Composition and antimicrobial activity of the essential oil and water extract from Japanese wild *Rosa rugosa*

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The essential oils and distilled-water extracts of *Rosa rugosa* flower, leaf, and fruit were analyzed by flame ionization detector-gas chromatography and mass spectrometer-gas chromatography. The principle components in the flower oil and water were citronellyl acetate, citronellol, and geranyl acetate, and citronellol and geraniol, respectively. For the leaf oil and water, it was γ -muurolene and geraniol and linalool, respectively. The main component in fruit water was terpinen-4-ol. Fruit oil, on the other hand, could not be extracted. Additionally, combining the *Rosa rugosa* distilled-water with one of the isoprenoids geraniol, farnesol, or citral showed higher antibacterial activity than the isoprenoid alone.

Key words: *Rosa rugosa*; hydrodistillation; essential oil; distilled water; antimicrobial activity;

1. INTRODUCTION

Roses have been a popular aesthetic since ancient times. Even today, their essential oils are regularly used for cosmetics, the most frequent being that from the extract of *Rosa damascene* [1], which is composed mostly of citronellol and geraniol [2].

R. rugosa, a native of northeastern Asia that includes the popular garden rose Pink Grootendorst, is also a popular species for this purpose [3, 4]. Because of its potential cosmetic and even pharmaceutical applications, the chemical components, composition, and phytochemistry of *R. rugosa* native to certain countries have been determined [5-9]. However, *R. rugosa* native to Japan is an exception. We therefore report here the chemical components of the essential oils and distilled water extracts from wild Japanese *R. rugosa*. Additionally, seeing that a number of studies have reported the antimicrobial activity of essential oil ingredients from a wide range of plants [10-16] and that we recently reported the antibacterial and antifungal activities of several isoprenoids [17], we investigated the antimicrobial activity of these components by conducting assays on the *rugosa* rose essential oil and distilled-water when combined with specific isoprenoids to identify potential synergistic effects, which may indicate cosmetic and/or pharmaceutical benefits.

2. EXPERIMENTAL

2.1 Plant materials

Wild *R. rugosa* flowers were collected in Ajigasawa, Aomori, Japan, on June, 2009. The flowers were hydrodistilled for 6 hours, resulting

in 0.145 g (0.0058% fresh distillate weight) and 0.64g (0.04%) of essential oils yielded from 2.5 kg of flower and 1.6kg of leaf, respectively.

2.2 Extraction of distilled-water

Distilled water extracts were extracted 4 times with hexane (300 ml hexane/L distilled water).

2.3 Analysis

Essential oils and distilled water extracts were analyzed by flame ionization detector-gas chromatography (GC-FID). The GC-FID analysis was performed using a Hitachi Gas Chromatograph G-5000A (Hitachi, Tokyo, Japan). A 60 ml/min nitrogen carrier flow rate was passed through a poly(alkylene glycol) column (30 m $\times$ 0.25 mm i.d.) using a 10 $^{\circ}$ C/min gradient that began with a 5 min hold at 40 $^{\circ}$ C and ended upon reaching 200 $^{\circ}$ C. Injector and detector temperatures were 150 $^{\circ}$ C and 250 $^{\circ}$ C, respectively. Mass spectrometer-gas chromatography (GC-MS) analysis was conducted on a JEOL Q1000GC-Mk II Mass Spectrometer, (Japan Electron Optics Laboratory Co. Ltd., Japan) that included an HP-5 column (30 m $\times$ ϕ 0.32 mm) using a 1 ml/min helium carrier flow rate. The temperature was programmed to deliver a 15 $^{\circ}$ C/min gradient that began with a 4.7 min hold at 50 $^{\circ}$ C and ended with a 2 min hold at 280 $^{\circ}$ C. Injector and GC-transfer line temperatures were both set to 200 $^{\circ}$ C.

Separated components were identified by comparing retention times and mass spectra with laboratory data and an NIST library (<http://www.nist.gov/srd/nist1a.cfm>). Accordance of the retention times in the GC-MS was

confirmed by co-injecting the reagent with an n-alkane ($C_7 - C_{40}$) retention index. The relative amounts of the components were calculated from their respective GC-MS peak areas.

2.4. Assay of antimicrobial activity

Antimicrobial assays were performed in accordance with the agar plate dilution method as recommended by the Japanese Society of Chemotherapy [18-20]. Bacteria were inoculated into 10 ml of Müller-Hinton Broth medium (MHB, Difco™, Becton Dickinson (BD), USA) and cultured at 37°C for 24 h without shaking. Each isoprenoid was dissolved in DMSO or a 5% aqueous solution of the surfactant HCO-50 (w/v) (Nikko Chemicals Company Ltd., Japan). Then, 120 µL of the dissolved isoprenoid solution was added to 12 ml Müller-Hinton Agar (MHA) culture medium. For combination assays, water obtained from the flower of rugosa rose by hydrodistillation was used instead of regular distilled water for the MHA culture medium. The optical density (OD) of bacterial cultures at 550 nm was determined using a Smart Spec™ 3000 (Bio-Rad Laboratories, USA) prior to performing colony counts. Cultures were adjusted to 1×10^6 colony-forming units (CFU)/ml, and colonies were counted after incubation at 37 °C for 20 h. Fungi were cultured in Sabouraud Dextrose agar medium (Eiken Chemical Co., Ltd., Japan) at 25-27 °C.

The Minimal Inhibitory Concentration (MIC) at which a molecule exerted antimicrobial activity was defined as less than 800 µg/mL, in accordance with the criterion set forth by the Japanese Society of Chemotherapy [20-22]. Media were sterilized using an STH 364 FA autoclave (Advantec Toyo Kaisha, Ltd.).

2.5. Bacterial strains

Gram-positive bacteria used were as follows: *Staphylococcus aureus* 209P, and *S. aureus* 834 (methicillin resistant - MRSA) Gram-negative strains were *E. coli* IFO-3806, and *Shigella sonnei*.

3. RESULTS AND DISCUSSION

Table 1 gives a summary of the components from essential oil and distilled water extracts from *R. rugosa* flower. We identified 27 components in the essential oil, the main being citronellyl acetate (12.1%), citronellol (7.7%) and geranyl acetate (5.5%). In the distilled water, 22 components were identified, with the main being citronellol (31.4%). Other sweet monoterpene alcohols like geraniol, nerol and linalool, had higher percentages in the floral water extract than in the essential oil. In contrast, acetates such as citronellyl acetate and geranyl acetate were proportionally less in the flower water extract than in the essential oil. Finally, the flower water extract saw a significant increase in the proportion of 2-phenylethanol, a popular ingredient used in fragrances. The increased alcohol percentages may imply pharmacological benefits, as we recently reported several

Table 1. Components of essential oil and distilled water extract from *R. rugosa* flower

No.	Components	EO (%)	DW ex. (%)	RT	RI	CI
1	β-myrcene	-	0.8	6:56	994	○
2	p-cymene	0.7	1.4	7:29	1027	○
3	limonene	1.6	3.7	7:34	1032	○
4	γ-terpinene	-	0.4	8:03	1063	-
5	acetophenone	-	0.6	8:10	1071	-
6	cis-linalool oxide	-	0.5	8:17	1078	-
7	linalool	0.5	9.0	8:38	1100	○
8	2-phenylethanol	0.9	8.1	8:50	1116	○
9	menthone	0.6	0.5	9:22	1158	○
10	terpinen-4-ol	-	0.7	9:40	1183	○
11	α-terpineol	-	5.2	9:49	1195	○
12	citronellol	7.7	31.4	10:13	1227	○
13	nerol	1.4	11.1			○
14	geraniol	1.5	13.4	10:30	1254	○
15	2-phenyl ethyl acetate	-	0.7	10:33	1260	-
16	citronellyl formate	1.4	-	10:42	1270	-
17	citronellyl acetate	12.1	0.8	11:29	1355	○
18	eugenol	-	4.1	11:34	1365	○
19	geranyl acetate	5.5	0.2	11:46	1385	○
20	methyl eugenol	0.5	2.3	11:58	1405	-
21	β-cedrene	0.9	-	12:08	1426	-
22	α-bergamotene	1.6	-	12:17	1443	-
23	epi-β-santalene	0.6	-	12:23	1454	-
24	γ-murolene	6.1	-	12:31	1469	-
25	α-curcumene	1.0	-	12:41	1489	-
26	2-tridecanone	6.0	-	12:45	1496	-
27	8-isopropyl-1,5-dimethyl cyclodeca-1,5-diene	5.5	-	12:53	1520	-
28	nerolidol	0.7	-	12:59	1524	-
29	caryophyllene oxide	0.3	-	13:32	1590	-
30	widdrol	-	0.4	13:46	1620	-
31	nerolidyl acetate	0.5	-	14:10	1672	-
32	β-bisabolol	0.5	0.1	14:21	1696	-
33	2-pentadecanone	1.4	-	14:23	1700	-
34	farnesol	1.0	0.2	14:35	1728	○
35	farnesal	0.4	-	14:44	1748	-
36	hexadecanol acetate	0.4	-	16:33	2008	-
-	n-alkane ($C_{21} \sim C_{27}$)	23.2	-	-	-	-
-	components less than 0.3%	0.6	0.6	-	-	-
Total		85.2	96.3			

Components less than 0.3%: α-pinene, α-terpinene, trans-rose oxide, neral, geraniol, hexadecanal

For tables 1-3: EO, yield (%) of essential oil

DWex, yeild (%) of distilled-water extract

RT, retention time

RI, retention index

CI: co-injection with authentic samples

Table 2. Components of essential oil and distilled water extract from *R. rugosa* leaf.

No.	Components	EO (%)	DW ex. (%)	RT	RI	CI
1	<i>trans</i> -linalool oxide	-	0.7	8:16	1077	-
2	<i>cis</i> -linalool oxide	-	0.5	8:30	1087	-
3	linalool	0.2	3.9	8:39	1101	○
4	nonanal	0.3	0.3	8:43	1107	-
5	terpinen-4-ol	tr	1.2	9:39	1181	○
6	2-methyl acetophenone	tr	0.3	9:44	1188	-
7	α -terpineol	tr	1.5	9:49	1195	○
8	citronellol	tr	0.4	10:13	1231	○
9	2-butanoic acid, hexyl ester	0.1	-	10:27	1253	-
10	geraniol	0.4	4.2	10:29	1256	○
11	piperitone	-	0.8	10:32	1260	-
12	geranial	-	0.5	10:41	1275	○
13	geranyl formate	tr	0.5	10:59	1303	-
14	4-hydroxy-3-methyl acetophenone	-	1.3	11:05	1313	-
15	4-methylene-2,8,8-trimethyl-2-vinyl-bicyclo[5.2.0]nonane	1.0	-	11:44	1382	-
16	β -cedrene	1.7	-	12:08	1426	-
17	α -cedrene	0.7	-	12:12	1434	-
18	bergamotene	1.2	-	12:16	1442	-
19	elemene	0.9	-	12:19	1447	-
20	α -amorphene	3.7	-	12:23	1455	-
21	γ -muurolene	10.4	-	12:31	1470	-
22	α -curcumene	1.2	-	12:41	1489	-
23	spathulenol	0.3	-	13:24	1574	-
24	caryophyllene oxide	0.8	-	13:32	1590	-
25	aromadendrene oxide	2.7	3.5	14:06	1663	-
26	α -bisabolol oxide	-	1.0	14:09	1670	-
27	Isolongifolol, methyl ether	-	0.8	14:11	1672	-
28	β -eudesmol	0.3	-	14:15	1683	-
29	α -bisabolol	1.6	-	14:20	1685	-
30	2,2,7,7-tetramethyl tricycle [6.2.1.0(1,6)]undec-4-en-3-one	3.1	0.9	14:27	1709	-
31	eudesma-5,11(13)-dien-8,12-olide	1.4	-	15:00	1784	-
32	isophytol	0.3	-	16:09	1948	-
33	parthenium	0.8	-	16:11	1960	-
34	phytol	1.9	-	17:13	2114	-
-	n-alkane (C ₂₃ ~C ₂₇)	1.5	-	-	-	-
-	components less than 0.3%	0.9	1.5	-	-	-
Total		37.2	23.6			

Components less than 0.3%: 1-octen-3-ol, β -myrcene, *p*-cymene, limonene, benzyl alcohol, *trans*-ocimene, benzenacetaldehyde, ocimene (isomer), 2-phenylethanol, methoxy citronellol, 2-nonenal, menthone, nonyl alcohol, decanal, neral, 2-butanoic acid, hexyl ether, nonanal dimethyl acetal, undecanal, 1,2-dihydro-1,1,6-trimethy naphthalene, eugenol, β -damascenone, 2,5-diisopropyl phenol, calamenene

Table 3. Components of essential oil and distilled water extract from *R. rugosa* fruit.

No.	Components	DW ex. (%)	RT	RI	CI
1	β -myrcene	1.5	6:52	992	○
2	limonene	0.5	7:31	1029	○
3	<i>trans</i> -ocimene	0.4	7:42	1041	-
4	ocimene	0.6	7:52	1052	-
5	<i>trans</i> -linalool oxide	0.4	8:14	1075	-
6	<i>cis</i> -linalool oxide	0.4	8:28	1089	-
7	linalool	15.6	8:37	1099	○
8	nonanal	0.8	8:41	1104	-
9	1-octen-3-yl-acetate	0.8	8:48	1113	-
10	borneol	1.0	9:30	1169	○
11	terpinen-4-ol	19.1	9:37	1179	○
12	α -terpineol	6.4	9:47	1192	○
13	citronellol	0.4	10:12	1230	○
14	nerol	0.9			○
15	geraniol	2.9	10:29	1256	○
16	linalyl acetate	3.4			○
17	lavandulyl acetate	6.6	10:51	1290	-
18	carvacrol	0.4	10:58	1302	-
19	neryl acetate	1.7	11:34	1365	○
20	geranyl acetate	3.2	11:45	1384	○
21	β -caryophyllene	5.1	12:10	1430	○
22	α -bergamotene	0.3	12:16	1442	-
23	β -farnesene	4.1	12:25	1458	-
24	γ -cadinene	1.7	12:59	1524	-
25	calamenene	0.3	13:03	1532	-
26	caryophyllene oxide I	0.3	13:20	1566	-
27	caryophyllene oxide II	3.5	13:36	1598	-
28	cubenol	0.3	13:50	1628	-
29	τ -cadinol	4.2	14:01	1652	-
30	<i>cis</i> -Z- α -bisabolene epoxide	0.7	14:10	1672	-
31	longipinocarvone	0.4	14:49	1759	-
32	6,10,14-trimethyl-2-pentadecanone	0.8	15:27	1847	-
-	n-alkane (C ₂₁ , C ₂₃ , C ₂₅ , C ₂₇)	1.4	-	-	-
-	components less than 0.3%	1.9	-	-	-
Total		92.3			

Components less than 0.3%: ethyl tiglate, *p*-cymene, γ -terpinene, camphor, nonyl alcohol, *p*-cymen-8-ol, carvenone, geranial, undecanal, α -caryophyllene, γ -muurolene, elemophilene, β -bisabolene, γ -eudesmol, cadalene

monoterpene alcohols such as geraniol have antimicrobial activity even at low concentrations[17].

Furthermore, geraniol, linalool and citronellol have a tendency to emit more sweet fragrance than their acetate compounds, suggesting the flower water may be more useful for cosmetic applications.

For the essential oil of leaf (Table 2), we identified 22 components, the main being γ -muurolene (10.4%). The leaf oil also contained geraniol (0.4%), but at a proportion significantly less than the amount in flower oil (1.5%). Rose like fragrance compounds such as geraniol and citronellol were lower than flower's, and it emitted grassy smell. We found 17 components in the water extract, the most prominent being geraniol (4.2%) and linalool (3.9%).

Furthermore, similar to *R. rugosa* flower, the proportional amounts of geraniol and linalool in the leaf water extract were greater than that in the essential oil.

The essential oil from *R. rugosa* fruit could not be extracted. Table 3 shows the 32 components identified from the fruit water extract, the main being terpinen-4-ol (19.1%) and linalool (15.6%). Additionally, the fruit water was found to contain several hydrophobic alkenes such as β -caryophyllene (5.1%) and β -farnesene (4.1%).

Upon identifying the main components of *R. rugosa*, we next considered potential pharmacological effects. On its own, we found the distilled-water from *R. rugosa* flower showed no antimicrobial activity against either Gram positive or Gram negative bacteria.

Table 4. Geraniol antimicrobial activity against bacteria when distilled-water from *R. rugosa* flower was used in the MHA culture medium.

geraniol ($\mu\text{g/ml}$) (+distilled water of <i>R. rugosa</i>)	Gram-positive bacteria		Gram-negative bacteria	
	<i>S. aureus</i>	MRSA	<i>E. coli</i>	<i>S. sonnei</i>
467	+	+	+	+
445	+	+	+	+
423	+	+	+	+
400	—	—	—	—
378	—	—	—	—

Table 5. Antimicrobial activity of farnesol when combined with distilled-water from *R. rugosa* flower for the MHA culture medium

farnesol ($\mu\text{g/ml}$) (+distilled water of <i>R. rugosa</i>)	Gram-positive bacteria		Gram-negative bacteria	
	<i>S. aureus</i>	MRSA	<i>E. coli</i>	<i>S. sonnei</i>
155	+	+	—	—
133	+	+	—	—
111	+	+	—	—
89	—	—	—	—
67	—	—	—	—

Table 6. Antimicrobial activity of citral when combined with distilled-water from *R. rugosa* flower for the MHA culture medium

citral ($\mu\text{g/ml}$) (+distilled water of <i>R. rugosa</i>)	Gram-positive bacteria		Gram-negative bacteria	
	<i>S. aureus</i>	MRSA	<i>E. coli</i>	<i>S. sonnei</i>
489	+	+	+	—
444	+	+	+	—
400	+	+	+	—
356	+	+	+	—
289	+	+	+	—
267	—	—	—	—
245	—	—	—	—
222	—	—	—	—

However, we have previously reported the antimicrobial activity of isoprenoids[17].

Therefore, to identify any synergistic effects, we conducted antibacterial assays by combining the distilled-water of *R. rugosa* flower and each of the three isoprenoids. This caused the MIC of geraniol in response to *S. aureus* and MRSA to decrease from 534 $\mu\text{g/ml}$ to 423 $\mu\text{g/ml}$ and in response to *E. coli* or *S. sonnei* to decrease from 445 $\mu\text{g/ml}$ to 423 $\mu\text{g/ml}$, indicating a stronger drug effect (Table 4). Similarly, the MIC for farnesol decreased from 184 $\mu\text{g/ml}$ to 111 $\mu\text{g/ml}$ against *S. aureus* or MRSA (Table 5). However, no change in the MIC was seen for farnesol against Gram negative bacteria when mixed with *R. rugosa* flower distilled water. This was also true for citral, where the MIC improved from 356 $\mu\text{g/ml}$ to 289 $\mu\text{g/ml}$ against Gram positive bacteria, but did not change against Gram negative bacteria like *E. coli*. However, no change in the MIC was seen for farnesol against Gram negative bacteria when mixed with *R. rugosa* flower distilled water. This was also true for citral, where the MIC improved from 356 $\mu\text{g/ml}$ to 289 $\mu\text{g/ml}$ against Gram positive bacteria, but did not change against Gram negative bacteria like *E. coli* (Table 6).

Therefore, we show that isoprenoids have a more potent drug effect when mixed with the flower water of *R. rugosa*. This is consistent with previous reports that have shown the oil of other plants produce a synergistic effect too[23-26].

4. CONCLUSIONS

We analyzed the components of *R. rugosa*, finding the principal components of the flower essential oil to be citronellyl acetate, citronellol, and geranyl acetate, while the principal components of the flower distilled water were citronellol and geraniol. The principal component of leaf essential oil was γ -muurolene while that of the leaf water was geraniol and linalool. While the essential oil of fruit could not be extracted, the principal component of fruit water was found to be terpinen-4-ol. More importantly, we found

synergistic effects when combining isoprenoids such as geraniol, farnesol, and citral with the distilled water of *R. rugosa* flower, suggesting potential drug applications.

5. ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Cosmetology Research Foundation (M.N.) and the municipal government of Ajigasawa (M. N.).

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(Received July 6, 2011; Accepted August 31, 2011)