J. Korean Wood Sci. Technol. 2017, 45(6): 682~688 pISSN: 1017-0715 eISSN: 2233-7180
https://doi.org/10.5658/WOOD.2017.45.6.682

Original Article

Essential Oil Analysis of *Illicium anistum* L. Extracts[1]

Hee-Jeong Min[2] · Chan-Soo Kim[3] · Hwa-Ja Hyun[3] · Young-Soo Bae[2,†]

ABSTRACT

Fresh japanese anise (*Illicium anisatum* L.) tree leaves were collected and ground after drying. The essential oils of the leaves were analyzed by gas chromatography-mass spectrometry (GC-MS) using headspace (HS) and solid phase-microextra (SPME) methods.

Volatile components of the leaves were identified 21 and 65 components in HS and SPME, respectively. The main components of the essential oils obtained by HS method were eucalyptol (36.7%), (+)-sabinene (15.61%), δ-3-carene (6.87%), α-pinene (6.07%), γ-terpinen (5.72%), α-limonene (5.26%), β-myrcene (4.13%), α-terpinene (4.04%) and β-pinene (3.73%). The other components were less than 3.5%. SPME method also showed that eucalyptol (17.88%) was main. The other were 5-allyl-1-methoxy-2 (13.29%), caryophyllene (6.09%), (+)-sabinene (5.60%), α-ocimene (4.89%) and β-myrcene (3.73%), and the rest were less amounts than 3.5%. This work indicated that many more volatile components were isolated, comparing to the previous literature data and that SPME method was much more effective than HS method in the analysis of the volatile components.

Keywords : Japanese anise tree (*Ilicium anisatum* L.), volatile components, headspace, solid phase-microextra, GC-MS analysis

1. INTRODUCTION

Many plants have bioactive components in their essential oils which consist of volatile and fragrant substances of plants (Guenther, 1948). Natural essential oils are usually mixtures of terpenoids (mainly monoterpenoids and sesquiterpenoids), aromatic and aliphatic compounds. Essential oils can be used for fine fragrances, flavouring and aromatherapy. The most common collection methods of these oils are steam distillation, extraction with solvents, and expression.

The development of chromatographic technique has allowed considerable progress in the study of the chemical composition of essential oil.

Gas chromatography (GC) or gas chromatography-mass spectroscopy (GC-MS) are used for

[1] Date Received June 15, 2017, Date Accepted July 31, 2017
[2] Department of Forest Biomaterials Engineering, College of Forest and Environmental Sciences, Kangwon National University, Chuncheon 24341, Republic of Korea
[3] Warm and Subtropical Forest Research Center, National Institute of Forest Service, Seogwipo 63582, Republic of Korea
[†] Corresponding author: Young-Soo Bae (e-mail: bae@kangwon.ac.kr, ORCID: 0000-0003-1108-9269)

qualitative and quantitative analysis of the volatiles (Zhao *et al*., 2005).

Headspace (HS) uses headspace gas injected directly onto a gas chromatographic column to analyze volatile compounds. On the other hand, solid phase-microextraction (SPME) provides many advantages over conventional sample preparation techniques like as solvent-free, inexpensive and rapid (Arthur and Pawliszyn, 1990).

Japanese star anise is a tree closely related to the chinese star anise. Since it is highly toxic, the fruit is not edible. Instead, the dried and powdered leaves are burned as incense in Japan.

The seed contains anisatin, shikimin and sikimitoxin, which cause severe inflammation of kidneys, urinary tract, and digestive organs (Yamada *et al*., 1968). However, recent studies have shown that the leaves and bark possess the inhibitory activities of aldose reductase, lipase, glycation, anti-elastase and anti-inflammatory as well as antioxidant activity (Kim and Oh, 1999; Kim and Kim, 2003; Kim and Kang, 2005; Kim *et al*., 2009).

The investigations on the leaf oil components of the tree was initiated by Eijkman (1885), who isolated safrol, eugenol and antethol. In domestic, the analysis of the essential oils of the tree was done by Lim (2008) and Kim (2009) who identified 35 and 52 components in the essential oils, respectively, and the main component was eucalyptol.

In this work, we investigated the essential oils of japanese anise tree leaves using headspace (HS) and solid phase-microextra (SPME) and compared with two methods to set the more effective analytical method up through the comparison of the previous analytical data for future functional and commercial use of the volatiles.

2. MATERIALS and METHODS

2.1. Plant material

Fresh japanese anise tree leaves were collected at Seogwipo, Jeju-do in January 2017, air dried for two weeks and then ground to fine particles to be analyzed.

2.2. Equipments

Two analytical methods, headspace (HS) and solid phase-microextraction (SPME), were used for analysis of the volatile components.

SPME were analyzed as follows : volatile components were absorbed by using 75 um Carboxen/PDMS fiber at 50℃ for 30 min using the leaves (8 g). After completion was the adsorption, the fiber inserted into the GC-MS injection port and then the fragrance components were desorbed at 200℃ for 5 minutes.

HS was performed with a Teledyne Tekmar HT3 (Static/Dynamic headspace System) and the operating conditions are shown in Table 1.

2.3. Analysis of the volatiles

Volatile components were analyzed by gas chromatography-mass spectrometry (GC-MS).

Table 1. Headspace conditions for analysis of volatile components

Variable	Value
Headspace mode	Constant
GC cycle time	30.00 min.
Valve oven temp.	100℃
Transfer line temp.	100℃
Standby flow rate	50 mℓ/min.
Platen/sample temp.	50℃
Platen temp equil. time	1.00 min.
Sample equil. time	30.00 min.
Pressurize time	2.00 min
Injection time	1.00 min

The sample was injected into the GC column and the oven temperature was maintained at 40℃ for 4 minutes and then it was increased until 240℃ at a rate of 10℃/min. The temperature of the injector and detector were 200℃ and 240℃, respectively and the carrier gas was He and the flow rate was 0.5℃/min. The electron ionisation condition was 70 eV, source temperature 250 and trap current 300 uA.

Isolated essential oils were injected on column and maintained 50℃ for 5 minutes. There were increased until 110℃ at a rate of 3℃/min and it was maintained for 10 minutes. Again there were increased to until 220℃ at a rate of 4℃/min and then it was maintained for 20 minutes. The temperature of the injector and detector (FID) were 270℃ and the carrier gas was He and the flow rate was 0.5℃/min.

3. RESULTS and DISCUSSION

Cook (1966) steam-distilled in the normal manner from the seeds and husks and then analyzed 37 volatiles components using GC-MS. The main component was cineole (18.1%), eucalyptol. The other major components were Linalool (10.1%), methyleugenol (9.8%), sesquiterpene hydrocarbon (7.2%), α-terpinyl acetate (6.8%), safrole (6.6%), terpinen-4-ol (3.9%), myristicin (3.5%) and α-terpineol (3.3%).

Lim (2008) identified 35 components of the leaves volatiles by GC-MS using TLC and preparative TLC for fractionation. The main components was also 1,8-cineole (45.32%), eucalyptol. The other major components were isopulegol (5.82%), 1-methyl-3-(1methylenthyl) cyclohexene (5.32%), linalool (5.23%), methylbutenol (5.23%), α-pinene (4.23%), 1-ρ-menthen-8-ylacetate (3.82%), β-phellandrene (3.24%), α-copaenen (1.94%), 2-hexene-1-ol (1.52%) and camphor (1.43%). Kim (2009) also reported 52 volatile components using GC-MS analysis on the leaves of japanese anise tree. The main component was also eucalyptol (21.8%) and the other components were sabinene (5.3%), α-terpineyl acetate (4.9%), kaurene (4.5%), isopimaradiene (3.2%), ent-pimara-8(14),15-diene (2.8%), 3,6-dimethoxy-2-

Table 2. Volatile compounds by Headspace analysis of *Illcium anisatum* leaves extracts

NO.	Retention time (min)	Constituent	Peak area (%)
1.	5.751	n-caproaldehyde	0.50
2.	7.037	2-hexenal	0.28
3.	8.590	α-phellandrene	0.93
4.	8.742	α-pinene	6.07
5.	9.095	camphene	0.79
6.	9.567	(+)-sabinene	15.61
7.	9.660	β-pinene	3.73
8.	9.863	β-myrcene	4.13
9.	10.200	α-thujene	0.59
10.	10.402	α-terpinene	4.04
11.	10.651	α-limonene	5.26
12.	10.760	eucalyptol	36.70
13.	10.932	β-ocimene	1.04
14.	11.149	γ-terpinen	5.72
15.	11.627	terpinolene	2.19
16.	11.876	δ-3-carene	6.87
17.	12.742	(+)-camphor	0.52
18.	13.085	γ-3-carene	0.17
19.	16.063	α-copaene	0.10
20.	16.686	β-caryophyllene	0.50
21.	17.583	myristicin	1.29

ethylbenzaladehyde (2.7%), safrol (2.7%), β-linalool (2.6%) and δ-cadienen (2.2%).

In this study, we have also characterized 21 and 65 components from the volatiles materials of the tree leaves, respectively, by GC-MS using headspace (HS) and solid phase-microextra (SPME) method.

In HS method, the volatiles were isolated 21 components and the main was eucalyptol (36.7%). The other major components were (+)-sabinene (15.61%), δ-3-carene (6.87%), α-pinene (6.07%), γ-terpinen (5.72%), α-limonene (5.26%), β-myrcene (4.13%), α-terpinene (4.04%) and β-pinene (3.73%). The rest were minor ones less than 3.5% (Table 2).

In SPME analysis (Table 3), we isolated 65 components from the volatiles of japanese anise leaves and the main component was also eucalyptol (36.7%). The other components were 5-allyl-1-methoxy-2 (13.29%), caryophyllene (6.09%), (+)-sabinene (5.60%), α-ocimene (4.89%), β-myrcene (3.73%) and the rest were also minor ones less than 3.5%. These finding mean that SPME analytical method may be an effective tool to identify volatile components of plant materials, including tree species.

Based on the above results, the components obtained from the two methods, HS and SPME,

Table 3. Volatile compounds by SPME analysis of *Illcium anisatum* leaves extracts

NO.	Retention time (min)	Constituent	Peak area (%)
1.	1.680	α-methypentenal	0.08
2.	1.722	isoprene	0.14
3.	2.604	2-butenal	0.12
4.	3.019	1-butene	0.14
5.	3.216	furan	0.06
6.	4.493	1,5-heptadien	0.08
7.	5.598	hexanal	0.49
8.	6.952	2-hexenal	1.34
9.	7.030	1,4-hexadiene	0.52
10.	7.855	cyclohexanal	0.04
11.	8.540	1,3-cycloheadiene	1.14
12.	8.701	α-pinen	2.00
13.	9.048	bicyclo heptane	0.37
14.	9.230	2-heptenal	0.04
15.	9.344	benzaldehyde	0.22
16.	9.551	(+)-sabinee	5.60
17.	9.634	β-pinene	1.56
18.	9.858	β-myrcene	3.73
19.	10.179	α-phellandrene	0.12
20.	10.387	α-terpine	0.79
21.	10.563	O-cymene	0.21
22.	10.771	eucalyptol	17.88
23.	10.906	ocimene	0.86
24.	11.139	γ-terpinene	1.44
25.	11.393	β-phelladrene	0.14
26.	11.617	α-terpinolen	0.87
27.	11.700	2-allyltoluene	0.10
28.	11.798	niobe oil	0.06
29.	11.907	3-carene	5.29
30.	12.727	(+)-camphor	0.72
31.	13.064	pseudolimonene	0.77
32.	13.484	α-ocimene	4.89
33.	14.854	rhyuno oil	0.97
34.	15.435	(+)-alloaromadendrene	0.05
35.	15.638	α-cubebene	1.27
36.	15.710	eugenol	2.43
37.	15.975	(+)-ylangene	0.17
38.	16.219	β-elemen	0.81
39.	16.297	eugenol methyl ether	0.21
40.	16.468	α-longipinene	0.05
41.	16.499	α-gurjunene	0.10
42.	16.712	caryophyllene	6.09

Table 3. To be Continued

NO.	Retention time (min)	Constituent	Peak area (%)
43.	16.774	α-elemene	0.64
44.	16.878	(+)-calarene	0.08
45.	16.919	(+)-aromadendrene	0.58
46.	16.987	γ-muurolene	0.30
47.	17.148	α-caryophyllene	1.71
48.	17.350	naphthalene	1.10
49.	17.412	(+)-cycloisosativene	0.13
50.	17.469	germacrene D	3.37
51.	17.661	5-allyl-1-methoxy-2	13.29
52.	17.724	γ-cadinene	0.43
53.	17.858	α-amorphene	1.90
54.	17.890	(+)-δ-cardinen	1.99
55.	17.952	(-)-calamenene	0.26
56.	18.076	cadina-1,4-dinene	0.32
57.	18.123	α-cadienen	0.51
58.	18.201	calacorene	0.09
59.	18.263	δ-gurjunene	0.05
60.	18.761	methoxyeugenol	0.24
61.	18.798	myristicin	0.64
62.	19.389	isoledene	0.20
63.	19.555	naphthalene	0.10
64.	19.586	isolcauren	0.14
65.	23.748	kaurene	0.62

were showed a big differences in the kinds of the volatiles, but the main was eucalyptol same as in the previous studies. This fact suggest that SPME method will be much more effective than HS method in the analysis of the volatile components because SPME are analyzed many components than HS by absorbing more volatile components.

4. CONCLUSION

Fresh japanese anise tree leaves were collected and ground after drying. The essential oils of the leaves were analyzed by gas chromatography-mass spectrometry (GC-MS) using headspace (HS) and solid phase-microextra (SPME) methods.

Volatile components of the leaves were characterized 21 and 65 components in HS and SPME, respectively. The main component of the essential oils in HS and SPME method was eucalyptol (36.7%) and this is the same as in previous literature data. The other major components from HS method were (+)-sabinene (15.61%), δ-3-carene (6.87%), α-pinene (6.07%), γ-terpinen (5.72%), α-limonene (5.26%), β-myrcene (4.13%), α-terpinene (4.04%) and β-pinene (3.73%). In SPME method, the major

compounds were 5-allyl-1-methoxy-2 (13.29%), caryophyllene (6.09%), (+)-sabinene (5.60%), α-ocimene (4.89%) and β-myrcene (3.73%). The rest were less than 3.5%. These facts indicated that many more volatile components were isolated, comparing to the previous studies, by SPME method and that SPME method will be much more effective than HS method in the analysis of the volatile components.

REFERENCES

Arthur, C.L., Pawliszyn, J. 1990. Solid-phase microextraction with thermal desorption using fused silica optical fibers. Analytical Chemistry 62: 2145～2148.

Cook, W.B., Howard, A.S. 1966. The essential oil of *Illicium anisatum* Linn. Canadian Journal of Chemistry 44(21): 2461～2464.

Kim, H.Y., Kang, M.H. 2005. Screening of Korean medicinal plants for lipase inhibitory activity. Phytotherapy Research 19: 359～361.

Kim, H.Y., Kim, K. 2003. Protein glycation inhibitory and antioxidative activities of some plant extracts in vitro. Journal of Agricultural and Food Chemeistry 51: 1586～1591.

Kim, J.Y., Kim, S.S., Oh, T.H., Baik, J.S., Song, G.P., Lee, N.H., Hyun, C.G. 2009. Chemical composition, antioxidant, anti-elastase, and anti-inflammatory activities of Illicium anisatum essential oil. Acta Pharmaceutica 59: 289～300.

Kim, H.Y., Oh, J.H. 1999. Screening of Korean forest plants for rat lens aldose reductase inhibition. Bioscience. *Biotechnology and Biochemistry* 63: 184～188.

Eijkman, J.F. 1885. Sur les principes constituants de l'Illicium religiosum. Recueil des Travaux Chimiques des Pays-Bas 4(2): 32～73.

Guenther, E.D. 1948. van Nostrand Company Inc. New York, p.12.

Lim, S.S., Lee, Y.S., Kim, H.M., Ahn, Y.H., Shin, K.H., Lee, S.H. 2008. GC/MS Analysis of Volatile Constituents from Broad-Leaved Indeciduous Trees. *Korean Journal of Plant Resources* 21(4): 237～248.

Yamada, K., Takada, S., Nakamura, S., Hirata, Y. 1968. Structures of anisatin and neoanisatin toxic sesquiterpenes from *Illicium anisatum*. Tetrahedron 24(1): 199～229.

Zhao, C.X., Liang, Y.Z., Fang, H.Z., Li, X.N. 2005. Temperature-programmed retention indices for gas chromatography-mass spectroscopy analysis of plant essential oils. Journal of Chromatography A 1096: 76～85.