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Analysis of Alkaloid Components and Essential Oils in the Jasmine Tea and Insecticidal Effects of Ethanolic Extracts of Jasmine Tea on House Dust Mite

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Abstract

GC/MS ingredient analysis and TD GC/MS analysis was performed to identify the components causing insecticidal activity among the variable components contained in jasmine tea. In the results, it was identified that the caffeine of 86.73% contained in the jasmine tea and the major essential oils in jasmine tea were delta-3-carene(23.61%), dl-limonene(16.35%), 2-beta-pinene(14.70%) and alpha-pinene, (-)-(12.17%). It was examined the insecticidal effect of the ethanolic extracts of jasmine tea on house dust mites by concentrations(1.0, 0.5, 0.25, 0.125, 0.0625 ㎎/40 ㎕). At the concentration of extract 1 ㎎/40 ㎕ and 0.5 ㎎/40 ㎕, a mortality rate of 100% was observed. At the concentration of extract 0.25 ㎎/40 ㎕, the mortality rate of 91.25% was observed. At the concentration of extract 0.125 ㎎/40 ㎕, the mortality rate of 40% was observed. These results suggest that jasmine tea ethanol extract shows a high insecticidal effect (>91.25%) at concentrations of 0.25 ㎎/40 ㎕ or higher. The insecticidal effects of the caffeine standard and 9 kinds of essential oil standards contained in the jasmine tea were measured in order to verify the insecticidal effect of jasmine tea. The caffeine showed a high insecticidal effect (>74.09%) at concentrations of 0.125 ㎎/40 ㎕ or higher. The delta-3-carene showed a high insecticidal effect (>94%) at concentrations of 0.125 ㎎/40 ㎕ or higher and a 70.84% insecticidal effect at a concentration of 0.0625 ㎎/40 ㎕. 2-beta-pinene, trans-caryophyllene, and gamma-terpinene showed high insecticidal effects (>80%) at concentrations of 0.25 ㎎/40 ㎕ or higher.

Keywords: *insecticidal effect, house dust mite, jasmine tea, alkaloid component, essential oil*

1. Introduction

Despise advances in modern medicine, hygiene pests such as ticks, mosquitoes, flies, cockroaches are having a negative impact on human life [1]. In particular, the house dust mite among hygiene pests is the most critical hygiene pests that cause allergies in humans by habitat with the human settlement [2]. Recent space that dust build up is expanding due to the increasing of the central heating structure and the use of bed and carpet by westernization of living conditions. Like this by the changes of the appropriate environment that house dust mite inhabits the numbers are increasing rapidly and the resulting damages are also a growing trend [3,4]. The damages caused by the mites are becoming more severe due to the more prosperous our standard of living [5]. Chemical control agents used till lately is effective but the use is limited in the home by the problem of residual toxicity [2, 6]. Therefore, there is a need for safe insecticides or repellents for use in the home. Tea has been one of the most popular drinks in the world for over 4,000 years [7]. Tea is manufactured in many parts of the world. Tea processing methods are classified by the degree of fermentation. Green tea is non-fermented, oolong and red tea

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are partially fermented, and black tea is completely fermented. Teas from different manufactures have their own characteristic color, taste, odor, and aroma [8]. Jasmine(Jasminum sambac Ait.), which belongs to the Oleaceae family, is one of the most important cultivated plant species in many countries for its ornamental, medicinal and edible values [9, 10]. Jasmine green tea is one of the most popular beverages consumed in China. Jasmine green tea is an excellent source of essential oil. In general, plant essential oils contain constituents predominantly of aromatic or terpenoid character. Essential oils have traditionally been used to impart flavoring or preservative effects to foods, or to instill fragrances in cosmetics and aromatherapy. Since ancient times, numerous civilizations have also valued essential oils for their therapeutic qualities in disease prevention and treatment. The ancient Egyptians buries essential oils with their pharaohs for medicinal uses in the afterlife. Later, the Greeks and Romans absorbed Egyptian practices of using essential oils in aromatherapy and expanded it to their baths for promotion of well-being. For instance, baths infused with the oils of jasmine, lavender, or ylang-ylang stimulated mental relaxation. Similarly, current interest in essential oils arises from the various bioactive effects they display, including antioxidant [11-13], anti-inflammatory [12], antimicrobial [13-15], antiviral [16-18], and anticarcinogenic [19]. This study aimed to test the insecticidal effect of jasmine tea ethanol extracts on house dust mites. First, it examined the insecticidal effect of the ethanolic extracts of jasmine tea on house dust mites by concentration. In addition, a GC/MS analysis and TD GC/MS (thermal desorption gas chromatography/mass spectrometry) analysis was performed to determine the content of alkaloid components and essential oils known to have an insecticidal effect in jasmine tea. Moreover, the insecticidal effect of jasmine tea extracts on house dust mites was determined by conducting an experiment with caffeine standard and essential oil standards.

2. Experimental Materials and Methods

2.1. Experimental Materials

2.1.1. Natural Materials: Jasmine tea was purchased online from Homerose Korea (http://www.homerose.co.kr/).

2.1.2. Mite: The house dust mites used in the experiment were *Dermatophagoides pteronyssinus*(*D. pteronyssinus*), the most common species in Korea. They were obtained from the parasitology lab at Chungbuk National University’s College of Veterinary Medicine.

2.2. Methods

2.2.1. Extraction: 30g jasmine tea with 300 ㎖ ethanol was extracted at room temperature for 24-hr and filtered. The process was repeated twice. The ethanol extract obtained from the previous step was decompressed and concentrated at 40±2℃, 30mmHg using an evaporator. This crude extract was used as the sample for the insecticidal experiment.

2.2.2. Culture of House Dust Mites: *D. pteronyssinus* was cultured using a medium that was specially prepared by mixing Ebioze powder and mouse feed in a 2:1 ratio, as shown in Table 1. The temperature and humidity were maintained at 25℃, 70%, respectively, using a thermo-hygrostat, and the medium humidity was set at 15%. House dust mites were observed using a stereoscopic microscope (OLYMPUS SZX7).

Table 1. Culture Medium for House Dust Mites

Instrument	Temperature	Relative humidity	Food	Medium humidity
Temperature and humidity chamber	25℃	70%	Ebioze powder: Mouse feed = 2:1	15%

2.2.3. Insecticidal Effects of Extracts on House Dust Mite: The insecticidal effect of the extract on house dust mites was measured using the residue thin film method, a type of direct contact method [20]. The samples (1 ㎎, 0.5 ㎎, 0.25 ㎎, 0.125 ㎎, and 0.0625 ㎎) were melted in 40 ㎕ ethanol and then put in a 2 ㎖ micro tube. They were shaken vigorously so that the sample was evenly spread on the wall of the tube, and then the solvent was volatilized. In each of the dried tubes, 25 house dust mites were inserted and then allowed to stand under dark conditions at 25℃ and 70% relative humidity. The control group was given only 40 ㎕ ethanol treatment. After 24 hr, each treatment group was examined for mortality using an stereoscopic microscope, and the individuals that did not move their appendages at all (even upon stimulation with a fine brush) were considered dead. In the control group treated with ethanol, less than 10% of individuals were dead.

2.2.4. GC/MS Analysis:

a. Preprocessing for GC/MS Component Analysis: The extracted and filtered from 300 ㎖ ethanol in 30g sample ground using Soxhlet at 60℃ for 6 hr was used as sample for GC/MS analysis.

b. GC/MS Component Analysis: The GC/MS used in this study was an Agilent 7890A GC/5975C MSD. For component separation, an Agilent 19091-436 UI (60 m× 250 um×0.25 ㎛) column was used. The column oven temperature was 100℃/5 min, 10℃/1 min, 2500℃/5min, and post 310℃/1 min . The temperature of the sample injection port was 250℃, and the split ratio for sample injection was 1:200. In terms of the setting for the mass spectrometer, a mass mode of scan and a mass range of 20amu− 550amu were selected for the analysis.

2.2.5. Essential Oils Analysis by TD GC/MS:

a. Sample pretreatment

1) Sample grinding: The sample for the essential oils analysis was used after being ground (cut) to a specific size (up to 5 ㎜) for efficiency of extraction.

2) Tenax tube condition: The Tenax tube used for volatile adsorption was used after 4 hr heat desorption during air supply at 350℃ using a high-purity (99.999%) Aux-controller 163 (GESTEL) heat desorption device. It was then cooled at room temperature and sealed. To prevent contamination in the atmosphere, it was stored in a separate closed storage area.

3) Sample processing: Jasmine tea(30g) was placed in a 3L Tedlar bag that was also filled with 2L high-purity nitrogen and stored in a 32℃ oven for two days. The same condition with 2L high-purity nitrogen without jasmine tea was also processed for a blank test.

4) Sample adsorption: The pretreated samples were adsorbed by 1 L each to the Tenax tube (Tenax TA Thermal Desorption Tube, Tenax glass, GESTEL) using a gas dilution system (Chemtopia) and immediately used as the samples for analysis.

b. Essential Oils analysis

The essential oils analysis was performed using a TD-GC/MS device (GC: Agilent 6890N, G1530N, and 2975 MASS) using the conditions described in Table 2.

3. Results

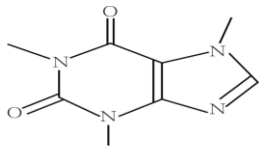
3.1. Compound Analysis in Jasmine Tea

3.1.1. Analysis by GC/MS: The identified compounds from jasmine tea were listed in Table 3, with their respective retention times(RT) and percent compositions(peak area %). The corresponding chromatograms were shown in Figure 1. The principle compound identified from jasmine tea was caffeine(86.73%). A key peak of retention time was shown at 10.879min., as shown in (A) in Figure 1 and its associated component was caffeine, as shown in the Table 3. Figure 1 (B) showed the mass spectrum of the caffeine portion (10.879 minutes) of jasmine tea ethanol extract, and (C) showed the mass spectrum of the authentic caffeine. It could know the fact that the two substances are identical, because to coincide with the two graphs. Caffeine(Table 4) is the most abundant alkaloid in tea. Caffeine is a bitter, white crystalline xanthine alkaloid found in various seeds, leaves, nuts, and berries. Caffeine ingested by insects when they feed on these plant parts functions as a natural pesticide that can paralyze and kill them. Caffeine is found in many plant species, where it acts as a natural pesticide, with high caffeine levels being observed in seedlings still developing foliage but lacking mechanical protection; caffeine paralyzes and kills certain insects feeding on the plant [21]. Therefore, caffeine is understood to have a natural function as both a natural pesticide and an inhibitor of seed germination of other nearby coffee seedlings, thus giving it a better chance of survival [22]. Thus, caffeine is known as a natural insecticide. In this study, the insecticidal effect of the caffeine standard on the house dust mite was measured because caffeine was assumed to appear the insecticidal effect on the house dust mite. The result was shown in 3.2.2.

Table 3. Compounds Identified from Jasmine Tea

Peak No.	Compound	Retention Time(min.)	Normalized Peak Area(%)
1	(1-(Cyclopenten-1-yl) ethenyloxy) trimethylsilsne	8.210	3.54
2	Caffeine	10.879	81.63
3	Caffeine	11.398	5.01
4	Phytol	12.400	1.34
5	alpha-Tocopherol	25.544	1.16
6	Stigmasta-7,16-dien-3-ol, (3.beta.,5.alpha.)-	31.786	4.80
7	Silikonfett	33.908	2.51

Table 4. Caffeine Identified from Jasmine Tea

Compound	Molecular Formula	IUPAC Name	Class of Alkaloids	Molecular Structure
Caffeine	$C_8H_{10}N_4O_2$	1,3,7-Timethylpurine-2,6-dione	Xanthine	

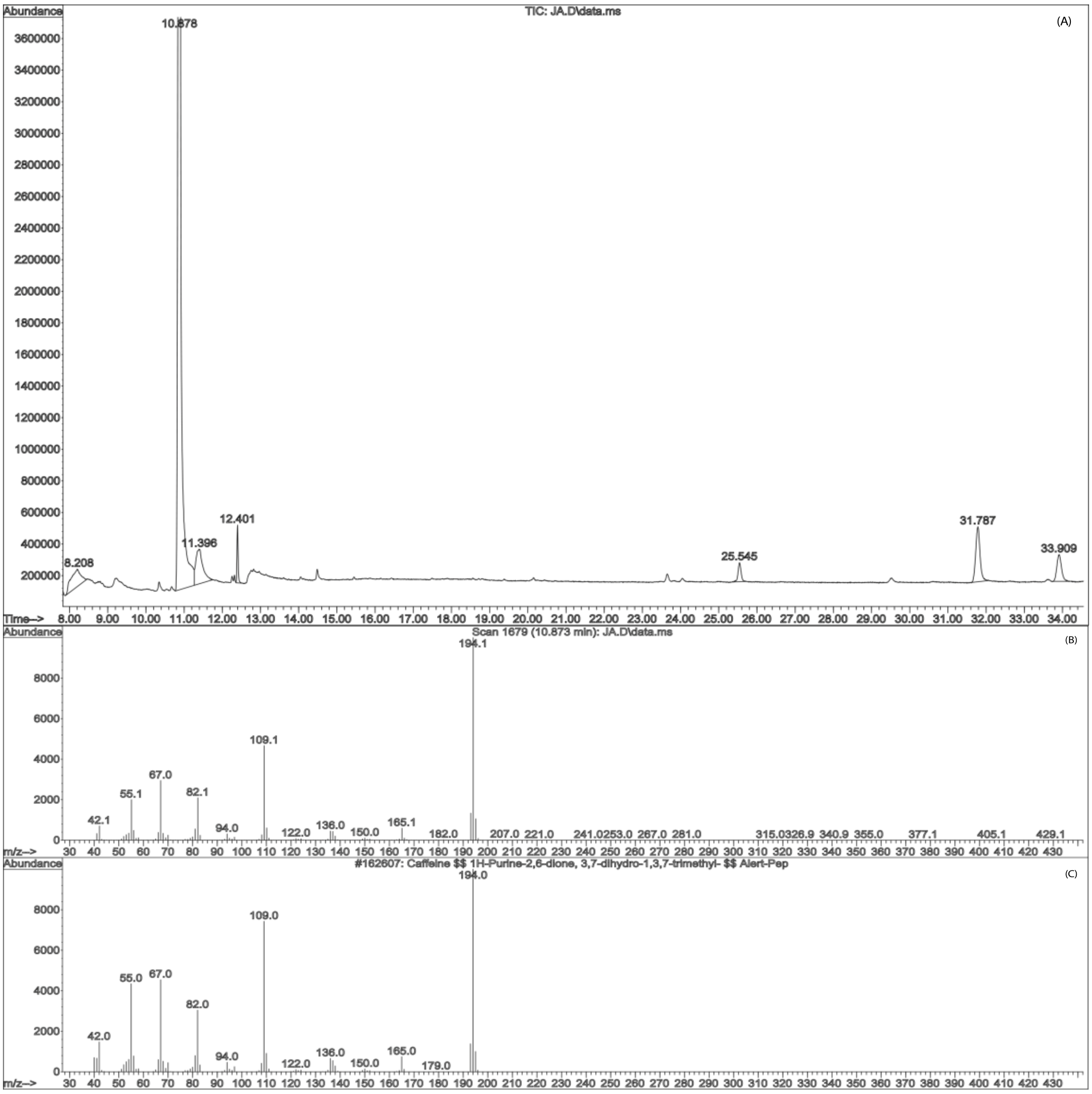


Figure 1. Total Ion Chromatograms of Compounds in Jasmine Tea (A), MS Spectra of Purified Caffeine from Jasmine Tea (B) and Authentic Caffeine (C)

3.1.2. Analysis by TD GC/MS: The total ion chromatogram of essential oils in the jasmine tea was presented in Figure 2. The figure showed high peaks between 13 minutes and 17 minutes, which correspond to the monoterpene compounds, including alpha-pinene, 2-beta-pinene, 1-phellandrene, delta-3-carene and dl-limonene as revealed by Table 5. The figure also showed low peaks between 13 minutes and 17 minutes, which correspond to the monoterpene compounds, including alpha-thujene, camphene, sabinene, beta-myrcene, gamma-terpinene and (+)-4-carene. TD GC/MS profile of the extracted flavor showed the presence of a wide range of compounds, including terpenoids, alcohol, aldehydes, ketones, heterocyclic and esters(Table 5). As outlined in Table 5, thirsty-three compounds in jasmine tea were identified. The major essential oils of jasmine tea were monoterpene such as delta-3-carene(23.61%), dl-limonene(16.35%), 2-beta-pinene(14.70%) and alpha-pinene, (-)-(12.17%). Monoterpenes in jasmine tea were contained about 86.78%. Therefore essential oils in jasmine tea were dominated by terpenoids. Among the non-terpenoids, ketones(1.40%), aldehydes(1.35%), esters(0.69%), ethers(0.62%) and alcohol(0.50%) was detected in somewhat lower amounts.

Terpenoids and phenylpropanoids form the major constituents of the essential oils. Terpenoids are the most diverse class of natural compounds consisting of more than 40,000 structurally different molecules, which have been isolated from animal and microbial species as well as from a wide variety of plants [23]. Therefore, terpenoids are a large group of phytochemicals, used by Chinese and Indian traditional medicine [24, 25]. Terpenoid compounds have been reported to be beneficial to the human body for anti-inflammatory, anti-oxidant, anti-cancer and anti-biotic effects [26]. The important classes of terpenoids present in plants are the volatile essential oils, triterpenoids, steroids and carotenoids. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure is C10H16 and they are called terpenes [27]. Many secondary plant metabolites are known for their insecticidal properties, and in many cases plants have a history use as home remedies to kill or repel insects [28]. It is known that some chemicals constituents of essential oils have insecticidal properties [29]. Many essential oils are composed of a variety of terpenoid compounds [30]. These substances are usually volatile and can be detected by the antennae or tarses of insects [29]. Essential oils isolated from plants consisting of mainly bioactive monoterpenes may have attractive or repellent effects. In some cases, they show an insecticidal action such as inhibition of molting and respiration, reduction in growth and fecundity, cuticle disruption, and effects on the invertebrate octopamine pathway [31, 32]. Essential oils are also effective repellents against some insect species [33] and their vapors and pure constituents also show toxic effects against larvae and adults of some insects [34-41]. Presence of volatile monoterpenes or essential oils in the plants provides an important defense stragy to the plants, particularly against herbivorous insect pests and pathogenic fungi [42]. These volatile terpenoids also play a vital role in plant-plant interactions and serve as attractants for pollinators [43]. Aromatic plants and their essential oils have been used since antiquity in flavor and fragrances, as condiment or spice, in medicines, as antimicrobial/insecticidal agents, and to repel insect or protect stored products [44-46]. In nature, essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides and also against herbivores by reducing their appetite for such plants. In the published paper [47], the insecticidal effect of essential oil standards on the house dust mites was measured because essential oils were assumed to appear the insecticidal effect on the house dust mites. As the result, it have been turned out that essential oils had the insecticidal effect on house dust mites (Figure 5).

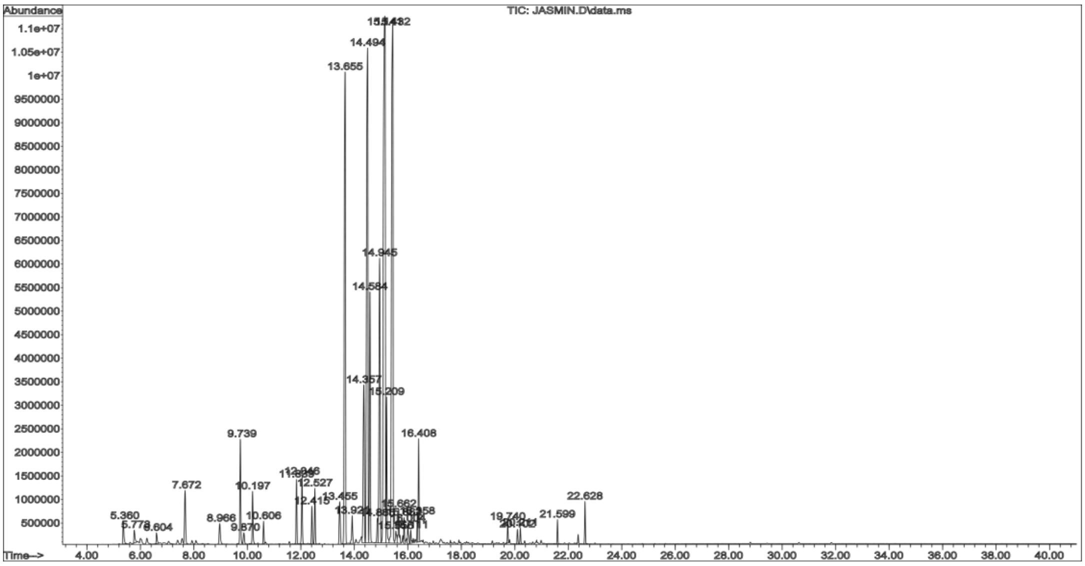


Figure 2. Total Ion Chromatograms Essential Oils in Jasmine Tea Analyzed by TD GC/MS

Table 5. Essential Oils in Jasmine Tea Analyzed by TD GC/MS

Peak No.	Compound	Group	Retention Time(min.)	Normalized Peak Area(%)
1	Methyl alcohol	Ⅱ	5.36	0.50
2	Acetone	Ⅳ	5.78	0.37
3	2-Butanone(CAS)	Ⅳ	6.61	0.22
4	Benzene(CAS)	Ⅰ	7.67	1.18
5	Methyl isobutyl ketone	Ⅳ	8.97	0.51
6	Toluene	Ⅰ	9.74	1.99
7	Butanoic acid, 2-methyl-, methyl ester(CAS)	Ⅵ	9.87	0.26
8	Hexanal(CAS)	Ⅲ	10.20	0.89
9	Acetic acid, butyl ester(CAS)	Ⅵ	10.61	0.43
10	Benzene, ethyl-(CAS)	Ⅰ	11.84	1.06
11	p-Xylene	Ⅰ	12.05	1.20
12	Styrene	Ⅰ	12.42	0.58
13	p-Xylene	Ⅰ	12.53	0.89
14	alpha-Thujene	Ⅰ	13.45	0.94
15	alpha-Pinene, (-)-	Ⅰ	13.65	12.17
16	Camphene	Ⅰ	13.92	0.53
17	Sabinene	Ⅰ	14.36	3.90
18	2-beta-Pinene	Ⅰ	14.49	14.70
19	beta-Myrcene	Ⅰ	14.59	4.71
20	1-Phellandrene	Ⅰ	14.94	7.03

21	delta-3-Carene	Ⅰ	15.14	23.61
22	dl-Limonene	Ⅰ	15.43	16.35
23	Heptane, 2,2,4,6,6-pentamethyl-octane, 2,2,6-trimethyl-	Ⅰ	15.56	0.32
24	Nonane, 3-methyl-5-propyl-	Ⅰ	15.66	0.73
25	gamma-Terpinene	Ⅰ	15.86	0.53
26	Hexane, 2,2,5-trimethyl-	Ⅰ	16.00	0.50
27	Octane, 3,6-dimethyl-	Ⅰ	16.11	0.21
28	(+)-4-Carene	Ⅰ	16.41	1.97
29	Piperitone oxide	Ⅳ	19.74	0.30
30	2-Propyl-2-pentenal	Ⅲ	20.10	0.22
31	2-Hexenal, (E)-(CAS)	Ⅲ	20.21	0.24
32	trans-Caryophyllene	Ⅰ	21.60	0.34
33	Furan, 2,3-dihydro-4-methyl-	Ⅴ	22.63	0.62

Ⅰ:hydrocarbon, Ⅱ:alcohol, Ⅲ:aldehyde, Ⅳ:ketone, Ⅴ:heterocyclic, Ⅵ:ester

3.2. Insecticidal Effect on House Dust Mite

3.2.1. Insecticidal Effect of Extract on House Dust Mite: The results of the measurements of the insecticidal effect of jasmine tea ethanol extract on house dust mites (D. pteronyssinus) were shown in Figure 3. As also shown in Figure 3, the extract showed a 100% insecticidal effect at the concentrations of 1 ㎎/40 ㎕ and 0.5 ㎎/40 ㎕ and showed a 91.25% insecticidal effect even at a concentration of 0.25 ㎎/40 ㎕. However, the insecticidal effect nearly disappeared at a concentration of 0.0625 ㎎/40 ㎕. These results suggested that jasmine tea ethanol extract showed a high insecticidal effect (>91.25%) at concentrations of 0.25 ㎎/40 ㎕ or higher.

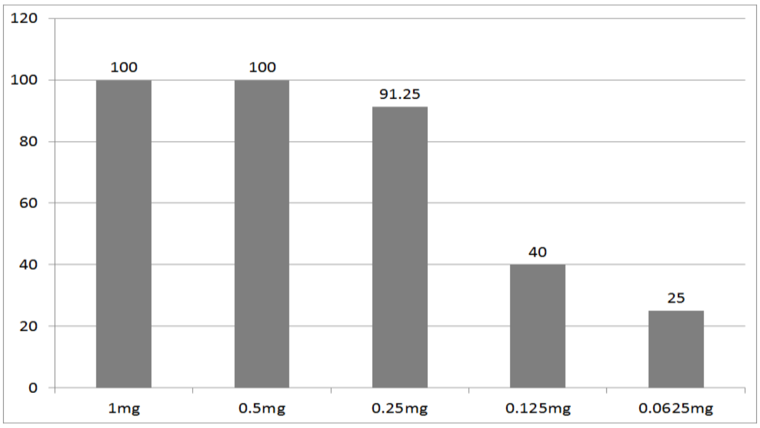


Figure 3. Insecticidal Effect of Ethanolic Extracts of Jasmine Tea on House Dust Mite

3.2.2. Insecticidal Effect of Caffeine Standard Contained in Jasmine Tea on House Dust Mite: As mentioned earlier, caffeine is known as a natural insecticide. The measurements from insecticidal effects of caffeine standard on house dust mite were shown in Figure 4. As shown in this Figure, the caffeine showed a 100% insecticidal effect at the concentrations of 1 ㎎/40 ㎕ and 0.5 ㎎/40 ㎕. The caffeine at the concentrations of 0.25 ㎎/40 ㎕ and 0.125 ㎎/40 ㎕ showed a insecticidal effect of 74.09% and 92.55%, respectively. These results suggested that caffeine showed a high insecticidal effect (>74.09%) at concentrations of 0.125 ㎎/40 ㎕ or higher. It was found that the insecticidal effect of jasmine tea ethanol extract on house dust mites was due to the effect of the caffeine contained in jasmine.

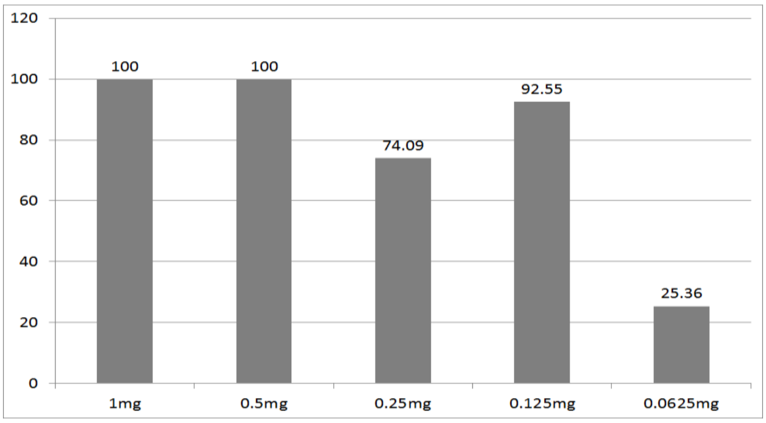


Figure 4. Insecticidal Effect of Caffeine Standard on House Dust Mite

3.2.3. Insecticidal Effect of Terpene Compound Standards Contained in Jasmine Tea on House Dust Mite: Terpene compounds contained in jasmine tea were shown in Table 6. As shown in Table 6, 11 kinds of monoterpene compounds and 1 kind of sesquiterpnene compound were found in the jasmine tea. Among them, the insecticidal effects of nine kinds of terpene compounds that were available for purchase were measured [47], and these were shown in Figure 5. As Figure 5 showed, delta-3-carene showed a high insecticidal effect (>94%) at concentrations of 0.125 ㎎/40 ㎕ or higher and a 70.84% insecticidal effect at a concentration of 0.0625 ㎎/40 ㎕. 2-beta-pinene, trans-caryophyllene, and gamma-terpinene showed high insecticidal effects (>80%) at concentrations of 0.25 ㎎/40 ㎕ or higher. These results indicated that the terpene compounds contained in jasmine tea had insecticidal effects on house dust mites.

Table 6. Terpene Compounds Contained in the Jasmine Tea

Terpene Compounds	
Monoterpene	alpha-Thujene
	alpha-Pinene
	Camphene
	Sabinene
	2-beta-Pinene
	beta-Myrcene
	1-Phellandrene
	delta-3-Carene
	dl-Llimonene
	gamma-Terpinene
	(+)4-Carene
Sesquiterpene	trans-Caryophyllene

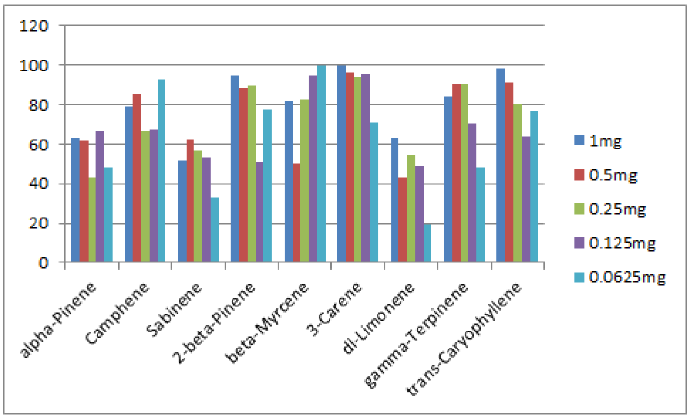


Figure 5. Insecticidal Effects of Essential Oil Standards Included in Jasmine Tea on House Dust Mite

4. Conclusion

The purpose of this study was to prove the insecticidal effect of jasmine tea on house dust mite. The insecticidal effect of the ethanolic extracts of jasmine tea on house dust mites by concentration was examined. GC/MS analysis and TD GC/MS analysis was performed to identify the compounds causing insecticidal activity among the variable components contained in jasmine tea. In order to verify the insecticidal effect of jasmine tea, the insecticidal effects of the authentic caffeine and 9 kinds of authentic essential oils were measured: alpha-pinene, camphene, sabinene, beta-pinene, beta-myrcene, delta-3-carene, dl-limonene, gamma-terpinene, and trans-caryophyllene.

In the results, the extract showed a 100% insecticidal effect at the concentrations of 1 ㎎/40 ㎕ and 0.5 ㎎/40 ㎕ and showed a 91.25% insecticidal effect even at a concentration of 0.25 ㎎/40 ㎕. However, the insecticidal effect nearly disappeared at a concentration of 0.0625 ㎎/40 ㎕. These results suggest that jasmine tea ethanol extract showed a high insecticidal effect (>91.25%) at concentrations of 0.25 ㎎/40 ㎕ or higher. It was found that caffeine of 86.73% contained in the jasmine tea by GC/MS analysis. Moreover, it was identified that the major volatile compounds of jasmine tea were delta-3-carene(23.61%), dl-limonene(16.35%), 2-beta-pinene(14.70%) and alpha-pinene, (-)-(12.17%) by TD GC/MS analysis. The insecticidal effect of the caffeine standard and 9 kinds of essential oil standards was measured. In the results, caffeine showed a high insecticidal effect (>74.09%) at concentrations of 0.125 ㎎/40 ㎕ or higher. The delta-3-carene showed a high insecticidal effect (>94%) at concentrations of 0.125 ㎎/40 ㎕ or higher and a 70.84% insecticidal effect at a concentration of 0.0625 ㎎/40 ㎕. 2-beta-pinene, trans-caryophyllene, and gamma-terpinene showed high insecticidal effects (>80%) at concentrations of 0.25 ㎎/40 ㎕ or higher. Therefore it was considered that the cause of insecticidal effect on the house dust mite was driven from a synergic effect between caffeine and essential oils contained in the jasmine tea.

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