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Insecticidal Activity of Extracts Isolated from *Syzygium Aromaticum*[1]

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ABSTRACT

This study separated the crude extract (70% ethanol) of and its three fractions (hexane, chloroform and ethyl acetate extracts) on the basis of polarity indexes, and examined for their insecticidal activities against aphid (*Uroleucon lactucicola*). For crude extraction, the 70% ethanol extract showed the best extract yield (58.0%) and insecticidal activity (69.0%) among the various concentrations tested (water, 30% ethanol, 50% ethanol, 70% ethanol and 95% ethanol). The major chemical compounds of different fractions (hexane, chloroform and ethyl acetate extracts) were identified as eugenol by head space-GC-MS analysis. The hexane extract showed the highest eugenol content (43.7%) and insecticidal activity (80.0%). The insecticidal activity is accordingly believed to be attributable to the eugenol component. This may provide a useful starting point for the development of bio-pesticides.

Keywords : insecticidal activity, *Syzygium aromaticum*, bio-pesticides

1. INTRODUCTION

Plant-derived extracts and their bioactive constituents have been suggested as alternatives to the most commonly used insecticides because many plants demonstrate insecticidal activities against insect pests (Kim *et al*. 2010; Lee *et al*. 2010). Plant volatile compound show wide and varied bioactivities in insect, ranging from toxicity to sublethal effects, including oviposition deterrence and antifeedant activity and attractant and repellent actions, as well as antimicrobial, antifungal and antitumor activities. Because of this, many studies have focused on essential oils, plant extracts, as new sources of pest control agents (Park *et al*. 2000, 2011). Natural pesticides, or pesticides derived from natural products, support both crop production and the environment by being effective in pest control, less toxic to non-target organisms and biodegradable at the same time, they may be safer than synthetic pesticides (Tsao *et al*. 2002).

Plants exhibiting aphid damage can have a variety of symptoms, such as decreased growth rates, mottled leaves, yellowing, stunted growth, curled leaves, browning, wilting, low yields and death. Until now, five species belong to the subgenus *Uroleucon* (*Uromelan*) have been reported in the Korea: *Uroleucon* (*Uromelan*) *lactucicola*, *Uroleucon* (*Uromelan*) gobonis, *Uroleucon* (*Uromelan*). *giganteum*, *Uroleucon* (*Uromelan*). *amamianum*, and *Uroleucon* (*Uromelan*)

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Cephalonopli (Matsumura 1917; Takahashi 1930; Takahashi 1962).

The damage of plants, and in particular commercial crops, has resulted in large amounts of resources and efforts being spent attempting to control the activities of aphids. The synthetic insecticides were useful for controlling aphid. However, their repeated use causes resistance, residual toxicity, and environmental pollution (Senthil-Nathan *et al*. 2009). There are many plant extracts and plant products that are eco-friendly and control aphids as effectively as chemical insecticides (Senthil-Nathan *et al*. 2009).

Syzygium aromaticum are native to the Maluku islands in Indonesia and used as a spice in cuisines all over the world. In Korea, cloves have been successfully used for asthma and various allergic disorders by oral administration (Kim *et al*. 1998). The activity in vitro of *S. aromaticum* extracts was demonstrated against pathogenic bacteria (Larhsini *et al*. 2001; Chaieb *et al*. 2007) and virus (Hussein *et al*. 2000). Moreover, the fungicidal activity of *S. aromaticum* was also demonstrated on several fungal species (Ranasinghe *et al*. 2002), fungi isolated from *onychomicosis* (Gayoso *et al*. 2005), *Saccharomyces cerevisiae* (Chami *et al*. 2005), *Candida* species (Chaieb *et al*. 2007).

There is no published report in the literature about the insecticidal activity of *S. aromaticum* against aphid (*Uroleucon lactucicola*). Therefore, the objective of our study was to evaluate the bio-insecticide potential of *S. aromaticum* and their major active chemical components. The present study was to evaluate insecticidal activity on aphid (*Uroleucon lactucicola*) of various extracts (*n*-hexane, chloroform, ethyl acetate and aqueous extracts) from the *S. aromaticum*.

2. MATERIALS and METHODS

2.1. Raw Material

S. aromaticum was purchased from Traditional Korean Medicine Pharmacy Market in Sancheong, Korea.

The *S. aromaticum* was dried in an oven at 40℃ for 2 days and finely powdered using a laboratory wiley Mill. The particles that passed through a 20-mesh sieve (850 ㎛) but were retained by a 40-mesh (450 ㎛) sieve were stored in a sealed plastic bag at -72℃.

2.2. Preparation of the Crude Extracts

The dried powder of *S. aromaticum* (300 g) was soaked in ethanol/water in a glass jar (2 L) at room temperature for more than 2 days and then the ethanol-water solution was decanted and filtered (Whatman No. 2) to give a crude extract. For extraction, water and four different concentrations (30%, 50%, 70%, and 95% by the volume) of ethanol were used. The crude extracts (70% ethanol) were then concentrated on a rotary vacuum evaporator (Buchi, Switzerland) under reduced pressure at 35～40℃, and freeze-dried. The yield of each extract was calculated as follows:

Extraction Yield (%)
= (weight of dry extract/weight of raw material) × 100

2.3. Fractionation of Crude Extracts

Fractionation of the crude extract (70% ethanol) was performed and respective fractions were obtained (Fig. 1) (Karawita *et al*. 2005). The Fractionation was sequentially divided into *n*-hexane, chloroform and ethyl acetate fractions for the chemical composi-

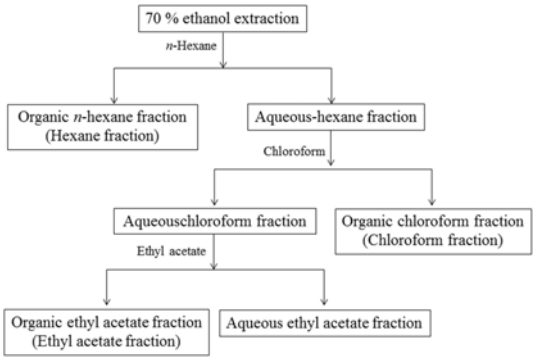

Fig. 1. Flow diagram of fractionation of *S. aromaticum.*

tion and bioassay. The different fractionation were concentrated using rotary vacuum evaporation at 45℃.

The different fractionations were labeled as organic *n*-hexane fraction (hexane fractionation), organic chloroform fraction (chloroform fractionation) and organic ethyl acetate fraction (ethyl acetate fractionation).

2.4. Analysis of Extracts from S. Aromaticum

The crude extract and different fractions of crude extract was analyzed (Sanz *et al*. 2001) by static headspace gas chromatography-mass spectrometry (headspace-GC-MS). The headspace-GC-MS analysis was performed with Clarus 600 gas chromatograph (Perkinelmer, CA, USA) equipped with a static headspace sampler (Perkinelmer, CA, USA). Ten milliliter vials containing 2 mℓ of crude extract and different fractions of crude extract were immediately sealed with silicone rubber Teflon caps. Each vial was equilibrated at 100℃ during 20 min in the static headspace sampler. Each vial was pressurized with carrier gas for 12 s, and 0.5 mℓ of medicinal herbs headspace sample was injected into a column DB-5MS (30 m × 0.25 mm × 0.25 ㎛ film thickness; HP Agilent, USA). The injector temperature was set at 260℃, and helium (1 mℓ/min) was the carrier gas. The initial column temperature of 35℃ was held for 5min, and then programmed at a rate of 5℃/min until reaching 280℃ and maintained at this temperature for further 10min, next programmed again at a rate of 10℃/min until reaching 300℃ and maintained at this temperature for further 14min. The mass spectrometer operated in the electron impact ionization mode (70 eV), with a scan range of 50 to 300 amu. The ion source temperature was set at 230℃. Identification of the constituents was performed on the basis of NIST library search. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

2.5. Bioassay

2.5.1. Insects

Aphid (*Uroleucon lactucicola*) was obtained from a single strain reared for several generations on *Triticumaestivum* in a climatic room at 24 ± 1℃ and a photo period of 14: 10 (light: dark). The insecticidal activity of plant extracts used was determined by direct contact application (Kim *et al*. 2003).

2.5.2. Filter Paper Contact Bioassay

The insecticidal activity was carried out for the crude extract and its various extracts against U.*lactucicola*. A dose of 1000 ppm of each extracts was applied to filter papers (Whatman No. 1). After drying under a fume hood for 2 min, each filter paper was placed in the bottom of a petri-dish (10 cm diameter × 4 cm), and then 20 adults of each test materials were placed in each petri-dish which was covered with a lid. Treated insects were held at 28 ± 1℃, 50 - 60% relative humidity, and a 16: 8 (light: dark) photoperiod. The insecticidal activity was determined at 48 h after treatment. Test insects were

Table 1. Effect of ethanol concentration on extract yield from *S. aromaticum*

Extraction method	Yield (%)	Insecticidal activity (%)
Water	38.2 ± 0.3[1)]	25.0 ± 3.3
30% ethanol	43.3 ± 0.5	52.0 ± 0.0
50% ethanol	47.3 ± 1.2	53.0 ± 5.8
70% ethanol	58.0 ± 1.7	69.0 ± 0.0
95% ethanol	54.2 ± 0.9	61.0 ± 0.0

[1)] Values are presented as means ± SD of three measurements

considered dead if appendages did not move when prodded with a fine brush. Negative control was prepared using distilled water. Eugenol (commercial oil)was used as a positive control with the tested insect. Oils used for the determination of insecticidal activity was dissolved in Tween 80 (0.5%) and was made up as distilled water. All treatments were replicated three times.

2.6. Statistical Analysis

The insecticidal activity was transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test at $P > 0.05$ (SAS 1990). Means (± SE) of untransformed data are reported.

3. RESULTS and DISCUSSION

3.1. Extraction Yields of Crude Extracts

Extraction is an important step for obtaining extracts with acceptable yields.

The extract yield of *S. aromaticum* was strongly dependent on the concentration used as shown in Table 1.

The extract yield of *S. aromaticum* was found to be 38.2%, 43.3%, 47.3%, 58.0% and 54.2% of the starting material for the water, 30% ethanol, 50% ethanol, 70% ethanol and 95% ethanol extracts, respectively. The yield of extract was maximized at 70% ethanol but declined considerably at higher ethanol concentrations. The insecticidal activity of the water, 30% ethanol, 50% ethanol, 70% ethanol and 95% ethanol extracts was 25.0%, 52.0%, 53.0%, 69.0%, 61.0%, respectively.

Ethanol was more effective in terms of extract yield and insecticidal activity than water, with 70% ethanol being the most efficient extraction solvent. These experimental results are in accordance with previous reports suggesting that a binary solvent system was superior to a mono-solvent system (water or pure ethanol) in terms of extraction yield (Chirinos *et al*. 2007).

Aqueous and solvent extraction is routinely used for the isolation of extractive substances from plant material. Both the extraction yield and chemical composition of extracts are strongly dependent on the extraction solvent used (Moure *et al*. 2001).

3.2. Chemical Composition of Crude Extracts

We characterized the chemical composition of crude extracts (70% ethanol extraction) of *S. aromaticum* using headspace-GC-MS. As shown in Table 2, the major chemical compounds found in extracts (Table 2) were eugenol (88.7%), caryophyllene (6.0%) and α-humulene (1.2%). These data are in agreement with the results from other studies (Prashar *et al*. 2006). The major component of *S. aromaticum*is nomally considered to be eugenol, along with β-caryophyllene and lesser amounts of other components such as benzyl alcohol, however the proportions vary widely. For example, Prashar *et al*. (2006) found the content of eugenoland β-caryophylleneto be 78% and 13%, whereas Pawar and Thaker (2006) found that the content of eugenol was 47.64%, and

Table 2. Chemical composition of crude extract from *S. aromaticum*

Constituent	R.T[1)](min)	Area (%)
α - Pinene	9.23	0.3
Camphene	9.71	0.2
dl - Limonene	12.03	0.7
Cineole	13.11	0.3
β -Ocimene	13.28	0.2
γ-Terpinene	14.70	-
3 - Carene	15.50	0.1
Linalool	15.52	0.2
β -Myrcene	15.53	0.2
Camphor	16.74	0.1
(+) −Menthone	17.02	0.1
(+) −Isomenthol	18.08	0.1
α - Terpineol	18.09	-
Carvone	20.20	-
Eugenol	24.88	88.7
Caryophyllene	25.68	6.0
α -Humulene	26.91	1.2
β -Farnesene	28.29	0.5
α - Selinene	27.97	-
δ- Cadinene	28.69	0.1
α -Cubebene	28.99	0.2
γ-Elemene	30.52	0.1
Total		99.3

1) R.T: Retention time
2) Not detected.

that of benzyl alcohol at 34.10%. Eugenol is used as an aromatic agent for food, and as a fragrance in the cosmetic industry, and it is also commonly used in dentistry for sedation of toothache, pulpitis, and dental hyperalgesia (Chaieb *et al*. 2007). Several biological actions of eugenol have previously been reported, namely antibacterial (Shokeen *et al*. 2008), and antifungal (Gayoso *et al*. 2005) activities.

3.3. Chemical Composition of Various Extracts

The detailed chemical compositions of the various extracts are presented in Table 3. The main chemical compounds found in the hexane fraction of *S. aromaticum* were eugenol (43.7%), androst-5-en-4-one (12.0%), 2-propenoic acid (8.3%), D-allose (8.0%), benzenepropanoic acid (5.2%), disulfide, bis (1-methylethyl) (3.7%) and vanillin (2.8%). The main compounds identified in the chloroform fraction were eugenol (16.5%), 5-methyl-z-5-docosene (8.1%), benzeneacetic acid (7.7%), vanillin (6.8%), 4-hydroxy-2- methoxycinnamaldehyde (6.1%), 2-propenoic acid (5.8%) and2-butenoic acid (5.2%), whereas the major chemical compounds found in the ethyl acetate fraction were ethyl 2-hydroxybenzyl sulfone (35.0%), eugenol (22.2%), α-amyrin (7.3%), β-amyrin trimethylsilyl ether (5.4%) and indole (4.1%).

Among the various extracts, the hexane fraction had highest eugenol contents.

D-allose (Gao*et al*. 2011), vanillin (Makni*et al*. 2011), 4-hydroxy-2-methoxycinnamaldehyde (stepan *et al*. 2008) and α-amyrin (Rajic *et al*. 2000) have been reported as active components contributing to anti-inflammatory and anti- fungal activities.

3.4. Insecticidal Activity Against U. Lactucicola Treated with Various Extracts using a Filter Paper Contact Bioassay

The insecticidal activity of *S. aromaticum* differed widely among the various extracts Table 4. Among the various extracts, the hexane fraction showed the highest insecticidal activity (80%). The insecticidal activity of chloroform fraction and ethyl acetate fraction was 57% and 63 = %, respectively. Significant differences were observed in the insecticidal activity

Table 3. Chemical composition of various extracts from *S. aromaticum*

Constituent	R.T[1] (min)	Hexanefraction(%)	Chloroform fraction(%)	Ethyl acetatefraction(%)
Dodecane	7.8	-[2]	-	1.9
2-allylphenol	11.3	1.0	3.7	0.6
Eugenol	12.8	43.7	16.5	22.2
Vanillin	13.4	2.8	6.8	-
Ethyl 2-hydroxybenzyl sulfone	13.8	-	-	35.0
Acetophenone	14.2	-	3.7	-
Heptadecane	14.5	0.4	-	-
D-allose	14.9	8.0	-	-
Carbamamidine	15.8	1.1	-	-
Trichloroacetic acid	16.3	1.8	1.5	-
Benzeneacetic acid	16.6	-	7.7	-
Disulfide, bis(1-methylethyl)	17.0	3.7	-	-
4-hydroxy-2-methoxycinnamaldehyde	17.6	-	6.1	0.6
Benzenepropanoic acid	18.1	5.2	-	-
2-propenoic acid	18.5	8.3	5.8	2.5
Androst-5-en-4-one	19.2	12.0	3.8	-
Aromadendrene oxide	19.9	0.6	3.9	0.8
Pentadecanoic acid	21.6	-	-	0.4
Tetratriacontane	24.4	1.3	0.7	-
Phenol	24.8	-	1.7	0.8
2-butenoic acid	26.1	-	5.2	-
Desacetylanguidine	26.7	0.7	-	-
5-methyl-z-5-docosene	27.0	-	8.1	-
Calcitriol	27.6	1.4	1.1	-
Trilostane	27.9	-	-	0.9
Tetrapentacontane	28.3	0.3	2.2	2.4
Indole	28.6	-	-	4.1
Azulene	28.8	-	-	3.4
Allylmethyldiphenylsilane	29.8	0.4	1.8	-
Butylenin	30.4	0.6	5.1	-
Docosane	31.3	-	3.5	-
3-benzofuranmethanol, 2,3-dihydro-3-methyl	31.7	0.5	3.2	-
Squalene	32.7	-	1.9	-
α -amyrin	33.8	-	-	7.3
β-amyrintrimethylsilyl ether	35.2	-	-	5.4
18-norabietane	36.9	-	-	2.4
Olean-12-en-28-oic acid	37.3	-	2.5	-
Propanoic acid	37.7	1.2	-	-
Longiverbenone	38.2	-	-	2.7
Total		95.0	96.5	93.4

[1] R.T: Retention time

[2] Not detected.

Table 4. Insecticidal activity of crude extract and various extracts from *S. aromaticum* by filter paper contact bioassay[1)]

Extract	Insecticidal activity (%)
Crude extract[2)]	70 ± 3.7[3)]
Hexane extract	80 ± 5.8
Chloroform extract	57 ± 5.8
Ethyl acetate extract	63 ± 5.8
Eugenol[4)]	75 ± 0.0
Control[5)]	23 ± 3.3

1) Insecticidal activity was observed for mortality 48 h after treatment
2) 70% ethanol extract
3) Each datum represents the mean of three replicates, each set up with 20 adults.
4) Positive control:commercial oil
5) Negative control

of the various extract against *U. lactucicola*.

The activity of the hexane fraction of *S. aromaticum* was similar to that recorded for the positive control (commercial oil: eugenol). Eeugenol was previously reported to be active against a range of plant pathogenic nematodes (Sangwan *et al*. 1990). A hexane extract of clove flower buds, which contains mainly eugenol (Guenther, 1950), was demonstrated to have a toxic contact effect on *S. zeamais* and *T. castaneum* and reduced their fecundity (Ho *et al*. 1994). Obeng-Ofori and Reichmuth (1997) also reported that eugenol was toxic to four coleopteran pests of stored products, namely, *S. zeamais*, *S. granarius*, *T. castaneum* and *P. truncatus*.

3.5. The Relationship between The Eugenol Content and Insecticidal Activity of Extracts

The Fig. 2 show the correlation between the eugenol content and insecticidal activity of the various extracts of *S. aromaticum*. The insecticidal activity

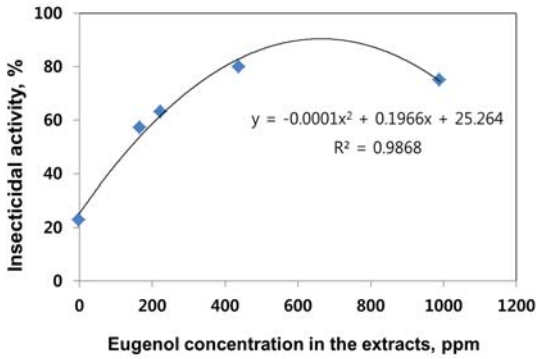

Fig. 2. Correlation of the insecticidal activity with eugenol of *S. aromaticum* extracts.

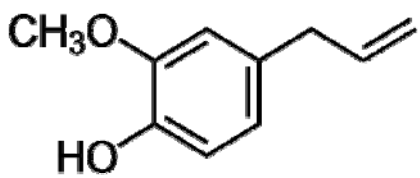

Fig. 3. Structures of eugenol.

of extracts was shown to be linearly correlated with eugenol content (correlation coefficient being 0.9868) (Fig. 2). It is accordingly believed that the insecticidal activity of the various extracts is due mainly to the presence of eugenol (Fig. 3). Further investigations examining the synergistic effect of eugenol and other minor components on the insecticidal activity of *S. aromaticum* are desirable.

4. CONCLUSION

In view of the environmental and health hazards posed by synthetic insecticides, the use of plant products as insecticides has gained increasing in recent years.

Extracts isolated from *S. aromaticum* are widely used and well known for their antibacterial and antifungal properties (Larhsini *et al*. 2001; Ranasinghe *et al*. 2002; Gayoso *et al*. 2005).

In this study, we reported the insecticidal activity of various extracts isolated from *S. aromaticum*. The chemical composition of the various extracts investigated in this study was similar to those reported previously (Srivastava *et al*. 2005; Chaieb *et al*. 2007), which show that eugenolis the main component of these extracts. The results of the present study indicated that the various extracts of *S. aromaticum* possess insecticidal activity against aphids (*U.lactucicola*). A comparison of the insecticidal activity and composition of the extracts, lead us to conclude that the activity of *S. aromaticum* is mainly due to its major compound eugenol.

Our results indicate that *S. aromaticum* and its components could be useful as a bio-insecticide for aphid (*U. lactucicola*) control. For the practical use of *S. aromaticum* and its constituents as novel insecticide, further study is necessary on the safety of these materials to humans, on development of formulations to improve the efficacy and stability and to reduce costs.

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