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Insecticidal Effect against House dust mite Using Ethanol Extract of *Theobroma cacao* L.

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Abstract

Background/Objectives: This study reviewed the insecticidal effect against house dust mite from ethanol extract of *Theobroma cacao* L..

Methods/Statistical analysis: The ethanolic extract of dried Theobroma cacao L. powder was used as the sample for the insecticidal experiment. The caffeine and monoterpene compounds, which are known to have an insecticidal effect among the components contained in Theobroma cacao L.., was analyzed using a gas chromatography-mass spectrometry and thermal desorption-gas chromatography/mass spectrometry.

Findings: Ethanolic Theobroma cacao L. extract showed 100% of excellent insecticidal effect at the concentration of 1.0 ㎎/40 ㎕ and 0.5 ㎎/40 ㎕, and showed 97.9% of good insecticidal effect even at the concentration of 0.25 ㎎/40 ㎕. By the results, it found that ethanolic Theobroma cacao L. extract has an insecticidal activity against house dust mite. By using a gas chromatography-mass spectrometry and thermal desorption-gas chromatography/mass spectrometry, the caffeine and monoterpene compounds, which are known to have an insecticidal effect among the components contained in Theobroma cacao L.., was identified.

Improvements/Applications: Through this study, it is thought that cacao extracts containing caffeine and monoterpene compounds having insecticidal effects can be used as natural insecticides.

***Keywords*:** insecticidal effect, house dust mite, *Theobroma cacao* L., gas chromatography-mass spectrometry, thermal desorption-gas chromatography/mass spectrometry.

1. Introduction

Recently, allergic diseases are on the rise due to the increase of various environmental pollution and changes in dietary patterns[1]. House dust mites are the most common indoor environmental allergens[2]. These breed in moist and warm environments, such as bedding, carpets, and furniture, and feed on dead skin or fungi that fall from the body of a person or animal. Various methods such as high-temperature steam cleaning, use of microfibers, and sterilization by chemical synthetic agents have been used to remove house dust mites, but an effective removal method has not been suggested. And the main chemical composition of pesticides commonly used is phthalthrin cyclopropanecarboxylate, benzyl benzoate, N, N-diethyl-m-toluamide [3]. It is pointed out that these ingredients are effective in combating house dust mites, but they become resistant when exposed continuously, and potentially destroy the atmospheric ozone layer, thereby potentially harming the environment and the human body[4]. Therefore, there is an urgent need to develop natural acaricides that utilize environmentally friendly natural extracts that are harmless to the human body and have strong acaricidal properties that can replace synthetic acaricides. Plant resources are divided into primary metabolites such as carbohydrates, proteins, fats and nucleic acids, and secondary metabolites produced through the biosynthetic process of the primary metabolites. Since these secondary metabolites have various physiological activities, they are used in natural medicines, functional foods, dietary supplements, natural pigments, natural flavors, natural pesticides, fungicides, and functional cosmetics[5]. These secondary metabolites include phenolic compounds, alkaloids, and terpene compounds[6]. Among natural products, basic plant components belonging to nitrogen-containing compounds are called alkaloids, and many have significant physiological effects. In addition, essential oils contained in natural products have long been used in perfumery and food fragrances and aromatherapy, and contain insecticidal active ingredients, most of which are known as monoterpenes. Until now, research on alkaloids mainly focuses on the separation of alkaloids contained in plants and analysis of their content, and studies on the insecticidal effects of house dust mites have been rarely conducted. Therefore, research on it is necessary. The final purpose of this study aims to verify the insecticidal effect on house dust mites using alkaloid-based *Theobroma cacao* L. known as a bioactive component. Therefore, I intend to research and develop materials that can protect the human body from harmful environments, such as house dust mites, by treating natural materials with insecticidal properties on fabric. As part of the study, I intended to examine the insecticidal effect of *Theobroma cacao* L. ethanol extract against house dust mite by concentration. In addition, caffeine and monoterpene compounds known to have an insecticidal effect among the

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components contained in *Theobroma cacao* L. was examined through GC / MS and TD GC / MS analysis.

2. Experiment

2.1. *Materials*

2.1.1. Theobroma cacao L.

Theobroma cacao L. used for this study was purchased from an online herbalist.

2.1.2. Mite

The house dust mites called *Dermatophagoides pteronyssinus*(*D. pteronyssinus*) were used in the experiment.

2.2. *Methods*

2.2.1. Extraction

Dried *Theobroma cacao* L. powder, at the liquor ratio of 1:20, were put to extracted at room temperature for 24 hr., and then filtered. The extracted/filtered extract was decompressed and concentrated using an evaporator, under 40±2℃ and 30mmHg. The crude extract thus obtained was used as the sample for the insecticidal experiment.

2.2.2. Breeding of House Dust Mites

For the breeding of *D. pteronyssinus*, a medium in which Ebioze powder and mouse feed mixed in a 2: 1 ratio was used. The environment of the incubator was maintained at a temperature of 25 ° C and a relative humidity of 75%.

2.2.3. Insecticidal Effects of Extracts against House Dust Mite

The residue thin film method, a type of direct contact method was utilized. 1 mg, 0.5 mg, 0.25 mg, 0.125 mg, and 0.00625 mg of samples were dissolved in 40 μl of ethanol, respectively, and put them in a 2 ml micro tube, shook well so that the samples were evenly deposited on the tube wall, and then volatilized the solvent. 25 house dust mites on cotton were placed in each dried tube, and then left at 25 ° C., a relative humidity of 70%, and dark conditions. The control treated only 40 μl of ethanol. After 24 hr., each treatment group was examined for mortality using a stereoscopic microscope.

2.2.4. Components Analysis

As described above, alkaloids are generally basic compounds contained in plants. This compound generally has physiological activity. Therefore, alkaloids are nitrogen-containing compounds and are defined as amine plant components that have remarkable physiological effects. *Theobroma cacao* L. used in this study contains caffeine of purine alkaloid[7], and is known to have an insecticidal effect[8]. As such, caffeine, which is known to have an insecticidal effect, was analyzed by GC / MS to see if it was contained in *Theobroma cacao* L. used in the experiment of this study. In addition, it was analyzed using TD GC / MS to see if the monoterpene compound, which is known to even have an insecticidal effect, is also contained in *Theobroma cacao* L..

2.2.5. Components Analysis by gas chromatography–mass spectrometry(GC/MS) Analysis[9]

100 g cacao powder was extracted with 300 mL of ethanol in a Soxhlet apparatus for 6 hours at 60 ° C., and then filted them. The extracted/filtered liquid were used as a sample for GC / MS analysis.

2.2.6. Analysis of volatile compounds using TD-GC-MS[10]

The essential oil analysis was performed using a thermal desorption gas chromatograph (TD-GC / MSD, GC: agilent 6890N, G1530N and 2975 MASS).

3. Results

3.1. *Insecticidal effect of Theobroma cacao L. ethanol extract against house dust mite*

[Table 1] showed the insecticidal effects of *Theobroma cacao* L. ethanol extract against house dust mite (*D. pteronyssinu*). As can be seen in [Table 1], ethanolic *Theobroma cacao* L. extract showed 100% of excellent insecticidal effect in the concentration of 0.5 ㎎/40 ㎕ and 1 ㎎/40 ㎕, respectively, and also showed excellent insecticidal effect of 97.09% at the concentration of 0.25mg/40 ㎕. Even when the concentration of the extract was 0.125mg, it showed an insecticidal effect of 75%. Like this, it was thought that the caffeine and monoterpene compounds, which are known to have an insecticidal action among the components contained in *Theobroma cacao* L., showed synergistic effects with each other. According to the literature[11], it was found that cacao beans contain 1.3-2% of alkaloids and 45-58% of fats and oils. It is known that the main components of alkaloids contained in cacao are theobromine and caffeine. In this study, too, it was confirmed that theobromine and caffeine was contained in *Theobroma cacao* L. as shown in Figure 2 and Table 2. As shown in [Figure 1], its chemical molecular formula is $C_8H_{10}N_4O_2$. It has insecticidal properties which causes sterility in some insects[12]. In the previous study[13], the caffeine has shown a 100% of insecticidal effect at the concentrations of 1 ㎎/40 ㎕ and 0.5 ㎎/40 ㎕, respectively. This result has suggested that caffeine has shown a high insecticidal effect. Therefore, it was thought that the caffeine contained in ethanolic *Theobroma cacao* L. extract affected the insecticidal effect against house dust mites. Not only this but it was believed that the monoterpene compounds such as 2-beta-pinene contained in *Theobroma cacao* L. also influenced the insecticidal effect against house dust mites. In the previous study[13], 2-beta-pinene, a type of monoterpene compound, has shown high insecticidal effects. Therefore, it was thought that the monoterpene compounds contained in *Theobroma cacao* L. affected the insecticidal effect against house dust mites. Therefore, caffeine and

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monoterpenes were confirmed by the following GC / MS and TD GC / MS component analysis.

Table 1: Insecticidal effect of ethanolic *Theobroma cacao* L. extract against house dust mite

Concentration (㎎/40㎕)	*Mortality(%)*[a]
1㎎	100
0.5㎎	100
0.25㎎	97.09
0.125㎎	75
0.0625㎎	30

a (remained mites/Total mites) × 100

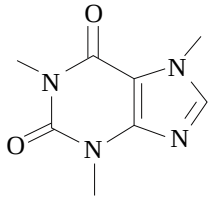
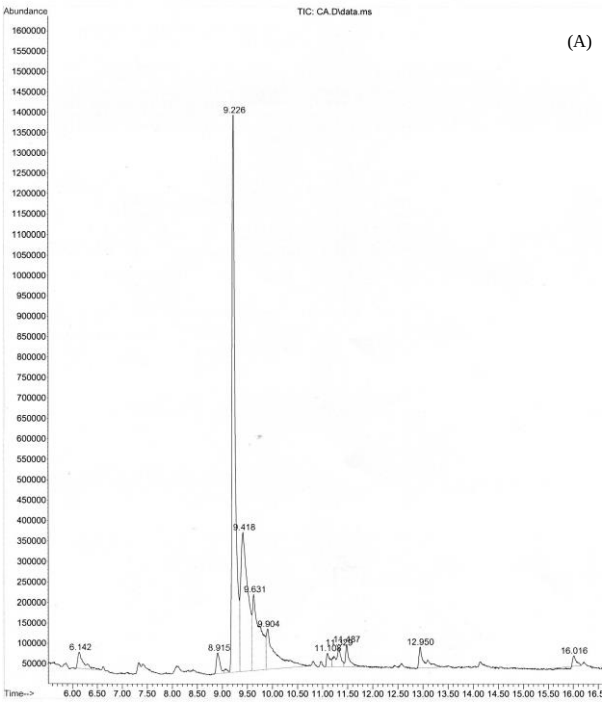


Figure 1. Structural formula of caffeine

3.2. *Analysis of Theobroma cacao L. by GC/MS*

As discussed above, *Theobroma cacao* L. ethanol extract showed a high insecticidal effect against house dust mites. To confirm the caffeine known to have an insecticidal effect among the components contained in *Theobroma cacao* L., the components were analyzed using GC / MS. The results were shown in [Figure 2] and [Table 2]. In [Figure 2] (A), the main peak appeared at the retention time of 9.226 minutes, and the component was caffeine, as shown in [Table 2]. (B) is the mass spectrum of the caffeine fraction (9.22 6 minutes) recovered from *Theobroma cacao* L. extract, and (C) showed the mass spectrum of the caffeine standard. Since it could be seen that the two graphs were identical, it was confirmed that the two substances were the same substance. Therefore, it was confirmed through the results of this study that *Theobroma cacao* L. extract contains caffeine, which has as an insecticidal activity.



(A)

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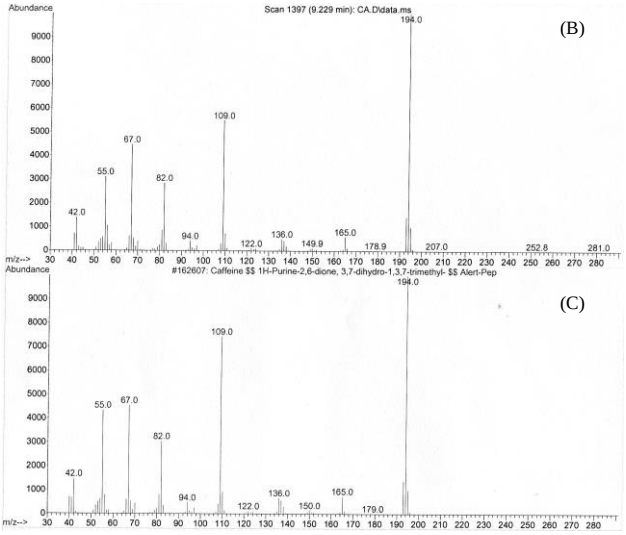

Figure 2. Total ion chromatograms of compounds in *Theobroma cacao* L. (A), MS spectra of purified caffeine from *Theobroma cacao* L. (B), authentic caffeine (C)

Table 2: Component identified from *Theobroma cacao* L.

Peak No.	*Compounds*	*tR(min.)*	*Peak area(%)*
1	Acetamine, 2-(2-hydroxyethoxy)-	6.140	2.25
2	Oxazolidine, 2-ethyl-	8.915	1.61
3	Caffeine	9.224	42.25
4	Theobromine (CAS)	9.416	23.26
5	Theobromine (CAS)	9.632	13.49
6	Caffeine (CAS)	9.905	8.86
7	Theobromine (CAS)	11.106	1.05
8	6-Octadecenoic acid, (z)-	11.328	2.38
9	Octadecenoic acid, ethyl ester (CAS)	11.485	1.88
10	9-Octadecenamide, (z)-	12.948	2.69
11	2-Ethylacridine	16.014	0.28

3.3. *Analysis of Theobroma cacao L. by TD GC/MS*

[Figure 3] showed the results of TD GC / MS measurement of *Theobroma cacao* L.. As could be seen in [Figure 3], peaks were high in the 7.06 minutes and 14.45 minutes, and the corresponding components were acetic acid and 2-β-pinene as shown in [Table 3]. Also, it was found that peaks were gathered between 5 minutes and 7 minutes, and between 13 minutes and 15 minutes. There were many aldehydes such as propanal, 2-methyl- and butanal, and 3-methyl- between 5 minutes and 7 minutes. It was found that the main components between 13 minutes and 15 minutes were mostly monoterpene hydrocarbons such as alpha-pinene, phenol, 2-beta-pinene, decane, and dl-limonene.

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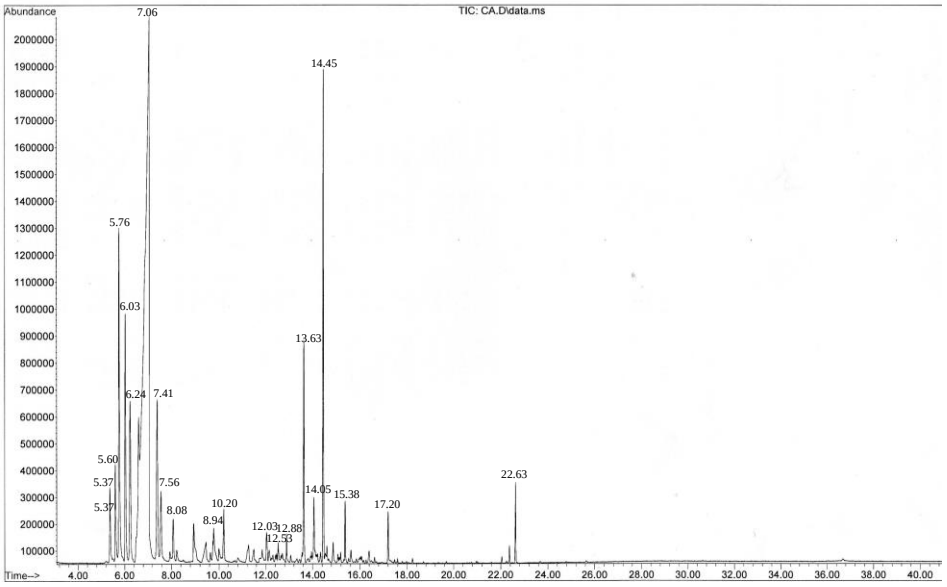

Figure 4. Total ion chromatograms of volatile components in *Theobroma cacao* L. analyzed by TD GC/MS

[Table 3] showed the MS data of the volatile components and the composition (% of peak area) of the volatile components contained in *Theobroma cacao* L.. As could be seen from [Table 3], acetic acid of the carboxylic acid was the main component of the volatile compounds with a peak area (%) of 49.72%, accounting for almost half of volatile compound contained in *Theobroma cacao* L.. A total of 27 volatile components were identified in *Theobroma cacao* L.. As shown in [Table 3], twenty-seven compounds in *Theobroma cacao* L. were identified. The major essential oils contained in *Theobroma cacao* L. were monoterpenes such as α-pinene(13.63%), 2-β-pinene(14.45%), dl-limonene(15.38%), and camphor(17.20). The monoterpenes contained in *Theobroma cacao* L. were 60.66%, showing more than half the content.

Table 3: Volatile components in *Theobroma cacao* L. analyzed by TD GC/MS

Peak No.	*Compound*	*Group*	*tR(min.)*	*Normalized peak area(%)*
1	acetaldehyde(CAS)	III	5.37	1.51
2	ethanol(CAS)	II	5.60	1.98
3	acetone	IV	5.76	6.70
4	acetic acid, methyl ester(CAS)	IV	6.03	4.61
5	isobutanal	III	6.24	3.94
6	2,3-butanedione(CAS)	IV	6.50	0.40
7	2-butanone(CAS)	IV	6.60	4.80
8	acetic acid(CAS)	V	7.06	49.72
9	butanal, 3-methyl-(CAS)	III	7.41	3.75
10	butanal, 2-methyl-(CAS)	III	7.56	1.67
11	pentanal	III	8.08	0.92
12	pentane, 1-methoxy-	I	8.94	1.19
13	propanoic acid, 2-methyl-(CAS)	V	9.46	0.93
14	toluene	I	9.75	0.27
15	2,3-butanediol	II	9.78	1.00
16	hexanal(CAS)	III	10.20	0.99
17	pentanoic acid(CAS)	V	11.26	0.62
18	p-xylene	I	12.03	0.57
19	p-xylene	I	12.53	0.30
20	nonane	I	12.88	0.44
21	alpha-pinene, (-)-	I	13.63	3.23
22	phenol	II	14.05	1.10
23	2-beta-pinene	I	14.45	6.39
24	decane	I	14.87	0.40

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25	dl-limonene	I	15.38	0.82
26	camphor	I	17.20	0.73
27	2-ethacrolein	III	22.63	1.02

I:hydrocarbon, II:alcohol, III:aldehyde, IV:ketone, V:carboxylic acid

4. Conclusion

In this study, in order to verify the insecticidal effect against house dust mites using alkaloid-based in *Theobroma cacao* L., known as a bioactive ingredient, the insecticidal effect against house dust mite of *Theobroma cacao* L. ethanol extract was examined by concentration. And caffeine and monoterpene compounds, which known to have an insecticidal effect among the components contained in *Theobroma cacao* L.,, were examined through GC / MS and TD GC / MS analysis. As a result, *Theobroma cacao* L. ethanol extract showed 100% of excellent insecticidal effect in the concentration of 0.5 ㎎/40 ㎕ and 1 ㎎/40 ㎕, respectively, and also showed excellent insecticidal effect of 97.09% at the concentration of 0.25mg/40 ㎕. Even when the concentration of the extract was 0.125mg, it showed an insecticidal effect of 75%. Thus, it was confirmed that the ethanol extract of *Theobroma cacao* L. showed an insecticidal effect against house dust mites. In addition, caffeine and four types of monoterpene compounds, which known to have an insecticidal effect, were identified from *Theobroma cacao* L..

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