Chemical Constituents of *YUZU* and *LIME* Essencial Oils and Their Antioxidative Activities

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In this examination, antioxidant activities and whitening effects of *yuzu*(*Citrus junos Sieb.ex* Tanaka) and *lime*(*Citrus aurantifolia S.*) essencial oils which are widely used in food flavors were studied. As a result, we found out that 1% to 2% concentration *yuzu* essencial oil contains equal antioxidant activity to □-tocopherol which is a substance commercially used as antioxidant. Also, from the result of tyrosinase activity inhibition test, an evaluation test on whitening effects, we found out that 1% to 2% concentration lime essencial oil contains higher inhibiting activation than arbutin which is a substance highly used in commercial cosmetics.

Keywords: *yuzu* essencial oil, *lime* essencial oil, DPPH radical scavenging effect, tyrosinase activity inhibition.

1. INTRODUCTION

Since ancient times, plants have been used as herbal medicines (Chinese medicines, Crude drugs) [1,2]. In Japan, plants with anti-bacterial action are used in seasonal cooking as a spice [3,4]. Also one of the characteristic ways of using herb is the herb bath. For example, on the boy's festival people take bath with iris leaves and on the winter solstice citruses (*yuzu*) were put into the bath.

Recently, as people get more health conscious, seasonal fruits produced in domestic and overseas are getting attention with their various physiological active substances.

However, the release of harmful freon gas to the atmosphere is causing a serious destruction of the ozone layer [5,6]. As a result, the middle wavelength (290 ~ 320nm) ultraviolet ray (UV-B), the most harmful sunbeam to the human body, is increasingly reaching to the earth [5]. The bad effects caused by UV-B are the developing pigment deposition to the skin that forms stain and flecks and promotion of the skin cancer and skin aging [7,8]. In reflection of the recent ecologic boom, many research on whitening effects and antioxidant activity of the plant origin element have been done and chemicals such as *arbutin*, *kojic acid* and *placenta* essence were found [9,10].

Therefore, we examined *yuzu* essencial (*Citrus junos Sieb. ex Tanaka*) [11,12] and *lime* essencial (Citrus *aurantifolia S.*) [13,14] that are widely used as flavor and fragrance, for the use in cosmetics combination-drug. This paper reports the result and the new findings from the experiments that have possibilities to be applied in the cosmetic combination-drug.

2. EXPERIMENTAL

2.1 Materials

Yuzu essencial oil (*Citrus junos Sieb.ex* Tanaka) harvested in Kochi prefecture and Mexican lime essencial oil (Citrus aurantifolia S.)were respecti-

vely extracted with diethyl ether and the solvent was distilled.

2.2 Analysis

These components were analyzed by using GC-MS (Hewlett Packerd : HP6890 GC, Hewlett Packerd HP 5972 MSD, column : TC-WAX, 60m x 0.25mm, 70 (5 min hold)~ 240°C (3°C / min), injection temp; 240°C, library: NIST(National Institute of Standards and Technology) Web Book).

2.3 Test of Biological Activity DPPH Radical Scavenging Effect [15)]

The test compounds (2 ml) were adjusted with ethanol solution to final concentration of 1 mM. Acetic acid buffer (1 mM) as added, and the mixture was warmed in tropical aquarium at 25°C. After 5 min, DPPH radical ethanol solution (1 ml, 0.2 mM) was measured with a spectrophotometer (517 nm). The DPPH (1,1-diphenyl-2-picrylhy-drazyl) radical scavenging rate of each sample was calculated and the 50% scavenging concentration based on the DPPH radical scavenging rate was also calculate based on the following formula : where **A** is the absorbance of the sample when a blank was substituted for ethanol, **B** the absorbance of the sample when a color contrast agent was substituted for ethanol in the DPPH radical ethanol solution, and **C** the absorbance of the color contrast agent alone.

$$\text{DPPH radical scavenging rate (\%)} = (1-(A-C)/B) \times 100$$

2.4 Reactive Oxygen-Species Scavenging Effect [16)]

A solution of individual compounds were prepared at the concentration determined previously (0.714 mmol) and tested for their reactive oxygen-species scavenging ability as described previously using the superoxide dismutase (SOD) test and Wako reagent (Wako Pure Chemical Industries Ltd.). The concentration of diformazan generated by the compounds was measured at 560 nm with a spectrophotometer.

2.5 Tyrosinase Activity Inhibition Test using Tyrosine as a Substrate [16)]

A solution of individual compounds that was used at a concentration of 0.08 mmol and tested for their ability to inhibit tyrosinase activity as described previously using tyrosinase (EC 1.14.18.,1 Sigma Chemical Co.,) as a substrate. Reading were taken at 475 nm with a spectrophotometer.

2.6 Tyrosinase Activity Inhibition Test using DOPA as a Substrate [16)]

A solution of 3,4-dihydroxy-L-phenylalanine (DOPA,Wako Pure Chemical Industries Ltd.) was prepared at the concentration determined previously (1.66 mmol), and the synthesized compounds were tested for their ability to inhibit tyrosinase activity as described previously using *L*-DOPA as a substrate.

3. Results and Discussion

The element contained in diethyl ether extract of *yuzu* and *lime* essencial oils was analyzed with GC-MS (Figure 1,2). As a result, the chemical structures of 52 compounds in the peak contained in *yuzu* essencial oil were explained. And the chemical structures of 73 compounds were explained from *lime* essencial oil (Table 1). Then for the practical use of the cosmetics combination medicine, an examination to evaluate the antioxidant activity of free radical had been done.

Free radical is a chemical, which is considered to be one of the factors that promote aging of the skin. We found that 1 % to 2 % concentration *yuzu* essencial oil contains equal antioxidant activity to □- tocopherol (100%), which is a substance often used in cosmetics in the market. This means that *yuzu* essencial oil contains that 96 ~ 100 % antioxidant activity (Table 2). On the other hand, we couldn't observe as much free radical value in *lime* essencial oil as we expected.

Lime essencial oil only showed 1/2 to 1/3 SC_{50} (50% Scaverging concentration) value to *yuzu* essencial oil. Therefore we found out that there is no antioxidant activity in *lime* essencial oil. Next,

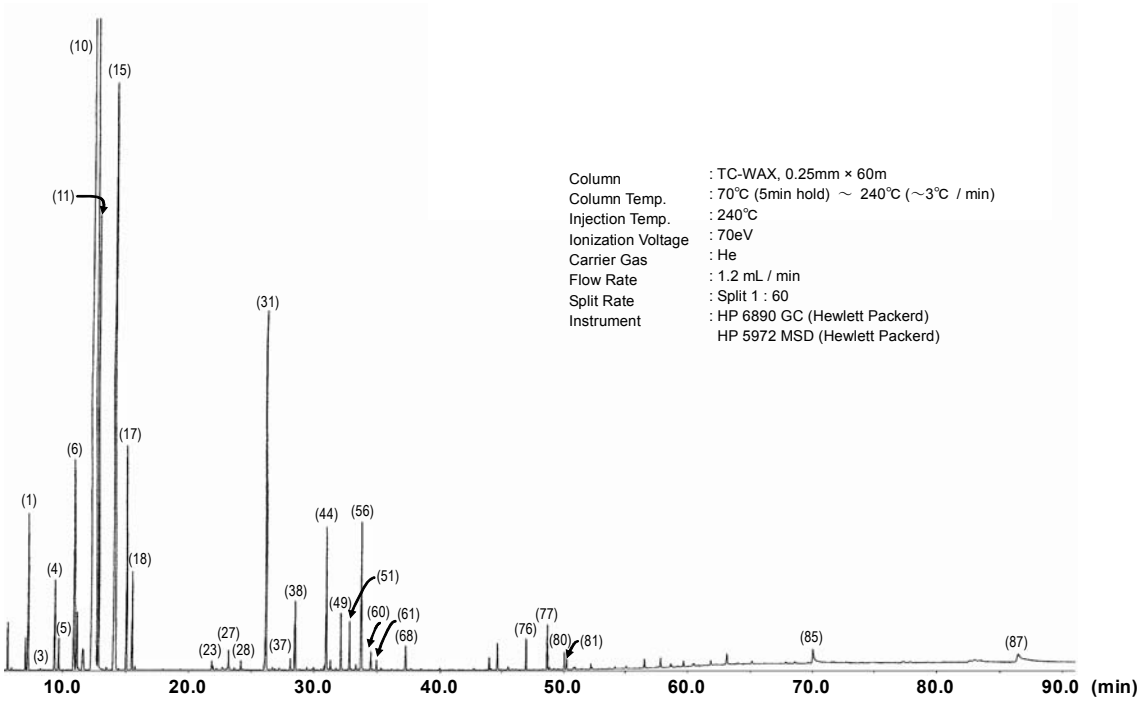


Fig. 1 Gas Chromatogram of *YUZU* Essence Oil (in Ether Extract)

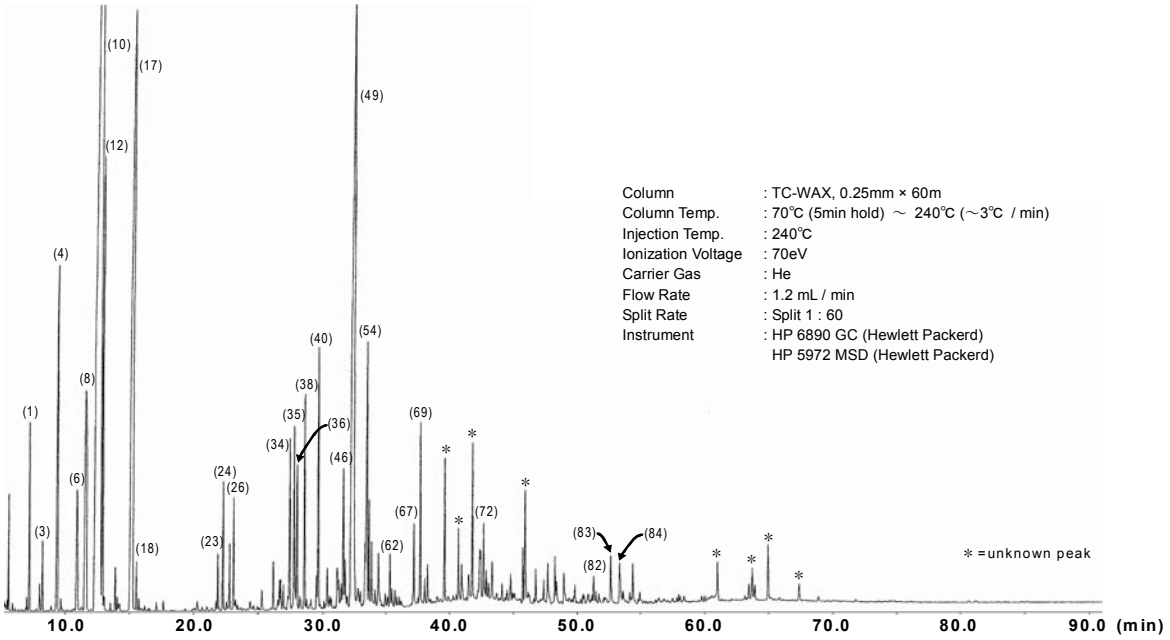


Fig. 2 Gas Chromatogram of *LIME* Essence Oil (in Ether Extract)

Table1 Major Components of *YUZU* and *LIME* Essence Oils (in ether Extract)

Peak No.	R.T. (min)	Compound	Peak area(%) YUZU OIL	Peak area(%) LIME OIL	Peak No.	R.T. (min)	Compound	Peak area(%) YUZU OIL	Peak area(%) LIME OIL
1	7.23	α-Pinene	1.68	0.85	45	31.32	α-Humulene	0.11	0.19
2	8.05	α-Fenchene	—	0.15	46	31.59	β-Terpineol	—	0.95
3	8.26	Camphene	0.01	0.37	47	31.73	Neral	0.03	0.38
4	9.38	β-Pinene	0.95	2.52	48	31.78	1,8-*p*-menthadien-4-ol	0.03	0.10
5	9.68	Sabinene	0.33	0.08	49	32.39	α-Terpineol	0.59	10.01
6	10.89	Myrcene	2.91	1.09	50	32.44	Borneol	—	1.61
7	11.25	α-Phellandrene	0.83	0.05	51	32.85	Germacrene D	0.55	0.15
8	11.53	1,4-Cineole	—	3.11	52	33.32	Neryl acetate	0.08	0.38
9	11.59	α-Terpinene	0.45	—	53	33.34	α-Muulorene	0.08	—
10	12.71	Limonene	55.80	26.55	54	33.48	β-Bisabolene	—	2.31
11	12.81	β-Phellandrene	4.69	—	55	33.65	Geraniol	—	0.63
12	12.87	1,8-Cineole	—	2.44	56	33.77	Bicyclogermacrene	1.84	—
13	13.48	*Cis*-β-Ocimene	0.03	0.04	57	33.84	Carvone	—	0.46
14	13.85	2,2-Dimethyl-5-(1-methyl-propenyl)tetrahydrofuran	—	0.21	58	34.12	α-Farnesene	—	0.16
					59	34.40	Geranyl acetate	—	0.38
15	14.02	γ -Terpinene	12.00	0.08	60	34.53	δ-Cadinene	0.19	—
16	14.20	*Trans*-β-Ocimene	0.01	0.04	61	34.98	β-Sesguiphellandrene	0.10	—
17	15.22	*p*-Cymene	2.22	9.77	62	35.31	*p*-Methyl phenylacetophenone	—	0.33
18	15.51	Terpinolene	0.92	0.22	63	35.33	*p*-Mentha-1(7),2-dien-8-ol	0.03	—
19	15.71	Octanal	—	0.06	64	35.46	Selina-3,7(11)-diene	—	0.14
20	16.18	Acetal	—	0.03	65	35.55	Myrtenol	—	0.04
21	17.64	6-Methyl-5-hepten-2-one	—	0.05	66	35.89	*p*-Mentha-1(7),8-dien-2-ol	—	0.09
22	20.29	Fenchone	—	0.05	67	37.22	Carveol (p-1)	0.39	0.59
23	21.88	Dehydro-*p*-cymene	0.08	0.30	68	37.26	Germacrene B	0.29	0.59
24	22.28	Limonene oxide (p-1)	—	0.70	69	37.72	*p*-Cymen-8-ol	0.03	1.14
25	22.79	Limonene oxide (p-2)	—	0.38	70	38.07	*Exo*-2-Hydroxy cineole	—	0.23
26	23.10	Epoxy terpinolene	—	0.62	71	38.29	Carveol (p-2)	—	0.33
27	23.24	δ-Elemene	0.24	0.09	72	42.67	Caryophyllene oxide	—	0.57
28	24.19	α-Copaene	0.10	—	73	42.74	Limonen-10-ol	0.02	0.10
29	24.39	Decanal	—	0.07	74	43.99	Oxa cyclotridec-10-en-2-one	0.14	0.10
30	25.29	Camphor	—	0.14	75	45.54	Methyl N-methylanthranilate	0.05	—
31	26.16	Linalool	4.52	0.23	76	46.99	Spathulenol	0.32	—
32	26.54	Octanol	—	0.08	77	48.70	Thymol	0.48	0.44
33	27.26	Iso-Butyric acid	0.03	0.09	78	48.91	Tau-Muurolol	0.03	0.22
34	27.42	Terpinen-1-ol	—	1.05	79	49.61	Carvacrol	0.02	0.04
35	27.75	Fenchyl alcohol	—	1.22	80	50.08	*Iso*-Spathulenol	0.18	—
36	27.98	α-Bergamotene	—	1.00	81	50.28	α-Cadinol	0.12	0.05
37	28.14	β-Elemene	0.12	0.14	82	51.30	Limonene diol	—	0.20
38	28.57	Terpinen-4-ol	0.91	1.27	83	52.65	Sobrerol	—	0.41
39	29.49	*p*-Mentha-2,8-dien-1-ol	0.03	0.18	84	53.35	δ-Hydroxy carvotanacetone	—	0.49
40	29.63	β-Terpineol	—	1.59	85	70.03	Hexadecanoic acid	0.57	—
41	30.33	Ascaridole	—	0.26	86	82.63	Oleic acid	0.08	—
42	30.63	Pinocarveol	0.02	0.09	87	86.48	Linoleic acid	0.92	—
43	30.83	Sesguisabinene	0.04	0.02	88	92.32	Linolenic acid	0.33	—
44	31.00	β-Farnesene	1.57	0.02	—	—	Others	1.91	18.89

the examination of antityrosinase activity had been done. Tyrosinase is a substance that will inhibit the biosynthesis of melanin, which is the cause of the stain and flecks. In *yuzu* essencial oil, either *L*-tyrosine or *L*-DOPA did not contain any inhibiting activation.

However, 1% to 2% concentration *Lime* essencial oil showed 68 ~ 87 % of inhibiting activation rates in *L*-DOPA. This result shows that *lime* essencial oil contain more inhibiting activation than *arbutin* (3mM=1.4 %), which is the comparative material. Also the inhibiting activation rate of *L*-tyrosine (Arbutin; 3mM=6.5%) was improved from 17.9 % to 31.5 %.

The results are shown in Table 3. We found that (17), the monoterpene hydrocarbons, and (6), have higher effectiveness of tyrosinase inhibitory activity than arbutin. On the other hand, (77) and (79), the aromatic compounds, due to the change of the concentration (1% to 2%) of *lime* essencial oil.Also,the antioxidant effect and whitening effectiveness of (6), (8), (17), (77) and (79), the chemical components (Figure 3) in the essence, were examined.shown equal figure to α-tocopherol, the comparative chemical, and higher antioxidant effect.

From these experiments, we cleared that *yuzu* and *lime* essencial oils have possibilities to be used as the cosmetic material, which has whitening effect and nailcare cosmetics and also in those cosmetics, we can expect high validity.

Table 2 DPPH Radical Scavenging Effect Assay and Inhibition of Tyrosinase Activity by *Yuzu* and *Lime* Essences (in Ether extract)

Essence Oil	DPPH radical scavenging effect		Tyrosinase inhibition (%)	
	Scavenging rate (%)[a)]	SC_{50}[b)]	*L*-Tyrosine	*L*-DOPA
Yuzu (2%)	100	34	N.D.[e)]	N.D.
Yuzu (1%)	95.7	75	N.D.	N.D.
Yuzu (2%) (water layer)	34.1	>400	N.D.	N.D.
Yuzu (1%) (water layer)	18.8	>400	N.D.	N.D.
Lime (2%)	79.9	92	31.5	87.3
Lime (1%)	86.6	151	17.9	69.2
Lime (2%) (water layer)	3.5	>400	N.D.	1.9
Lime (1%) (water layer)	2.4	>400	N.D.	N.D.
α-tocopherol[c)]	100	9	N.D.	N.D.
Arbutin [d)]	N.D.	N.D.	6.5	1.4

a) Concentration 0.4% (W / W).

b) 50% Scavenging Concentration $x10^{-3}$ % (W / W).

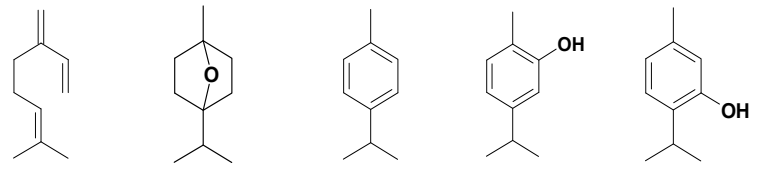
c) Concentration 0.2 mM. d) Concentration 3.0 mM.

e) N.D. : No detected.

Table 3 DPPH Radical Scavenging Effect Assay and Inhibition of Tyrosinase Activity by Monoterpenoids

Compound	DPPH radical scavenging effect		Tyrosinase inhibition (%)	
	Scavenging rate (%)[a)]	SC_{50}[b)]	*L*-Tyrosine	*L*-DOPA
(6)	N.D.	N.D.	6.6	9.9
(8)	N.D.	N.D.	7.0	N.D.
(17)	N.D.	N.D.	4.8[e)]	14.4
(77)	95.8	12	N.D.	N.D.
(79)	94.8	13	N.D.	N.D.
α-Tocopherol[c)]	100.0	9	N.D.	N.D.
Arbutin[d)]	N.D.	N.D.	6.5	1.4

a) Concentration 0.4% (W / W).
b) 50% Scavenging Concentration $x10^{-3}$ % (W / W).
c) Concentration 0.2 mM. d) Concentration 3.0 mM.
e) N.D. : No detected.



(6) (8) (17) (77) (79)

Fig. 3 Chemical Structures of Compound in *Yuzu* and *Lime* Essence Oils

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