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ORIGINAL ARTICLE

Fragrance and Metabolite Components of Flowers from Korean Native *Apocynum lancifolium* Russanov

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Abstract

This study characterizes the volatile aromatic and metabolite components of domestic native *Apocynum lancifolium* blossom. The accurate characterization of fragrances collected from the blossom was carried out using gas chromatography-mass. A total of 70 chemical components were identified, including ketones of acetophenone (29.22%), phenylethyl alcohol (10.54%), methyl-benzenemethanol (8.43%), benzyl alcohol (7.97%), natural bicyclic sesquiterpene types of caryophyllene (6.08%), gurjunene (6.20%), humulene (1.90%), and ocimene (1.04%). Overall, the content of ketones, alcohols, and terpenes was higher than that of others. The major metabolite components were pentanoic acid, malic acid, fructofuranoside, quinic acid, tagatose, sorbose, galactose, inositol, galactaric acid, glucopyranoside, and octadecenoic acid.

Key words : *Apocynum lancifolium*, Acetophenone, Caryophyllene, Gurjunene, Metabolite

1. Introduction

Apocynum lancifolium or *Apocynum venetum* is commonly used as a native plant because of its beautiful appearance, fragrant scent, and excellent landscape aesthetics when planted in idle lands (Butterweck et al., 2001). Lately, it has been receiving attention after it was found that the scent of the flowers functions as an antidepressant. *A. lancifolium* is a perennial herbaceous or half-shrub plant, usually known as Napoma, with a length of 1 to 2 meters. Since it belongs to the family *Apocynaceae, A. lancifolium* leaks white fluids from wounds, and blooms between June and August (He et al., 1992). As a plant that has been used in traditional medicine since the 15th century in China (Xie et al., 2012), *A. lancifolium* shows good medicinal effects when its leaf extracts are used, acting as an antihypertensive agent (Kim et al., 2000; Kwan et al., 2005), cholesterol-lowering agent (Kim et al., 1998$_b$), and anxiolytic agent (Grundmann et al., 2007; Grundmann et al., 2009). It has also been used in the treatment of atherosclerosis (Lv et al., 2016) and myocardial ischemia-reperfusion injury (Wang et al., 2015). Tea made from the leaves of this plant has been put to practical use as a health drink in China, Japan and other East Asian countries and, more recently, in North America (Xiang et al., 2012). In addition, the extract of wild *A. lancifolium* is attracting attention in

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Fig. 1. The seedlings in pots (left) and the plants planted on the ground (right) in a greenhouse for analysis of *A. lancifolium*.

Korea as one of the representative wild plants expected to improve health, by serving as a food additive and natural product for cosmetic purposes, and acting as an antioxidant, anti-inflammatory agent, and astringent agent (Park and Lee, 2021). As described above, research on the chemical composition and medicinal effects of *A. lancifolium* leaves is known at home and abroad, and studies on metabolites and fragrance components of flowers are lacking despite the pleasant fragrance of *A. lancifolium*. Therefore, this study was conducted to prepare the foundation for securing genetic resources, ecotourism resources, and medicinal resources for farm households, through the analysis of the aromatic and metabolite components of *A. lancifolium* blossom.

2. Materials and Methods

2.1. Plant materials

Seeds of plants used for analysis were collected from Yeongjong Island, Incheon in October 2019. They were sown in a seedling container in a 50% shaded greenhouse in April 2020, and after germination, were planted on the ground of the greenhouse in June in Bibongmyeon, Hwaseongsi. All samples were collected at the flowering stage in June 2021, and stored in a −20℃ deep freezer until their usage for the analysis (Fig. 1).

For the analysis of fragrance components, 2 g of flower sample + 3 g of NaCl (added without dissolving the powder reagent salt) in a SPME amber vial was freeze-fractured, vortexed with the HS−SPME−GC/MS system (TSQ 8000, Thermo Fisher Scientific Inc., USA), and used with the SPME fiber conditions set as SPME fiber coated with divinylbenzene/ polydimethylsiloxane (DVB/PDMS, 50/30 mm, 10 mm fiber length, SUPELCO Co., USA). For metabolite analysis, 20 ㎎ of the powdered sample (lyophilized after pulverization) was ultrasonically extracted with 1 mL of 70% methanol, and filtered with a 0.2 μL syringe. After drying the filtered sample with 150 μL speed vacuum, 50 μL of 20,000 ppm methyl hydroxyl chloride amine in pyridine was added to the dried sample, and reacted in an oven at 30℃ for 90 min. After the reaction, 50 μL of BSTFA + 1% TMCS solution and 30 μL of internal standard (1000 ppm fluoranthene) were added to the sample vial, followed by vortexing. The sample was analyzed by GC-MS in a

Table 1. Analysis conditions of volatile organic compounds and metabolite components by GC-MS

Items		VOCs	Metabolite
Split Ratio		10 : 1	60:1
Detector	Gas chromatograph	Trace 1310	Trace 1310
	Mass spectrometer	TSQ 8000: Triple quadrupole	ISQ LT single quadrupole
Column		DB-wax (60 m×0.25 ㎜, 0.50 ㎛), Agilent technologies	DB-5ms (60 m×0.25 ㎜, 0.25 ㎛), Agilent technologies
Carrier GAS and Inlet temperature, column Flow		He, 230℃, 2 mL/min	He, 300℃, 1.5 ㎖/min
Temperature program	Initial temperature	40℃ (5 min)	50℃ (2 min)
	Heating rate	-	180℃, (8 min)
	Final temperature	240℃, (10 min)	320℃, (10 min)

GC vial + insert after reaction in an oven at 60℃ for 30 min.

2.2. Component Analysis

The equipment conditions of gas chromatography -mass spectrometry (GC/MS) used for the analysis of fragrance components and metabolites are shown in Table 1, and the analysis was performed by NICEM at Seoul National University. This data was obtained from analysis by GC-MS, and the component analysis of each peak separated in the Total Ionization Chromatogram (TIC) by GC-MS was carried out by analyzing and comparing with the standard NIST Mass Spectral Library (W8NO. L, Agilent Co.) The substance was estimated based on the mass spectrometry data in the literature, and the quantification of the identified volatile fragrance components was represented as the relative peak area (%) of each compound.

3. Results and Discussion

A total of 70 fragrance components were analyzed, and a substance with a library matching rate of 70% was judged to be a statistically significant fragrance component in the flower of *A. lancifolium* (Table 2). Among these fragrance components, 16 types of alcohols, 14 hydrocarbons, 11 terpenes, 10 acids, 6 ketones, 5 aldehydes, 3 types of ester components, 2 types of nitriles and furans, 1 type of amides, and phenylpropenes, were analyzed (Table 3). The major volatile compounds of *A. lancifolium* consisted of ketones (32.38%), alcohols (29.30%), terpenes (17.83%), and hydrocarbons (11.52%), while minor ones consisted of amides (0.18%), acids (4.63%), and aldehydes (2.33%), etc. (Figure 3). The mass spectrum of the compound corresponding to CAS# (Chemical Abstract Service Registration Number) was well matched and identified as shown in Table 2. Fig. 2 shows the GC chromatogram obtained by analyzing the fragrance component of the *A. lancifolium*. Table 4 indicates that 14 of the 70 components occupy more than 1% of the peak area. These 14 main components are ethanol, 1-butanol, 3-methyl-(impure), ocimene, (1R,2R,3R,4R)-1,2:3,4-Bis (isopropylidenedioxy)-5-(2', 2'-dimethylpropylidene) cyclopentane, gurjunene, 2,6-nonadienal, (E,Z)-caryophyllene, acetophenone, caryophyllene, humulene, methyl benzenemethanol, benzyl alcohol, and phenylethyl alcohol. Among them, the ketone type substance acetophenone accounted for the largest amount (29.22%), followed by alcohol type substances such as phenylethyl alcohol (10.54%), methyl-benzenemethanol (8.43%), benzyl alcohol (7.97%), terpene type substances such as caryophyllene (6.08%), gurjunene. (6.20%), and humulene (1.90%)

Table 2. List of discriminative fragrant compounds of *A. lancifolium* by GC-MS and identification based on library matching

No	RT (min)	Compound	Area ratio (%)	Cas#*
1	6.61	Hexamethyl-cyclotrisiloxane	0.83	541-05-9
2	9.79	Ethanol	1.09	64-17-5
3	10.34	Octamethyl-cyclotetrasiloxane	0.63	556-67-2
4	12.71	Pinene	0.70	80-56-8
5	14.46	3-Methyl-butanoic acid ethyl ester	0.17	108-64-5
6	15.06	Hexanal	0.33	66-25-1
7	15.16	Decamethyl-cyclopentasiloxane	1.46	541-02-6
8	15.30	2-Methyl-butanenitrile	0.18	18936-17-9
9	15.86	2-Pinene	0.30	127-91-3
10	16.68	3-Methyl-butanenitrile	0.54	625-28-5
11	17.49	1-Penten-3-ol	0.30	616-25-1
12	19.02	1-Butanol, 3-methyl-(impure)	1.39	123-51-3
13	19.54	5-Methyl-, methyl esterhexanoic acid	0.34	2177-83-5
14	20.05	Dodecamethyl-cyclohexasiloxane	1.75	540-97-6
15	20.61	Ocimene	1.04	13877-91-3
16	21.87	Pentanoic acid, octyl ester	0.55	5451-85-4
17	22.26	Trans-2-(2-pentenyl)furan	0.18	70424-14-5
18	22.59	2-Penten-1-ol	0.27	1576-95-0
19	22.65	4-Hexen-1-ol, acetate	0.28	72237-36-6
20	22.84	2-Pentenoic acid, 4,4-dimethyl-, methyl ester	0.69	16812-85-4
21	23.11	2-Heptenal	0.16	57266-86-1
22	23.19	Trans-2-(2-pentenyl)furan	0.33	70424-14-5
23	23.36	5-Hepten-2-one, 6-methyl-	0.20	110-93-0
24	23.50	1-Hexanol	0.21	111-27-3
25	23.84	2-Feptenoic acid, 3-methyl-, methyl ester	0.33	50652-81-8
26	24.42	Tetradecamethyl-cycloheptasiloxane	0.46	107-50-6
27	24.53	3-Hexen-1-ol	0.78	928-96-1
28	24.98	Nonanal	0.23	124-19-6
29	25.54	Cyclotetrasiloxane,octamethyl	0.38	556-67-2
30	25.97	7-Tetradecene	0.21	10374-74-0
31	26.24	1-Octen-3-ol	0.23	3391-86-4
32	26.36	Linalool oxide	0.24	5989-33-3
33	27.03	4-Hydroxy-2-pentanone	0.46	4161-60-8
34	27.16	Trans-linalool oxide	0.18	34995-77-2
35	27.28	(1R,2R,3R,4R)-1,2:3,4-Bis(isopropylidenedioxy)-5-(2',2'-dimethylpropylidene) cyclopentane	2.17	NA
36	27.48	5-Chloro-3-(p-ethylphernyl)-2-nitrothiophene	0.42	NA
37	27.94	(S)-3-Ethyl-4-methylpentanol	0.76	NA

Table 2. Continued.

No	RT (min)	Compound	Area ratio (%)	Cas#*
38	28.11	7,8-Bis(trimethylsilyl)benzo(5,6-g)-1H,3H-quinazoline-2,4-dione	0.25	80964-27-8
39	28.67	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6,7-endo,endo,diol	0.15	56084-15-2
40	28.86	**L**inalool	0.29	78-70-6
41	29.01	Gurjunene	6.20	489-40-7
42	29.36	Gurjunene	0.49	489-40-7
43	30.38	2,6-Nonadienal, (E,Z)-	1.43	557-48-2
44	30.86	Caryophyllene	6.08	87-44-5
45	31.98	(E)-famesene	0.41	18794-84-8
46	32.10	Alloaromadendrene	0.27	25246-27-9
47	32.28	Acetophenone	29.22	98-86-2
48	32.53	Benzoic acid, ethyl ester	0.87	93-89-0
49	32.70	Caryophyllene / humulene	1.90	6753-98-6
50	33.49	2-Hydroxy-2-(p-methoxyphenyl)-3-hydroxycarbonyl-3-met hyl-butane	0.95	27925-42-4
51	33.69	Isocaryophillene	0.89	NA
52	33.82	2,6,6,9-Tetramethyl-,(1R,2S,7R,8R)-tricyclo[5.4.0.0(2,8)]undec-9-ene	0.16	5989-08-2
53	33.98	Farnesene	0.77	502-61-4
54	34.39	(3S)-3-Phenyl-2,3-dihydro-1 h-isoindol-1-one	0.52	NA
55	34.59	Cadinene	0.17	483-76-1
56	35.35	Methyl salicylate	0.82	119-36-8
57	35.70	Methyl-benzenemethanol	8.43	98-85-1
58	35.98	1-(2-Hydroxyphenyl)-ethanone	1.32	118-93-4
59	36.32	Hexanoic acid	0.22	142-62-1
60	37.20	Benzyl alcohol	3.97	100-51-6
61	37.35	Acetic acid, diethyl-	0.40	88-09-5
62	37.57	Pentanoic acid, phenylmethyl ester	0.34	10361-39-4
63	38.01	Phenylethyl alcohol	10.54	60-12-8
64	38.69	2-Pentenoic acid, 2,3-dimethyl-	0.72	122630-51-7
65	40.20	Pentadecanal-	0.18	2765-11-9
66	43.27	Eugenol	0.51	97-53-0
67	43.62	N-phenyl-formamide	0.18	103-70-8
68	45.34	Heptaethylene glycol	0.19	5617-32-3
69	45.91	Dimethyl phthalate	0.20	131-11-3
70	54.89	Benzyl benzoate	0.19	120-51-4

*CAS# (chemical abstract service registration number)

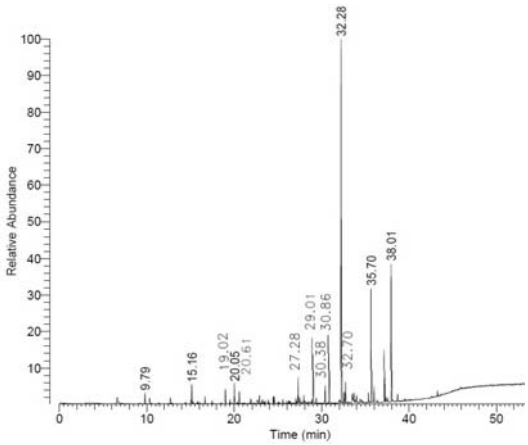

Fig. 2. GC chromatogram of the volatile compounds of flowers according to retention times in *A. lancifolium*. Bold numbers on the chromatogram are the peak retention times identified.

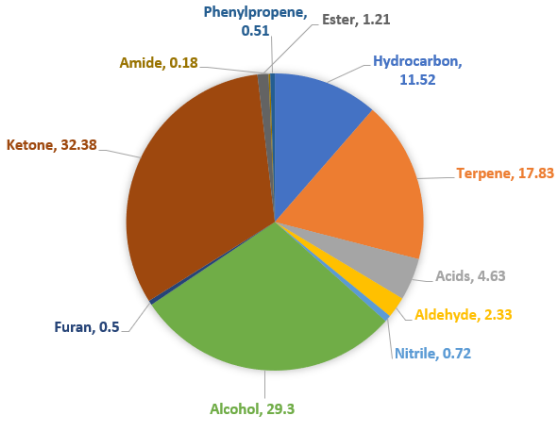

Fig. 3. Relative content (%) of volatile compounds in *A. lancifolium*.

Table 3. The classification of compounds in *A. lancifolium* by GC-MS.

Classification (Total number)	Compound name
Hydrocarbon (14)	Hexamethyl-cyclotrisiloxane, Octamethyl-cyclotetrasiloxane, Decamethyl-cyclopentasiloxane, 7-Tetradecene, Isocaryophillene, Dodecamethyl- Cyclohexasiloxane, Cadinene, ocimene, Tetradeca methyl-cycloheptasiloxane, 1,1,1,3,3,5,5,7,7-Nonamethyl tetrasiloxane, (1R,2R,3R,4R)-1,2:3,4-Bis (isopropylidenedioxy)-5-(2',2'-dImethylpropylidene)cyclopentane, 5-Dhloro-3-(p-ethylphernyl)-2-nitrothiophene, 2-Hydroxy-2-(p-methoxyphenyl)-3-hydroxycarbonyl-3-methyl-butane, 2,6,6,9-tTtramethyl-,(1R,2S,7R,8R)-tricyclo[5.4.0.0(2,8)]undec-9-ene
Terpene (11)	Pinene, gurjunene, Gurjunene, 2-pinene, Sabinene, Caryophyllene, (E)-famesene, Alloaromadendrene, Caryophyllene, Humulene, Farnesene, Linalool oxide, Trans-linalool oxide, **L**inalool
Acid (10)	3-Methyl-butanoic acid ethyl ester, Hexanoic acid, 5-Methyl- methyl ester, Pentanoic acid, octyl ester, 2-Pentenoic acid, 4,4-Dimethyl-, Methyl ester, 2-Feptenoic acid, 3-Methyl-, Methyl ester, Benzoic acid, Ethyl ester, Hexanoic acid, Acetic acid, Diethyl- pentanoic acid, Phenylmethyl ester, 2,3-Dimethyl-2-pentenoic acid
Aldehyde (5)	Hexanal, 2-heptenal nonanal, 2,6-Nonadienal, (E,Z)-pentadecanal
Nitrile (2)	2-Methyl-butanenitrile, 3-Methyl-butanenitrile
Alcohol (16)	Ethanol, 1-Penten-3-ol, 1-Butanol, 3-Methyl-(impure) -Penten-1-ol, 4-Hexen-1-ol, Acetate, 1-Hexanol, 3-Hexen-1-ol, 1-Octen-3-ol, Alcohol, Benzyl alcohol, 4-Hydroxy-2-pentanone, 7,8-Bis(trimethylsilyl)benzo(5,6-g)-1h,3H-quinazoline-2,4-dione, Phenylethyl, (S)-3-Ethyl-4-methylpentanol, Heptaethylene, glycol1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6,7-Endo,endo,diol,Methyl-benzene methanol
Furan (1)	Trans-2-(2-pentenyl)furan
Ketone (6)	5-Hepten-2-one, Acetophenone, Benzoic Acid, Ethyl ester, 6-Methyl-7,8-bs(trimethylsilyl)benzo (5,6-g)-1H,3H-quinazoline-2,4-dione, (3S)-3-Phenyl-2,3-Dihydro-1-H-isoindol-1-one, 1-(2-Hydroxyphenyl)-ethanone
Ester (3)	Methyl salicylate, Dimethyl phthalate, Benzyl benzoate
Amide (1)	N-phenyl-formamide
Phenylpropene (1)	Eugenol

Table 4. The major fragrant compounds of flowers and its peak area (%) by GC-MS

No.	Retention time	Compounds*	Classification	Area(%)
1	9.79	Ethanol	Alcohol	1.09
2	15.16	Decamethyl-cyclopentasiloxane	Hydrocarbon	1.46
3	19.02	1-Butanol, 3-methyl-(impure)	Alcohol	1.39
4	20.05	Dodecamethyl-cyclohexasiloxane	Alcohol	1.75
5	20.61	Ocimene	Hydrocarbon	1.04
6	27.28	(1R,2R,3R,4R)-1,2:3,4-Bis (isopropylidenedioxy)-5 -(2',2'-dimethylpropylidene)cyclopentane	Hydrocarbon	2.17
7	29.01	Gurjunene	Terpene	6.20
8	30.38	(E,Z)-2, 6-Nonadienal	Aldehyde	1.43
9	30.86	Caryophyllene	Terpene	6.08
10	32.28	Acetophenone	Ketone	29.22
11	32.70	Caryophyllene	Terpene	1.90
12	35.70	Benzenemethanol	Alcohol	8.43
13	37.20	Benzyl alcohol	Alcohol	3.97
14	38.01	Phenylethyl alcohol	Alcohol	10.54

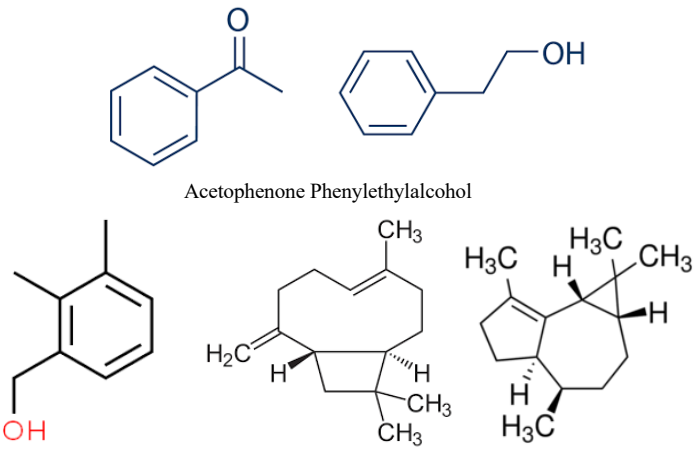

Fig. 4. The chemical structures of the most abundant aromatic components in A. lancifolium.

(Table 4). Those in parentheses indicate the peak area (%).

The phenylpropene type (eugenol), the acids (acetic acid, butanoic acid, hexanoic acid, pentanoic acid, 2-heptenoic acid, benzoic acid), and trans-2-(2-pentenyl) furan were identified Among the esters, methyl salicylate, dimethyl phthalate, and benzyl benzoate were identified (Table 3).

Acetophenone (chemical formula $C_6H_5COCH_3$) is the most abundant of all components found in the analysis. It is one of the aromatic ketone ingredients, and is a colorless organic compound that is used as an

Table 5. List of discriminative metabolites of flowers and its peak area (%) by GC-MS

No.	Retention time	Compounds*	Classification	Area(%)
1	17.68	Pentanoic acid	O[z]	1.33
2	27.98	DL-malic acid,	O	2.48
3	38.02	Fructofuranoside	S[y]	1.02
4	42.05	Penta-qunic acid	O	3.13
5	42.65	D-(-)-tagatose	S	7.85
6	43.10	L-(-)-sorbose	S	5.46
7	43.80	D-galactose	S	25.06
8	44.63	D-galactose	S	4.07
9	48.17	Inositol	S	12.66
10	48.68	Galactric acid	O	2.02
11	51.45	Inositol	S	3.04
12	62.03	A-D-glucopyranoside	S	6.87
13	72.57	9-Octadecenoic acid	O	7.24
14	72.63	9-Octadecenoic acid	O	6.43

*: above 1% of total
[z] Organic acids
[y] Sugar and sugar derivative

intermediate in chemical reactions to produce the sweet fragrance of cherry, one of the main ingredients of perfume, as well as pharmaceuticals, resins, seasonings, teardrops, and sleeping pills. This substance is also one of the essential oil components extracted from the root of *Cyanachum paniculatum*, which has been reported to have antibacterial activity against human intestinal microflora, and acaricidal activity against small mites (Lee et al., 2018). Because of its antibacterial activity, the market demand for acetophenone is increasing, especially after the COVID-19 pandemic (https://www.radiantinsights.com/research/global-3-hydroxy-acetophenone-cas-121-71-1-market-2018-2023/request-sample). The most abundant fragrance component after acetophenone was phenylethyl alcohol, followed by benzenemethanol, and a large amount of alcohol-based components. The content of ketones and alcohols among the volatile fragrance components is high, and thus, these two components are believed to play an important role in the fragrance characteristics of the flower. In addition, volatile terpenes such as gurjunene, pinene, sabinene, caryophyllene, humulene, alloaromadendrene, farnesene, and linalool were also found, and are the representative aromatic components of the plant as well. Owing to such high quantities of these fragrance components, *A. lancifolium* has high potential of being a fragrance resource.

The GC-MS run time for the metabolite analysis of the *A. lancifolium* was 100 minutes, and the main components consist of sugars, sugar derivatives, and organic acids, including pentanoic acid, DL-malic acid, fructofuranoside, quinic acid, D-(-)-tagatose, L-(-)-sorbose, galactose, inositol, galactaric acid, à-D-glucopyranoside, and 9-octadecenoic acid (Fig. 5). D-galactose, which accounted for the most (29.13%) of the total components, made up 25.06% and 4.07% at retention times of 43.8 min and 44.63 min, respectively. Next, Inositol accounted for 15.70% of the total components, and made up 12.66% and 3.04% at retention times of 48.17 minutes and 51.45 minutes, respectively, 9-octadecenoic acid was analyzed over retention time of 72.57 minutes to 72.63 minutes,

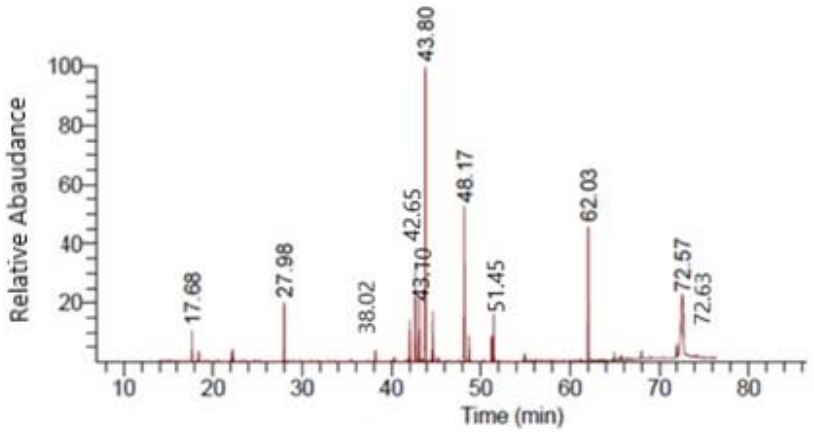

Fig. 5. GC chromatogram of the metabolites of flowers according to retention time in *A. lancifolium*. Bold numbers on the chromatogram are the peak retention times identified.

accounting for 13.47% of the total components, D-(-)-tagatose accounted for 7.85%, and à-D-glucopyranoside accounted for 6.87% (Table 5).

Among the components of *A. lancifolium*, galactose is a natural sugar known to be a constituent of lactose, with a chemical structure consisting of aldehyde-type hexose $C_6H_{12}O_6$. It also exists in nature as a constituent of polysaccharides such as galactan and galactomannan. Galactaric acid protects against oxidative stress and slows down skin aging, and pentanoic acid and malic acid are representative antioxidants. Inositol is a sugar alcohol with a 6-membered carbon ring, with a chemical formula of $C_6H_6(OH)_6$. These compounds are highly crystalline and sweet like sugar. Certain fatty acids, such as 9-octadecenoic acid, are known to be antioxidants and antibacterial substances. According to literature, flavonol and flavonol glycosides are the main compounds present in the leaves of Chinese *A. lancifolium* (Wenyan et al., 2012), and these compounds have been especially studied for their antidepressant effect, anti-oxidant effect, and toxin neutralizing effect on the nicotine component of tobacco (Butterweck et al., 2001; Bourin et al., 2009). The extensive pharmacological activity of Chinese *A. lancifolium* is known to be because of various flavonoid components (Xie et al., 2012), and the leaves of the plant have been reported to contain various substances, such as minerals, flavonol, flavonol glycosides, flavan-3-ols, and organic acids, along with a large amount of fiber, salt, magnesium, calcium, iron, manganese, potassium, copper, and aluminum (Fan et al., 2006; Wang, 2017). Fragrance components are produced by chemical and physical interactions within the plant, and the degree of fragrance expression varies depending on the compound composition, concentration, and matrix. Therefore, the correlation with the fragrance component through metabolite analysis is important (Jo et al., 2010). In addition, fragrance is known to be directly influenced by the amount of product, differences in varieties, plant parts, surrounding environment, and growth conditions, as enzymes act by the expression of genes in the substrate of each plant. Fragrances are produced by the complex interaction of various components and pathways, and are also influenced by primary metabolic components such as sugars and amino acids. Therefore, an in-depth study is needed on the relationship between

environmental factors like climate and soil, the production of fragrance components and metabolites of *A. lancifolium*, the effects of fragrance components on the human body, and the comparison of fragrance components and metabolites of Chinese species.

The details of the volatile fragrance components and metabolites of *A. lancifolium* found in this study can be used not only as basic data for the development of eco-tourism resources and fragrance for incense but will also be useful as basic data for the development of medicinal materials. In addition, the metabolites found in this study are considered highly valuable as medicinal resources, and can be efficiently utilized for the cultivation of cash crops.

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