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三種臺灣產植物之萃取物對埃及斑蚊幼蟲的殺蟲活性

Larvicidal activity of extracts from three Taiwanese plants

on *Aedes aegypti*

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MASTER'S THESIS ACCEPTANCE CERTIFICATE
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本論文係 黃麟傑 (R10632013) 在國立臺灣大學 昆蟲學研究所 完成之碩士學位論文，於民國 113 年 7 月 3 日承下列考試委員審查通過及口試及格，特此證明。

The undersigned, appointed by the Graduate Institute of Entomology on 3 (date) July (month) 2024 (year) have examined a Master's Thesis entitled above presented by HUANG, LIN-JIE (R10632013) candidate and hereby certify that it is worthy of acceptance.

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摘要



登革熱一直是臺灣重要的公共衛生問題，此病已在近數十年來爆發過多次疫情。病媒蚊防治能夠防止或減緩登革熱疫情擴散，而使用合成殺蟲劑的化學防治是常用的防治手段。然而，過度使用合成殺蟲劑導致了害蟲抗藥性、危害非目標生物、長期汙染等問題。取自植物的殺蟲化學物質被認為是良好的化學防治藥劑替代品，因為藉由其複雜的作用機制、對人畜可能較為安全、可生物分解等特性能彌補合成殺蟲劑的問題。本研究目標為測試茵陳蒿(*Artemisia capillaris*)、棕葉狗尾草(*Setaria palmifolia*)、血桐(*Macaranga tanarius*)的萃取物對登革熱病媒蚊埃及斑蚊(*Aedes aegypti*)幼蟲的毒性。本研究室先前的研究顯示這些植物的萃取物對臺灣蚊蠓(*Forcipomyia taiwana*)具有忌避效果，但是其對蚊科的效果尚未被探討過。萃取物由這些植物的葉片經過冷凍、磨粉、以己烷和乙酸乙酯萃取而成，並在實驗室條件下，以不同的濃度測試其對埃及斑蚊幼蟲的殺蟲效果。測試結果顯示僅有茵陳蒿的己烷萃取物能作為有效的蚊蟲幼蟲殺蟲劑，其能夠快速擊暈幼蟲，且其在 24 與 48 小時內對埃及斑蚊幼蟲的半數致死濃度分別為 187.60 ppm (v/v)與 128.31 ppm。以氣相層析質譜儀分析各萃取物的化學成分的結果也顯示，和其他殺蟲效果較弱的植物萃取物相比，茵陳蒿己烷萃取物含有最多種被指出具殺蟲活性的化學物質。本研究的延伸方向包含測試化學協同作用的存在與否、探究殺蟲的作用機制、開發可實地應用的蚊蟲幼蟲殺蟲劑劑型。本研究也能對從植物中找尋殺蟲劑的生物探勘做出貢獻。

關鍵詞：埃及斑蚊、殺幼蟲劑、植物萃取物、茵陳蒿、棕葉狗尾草、血桐

Abstract



Dengue is a major public health concern in Taiwan, with multiple outbreaks in recent decades. Mosquito vector control is an effective way of preventing or mitigating the spread of the disease, and chemical control with synthetic insecticides is a common practice. Overuse of synthetic insecticides had led to problems such as resistance, non-target effects, and long-term pollution. Insecticidal chemicals derived from plants are considered good alternative options for chemical control agents due to their complex modes of action, potentially better safety to humans and domestic animals, and biodegradability. This study aimed to test the larvicidal effects of leaf extracts of capillary wormwood (*Artemisia capillaris*), palmgrass (*Setaria palmifolia*), and parasol leaf tree (*Macaranga tanarius*) against dengue vector yellow fever mosquito (*Aedes aegypti*). A previous study in our laboratory suggested that extracts from these plants have repellency effects against biting midges (*Forcipomyia taiwana*), but their effects against mosquitoes were yet to be explored. The plant leaves were frozen, ground into powder, and extracted with hexane and ethyl acetate. These extracts were tested in laboratory assays against larval *Aedes aegypti* at different concentrations. Only *A. capillaris* hexane extract was found to be an effective larvicide, causing fast knockdown and having an LC₅₀ of 187.60 ppm (v/v) at 24 hours of exposure and 128.31 ppm at 48 hours of exposure. Gas-



chromatography mass-spectrometry (GC-MS) determined chemical compositions of the tested plant extracts found that, compared to other less effective extracts, *A. capillaris* hexane extract contains the most chemicals reported to be insecticidal. This study may be further extended by checking for synergistic effects, exploring the modes of action, and developing mosquito larvicide formulations for field use, and the study can contribute to the bioprospecting for pesticides in plant-derived chemicals.

Keywords: *Aedes aegypti*, larvicide, plant extract, *Artemisia capillaris*, *Setaria palmifolia*, *Macaranga tanarius*

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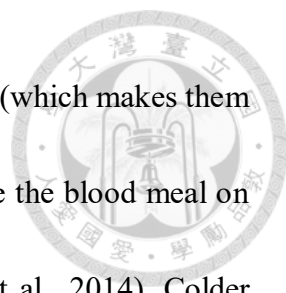


Introduction

I. Importance of dengue, its vector, and vector control

In Taiwan, mosquito (Culicidae) bites are not merely a common nuisance, but a major public health concern, as mosquitos are capable of vectoring various pathogens through the process of taking blood meals from vertebrate hosts, including humans and domesticated animals like dogs and pigs. Among the mosquito-borne diseases that have occurred or are of concern in Taiwan, dengue is the one whose information is most commonly disseminated by central and local government health departments. Even though the presence of the disease is not constant in Taiwan, outbreaks originating from imported cases occurred multiple times throughout the recent decades. Anti-viral medications against dengue virus do not yet exist (Lee et al., 2023), and the two extant vaccines for dengue virus are not yet approved in Taiwan, one of which is only situationally effective (Taiwan Centers for Disease Control, 2024). These constraints leave vector control the only effective option to prevent or mitigate the threat and damage of dengue in Taiwan.

Dengue are vectored by two species of mosquitoes in Taiwan: Yellow fever mosquito (*Aedes aegypti*) and Asian tiger mosquito (*Ae. albopictus*). *Ae. aegypti* is considered the more potent vector because of multiple factors, such as stronger preference to indoor



environment and human hosts, higher alertness during blood feeding (which makes them more likely to be disturbed and fly away from the host, and continue the blood meal on another host), and longer adult lifespan (Chow et al., 2005; Yang et al., 2014). Colder winter temperatures likely restrict the distribution range of *Ae. aegypti*, confining it to areas south of the Tropic of Cancer in Chiayi County in Taiwan, while *Ae. albopictus* is present in all areas below the altitude of 1500 m in the whole island of Taiwan. Such difference in distribution ranges is believed to be the reason why the frequency and intensity of dengue outbreaks are higher in southern Taiwan compared to northern Taiwan (Tsai & Teng, 2016). *Ae. aegypti* are known to mainly utilize water-filled artificial containers as breeding sources where their larvae grow (Chow et al., 2005). This means, at the larval stage their habitats are restricted to small bodies of water. For this reason, plus the fact that mosquitoes are generally more vulnerable in the larval stage, it would be ideal to target the larvae by applying chemical controls. Compared to adulticides, such container-based larvicides would have higher insecticidal efficacy and better specificity, along with smaller contamination areas (Kala et al., 2020).

II. Concerns regarding synthetic insecticides

Aside from habitat modification (removal of breeding sources), chemical control with synthetic insecticides is the most efficient way of controlling dengue vectoring



mosquitoes in both time and economic aspects. However, the overuse and improper use of synthetic insecticides have led to a number of concerns:

(1) Resistance: Repeated use of the same insecticide or insecticides with similar modes of action will eliminate susceptible strains of mosquitoes, while strains that are able to survive the exposure of such insecticides (such as ones with mutated molecular receptor sites, stronger metabolism that can detoxify insecticides, and ecologies or behaviors that make them less likely to be exposed to insecticides) keep reproducing and take up larger proportions of the local mosquito population, making the use of the said insecticide type ineffective. It has been reported that *Ae. aegypti* collected from the field in several districts in Kaohsiung City show various levels of resistance to commonly used insecticides, including multiple pyrethroids, multiple organophosphates, fipronil, and imidacloprid (Pai et al., 2023).

(2) Non-target effects: The toxic effects of insecticides (or any pesticides) normally do not only affect target organisms. Besides target pests, pesticides may also impact the health of neutral or beneficial organisms, or even humans when they are exposed to these chemicals. This can cause detrimental effects to the ecosystem, economy, and the health and welfare of humans. For example, cypermethrin is a commonly used insecticide used to control mosquitoes, but it is also highly toxic to fish (Ullah et al., 2018), making the use of the insecticide a potential threat to captive fishes in fish tanks, fish ponds, or fish

farms, and wild fishes in the natural waterbodies.

(3): Long-term pollution: The use of insecticides (or any pesticides) is a practice of intentionally polluting the environment. This makes it imperative that the effects of pesticides should be manageable, ensuring that the effect will only affect the space and time where the target pests are present, and to minimize non-target effects as much as possible. Some synthetic insecticides persist in the environment for too long, and spread out of the range they were applied in, causing unwanted non-target effects outside of the desired space and time. Some may even go through biomagnification, causing the synthetic pesticides to accumulate in high level consumers in the food web. In South Africa, where DDT was still actively used for mosquito control, DDT was found with high concentrations in African tigerfish (*Hydrocynus vittatus*, Characiformes: Alestidae), and human consumption of the contaminated fish can lead to high risk of cancer (Gerber et al., 2016).

(4) Regulations: Due to the aforementioned concerns, the regulations on synthetic insecticides are getting stricter, and some insecticides are or will eventually be banned. In Taiwan, chlorpyrifos, an insecticide that was originally approved to be used for mosquito control, was banned since April 2024 as an environmental pesticide, due to its risk to human health (Taiwan Environment Protection Administration, 2022), such as its link to damage to neural development (Nandi et al., 2022). Bans such as this suggest that the

options of synthetic insecticides to control disease-vectoring mosquitoes will start to run out.



III. Plant-derived chemicals as dengue vector control agent

Many plants produce chemicals that are toxic to insects as defensive mechanisms against herbivorous insects, and there's a long history of humans using these chemicals on the control of biting insects like mosquitoes (Pavela et al., 2019). These chemicals can usually be extracted from plant materials with distillation and/or solvents, yielding plant extracts such as essential oils. The advantages of using plant extracts as alternative chemical control agent over synthetic insecticides are:

(1) Higher difficulty of resistance development: The plant extracts are composed of multiple compounds, acting in multiple modes of action, and different compounds in a plant extract may have synergistic effects. Compared with a singular mode of action, against which one mutation may be enough to change the target pest's susceptibility, multiple mutations may be required for a strain of target pest to be able to survive all the complex insecticidal mechanisms in a plant extract, which is much less likely to happen (Rattan, 2010).

(2) Possible better safety to humans: Although many plant extracts are highly toxic to insects, many of them are also used as medicines, fragrances, and even food additives by

humans, as they have little to no noticeable toxicity against humans. The difference of toxicity to different species can be used as a selective chemical control method, suppressing mosquito populations while maintaining the safety of the humans exposed to the control agents. However, the risks to humans, domestic animals, and other non-target organisms for each plant extract should be tested for confirmation (Pavela et al., 2019).

(3) Biodegradable: Due to the plant-derived nature of plant extracts, the chemical compounds in them are biodegradable, preventing the concern of long-term pollution.

(4) Relaxed regulations and higher public acceptance: Due to past cases of synthetic insecticides causing substantial or potential damage to the human body or environment, the use of synthetic insecticides is under stricter regulations and is commonly seen as potential danger to health and environment by the general public. However, in some countries, regulations on insecticides that are considered to have lower risk to human health and the environment are getting relaxed, making the development of insecticides based on plant-derived chemicals more promising (Isman, 2015). Furthermore, they are often considered as the “safer options” by the general public. Although the risk to human health of synthetic insecticides use can be minimized with proper regulations and operation instructions, residents of epidemic areas might still refuse synthetic insecticide use because of fear. As breeding sources of *Ae. aegypti* are mainly water-filled containers in urban environments, the cooperation of local residents is an important part of dengue

vector control.



Nonetheless, there are also considerable drawbacks to using plant extracts as chemical control agents:

(1) Unstable quality: As plant extracts are derived from living organisms, the quantity and quality of each batch of plant extracts produced is not consistent. The concentration of active ingredients are highly dependent on factors such as growth stages and environmental conditions, but optimizations of such factors are underdeveloped (Damalas & Koutroubas, 2020).

(2) Short persistence: The chemically less stable and biodegradable nature of chemicals in plant extracts prevent them from causing long-term pollution, yet also means that plant extracts may not persist long enough in the environment to control target pests in a preferable period of time, resulting in a requirement of higher application rates (Damalas & Koutroubas, 2020).

(3) Higher cost: Unlike synthetic insecticides that can be mass-produced with relative ease, only requiring the chemical materials and synthesis processes that are generally well-established; the production of plant extracts is much more complicated, requiring growing or collecting plant materials for extraction, whose quantity and quality can be uncertain. The lower chemical stability of plant extracts can also increase the cost, as shorter shelf life limits the cost-effectiveness, and the low environmental persistence

leads to a need for repeated application to reach the desired control efficacy (Damalas & Koutroubas, 2020).



This study sought to identify new plant extracts for larvicidal use against *Ae. aegypti*. The species of plants chosen for this study have recorded traditional medicinal uses by aboriginal peoples in Taiwan as well as other countries, and are all obtainable in Taiwan. In a previous study done in our laboratory, a selection of extracts of these plants showed strong repellent or knockdown effects against biting midges (*Forcipomyia taiwana*, Ceratopogonidae). While some of these potent extracts had already been tested against mosquitoes before, others had not, making the extracts of these plant our high-priority materials for mosquito larvicidal tests (Guo et al., 2023).

The species include: (1) Capillary wormwood (*Artemisia capillaris*, Asteraceae), palmgrass (*Setaria palmifolia*, Poaceae), and parasol leaf tree (*Macaranga tanarius*, Euphorbiaceae). As these plants are all available in Taiwan, they can be readily procured for plant extract production, even on a small scale by individuals trying to make their own botanical repellents.

Materials and Methods

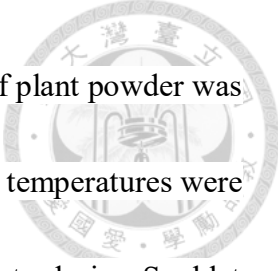


I. Plant sample collection

The plant materials were originally collected for the previous study on biting midges (Guo et al., 2023) as follows: *A. capillaris* and *S. palmifolia* on August 6th, 2021, in Taipingshan National Forest Recreational Area (24°29'52" N 121°32'06" E); *M. tanarius* on October 27th, 2020, in National Taiwan University campus (25°01'07.3" N 121°32'36.3" E). *A. capillaris* and *S. palmifolia* were identified by Taipingshan National Forest Recreational Area staffs Lee, Chi-Chun and Tsai, Ming-Zhi; *M. tanarius* was identified by identification marker plaque. Voucher specimens of the collected plants were made and preserved in National Taiwan University Herbarium. The plant leaves were cut with gardening shears, collected in sealable plastic bags, and preserved as frozen samples (kept under -4 °C or -20 °C) before extraction.

II. Plant sample extraction

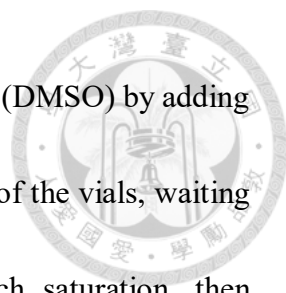
The plant extraction was done for the previous study (Guo et al., 2023), but the methods are summarized here to facilitate understanding. In brief, for each plant species, frozen plant leaf sample was ground into powder with a Chinese medicine grinder (Yuzako Co., Ltd., Changhua County, Taiwan), and 50 grams of powder was then put into a custom-made Soxhlet extractor for extraction. Each sample was first extracted with



hexane heated to 70 °C for 24 hours, then the remaining solid phase of plant powder was extracted again with ethyl acetate heated to 78 °C for 24 hours. These temperatures were chosen because the solvents need to be heated to their boiling points during Soxhlet extraction (Redfern et al., 2014). Each of the resulting plant extract oils typically consisted of a light phase (usually green, containing essential oils) and a heavy phase (usually orange colored, containing proteins, waxes, and water), which were separated using a separator funnel. An exception was the hexane extract of *A. capillaris*, which did not separate into a light phase and heavy phase and was yellow-brown, whereas the other extracts mentioned had a distinct, dark green light phase. Then the oil in the light phase was separated from the solvent under a vacuum using a rotary evaporator, and stored in amber vials in the dark at room temperature for later use. These stored plant extracts all had noticeable scents even at the time of the present experiment.

III. Plant extract solution preparation

The plant extracts chosen for this study were *A. capillaris* hexane and ethyl acetate extract, *S. palmifolia* hexane and ethyl acetate extract, and *M. tanarius* ethyl acetate extract. The *M. tanarius* hexane extract produced in the previous study (Guo et al., 2023) had depleted and could not be included. The plant extracts were dried in their storage vials under room temperature, under a fume hood to speed up the process if needed. The



dry residues of plant extracts were dissolved with dimethyl sulfoxide (DMSO) by adding a small amount (approximately 2 ml each time) of DMSO into each of the vials, waiting for DMSO to dissolve the plant extract solid residues and reach saturation, then transferring the resulting solutions out as 100% stock solutions (Pavela, 2008). Due to differences of dissolving speed of ethyl acetate extracts and hexane extracts, the waiting times were 30 to 40 minutes and 24 hours, respectively. The process was repeated until most (but not all) solid residues were dissolved, to prevent getting unsaturated solutions. These stock solutions were then diluted with DMSO to the desired concentrations.

IV. Mosquito rearing

The mosquitoes were an *Ae. aegypti* strain sourced in Tainan City by Taiwan CDC, provided by Dr. Tsai, Kun-Hsien of the Vector-borne Infectious Diseases Lab, College of Public Health, National Taiwan University. The larvae used for bioassays were kept in RO water inside an incubator set with the conditions of 28 °C, 80% relative humidity, and 16:8h (light/dark) light cycle, fed *ad libitum* with crushed goldfish feed every day. The adult mosquitoes were kept in BugDorm® collapsible insect rearing cages, fed with 10% w/v sugar water changed twice a week, and the blood meals were provided by putting the author's forearm in the cage for 15 to 20 minutes once a week (Peach et al., 2019). The oviposition site was prepared with a 125 ml plastic cup approximately 20% full with RO

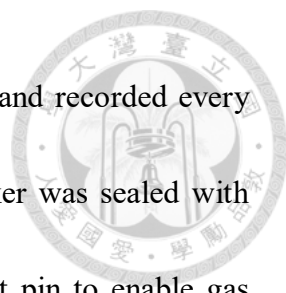
water and lined with filter paper on the inner side. The filter papers with eggs were collected twice a week, dried under room temperature, marked with the collection date, and stored in a plastic box inside Ziploc bags for future use.



V. Larvicidal assay

Based on guidelines provided by WHO (World Health Organization, 2005), the larvicidal bioassays were conducted in 250 ml beakers. Each beaker contained RO water added to reach a total of 100mL after the addition of 25 late third-instar to early forth-instar (three to five days old, ideally four days old) *Ae. aegypti* larvae, five grains of goldfish feed as larval food, and either 0.1mL DMSO (negative control), 0.1mL of DMSO-plant extract solution, or 0.2mL of 50% v/v DMSO aqueous solution suspended with VectoBac WDG, a commercially available *Bacillus thuringiensis israelensis* (*Bti*) mosquito larvicide product, producing a ratio of six g/l as suggested by the product instructions (positive control) (Manzano et al., 2020). The *Bti* product does not require DMSO to apply, but DMSO was added for consistency with negative control and experimental groups. The *Bti* suspension used in the positive control group was stirred with a spatula before each use. Each plant extract-concentration combination and both types of control groups in every bioassay were tested in triplicates.

Once the beakers were all set, they were placed in an incubator with the same



conditions as larva rearing. The status of the larvae was examined and recorded every hour for the first 6 hours since the bioassay began, then each beaker was sealed with plastic wrap and 25 holes poked into the wrap with a No. 5 insect pin to enable gas exchange while preventing outside contamination, overly rapid evaporation, and adult mosquitoes escaping in the following days. The statuses of the larvae were then recorded every 24 hours. For initial tests, the observations were done until 10 days (240 hours) after each bioassay began; for further hit-and-trial and toxicity determining tests, the observations were done until four days (96 hours) after each bioassay began. Three grains of goldfish feed were added as supplementary food to each beaker containing living larvae every 24 hours since the 48th hour after the bioassay began, until (1) the beaker contained at least one adult mosquito capable of flying, (2) no living larvae were seen in the beaker, or (3) the bioassay ended.

The statuses recorded were as follows. (1) Alive: Larva shows locomotion ability and is able to reach the water surface. (2) Inactive: Larva is seen moving or twitching, but lost locomotion ability or sinks to the bottom of the beaker when resting. This status also includes individuals that appear moribund, which sink to the bottom the beaker, curl to the dorsal side, and whose only visible movements are twitching every few seconds and/or mouthpart movements. (3) Dead: Larva does not respond to vibration and gentle probing with a disposable dropper, or shows signs of decay, such as blackening. Any

missing larvae after the first six hours were considered dead as well, due to the larvae's tendency of cannibalizing their dead. While some suggest counting head capsules to check for cannibalism, this was not feasible as the shed head capsules of molted larvae could be mistaken for dead larvae.

The set of plant extract concentrations used in initial tests were 500 ppm (99.9 ml of RO water, 0.05 ml of 100% stock solution, 0.05 ml of DMSO in each beaker) and 1000 ppm (99.9 ml of RO water and 0.1 ml of 100% stock solution).

The data obtained were analyzed by Dr. Tsai, Chen-An in Division of Biometrics, Department of Agronomy, College of Bio-resources & Agriculture, National Taiwan University, using R 4.4.1. The effects of dose and time were analyzed with two-way repeated measures ANOVA, and Tukey's HSD tests was used for *post hoc* analyses on the dose effect on the observation of each day.

VI. Toxicity determination

To determine the LC_{50} and LC_{90} of the most effective test compound, *A. capillaris* hexane extract, the concentrations tested were decided as 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm after a series of hit-and-trial larvicidal assays. The recorded mortality rates of each of the concentrations on the 24th and 48th hours after the bioassay began were organized for LC_{50} and LC_{90} calculations.

Toxicity analyses were also done by Dr. Tsai Chen-An with logit dose-response according to previous studies (Finney, 1971; Russell et al., 2007; Wheeler et al., 2006), using R 4.4.1 to calculate LC₅₀ values, LC₉₀ values and their 95% confidence interval.



As the World Health Organization does not have standards for evaluating the effectiveness of plant-derived mosquito larvicides, some researchers have set the standards on their own. Komalamisra et al. (2005) defined the effectiveness of a plant extract against mosquito larvae as highly active when the LC₅₀ is below 50 ppm; moderately active when the LC₅₀ is between 50 ppm and 100 ppm; effective when the LC₅₀ is between 100 and 750 ppm; and ineffective when the LC₅₀ is above 750 ppm (Komalamisra et al., 2005). For ease of measurement, this study used the v/v measurement of ppm (μl/l) instead of w/v measurement (mg/l) in Komalamisra et al.'s study.

VII. Plant extract chemical composition analysis

This analysis was done for the previous study (Guo et al., 2023), and the detailed methods are described there. In brief, the chemicals in the plant extracts were identified and had their relative abundance measured using an Agilent Technologies 7890A-5975C gas chromatography mass spectrometer (GC-MS) in National Taiwan University. The identification of chemicals was done based on a NIST library search.



For this study, the chemicals detected in the plant extracts were checked for insecticidal efficacy or presence in reported insecticidal plant products by searching the online literature. Confirmed or potentially insecticidal chemicals were then listed based on their presence and abundance in each plant extract. To compare the potential for insecticidal efficacy based on chemical composition, for each extract the count of kinds and total area percentage of all the chemicals with known or possible insecticidal activity as reported in literature were summed up.

Results

I. Larvicidal assays

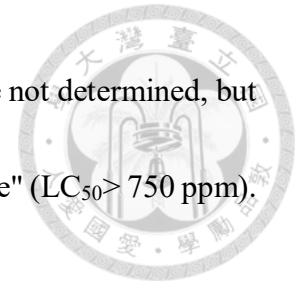
The results of the initial tests suggest concentration-dependent responses of *Ae. aegypti* larvae to all of the selected plant extract solutions, with growth delay, feeding inhibition, and mortality observed at various levels. *A. capillaris* ethyl acetate extract had no significant larvicidal effect and only showed significantly delayed adult emergence within the 4th and 5th day of observation (Figure 1a; Figure 2a). *A. capillaris* hexane extract showed the strongest larvicidal effect in the initial tests (Figure 1b; Figure 2b), putting all larvae into an inactive state within one hour and killing all larvae within 24 hours in both tested concentrations. *S. palmifolia* ethyl acetate extract, *S. palmifolia*

hexane extract, and *M. tanarius* ethyl acetate extract all had significant effects on the mortality rates and adult emergence rates, but the effects were considered low because of the prolonged larva-killing time and observed adult emergence under the high concentration treatments of 500 ppm and 1000 ppm (Figure 1c-e; Figure 2c-e). Thus, further tests to determine LC₅₀ and LC₉₀ values were only done on *A. capillaris* hexane extract.

Through a series of hit-and-trial tests, the concentrations used to determine the toxicity of *A. capillaris* hexane extract against *Ae. aegypti* larvae were determined to be 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm; as nearly no mortality was observed at 10 ppm and 25 ppm, yet nearly all larvae died within 24 hours at 300 ppm. The lethal concentrations were calculated based on the experimental data of three toxicity determination tests (Figure 3), plus data from one hit-and-trial test with 300 ppm included *post hoc* as a correction. The LC₅₀ values were as follows: 187.60 ppm at 24 hours, with a 95% confidence interval of (172.14, 203.07); and 128.31 ppm at 48 hours, with a 95% confidence interval of (118.71, 137.90) (Figure 4). The LC₉₀ values were as follows: 526.24 ppm at 24 hours, with a 95% confidence interval of (410.15, 642.33); and 332.26 ppm at 48 hours, with a 95% confidence interval of (281.90, 382.62) (Figure 4)

Based on the standards set by Komalamisra et al. (2005), the efficacy of *A. capillaris* hexane extract falls in the category of "effective" plant extracts (100 ppm <

LC₅₀ < 750 ppm). The LC₅₀ values of other plant extracts tested were not determined, but judging by the initial test results, they should be classed as "ineffective" (LC₅₀ > 750 ppm).



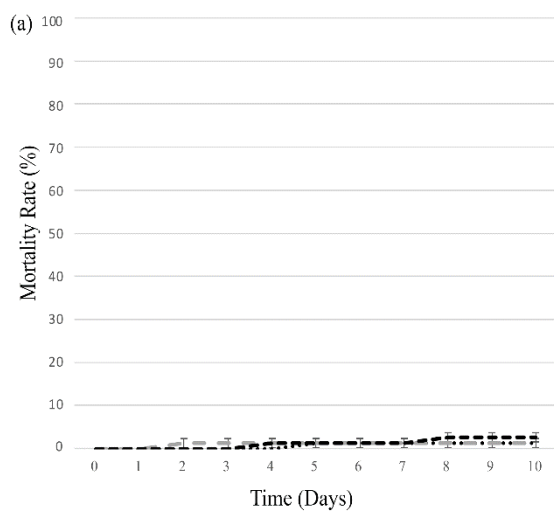
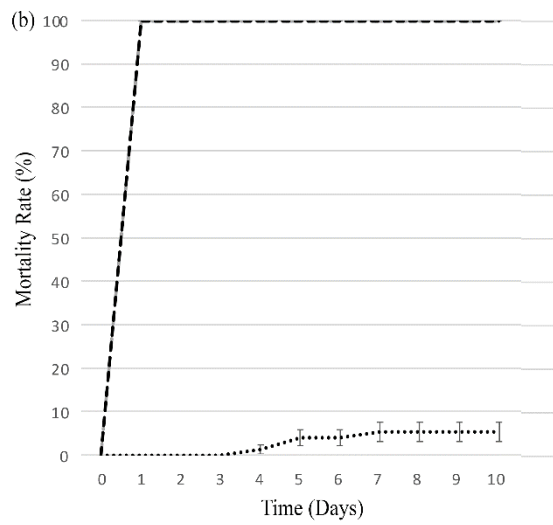
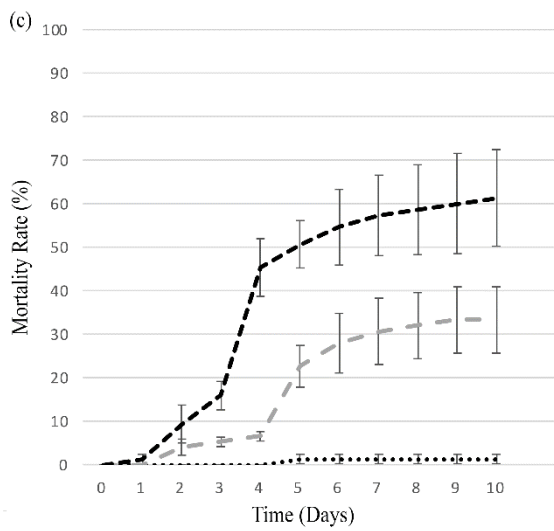
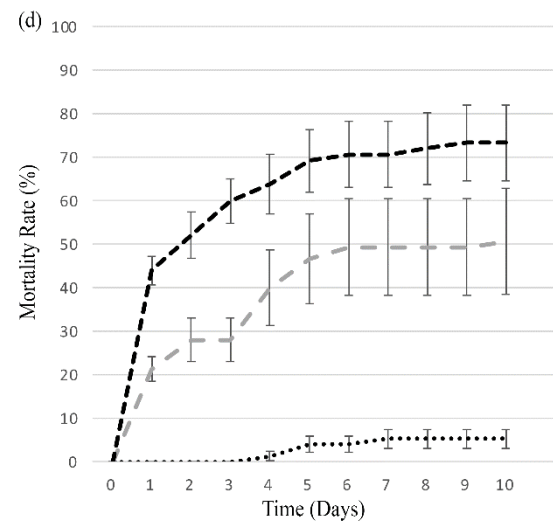
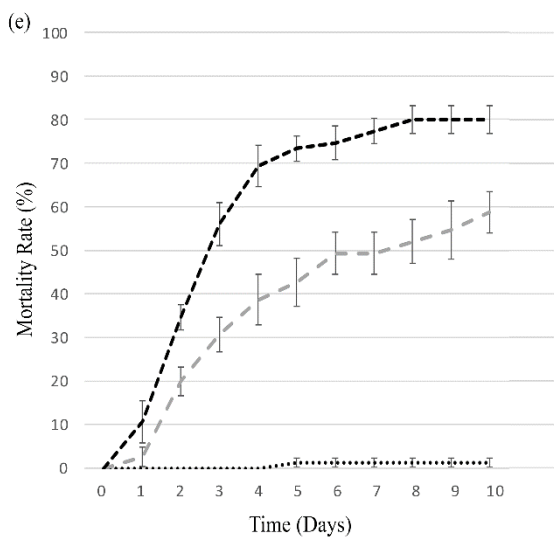
II. Effects of A. capillaris hexane extract on Ae. aegypti larvae

When exposed to sufficient concentrations (around 100 to 150 ppm or more) *A. capillaris* hexane extract, some of the *Ae. aegypti* larvae turned inactive within one hour. The inactive larvae were either unable to float to the surface, or completely lost locomotion ability, only twitching on the bottom of the beaker. Even at lower concentrations such as 50 ppm, within the first few hours all larvae showed visible lethargy with slowed down movements, and some larvae exhibited behavioral abnormalities such as repeatedly touching their rear end with their mouthparts, being trapped on the surface, and taking a dorsal side-down position (with the breathing tube pointing downwards). These effects could be observed in the *A. capillaris* hexane extract groups earlier than in positive control groups, where inactivity occurred after two to three hours, just as described on the *Bti* product label. However, unlike the *Bti* positive control groups, in which all inactive larvae would subsequently die, some of the larvae treated with *A. capillaris* hexane extract at these concentrations (50 to 250 ppm) were observed recovering 24 hours after treatment, even including a few individuals that originally appeared moribund.



III. Known insecticidal compounds in the plant extracts

The chemical compositions of the plant extracts were analyzed in the previous study (Guo et al, 2023), and the data from that study are listed in appendix table, along with new data on whether or not each chemical is reported in insecticidal plant products from published studies. For improved readability and ease of analysis, Table 1 shows only the chemicals reported in insecticidal plant products, listing their abundance (represented by the area percentages of the chemicals in the GC-MS analysis graphs) in each of the tested plant extracts. Of the top 20 most abundant compounds in each extract, *A. capillaris* hexane extract had the most shared with known insecticidal plant extracts (16/20, comprising 32.67% of the extract), of which seven (comprising 8.29% of the extract) had been tested as pure compounds in prior studies and are known to be insecticidal. Eleven of these 16 compounds were not found in other extracts, including five with verified insecticidal activity: D-limonene, linalool, nerolidol, nonacosane, and γ -Terpinene.



..... Negative Control
 - - - 500 ppm
 - . - 1000 ppm

Figure 1. Daily larval/pupal mortality rate of *Ae. aegypti* in the initial tests. In the experimental groups, the larvae were under the exposure of 500 ppm and 1000 ppm of (a) *A. capillaris* ethyl acetate extract (b) *A. capillaris* hexane extract (c) *S. palmifolia* ethyl acetate extract (d) *S. palmifolia* hexane extract (e) *M. tanarius* ethyl acetate extract.

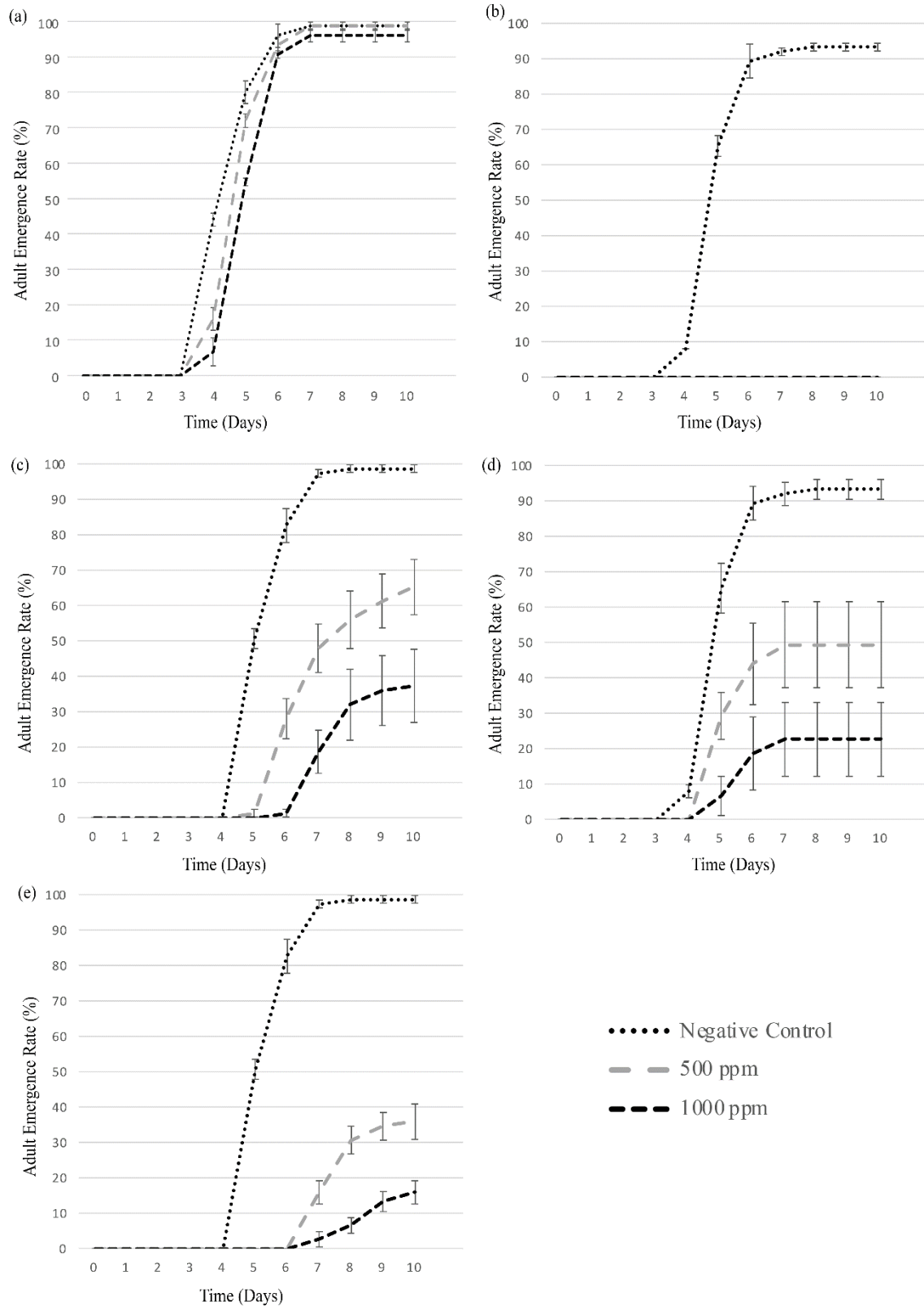
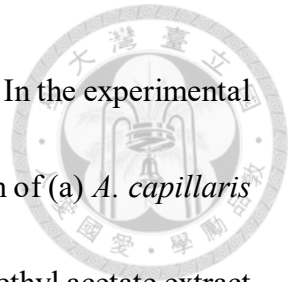


Figure 2. Daily adult emergence rate of *Ae. aegypti* in the initial tests. In the experimental groups, the larvae were under the exposure of 500 ppm and 1000 ppm of (a) *A. capillaris* ethyl acetate extract (b) *A. capillaris* hexane extract (c) *S. palmifolia* ethyl acetate extract (d) *S. palmifolia* hexane extract (e) *M. tanarius* ethyl acetate extract.



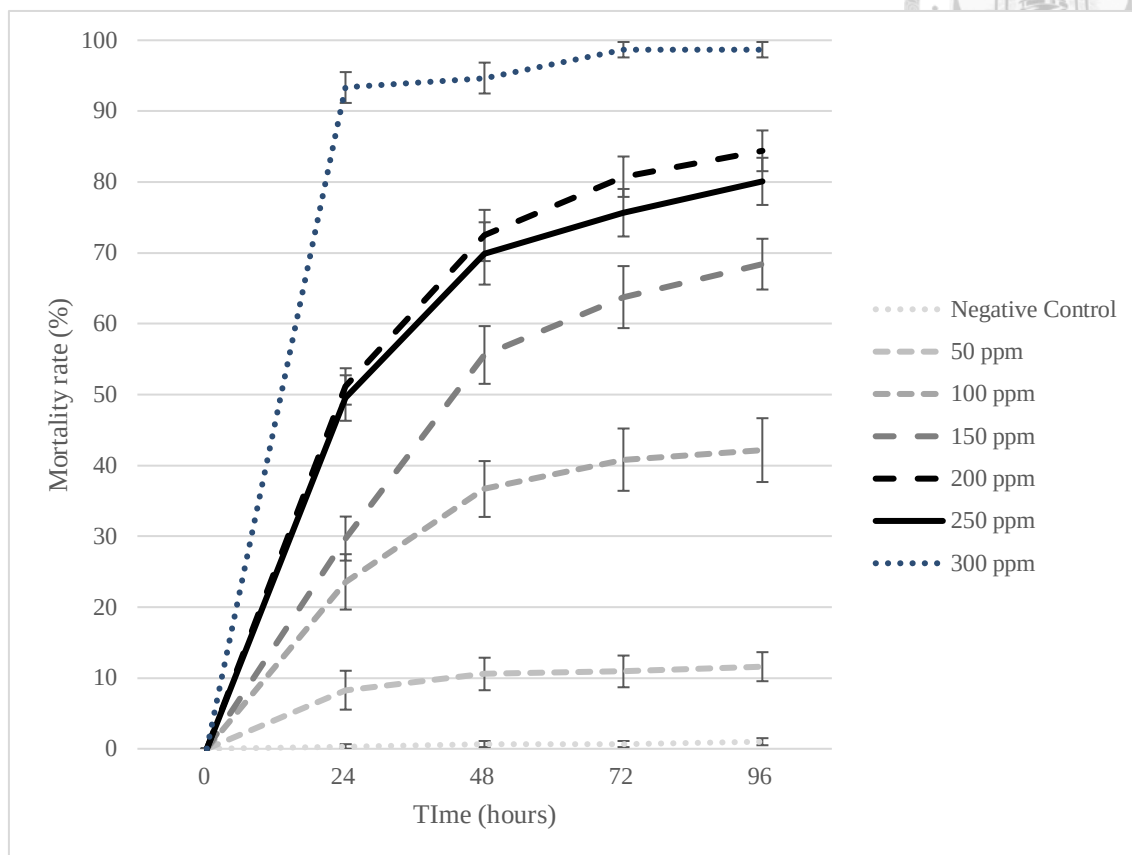


Figure 3. Larval/pupal mortality rate of *Ae. aegypti* exposed to *A. capillaris* hexane extract in three toxicity determining tests and one hit-and-trial test added for *post hoc* correction.

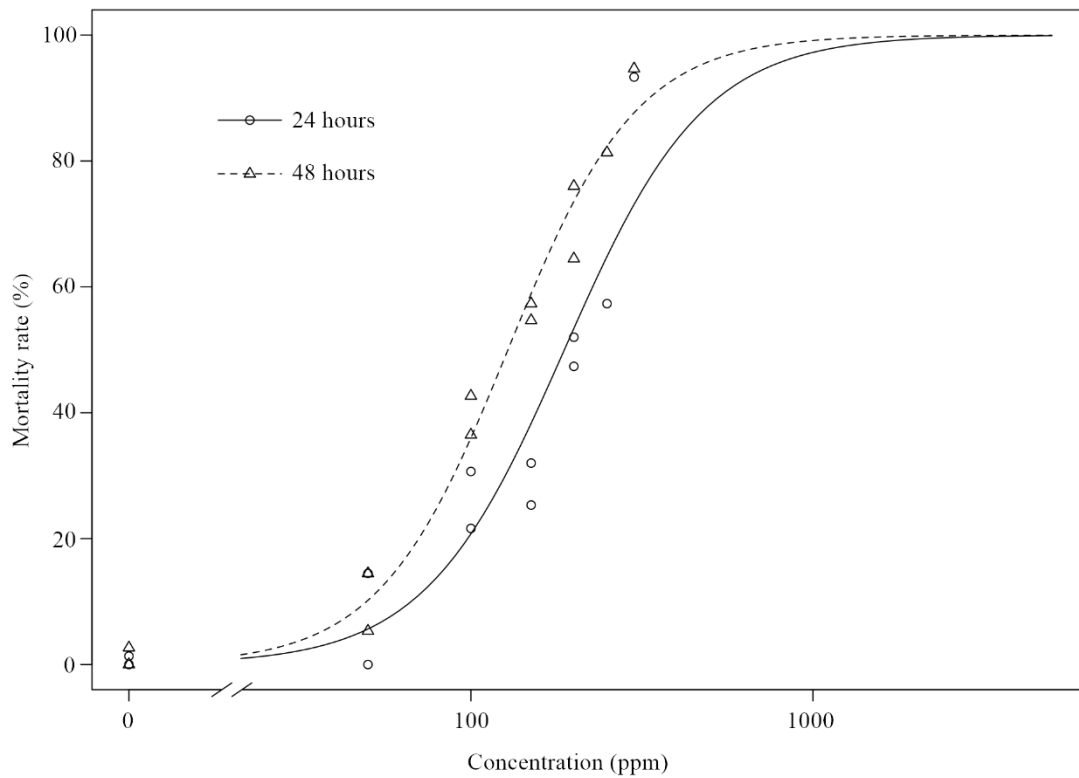


Figure 4. The dose-response curves of larval mortality rate of *Ae. aegypti* exposed to *A. capillaris*, graph made by Dr. Tsai, Chen-An using logit dose response analysis.

Table 1. The list of chemicals previously reported from insecticidal plant products also found in the tested plant extracts and their area abundance. This data is organized from the contents of Appendix 1. Chemicals marked with an asterisk and grey background were tested individually in the literature and have known insecticidal activity: the others are hypothesized to be insecticidal due to their presence in a plant with verified insecticidal activity. A dash means the chemical was not in the top 20 most abundant chemicals for that plant extract. “Unique” denotes chemicals that were found in the list of only one of the plant extracts tested in this study.

Chemical \ Plant extract	<i>A. capillaris</i> ethyl acetate extract	<i>A. capillaris</i> hexane extract	<i>S. palmifolia</i> ethyl acetate extract	<i>S. palmifolia</i> hexane extract	<i>M. tanarius</i> ethyl acetate extract
*1,2,3-Benzenetriol	-	-	-	-	1.0503%
1-Benzyl-3-[2-(4-methoxyphenyl)ethyl]thiourea	-	4.1575%	-	-	-
*Caryophyllene	4.153%	2.527%	-	-	-
Chavibetol	-	0.7164%	-	-	-
*Coumaran	-	-	0.7215%	-	-
6,7-Dimethoxyquinoxaline-2,3-diol	1.3173%	-	-	-	-
β-Eudesmol	-	3.5376%	-	-	-
Hexadecanamide	-	-	-	1.1571%	0.862%
*n-Hexadecanoic acid	1.2237%	-	3.9533%	1.3407%	1.1375%
*Humulene	1.4466%	1.1003%	-	-	-
*D-Limonene	-	0.6105%	-	-	-
*Linalool	-	1.133%	-	-	-
*Linoleic acid	-	-	-	0.6507%	-
α-Linolenic acid	1.1107%	0.7634%	3.1214%	4.112%	1.517%

Lupenone	-	-	-	-	1.6301%
2-Monostearin	1.8262%	7.3764%	4.5288%	-	-
γ -Muurolene	-	1.3174%	-	-	-
*Nerolidol	-	0.6598%	-	-	-
*Nonacosane	-	1.0076%	-	-	-
1,3,12-Nonadecatriene	-	-	0.8455%	-	-
trans- β -Ocimene	-	1.2037%	-	-	-
Octadecanamide	-	-	-	0.6986%	-
Octadecanoic acid	1.1924%	-	2.0521%	-	0.8929%
*9-Octadecyne	-	-	1.437%	-	-
Oleamide	5.3389%	3.5414%	11.9132%	17.6878%	8.4602%
*Phytol	-	-	3.0375%	4.8242%	1.5815%
*(+)- α -pinene	-	-	0.7564%	-	-
Scopoletin	2.2835%	-	-	-	-
Stigmasterol	-	-	1.6389%	2.8276%	-
* γ -Terpinene	-	1.2533%	-	-	-
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	-	-	-	0.3103%	-
β -turmerone	-	1.6614%	-	-	-
Total # of potentially insecticidal chemicals	9	16	11	9	8
Area percentage	19.8923%	32.5667%	34.0056%	33.609%	17.1315%
Total # of chemicals with verified insecticidal activity	3	7	5	3	3
Area percentage	6.8233%	8.2915%	9.9057%	6.8156%	3.7693%
Number of unique chemicals	2	11	4	3	2
Area percentage	3.6008%	17.2582%	3.7604%	1.6596%	2.6804%
Unique chemicals with verified insecticidal activity	0	5	3	1	1
Area percentage	0%	4.6642%	2.9149%	0.6507%	1.0503%

Discussion



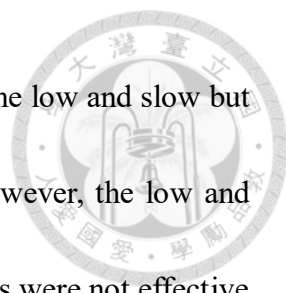
Judging by the larvicidal assay results, exposure to *A. capillaris* hexane extract can quickly cause inactivity in *Ae. aegypti* larvae, even more rapidly than *Bti*. However, the observed phenomenon of larvae recovering from inactivity suggest the low persistence of *A. capillaris* hexane extract in the tests, allowing the mosquito larvae to survive if the extract did not cause mortal damage to them in the first few hours. Larvae dying after more than 24 hours of exposure thus were likely directly or indirectly killed by damage taken during the initial hours of exposure, but the level of damage was not high enough to kill them within 24 hours and they only succumbed later. The extract could thus be potentially used as a fast-acting larvicide, so long as the dose is high enough (at least 300 ppm, ideally higher), though this would need to be verified with field trials. The other chemicals are not effectively larvicidal, at least not in a field viable dose.

Comparing the compounds found in GC-MS analyses with the information in the literature online, *A. capillaris* hexane extract was found to have the most (16 out of 20) compounds present in other plant extracts/essential oils with known insecticidal effects, seven of which were tested individually and reported insecticidal: caryophyllene (Govindarajan et al., 2016), γ -terpinene (Gong & Ren, 2020), linalool (Fujiwara et al., 2017), humulene (Govindarajan & Benelli, 2016), nonacosane (Junhirun et al., 2018; Silva et al., 2023), nerolidol (Di Campli et al., 2012), and d-Limonene (Tripathi et al.,

2003). Only two of the confirmed insecticidal compounds in *A. capillaris* hexane extract (caryophyllene and humulene) were also found in *A. capillaris* ethyl acetate extract, and the other five were only found in *A. capillaris* hexane extract.



Other plant extracts contained fewer chemicals with known or hypothesized insecticidal effects. *A. capillaris* ethyl acetate extract has nine chemicals found in reported insecticidal plant extracts, three of which were confirmed insecticidal: caryophyllene, humulene and n-hexadecanoic acid (Ragavendran & Natarajan, 2015); *S. palmifolia* ethyl acetate extract has 11, five of which were confirmed insecticidal: n-hexadecanoic acid, phytol (Benelli et al., 2020), 9-octadecyne (Dhivya et al., 2018), (+)- α -pinene (Pohlit et al., 2011), and coumarin (Rajashekar, 2016); *S. palmifolia* hexane extract has nine, three of which were confirmed insecticidal: phytol, n-hexadecanoic acid, and linoleic acid (Ramsewak et al., 2001); *M. tanarius* ethyl acetate extract has eight, three of which were confirmed insecticidal: phytol, n-hexadecanoic acid, and 1,2,3-benzenetriol (Amala & Jeyaraj, 2014). N-hexadecanoic acid is on the top 20 list of every plant extract used except the most effective *A. capillaris* hexane extract; phytol, which was reported toxic to aphids (Benelli et al., 2020), can be found in the extracts from *S. palmifolia* and *M. tanarius*. Stigmasterol was found in both extracts from *S. palmifolia*. The chemical was found cytotoxic to cotton leafworm (*Spodoptera litura*, Lepidoptera: Noctuidae) cells, but did not cause obvious mortality when applied to *Ae. albopictus* larvae (Huang et al., 2009).



The presence of these known insecticidal chemicals likely explains the low and slow but noticeable larvicidal effects of those extracts in the initial tests. However, the low and slow effects suggest either these chemicals/combinations of chemicals were not effective against *Ae. aegypti* larvae, or that these plant extracts are not good sources of these insecticidal chemicals.

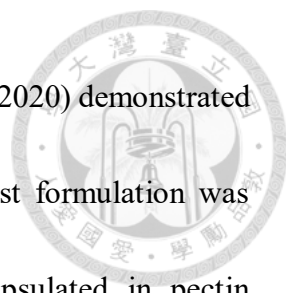
Unexpectedly, the *M. tanarius* ethyl acetate extract appeared to be the second most effective plant extract in the initial tests, but its proportions of putatively or known insecticidal chemicals were lower than the others. This might suggest that some compounds in the extract have considerable insecticidal effects that may or may not have been reported before, or that synergistic effects caused a stronger larvicidal effect than expected. Alternatively, the residual ethyl acetate used as the initial solvent in the extract had affected the results, as ethyl acetate is insecticidal, and, unlike other samples, the *M. tanarius* ethyl acetate extract was still dissolved in ethyl acetate after storage while the other ethyl acetate extracts had become dry residues during storage. The fume hood was used to evaporate the ethyl acetate in the *M. tanarius* sample, but this removal may not have been as thorough as expected. Nonetheless, *M. tanarius* ethyl acetate extract is not nearly as effective as the *A. capillaris* hexane extract, and likely is not worth further investigation.

Oleamide was present in all of the plant extracts used, and is reported in plant-

derived insecticidal products, but it is also non-volatile and can leak out from laboratory plastic wares (McDonald et al., 2008), which means it might be unavoidable contamination from plastic wares used in the extraction process. In any case, the low effectiveness of most of the extracts tested suggest that oleamide was not a significantly larvicidal component.

Now that *A. capillaris* hexane extract has been identified as an effective larvicide, further studies can go into several directions. One is to separate the plant extract into partitions and test whether their efficacy will be higher or lower than the whole plant extract to determine if the component chemicals should be purified for higher efficacy (and identify the active compounds) or if the whole plant extract should be used due to synergistic effects (Ghosh et al., 2012). Another direction is to seek insights on the modes of actions of the effective plant extract or its partitions. Methods such as testing for differences of gene expressions of control and affected larvae (Muturi et al., 2020), or using microscopy to check for tissue damages in affected larvae can be used to explore modes of actions (Kala et al., 2020).

A third research direction is to design formulations. Given that plant extracts have the aforementioned drawbacks as chemical control agents, it is crucial to design formulations that can both strengthen and lengthen their efficacy, and make them easy to use. Encapsulation with polymer materials appears to be a promising approach, as it this



enables slow release of the essential oils. A study in India (Kala et al., 2020) demonstrated an easy-to-use essential oil mosquito larvicide formulation: the test formulation was Himalayan cedar (*Cedrus deodara*, Pinaceae) essential oil encapsulated in pectin nanoparticles, which were then bound to cotton tea bags and used as a larvicide against the malaria vector *Anopheles culicifacies*. This larvicide was applied by dropping a treated tea bag into waterbodies infested by their larvae. Given that a majority of breeding sources of *Ae. aegypti* are water-filled artificial containers, a plant extract formulation with similar application methods would be a fitting method of *Ae. aegypti* larva control. The user simply needs to take out one portion of the solid formulation and drop it in an infested container. It barely requires any training to apply, enabling the residents of epidemic areas or high-risk areas to do vector control by themselves.

The forth direction is synthesizing the effective chemicals or their analogues as novel synthetic insecticides. The active ingredients in the plant extracts or their analogues with similar effects might be synthesized, eliminating the need for harvesting plants and complex extraction procedures for oil production. These synthesized compounds can be used to produce novel synthetic insecticides, either in the form of conventional insecticides with singular active ingredients, or artificial essential oils as mixtures of multiple compounds with synergistic effects. If the synthesized compounds are the same as the active compounds in the plant extracts, the resulting products should have some of

the same benefits of chemicals derived from natural plants like lower human health risk and biodegradability, but with better stability and lower cost.



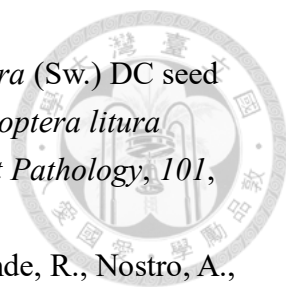
The final research direction is discovering more plant species with effective extracts.

Researchers can find plants containing chemicals with desired insecticidal effects more efficiently by referencing previous studies. The approaches can be based on ethnobotany like the original *F. taiwana* study (Guo et al., 2023), or the use of previously known insecticidal plants against different insect targets as was done in this study. Approaches to find novel plants with possible insecticidal activity can be based on taxonomy (finding plant species closely related to the ones found to be effective) or the chemical composition of the plant extracts. Data on effective botanical insecticides can thus help people in different geographical regions find locally available plants with desired toxic effects against their local pests.



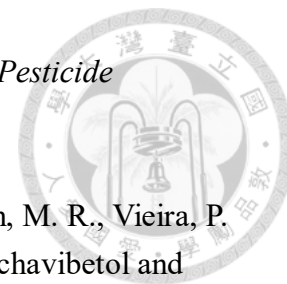
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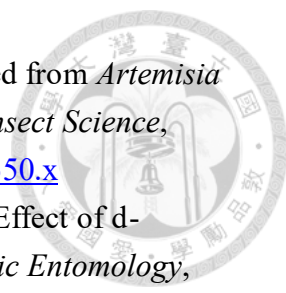
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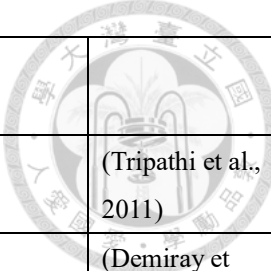
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Appendix



Appendix 1. The top 20 most abundant chemicals in (a) *A. capillaris* ethyl acetate extract (b) *A. capillaris* hexane extract (c) *S. palmifolia* ethyl acetate extract (d) *S. palmifolia* hexane extract (e) *M. tanarius* ethyl acetate extract. CAS numbers of chemicals were provided by the GC-MS software. Area percentage was calculated by the area below the peak, and if the chemical had multiple peaks, the areas were summed. Quality is the estimated confidence of correct identification out of 100% given by the software. A quality value below 50 is considered suspect. Chemical data were obtained during the previous study (Guo et al., 2023), and the literature survey to check for known insecticidal activity was done in this study. “Yes” marked with an asterisk means the chemical was tested individually and showed insecticidal activities in the associated reference.

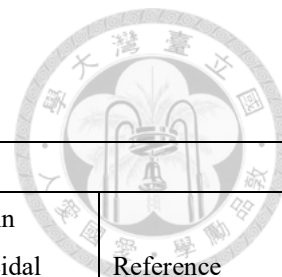
(a) <i>A. capillaris</i> ethyl acetate extract					
Chemical	CAS	Area percentage	Quality	Found in insecticidal plant extracts?	Reference
Toluene	108-88-3	20.588	91	No	
1-m-Tolyl-piperazine	41186-03-2	13.3775	38	No	
Sakuranin	529-39-5	6.3126	94	No	
Oleamide	301-02-0	5.3389	98	Yes	(Reyes-Ávila et al., 2023)
1-Ethoxybutane	628-81-9	4.7357	90	No	
2-Vinylnaphthalen	827-54-3	4.6208	60	No	
Butyl acetate	123-86-4	4.571	83	No	
2-Palmitoylglycerol	23470-00-0	4.1674	91	No	
Caryophyllene	87-44-5	4.153	99	Yes*	(Govindarajan et al., 2016)



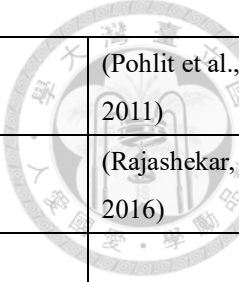
Methyl N-(4-methylphenyl)alaninate	1000305-34-4	2.5208	42	No	
Scopoletin	92-61-5	2.2835	78	Yes	(Tripathi et al., 2011)
2-Monostearin	621-61-4	1.8262	72	Yes	(Demiray et al., 2013)
Coniferol	1000297-95-5	1.8081	97	No	
Humulene	6753-98-6	1.4466	98	Yes*	(Govindarajan & Benelli, 2016)
6,7-Dimethoxyquinoxaline-2,3-diol	4784-02-5	1.3173	87	Yes	(Jadhav, 2017)
n-Hexadecanoic acid	57-10-3	1.2237	99	Yes*	(Ragavendran & Natarajan, 2015)
Octadecanoic acid	57-11-4	1.1924	92	Yes	(Okonkwo & Onyeji, 2018)
4,8-Ethano-4H-1,3-benzodioxin, hexahydro-	57790-44-0	1.1717	25	No	(Muthusamy et al., 2021)
α -Linolenic acid	463-40-1	1.1107	99	Yes	(Ragasa et al., 2014)
5-Ethyl-2-furaldehyde	23074-10-4	0.8700	27	No	

(b) <i>A. capillaris</i> hexane extract					
Chemical	CAS	Area percentage	Quality	Found in insecticidal plant extracts?	Reference
2-Vinylnaphthalen	827-54-3	31.3536	60	No	
2-Palmitoylglycerol	23470-00-0	14.6312	91	No	
2,4-Pentadiynylbenzene	41268-41-1	7.4923	87	No	
2-Monostearin	621-61-4	7.3764	91	Yes	(Demiray et al., 2013)
1-Benzyl-3-[2-(4-	1000302-33-4	4.1575	60	Yes	(Abdul Fattah

methoxyphenyl)ethyl]thiourea					et al., 2018)
Oleamide	301-02-0	3.5414	99	Yes	(Reyes-Ávila et al., 2023)
β -Eudesmol	473-15-4	3.5376	99	Yes	(Acharya et al., 2021)
Caryophyllene	87-44-5	2.527	99	Yes*	(Govindarajan et al., 2016)
N-(2,6-Dimethylphenyl)-4,6-dimethoxy-1,3,5-triazin-2-amine	27315-23-7	2.485	41	No	
β -turmerone	1000374-15-9	1.6614	96	Yes	(Ajaiyeoba et al., 2008)
γ -Murolene	30021-74-0	1.3174	90	Yes	(Lawal et al., 2014)
γ -Terpinene	99-85-4	1.2533	96	Yes*	(Gong & Ren, 2020)
trans- β -Ocimene	3779-61-1	1.2037	96	Yes	(Kiran et al., 2006)
Linalool	78-70-6	1.133	97	Yes*	(Fujiwara et al., 2017)
Humulene	6753-98-6	1.1003	97	Yes*	(Govindarajan & Benelli, 2016)
Nonacosane	630-03-5	1.0076	99	Yes*	(Junhirun et al., 2018; Silva et al., 2023)
α -Linolenic acid	463-40-1	0.7634	99	Yes	(Ragasa et al., 2014)
Chavibetol	501-19-9	0.7164	98	Yes	(Niculau et al., 2018; Rai et al., 2019)
Nerolidol	40716-66-3	0.6598	91	Yes*	(Di Campi et al., 2012)
D-Limonene	5989-27-5	0.6105	99	Yes*	(Tripathi et al., 2003)



(c) <i>S. palmifolia</i> ethyl acetate extract					
Chemical	CAS	Area percentage	Quality	Found in insecticidal plant extracts?	Reference
Toluene	108-88-3	25.672	91	No	
Oleamide	301-02-0	11.9132	98	Yes	(Reyes-Ávila et al., 2023)
2-Palmitoylglycerol	23470-00-0	11.7297	81	No	
1-Ethoxybutane	628-81-9	8.6298	90	No	
Phytol acetate	1000375-01-4	5.7260	74	No	
2-Monostearin	621-61-4	4.5288	80	Yes	(Demiray et al., 2013)
Butyl acetate	123-86-4	4.5021	83	No	
n-Hexadecanoic acid	57-10-3	3.9533	99	Yes*	(Ragavendran & Natarajan, 2015)
α -Linolenic acid	463-40-1	3.1214	99	Yes	(Ragasa et al., 2014)
Phytol	150-86-7	3.0375	99	Yes*	(Benelli et al., 2020)
Octadecanoic acid	57-11-4	2.0521	98	Yes	(Okonkwo & Onyeji, 2018)
p-Xylene	106-42-3	1.9874	97	No	
Stigmasterol	83-48-7	1.6389	96	Yes	(Huang et al., 2009)
9-Octadecyne	35365-59-4	1.437	50	Yes*	(Dhivya et al., 2018)
2-(1,3-Benzothiazol-2-ylsulfanyl)-1-(3,5-dimethyl-1H-pyrazol-1-yl)ethanone	155670-84-1	0.9831	43	No	
1,3,12-Nonadecatriene	1000131-11-1	0.8455	64	Yes	(Khan et al., 2019)
Bisphenol A diglycidyl ether	1675-54-3	0.8208	50	No	(Marqueño et al., 2019)



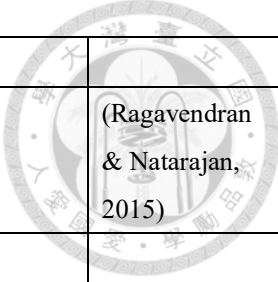
(+)- α -pinene	7785-70-8	0.7564	96	Yes*	(Pohlit et al., 2011)
Coumaran	496-16-2	0.7215	78	Yes*	(Rajashekar, 2016)
2,6-Dimethyl-1-nonen-3-yn-5-yl valerate	1000292-49-0	0.6642	44	No	

(d) *S. palmifolia* hexane extract

Chemical	CAS	Area percentage	Quality	Found in insecticidal plant extracts?	Reference
2-Palmitoylglycerol	23470-00-0	37.3255	91	No	
Oleamide	301-02-0	17.6878	98	Yes	(Reyes-Ávila et al., 2023)
1-Monostearin	123-94-4	14.3362	91	No	
Phytol	150-86-7	4.8242	93	Yes*	(Benelli et al., 2020)
α -Linolenic acid	463-40-1	4.112	99	Yes	(Ragasa et al., 2014)
1,54-Dibromotetrapentacontane	1000156-09-4	3.0564	93	No	
Stigmasterol	83-48-7	2.8276	97	Yes	(Huang et al., 2009)
2,6-Di-tert-butyl-dimethylamino-p-cresol	88-27-7	2.4842	42	No	(Michałowicz & Duda, 2007)
Vitamin E	59-02-9	2.273	99	No	
Phytol acetate	1000375-01-4	2.0263	59	No	
n-Hexadecanoic acid	57-10-3	1.3407	99	Yes*	(Ragavendran & Natarajan, 2015)
Hexadecanamide	629-54-9	1.1571	90	Yes	(Hwang & Mulla, 1980)
β -Sitosterol	83-46-5	1.1381	90	No	(Perera & Wijerathna,

					2019)
Z,Z-10,12-Hexadecadien-1-ol acetate	1000130-89-5	0.9957	90	No	(Baker & Longhurst, 1981)
1-Chloroeicosane	42217-02-7	0.8745	83	No	
2-Tetradecyloxirane	7320-37-8	0.8706	42	No	
Octadecanamide	124-26-5	0.6986	93	Yes	(Zayed et al., 2014)
Linoleic acid	60-33-3	0.6507	99	Yes*	(Ramsewak et al., 2001)
1,4-Diphenyl-2-butene	13657-49-3	0.4393	43	No	
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	102608-53-7	0.3103	43	Yes	(Kareem & Annon, 2016)

(e) <i>M. tanarius</i> ethyl acetate extract					
Chemical	CAS	Area percentage	Quality	Found in insecticidal plant extracts?	Reference
Toluene	108-88-3	22.6398	91	No	
Doxazosin impurity F	23680-84-4	10.0984	25	No	
Oleamide	301-02-0	8.4602	98	Yes	(Reyes-Ávila et al., 2023)
1-Ethoxybutane	628-81-9	7.9571	90	No	
Vitamin E	59-02-9	5.1513	99	No	
Butyl acetate	123-86-4	4.1282	83	No	
Phytol acetate	1000375-01-4	4.1156	74	No	
1-Monostearin	123-94-4	2.0734	90	No	
Lupenone	1617-70-5	1.6301	81	Yes	(Esquivel-Chi et al., 2024)
Phytol	150-86-7	1.5815	99	Yes*	(Benelli et al., 2020)
α -Linolenic acid	463-40-1	1.517	99	Yes	(Ragasa et al., 2014)
p-Xylene	106-42-3	1.2172	97	No	
(17 β)-2,3,4-Trimethoxyestra-	4314-54-9	1.1771	48	No	



1,3,5(10)-trien-17-ol					
n-Hexadecanoic acid	57-10-3	1.1375	99	Yes*	(Ragavendran & Natarajan, 2015)
1-[(2-Phenylcyclopropyl)sulfonyl]-1,2,3,4-tetrahydroquinoline	17299-24-0	1.1267	60	No	
Eicosane	112-95-8	1.0725	97	No	
1,2,3-Benzenetriol	87-66-1	1.0503	91	Yes*	(Amala & Jeyaraj, 2014)
Longifolenaldehyde	19890-84-7	0.9844	95	No	
Octadecanoic acid	57-11-4	0.8929	94	Yes*	(Okonkwo & Onyeji, 2018)
Hexadecanamide	629-54-9	0.862	95	Yes*	(Hwang & Mulla, 1980)