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Larvicidal potential of selected indigenous lichens against three mosquito species–*Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi*

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ABSTRACT

Objective: Mosquitoes are the major transmitting vectors of serious human diseases, causing millions of deaths every year with undesirable effects, including toxicity to non-target organisms. Some plants with insecticidal properties have been used in recent years for the control of a variety of pest insects and vectors. In the quest for alternative natural biological control agents against mosquito larvae lichens were selected.

Method: Larvicidal activity was assessed with methanolic extracts of *Parmotrema reticulatum, Parmotrema kamatti, Parmotrema tinctorum, Parmelia erumpens, Leptogium papilosum*, and *Roccella montagnei* against *Aedes aegypti, Anopheles stephensi*, and *Culex quinquefasciatus*. The standard WHO protocols with minor modifications were adopted and the bioassay was evaluated at the concentrations of 100–500 µg/mL for each lichen. Since all the lichen extracts showed complete mortality against *C. quinquefasciatus* in 100 µg/mL, the concentrations were decreased to 100, 50, 25, 12.5, and 6.25 µg/mL for *C. quinquefasciatus*. Larval mortality was observed for 24 h after treatment.

Results: All the lichen extracts exhibited activity against third instar larvae of *A. aegypti* and *A. stephensi* at 100 µg/mL, and 100% mortality was observed against the vector *C. quinquefasciatus* at 100 µg/mL. The highest larvicidal activity was found with *L. papilosum* against *A. aegypti* (LC_{50} = 81.127 µg/mL) and *A. stephensi* (LC_{50} = 89.10 µg/mL). Similarly, *P. tinctorum* and *R. montagnei* when tested against *C. quinquefasciatus* with minimum concentration <100 µg/mL exhibited significant activity with LC_{50} values of 5.32 and 6.97 µg/mL.

Conclusion: The bioassay results revealed larvicidal potential of lichens especially against *C. quinquefasciatus* with high mortality even at lower concentration. Hence, lichens can be used as an ideal sustainable approach for the control of lymphatic filariasis caused by vector *C. quinquefasciatus*.

1. Introduction

One of the most dangerous creature mosquitoes in the world are referred to as “flying syringes”, which can transmit more diseases than any other groups of arthropods. These tiny creatures have the potential lethal capacity to affect and kill millions of people throughout the world. Mosquito like *Aedesaegypti, Anopheles stephensi*, and *Culexquinquefasciatus* are vectors for the pathogens of various diseases like dengue, chikungunya, yellow fever, malaria, filariasis, and Japanese encephalitis. More than 700 million people suffering from these diseases were annually died (Taubes, 1997). Mosquito borne diseases affect major commercial income and labour outputs mainly in tropical and subtropical countries but other parts of the world is still not free from vector-borne diseases (Fradin & Day, 2002).

Yellow fever and chikungunya caused by *A. aegypti* vector are widely distributed in tropical, subtropical zones (Hales, Wet, Maindonald, & Woodward, 2002), and India (Taubitz et al., 2007), where *C. quinquefasciatus* causing lymphatic filariasis, which is extensively dispersed in tropical zones affecting 120 million people worldwide with 44 million people having similar chronic manifestation (Govindarajan, Rajeswary, Hoti, & Benelli, 2015).

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Vector control is by far the most successful method for reducing incidences of mosquito-borne diseases (Lima, Maia, Sousa, Morais, & Freitas, 2006). Chemical pesticide is the most effective way against mosquitoes. In the context of increasing trend to use more powerful synthetic insecticides to achieve immediate results in the control of mosquitoes, which ultimately has developed physiological resistance by the vectors and its increased toxicity to non-target organism are noteworthy (Liu et al., 1997).

However, high cost of synthetic pyrethroids, environment and food safety concerns, unacceptability and toxicity of many organophosphates and organochlorines, and a global increase in insecticidal resistance have been argued for stimulating research towards the development of potential insecticides of botanic origin (Luize et al., 2003; MacNeil, Sumba, Lutzke, Moormann, & Rochford, 2003). Thus, the Environmental Protection Act in 1969 has framed a number of rules and regulations to check the application of chemical control agents in nature (Mansour, Messeha, & el-Gengaihi, 2000). Hence, an alarming surge of physiological resistance in the vectors has led to search for the new tools that could be a specific and ecofriendly target. This surge has laid a way for botanicals as naturally occurring insecticides.

The use of biological products is one of the best alternatives for the mosquito control and many plant products have been tried in earlier days before the discovery of chemical pesticides. Hence, the search for biological preparations and pure compounds that do not produce adverse effects in the non-targeted organisms, along with the benign environmental characteristics, remain a top priority research for the scientists, associated with the development of alternative vector control measures (Meshram, Kulkarni, & Joshi, 1996; Mohan, Sharma, & Srivastava, 2005).

Lichens are stable, ecologically obligate, and self-supporting composite organisms, comprised of a fungal and an algal partner. Lichens have worldwide distribution from artic to tropical regions and also from plains to mountains. Lichens have been used as food and folk medicine in many countries over a considerable period of time. Lichens have been used all over the world as medicine and the secondary metabolites, and have a variety of biological activities like anti-oxidant, antibiotic, antiviral, antimycobacterial, antimutagenic, antitumoral, analgesic, antitermite, cytotoxic, insecticidal, antimicrobial, antiherbivore, antiprotozoal, enzyme inhibitory, wound healing, anti-inflammatory, and antipyretic properties (Boustie, Tomasi, & Grube, 2011; Vivek, Yashoda, Manasa, Prashith, & Vinayaka, 2014).

2. Materials and methods

2.1. Lichen collection, identification, and authentication

The *Parmotrema reticulatum* (*Pr*), *P. kamatti* (*Pk*), *P. tinctorum* (*Pt*), *Parmelia erumpens* (*Pe*), *Leptogium papilosum* (*Lp*), and *Rocella montagnei* (*Rm*) samples were collected during February to March 2017 from Yercaud hills at an altitude of 1300 MSL. Identification of these lichens was done by morphological, anatomical, and chemical tests. Finally the lichen samples were authenticated and the specimen samples are kept in the Herbarium of Indian Lichenological Society, Lichenology Laboratory Plant Diversity, CSIR-National Botanical Research Institute (Govt. of India), Lucknow, U.P., India and K.S.Rangasamy College of Technology lichen herbarium, Tiruchengode, Namakkal District, TN.The lichens are shade dried to remove the moisture content and were stored for the further use.

2.2. Extraction

Twenty−five grams of powdered lichen samples were taken separately and mixed with 500 mL of methanol and then magnetically stirred in a separate container for overnight at room temperature. The residue was removed by filtration. The filtrate was concentrated under reduced pressure in a rotary evaporator at (60 ± 10) °C to yield up to 10% of crude extract and the resultant extract was used for further studies (Syed Zameer Ahmed, Sidhra, Ponmurugan, & Senthil Kumar, 2016).

2.3. Mosquito larvae

The third instar larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* colonies were obtained from Centre for Research in Medical Entomology, Madurai. The larvae were fed with powdered dog biscuit and yeast in the ratio 3:1 and maintained at (27 ± 2) °C and (80 ± 2)% RH with a photoperiod of 14:10-h light and dark cycles (Sharma & Saxena, 1994).

2.4. Larvicidal bioassay

One gram of crude extract of lichens was first dissolved in 100 mL of methanol (stock solution). From the stock solution, different concentrations ranging from 100 to 500 µg/mL were prepared with tap water. Experiments were conducted for 24 h at room temperature (28 ± 2) °C. The larvicidal activity was assessed by the procedure of the WHO (1996) with some modifications as stated by Mohan Roopan et al. (2013). For bioassay test, larvae were taken in five batches of 20 in 249 mL of water and 1.0 mL of the desired lichen extract concentration. The control was set up with methanol and distilled water. The mortality of larvae were calculated after 24 h of exposure and the percentage mortality was reported from the average of five replicates.

2.5. Statistical analysis

The average larval mortality data were taken for calculating LC_{50}, LC_{90}, and other statistics at 95% fiducial limits of the upper confidence limit (UCL) and lower confidence limit (LCL), and chi-square values were calculated using probit analysis method by SPSS tool.

3. Results

The larvicidal activity of six different lichen species were demonstrated in the present study against third instar larvae of *A. aegypti*, A. stephensi, and C. quinquefasciatus. Results were observed after 24 h for the methanolic extracts of *Pr, Pk, Lp, Pe, Pt*, and *Rm* against the third instar larvae of *A. aegypti* at various concentrations (100, 200, 300, 400, and 500 µg/mL) and found to have potent larvicidal activity which is exemplified in Table 1.

The lowest larvicidal activity against third instar larvae of *A. aegypti* was found in *Pr, Rm* (LC_{50} = 417.082 and 640.94 µg/mL; LC_{90} = 854 and 840.835 µg/mL) and moderate larvicidal activity was found in *Pk, Pe* (LC_{50} = 296.323 and 341.041 µg/mL; LC_{90} = 669.076 and 635.59 µg/mL) and the highest larvicidal activity was found in *Pt, Lp* (LC_{50} = 201.131 and 81.127 µg/mL; LC_{90} = 289.818 and 134.745 µg/mL). The obtained LC_{50} and LC_{90} correspond to the obtained the percentage mortality which can be observed in Fig. 1.

Table 2 elucidated the potent larvicidal activity of the methanolic extracts of *Pr, Pk, Lp, Pe, Pt*, and *Rm* observed at various concentrations(100, 200, 300, 400, and 500 µg/mL) after 24 h against third instar larvae of *A. stephensi*. The lowest larvicidal activity against third instar larvae of *A. stephensi* was found in *Pe, Pt* (LC_{50} = 112.035 and 156.239 µg/mL; LC_{90} = 619.953 and 517.576 µg/mL) and moderate larvicidal activity was found in *Pk, Lp* (LC_{50} = 153.276 and 89.107 µg/mL; LC_{90} = 443.956 and 244.031 µg/mL) and the highest larvicidal activity was found in *Pr, Rm* (LC_{50} = 102.054 and 127.362 µg/mL; LC_{90} = 145.403 and

Table 1
Larvicidal activity of methanolic extract of different lichen species against third instar larvae of *Aedes aegypti*.

Species	LC_{50} (UCL–LCL) / (μg · mL^{-1})	LC_{90} (UCL–LCL) / (μg · mL^{-1})	χ^2 (df = 4)
P. reticulatum	417.082 (504.33–298.26)	854.000 (982.02–692.29)	1.735
P. kamatti	296.323 (430.37–190.39)	669.076 (894.10–539.81)	0.826
L. papilosum	81.127 (101.51–4.13)	134.745 (235.98–108.66)	1.194
P. erumpens	341.041 (438.83–279.10)	635.590 (955.28–498.52)	3.277
P. tinctorum	201.131 (254.41–143.09)	289.818 (544.31–233.43)	1.163
R. montagnei	640.940 (805.76–224.52)	840.835 (664.16–925.54)	6.254

Control–nil mortality. Significant at $P < 0.05$ level. LC_{50}: lethal concentration that kills 50% of exposed larvae; LC_{90}: lethal concentration that kills 90% of exposed larvae; UCL: upper confidence limit; LCL: lower confidence limit; χ^2: chi-square; df: degree of freedom.

Table 2
Larvicidal activity of methanolic extract of different lichen species against third instar larvae of *A. stephensi*.

Species	LC_{50} (UCL–LCL) / (μg · mL^{-1})	LC_{90} (UCL–LCL) / (μg · mL^{-1})	χ^2 (df = 4)
P. reticulatum	102.054 (157.46–34.74)	145.403(231.57-72.82)	0.511
P. kamatti	153.276 (214.18–70.62)	443.956(1403.81-304.09)	4.456
L. papilosum	89.107 (137.68–13.03)	244.031(678.59-164.45)	2.682
P. erumpens	112.035 (193.06–0.19)	619.953(2019.38-338.83)	1.733
P. tinctorum	156.239 (224.92–59.80)	517.576(2597.21-335.48)	3.249
R. montagnei	127.362 (215.34–31.67)	485.739(1975.85-295.48)	2.742

Control–nil mortality. Significant at $P < 0.05$ level. LC_{50}: lethal concentration that kills 50% of exposed larvae; LC_{90}: lethal concentration that kills 90% of exposed larvae; UCL: upper confidence limit; LCL: lower confidence limit; χ^2: chi-square; df: degree of freedom.

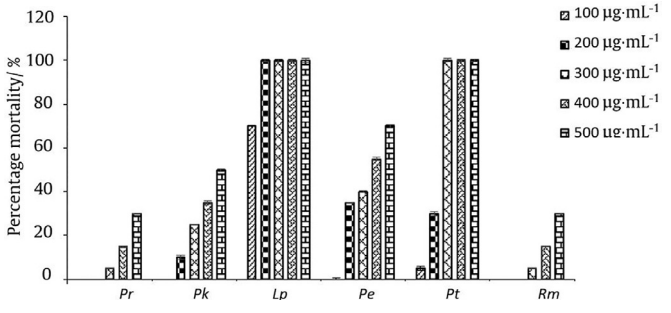

Fig. 1. Graphical representation of percentage mortality of methanolic extracts of different lichen species against third instar larvae of *Aedes aegypti*.

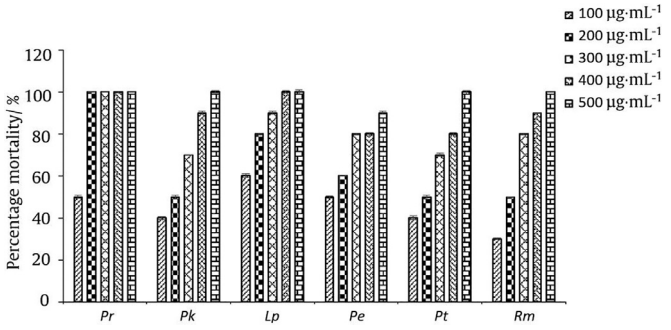

Fig. 2. Graphical representation of percentage mortality of methanolic extracts of different lichen species against third instar larvae of *A. stephensi*.

485.739 μg/mL). The percentage mortality for each concentration of different lichens can be observed in Fig. 2 and the obtained values correlates with LC_{50} and LC_{90} value of each lichens.

Similarly, all the lichen extracts exhibited complete mortality in 100 μg/mL against third instar larvae of *C. quinquefasciatus*, so lower concentration (100, 50, 25, 12.5, and 6.25 μg/mL) were taken. Table 3 illustrated the results of the larvicidal activity of methanolic extracts of *Pr, Pk, Lp, Pe, Pt*, and *Rm* against third instar larvae of *C. quinquefasciatus* observed after 24 h. The lowest larvicidal activity against third instar larvae of *C. quinquefasciatus* was found in *Pk, Pe* (LC_{50} = 13.202 and 9.251 μg/mL; LC_{90} = 64.913 and 44.175 μg/mL) and moderate larvicidal activity was found in *Pr, Lp*

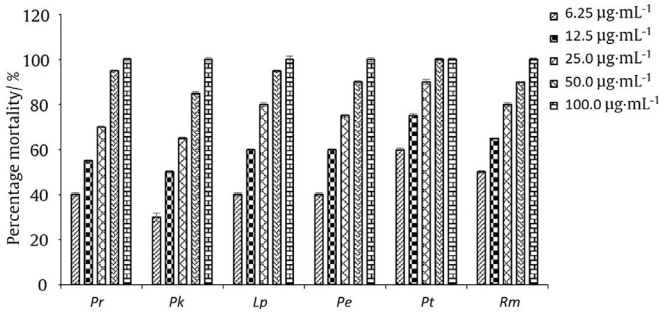

Fig. 3. Graphical representation of percentage mortality of methanolic extracts of different lichen species against third instar larvae of *C. quinquefasciatus*.

(LC_{50} = 9.999 and 8.974 μg/mL; LC_{90} = 42.407 and 40.677 μg/mL) and the highest larvicidal activity was found in *Pt, Rm* (LC_{50} = 5.324 and 6.971 μg/mL; LC_{90} = 20.937 and 35.207 μg/mL). Fig. 3 illustrated the % mortality obtained for the various concentrations of each lichen and correlated to the obtained LC_{50} and LC_{90}.

4. Discussion

Agricultural crops get directly affection by insect vectors and cause severe damage, resulting in revenue loss, especially mosquitoes directly transmit diseases (WHO, 2010). Prevention and control of mosquitoes are important to reduce the vector-borne disease incidence. Many control measures have been applied to reduce mosquito population in which larvae are annihilate at different stages to prevent the establishment of mosquito population. Larval source management (LSM) is particularly valuable in regions where the primary mosquito vectors are exophilic, so usage of indoor residual spraying was less effective (WHO, 2014). In order to prevent the completion of development of the immature stages of mosquitoes, the LSM maintains the aquatic habitats that are potential larval habitats for mosquitoes (Reddy et al., 2011).

Synthetic chemicals are proved to be effective, but they also pose potential threat to the environment and human health (Bagavan, Rahuman, Kamaraj, & Geetha, 2008). Therefore, eco-friendly alternatives are now preferred for the safe control of mosquitoes. The results of pesticidal and phytochemical screenings of a number of plants based on traditional knowledge strongly

Table 3
Larvicidal activity of methanolic extract of different lichen species against third instar larvae of *C. quinquefasciatus*.

Species	LC_{50} (UCL–LCL) / (μg · mL^{-1})	LC_{90} (UCL–LCL) / (μg · mL^{-1})	χ^2 (df = 4)
P. reticulatum	9.999 (14.07–5.80)	42.407 (96.14–28.16)	2.961
P. kamatti	13.202 (18.73–8.03)	64.913 (167.31–40.76)	2.175
L. papilosum	8.974 (12.59–5.09)	40.677 (115.72–25.50)	1.507
P. erumpens	9.251 (13.43–4.84)	44.175 (107.62–28.66)	1.178
P. tinctorum	5.324 (8.19–1.72)	20.937 (48.37–14.268)	1.230
R. montagnei	6.971 (10.99–2.57)	35.207 (75.41–23.94)	1.103

Control–nil mortality. Significant at $P < 0.05$ level. LC_{50}: lethal concentration that kills 50% of the exposed larvae; LC_{90}: lethal concentration that kills 90% of the exposed larvae; UCL: upper confidence limit; LCL: lower confidence limit; $\chi 2$: chi-square; df: degree of freedom.

indicated that plants possess pesticidal properties that can be harnessed cheaply for the use in agriculture and related fields. The phytochemicals from plant origin were proved to be effective due to multiple modes of action (Vinayaka, Krishnamurthy, Praveen kumar, Sudharshan, & Chinmaya, 2009). Research area of lichen is a flourishing field and it is found to contain many properties. Hence, the methanolic extracts of *Pr, Pk, Lp, Pe, Pt*, and *Rm* were further studied for larvicidal activity against *A. egypti, A. ephensi*, and *C. quinquefasciatus* to complement in this research.

High mortality was observed in dichloromethane, ethyl acetate, and acetone extract of *R. montagani* against *C.quinquefasciatus* at test concentrations from 125 to 1000 μg/mL. At 250 μg/mL concentration the mortality was 60% in dichloromethane and 50% in acetone extracts of *Rm*, and the abnormal behaviours of the larvae such as circular movements near the periphery of the beaker opposed to normal zig-zag motion in the control sets were also reported (Balaji, Sakthivadivel, Bharath, & Hariharan, 2012). The dichloromethane extract of *Rm* was reported to have significant LC_{50} value of 126.16 μg/mL at 24 h, but all the lichens collected from Yercaud displayed the opposite effect. One hundred percent mortality at 100 μg/mL and significant LC_{50} values were observed in methanolic extracts of *Pt* and *Rm* at 5.324 and 6.971 μg/mL respectively at 24 h with abnormal behaviour of larvae indicating the toxic effect of the test solution against larval nervous system (Murugesan et al., 2003).

Previous investigation tested with *Pt* and *Rm* on *A. aegypti* for larvicidal activity stated with the extract represented 100% mortality at 5 mg/mL concentration (Vinayaka et al., 2009). The results of the present study strongly corroborated with the previous reports by confirming the activity of *Pt* and *Rm* with 100% mortality at 500 μg/mL, and the LC_{50} value was found to be 201.131 μg/mL. Furthermore, few isolated compounds were reported to exhibit significant mosquito larvicidal activity against the second instar larvae of *A. aegypti* at 10 μg/mL: cabraleadiol monoacetate (90% moribund after 24 h), 4-O-methylcryptochlorophaeic acid (60% dead after 24 h), lichexanthone (80% moribund after 24 h) isolated from *Pyxine consocians*, and 3,6-dimethyl-2–hydroxy–4-methoxybenzoic acid (90% moribund after 24 h) isolated from *Heterodermia leucomelos*, which suggests that the lichens have great larvicidal potential against the *A. aegypti* and it can be used as a biological larvicide to prevent the spread of dengue and chikungunya (Kathirgamanathar, Ratnasooriya, Baekstrom, Andersen, & Karunaratne, 2006).

It is found that botanical derivatives possessing mosquitocidal properties directly attack on the nervous system and affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae (Mann & Kaufman, 2012), which are the most probable reason for larval death. This could be due to the presence of phytoconstituent within the lichen samples. Previous reports stated that alkaloids among the active molecules are effective against mosquito larvae (Liu, Liu, du, & Deng, 2012). Alkaloids are nitrogenous compounds that show insecticidal properties at low concentration and the mode of action on insect vectors varies with the structure of their molecules, but many are reported to affect acetylcholinestrase (AChE) or sodium channels for inhibition of AChE activity, which is responsible for terminating the nerve impulse transmission through synaptic pathway. Alkaloids constrict the blood vessels and depress autonomic nervous system activity, thereby contributing to the effectiveness of insecticide in killing the larvae of mosquitoes and disrupting the life cycle of the mosquito.

5. Conclusion

The present investigation reports the larvicidal potential of lichens effective even at very low concentration and could be a better alternative for chemical insecticides against diseases spreading mosquito larvae.

Conflict of interest

The authors declare no conflict of interest.

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References

Bagavan, A., Rahuman, A. A., Kamaraj, C., & Geetha, K. (2008). Larvicidal activity of saponin from *Achyranthes aspera* against *Aedesaegypti* and *Culexquinquefasciatus* (Diptera: Culicidae). *Parasitology Research, 103*, 223–229.

Balaji, P., Sakthivadivel, M., Bharath, P., & Hariharan, G. N. (2012). Larvicidal activity of various solvent extracts of lichen *RoccellaMontagnei* against filarial vector *CulexQuinquefasciatus*. *Drug Discovery, 2*(6), 36–39.

Boustie, J. B., Tomasi, S., & Grube, M. (2011). Bioactive lichen metabolites: Alpine habitats as an untapped source. *Phytochemistry Reviews, 10*, 287–307.

Fradin, M. S., & Day, J. F. (2002). Comparative efficacy of insect repellents against mosquitoes bites. *New England Journal of Medicine, 347*, 13–18.

Govindarajan, M., Rajeswary, M., Hoti, S. L., & Benelli, G. (2015). Ovicidal activity of *Pithecellobium dulce* (Family: Fabaceae) leaf and seed extracts against fi lariasis vector mosquito *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Medicinal Herbs and Ethnomedicine, 1*, 116–119.

Hales, S., Wet, N. D., Maindonald, J., & Woodward, A. (2002). Potential effect of population and climate changes on global distribution of dengue fever: An empirical model. *Lancet, 360*, 830–834.

Kathirgamanathar, S., Ratnasooriya, W. D., Baekstrom, Peter, Andersen, RaymondJ., & Karunaratne, V. (2006). Chemistry and bioactivity of physciaceae lichens *Pyxineconsocians* and *Heterodermia leucomelos*. *Pharmaceutical Biology, 44*(3), 217–220.

Lima, M. G., Maia, I. C., Sousa, B. D., Morais, S. M., & Freitas, S. M. (2006). Effect of stalk and leaf extracts from Euphorbiaceae species on *Aedes aegypti* (Diptera, Culicidae) larvae. *Revista do Instituto de Medicina Tropical de São Paulo, 48*(4), 211–214.

Liu, S. Y., Sporer, F., Wink, M., Jourdane, J., Henning, R., Li, Y. L., & Ruppel, A. (1997). Anthraquinones in *Rheum palmatum* and *Rumexdentatus* (Polygonaceae), and phorbol esters in *Jatrophacurcas* (Euphorbiaceae) with molluscicidal activity against the schistosome vector snails Oncomelania, Biomphalaria, and Bulinus. *TM IH Trop Med Int Health, 2*, 179–188.

Liu, Z. L., Liu, Q. Z., du, S. S., & Deng, Z. W. (2012). Mosquito larvicidal activity of alkaloids and limonoids derived from *Evodiarutaecarpa* unripe fruits against *Aedesalbopictus* (Diptera: Culicidae). *Parasitology Research, 111*(3), 991–996.

Luize, P. S., Ueda-Nakamura, T., Zimmermann, A., Vidoti, G. J., DiasFilho, B. P., Morgado-Diaz, J. A., & Nakamura, C. V. (2003). Ultrastructural alterations induced by AZ-7, a compound from *Pedilanthustithymaloides*, on Amastigote forms of Trypanosoma cruzi. *ActaMicrosc, 12*, 319–320.

MacNeil, A., Sumba, O. P., Lutzke, M. L., Moormann, A., & Rochford, R. (2003). Activation of the Epstein–Barr virus lytic cycle by the latex of the plant *EuphorbiatirucalliBr*. *Journal of Cancer, 88*(10), 1566–1569.

Mann, R. S., & Kaufman, P. E. (2012). Natural product pesticides: Their development, delivery and use against insect vectors. *Mini-Reviews in Organic Chemistry, 9*, 185–202.

Mansour, S. A., Messeha, S. S., & el-Gengaihi, S. E. (2000). Botanical biocides .4. Mosquitocidal activity of certain *Thymus capitatus* constituents. *Journal of Natural Toxins, 9*(1), 49–62.

Meshram, P. B., Kulkarni, N., & Joshi, K. C. (1996). Antifeedant activity of *Azadirachtaindica* and *Jatrophacurcas* against *Papiliodemoleus* L. *Journal of Environmental Biology, 17*, 295–298.

Mohan, L., Sharma, P., & Srivastava, C. N. (2005). Evaluation of *Solanumxanthocarpum* extracts as mosquito larvicides. *Journal of Environmental Biology, 26*(2), 399–401.

Mohan Roopan, S., Rohit, Madhumitha, G., Abdul Rahuman, A., Kamaraj, C., Bharathi, A., & Surendra, T. V. (2013). Low-cost and eco-friendly phyto-synthesis of silver nanoparticle using Cocos nicifera coir extract and its larvicidal activity. *Industrial crops and products, 43*, 631–635.

Murugesan, S., & Thilagavathy, D. (2003). Larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemonemexicana* L seed. *Bioresource Technology, 89*(2), 213–216.

Reddy, M. R., Overgaard, H. J., Abaga, S., Reddy, V. P., Caccone, A., Kiszewski, A., & Slotman, M. A. (2011). Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malaria Journal, 10*, 184.

Sharma, M., & Saxena, R. C. (1994). Phytotoxicologial evaluation of Tegetes erectes on aquatic stages of *Anophelesstephensi*. *Indian Journal of Malariology, 31*, 21–26.

Syed Zameer Ahmed, K., Sidhra, S., Ponmurugan, P., & Senthil Kumar, B. (2016). Ameliorative potential of *Solanum trilobatum* leaf extract and fractions on lipid profile and oxidative stress in experimental diabetes. *Pakistan Journal of Pharmaceutical Science, 29*(5), 1571–1578 16.

Taubes, G. A. (1997). *Mosquito bites back* (pp. 40–46). New York: Times Magazine. 24 August.

Taubitz, W., Cramer, J. P., Kapaun, A., Pfeffer, M., Drosten, C., Dobler, G., Burchard, G. D., & Löscher, T. (2007). Chikungunya fever in travelers: clinical presentation and course. *Clinical Infectious Diseases, 45*(1), 1–4.

Vinayaka, K. S., Krishnamurthy, Y. L., Praveen kumar, S. V., Sudharshan, S. J., & Chinmaya, A. (2009). Larvicidal and Wormicidal efficacy of methanolic extracts of five macrolichens collected from Bhadra wildlife sanctuary. *Biomedicine, 29*(4), 327–331.

Vivek, M. N., Yashoda, K., Manasa, M., Prashith, K. T. R., & Vinayaka, K. S. (2014). Radical scavenging and antibacterial activity of three Parmotrema species from Western Ghats of Karnataka, India". *Journal of Applied Pharmaceutical Science, 4*(3), 86–91.

WHO (2010). *Malaria fact sheets No. 94*. Geneva: WHO media centre WHO Report.

WHO (2014). *A global brief on vector-borne diseases [Document number: HO/DCO/WHD/2014.1]* Geneva, Switzerland.