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# REFUGIA PLANTS WITH VOLATILE COMPOUNDS IN OIL PALM PLANTATION AREAS USING GC-MS ANALYSIS

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## SUMMARY

Refugia is a flowering plant often found in oil palm plantation areas, cultivated and grown wild, with pink and bright yellow flowers. The most common types of refugia plants belonged to the species *Antigonon leptosus, Turnera ulmifolia, Cassia tora, Crotalaria pallida,* and *Crotalaria retusa*. However, currently these types of refugia often become planted as edge plants to attract natural enemy insects such as predatory insects. The natural enemies' attraction to plants is due to the flower shape, color, and the volatile compounds released by the flowers. The following study aimed to determine the flower volatile compounds and their function by interaction between plants and their environment using the glass chromatography-mass spectrometry (GC-MS) analysis. The results revealed the production of various types of secondary metabolite compounds by each refugia, such as octadecanoic acid, 1,3,4,5-tetrahydroxy cyclohexanecarboxylic acid, 9-octadecanoic acid, pentadecanoic acid, hexadecanoic acid, tetracosamethyl cyclododecasiloxane, and ester with different percentages. The ester content was 17.35% in *A. leptosus*, 56.91% in *T. ulmifolia*, 30.54% in *C. tora*, 36.35% in *C. pallida*, and 14.39% in *C. retusa*. This ester compound provides an aroma to the flowers, and it attracts natural enemies to make the refugia their microhabitat.

**Keywords:** Refugia plants, insect species, volatile compounds, GC-MS analysis, oil palm, microhabitat, flowers

**Key findings:** Refugia has various colors, shapes, and volatile compounds, such as acids—octadecanoic, 1,3,4,5-tetrahydroxy cyclohexanecarboxylic, 9-octadecenoic, pentadecanoic, and hexadecanoic, tetracosamethyl cyclododecasiloxane, and ester compounds. The ester content was the highest in the species *T. ulmifolia* (56.91%).

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## INTRODUCTION

Refugia plants can provide shelter, food sources, and other resources for natural enemies, such as predators and parasitoids. Refugia plants grow around the fields of cultivated crop plants, with the potential to act as microhabitats for natural enemies (Septariani *et al.*, 2019). Secondary metabolites are biosynthetic compounds derived from primary metabolites, generally produced by organisms, which are useful for self-defense from the environment, as well as from the attack of other organisms. Meanwhile, the primary metabolites produced by the organisms through basic metabolism aid the growth and development of concerned organisms (Sianipar, 2019).

The refugia attract natural enemies due to compounds produced by the plants. The plant's volatile compounds can be kairomones derived from terpenoid groups and attractants for insects, aromatic derivative compounds, alcohols, aldehydes, esters, acids, and sulfur compounds. Plant aromas dominate the atmospheric chemical environment that encompasses terrestrial communities, where hundreds of plant species with a distinctive spectrum of chemical aromas can provide several important signals that stimulate behavioral patterns, leading to the search for preferred oviposition sites, suitable food sources, finding suitable mates, and shelter (Metcalf and Kogan, 2008). Volatile compounds contained in plants can exhibit various biological activities in insects, such as inhibition to eat, refusal to lay eggs, inhibition of growth and development, and death (Dadang and Prijono, 2008).

Volatile compounds released by flowers generally serve as a means of communication between plants and other organisms. Plants release volatile compounds for insects to receive as signals regarding the location of food sources, mates, and natural enemies. Therefore, the insects can easily recognize the host plants and will determine them as hosts if the plants' nutritional components meet their needs. Volatile compounds can produce aromas that are volatile in nature, and their scent can influence the animal's behavior (Masriany *et al*., 2020).

In the presented study, the said strategy, used to identify the chemical compounds in the refugia plants typically planted on the edges of oil palm plantations, sustained analysis for their bioactive compounds using gas chromatography-mass spectrometry (GC-MS). Similarly, the research sought to determine the chemical compounds attractive to natural enemy insects.

## MATERIALS AND METHODS

Qualitative and quantitative phytochemical examinations are vital to determine the active compounds that can provide beneficial and toxic effects from the refugia plants. The extraction of volatile compounds happened at the Phytochemistry Laboratory, Faculty of Pharmacy, University of North Sumatra, Indonesia. The refugia plants used were *T. ulmifolia, A. leptosus*, *C. pallida, C. retusa,* and *C. tora*. Three kilograms of refugia collected underwent drying and grinding before extracting the volatile compounds from the refugia flowers. The study of secondary metabolite compounds contained in refugia plants proceeded through phytochemical tests, carried out via qualitative analysis to determine the active compounds in the plants. This refugia extraction further received analysis using GC-MS (Mahmiah *et al.,* 2017).

The use of GC-MS to identify the different compounds in samples specifically applied the gas liquid chromatography and mass spectrometry methods. GC-MS helped determine the molecular weight of a compound with fragmentation as one of the structural analyses. The compound analysis method using GC-MS has a higher sensitivity to volatile compounds. The GC-MS analysis provides crucial information about the different chemical compounds that are volatile, non-ionic, and thermally stable, as well as having a relatively low molecular weight (Revathi *et al.*, 2014).

**Compounds extraction and GC-MS analysis**

In the extraction method, employing the maceration technique applied the methanol solvent with three repetitions. After obtaining the maceration results, a distillation process followed to separate the solvent and obtain the extraction results of the volatile compounds, possibly up to 300 ml in each flower. Each extraction proceeded with doses of 15 and 20 ml.

The GC-MS analysis, as carried out, used a 6890 N Network GC System (Agilent Technologies), equipped with a DB5 capillary column (30 m, 0.25 mm, 0.25 µm layer thickness, and a 5973 mass spectrometer, which acted as a detector). The chromatogram conditions used included helium carrier gas with a flow rate of 0.7 mL/min; column temperature initially kept at 180 °C for 5 min, then increasing the temperature to 180 °C–260 °C at 3 °C/min and 260 °C–280 °C at 3 °C/min and 15 °C/min; and finally, increasing to 280 °C at 5 min with an injector temperature of 280 °C and a detector temperature of 290 °C. Mass spectrometry operating parameters included ionization potential of 70 eV with ion source temperature of 290 °C; quadrupole temperature of 100 °C with solvent deceleration of 7.0 min; scanning speed of 2000 amu/second with a scanning range of 30–600 amu and EV voltage of 3,000 volts (Nusaibah *et al*., 2016).

**Analysis**

Gas chromatography has wide applications that can serve as a separation and analysis of a mixture of several components. The results of gas chromatography show a chromatogram of methanol extract. Moreover, identification of each peak in the chromatogram ensued by matching the MS spectrum of each peak with the Wiley database to determine the type of compound (Hartono *et al*., 2017).

## RESULTS AND DISCUSSİON

**GC-MS analysis in refugia**

In each refugia species, the secondary metabolite compounds obtained through extraction were evidently the type of volatile compounds that can attract insects. Several types of refugia appeared growing on the edge of oil palm plantations, and their flowers can attract the natural enemy insects (Figure 1). Types of plants that can benefit as refugia plants include flowering plants, broadleaf weeds, wild plants planted or growing on their own in the planting area, and vegetables (Horgan *et al.*, 2016).

***Antigonon leptosus***

The *A. leptosus* is a pink type of refugia that blooms all day with small flowers, and the survey results revealed natural enemies, such as predators and pollinators, came to this refugia. Fardani *et al*. (2020) reported plants cultivated on the edge of the main road along the oil palm plantations were commonly useful as a food source and alternative for parasitoids and predators of oil palm pests, providing honey for these insects. Most of the insect visitors to *A. leptosus* flowers were the predators of the orders Diptera and Hemiptera.

**Table 1.** Volatile compound content in *A. leptosus.*

| No. | RT | Quality | Compound | Percentage (%) |
|---|---|---|---|---|
| 1 | 4.749 | 91 | ethanol, 2-butoxy | 1.00 |
| 2 | 28.358 | 16 | 1-methylethyl 3-methylbutanoate | 4.91 |
| 3 | 30.944 | 80 | 1,3,4,5-tetrahydroxy cyclohexanecarboxylic acid | 63.13 |
| 4 | 31.358 | 46 | propanoic acid, 2-methyl, decyl ester | 17.35 |
| 5 | 32.985 | 55 | cis-13-octadecenoic acid | 1.45 |
| 6 | 33.585 | 81 | 1,2-benzenedicarboxylic acid | 1.39 |
| 7 | 35.109 | 98 | 9,10- anthracenedione, 1,8-dihydroxy-3-methoxy-6-methyl | 3.93 |

The GC-MS analysis of *A. leptosus* showed seven peaks of compounds identified in the extraction results of *A. leptosus*, with the following compounds (Table 1): ethanol, 2-butoxy; 1-methylethyl 3-methylbutanoate; 1,3,4,5-tetrahydroxy, -cyclohexanecarboxylic acid; propanoic acid, 2-methyl decyl ester; cis-13-octadecenoic acid; 1,2-benzenedicarboxylic acid; and 9,10-anthracenedione, 1,8-dihydroxy-3-methoxy-6-methyl. The GC-MS analysis showed a chlorogenic acid content of 63%, and this acid was a cinnamic ester, which came from the formal condensation of the carboxy group of trans-caffeic acid with the 3-hydroxy group of quinic acid. Chlorogenic acid is an intermediate metabolite in lignin biosynthesis. Chlorogenic acid acts as a plant metabolite and food component; it is also an ester of cinnamic and tannin. Based on secondary metabolite tests, *A. leptosus* contains terpenoid compounds. In *A. leptosus*, the ester content was 17.35%. Masriany *et al*. (2020) reported that secondary metabolites acting as attractive substances for insects were the compounds obtained from the terpenoid group.

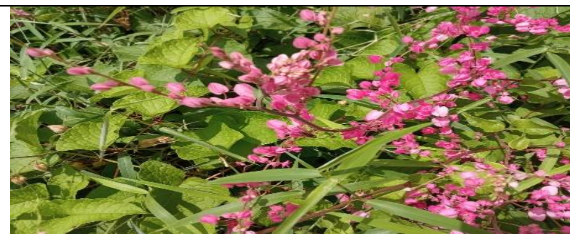
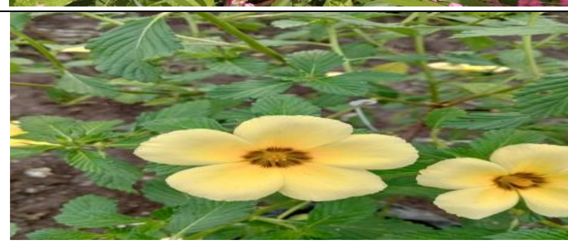
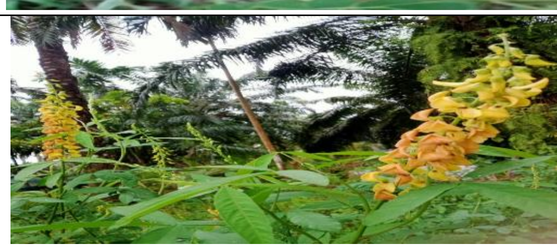
| | | |
|---|---|---|
| 1 | *Antigonon leptosus* |  |
| 2 | *Turnera ulmifolia* |  |
| 3 | *Cassia tora* |  |
| 4 | *Crotalaria pallida* |  |
| 5 | *Crotalaria retusa* |  |

**Figure 1.** Refugia plants grown in the oil palm plantations.

### *Turnera ulmifolia*

The *T. ulmifolia* plant contains chemical compounds including terpenoids, flavonoids, steroids, benzenoids, alkaloids, lipids, sitosterol, stigmasterol, cyanogenic glycosides, and essential oils. The GC-MS analysis of *T. ulmifolia* refugia showed 19 compound peaks identified as the following compounds (Table 2): 3-Cyclopentene-1- Carboxaldehyde; ethanol, 2-butoxy-; pyrazine, 2,6-dimethyl-; gamma-butyrolactone; delta2-1,24-triazoline-5-thione; phentadecane; tetracosamethyl-cyclododecasiloxane; 6-Oxabicyclo 3.2,1 Oct-3-ene,4,7,7-Trimethyl-; 2-(Trimethylsilyl) Benzenethiol; 1-Buten-3-one,1-(1-acethyl-5,5-dimethylcyclopentyhl)-; 2-(4-Hydroxybutyl) Cyclohexanol; hexadecanoic acid, methyl ester; 9-octadecenoic acid (Z)-, methyl ester; octadecanoic acid methyl ester; Oxacyclotetradecane-2,11-dione, 13-methyl-; 9,12-Octadecadienoic acid, methyl ester; Butyl 9-tetradecenoate; 2,3-Dihydroxypropyl elaidate; and 2-Methyl-z,z-3, 13-octadecadienol.

The GC-MS analysis revealed *T. ulmifolia* flowers mostly contain the ester (56.91%) and unsaturated fatty acids (31.89%), widely distributed and abundant in nature. These fatty acids can be suitable commercially in manufacturing the oleates and lotions, as well as pharmaceutical solvents. Secondary metabolites are biosynthetic compounds derived from primary metabolites, which are generally produced by organisms useful for their self-defense in the environment. The substances produced by the organisms through basic metabolism support the growth and development of the organism concerned, which are primary metabolites (Sianipar, 2019).

### *Cassia tora*

The GC-MS analysis of *C. tora* refugia displayed 25 compound peaks in the extraction results (Table 3): Cis-vaccenic acid; 9-Octadecenoic acid,(E)-; oleic acid; hexadecanoic acid, methyl ester; Cyclopropeneoctanal,2-octyl; 2-Methyl-Z,Z-

**Table 2.** Volatile compound content in *T. ulmifolia.*

| No. | RT | Quality | Compound | Percentage (%) |
|---|---|---|---|---|
| 1 | 4.039 | 72 | 3-Cyclopentene-1- Carboxaldehyde | 6.32 |
| 2 | 4.735 | 91 | ethanol, 2-butoxy- | 9.70 |
| 3 | 4.845 | 87 | pyrazine, 2,6-dimethyl- | 3.42 |
| 4 | 4.990 | 64 | gamma-butyrolactone | 1.20 |
| 5 | 6.224 | 38 | delta2-1,24-triazoline-5-thione | 3.54 |
| 6 | 22.159 | 95 | phentadecane | 1.51 |
| 7 | 26.862 | 50 | tetracosamethyl-cyclododecasiloxane | 1.12 |
| 8 | 27.869 | 40 | 6-Oxabicyclo3,2,1Oct-3-ene,4,7,7-Trimethyl- | 1.15 |
| 9 | 28.655 | 43 | 2-(Trimethylsilyl)Benzenethiol | 2.03 |
| 10 | 29.048 | 70 | 1-Buten-3-one,1-(1-acethyl-5,5-dimethylcyclopentyhl)- | 1.94 |
| 11 | 29.655 | 90 | 2-(4-Hydroxybutyl)Cyclohexanol | 1.22 |
| 12 | 29.792 | 99 | hexadecanoic acid, methyl ester | 15.73 |
| 13 | 30.923 | 99 | 9-octadecenoic acid (Z)-, methyl ester | 31.89 |
| 14 | 31.054 | 98 | octadecanoic acid, methyl ester | 7.63 |
| 15 | 31.268 | 91 | Oxacyclotetradecane-2,11-dione,13-methyl- | 1.97 |
| 16 | 31.413 | 95 | 9,12-Octadecadienoic acid, methyl ester | 1.66 |
| 17 | 32.082 | 70 | Butyl 9-tetradecenoate | 2.04 |
| 18 | 32.144 | 59 | 2,3-Dihydroxypropyl elaidate | 1.53 |
| 19 | 33.088 | 84 | 2-Methyl-z,z-3,13-octadecadienol | 1.75 |

**Table 3.** Volatile compound content in *C. tora.*

| No. | RT | Quality | Compound | Percentage (%) |
|---|---|---|---|---|
| 1 | 29.082 | 89 | Cis-Vaccenic acid | 1.27 |
| 2 | 29.455 | 93 | 9-Octadecenoic acid,(E)- | 1.41 |
| 3 | 29.668 | 53 | oleic acid | 2.26 |
| 4 | 29.820 | 99 | hexadecanoic acid, methyl ester | 6.42 |
| 5 | 30.392 | 97 | Cyclopropeneoctanal,2-octyl | 6.40 |
| 6 | 30.496 | 95 | 2-Methyl-Z,Z-3,13-octadecadienol | 1.08 |
| 7 | 30.965 | 99 | 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- | 22.59 |
| 8 | 31.068 | 97 | Methyl 16-methyl-heptadecanoate | 4.15 |
| 9 | 31.192 | 95 | 2,3-Dihydroxypropyl(9Z)-9-Octadecenoate | 2.63 |
| 10 | 31.296 | 95 | 2-Methyl-Z,Z-3,13-Octadecadienol | 1.56 |
| 11 | 31.392 | 94 | 2-Methyl-Z,Z-3,13-Octadecadienol | 3.30 |
| 12 | 31.641 | 86 | Octadec-9-enoic acid | 4.50 |
| 13 | 31.792 | 95 | Octadec-9-enoic acid | 2.42 |
| 14 | 31.937 | 92 | erucic acid | 1.33 |
| 15 | 32.034 | 93 | 2,3-Dihydroxypropyl elaidate | 2.69 |
| 16 | 32.158 | 96 | 2,3-Dihydroxypropyl elaidate | 2.00 |
| 17 | 32.385 | 94 | 1-Nonadecene | 1.96 |
| 18 | 32.640 | 94 | 13,Methyloxacyclotetradecane-2,11-Dione | 2.11 |
| 19 | 34.992 | 90 | 2,6,10-Dodecacatrioen-1-01,3,7,11-trimethyl- | 2.58 |
| 20 | 40.908 | 78 | 2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-6-Chromanol | 1.04 |
| 21 | 47.231 | 83 | Ergost-4-EN-3ONE,(24R)- | 3.55 |
| 22 | 47.576 | 74 | Ergosta-8,25-DIEN-3-ONE,14,24-Dimethyl- | 2.39 |
| 23 | 48.217 | 95 | 4,22-Stigmastadiene-3-one | 2.84 |
| 24 | 50.451 | 93 | Stigmast-4-EN-3-ONE | 11.84 |
| 25 | 51.030 | 55 | OLEIC Acid, Propyl Ester | 1.53 |

3,13-octadecadienol; 9,12,15- octadecatrienoic acid, methyl ester, (Z,Z,Z)-; Methyl 16-methyl-heptadecanoate; 2,3-Dihydroxypropyl(9Z)-9-Octadecenoate; 2-Methyl-Z,Z-3,13-Octadecadienol; 2-Methyl-Z,Z-3,13-Octadecadienol; Octadec-9-enoic acid; Octadec-9-enoic acid; erucic acid, 2,3-Dihydroxypropyl elaidate; 2,3-Dihydroxypropyl elaidate; 1-Nonadecene; 13,Methyloxacyclotetradecane-2,11-Dione; 2,6,10-Dodecacatrioen-1-01,3,7,11-trimethyl-; 2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-6-Chromanol; Ergost-4-EN-3ONE,(24R)-; Ergosta-8,25-DIEN-3-ONE,14,24-Dimethyl-; 4,22-Stigmasta diene-3-one; Stigmast-4-EN-3- one; oleic acid; and propyl ester.

The *C. tora* contains ester (30.54%) and methyl linolenate content (22.59%). This compound is a fatty acid methyl ester derived from alpha-linolenic acid. Methyl linolenate acts as a plant metabolite and insect attractant. Methyl linolenate has a functional relation to alpha-linolenic acid, and this compound attracts the insects to refugia plants. In plants, the secondary metabolites have several functions, including acting as an attractant (attracting pollinating insects), protecting against environmental stress, protecting against pests/diseases (phytoalexin), protecting against ultraviolet rays, and acting as a growth regulator to compete with other plants (allelopathy). In short, secondary metabolites are beneficial to organisms to interact with their environment (Supriyono, 2007).

### *Crotalaria pallida*

The GC-MS analysis detailed *C. pallida* refugia with 26 compound peaks identified through extraction results (Table 4). These comprised

**Table 4.** Volatile compound content in *C. pallida.*

| No. | RT | Quality | Compound | Percentage (%) |
|---|---|---|---|---|
| 1 | 15.836 | 35 | Propanedioic acid, diethyl ester | 1.38 |
| 2 | 29.075 | 47 | 11,13-Dimethyl-12-tetradecen-1-01 acetate | 7.77 |
| 3 | 29.172 | 45 | Estra-1,3,5 (10)-trien-17 beta-0L | 2.40 |
| 4 | 29.296 | 25 | (9E)-9-octadecenoic acid | 2.21 |
| 5 | 29.448 | 53 | 2-Aminoethanethiol hydrogen sulfate (ester) | 1.89 |
| 6 | 29.668 | 45 | 1-tert-Butoxy-2-methylbenzene | 5.01 |
| 7 | 29.813 | 99 | hexadecanoic acid, methyl ester | 9.03 |
| 8 | 30.275 | 81 | oleic acid | 1.39 |
| 9 | 30.406 | 93 | Octadec-9-enoic acid | 1.76 |
| 10 | 30.910 | 99 | 9,12-Octadecadienoic acid, methyl ester | 18.47 |
| 11 | 31.061 | 97 | octadecanoic acid, methyl ester | 5.59 |
| 12 | 31.275 | 90 | Octadec-9-Enoic Acid | 4.42 |
| 13 | 31.420 | 90 | Octadec-9-Enoic Acid | 2.80 |
| 14 | 31.558 | 87 | Oleic Acid | 1.74 |
| 15 | 31.640 | 76 | (9Z)-9-Octadecenamide | 3.38 |
| 16 | 31.958 | 64 | cyclopentadecanone | 1.62 |
| 17 | 32.033 | 94 | 2,3-dihydroxypropyl elaidate | 1.36 |
| 18 | 32.158 | 95 | Methyl (9E)-9-Octadecenoate | 3.49 |
| 19 | 32.392 | 95 | Octadecenoic acid, (E) | 2.93 |
| 20 | 32.599 | 90 | 9-Octadecenamide | 3.20 |
| 21 | 32.964 | 52 | S-(2-(N,N-Dimethylamino)ethyl)morpholine-N-carbonylthiocarbohydroximate | 1.54 |
| 22 | 33.344 | 93 | Methyl dihydromalvalate | 1.18 |
| 23 | 46.127 | 70 | urs-12-ene | 1.71 |
| 24 | 47.051 | 86 | alpha-amyrin | 5.26 |
| 25 | 47.244 | 80 | Octadec-9-enoic acid | 1.13 |
| 26 | 50.409 | 95 | Stigmast-4-en-3-one | 4.03 |

Propanedioic acid diethyl ester; 11,13-Dimethyl-12-tetradecen-1-01 acetate; Estra-1,3,5(10)-trien-17 beta-0L; (9E)-9-octadecenoic acid; 2-Aminoethanethiol hydrogen sulfate (ester); 1-tert-Butoxy-2-methylbenzene; and hexadecanoic acid, methyl ester. Other compounds also included oleic acid, octadec-9-enoic acid; 9,12-octadecadienoic acid methyl ester; octadecanoic acid methyl ester;, Octadec-9-Enoic Acid; Octadec-9-Enoic Acid; oleic acid; (9Z)-9-octadecenamide; cyclopentadecanone; 2,3-dihydroxypropyl elaidate; methyl (9E)-9-octadecenoate; octadecenoic acid, (E); 9-octadecenamide; S-(2-(N,N-dimethylamino)ethyl) morpholine-N-carbonylthiocarbohydroximate; Methyl dihydromalvalate; urs-12-ene; alpha-amyrin; Octadec-9-enoic acid; and stigmast-4-en-3-one.

In *C. pallida* refugia the linoleic acid content was 18.47%. Linoleic acid acts as a plant metabolite, *Daphnia galeata* metabolite, and algae metabolite. Linoleic acid is an omega-6 fatty acid and octadecadienoic acid, as well as a conjugate acid of linoleic acid and the ester content (36.35%) in *C. pallida*.

***Crotalaria retusa***

The GC-MS analysis of *C. retusa* refugia showed 14 peaks of different compounds identified through extraction results (Table 5): Ethanone,1-(1-methylcyclopentyl)-; 5H-1–Pyrindine; 2-Hydroxy-2-methyl-but-3-enyl, 2-methyl-2(Z)-butenoate; 2-Methoxy-4-

**Table 5.** Volatile compound content in *C. retusa.*

| No. | RT | Quality | Compound | Percentage (%) |
|---|---|---|---|---|
| 1 | 4.562 | 64 | Ethanone,1-(1-methylcyclopentyl)- | 7.02 |
| 2 | 5.617 | 93 | 5H-1-Pyrindine | 1.43 |
| 3 | 7.893 | 53 | 2-Hydroxy-2-methyl-but-3-enyl, 2-methyl-2(Z)-butenoate | 43.82 |
| 4 | 12.982 | 94 | 2-Methoxy-4-vinylphenol | 1.49 |
| 5 | 30.606 | 35 | Ethanol,2,2-(1,2-ethanediylbis (oxy)) bis-diacetate | 3.38 |
| 6 | 30.909 | 43 | 1,3-Dioxolane acid, ethyl ester | 1.51 |
| 7 | 31.475 | 99 | Hexadecanoic acid, ethyl ester | 9.74 |
| 8 | 31.509 | 50 | 3-Methyldodecanoic acid | 2.98 |
| 9 | 31.716 | 49 | Ethanol, 2,2 –(1,2-ethanediylbis(oxy)) bis-, diacetate | 10.21 |
| 10 | 32.557 | 99 | 9,12-octadecadienoic acid, ethyl ester | 1.13 |
| 11 | 33.688 | 99 | 3,4-Dihydroxy-3,4,5-trimethyl-4,5,8,10,12,13,13a,13b-octahydro-2H-(1,6) dioxacycloundecino (2,3,4-gh) pyrrolizine-2,6 (3H)-dione | 3.91 |
| 12 | 35.088 | 97 | 9,12-Octadecadienoic acid (Z,Z)- 2-hydroxy-1-(hydroxymethyl) ethyl ester | 2.01 |
| 13 | 40.259 | 99 | gamma-sitosterol | 1.25 |
| 14 | 40.010 | 52 | Dehydro-cohumulinic acid | 2.36 |

vinylphenol; Ethanol,2,2-(1,2-ethanediylbis[oxy]) bis-, diacetate; 1,3-dioxolane acid ethyl ester; hexadecanoic acid, ethyl ester; 3-Methyldodecanoic acid; Ethanol, 2,2–(1,2-ethanediylbis[oxy]) bis-, diacetate; 9,12-octadecadienoic acid ethyl ester; 3,4-dihydroxy-3,4,5-trimethyl-4,5,8,10,12,13,13a,13b-octahydro-2H-(1,6) dioxacycloundecino (2,3,4-gh) pyrrolizine-2,6 (3H)-dione; 9,12-octadecadienoic acid (Z,Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester; gamma-sitosterol; and dehydro-cohumulinic acid. In *C. retusa* refugia, the ester content was 14.39%.

The secondary metabolite compounds produced by refugia plants, such as octadecanoic acid, 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid, quinic acid, or 9-octadecenoic acid, are potential biochemical compounds in plants as self-defense. Meanwhile, pentadecanoic acid (CAS) is one of the hydrocarbon compounds that can attract several predators from the Reduviidae group (Kumar and Ambrose, 2014). Plant parts can release chemical compounds that attract predatory insects, such as 9,12-octadecadienoic acid hexadecanoic acid ethyl ester, which also become a means of communication between plants and natural enemy insects (Hariyadi and Nanda, 2023).

The secondary metabolites' functions allow them to defend themselves against unfavorable environmental conditions, overcome pests and diseases, and attract pollinators for pollinating flowers of refugia. *A. leptosus, T. ulmifolia, C. tora, C. pallida,* and *C. retusa* release these metabolite compounds as an interaction between the plant and its environment. In plants, the flavonoids have various important roles, such as providing color and aroma to the flowers and fruits, as signaling molecules, and as detoxification agents. Apart from that, flavonoids are also vital in protecting plants from biotic and abiotic stress factors, as well as filtering UV rays, causing more flavonoids in many plant leaf organs (Panche *et al.*, 2016). Saponin is a glycoside found in various parts of plants, and its chemical structure consists of glycones and aglycones. The aglycone part is sapogenin, while the glycone part consists of glucose, fructose, and other types of sugar (Nurzaman *et al*., 2018).

Triterpenoids are the plant's secondary metabolite compounds in the form of terpenoid derivatives, which have a carbon skeleton composed of six isoprene units (2-methylbuta-1,3-diene). Triterpenoid compounds can be cyclic and acyclic. Triterpenoids were also usually composed of aldehyde, alcohol, and carboxylic acid groups (Widiyati, 2006).

Steroids are one of the secondary metabolite compounds in plants, which can categorically be the main class of plant phytochemicals besides phenolics, terpenoids, essential oils, alkaloids, and polypeptides (Bialangi *et al*., 2018).

Alkaloids are one of the secondary metabolic compounds produced by the plants. This compound is prevalent in various plant organs, such as leaves, bark, twigs, and seeds (Aksara *et al.*, 2013). In plants, alkaloids have an important defensive role against biotic and abiotic disorders. Alkaloids are toxic to pathogens and predators. They also play a crucial role in helping plants survive stressful conditions due to drought stress, water stress, and extreme temperatures. Additionally, certain types of alkaloids can attract pollinators, and with the small amounts of toxic alkaloids, these can kill pathogens in pollinators' bodies (Matsuura and Fett-Neto, 2017).

The refugia plants used in this research are types of refugia that contain secondary metabolite compounds desirable for plants to adapt to their environment, self-defense, attract natural enemy insects, and serve as growth regulators. Hence, this refugia is excellent for cultivation in plantation areas as an effort to conserve the environment. Tannins are the complex organic compounds found in plants as secondary metabolite products. Tannins comprise phenolic compounds, which are difficult to crystallize and separate, and also contain protein that is difficult to precipitate. Moreover, tannins have polyphenolics compounds with antibacterial, antioxidant, and astringent activities (Desmiaty *et al*., 2008). The ester compounds can attract insects apart from refugia morphology. Various types of natural insect enemies exist in refugia flowers in oil palm plantation areas that are beneficial, i.e., *Sycanus* sp., as predators and for the pollination process of plants, such as bees, butterflies, and beetles.

## CONCLUSIONS

Various refugia species' assessments for metabolite compounds included *Antigonon leptosus, Turnera ulmifolia, Cassia tora, Crotalaria pallida,* and *Crotalaria retusa,* containing alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenes/steroids. The secondary metabolite compounds produced by refugia function as self-defense and a means of communication between plants and their environment, such as octadecanoic acid, 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid, 9-octadecanoic acid, pentadecanoic acid, hexadecanoic acid, tetracosamethyl cyclododecasiloxane, and ester compounds. The ester content was 17.35% in *A. leptosus*, 56.91% in *T. ulmifolia*, 30.54% in *C. tora*, 36.35% in *C. pallida*, and 14.39% in *C. retusa*. The ester compounds provide aroma to the refugia to attract various natural insect enemies.

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