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Insecticidal Effects from Ethanol Extracts of Root Peel, Stem Peel and Fruit Peel of Pomegranate (*Punica granatum* L.) on House Dust Mite

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Abstract

The purpose of this study was to prove insecticidal effect from ethanol extracts of root peel, stem peel and fruit peel of pomegranate(Punica granatum L.*) on house dust mite. The insecticidal effects of each extracts on house dust mit were applied by direct contact method at different concentrations(1.0, 0.5, 0.25, 0.125, 0.0625㎎/40㎕) and exposure time of 24hours. At the concentration of extract 1㎎/40㎕, a complete mortality of 100% was observed from the root peel, stem peel, and fruit peel of pomegranate. At the concentration of extract 0.5㎎/40㎕, the high mortality of 88.00%, 100% and 93.10% was observed from the root peel, stem peel and fruit peel of pomegranate, respectively. At the concentration of extract 0.25㎎/40㎕, the highest mortality of 89.50% was observed from the root peel, followed by 83.25% in stem peel and 57.00% in fruit peel. At the concentration of extract 0.125㎎/40㎕, 68.30%, 55.00% and 69.57% of mortality were observed from root peel, stem peel and fruit peel of pomegranate, respectively. By the results, the ethanol extracts of root peel, stem peel and fruit peel of pomegranate were shown to present a highly powerful insecticidal effect on house dust mite. GC/MS analysis was used to identify pseudopelletierine, which is known as insecticidal active component in pomegranate. As the result, pseudopelletierine was found in the root peel. In order to verify the insecticidal effect of pomegranate, the insecticidal effects of the authentic pseudopelletierine and 11 terpene compounds contained in root peel, stem peel and fruit peel of pomegranate were measured. From the results, pseudopelletierin presented a higher insecticidal effect on house dust mite at the concentration higher than 0.5㎎/40㎕. The camphor showed a great mortality over 90% at all concentrations and delta-3Carene also showed a high mortality, which was higher than 70% at all concentrations. The beta-Pinene, trans-Caryophyllene, gamma-Terpinene showed the mortality of 80% or higher at the concentration ranged from 1㎎/40㎕ to 0.125㎎/40㎕, and camphene showed the mortality of 60% or higher at all concentrations.*

Keywords: *insecticidal effect, house dust mite, root peel, stem peel and fruit peel of Pomegranate, ethanol extract, gas chromatography-mass spectrometry*

1. Introduction

Dermatophagoides pteronyssinus is the world's most common house dust mite species, accounting for 60~90% of all indoor dust mites. This *Dermatophagoides pteronyssinus* is mostly found in textile goods, including bedding, chair, sofa, quilting or carpet. The house dust mites feed and grow themselves on dead skin cells or dandruff that humans or animals shed every day. Globally, it is well known for one of main factors causing the respiratory symptoms such as asthma, rhinitis or atopy, and

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also allergic symptoms [1]. There were many attempts to use diverse methods, such as micro fiber fabrics, high-temperature steam cleaner or chemical compositions, in order to remove the house dust mists; however, the powerful, effective removal approaches have not yet been proposed [2]. Especially the varied types of chemical insecticides are highly harmful to human body at its higher concentration, and for this reason, they are not widely preferred [3]. Thereby, a study should be undertaken to investigate the insecticidal effects of the natural components with less side effects on human body, instead of the chemical synthetic components. The natural component has physiological active components such as phenol compounds, terpene compounds and alkaloids on a wide range, displaying physiological activity against the harmful insects[4]. These physiological active components are extensively diverse upon the type of plant. Especially they react as toxins, inhibitor, repellent, growth inhibitor or feeding inhibitor targeting against arthropods, playing a key role in preventing sanitary insects [5]. Since the natural components are biodegraded into non-toxic materials, which is highly developable into an eco-friendly insecticide; therefore, its related study and development need to be urgently undertaken. Unfortunately, the study on insecticidal effects of pomegranate (*Punica granatum* L.) on house dust mist was rarely performed. The pomegranate, *Punica granatum* L. belongs to the punicaceae family and is grown in many countries, especially in the Mediterranean region, Iran, India, Pakistan, Afghanistan, Saudi Arabia, and in the subtropical areas of south America[6]. Pomegranate is one of the most investigated fruits in recent years. Numerous studies have been published regarding antioxidant[7], anti-hypertensive[8], and anti-cancer[9] effects of pomegranate juice. Antibacterial, anti-inflammatory, and anti-allergic activities of pomegranate peel extracts have been studied[11]. Antimicrobial activities of pomegranate peel extracts have been proved against staphylococcus aureus, Listeria monocytogenes, Escherichia coli and Yersinia enterocolitica[10]. Hydro alcoholic extracts of pomegranate peels showed anti-diabetic activities by significantly reducing blood glucose level of normal and diabetic rats[12-13]. In general, the pomegranate has been widely used in removing parasites as a parasiticide, and also used in treating ulcer, diarrhea, hyperacidity, dysentery, bleeding and microbial infection. Moreover, this also has been used as a fever reducer [14-15]. Root peel, stem peel and branch peel of pomegranate are well known for its effectiveness in eradicating tapeworms. 30g of pomegranate peel is to be boiled at water 500㎖ and taken at empty stomach, three times a day to treat the tapeworms in the body. This peel also can be burnt or its boiled water can be smeared on water insects for their eradication; and it is also effective in removing bad breath [16]. The main components in root peel of pomegranate are piperidine alkaloids - Isopelletierine, pseudopelletierine, methyl pelletierine and methyl isopelletierine. The total contents of alkaloids in root peel are about 0.6%, followed by 0.5% in stem peel and 0.4% in branch peel. The fruit peel of pomegranate contains of tannin, sugars, resines, mannitol, inulin, succinic acid, oxalic acid, pectin, Ca, and isoquercetin [17-23]. From ancient times, pomegranate is widely used as natural dye and the key ingredient of the color dye is Tannin. Tannin dyes colors especially from brown to dark brown - light brown by aluminum mordant, yellow brown by copper mordant, blackish brown by steel mordant, dark brown by combining steel and alkali, producing a diverse color [24].

The purpose of this study is to prove the insecticidal effects of pomegranate, which is alkaloids natural dye known as physiologically active material; ultimately, it is to scientifically identify the potentiality of pomegranate as the natural dye with insecticidal effects on house dust mite. In this study, the natural insecticidal dye is to be used on a fabric to investigate and develop the material capable of protecting human body from the harmful environment such as house dust mite. As a part of the study, the pomegranate was divided into 3 parts: root peel, stem peel and fruit peel. Each part was extracted with ethanol, and the insecticidal effects of such extracts on house dust

mite were investigated at different concentration. GC/MS ingredient analysis was performed to identify the component taking insecticidal activity among the variable components contained in the root peel, stem peel and fruit peel of pomegranate. Also, the insecticidal effects of piperidine alkaloids, the pseudopelletierine and 11 terpene compounds were measured: limonene, alpha-pinene, beta-pinene, camphor, camphene, sabinene, beta-myrcene, delta-3-carene, gamma-terpinene, ortho-cymene and trans-caryophyllene.

2. Experimental Materials and Methods

2.1. Experimental Materials

2.1.1. Natural Materials: Root peel and stem peel were derived from pomegranate tree purchased from pomegranate garden located in Goheung, 2010. The fresh fruit peel was derived from pomegranate purchased in E-mart, Jechoen branch, 2012. According to literature reference [6], the root peel and stem peel of pomegranate are known as insecticide and their valid ingredients are highly volatile, requiring a fresh material for experiment; for this reason, the fresh root peel, stem peel and fruit peel of pomegranate were used as a sample in this study.

2.1.2. Mite: House dust mite used in this experiment was *Dermatophagoides pteronyssinus*, the most common species found in Korea, which was received from parasitology laboratory in Veterinary college of Chungbuk National University.

2.2. Experimental Methods

2.2.1. Extraction: 300㎖ of ethanol was added to each 30g of root peel, stem peel and fruit peel of pomegranate and they were extracted at the room temperature for 24 hours, and filtered. This process was repeated through a couple of times. The ethanol extract obtained from the previous step was decompressed and concentrated at 40±2℃, 30mmHg, respectively by using a concentrator to obtain the ethanol extract. This crude extract was used as a sample for testing insecticide.

2.2.2. Breeding of House Dust Mite: The culture media with Ebioze powder and mite sample mixed in the ratio of 2:1 was used to breed *Dermatophagoides pteronyssinus*. At here, a thermohygrostat was used to maintain the temperature 25℃ and humidity 70%. The breeding plastic box (20.5×20.5×20.5㎝) was maintained at a relative humidity and filled with the saturated NaCl solution to prevent mites from escaping. A small plastic box (12.5×12.5×7.0㎝) containing the media and house dust smite was inserted into above-mentioned larger box and then cultured for 2-4 weeks. The grown imaginal house dust mite was used in the experiment. The stereoscopic microscope (OLYMPUS SZX7) was used to observe the house dust mite.

2.2.3. Insecticidal Effects of Extracts on House Dust Mite: A type of direct contact method [24] was used to measure the insecticidal effects of extracts on house dust mite. 1㎎, 0.5㎎, 0.25㎎, 0.125㎎ and 0.0625㎎ of each sample was melted in 40㎕ of ethanol, and placed into a micro 2㎖ tube. This tube was well shaken for samples to be evenly smeared on the tube and the remaining solvent was volatilized. 25 mites were placed into each dried tube and left at the temperature 25℃ and humidity 70% under dark condition. The control group was treated with 40㎕ of ethanol only. The stereoscopic microscope (×20) was used to investigate the insecticidal activity of each treatment group after 24 hours. A fine brush was used to touch the mites, and the paper

placed under the mite stayed inactive were considered dead. Less than 10% of control group treated with the ethanol only was found dead.

2.2.4. GC/MS Ingredients Analysis: Alkaloids refer to nitrogenous compounds and are defined as the aminic plant component with a significant physiological function [26]. The alkaloids are also contained in the root peel, stem peel and fruit peel of pomegranate and according to the previous literature [16], 0.6%, 0.5% and 0.4% of alkaloids are contained in the root peel, stem peel and branch peel of pomegranate, respectively. Main alkaloids are pelletierine and pseudopelletierine, and they are toxic to tapeworms. These tapeworms are paralyzed by the toxicity, ultimately dead. [16]. Thereby, a GC/MS analysis was performed to identify whether the root peel, stem peel and fruit peel of pomegranate used in this study contained the insecticidal components, such as pelletierine, pseudopelletierine, etc.

a. Preprocessing for GC/MS Ingredients Analysis: 300㎖ of ethanol was added to 30g of each sample and then extracted at temperature 60℃ for 6 hours using a Soxhlet's extractor and then filtered. Each filtered sample was used as a sample for GC/MS analysis.

b. GC/MS Ingredients Analysis: GC/MS used in this experiment was Agilent 7890A GC/5975C MSD. Agilent 19091-436 UI (60 m× 250 ㎛× 0.25 ㎛) column was used in decomposition. The column oven temperature was at 100 ℃/5 min, 10 ℃/min, 2500 ℃/5 min and Post 310 ℃/1 min. The temperature of an inlet in sample was 250℃ and the sample was injected in the division ratio of 1:200. In the analysis, the mass mode in the mass spectrometer was set at scan and the mass range was set from 20 amu to 555 amu.

3. Results

3.1. Yield Rate of Extract

300㎖ of ethanol was added to each 30g of root peel, stem peel, and fruit peel of pomegranate and they were extracted at the room temperature for 24 hours, and filtered. This process was repeated through a couple of times. The ethanol extract obtained from the previous step was decompressed and concentrated at 40±2℃, 30mmHg, respectively by using a concentrator to obtain the ethanol extract. This crude extract was used as a sample for testing insecticide. The yield rate of crude extract was shown in table 1. Here, the highest yield as 10.551g was observed from the fruit peel of pomegranate. The yield of ethanol extracts – root peel and stem peel of pomegranate was shown to be relatively low as 0.769g and 1.635g, respectively.

Table 1. Yield of Ethanol Extract of Pomegranate's Root Peel, Stem Peel and Fruit Peel

Plant Species	Family Name	Korean Name	Yield(%)[a]
Punica granatum L.	*Punicacea*	Root peel	0.769g
		Stem peel	1.635g
		Fruit peel	10.551g

[a] (weight of extract / weight of test plant) × 100.

3.2. Insecticidal Effects of Extracts on House Dust Mite

A type of direct contact method was used to measure the insecticide effects of extracts from root peel, stem peel, and fruit peel of pomegranate on house dust mite and its results were shown in table 2. As can be seen in this table, At the concentration of extract 1㎎/40㎕, 100% of a complete mortality was observed from the root peel, stem peel, and fruit peel of pomegranate. At the concentration of extract 0.5㎎/40㎕, the high mortality of 88.00%, 100% and 93.10% were observed from the root peel, stem peel and fruit peel of pomegranate, respectively. At the concentration of extract 0.25㎎/40㎕, the highest mortality of 89.50% was observed from the root peel, followed by 83.25% in stem peel and 57.00% in fruit peel. At the concentration of extract 0.125㎎/40㎕, 68.30%, 55.00% and 69.57% of mortality were observed from root peel, stem peel and fruit peel of pomegranate, respectively, whereas nearly no insecticidal effects as 6.67%, 11.67% and 10.00%, were observed from root peel, stem peel and fruit peel of pomegranate at the concentration of extracts 0.0625㎎/40㎕. As described above, a considerably high mortality ranged from 83.25% to 100% was observed at the concentration higher than 0.25㎎/40㎕. Thus what extracts of root peel, stem peel and fruit peel of pomegranate presented the insecticidal effects on house dust mite was considered that was driven from a synergic effect between pseudopelletierine, which was known for insecticidal activity, identified from the GC/MS ingredient analysis (3.3) and terpene components, which was also known for insecticidal activity, confirmed through TD GC/MS aromatic ingredient analysis conducted in the previous study [27]. Their measurements of insecticidal effects were shown in Table 6 and 8.

Table 2. Insecticidal Effect on Ethanol Extract of Pomegranate's Root Peel, Stem Peel and Fruit Peel on House Dust Mite

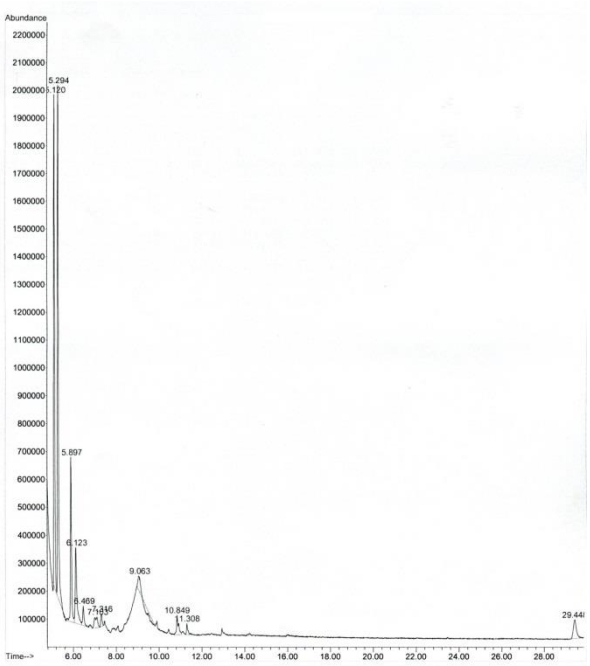
Concentration (㎎/40㎕))	Samples	Mortality(%)[a]
1	Pomegranate's root peel	100
	Pomegranate's stem peel	100
	Pomegranate's fruit peel	100
0.5	Pomegranate's root peel	88.00
	Pomegranate's stem peel	100
	Pomegranate's fruit peel	93.10
0.25	Pomegranate's root peel	89.50
	Pomegranate's stem peel	83.25
	Pomegranate's fruit peel	57.00
0.125	Pomegranate's root peel	68.30
	Pomegranate's stem peel	55.00
	Pomegranate's fruit peel	69.57

0.0625	Pomegranate's root peel	6.67
	Pomegranate's stem peel	11.67
	Pomegranate's fruit peel	10.00

[a] (remained mites/Total mites) × 100

3.3. GC/MS Ingredient Analysis for Root Peel, Stem Peel and Fruit Peel of Pomegranate

As can be seen above, the ethanol extracts of root peel, stem peel and fruit peel of pomegranate were shown to present a highly powerful insecticidal effect. GC/MS analysis was used to identify pseudopelletierine, which is known as insecticidal active component in pomegranate; and their results were shown in Table 3, 4 and 5. As presented in these tables, 11 different types of ingredients were confirmed in the root peel of Pomegranate. Among the identified ingredients, 4 types were alkaloids and they were pelletierine, mepivacaine, pseudopelletierine and aniline, 4-fluoro-N-[2-(2-thienoyl)-ethyl]. 6 different ingredients were found in the stem peel and only one 1-aza-2,4-diphenyl-3- methoxycarbonyl-cyclopent-1-ene was found to be alkaloids. 8 types of ingredients were observed in the fruit peel of pomegranate and only one 2-p-nitrophenyl-1,3,4-oxadiazol-5-one was found for alkaloids. As mentioned above, pseudopelletierine, which is known as an insecticidal component, was observed in the root peel of pomegranate only. A key peak of remaining time was shown at 5.907min., as shown in (A) in Figure 1 and its associated component was pseudopelletierine, as shown in the Table 3. (B) in Figure 1 illustrated the mass spectrum of pseudopelletierine fraction (5.907min.) purified from the extracts of root peel, and (C) in Figure 1 presented the mass spectrum of the authentic pseudopelletierine. Two graphs presented in (B) and (C) were in consistency each other, considering two components are same materials. The molecular formula of pseudopelletierine was shown in Figure 2. Pseudopelletierin refers to piperidine alkaloids with the piperidine inside the molecule, as shown in Figure 3.



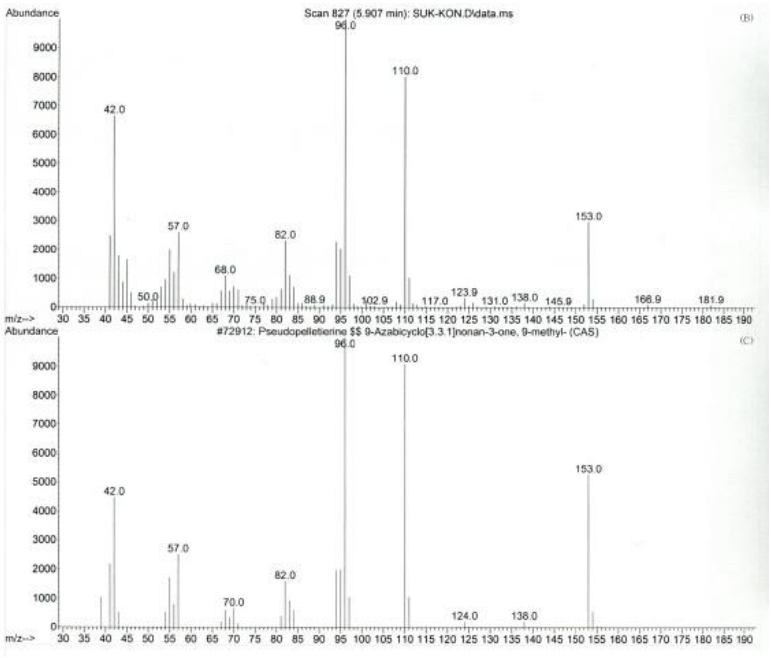


Figure 1. Total Ion Chromatograms of Compounds in the Root Peel of Pomegranate (A), MS Spectra of Purified Pseudopelletierine from the Root Peel of Pomegranate (B) and Authentic Pseudopelletierine (C)

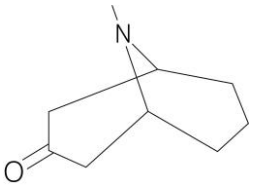


Figure 2. Structural Formula of Pseudopelletierine

Figure 3. Structural Formula of Piperidine

Table 3. Compounds Identified from the Root Peel of Pomegranate

Peak No.	Compounds	tR(min.)	Peak Area(%)
1	Pelletierine	5.12	31.37
2	Mepivacaine	5.30	34.37
3	Pseudopelletierine	5.90	12.34
4	1,5-anhydro-arabino-furanose	6.12	8.74
5	Propanoic acid, 2-(aminooxy)-	6.47	1.98
6	2-Bromoethanol	7.10	2.36
7	exo-1,2-O-Ethylidene-α-d-erythrofuranose	7.32	1.13

8	D-glycero-D-manno-Heptitol	9.06	-0.19
9	Aniline, 4-fluoro-N-[2-(2-thienoyl)-ethyl]-	10.85	2.64
10	7-Isopropyl-4a-methyloctahydro-2(1H)-naphthalenone	11.31	1.16
11	γ-Sitosterol	29.45	4.10

Table 4. Compounds Identified from the Stem Peel of Pomegranate

Peak No.	Compounds	tR(min.)	Peak Area(%)
1	(2R, 3R)-(2-2H1)-1,3-Butanediol	5.94	4.97
2	Benzenemethanethiol 1-aza-2,4-diphenyl-3-methoxycarbonyl-cyclopent-1-ene	8.65 8.61	14.22
3	1H-Indole, 2-methyl-3-phenyl-	11.32	3.54
4	3-Phenyl-1,2,4-benzotriazine	12.95	2.30
5	β-Sitosterol	29.46	12.19
6	Friedelan-3-one	38.80	62.78

Table 5. Compounds Identified from the Fruit Peel of Pomegranate

Peak No.	Compounds	tR(min.)	Peak Area(%)
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	7.19	1.95
2	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	7.32	56.26
3	propanamide, N,N-dimethyl-	7.91	3.81
4	Estra-1,3,5(10)-trien-17-β-ol	8.15	4.98
5	β-D-Glucopyranose, 1,6-anhydro-	8.50	15.59
6	1,6-anhydro-β-D-gluco-furanose	9.04	9.40
7	Lactose	9.28	5.15
8	2-p-Nitrophenyl-1,3,4-oxadiazol-5-one	31.83	2.86

3.4. Insecticidal Effects of Authentic Pseudopelletierine on House Dust Mite

The measurements from insecticidal effects of pseudopelletierine on house dust mite were shown in Table 6. As shown in this Table, 100% of a complete mortality was observed at concentration 1㎎/40㎕, and 73.06% of mortality was observed at concentration 0.5㎎/40㎕. However, no mortality was observed at all concentrations lower than 0.25㎎/40㎕. As mentioned above, pseudopelletierin presented a higher insecticidal effect on house dust mite at the concentration higher than 0.5㎎/40㎕.

Table. 6 Insecticidal Effects of Authentic Pseudopelletierine on House Dust Mite

Authentic	Mortality(%)[a]				
	1㎎/40㎕	0.5㎎/40㎕	0.25㎎/40㎕	0.125㎎/40㎕	0.0625㎎/40㎕
Pseudophyllene	100.00	73.06	10.61	0.00	0.00

[a] (remained mites/Total mites) × 100

3.5. Insecticidal Effects of Authentic Terpene Compounds on House Dust Mite

Terpene compounds contained in root peel, stem peel and fruit peel of pomegranate, which was identified in the previous study [27], were shown in Table. 7. As shown in Table 7, 15 types, 2 types and 4 types of terpene compounds were found in the root peel, stem peel and fruit peel of pomegranate, respectively. Among those types, 11 purchasable terpene compounds were measured for their insecticidal effects, as shown in the table 8. As can be seen from table 8, the camphor showed a great mortality higher than 90.00% at all concentrations and delta-3Carene also showed a high mortality, which was higher than 70.00% at all concentrations. The beta-Pinene, trans-Caryophyllene, gamma-Terpinene showed the mortality of 80.00% or higher at the concentration ranged from 1㎎/40㎕ to 0.125㎎/40㎕, and camphene showed the mortality of 60.00% or higher at all concentrations. As shown in the 3.4 and 3.5, It was considered that cause of insecticidal effect of the extract of each part on the house dust mite was driven from a synergic effect between pseudopelletierine and terpene compounds contained in the root peel, stem peel and fruit peel of pomegranate.

Table 7. Terpene Compounds Contained in the Root Peel, Stem Peel and Fruit Peel of Pomegranate

Terpene Compounds		Pomegranate
Monoterpene	Limonene	Root peel
	alpha-Pinene	Root peel
	Camphor	Root peel
	Camphene	Root peel
	Sabinene	Root peel
	beta-Myrcene	Root peel
	delta-3-Carene	Root peel
	gamma-Terpinene	Root peel, Fruit peel
	alpha-Thujene	Root peel
	2-beta-Pinene	Root peel
	1-phellandrene	Root peel
	alpha-Terpinolene	Root peel
	alpha-Amorphene	Root peel

	beta-Thujene	stem peel, Fruit peel
	beta-Phellandrene	Fruit peel
	ortho-Cymene	Root peel, Fruit peel
	para-Cymene	stem peel
Sesquiterpene	trans-Caryophyllene	Root peel

Table 8. Insecticidal effects of authentic terpene compounds on house dust mite

Specimens		Mortality(%)[a]				
		1㎎/ 40㎕	0.5㎎/ 40㎕	0.25㎎/ 40㎕	0.125㎎/ 40㎕	0.0625㎎/ 40㎕
Monoterpene	Limonene	63.29	43.25	54.55	49.11	19.62
	alpha-Pinene	63.14	61.58	43.30	66.45	48.33
	beta-Pinene	95.00	88.56	89.96	50.82	77.45
	Camphor	100.00	98.72	100.00	94.16	92.58
	Camphene	79.13	85.63	66.90	67.78	92.42
	Sabinene	51.31	62.46	56.48	53.33	33.24
	beta-Myrcene	82.00	50.18	82.58	94.74	100.00
	delta-3-Carene	100.00	95.83	94.21	95.30	70.84
	gamma-Terpinene	84.02	90.63	90.48	70.50	48.05
	ortho-Cymene	100.00	82.56	53.36	55.54	60.83
Sesquiterpene	trans-Caryophyllene	98.04	91.47	80.37	63.96	77.02

4. Conclusion

Each part of pomegranate - root peel, stem peel and fruit peel was extracted with ethanol and the insecticidal effects of those extracts on house dust mite were investigated at different concentration. GC/MS ingredient analysis was to be performed to identify the component taking insecticidal activity among the variable components contained in the root peel, stem peel and fruit peel of pomegranate. In order to verify a higher insecticidal effect of pomegranate, the insecticidal effect of the authentic pseudopelletierine and authentic 11 terpene compounds was to be measured: limonene, alpha-pinene, beta-pinene, camphor, camphene, sabinene, beta-myrcene, delta-3-carene, gamma-terpinene, ortho-cymene, and trans-caryophyllene.

As a result, 100% of a complete mortality was shown at the concentration 1㎎/40㎕ of the extract of each part. A high mortality as higher than 88.00% was observed at the concentration 0.5㎎/40㎕ of the extract of each part while mortality from 55.00 to 69.57% was observed at the concentration 0.125㎎/40㎕ of the extract of each part. Therefore, it was confirmed that the insecticide of ethanol extracts from root peel, stem peel and fruit peel of pomegranate was particularly effective against the house dust mite. GC/MS analysis was performed to identify the pseudopelletierine in each part of pomegranate, the component well-known for taking insecticidal effect; and as a result, pseudopelletierine component was found in the root peel. From

measurements of the insecticidal effect of the authentic pseudopelletierine and authentic 11 terpene compounds, pseudopelletierin presented a higher insecticidal effect on house dust mite at the concentration higher than 0.5㎎/40㎕. The camphor showed a great mortality higher than 90.00% at all concentrations and delta-3Carene also showed a high mortality, which was higher than 70.00% at all concentrations. The beta-Pinene, trans-Caryophyllene, gamma-Terpinene showed the mortality of 80.00% or higher at the concentration ranged from 1㎎/40㎕ to 0.125㎎/40㎕, and camphene showed the mortality of 60.00% or higher at all concentrations. Therefore It was considered that cause of insecticidal effect on the house dust mite was driven from a synergic effect between pseudopelletierine and terpene compounds contained in the root peel, stem peel and fruit peel of pomegranate.

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