Chemical Constituents and Antifeedant Activity of Essential Oils from Four Selected Malaysian Local Plants against the Invasive Red Palm Weevil Larvae

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Abstract: *Red palm weevil, a significant pest affecting oil palm cultivation, necessitates eco-friendly control strategies due to the environmental and health risks posed by synthetic insecticides. This study explores the efficacy of essential oils from lemongrass, gelam, pandan, and beach vitex as sustainable alternatives. Employing hydrodistillation for oil extraction, this study assessed the total phenolic content (TPC) and total flavonoid content (TFC) using gallic acid and quercetin standards, respectively. GC-MS analysis was conducted to identify the chemical constituents. The antifeedant activity was evaluated through food consumption, larval weight changes, and the feeding deterrent index (FDI) in sago food substrate experiments. Findings show oil yields of 0.42, 0.24, 0.04, and 0.03% w/w for lemongrass, gelam, beach vitex, and pandan, respectively, with gelam exhibiting the highest TPC and TFC (12.3 ± 0.36 and 10.8 ± 0.03). Significant constituents identified include β-citral and citral in lemongrass, terpinolene in gelam, α-pirene in vitex, and phytol in pandan. Lemongrass and gelam displayed notable antifeedant effects, with FDI ranging from 24 to 28%, suggesting their potential as alternatives for managing the red palm weevil. This research highlights the potential of lemongrass and gelam oils as environmentally friendly and effective alternatives to synthetic insecticides in combating the invasive red palm weevil.*

Keywords: *phytochemical; GC-MS analysis; bioefficacy; botanical pesticides;* Rhynchophorus ferrugineus

■ INTRODUCTION

The red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae), is recognized as a category-A2 quarantine insect pest that destroys palm species worldwide, including in Malaysia [1]. It is denominated the "silent killer of palm" in many reports from different countries due to the borer's characteristic that could conceal its entire life cycle within a host plant while at the same time leaving no noticeable symptom on the infested plant during the early infestation stage. Once symptoms like an umbrella-shaped crown are spotted, the plant is incurable, while RPWs within this dying plant are fit and set to attack the next host plant [2]. Hence, there is an urgent need to protect the palm species in our nation from this serious pest, especially since Malaysia plays a crucial role as one of the world's largest palm oil exporters, fulfilling the growing global demand for oils and palm oil products.

The current RPW integrated pest management in Malaysia is heavily reliant on chemical control, which involves trunk injection and fumigation methods. Synthetic insecticides such as methamidophos and cypermethrin are applied; however, these insecticides are highly toxic and persistent. Besides deteriorating the environment and human health, it was also reported that these insecticides are still detectable in the harvested crops, which led to a setback in crop trading for three months [3]. On the other hand, RPWs could develop

resistance after long-term exposure to similar synthetic insecticides that are applied in management [4]. To address these problems, many natural products have been studied and explored in recent years, which facilitates the discovery of alternatives that can replace synthetic insecticides [5].

Among many natural products, plant-derived products are an excellent choice for studying the discovery of synthetic insecticide alternatives. Plants develop unique metabolites that carry out varying functions for interspecies interaction. For example, de Souza et al. [6] revealed the relationship between allelochemicals from herbivore-infested plants and the predator of this herbivore. Filiferol, a chemical in *Washingtonia filifera*, exhibits a larvicidal effect against RPW, resulting in *W. filifera* displaying a specialized natural resistance to a pest that infests more than 30 palm species globally [7]. Besides, plant-derived products are almost entirely safe and non-persistent, having a minimal effect on non-targeted organisms and ecosystems [8].

Essential oil (EO), a plant secondary metabolite, is one of the promising natural products that has been certified by numerous studies, demonstrating various methods such as repellency, ovicidal behavior, and feeding deterrence towards insect pests [9-10]. For example, various species of mosquitoes, houseflies, and store product pests have been proven to be weakened by EOs [11-13]. These target-specific effects of EO are contributed to by the presence of certain chemical constituents within it. Therefore, revealing the chemical compositions of EOs could provide clearer information to evaluate their effect on the target insect. In this study, we aimed to determine the chemical constituents of four local plant EOs, which included lemongrass (*Cympobogon citratus*), gelam (*Melaleuca cajuputi*), beach vitex (*Vitex rotundifolia*), and pandan (*Pandanus amaryllifolius*), as well as the effectiveness of these EOs as antifeeding deterrents against RPW third instar larvae. Lemongrass and pandan plants were selected because they are easily accessible within the area, while gelam and beach vitex were chosen as exclusive plants that grow near accessible melaleuca forests and beachesides, respectively. The determination of the chemical constituents of these plants was a valuable result of this study. Then, the larval stage was selected for this study because RPW remains in this stage for the longest duration of its life cycle. In addition, RPW in the larval stage heavily relies on feeding behavior for its growth [14]. Hence, it is evident that deteriorating its feeding habits through EO is enough to affect its survival and development adversely.

■ EXPERIMENTAL SECTION

Materials

The four plant samples were obtained through the purchase of or wild sample collections within Terengganu State, Malaysia. Fresh leaves of lemongrass and pandan were purchased from Suraya Grocery (5°23'45.3"N 103°05'38.3"E) and Pasar Nelayan (5°26'06.2"N 103°03'52.3"E), respectively. In contrast, leaf samples of gelam and beach vitex were collected from Melaleuca Forest in Bari Besar, Permaisuri (5°33'14.6"N 102°52'15.8"E) and the coastal side of Pantai Tok Jembal, Kuala Nerus (5°24'19.0"N 103°05'52.1"E), respectively. The chemicals used in the study were *n*-hexane for analysis ($CH_3(CH_2)_4CH_3$, Merck, Germany), Triton X-100 (*t*-Oct-C_6H_4-$(OCH_2CH_2)_xOH$, x = 9–10, Merck, Germany), and distilled water, which functioned as solvents in different experiments.

Instrumentation

The hydrodistillation process was conducted using Clevenger's apparatus set up with a five-liter heating mantle (MTOPS MS-E107). Gas chromatography-mass spectrometry (GC-MS) analysis was performed using a GC-2010 Plus Shimadzu and a GC-MS-QP2010 Ultra Shimadzu, as described in the next section. The larval development observations were aided by a digital vernier caliper (A2583, ChemBio Technology) and an electronic analytical weight balance (Sartorius AX224) to determine its instar stages.

Procedure

Essential oil extraction

About 3 kg of lemongrass and gelam leaves, 6 kg of pandan leaves, and 7 kg of pandan leaves were used to extract the oil. The fresh leaves were washed with distilled water and then dried using a paper towel before

being cut into smaller pieces and subjected to hydrodistillation. Each plant sample was transferred into a heating mantle, and then distilled water was added to cover it. The condenser and Clevenger apparatus were assembled for the collection of EO. Hydrodistillation was conducted for a duration of 2 h. EO was collected directly, while the distillate was processed through a separatory funnel using hexane as a solvent. These procedures were repeated until there were sufficient EOs for further experimentation and analysis. The total oil yield was recorded and expressed as a percentage of oil per weight of the fresh sample (% w/w). EOs were kept inside the refrigerator in pure oil form for the storage process. The pure oil was diluted into 5 and 10% (v/v) using distilled water with Triton X-100 as an emulsifier for the antifeedant analysis.

Insect sampling and rearing process

For three months, pheromone mass trapping for wild adult RPW was conducted around August 2022. The sampling procedure was referred to Yan et al. [15] with slight modifications. Ten pheromone traps were installed in the Kuala Nerus district of Terengganu (5°22'17.6"N, 103°04'52.5"E). The trap was designated using a 5-L polypyrene bucket that was drilled with four holes in its upper part and covered with a lid tied with a hanging rope to hang a pheromone lure (Ferrolure P028+, ChemTica Int., Costa Rica). Food bait, pineapple slices, and water were filled into the traps, which were also replenished simultaneously with the time when trapped RPWs were collected each week. All the collected RPWs were transferred and reared in the Ecology Lab at Universiti Malaysia Terengganu.

Sugarcane (*Saccharum officinarum*) was used as a food source and egg-laying substrate. It was cut into segments, approximately 10 cm in length, sliced longitudinally, and then placed in a ventilated plastic container (10 cm diameter × 5 cm height) containing wild adult RPWs. Food substrates were replaced once every four days, and the fiber part of the fed sugarcane was ripped to obtain eggs or larvae. A sago (*Metroxylon sagu*) stem, cut into cubes (8 cm^3), was provided as a food source for neonate larvae. Each larva was reared separately in a ventilated plastic container (3.6 cm diameter × 3.2 cm height) to prevent cannibalistic behavior, which could significantly reduce larval survivorship. These larvae were observed for food substrate replacement and their molting process until they achieved the desirable instar stages.

Quantification of total phenolic and flavonoid contents of essential oil

Total phenolic content (TPC) and total flavonoid content (TFC) for all four extracted plant EOs were determined using the Varian UV-vis Spectrophotometer CARY 50 CONC, aided by the software CaryWinUV Concentration Application Ver 5.0.0.999 (Agilent Technologies Inc.). All EO test samples and standards (gallic acid and quercetin) were performed in triplicate.

TPC was estimated using the Folin-Ciocalteu method, as described by Kamboj et al. [16], with some modifications. A 1 mL of the EO test sample was mixed with 1 mL of 10% Folin-Ciocalteu reagent in a test tube. A 2 mL of 7.5% Na_2CO_3 were added to the test tube and shaken gently. The mixture was then allowed to incubate in a dark place for 90 min at room temperature until an intense blue color developed. Then, the absorbance of the sample was measured at 750 nm. The spectrophotometric blank unit was performed using a reagent blank with solvent. Gallic acid (i.e., 0.001, 0.01, 0.02, 0.04, and 0.06 mg/mL) was used as a standard. TPC was expressed as mg of gallic acid equivalent (GAE)/g of plant.

TFC was estimated using an $AlCl_3$ colorimetric assay [17] with some modifications. A 2 mL of the EO test sample were mixed with 2 mL of a 2% $AlCl_3$ solution. Then, the test tube was shaken gently. The mixture was stored in a dark place for 1 h at room temperature until a yellow color was developed. The absorbance was then measured at 430 nm. The spectrophotometer blank unit was performed using a reagent blank with solvent. Quercetin (i.e., 0.001, 0.01, 0.02, 0.04, and 0.06 mg/mL) was used as a standard. TFC was expressed as mg of quercetin equivalent (QE)/g of plant.

Gas chromatography-mass spectrometry analysis

All four extracted EOs were diluted into 1 µL samples using hexane as a solvent for further analysis. These samples were sent to the Centre of Research and Field Service, Universiti Malaysia Terengganu, Malaysia

(CRaFS, UMT) for GC-MS analysis. The analysis of extracted EOs was performed using the following setup: GC-2010 Plus Shimadzu and GC-MS-QP2010 Ultra Shimadzu, equipped with a 30 m × 0.25 mm × 0.25 μm film thickness fused silica capillary column SLB™-5ms. Helium gas was used as the carrier gas at a flow rate of 13.6 mL/min. The temperature was initially programmed to 50 °C for 1 min, then gradually increased to 300 °C at 5 °C/min, held for 5 min, and finally increased to 320 °C at 5 °C/min and maintained for 5 min. The electron ionization mode, with an ionization energy of 70 eV, was used for detection. The detected constituents were identified and referred to the NIST11 Mass Spectral Library.

Antifeedant activity of essential oils

Third instar larvae were selected as the experimental subject, according to counts of molting or Dyar's ratio head capsule measurement by digital vernier calipers [18]. Each larva was acclimatized and starved separately for 6 h. The sago stem was cut into a cube (8 cm^3), and a small hole was drilled as an initial tunnel for the larvae to burrow into. The initial weight of starved larvae and sago cubes was measured. The sago cube was either treated with 0.7 mL of negative control (solvent with emulsifier) or diluted plant EOs at concentrations of 5 or 10% (v/v) using a dropper, then transferred into a ventilated plastic container (3.6 cm diameter × 3.2 cm height) along with one starved larva. Two concentrations, 5 and 10%, were selected after a preliminary range test, as well as for minimizing the use of treatments. All containers (with treated sago stems and larvae) were covered with a cap and placed in a dark place. The conditions of the sago cubes and larvae were observed daily for a duration of 5 days. After the fifth day, the weight of sago cubes and larvae was measured, and the mortality of larvae was also recorded. Feeding deterrence index (FDI) was calculated by Eq. (1) [19];

$$\text{FDI} = \frac{\text{C} - \text{T}}{\text{C}} \times 100\% \quad (1)$$

where C = weight of food consumed in control and T = weight of food consumed in treatment.

Data analysis

All data were statistically analyzed using the mean values from the three replications in each treatment. The results were recorded only if the larva survived for at least three days. Datasets were sorted and analyzed using Microsoft Excel and the IBM SPSS Statistics program, version 20. Average values of EO yield per fresh sample were calculated. The Shapiro-Wilk method was applied to test the normality of datasets. The weight of sago cube leftovers and the weight of larvae were compared through ANOVA, along with Tukey's post hoc test.

■ RESULTS AND DISCUSSION

Essential Oil Yield

The EO yield for lemongrass, gelam, beach vitex, and pandan was 0.42, 0.24, 0.04, and 0.03% w/w, respectively. The amount of oil collected from hydrodistillation using a fresh plant sample was low. Faria and Barbosa [20] and Soliman et al. [21] obtained 0.33 and 0.66% of lemongrass EO from previous studies that applied similar distillation methods. Gelam fresh leaves can provide only 0.4 to 1.2% of oil [22]. Beach vitex oil collected from Vietnam recorded a 0.09% oil yield [23]. Last, only 0.002% oil was obtained from hydrodistillation using fresh pandan leaves from Myanmar [24].

Hanaa et al. [25] suggested that different sample drying or extraction methods may affect the amount of EO obtained from plants. For example, dried and ground lemongrass samples were able to provide 0.9 to 2.7% EO through hydrodistillation [26]. Zakaria et al. [27] reported a minimum oil yield of 15.9% using a freeze-dried and powdered pandan sample. For different distillation methods, Sakasegawa et al. [28] reported collecting EO in the range of 3.6 to 9.1% after conducting a 16-h steam distillation using fresh gelam leaves.

Chemical Constituents of Essential Oils

Some plants contained chemicals that deterred insects and emitted a foul odor. EOs are highly concentrated, fragrant chemicals derived from plants. They are unstable and are commonly referred to as the "core" of the plant from which they originate. These chemicals are crucial for plant defense against many pest species in nature [29]. EOs consist of various chemical compositions. Phenolic and flavonoid molecules are significant as they are produced as secondary metabolites

Table 1. Total phenolic and flavonoid content for the four plants' essential oils

Essential oil	Oil yields (%)	TPC (mg GAE/g)	TFC (mg QE/g)
Lemongrass	0.42	5.2 ± 0.68^{b}	0.6 ± 0.05^{b}
Gelam	0.24	12.3 ± 0.36^{a}	10.8 ± 0.03^{a}
Beach vitex	0.04	3.4 ± 0.30^{b}	0.4 ± 0.10^{b}
Pandan	0.03	1.2 ± 0.45^{c}	0.3 ± 0.06^{b}

Different alphabets after the value indicate statistically significant differences (n = 3, $p < 0.05$) among the essential oils with similar phytochemical content

in response to a defense mechanism against plant-eating insects. Table 1 displays the TPC and TFC of the four plant EOs. Gelam has significantly higher quantities of TPC and TFC compared to the other three ($p < 0.05$). Lemongrass and beach vitex were second and third, respectively, with pandan having the lowest value.

Evolutionary mechanisms could influence the amounts of phenolic and flavonoid compounds in plants. Plants have developed these chemicals as part of their defense mechanisms against viruses, herbivores, and environmental stresses [30-31]. Environmental factors, including soil composition, temperature, light intensity, and the availability of nutrients and water, can influence the production of phenolics and flavonoids in plants [32]. Therefore, various plant species within the same genus may develop unique and specific compositions in varying conditions or environments [33]. Phenolic and flavonoid molecules are crucial for the insecticidal effects of EOs. Phenolic compounds in essential oils are believed to play a crucial role in chemical defense mechanisms by exhibiting antifeeding, digestibility-reducing, and other actions against herbivores or insects [34-35].

Besides, previous studies have shown a variety of TPC and TFC ranges in these plants, depending on the extraction methods, parameters, or plant parts. Godwin et al. [36] recorded TPC and TFC ranges in lemongrass around 1.3 to 7.3 mg GAE/g and 6.9 to 12.9 μg QE/g, respectively. However, it was possible to obtain higher TPC values while using the ethanolic extraction method (67 mg GAE/g) [37] and the methanolic extraction method (9.68 to 43.17 mg GAE/g) [38]. For gelam, Khongsai et al. [39] reported 4.37 mg GAE/g of TPC and 0.47 mg QE/g of TFC using an aqueous extraction method. Then, TPC and TFC were found to be higher in the flower part (55 mg GAE/g and 19.6 mg QE/g) [40] and wood part (23.2 mg GAE/g and 7.55 mg QE/g) [41]. There was only one study from Korea [42] that recorded the TPC and TFC values of beach vitex in methanolic extract (35.52 mg GAE/g and 38.07 mg QE/g). In contrast, many studies have been conducted on the species *V. trifolia*. The same applies to the pandan plant; ethanolic and methanolic extracts showed a higher TPC and TFC, yet this plant still contained fewer compounds than the other three plants mentioned above [43-44].

The detected constituents from the GC-MS analysis were identified based on the characteristics of their molecular ions and fragments, as well as those of reference compounds available in the NIST11 Mass Spectral Library. According to the results, the retention times for each EO were different, indicating differences in compound variations in different EOs. In lemongrass EO (Fig. 1(a)), it did not detect other substances after 28.0 min. Chemical constituents in gelam (Fig. 1(b)) and vitex EOs (Fig. 1(c)) continued to reveal themselves until 35.0 and 39.0 min, respectively. For pandan EO (Fig. 1(d)), no substance was detected until 29.0 min. Chemical constituents for the four plant EOs in this study were determined and presented in Table 2. Most EOs were presented with one or two dominant substances, except for vitex EO. Citral (27.6%) and β-citral (42.40%) were the dominant substances in lemongrass EO; terpinolene (24.48%) was the dominant substance in gelam EO; and phytol (63.54%) was the major compound in pandan EO. In the analysis, no substance in vitex EO reached more than 20.0% of its peak area; however, vitex EO contained the highest amount of 24 chemical substances among the other three EOs (lemongrass: 11 substances, gelam: 23 substances, and pandan: 4 substances).

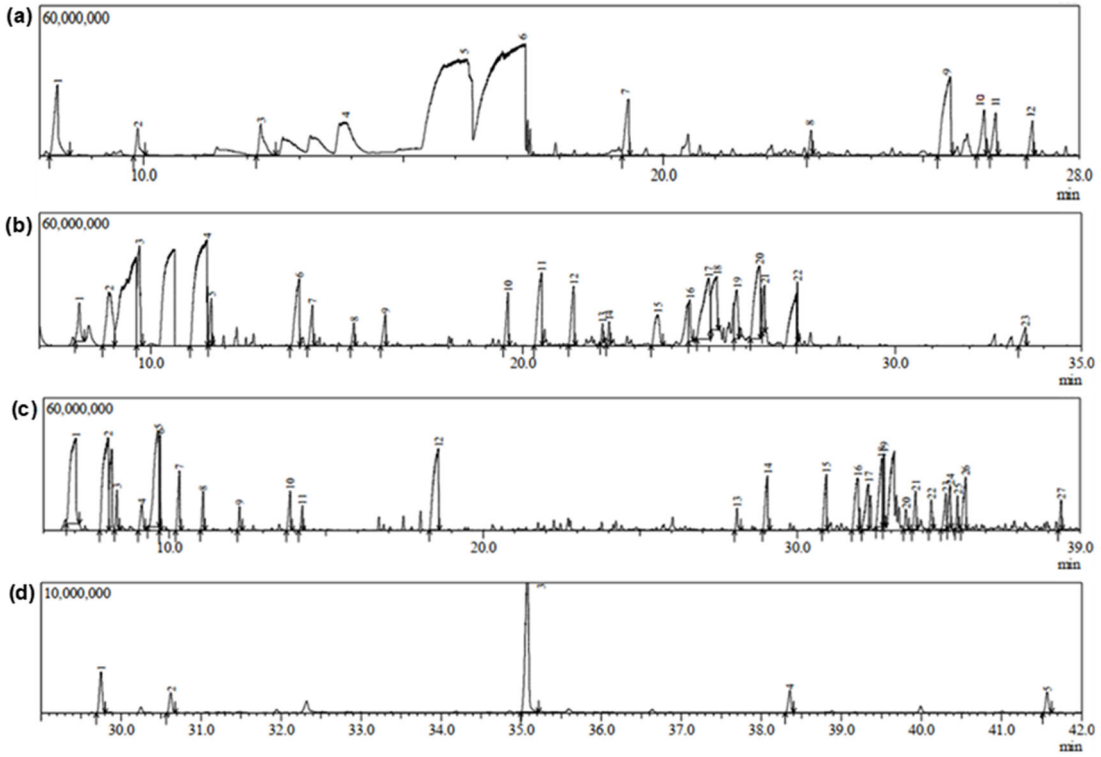

Fig 1. Total ion chromatogram of GC-MS analysis of lemongrass, gelam, vitex and pandan EO respectively. There were (a) 12 peaks in lemongrass EO, with one similar substance (peaks 11 and 12 detected the same substance); (b) 23 peaks were detected in gelam EO; (c) 27 peaks in vitex EO, while three peaks (22, 23, and 26) were classified as the same substance; and (d) 5 peaks with one similar substance that were detected in pandan EO (peaks 1 and 2 detected the same substance)

Table 2. Chemical constituents of four plant essential oils

Plant	Retention time (min)	Compound name	Molecular formula	Molecular mass (g/mol)	Area (%)
Lemongrass	8.35	β-Myrcene	$C_{10}H_{16}$	136	4.51
	9.89	α-Ocimene	$C_{10}H_{16}$	136	1.27
	12.26	(4*E*,6*Z*)-Allo-ocimene	$C_{10}H_{16}$	136	2.33
	13.90	Limonene 1,2-epoxide	$C_{10}H_{16}O$	152	4.95
	16.06	β-Citral	$C_{10}H_{16}O$	152	42.20
	17.35	Citral	$C_{10}H_{16}O$	152	27.60
	19.33	Geranyl acetate	$C_{12}H_{20}O_2$	196	2.91
	22.84	δ-Cadinene	$C_{15}H_{24}$	204	0.72
	25.52	Selina-6-en-4-ol	$C_{15}H_{26}O$	222	7.96
	26.17	α-Cadinol	$C_{15}H_{26}O$	222	2.32
	26.40	Juniper camphor	$C_{15}H_{26}O$	222	3.23
Gelam	8.08	β-Pinene	$C_{10}H_{16}$	136	2.25
	8.88	α-Phellandrene	$C_{10}H_{16}$	136	6.98
	9.69	D-Limonene	$C_{10}H_{16}$	136	5.82
	11.51	Terpinolene	$C_{10}H_{16}$	136	24.48
	11.63	Linalool	$C_{10}H_{18}O$	154	1.84
	13.99	Terpinene-4-ol	$C_{10}H_{18}O$	154	5.62

Plant	Retention time (min)	Compound name	Molecular formula	Molecular mass (g/mol)	Area (%)
	14.34	L-α-Terpineol	$C_{10}H_{18}O$	154	1.95
	15.46	β-Citral	$C_{10}H_{16}O$	152	0.91
	16.30	α-Citral	$C_{10}H_{16}O$	152	1.47
	19.60	(-)-β-Elemene	$C_{15}H_{24}$	204	2.48
	20.50	Caryophyllene	$C_{15}H_{24}$	204	5.83
	21.36	Humulene	$C_{15}H_{24}$	204	3.26
	22.14	β-Selinene	$C_{15}H_{24}$	204	0.59
	22.31	α-Selinene	$C_{15}H_{24}$	204	0.74
	23.62	Elemol	$C_{15}H_{26}O$	222	3.20
	24.49	Caryophyllene oxide	$C_{15}H_{24}O$	220	1.66
	24.99	Guaiol	$C_{15}H_{26}O$	222	9.06
	25.21	8-Fluoro-5,6-dimethoxy-α-tetralone	$C_{12}H_{13}FO_3$	224	5.92
	25.75	γ-Eudesmol	$C_{15}H_{26}O$	222	3.34
	26.35	β-Eudesmol	$C_{15}H_{26}O$	222	8.74
	26.49	Bulnesol	$C_{15}H_{26}O$	222	1.86
	27.37	Farnesol	$C_{15}H_{26}O$	222	0.82
	33.50	2-Isopropyl-10-methylphenanthrene	$C_{18}H_{18}$	234	1.19
Vitex	7.03	(+)-α-Pinene	$C_{10}H_{16}$	136	13.09
	8.06	α-Fenchene	$C_{10}H_{16}$	136	12.41
	8.35	β-Myrcene	$C_{10}H_{16}$	136	1.40
	9.13	(+)-4-Carene	$C_{10}H_{16}$	136	3.26
	9.65	D-Limonene	$C_{10}H_{16}$	136	11.43
	9.72	Eucalyptol (cineole)	$C_{10}H_{18}O$	154	3.00
	10.34	γ-Terpinene	$C_{10}H_{16}$	136	2.85
	12.25	(4*E*,6*Z*)-Allo-ocimene	$C_{10}H_{16}$	136	0.81
	13.86	Terpinene-4-ol	$C_{10}H_{18}O$	154	1.90
	14.25	α-Terpineol	$C_{10}H_{18}O$	154	1.01
	18.57	α-Terpineol acetate	$C_{10}H_{20}O_2$	196	10.26
	28.08	1a,2,5,5-Tetramethyl-cis-1a,4a,5,6,7,8-hexahydro-gamma-chromene	$C_{13}H_{22}O$	194	0.93
	29.06	Androsta-4,6-dien-17-ol-3-one acetate	$C_{21}H_{28}O_3$	328	3.40
	30.93	(+)-Hibaene	$C_{20}H_{32}$	272	3.20
	31.93	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	220	4.70
	32.27	β-iso-methyl ionone	$C_{14}H_{22}O$	206	3.84
	32.70	Diethylethoxy(2-methylbutoxy)-silane	$C_{11}H_{26}O_2Si$	218	5.92
	32.75	8-(1,4,4a,5,6,7,8,8a-Octahydro-2, 5, 5,8a-tetramethylnaphth-1-yl)-6-methyl-oct-5-en-2-ol	$C_{23}H_{40}O$	332	2.60
	33.46	3-*O*-Acetyl-6-methoxy-cycloartenol	$C_{33}H_{54}O_3$	498	0.90
	33.77	Isophyllocladene	$C_{20}H_{32}$	272	1.93
	34.27	Thunbergol	$C_{20}H_{34}O$	290	6.56
	34.87	Androstadienone	$C_{19}H_{26}O$	270	2.18
	35.11	Phytol	$C_{20}H_{40}O$	296	1.17
	38.41	1-Acetoxy-3,7-dimethyl-6,11-undecadiene	$C_{16}H_{28}O_2$	252	1.23
Pandan	29.75	Phytol acetate	$C_{22}H_{42}O_2$	338	21.4
	35.08	Phytol	$C_{20}H_{40}O$	296	63.54
	38.36	Octacosane	$C_{28}H_{58}$	394	7.71
	41.57	Eicosane	$C_{20}H_{42}$	282	7.36

The two citral isoprene units, either *E*-isomer (known as α-citral, *trans*-citral, or geranial) or *Z*-isomer (known as β-citral, *cis*-citral, or neral), are common substances in *Cymbopogon* species. These substances are also present in different plants, including citrus species. The presence of this aroma compound could exhibit a lemon or sour scent. 1,8-cineole (eucalyptol) and terpinolene were the dominant substances in *Melaleuca* species. The results of this study were similar to those of a previous study [28], which utilized *Melaleuca* EO from Dungun, Terengganu, Malaysia. Cineole was not detected, while the oils were composed of approximately 20% terpinolene, which was the dominant substance.

Beach vitex was believed to have been used as a medicinal plant for many years in European and Asian countries. From previous studies, 1,8-cineole and α-pinene were the major constituents in its EO [28]. Alternatively, Van et al. [23] recorded that sclareol was the principal constituent (29.02%) in *Vitex* EO. Plant growth factors, such as environmental quality, possibly cause the difference in constituents between each study. Phytol was found to be the most abundant substance in pandan EO. Results from Chen and Ge [45] and Mar et al. [24] supported phytol as the major constituent in pandan EO at 42.15 and 21.35%, respectively. Pandan was often applied in culinary settings due to the aromatic compound 2-acetyl-1-pyrroline, which provides flavor and fragrance. However, this substance was not detected during the GC-MS analysis, which may be due to the extraction method used in this study [46].

Antifeedant Activity of EOs Against RPW Larvae

The food consumed by the larvae can reflect the effectiveness of EO in inhibiting their growth. The lower the consumption value, the stronger the antifeedant activity of EO. The findings in Table 3 showed that the range of daily feeding amounts in the treatment was between 0.062 and 0.078 g/day, compared to 0.086 g/day for the negative control, indicating lower food consumption by the larvae in the presence of EOs. There were statistically significant differences between the daily rates of consumption of negative control, lemongrass ([5%; df = 17; F = 6.843, p = 0.019]), and gelam (df = 22; F = 6.186, p = 0.008).

The weight changes of the larvae were also recorded to determine their growing pattern within the experimental duration (Table 3). The higher the weight change value indicated, the greater the larvae's growth. The larval weight treated with 10% lemongrass essential oil was significantly lower than other treatments (df = 24; F = 6.310, p = 0.003). The negative value implied that lightweight larvae were weakening over time, as they were unable to grow in certain circumstances. In a separate study, the mortality of larvae that consumed sago food substrates treated with 5 and 10% lemongrass EO was 56 and 78%, respectively (data not provided). Mortality in the 10% gelam EO treatment was 44%, while no mortality occurred in the negative control or the other EO treatments (data not provided). During the experimental observations, larvae in both 5 and 10% (v/v) lemongrass treatments were tunneled out of the food substrate.

Table 3. Mean value and standard error for daily consumption and weight changes of RPW larvae

Treatment	Concentration (% v/v)	Consumption (g/day)	Weight change (%)	FDI (%)
Control	-	0.086 ± 0.004 ^{a}	20.96 ± 4.3	-
Lemongrass	5	0.062 ± 0.008 ^{bc}	2.99 ± 23.5	27.4
	10	0.065 ± 0.001 ^{bc}	−42.36 ± 11.8*	24.2
Gelam	5	0.062 ± 0.006 ^{bc}	14.80 ± 6.1	27.5
	10	0.064 ± 0.006 ^{bc}	7.64 ± 4.0	24.9
Vitex	5	0.074 ± 0.005 ^{ac}	29.55 ± 11.3	13.6
	10	0.076 ± 0.007 ^{ac}	20.90 ± 7.8	11.7
Pandan	5	0.077 ± 0.007 ^{ac}	21.90 ± 6.4	10.6
	10	0.074 ± 0.006 ^{ac}	39.72 ± 10.8	14.2

Symbol * indicates a significantly lower result compared with all other treatments (p < 0.05). The same small letter suggests that there was no significant difference between treatments (p > 0.05). FDI is an abbreviation for Feeding Deterrent Index

In contrast, those concealed within the food substrate died in the first two days (data not provided). The larvae that survived would consume the food substrate from the outer surface instead of hiding within it. This phenomenon was not observed in the other treatments.

The FDI was calculated using the amount of sago substrate consumed by the control and treatments. It quantifies the effectiveness of a plant's chemical defenses in reducing the feeding behavior of herbivores. A high FDI suggests that the test substance is effective at deterring the herbivore from feeding. Conversely, a low FDI indicates that the test substance is ineffective at deterring feeding. Thus, from Table 3, two different ranges were found within the FDI of the treatments: 10–15% were grouped by vitex and pandan EOs, while lemongrass and gelam EOs were categorized in the 24–28% FDI range. The effectiveness of the antifeeding effect can be further ranked from weakest to strongest as follows: pandan EO, vitex EO, gelam EO, and then lemongrass EO.

The promising results from lemongrass EO that could cause a weakening and lethal effect may be due to the high amount of citral within, as its major constituent. Kareim et al. [47] reported the oviposition-deterrent effect of lemongrass EO on RPW. Additionally, citral has been proven to repel many pest insects, including *Myzus persicae* [48], *Musca domestica* [49], *Ulomoides dermestoides* [50], *Helicoverpa armigera*, and *Spodoptera litura* [51]. Among the phenolic and flavonoid compounds found in plants, carvacrol, linalool, α-pinene, menthol, cinnamaldehyde, eugenol, 1-8 cineole, geraniol, and limonene are some of the components of EOs that have shown insecticidal activity against different pests [52-54].

Insecticidal and behavior disruption activities of the other three EOs were also evaluated from previous studies; however, these EOs have not been tested against RPW yet. Gelam extracts were reported to cause mortality against *Sitophilus zeamais* and *Tribolium castaneum* [55] and repel aphids and mealybugs [54]. Vitex extracts were found to exhibit larvicidal activity against *Aedes aegypti* [28] and deter cockroaches [56]. Meanwhile, pandan extracts could repel *Blattella germanica* [57] and *Sitophilus oryzae* [58], they also caused mortality against *Plutella xylostella* larvae [59].

■ CONCLUSION

This research establishes a clear relationship between the chemical constituents of EOs from lemongrass, gelam, pandan, and beach vitex and their antifeedant activity against the red palm weevil. The total bioactive compounds (phenolic and flavonoid) could be helpful in preliminary experimental tests, which hypothesize their effectiveness. However, the presence of specific compounds, such as β-citral and citral in lemongrass and terpinolene in gelam, is more closely correlated with the observed high antifeedant efficacy of these oils. This study highlights that the effectiveness of these plant-derived oils as insect deterrents is linked to their unique chemical profiles; also, EOs with higher total phenolic and flavonoid content show a direct association with increased antifeedant activity. This relationship highlights the potential for leveraging targeted chemical constituents within essential oils to develop effective, eco-friendly pest management solutions, providing a promising avenue for reducing the environmental impact of synthetic insecticides in agriculture.

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■ CONFLICT OF INTEREST

The authors have no conflict of interest.

■ AUTHOR CONTRIBUTIONS

Karh Yan Tay carried out the research, analyzed the data, and wrote the manuscript. Faizatul Shimal

Mehamod, Wahizatul Afzan Azmi, Nor Omaima Harun, and Azila Adnan supervised the research progress (Karh Yan Tay and Faizatul Shimal Mehamod in plant chemical compounds determination; Karh Yan Tay and Wahizatul Afzan Azmi identified and reared insects *in vitro* conditions) and revised the manuscript. Hazlina Ahamad Zakeri conceptualized and designed the research, provided the theoretical framework, and revised the manuscript. All authors read and agreed to the final version of this manuscript.

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