LC50 values of 17.47, 76.04, 18.60, 119.93, 26.62, 18.43, 64.87, 34.06, 13.63, and 50.39 ppm) and acetone, chloroform, ethyl acetate and methanol extracts of A. squamosa, acetone and methanol extracts of G. superba, chloroform, ethyl acetate, methanol extract of P. daemia, ethyl acetate and methanol extracts of P. emblica against Cx. tritaeniorhynchus (LC50 = 106.41, 63.81, 60.01, 78.21, 87.25, 92.43, 124.73, 31.94, 76.64, 69.09, and 54.82 ppm), respectively[34].

Karunamoorthi and Ilango[35] have reported that the LC50 and LC90 values of methanol leaf extracts of Croton macrostachyus (C. macrostachyus) were 89.25 and 224.98 ppm, respectively against late third instar larvae of malaria vector, Anopheles arabiensis (An. arabiensis). Bagavan et al[36] have reported that peel chloroform extract of Citrus sinensis (C. sinensis), leaf ethyl acetate extracts of Ocimum camun (O. camun), and Ocimum sanctum (O. sanctum) and leaf chloroform extract of Rhinacanthus nasutus (R. nasutus) against the larvae of An. subpictus (LC50 = 58.25, 88.15, 21.67 and 40.46 ppm) and peel methanol extract of C. sinensis, leaf methanol extract of O. camun, ethyl acetate extracts of O. sanctum and R. nasutus against the larvae of Cx. tritaeniorhynchus (LC50 = 38.15, 72.40, 109.12 and 39.32 ppm), respectively.

Table 2

| Botanical name | Solvents  | Species | LC50±SE (μg/mL) | LCL – UCL | LC90±SE (μg/mL) | LCL – UCL | χ² (df = 4) |
|---------------|-----------|---------|-----------------|-----------|-----------------|-----------|-------------|
| A. precatorius | Ethyl acetate | An. vagus | 19.31±1.30(21.86-16.77) | 71.71±7.43(86.28-57.15) | 71.71±7.43(86.28-57.15) | 7.24 |
| Acetone | An. vagus | 93.94±6.43(106.55-81.34) | 413.27±46.94(505.27-321.26) | 16.52 |
| Methanol | An. vagus | 99.20±7.70(114.29-84.10) | 486.35±59.14(602.26-370.44) | 5.39 |
| C. bonplandianum | Ethyl acetate | An. vagus | 119.33±9.41(137.78-100.89) | 748.50±107.35(958.91-538.09) | 14.56 |
| C. dactylon | Ethyl acetate | An. vagus | 68.12±4.25(76.46-59.80) | 241.46±24.79(290.01-192.88) | 12.38 |
| M. paradisiaca | Chloroform | An. vagus | 35.18±3.19(41.43-28.93) | 311.55±57.94(450.49-207.99) | 4.04 |
| S. aromaticum | Hexane | An. vagus | 35.18±3.19(41.43-28.93) | 311.55±57.94(450.49-207.99) | 4.04 |
| Ether acetate | An. vagus | 90.6±4.35(96.43-84.78) | 362.61±45.92(432.20-292.59) | 8.87 |
| Ethyl acetate | An. vagus | 32.6±2.86(38.20-33.32) | 662.61±45.92(722.77-452.45) | 4.19 |
| Ethyl acetate | Ar. subalbatus | Cx. vishnui | 136.84±12.98(162.28-111.39) | 241.46±24.79(290.01-192.88) | 12.38 |
| Methanol | An. vagus | 41.3±2.83(46.68-35.59) | 1383.2±217.33(224.61-141.24) | 11.7 |
| Methanol | Cx. vishnui | 36.2±2.86(38.20-33.32) | 662.61±45.92(722.77-452.45) | 4.19 |

Control – Nil mortality, LC50 – Lethal concentration that kills 50% of the exposed parasite, LC90 – Lethal concentration that kills 90% of the exposed parasite, UCL = upper confidence Limit, LCL = lower confidence Limit, χ² – Chi-square, df – degree of freedom. Significant at P<0.05 level.
respectively. The highest larval mortality was found in the leaf ethyl acetate of Aegle marmelos (A. marmelos), Eclipta prostrata (E. prostrata), hexane and methanol extracts of Andrographis paniculata (A. paniculata), and Cocculus hirsutus (C. hirsutus) with LC₅₀ values of 167.00, 78.28, 67.24, and 142.83 ppm, respectively[37]. The LC₅₀ values for carbon tetrachloride, methanol and petroleum ether extracts of Euphorbia hirta (E. hirta) against the larvae of An. stephensi were 11 063.00, 19 280.00 and 9693.90 ppm after 24 h, and the same extracts showed LC₅₀ values of 10 922.00, 18 476.00 and 7 752.80 ppm after 48 h, respectively[38]. Batabyal et al.[39] reported that the carbon tetrachloride extract of Ricinus communis (R. communis) with LC₅₀ at 144.11 and 92.44 ppm and LC₉₀ at 432.42 and 352.89 ppm after 24 and 48 h, respectively against the larvae of filarial vector, Cx. quinquefasciatus. Singh et al.[40] have reported that the LC₅₀ values of hexane extract obtained from leaves of Eucalyptus citriodora (E. citriodora) against 11th instar larvae of An. stephensi, Cx. quinquefasciatus and Ae. aegypti were 69.86, 81.12 and 91.76 ppm, respectively against 24 h, and 26.7, 29.9 and 38.8 ppm respectively after 72 h. The extracts of Myrtus communis (M. communis) were found to be the most toxic with LC₅₀ values of 16 mg litre⁻¹ against fourth—instar larvae of Culex pipiens molestus (Cx. pipiens molestus)[41].

The LC₅₀ value of petroleum ether extracts of Jatropha curcas (J. curcas), Pedilanthus tithymaloides (P. tithymaloides), Phyllanthus amarus (P. amarus), E. hirta and Euphorbia tirucalli (E. tirucalli) were 8.97, 55.26, 90.92, 272.36, and 4.25 ppm, respectively, against Ae. aegypti and 11.34, 76.61, 113.40, 424.94, and 5.52 ppm, respectively, against Cx. quinquefasciatus[42]. This has been observed earlier by Kamaraj et al.[22] that the highest larval mortality was found in leaf petroleum ether, flower methanol extracts of Cassia auriculata (C. auriculata), flower methanol extracts of Luecas aspera (L. aspera) and R. nasutus, leaf and seed methanol extracts of Solanum torvum (S. torvum) and leaf hexane extract of Vitex negundo (V. negundo) against the larvae of Anopheles subpictus (An. subpictus) (LC₅₀ = 44.21, 44.69, 53.16, 41.07, 35.32, 28.90 and 44.40 ppm) and against the larvae of Cx. tritaeniorynchus (LC₅₀ = 69.83, 51.29, 81.24, 71.79, 44.42, 84.47 and 65.35 ppm), respectively. The bioassay—guided fractionation of Achyranthes aspera (A. aspera) led to the separation and identification of a saponin as a potential mosquito larvicidal compound with LC₅₀ value of 18.20 and 27.24 ppm against Ae. aegypti and Cx. quinquefasciatus, respectively[43].

Kamaraj et al.[44] have reported that the peel methanol extract of C. sinensis, leaf and flower ethyl acetate extracts of O. canum against the larvae of An. stephensi (LC₅₀ = 95.74, 101.53, 28.96; LC₉₀ = 303.20, 492.43 and 168.05 ppm), respectively. The highest larval mortality was found in methanol extract of O. canum, R. nasutus and acetone extract of O. sanctum against the larvae of Ae. aegypti (LC₅₀ = 99.42, 94.43 and 81.56 ppm) and against Cx. quinquefasciatus (LC₅₀ = 44.54, 73.40 and 38.30 ppm), respectively[45]. The R. communis seed extract exhibited larvicidal effects with 100% mortality at the concentrations of 32–64 μg/mL, and showed LC₅₀ values 7.10, 11.64 and 16.84 μg/mL against the larvae of Cx. quinquefasciatus, An. stephensi and Aedes albopictus (Ae. albopictus), respectively[46].

In conclusion, an attempt has been made to evaluate the larvicidal activity of plant extracts against An. vagus, Ar. subalbatus and Cx. vishnui. The results reported here open the possibility of further investigations of efficacy on their larvicidal properties of natural product extracts. The isolation and purification of crude and seed methanol of A. precatorius, flower methanol of M. paradisiaca, flower bud hexane extracts of S. aromaticum are in progress.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to C. Abdul Hakeem College Management, Dr. S. Mohammed Yousuff, Principal, Dr. K. Abdul Subhan, HOD of Zoology Department for their help and suggestion.

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