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Mosquito larvicidal activity of *Hyptis capitata* leaves ethanolic extract and fraction against *Culex quinquefasciatus*

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ABSTRACT. Larvicidal potential of *Hyptis capitata* grown in Indonesia has not been extensively studied. Its leaves are extracted with the maceration method using ethanol as the solvent. Furthermore, this study aims to use the ethanolic leaf extract of the *H. capitata* for larvicidal assays against instar III/IV larvae of *Culex quinquefasciatus* with different concentrations, including 1000, 500, 250, 125, and 62.5 µg/mL. Fractionation of the extract was carried out by vacuum liquid chromatography, and obtained four fractions, namely fractions F1, F2, F3, F4. Fractions were also used for the larvicidal assay. The constituents of the extract were then analyzed with the GC-MS method to predict the components involved in its toxicity. Larvicidal data obtained were analyzed using regression analysis to determine the LC50 value. Analysis of variance was carried out with one-way ANOVA using Tuckey HSD-test on the SPSS ver. 26 at 95% confidence and significance P<0.05. *H. capitata* ethanolic leaf extract has a higher level of toxicity than its fraction, with LC50 value 990.90 µg/mL. *H. capitata* ethanolic leaf extract are capable of providing a toxic effect on larvae, with a mortality percentage of up to 50% more. Some compounds that were assumed to play a role in its toxicity include pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester; 1-heptadecyne; 9-tetradecen-1-ol, acetate; oxyrane, deodecyl-; 9,12,15-octadecatrienal; and 6,11-dimethyl-2,6,10-dodecatrien-1-ol. These finding indicate that the ethanolic leaf extract of *H. capitata* has the potential to be developed as a biolarvicidal agent against *C. quinquefasciatus*.

Keywords: biolarvicidal; *Culex quinquefasciatus*; GC-MS; level of toxicity; potential of knobweed

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INTRODUCTION

Mosquitoes are one of the insects, which play a role in the spread of various diseases among humans and pets, and causing different health problems (Gouge *et al.*, 2019). Unlike the male mosquitoes, the females need blood for the development of their eggs (Nikbakhtzadeh *et al.*, 2017). Furthermore, there are several species of the insect, but only a few can cause diseases, such as *Culex quinquefasciatus*. This species is the main vector for the nematode *Wuchereria bancrofti*, which causes Bancroftian filariasis (Simonsen & Mwakitalu, 2013). Bancroftian filariasis is the main cause of lymphatic filariasis, with a percentage reaching 90% (Pattanshetty *et al*., 2010; Irvine *et al*., 2017). Globally there are around 25 million men affected by lymphatic filariasis in 2020. In 50 countries, there are 863 million people who are taking preventive therapy to limit the spread of infection (World Health Organization, 2022).

Several efforts have been made to curtail the activities of mosquitoes, such as the use of insecticides, which is expected to have a negative effect on pests, with no harmful effect on environment or non-target organisms (Ghosh *et al.*, 2012; The World Bioprotection Forum, 2021). However, insecticides have negative impact due to their chemical components that are harmful to humans and the surrounding environment (Nicolopoulou-Stamati *et al.*, 2016). Therefore, it is necessary to develop new variants of insecticides that are harmless and more environmentally friendly, such as bioinsecticides. Biological insecticide contains basic ingredients obtained from plants including chemicals (bioactive) that are toxic to insects but are easily biodegradable in nature and relatively safe (Demirak *et al.*, 2022).

Natural materials from plants contain secondary metabolites that can repel mosquitoes, hence, they are often used as repellants (Hansen *et al.*, 2016). Repellant activity is expected to reduce or prevent mosquitos' bites. In addition to repellant activity, one of the ways that can also be used to

reduce mosquito population is to target their larvae. Currently, one that is being widely researched is plant-based larvicidal (Ghosh *et al.,* 2012; Pavela *et al.,* 2019).

H. capitata is one of plants that widely used as traditional medicine ingredients. This plant has also been used to treat various health problems such as wounds, diabetes and fever (To'bungan *et al.*, 2022a). The methanolic extract of *H. capitata* leaves grown in Thiruvananthapuram, India, showed an LC_{50} value of 11.15 mg/mL. The plant has the potential to be developed as a biolarvicidal against *C. quinquefasciatus* (Sumitha *et al.,* 2021), but the larvicidal activity of this species that growing in Indonesia has not been explored. The difference of solvent and environmental factors where a plant grows can affect its secondary metabolites content (Widyawati *et al.,* 2014; Felhi *et al.,* 2017; Yang *et al.,* 2018). Therefore, it is necessary to investigate the potential of *H. capitata* grown in Indonesia to be developed as a biolarvicidal.

MATERIALS AND METHODS

H. capitata leaves were taken around the oil palm plantation area, Bungapati village, Tana Lili District, North Luwu Regency, South Sulawesi. Identification of the sample was then carried out at Laboratory of the plant systematics, Universitas Gadjah Mada, with certificate number 014535/S.Tb/III/2019 (To'bungan *et al.,* 2022b). Subsequently, herbarium *H. capitata* was stored in the laboratory of Techno-bio Industry, Faculty of Biotechnology, Universitas Atma Jaya, Yogyakarta. This study begins with extraction of phytochemical compounds from leaves of *H. capitata* with absolute ethanol. The larvicidal test was started by testing the larvicidal activity of the ethanolic extract of the leaves of *H. capitata*. The ethanolic extract of the leaves then fractionated to compare the larvicidal potency of the extract and its fraction, explore the compounds that may play a role in larvicidal activity, as well as to determine the interactions of the compounds that may occur.

Leaves extraction. The leaves powder was extracted with the maceration method using absolute ethanol as solvent. Extraction procedure have been published previously (To'bungan *et al.,* 2022a).

Fractionation. Fractionation was carried out using a vacuum liquid chromatography method, followed by thin layer chromatography to monitor the chromatogram pattern. There are 8 eluent compositions of vacuum liquid chromatography which are used to separate the compounds contained in the ethanolic leaf extract. Fractionation has produced 4 fractions, called fractions F1, F2, F3, F4. The fractionation procedure and data for grouping fractions (F1-F4) have been published previously (To'bungan *et al.,* 2022a).

Larvicidal test. The samples used were the larvae of the Surabaya strain of *C. quinquefasciatus* F13 mosquito that reared in Laboratory of Entomology, Institute of Tropical Disease, Universitas Airlangga. The leaf extract and fraction were dissolved with 1% Tween 20 into various concentration series, including 1000, 500, 250, 125, and 62.5 µg/mL. A total of 25 instar III/IV larvae were placed into a becker glass, containing 50 ml of the test solution per concentration with 3 replications. This process was also carried out for the negative control, containing water + tween. The larvicidal activity was observed during the 24-hour treatment, with room temperature 21-25˚C, and humidity 50-64%, after which the number of dead larvae was calculated and the percentage of mortality was determined with formula: Mortality (%) = (Number of dead larvae/number of larvae introduced) × 100% (Panneerselvan *et al.,* 2012).

Analysis of secondary metabolite content with GC-MS. The GC-MS analysis was carried out at the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada. GC-MS procedure have been published previously (To'bungan *et al.,* 2022a).

Data analysis. The data collected were analyzed with the regression analysis to determine the LC_{50} value. Analysis of variance was carried out with one-way ANOVA using Tuckey HSD-test on the SPSS ver. 26 at 95% confidence level, and significance $P<0.05$.

RESULTS AND DISCUSSION

The larvicidal test results showed that the *H. capitata* ethanolic leaf extract treatment with variations in concentration against *C. quinquefasciatus* larvae caused different percentages of larval mortality with an LC_{50} value of 990.90 µg/mL (0.99 mg/mL), as shown in Table 1. LC_{50} values <1000 µg/mL were categorized as toxic. This finding indicates the toxicity of the ethanolic extract of *H. capitata* is higher than its methanolic extract against *C. quinquefasciatus* larvae, (LC_{50} 11.15 mg/mL) which had been previously studied by Sumitha *et al.*, 2021. The differences solvents for extracting and the location where plant grown affect the extractable phytochemical compounds (Widyawati *et al.*, 2014; Sampaion *et al.*, 2016; Felhi *et al.*, 2017; Yang *et al*., 2018). An effort to obtain an effective solvent are needed to extract phytochemical compounds that act as larvicidal. The effect of variations in concentrations on larval mortality is presented in Fig. 1. Treatment with a concentration of 1000 ug/mL caused the greatest larval mortality, with mortality percentage of 50.67%. Toxic effect indicated by the percentage of larval mortality (Kurniawan & Ropiqa, 2021).

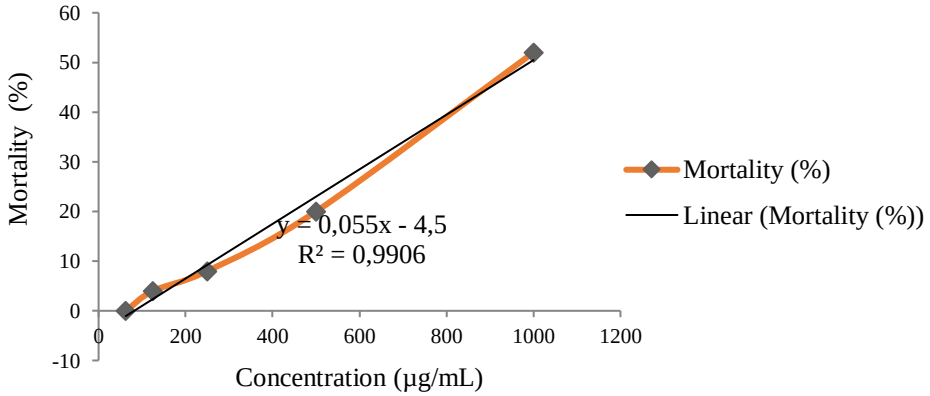

Fig. 1. Larvicidal activity of *Hyptis capitata* ethanolic leaf extract against *Culex quinquefasciatus*

In the control group in Table 1., there were no larval deaths, and indicates that the mortality in the treatment group was caused by the ethanolic leaf extract treatment. Furthermore, no larva mortality was recorded in the group that treated with a concentration 62.5 µg/mL. This shows that, the treatment at these concentrations has not been able to have a toxic effect on the larvae. Mortality of mosquito larvae exposed to the extract, can influenced by the content of phytochemical compounds (Senthil-Natan, 2020).

Table 1. Larvicidal activity of *Hyptis capitata* ethanolic leaf extract against *Culex quinquefasciatus*

Concentration (µg/mL)	Mortality (%)	LC_{50} (µg/mL)
Control	0	
1000	50.67 ± 3.53^{c}	
500	17.33 ± 1.33^{b}	990.90
250	8.00 ± 2.31^{a}	
125	2.67 ± 1.33^{a}	
62.5	0	

Note: The LC_{50} value is represented by mean ± standard error of three replications. Different superscript alphabetic letters were significantly different at $p < 0.05$ by the Tukey HSD-test.

Fractionation was conducted to investigate whether the compounds contained in *H. capitata* work antagonistically or synergistically in larvicidal activity. The results of the larvicidal test fractions F1-F4 showed that, the LC_{50} value of fraction was greater than the LC_{50} value of the ethanolic leaf extract. The results of the larvicidal activity of fractions F1-F4 can be seen in Table 2. The level toxicity of fractions F1-F4 against *C. quinquefasciatus* larvae showed a decrease compared to the toxicity of the crude extract. Biological activity, including toxicity, was influenced by the content of compounds in the extract and fraction (Lopes *et al*., 2013; Altemimi *et al.*, 2017).

Table 2. Larvicidal activity of F1-F4 against *Culex quinquefasciatus*

Treatment	LC_{50} ± SE (µg/mL)
F1	2310.47 ±28.83[c]
F2	1842.23 ± 96.01[b]
F3	1592.94 ± 19.20[a]
F4	1429.32 ±66.04[a]

Note: The LC_{50} value is represented by mean ± standard error of three replications. Different superscript alphabetic letters were significantly different at $p < 0.05$ by the Tukey HSD-test.

Due to the toxicity of the *H. capitata* leaf ethanol extract which is more toxic than its fraction, an investigation of the secondary metabolite content was carried out on the leaf ethanolic extract. The chromatogram GC-MS of *H. capitata* ethanolic leaf extract shows the presence of 68 peaks, as shown in Fig. 2.

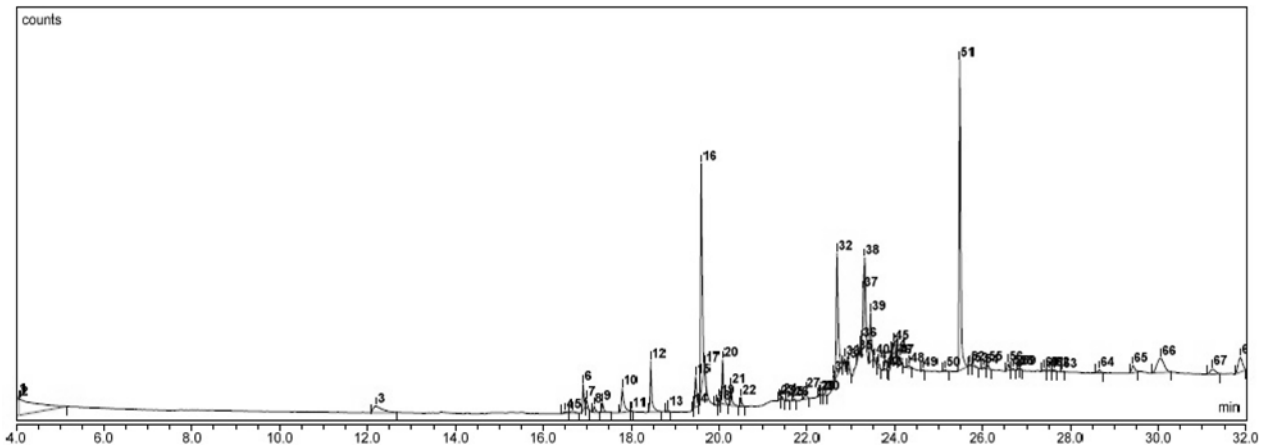

Fig. 2. Chromatogram of metabolite compounds from the leaves ethanolic extract of *Hyptis capitata* with 68 detected peaks

Furthermore, there are 18 compounds with a similarity index above 700, which are assumed to be contained in the extract, as shown in Table 3.

Table 3. Metabolite compounds of *Hyptis capitata* ethanolic leaf extract

No.	Retention time	% Area	Formula	Compound
1	4.02	1.27	$C_4H_6N_6O_3$	2,4,6,8-Tetraazabicyclo[3.3.0]octan-3-one, 7-nitroimino-
2	12.19	2.61	$C_4H_6N_2O_2$	Tetrahydropyrrole-3-amino-2,5-dione
3	16.66	0.28	$C_6H_7N_3O_2$	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-
4	16.90	1.39	$C_{17}H_{32}$	1-Heptadecyne
5	16.98	0.50	$C_9H_{18}O$	1,2-Epoxynonane
6	17.16	0.45	$C_8H_{14}O_2$	9-Oxabicyclo[4.2.1]nonan-2-ol
7	17.35	0.75	$C_{14}H_{28}O$	E-7-Tetradecenol
8	18.44	3.43	$C_{20}H_{40}O_2$	Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester
9	18.83	0.13	$C_{10}H_{18}O$	3-Decyn-2-ol
10	19.41	0.11	$C_{16}H_{30}O_2$	9-Tetradecen-1-ol, acetate, (E)-
11	19.59	16.10	$C_{14}H_{28}O$	Oxirane, dodecyl-
12	19.57	0.25	$C_{17}H_{32}O_2$	10-Methyl-E-11-tridecen-1-ol propionate
13	20.08	2.73	$C_{18}H_{30}O$	9,12,15-Octadecatrienal
14	21.38	0.07	$C_8H_{16}O_8$	I-Gala-l-ido-octose
15	23.54	0.16	$C_7H_{11}NO_2$	4,4-Ethylenedioxy-pentanenitrile
16	24.64	0.08	$C_5H_{10}O_5$	DL-Arabinose
17	25.48	20.05	$C_{14}H_{24}O$	6,11-Dimethyl-2,6,10-dodecatrien-1-ol
18	31.23	1.13	$C_{16}H_{30}Si$	1-Ethenyl-3-(1-hexenyl)-4-trimethylsilylcyclopentane

Larvicidal activity test show that ethanolic leaf extract was toxic against *C. quinquefasciatus* larvae. Treatment of *H. capitata* leaf ethanolic extract against *C. quinquefasciatus* larvae showed

more toxic LC_{50} value than the methanolic leaf extract conducted by Sumitha *et al.,* (2021). Solvents with different polarities will extract different phytochemical compounds. This causes differences biological activity of the test material (Lopes *et al.*, 2013; Widyawati *et al.*, 2014; Felhi *et al.*, 2017). The difference in environmental conditions where plants grow is also one of the causes of differences in the content of secondary metabolites (Sampaio *et al.,* 2016).

Production of secondary metabolites is influenced by biochemical and physiological conditions of plants, which are strongly influenced by environmental factors such as light, soil water content, soil fertility, salinity, carbon dioxide and temperature (Pant *et al.,* 2021). Sumitha *et al.,* (2021), stated that phytol, squalene and tocopherol played a role in the toxicity of the methanol extract of *H. capitata* leaves on *C. quinquefasciatus* larvae. The difference in the solvent used is also thought to affect the type of compound extracted. Several compounds that are thought play a role in the toxic activity of the ethanolic leaf extract of *H. capitata* against *C. quinquefasciatus* larvae, based on Table 2. namely pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester; 1-heptadecyne; 9-tetradecen-1-ol, acetate; oxyrane, deodecyl-; 9,12,15-octadecatrienal; and 6,11-dimethyl-2,6,10-dodecatrien-1-ol.

Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester, also known as Pristanic acid (Roca-Saavedra *et al.,* 2017). Pristanic acid is a product of phytol metabolism (Bobe *et al.,* 2020). Furthermore, phytol has a cytotoxicity effect against some cancer cell lines (Pejin *et al*. 2014; Kim *et al.,* 2015; Thakor *et al.,* 2017). It also presence in *Calotropis gigantea* leaves as larvicidal activity against *Spodoptera litura* larvae (Babu *et al.,* 2016). Several studies have also reported the cytotoxicity effect of pristanic acid against brain cancer cell lines (Kruska & Reiger, 2011), but its effect on mosquito larvae and other insects has not been explored. Bobe *et al.,* (2020) stated that phytol and its metabolites have in vitro anticancer activity and can cause mortality in experimental animals. Therefore, it is possible that pristanic acid also plays a role in larvicidal activity of ethanolic leaf extract.

The 1-Heptadecyne is also present in the aqueous extract of *Momordica charantia* leaves, and the LC_{50} of its larvicidal activity against *C. quinquefasciatus* was 42.63 mg/L (Gandhi *et al.,* 2017). 9-Tetradecen-1-ol, acetate, (E), is a carboxylic ester compound (National Center for Biotechnology information, 2022a), which is also contained in the essential oil of *Mentha piperita*. A previous study reported that it is plays a role in the insecticidal effect of *M. piperita* against *Sitophilus oryzae* L. (Mackled *et al.,* 2019). The presence of both in the ethanolic leaf extract of *H. capitata* thought to contribute to the toxicity against *C. quinquefasciatus*. The GC-MS fraction analysis of *Plectranthus amboinicus* showed that oxyrane, deodecyl- is one of its main components, and it is suspected to contribute to the mosquitocidal activity against instar 4 *Aedes aegypti* larvae (Paramasivam *et al.,* 2022). Its relatively high content in the ethanolic leaf extract of *H. capitata*, supports the alleged involvement in the larvicidal activity of *H. capitata*.

The 9,12,15-Octadecatrienal is a fatty aldehyde (National Center for Biotechnology Information, 2022b). Several studies revealed that it can act as an antibacterial (Ardalan *et al.,* 2017; Padma *et al.,* 2019). It is also present in the petroleum ether extracts of *Lagenaria siceraria* leaves, which are toxic to *Spodoptera littoralis* (Abaza, 2020). A previous study predicted that the extract can be developed as a bioinsecticide to control *S. littoralis*. Based on the results of these previous studies, this compound is predicted play a role in the larvicidal activity of the ethanolic leaf extract of *H. capitata*.

Table 2. shows that 6,11-Dimethyl-2,6,10-dodecatrien-1-ol has the largest percentage area compared to other compounds. It is one of the most dominant components of *Pavetta indica* L. aerial parts, that has cytotoxicity activity against breast cancer cell MDA-MB-231 (Thi-Kim *et al.,* 2019). The toxic effect of this compound against mosquito larvae or other insects has not been reported. However, based on its large percentage in *H. capitata* ethanolic leaf extract and cytotoxicity to cancer cells, it is assumed to be toxic against *C. quinquefasciatus* larvae.

Another species of *Hyptis* plant that has also been reported to have larvicidal activity is *H. suaveolens*. Its ethanolic and aqueous leaf extract showed toxicity against Anopheles mosquito larvae with LC_{50} of 316.22 and 323.59 mg/L, respectively (Dakum *et al.,* 2021). When comparing the IC_{50}

value larvicidal test of *H. capitata* extract and fraction, it can be seen that the toxicity level of the fraction is lower. The phytochemical compound in fractions that have been divided into several fractions, no longer causes toxic effects on the *C. quinquefasciatus* mosquito larvae. This is shows that the components contained in the crude extract synergize with each other in providing toxicity effects. Therefore, the crude extract of *H. capitata* is recommended as an alternative larvicidal. Based on the potential of *H. capitata* to be developed as a natural larvicidal agent, it is necessary study its effects on non-target organisms as well as measure the safety in the future.

CONCLUSION

Ethanolic leaf extract of *Hyptis capitata* has the potential to be developed as a biolarvicidal agent against *C. quinquefasciatus*. *H. capitata* ethanolic leaf extract toxicity level against larvae of *Culex quinquefasciatus* is 990.90 µg/mL. *H. capitata* ethanolic leaf extract are capable of providing a toxic effect on larvae, with a mortality percentage of up to 50% more. Some compounds that are assumed to play a role in its activity include pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester; 1-heptadecyne; 9-tetradecen-1-ol, acetate; oxyrane, deodecyl-; 9,12,15-octadecatrienal; and 6,11-dimethyl-2,6,10-dodecatrien-1-ol. However, further studies are advised on its effects on non-target organisms.

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REFERENCES

Abaza AM. 2020. Phytochemical and insecticidal evaluation of agro-waste of *Lagenaria siceraria* (cucurbitaceae) plant against the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Egyptian Journal of Plant Protection Research Institute*. vol 3(2): 777–785.

Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*. vol 6(4): 1–23. doi: https://doi.org/10.3390/plants6040042.

Ardalan P, Arsalan P, Nazim M. 2017. Antibacterial and antioxidant activities of the volatile composition of the flower and fruit of *Solanum sisymbriifolium* (Litchi Tomato). *Pharmaceutical Sciences.* vol 23: 66–71. doi: https://doi.org/10.15171/PS.2017.10

Bobe G, Zhang Z, Kopp R, Garzotto M, Shannon J, Takata Y. 2020. Phytol and its metabolites phytanic and pristanic acids for risk of cancer: current evidence and future directions. *European Journal of Cancer Prevention*. vol 29(2): 191–200. doi: https://doi.org/10.1097/CEJ.0000000000000534.

Demirak MŞŞ, Canpolat E. 2022. Plant-based bioinsecticides for mosquito control: Impact on insecticide resistance and disease transmission. *Insects*. vol 13(2): 1–24. doi: https://doi.org/10.3390/insects13020162.

Dakum YD, Amajoh CN, Ombugadu A, Istifanus G, Agwom F, Joseph ST, Jwanse IR, Lapang PM, Kopdorah SW, Pam DD. 2021. Larvicidal efficacy and gc-ms analysis of *Hyptis suaveolens* leaf extracts against anopheles species. *International Journal of Biochemistry Research & Review*. vol 30(1): 8–19. doi: https://doi.org/10.9734/ijbcrr/2021/v30i130241.

Felhi S, Daoud A, Hajlaoui H, Mnafgui K, Gharsallah N, Kadri A. 2017. Solvent extraction effects on phytochemical constituents' profiles, antioxidant and antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peels fruits. *Food Science and Technology*. vol 37(3): 483–492. doi: https://doi.org/10.1590/1678-457x.23516.

Gandhi PR, Jayaseelan C, Mary RR, Mathivanan D, Suseem SR. 2017. Acaricidal, pediculicidal and larvicidal activity of synthesized zno nanoparticles using *Momordica* charantia leaf extract against blood feeding parasites. *Experimental Parasitology*. vol 181: 47–56. doi: https://doi.org/10.1016/j.exppara.2017.07.007.

Gouge DH, LiS, Walker K, Sumner C, Nair S, Olson C, Ramberg F. 2017. *Mosquitoes: Biology and Integrated Mosquito Management*. Tucson: The University of Arizona, pp. 1–12.

Ghosh A, Chowdhury N, Chandra G. 2012. Plant extracts as potential mosquito larvicides. *Indian Journal of Medical Research*. vol 135(5): 581–598.

Hansen SC, Stolter C, Imholt C, Jacob J. 2016. Plant secondary metabolites as rodent repellents: A systematic review. *Journal of Chemical Ecology*. vol 42(9): 970–983. doi: https://doi.org/10.1007/s10886-016-0760-5.

Irvine MA, Stolk WA, Smith ME, Subramanian S, Singh BK, Weil GJ, Michael E, Hollingsworth TD. 2017. Effectiveness of a triple-drug regimen for global elimination of lymphatic filariasis: a modelling study. *The Lancet Infectious Diseases*. vol 17(4): 451-458. doi: https://doi.org/10.1016/S1473-3099(16)30467-4.

Kim CW, Lee HJ, Jung JH, Kim YH, Jung DB, Sohn EJ, Lee JH, Woo HJ, Baek NI, Roca YC, Kim SH. 2015. Activation of caspase-9/3 and inhibition of epithelial mesenchymal transition are critically involved in antitumor effect of phytol in hepatocellular carcinoma cells. *Phytotherapy Research*. vol 29: 1026–1031. doi: https://doi.org/10.1002/ptr.5342.

Kruska N, Reiser G. 2011. Phytanic acid and pristanic acid, branched-chain fatty acids associated with Refsum disease and other inherited peroxisomal disorders, mediate intracellular Ca^{2+} signaling through activation of free fatty acid receptor GPR40. *Neurobiology of Disease*. vol 43(2): 465–472. doi: https://doi.org/10.1016/j.nbd.2011.04.020.

Kurniawan H, Ropiqa M. 2021. Uji toksisitas ekstrak etanol daun ekor kucing (*Acalypha hispida* Burm.f.) dengan metode *brine shrimp lethality test* (BSLT). *Journal Syifa Sciences and Clinical Research*. vol 3(2): 52–62. doi: https://doi.org/10.37311/jsscr.v3i2.11398.

Lopes AP, Bagatela BS, Rosa PCP. 2013. Antioxidant and cytotoxic effects of crude extract, fractions and 4-nerolidylcathecol from aerial parts of *Pothomorphe umbellata* L. (Piperaceae). *BioMed Research International*. vol 2013: 1–5. doi: https://doi.org/10.1155/2013/206581.

Mackled MI, El-hefny M, Bin-jumah M, Wahba TF. 2019. Assessment of the toxicity of natural oils from *Mentha piperita*, *Pinus roxburghii*, and *Rosa* spp. against three stored product insects. *Processes*. vol 7(861): 1–15. doi: https://doi.org/10.3390/pr7110861.

Mahesh-Babu S, Baranitharan M, Dhanasekaran S, Thushimenan S, Kovendan K, Jeyasankar A. 2016. Chemical compositions, antifeedant and larvicidal activity of *Pongamia pinnata* (L.) against polyphagous field pest, *Spodoptera litura*. *International Journal of Zoological Investigations*. vol 2(1): 48–57.

Meyer BN, Ferrigni NR, Putnam JE. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*. vol 45(1): 31–34. doi: https://doi.org/10.1055/s-2007-971236.

National Center for Biotechnology Information. 2022a. PubChem Compound Summary for CID 5363249, (E)-9-Tetradecen-1-ol acetate. Bethesda: NCBI. https://pubchem.ncbi.nlm.nih.gov/.

National Center for Biotechnology Information. 2022b. PubChem Compound Summary for CID 5283384, 9,12,15-Octadecatrienal. Bethesda: NCBI. https://pubchem.ncbi.nlm.nih.gov/.

Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, Hens, L. 2016. Chemical pesticides and human health: The urgent need for a new concept in agriculture. *Frontiers in Public Health.* vol 4: 1–8. doi: https://doi.org/10.3389/fpubh.2016.00148.

Nikbakhtzadeh MR, Buss GK, Leal WS. 2016. Toxic effect of blood feeding in male mosquitoes. *Frontiers in Physiology*. vol 7: 1–7. doi: https://doi.org/10.3389/fphys.2016.00004.

Padma M, Ganesan S, Jayaseelan T, Azhagumadhavan S, Sasikala P, Senthilkimar S, Mani P. 2019. Phytochemical screening and GC–MS analysis of bioactive compounds present in ethanolic leaves extract of *Silybum marianum* (L). *Journal of Drug Delivery and Therapeutics*. vol 9(1): 85–89. doi: https://doi.org/10.22270/jddt.v9i1.2174.

Pant P, Pandey S, Dall'Acqua S. 2021. The influence of environmental conditions on secondary metabolites in medicinal plants: a literature review. *Chemistry and Biodiversity*. vol 18(11): 1–42 doi: https://doi.org/10.1002/cbdv.202100345.

Panneerselvam, C., Murugan, K., Kovendan, K., Kumar, P.M. 2012. Mosquito larvicidal, pupicidal, adulticidal, and repellent activity of *Artemisia nilagirica* (Family: Compositae) against *Anopheles stephensi* and *Aedes aegypti*. *Parasitology Research.* vol 111: 2241–2251. doi: https://doi.org/10.1007/s00436-012-3073-9.

Paramasivam D, Balasubramanian B, Park S, Alagappan P, Kaul T, Liu W, Pachiappan P. 2022. Phytochemical profiling and biological activity of *Plectranthus amboinicus* (Lour.) mediated by various solvent extracts against *Aedes aegypti* larvae and toxicity evaluation. *Asian Pacific Journal of Tropical Medicine*. vol 13(11): 494–502. doi: https://doi.org/10.4103/1995-7645.295360.

Pattanshetty S, Kumar A, Kumar R, Rao CR, Badiger S, Rashmi R, Kamath S. 2010. Mass drug administration to eliminate lymphatic filariasis in Southern India. *Australasian Medical Journal AMJ*. vol 3(13): 847-850. doi: http//dx.doi.org/10.4066/AMJ.2010.479

Pavela R, Maggi F, Lannarelli R, Benelli G. Plant extract for developing mosquito larvicides: from laboratory to the fiels, with insight on modes of action. 2019. *Acta Tropica*. vol 193: 236–271. https://doi.org/10.1016/j.actatropika.2019.01.019.

Pejin B, Kojic V, Bogdanovic G. 2014. An insight nto the cytotoxic activity of phytol at *in vitro* conditions. *Natural Product Research*. vol 28: 2053–2056. doi: https://doi.org/10.1080/14786419.2014.921686.

Roca-Saavedra P, Mariño-Lorenzo P, Miranda JM, Porto-Arias JJ, Lamas A, Vazquez BI, 2017. Phytanic acid consumption and human health, risks, benefits and future trends: a review. *Food Chemistry*. vol 221: 237–247. doi: https://doi.org/10.1016/j.foodchem.2016.10.074.

Sampaio BL, Edrada-Ebel R, Da Costa FB. 2016. Effect of the environment on the secondary metabolic profile of *Tithonia diversifolia*: A model for environmental metabolomics of plants. *Scientific Reports*. vol 6: 1–11. doi: https://doi.org/10.1038/srep29265.

Senthil-Nathan, S. 2020. A review of resistance mechanisms of synthetic insecticides and botanicals, phytochemicals, and essential oils as alternative larvicidal agents against mosquitoes. *Frontiers in Physiology*. vol 10: 1–21. doi: https://doi.org/10.3389/fphys.2019.01591.

Simonsen PE, Mwakitalu ME. 2013. Urban lymphatic filariasis. *Parasitology Research*. vol 112(1): 35–44. doi: https://doi.org/10.1007/s00436-012-3226-x

Sumitha V, Mini I, Nair LS. 2021. Larvicidal efficacy of *Hyptis capitata* Jacq. against *Culex quinquefasciatus* mosquito (Culicidae). *International Journal of Mosquito Research*. vol 8(4): 42–46.

Thakor P, Subramanian RB, Thakkar SS, Ray A, Thakkar VR. 2017. Phytol induces ROS mediated apoptosis by induction of caspase 9 and 3 through activation of TRAIL, FAS and TNF receptors and inhibits tumor progression factor glucose 6 phosphate dehydrogenase in lung carcinoma cell line (A549). *Biomedicine & Pharmacotheraphy*. vol 92: 491–500. doi: https://doi.org/10.1016/j.biopha.2017.05.066.

The World Bioprotection Forum. 2021. Biopesticides: Natural alternatives to toxic chemicals for pest and disease control. Swansea: World BioProtection Forum. https://www.worldbioprotectionforum.com/.

Thi-Kim Nguyen Y, Moon JY, Ryu JY, Eum S, Bach TT, Cho SK. 2019. Methanol extract of aerial parts of *Pavetta indica* L. enhances the cytotoxic effect of doxorubicin and induces radiation sensitization in mda-mb-231 triple-negative breast cancer cells. *Molecules.* vol 24(12): 1–15. doi: https://doi.org/10.3390/molecules24122273.

To'bungan N, Jati WN, Zahida F. 2022a. Acute toxicity and anticancer potential of knobweed (*Hyptis capitata*) ethanolic leaf extract and fraction. *Plant Science Today*. vol 9(4): 955-962. doi: https://doi.org/10.14719/pst.1847.

To'bungan N, Pratiwi R, Widyarini S, Nugroho LH. 2022b. Cytotoxicity extract and fraction of knobweed (*Hyptis capitata*) and its effect on migration and apoptosis of t47d cells. *Biodiversitas*. vol 23(1): 572–580. doi: https://doi.org/10.13057/biodiv/d230162.

Widyawati S, Budianta TDW, Kusuma FA, Wijaya EL. 2014. Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* less leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research*. vol 6(4): 850–855.

World Health Organization. 2022. Lymphatic filariasis. Geneva: WHO. https://www.who.int/.

Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q. 2018. Response of plant secondary metabolites to environmental factors. *Molecules.* vol 23(4): 1–26. doi: https://doi.org/10.3390/molecules23040762.