parasitologia

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Larvicidal Effect of *Vitex ovata* Thunb. (Lamiales: Lamiaceae) Leaf Extract towards *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) (Diptera: Culicidae)

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Citation: Aziz, M.; Hashan Arif, E.I.; Muhammad Dimyati, N.I.; Ishak, I.H.; Hamdan, R.H.; Syazwan, S.A.; Peng, T.L. Larvicidal Effect of *Vitex ovata* Thunb. (Lamiales: Lamiaceae) Leaf Extract towards *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) (Diptera: Culicidae). *Parasitologia* **2021**, *1*, 210–217. https://doi.org/10.3390/parasitologia1040022

Academic Editor: Romain Pigeault

Received: 3 August 2021
Accepted: 29 September 2021
Published: 11 October 2021

Abstract: According to the WHO there are more than 700,000 deaths every year involving vector-borne diseases such as malaria, dengue, Chagas disease, yellow fever and Japanese encephalitis. *Aedes aegypti*, the principal vector of the dengue virus, is of great concern in various parts of the world, especially in tropical and subtropical countries. Vector control through insecticide application is one of the best ways to control the disease's transmission. Thus, insecticide resistance in *Ae. aegypti* poses a significant threat to public health worldwide. The use of plant natural product-based insecticides that are less harmful to the environment, and without known resistance development, constitutes an alternative to chemical insecticides. Given this, a methanolic extract from *Vitex ovata* plants was prepared and tested for its larvicidal effect against *Ae. aegypti*. A susceptibility test on *Ae. aegypti* larvae was conducted using the standard WHO method. Results showed that the methanolic extract of *V. ovata* had larvicidal activity against *Ae. aegypti* with LC_{50} values of 2114 mg/L, and achieved 84% mortality with the highest concentration at 10,000 mg/L. This study showed that the crude extract of *V. ovata* bioactive molecules could be potentially developed as biolarvicides for *Aedes* mosquito vector control. This study recommends future research on using different solvents in the isolation of active ingredients from *V. ovata*, identification of phytochemicals with larvicidal properties, a toxicity study and lastly, an evaluation of the effectiveness of controlling *Aedes* in small-scale field trials for environmentally safe botanical insecticide intervention.

Keywords: methanolic extract; natural product-based insecticides; LC_{50}; mosquitoes

1. Introduction

Aedes (*Stegomyia*) *aegypti* (Linnaeus, 1762), the principal vector of the dengue virus, is of great concern in various parts of the world, especially in tropical and subtropical countries [1]. The worldwide distribution of dengue epidemics includes over 100 countries, and up to 50–100 million infections are now estimated to occur annually [2]. In Malaysia, the number of dengue cases in 2019 was reported to be 127,407, with 176 deaths [3]. The increasing of this vector-borne disease is of great concern, as the incidence of dengue has increased 30-fold over the last 50 years [2]. Other than the dengue virus, *Aedes* (*Stegomyia*) *aegypti* s also known to transmit yellow fever virus, chikungunya virus, and Zika virus, putting more than half of the world's population at risk.

Vector control through insecticide application is one of the best ways to control disease transmission. Most of the control measures for dengue vectors target adults by using permethrin, deltamethrin, and malathion, or using larvicide such as temephos and *Bacillus thuringiensis israelensis* [4]. The use of these insecticides is practiced by government operators, private companies and even household communities. Intense exposures of insecticide have contributed to insecticide resistance in *Aedes* populations. Evidence of resistance towards permethrin and temephos has been recorded from both *Ae. aegypti* and *Ae. albopictus* (Skuse, 1894) in a few localities of Malaysia [5–7].

Given insecticide resistance and the limited success of biocontrol programs on *Aedes* [8], a search for new insecticides has become necessary. The use of botanical insecticides has a long history and their use is as a safer solution to control various types of insect pests. Naturally occurring chemicals extracted from plants act quickly (compared to biological control), degrade rapidly and usually have low mammalian toxicity [9,10]. Botanical insecticides affect various insects differently and can be classified into six groups: repellents, feeding deterrents/antifeedants, toxicants, growth retardants, chemosterilants, and attractants [11,12].

Alternative uses of bio-insecticides derived from plants might provide a more suitable and sustainable solution against *Ae. aegypti*. Following this safer and greener alternative concept, the *Vitex ovata* Thunb. (synonym: *Vitex rotundifolia* L.f.) plant, which is ubiquitous at the shoreline of Malaysia, was evaluated. *Vitex ovata* was chosen considering several species from this plant genus were known to have larvicidal properties [13]. Leaf extract from *V. trifolia* L. consists of 89 phytochemical components, with the major component identified as eucalyptol, which is known as an insect repellant. *Vitex ovata*, also known as *V. rotundifolia* [14], possesses a biological compound named rotundial with potent repelling activity against *Ae. aegypti* [15]. Nevertheless, a study on the efficacy of *V. ovata* leaf extract as a larvicide against *Ae. aegypti* is not yet available. Hence, the purpose of this study was to evaluate the effectiveness of *V. ovata* crude extracts against the *Ae. aegypti* as a larvicide.

2. Results

The larvicidal effects of *V. ovata* methanolic extracts were concentration dependent. The average mortality of *Ae. aegypti* from three replicates for each of the concentrations are shown in Table 1. The entire larvicidal bioassay showed a significant increase in mortality with extract concentration ($F = 38.68$, $p < 0.001$). The two highest concentrations tested, 5000 mg/L and 10,000 mg/L, had the highest larvicidal activities, with 76% and 84% mortality achieved within 24 h, respectively (statistics showed no difference in mortality between the two concentrations). The lowest mortality of the larvae caused by the extract was 34% at a concentration of 1250 mg/L. No mortality was observed in the controls. In this study, the LC_{50} and LC_{90} values of *V. ovata* methanolic extracts were 2114 and 13,902 mg/L, respectively (Figure 1).

Table 1. Mortality of *Ae. aegypti* larvae exposed to *V. ovata* methanolic extract.

Concentration (mg/L)	Actual Larvae Mortality n ± SE	Actual Larvae Mortality (%)	Arcsine Transformed Larvae Mortality (%)
10,000	21.00 ± 0.89	84.00 ± 3.55	67.59 ± 2.64 [a]
5000	19.00 ± 0.74	76.00 ± 2.95	61.31 ± 2.21 [a]
2500	13.90 ± 0.50	55.67 ± 2.00	48.30 ± 1.17 [b]
1250	8.40 ± 1.16	33.67 ± 4.66	35.29 ± 2.85 [c]

Within column, means without common letters differ at $p < 0.05$. Different letters used here just an indication to show whether the means are same or different from each other.

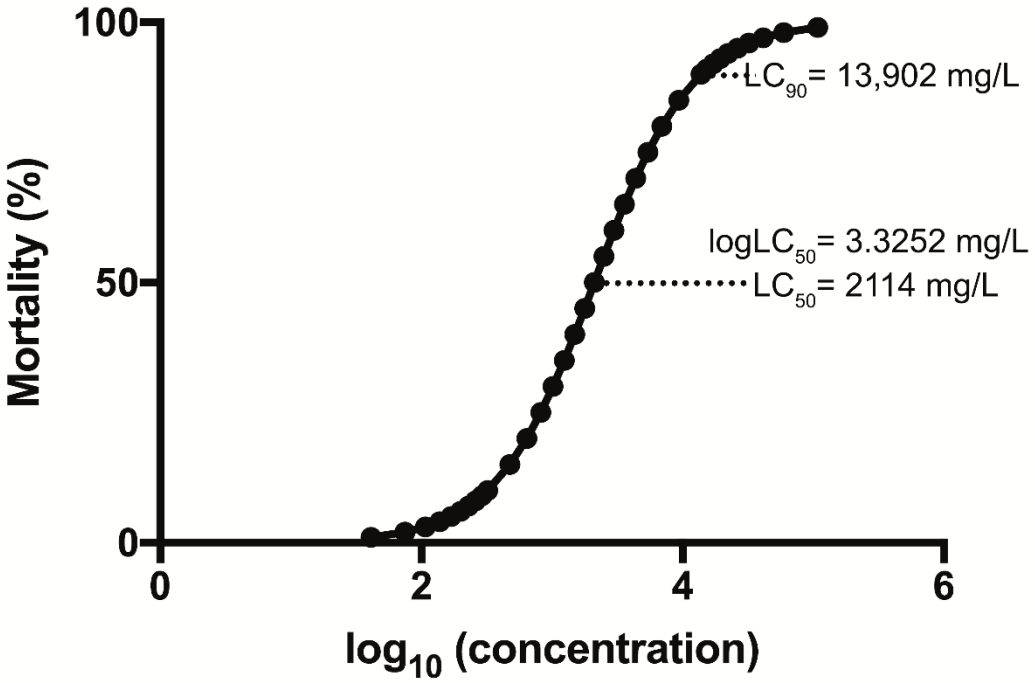

Figure 1. Concentration-response curve for the larvicidal effect of *V. ovata* extract on *Ae. aegypti*.

Under microscopic observation, the larvae treated with the plant extract generally appeared darker, with the thorax and upper abdominal segment prominently dark in colour, compared to the larvae from the control groups. The lower abdominal segments of the treated larvae were as clear as the control larvae. Besides, the anal gills of the treated larvae had a dark discolouration compared to the larvae from the control group (Figure 2).

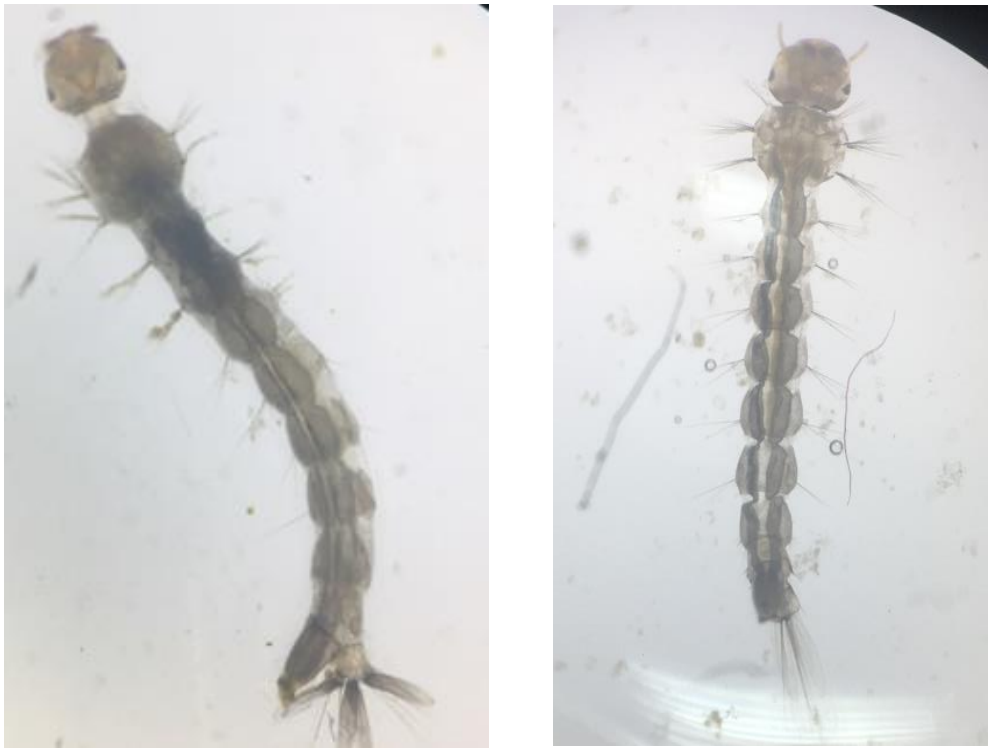

Figure 2. *Aedes aegypti* larva treated with *V. ovata* extract (**Left**) and control larva (**Right**) showing difference in the colouration of their midgut, in which the plant extract (greenish material) was visible in the treated larvae after 24 h.

3. Discussion

This study showed that the *V. ovata* methanolic extract might have larvicidal properties that can kill *Ae. aegypti* larvae. The crude extract of *V. ovata* used could be an innovative application as an alternative to synthetic chemical insecticides. The highest mortality of 68% achieved in this study was considered less effective. A larvicide is said to be effective if the mortality of larvae reaches at least 90% within 24 h [16]. Several authors have developed criteria to characterize mosquito larvicide potency for essential oils derived from plants, generally considered products with $LC_{50} < 100$ mg/L as exhibiting a significant larvicidal effect [17–21]. However, there is no standard criterion established by the WHO for determining the larvicidal activity of natural products.

Several species of plants under *Vitex* are known to have larvicidal activity against mosquitoes. Several studies had been conducted on *Vitex agnus castus* L., *V. altissima* L.f., *V. grandifolia* Gürke, *V. negundo* L., *V. peduncularis* Wall. ex Schauer, *V. rotundifolia* Michx., *V. schiliebeni* Moldenke and *V. trifolia* L. against mosquito species such as *Culex quinquefasciatus* (Say, 1823), *C. pipiens* (Linnaeus, 1758), *Anopheles stephensi* (Liston, 1901), *A. gambiae* (Giles 1902), *Ae. aegypti*, and *Ae. albopictus* [22]. Overall, crude extracts are less effective than the essential oils of most plant extracts and less effective compared with only active compounds used [23,24]. Crude plant extracts might have lower killing power; however, the botanical blends of chemical compounds leave very little chance for the pests to develop resistance to such constituents.

The dark materials observed in the midgut of the larvae were similar to those observed in previous studies [25,26]. This indicated that the *Aedes* larvae ingested the *V. ovata* plant extract, suggesting the killing mechanism of this plant extract against the mosquito larvae. The absence of microorganisms in the leaf extract reveals that no bacteria or fungus were introduced in the larval environment together with the extract. Thus, the darkening of the *Aedes* larvae was not related to midgut melanization after incubation with the extract [27]. Therefore, the mortality of the larvae could be attributed to the extract components solely with deleterious effects in the midgut such as vacuolization, microvilli damage, and cell lysis, which eventually impair the gut homeostasis and extensive tissue disorganization [28,29].

The major constituents of *V. rotundifolia* essential oil are α-pinene and 1.8-cineole. These two monoterpenes have been demonstrated as eco-friendly larvicides against mosquitoes and houseflies [30,31]. However, contradictory results obtained from Waliwitiya et al. [32] and Huang et al. [33], concerning α-pinene and 1.8-cineole being the principal larvicidal components of *V. rotundifolia* against *Ae. aegypti* remain to be confirmed. The fruit is, by far, the most studied part of *V. rotundifolia*, followed by the leaves [34,35].

Chemical constituents of *V. ovata* methanolic leaf extract are not available. The bioactivity of plant phytochemicals can vary significantly depending on plant species, plant part, and even the type of solvent used in extraction [36]. Thus, further studies on the phytochemistry of the *V. ovata* methanolic leaf extract are required to elucidate this plant's larvicidal potential further.

As mentioned above, the result from this study showed that *V. ovata* possess larvicidal components. However, methanol may not fully extract these components, as research has proved that solvent polarity mediates extraction yield and phytochemical contents [37–40]. A series of polar and nonpolar solvents tested to increase the extraction efficiency of phytochemicals with larvicidal properties from *V. ovata* might be an excellent approach to avoid missing out on any valuable compounds derived from the plant.

Many other factors could determine whether a plant extract is suitable to proceed to a field trial and eventual commercialization. For instance, the toxicity of *V. ovata* should be tested in-vitro and in-vivo to make sure it is environmentally safe does not cause toxicity to nontargeted organisms. Even though phytochemicals are relatively safe [41], a high dose d could still be harmful [42]. Adequacy of the plant source is a primary concern when moving to commercialization. In this study, roughly 500 g of live *V. ovata* plant (leaves)

could produce 50 mL of the crude extract. Countries in which *V. ovata* is abundant could consider this plant for developing the bio-insecticide as suggested.

4. Materials and Methods

4.1. Plant Methanolic Extract Preparation

Whole plants of *V. ovata* (Figure 1) were collected from Pantai Sabak, Kota Bharu, Kelantan, Malaysia (6°9′59.6952″ N, 102°20′31.4988″ E), and the species were identified based on a morphological view of phyllotaxis. Next, the leaves of *V. ovata* were removed and washed under running water to remove soil. The cleaned leaves were then dried in an oven (50 ± 1 °C) for 24 h. The dried samples were pulverized into fine powders using an electrical grinding machine (Faber FBG-460 K, Faber, Fabriano, Italy). A total of 100 g of fine powder was put into conical flasks, and one litre of methanol was poured into the flask. The mixture was stirred every 24 h using a magnetic stirrer, in a process that lasted for 3 days. The mixture was then filtered using Whatman No. 1 filter paper to remove the sample waste and was evaporated in a rotary vacuum evaporator at 40 °C to obtain the crude extract. The concentrated extract was then stored in the refrigerator (4 °C) for further use. Extract purity was checked by streaking the extract on nutrient agar (NA) with a sterile swab and incubated at 37 °C for 24 h to ensure no contamination.

4.2. Mosquito Larvae Rearing

Ae. aegypti eggs were obtained from the Vector Control Research Unit (VCRU) at the University Sains Malaysia (USM), Penang, Malaysia. The eggs were hatched in dechlorinated water for 24 h and maintained at 28 ± 2 °C (room temperature), a pH of 6.9 to 7.0, and dissolved oxygen from 5.5 to 6.1 mg/L in the laboratory. Ground cat food was used as the food source for the larvae. After five to six days, the early 4th instar larvae were collected for the larvicidal bioassay.

4.3. Larvicidal Bioassay

Larvicidal bioassays were performed following the standard World Health Organization [43] guidelines on larval susceptibility test methods (distilled water and plant solution). The bioassays were carried out using 25 early 4th instar *Ae. aegypti* larvae (homogeneous population consisting of 5 mm to 6 mm in body length). The bioassays were replicated three times for each concentration, with methanol CH_3OH as a solvent for the control. During the testing period, ground cat food was provided. In this study, four concentrations of 1250 mg/L, 2500 mg/L, 5000 mg/L and 10,000 mg/L were tested against the larvae. The control solutions were prepared with 1 mL of distilled water and 10% of the respective methanol solvent for each experimental replicate. The solvent control was to ensure that the mortality results were not due to the solvent. The experiments were conducted at a room temperature of 28 ± 2 °C, and larvae mortalities were recorded after 24 h. Death was considered as immobilization and total absence of movement from the larvae, even after touch. The data were analyzed using probit analysis for computing LC_{50}, and LC_{90} and one-way ANOVA was performed on data after arcsine transformation to discriminate the difference between the concentrations used (IBM SPSS Statistics 24, IBM, New York, NY, USA).

4.4. Microscopic Observation

The tested and control *Ae. aegypti* larvae were observed under a dissecting microscope for comparison on the physical changes.

5. Conclusions

Nowadays, environmental safety is considered to be of utmost importance. Insecticide that is eco-friendly with an acceptable mortality on the target organisms to ensure the pest population under the threshold level should be promoted. Leaf extract of *V. ovata* showed larvicidal activity on *Ae. aegypti* and may be developed as bio-larvicides for *Aedes* mosquito vector control. Since *V. ovata* is widely distributed in Malaysia, commercial exploitation

could provide an important step in developing a novel plant-based insecticide. Screening locally available medicinal plants for bio-insecticides could generate local employment, minimize dependence on imported products, and encourage local efforts to enhance the public health system.

Author Contributions: Conceptualization, T.L.P.; methodology, I.H.I.; formal analysis, S.A.S.; investigation, M.A., E.I.H.A. and N.I.M.D.; writing—original draft preparation, T.L.P. and M.A.; writing—review and editing, R.H.H.; supervision, T.L.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: The authors would like to thank the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, for providing facilities and support this project.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. McKerr, C.; Lo, Y.-C.; Edeghere, O.; Bracebridge, S. Evaluation of the National Notifiable Diseases Surveillance System for Dengue Fever in Taiwan, 2010–2012. *PLoS Negl. Trop. Dis.* **2015**, *9*, e0003639. [CrossRef]
2. World Health Organization (WHO). Dengue and Severe Dengue. 2019. Available online: https://www.who.int/news-room/q-a-detail/dengue-and-severe-dengue (accessed on 31 July 2021).
3. World Health Organization (WHO). Update on The Dengue Situation in The Western Pacific Region. 2020. Available online: https://iris.wpro.who.int/bitstream/handle/10665.1/14461/Dengue-20200102.pdf (accessed on 31 July 2021).
4. Ishak, I.H.; Jaal, Z.; Ranson, H.; Wondji, C.S. Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. *Parasites Vectors* **2015**, *8*, 181. [CrossRef]
5. Rahim, J.; Ahmad, A.H.; Ahmad, H.; Ishak, I.H.; Rus, A.C.; Maimusa, H.A. Adulticidal Susceptibility Evaluation of *Aedes albopictus* Using New Diagnostic Doses in Penang Island, Malaysia. *J. Am. Mosq. Control Assoc.* **2017**, *33*, 200–208. [CrossRef] [PubMed]
6. Elia-Amira, N.M.R.; Chen, C.D.; Low, V.L.; Lau, K.W.; Haziqah-Rashid, A.; Amelia-Yap, Z.H.; Lee, H.L.; Sofian-Azirun, M. Adulticide Resistance Status of *Aedes albopictus* (Diptera: Culicidae) in Sabah, Malaysia: A Statewide Assessment. *J. Med. Èntomol.* **2019**, *56*, 1715–1725. [CrossRef] [PubMed]
7. Wan Najdah, W.M.A.; Ahmad, R.; Nor, Z.M.; Hassan, A.F. Spatial distribution, enzymatic activity, and insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* from dengue hotspot areas in Kuala Lumpur and Selangor, Malaysia. *Serangga* **2020**, *25*, 65–92.
8. Benelli, G. Research in mosquito control: Current challenges for a brighter future. *Parasitol. Res.* **2015**, *114*, 2801–2805. [CrossRef] [PubMed]
9. Ghosh, A.; Chowdhury, N.; Chandra, G. Plant extracts as potential mosquito larvicides. *Indian J. Med. Res.* **2012**, *135*, 581–598. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3401688/ (accessed on 31 July 2021).
10. Silva-Aguayo, G. Botanical Insecticides. 2013. Available online: https://ipmworld.umn.edu/silva-aguayo-botanical (accessed on 31 July 2021).
11. Rattan, R.S. Mechanism of action of insecticidal secondary metabolites of plant origin. *Crop. Prot.* **2010**, *29*, 913–920. [CrossRef]
12. Rajashekar, Y.; Bakthavatsalam, N.; Shivanandappa, T. Botanicals as Grain Protectants. *Psyche A J. Èntomol.* **2012**, *2012*, 646740. [CrossRef]
13. Chandrasekaran, T.; Thyagarajan, A.; Santhakumari, P.G.; Pillai, A.K.B.; Krishnan, U.M. Larvicidal activity of essential oil from *Vitex negundo* and *Vitex trifolia* on dengue vector mosquito Aedes aegypti. *Rev. Soc. Bras. Med. Trop.* **2019**, *52*, e20180459. [CrossRef]
14. Munir, A.A. A taxonomic revision of the genus *Vitex* L (Verbenaceae) in Australia. *J. Adelaide Bot. Gard.* **1987**, *10*, 31–79. Available online: http://www.jstor.org/stable/23890926 (accessed on 3 June 2021).
15. Watanabe, K.; Takada, Y.; Matsuo, N.; Nishimura, H. Rotundial, a New Natural Mosquito Repellent from the Leaves of Vitex rotundifolia. *Biosci. Biotechnol. Biochem.* **1995**, *59*, 1979–1980. [CrossRef]
16. Sutiningsih, D.; Nurjazuli, N.; Nugroho, D.; Satoto, T.B.T. Larvicidal Activity of Brusatol Isolated from *Brucea javanica* (L) Merr on *Culex quinquefasciatus*. *Iran. J. Public Health* **2019**, *48*, 688–696. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6500525/ (accessed on 3 June 2021). [CrossRef] [PubMed]

17. Komalamisra, N.; Trongtokit, Y.; Rongsriyam, Y.; Apiwathnasorn, C. Screening for larvicidal activity in some Thai plants against four mosquito vector species. *Southeast Asian J. Trop. Med. Public Health* **2005**, *36*, 1412–1422. Available online: https://pubmed.ncbi.nlm.nih.gov/16610643/ (accessed on 3 June 2021). [PubMed]
18. Kiran, S.R.; Bhavani, K.; Devi, P.S.; Rao, B.R.; Reddy, K.J. Composition and larvicidal activity of leaves and stem essential oils of Chloroxylon swietenia DC against *Aedes aegypti* and *Anopheles stephensi*. *Bioresour. Technol.* **2006**, *97*, 2481–2484. [CrossRef]
19. Massebo, F.; Tadesse, M.; Bekele, T.; Balkew, M.; Gebre-Michael, T. Evaluation on larvicidal effects of essential oils of some local plants against *Anopheles arabiensis* Patton and *Aedes aegypti* Linnaeus (Diptera, Culicidae) in Ethiopia. *Afr. J. Biotechnol.* **2009**, *8*, 4183–4188. [CrossRef]
20. Magalhães, L.A.M.; Da Paz Lima, M.; Ortiz Mayo Marques, M.; Facanali, R.; Pinto, A.C.D.S.; Pedro Tadei, W. Chemical Composition and Larvicidal Activity against Aedes aegypti Larvae of Essential Oils from Four *Guarea* Species. *Molecules* **2010**, *15*, 5734–5741. [CrossRef]
21. Dias, C.N.; Moraes, D.F.C. Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: Review. *Parasitol. Res.* **2013**, *113*, 565–592. [CrossRef]
22. Lukminati, N.; Kardela, W.; Ifora. Larvicidal activity and efficacy of *Vitex* species: A review. *Sci. Int.* **2020**, *32*, 493–500.
23. Jantan, I.; Ping, W.O.; Visuvalingam, S.D.; Ahmad, N.W. Larvicidal Activity of the Essential Oils and Methanol Extracts of Malaysian Plants on *Aedes aegypti*. *Pharm. Biol.* **2003**, *41*, 234–236. [CrossRef]
24. Kannathasan, K.; Senthilkumar, A.; Venkatesalu, V. Mosquito larvicidal activity of methyl-p-hydroxybenzoate isolated from the leaves of *Vitex trifolia* Linn. *Acta Trop.* **2011**, *120*, 115–118. [CrossRef] [PubMed]
25. Ravi, R.; Zulkrnin, N.S.H.; Rozhan, N.N.; Yusoff, N.R.N.; Rasat, M.S.M.; Ahmad, M.I.; Ishak, I.H.; Amin, M.F.M. Chemical composition and larvicidal activities of *Azolla pinnata* extracts against Aedes (Diptera: Culicidae). *PLoS ONE* **2018**, *13*, e0206982. [CrossRef]
26. Ravindran, D.R.; Bharathithasan, M.; Ramaiah, P.; Rasat, M.S.M.; Rajendran, D.; Srikumar, S.; Ishak, I.H.; Said, A.R.; Ravi, R.; Amin, M.F.M. Chemical Composition and Larvicidal Activity of Flower Extracts from *Clitoria ternatea* against Aedes (Diptera: Culicidae). *J. Chem.* **2020**, *2020*, 3837207. [CrossRef]
27. Yassine, H.; Kamareddine, L.; Osta, M.A. The Mosquito Melanization Response Is Implicated in Defense against the Entomopathogenic Fungus *Beauveria bassiana*. *PLoS Pathog.* **2012**, *8*, e1003029. [CrossRef] [PubMed]
28. Gusmão, D.S.; Páscoa, V.; Mathias, L.; Vieira, I.J.C.; Braz-Filho, R.; Lemos, F.J.A. *Derris* (Lonchocarpus) *urucu* (Leguminosae) Extract Modifies the Peritrophic Matrix Structure of *Aedes aegypti* (Diptera: Culicidae). *Mem. Inst. Oswaldo Cruz* **2002**, *97*, 371–375. [CrossRef]
29. Procópio, T.F.; Fernandes, K.M.; Pontual, E.V.; Ximenes, R.M.; de Oliveira, A.R.C.; de Santana Souza, C.; de Albuquerque Melo, A.M.M.; do Amaral Ferraz Navarro, D.M.; Paiva, P.M.G.; Martins, G.F.; et al. *Schinus terebinthifolius* Leaf Extract Causes Midgut Damage, Interfering with Survival and Development of *Aedes aegypti* Larvae. *PLoS ONE* **2015**, *10*, e0126612. [CrossRef]
30. Kumar, P.; Mishra, S.; Malik, A.; Satya, S. Housefly (*Musca domestica* L.) control potential of *Cymbopogon citratus* Stapf. (Poales: Poaceae) essential oil and monoterpenes (citral and 1,8-cineole). *Parasitol. Res.* **2013**, *112*, 69–76. [CrossRef]
31. Govindarajan, M.; Rajeswary, M.; Hoti, S.L.; Bhattacharyya, A.; Benelli, G. Eugenol, α-pinene and β-caryophyllene from *Plectranthus barbatus* essential oil as eco-friendly larvicides against malaria, dengue and Japanese encephalitis mosquito vectors. *Parasitol. Res.* **2015**, *115*, 807–815. [CrossRef]
32. Waliwitiya, R.; Kennedy, C.J.; Lowenberger, C.A. Larvicidal and oviposition-altering activity of monoterpenoids, trans-anithole and rosemary oil to the yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae). *Pest Manag. Sci.* **2009**, *65*, 241–248. [CrossRef]
33. Huang, H.-T.; Lin, C.-C.; Kuo, T.-C.; Chen, S.-J.; Huang, R.-N. Phytochemical composition and larvicidal activity of essential oils from herbal plants. *Planta* **2019**, *250*, 59–68. [CrossRef]
34. Meena, A.K.; Niranjan, U.S.; Rao, M.M.; Padhi, M.M.; Babu, R. A review of the important chemical constituents and medicinal uses of Vitex genus. *Asian J. Tradit. Med.* **2011**, *6*, 54–60.
35. Rani, A.; Sharma, A. The genus Vitex: A review. *Pharmacogn. Rev.* **2013**, *7*, 188–198. [CrossRef] [PubMed]
36. Shaalan, E.; Canyon, D.; Younes, M.W.F.; Abdel-Wahab, H.; Mansour, A.-H. A review of botanical phytochemicals with mosquitocidal potential. *Environ. Int.* **2005**, *31*, 1149–1166. [CrossRef] [PubMed]
37. Abidin, L.; Mujeeb, M.; Mir, S.R.; Alam Khan, S.; Ahmad, A. Comparative assessment of extraction methods and quantitative estimation of luteolin in the leaves of *Vitex negundo* Linn. by HPLC. *Asian Pac. J. Trop. Med.* **2014**, *7* (Suppl. S1), S289–S293. [CrossRef]
38. Iloki-Assanga, S.B.; Lewis-Luján, L.M.; Lara-Espinoza, C.L.; Gil-Salido, A.A.; Fernandez-Angulo, D.; Rubio-Pino, J.L.; Haines, D.D. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. *BMC Res. Notes* **2015**, *8*, 396. [CrossRef] [PubMed]
39. Wakeel, A.; Jan, S.A.; Ullah, I.; Shinwari, Z.K.; Xu, M. Solvent polarity mediates phytochemical yield and antioxidant capacity of *Isatis tinctoria*. *PeerJ* **2019**, *7*, e7857. [CrossRef]
40. Nawaz, H.; Shad, M.A.; Rehman, N.; Andaleeb, H.; Ullah, N. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Braz. J. Pharm. Sci.* **2020**, *56*, 17129. [CrossRef]
41. Altemimi, A.; Lakhssassi, N.; Baharlouei, A.; Watson, D.G.; Lightfoot, D.A. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants* **2017**, *6*, 42. [CrossRef]

42. American Chemical Society. High Doses of Phytochemicals, Including Flavanoids, in Teas and Supplements Could Be Unhealthy. *ScienceDaily*. 2007. Available online: www.sciencedaily.com/releases/2007/04/070430224756.htm (accessed on 12 September 2021).
43. World Health Organization. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. 2005. Available online: https://apps.who.int/iris/handle/10665/69101 (accessed on 31 July 2021).