Volatile Components of Essential Oil from Cultivated *Myrica gale* var. *tomentosa* and Its Antioxidant and Antimicrobial Activities

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Abstract: Aromatic components in the essential oil prepared from the leaves of cultivated *Myrica gale* var. *tomentosa* were compared with those from oil derived wild plants by using gas chromatography-mass spectroscopy (GC/MS). We found that essential oils from both the wild and cultivated plants contained similar aromatic components such as β -elemenone, selina 3,7(11)-diene, myrcene, limonene, cymene, 1,8-cineole, and β -pinene, but the content ratio of the oil was significantly different, which might yield differences in the aromatic properties. The aroma impact components of the essential oils were also determined using GC/MS-Olfactometry (GC/MS-O) and aroma extract dilution analysis. Eight aromatic compounds, including linalool, limonene, and 1,8-cineole, were shown to contribute to the aromatic properties of cultivated *M. gale* var. *tomentosa*. The strongest aromatic note was defined as linalool, followed by limonene, 1,8-cineole, and β -elemenone. The essential oil, ethanol (EtOH), 1,3-butylene glycol (BG), and 1,3-propanediol (PD) extracts prepared from the leaves of cultivated *M. gale* var. *tomentosa* also showed antioxidant and antimicrobial activities, that is, they demonstrated scavenger activity against hydroxyl and superoxide radicals in the aqueous phase, and showed inhibitory effects on lipid peroxidation. The essential oil extracts also exhibited antimicrobial activity against gram-positive bacteria, with the lowest minimum inhibitory concentration value against *Bacillus subtilis*. In conclusion, the essential oil and solvent extracts from cultivated *M. gale* var. *tomentosa* have a potential for utilization as food and cosmetic ingredients.

Key words: aroma-impact compound, antioxidant, antimicrobial, essential oil, *Myrica gale* var. *tomentosa*

1 INTRODUCTION

Myrica gale and other members of the Myricaceae plant family are aromatic shrubs, and are widely distributed in the northern hemisphere. *Myrica gale* grows about 1 m high with oblanceolate green leaves, dentate at the top and marked with resinous points. The fruit is a waxy resinous winged drupe. The plant is used in traditional medicine in Northern Europe and North America¹. The dried leaves of *M. gale* are also used as a flavoring agent for beer, and a perfuming agent for linen. Moreover, dried leaves and fruits are used as a spice for soups and stews². However, the commercial applications of *M. gale* have not spread to Northern Europe and America, because of the lack of available resources. In Asia, *M. gale* var. *tomentosa* grows in the wild, in the marshes in Japan, especially in Hokkaido, the northern island of Japan. Commercial use of *M. gale*

var. *tomentosa* in Japan has also not been available.

Recently, we developed a mass propagation system of *M. gale* var. *tomentosa* by using a shoot tip culture technique. This tissue culture system is ideal for propagating plants with genetic homogeneity.

Using our method, 2 hundred million seedlings can be obtained from one seedling in one year³. Therefore, *M. gale* var. *tomentosa* is expected to become a new resource owing to its aromatic and medicinal characteristics.

The essential oils of flowers and leaves from *M. gale*, and other plants of the same family, have been collected in Europe and Northern America, and characterized^{2,4-8}. Anticancer, insect-repelling, and antifungal activities of the essential oils of these plants have been reported^{6,8,9}.

In the present paper, we compared the volatile components in the leaves of cultivated *M. gale* var. *tomentosa*

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with those of wild plant by using gas chromatography-mass spectrometry (GC/MS). We also compared the characteristic aromatic compounds (aroma-impact compounds) in the essential oils of cultivated *M. gale* var. *tomentosa* with wild-type plants by GC-Olfactometry (GC-O). Moreover, antioxidant and antimicrobial activities of essential oil and plant extracts were conducted to investigate their potential for use as natural food additives and/or cosmetic resources.

2 Materials and methods

2.1 Chemicals and solvent

2,2-Diphenyl-1-picrylhydrazyl were purchased from Sigma-Aldrich (St. Louis, USA). Methanol (MeOH), dimethyl sulfoxide (DMSO), disodium hydrogen phosphate, sodium dihydrogenphosphate anhydrous, tert-butyl hydroxyanisole (BHT), linalool and chloroform were obtained from Kanto Chemical Co (Tokyo, Japan). 1,3-Propanediol was from DuPont Tate & Lyle Bio Products (Tokyo, Japan), and 1,3-butylene glycol was from Kokyu Alcohol Kogyo Co (Chiba, Japan). SOD assay kit-WST was from Dojindo Laboratories (Kumamoto, Japan). β -carotene, polyoxyethylene (20) sorbitan monopalmitate, linoleic acid, cymene, nerolidol, and terpinene-4-ol were from Wako Pure Chemical Industries (Tokyo, Japan). α -Pinene, β -pinene, and limonene were from Tokyo Chemical Industry Co (Tokyo, Japan). 1,8-Cineole and isoamyl isovalerate were from Soda Aromatic Co. (Tokyo, Japan).

2.2 Plant materials

Wild *M. gale* var. *tomentosa* plants were collected in 2009 in Tomakomai, Hokkaido, Japan. Apical buds (ca. 1.0 mm) of the wild plants were immersed in 70% ethanol for 30 s and washed with sterilized water. They were further disinfected in 1% sodium hypochlorite solution containing 0.1% Tween 20 for 10 min, before being washed 3 times with sterilized water. Shoot tips cut from buds that were approximately 0.5 cm long were cultured on Woody Plant solid medium¹⁰⁾ supplemented with 0.6 mg/l of 6-benzylaminopurine at 25°C with 16 h of continuous light for 1 month. The shoots were transplanted into the Woody Plant solid medium supplemented with 0.75 mg/l of 1-naphthylacetic acid for root initiation. The same culture conditions were used for the shoot-tip culture. The explants obtained via shoot-tip culture were transplanted into pots containing a mixture of vermiculite and perlite (1:1, w/w) and were acclimatized for 3 months in a green house. The plants were then transplanted to fields at the Forestry Research Institute, Hokkaido, Japan, for 3 years³⁾. Leaves collected from these plants represent cultivated *M. gale* var. *tomentosa*. Leaves collected from wild and cultivated plants were immediately dried in a drying machine at 40°C for 3 days.

2.3 Preparation of essential oil

Essential oils and aroma water were obtained from dried leaves (100 g) by hydrodistillation with 2,000 ml of distilled water for 1 h.

2.4 Solvent extraction

Dried leaves (100 g) were extracted with 500 ml ethanol (EtOH), 1,3-butylene glycol (BG) or 1,3-propanediol (PD) (w/v) at room temperature for 5 days, followed by filtering through filter paper (No. 2, Advantec, Tokyo, Japan). The filtrates were used as the BG and PD extracts, respectively.

2.5 Gas chromatography-mass spectrometry

An Agilent 7890A gas chromatograph equipped with a 5975C mass spectrometer was used to analyze volatile compounds. A DB-WAX column (30 m $\times$ 0.25 mm i.d., 0.25 film thickness; J&W Scientific) was used for the analysis. The oven temperature was held at 60°C for 0.5 min and then increased to 250°C at the rate of 3°C/min. The injector and ion source temperature were set at 250°C and 230°C, respectively. Helium was used as the carrier gas at a flow rate of 1.00 ml/min with a split ratio of 30:1 for a 1- μ l sample injection of oil. Electron impact (EI) spectra were recorded at 70 eV, in an m/z range of 30-400. The compounds were identified by their GC retention indices that were calculated from the retention time in relation to those of a series of C₈-C₃₀ *n*-alkanes on a 30-m DB-Wax capillary column and by computer matching using AromaOffice 2D (Gestel, Nishikawa). The identification of the compounds was further confirmed by comparison of their fragmentation patterns with those in the literature, and by co-injection with authentic compounds, when possible.

2.6 Gas chromatography-mass spectrometry/olfactometry

GC/MS-O was performed using an Agilent 7890A-5975C mass spectrometer equipped with an olfactory detection port (ODP2; Gestel). The analytical conditions were the same as those used for the GC/MS analysis.

2.7 Aroma extract dilution analysis

The characteristics of aromatic compounds in the essential oil were determined by aroma extraction dilution analysis (AEDA)¹¹⁾. Essential oil from dried leaves of cultivated plants was diluted at 30% (v/v) with *n*-hexane and subsequently diluted two-fold. The aromatic compounds were separated by GC/MS-O on a DB-WAX capillary column and were sniffed by 3 panelists. The strength of the compounds was expressed by using flavor dilution (FD) factors. FD factors of the characteristic aromatic compounds were determined by the highest dilution factors at which their odors were detectable.

2.8 DPPH radical scavenging assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured using the spectrophotometric method¹²⁾ with slight modifications. A freshly prepared solution of 100 mM DPPH in MeOH was used. Sample solutions (100 μ l) at concentrations of 5–400 mg/ml and 400 μ l of 100 mM Tris HCl buffer (pH 7.4) were mixed with 100 mM DPPH solution (500 μ l). The mixture was shaken well and then incubated in the dark for 20 min at 30°C after which the absorbance at 517 nm was measured.

The anti-oxidative activity of the sample was expressed as inhibition of the DPPH radical quenching calculated using the following equation:

$$\text{Inhibition (\%)} = [(A_{517(\text{control})} - A_{517(\text{sample})}) / A_{517(\text{control})}] \times 100$$

The IC₅₀ value was calculated from an inhibition curve. The assays were carried out in triplicate. Gallic acid and ascorbic acid were used as positive controls.

2.9 Superoxide dismutase-like activity assay

The superoxide dismutase (SOD)-like activity was determined using a SOD Assay Kit-WST (Dojindo Laboratories, Kumamoto, Japan) following the manufacturer's instructions. Briefly, samples were mixed with xanthine oxidase and tetrazolium salt in 96-well microplates for 20 min at 37°C. Thereafter, the absorbance was measured at 440 nm by using a microplate reader. The percentage of inhibition was calculated as follows:

$$\text{Inhibition (\%)} = [(A_{440(\text{control})} - A_{440(\text{sample})}) / A_{440(\text{control})}] \times 100$$

The IC₅₀ value was calculated from an inhibition curve. Assays were carried out in triplicate, and gallic acid and ascorbic acid were used as positive controls.

2.10 β -Carotene bleaching assay

β -Carotene (10 mg) was dissolved in 10 ml of chloroform. An aliquot (0.2 ml) of this solution was added into a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. Chloroform was removed under reduced pressure at 40°C for 5 min, and water (50 ml) was added to the residue with vigorous agitation in order to form an emulsion. The emulsion (5 ml) was added to a tube containing 0.2 ml of the essential oil, aroma water, and extract solutions. Absorbance at 470 nm was measured immediately after mixing, and the test emulsion was incubated at 50°C for 120 min. Absorbance at 470 nm of the test emulsion was measured every 15 min. BHT was used as the positive control. The antioxidant activities of the samples were evaluated using the following formula:

$$\text{Inhibition (\%)} = (A_t - C_t) / (C_0 - C_t) \times 100$$

where A_t and C_t are the final (120 min) absorbance of the test sample and control, respectively, and C_0 is the absorbance for the control.

2.11 Determination of total phenolic content

Total phenolic contents were determined using the Folin-Ciocalteu method as described by Singleton *et al.*¹³⁾, using gallic acid as a standard. Sample solutions were prepared by dissolving the sample with MeOH at a concentration of 1.0 mg/ml. The solutions (100 μ l) were then mixed with Folin-Ciocalteu reagent (200 μ l) and incubated for 30 min at room temperature. After addition of 1N NaOH (500 μ l), absorbance at 750 nm was measured. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of the sample. These assays were also carried out in triplicate.

2.12 Antimicrobial activity

2.12.1 Antimicrobial screening

Anti-microbial activities against *Staphylococcus aureus* NRIC 1135, *Escherichia coli* NRIC 1999, *Saccharomyces cerevisiae* NRIC 1410, *Bacillus subtilis* JCM 1465, and *Candida albicans* JCM 2085 were tested using the paper disc diffusion assay. *S. aureus*, *E. coli* and *B. subtilis* were grown on Nutrient Broth medium and *S. cerevisiae* and *C. albicans* were grown on yeast peptone dextrose (YPD) medium for 24 h to yield a final concentration of 10^6 – 10^7 CFU/ml. The microbial suspension (0.1 ml) was spread onto Nutrient Agar (*S. aureus*, *E. coli*, and *B. subtilis*) or YPD Agar (*S. cerevisiae* and *C. albicans*) plates, respectively. Sterile filter paper discs (Advantec, 1.6 mm in diameter) were placed on the surface of the microbial agar plates. The essential oils and solvent extracts (10 mg/ml) were dissolved in 50% DMSO and were applied onto the paper discs. Kanamycin (100 μ g/ml; Wako Pure Chemical Industries, Japan) and/or aureobasidin A (3.0 μ g/ml; Takara Bio Inc., Japan) were used as positive controls against bacterial strains and yeast strain, respectively. The solvent was used as a negative control. The plates were incubated at 25°C (*C. albicans*), 30°C (*S. cerevisiae* and *B. subtilis*), and 37°C (*S. aureus* and *E. coli*) for 24 h. The diameter (width) of the inhibitory zone was measured after 24 h of incubation.

2.12.2 Determination of minimum inhibitory concentrations

Micro-well dilution broth susceptibility assays were used to determine the minimum inhibitory concentrations (MICs). Stock solutions of the essential oil were prepared in 50% DMSO (100 μ g/ml) and then serially diluted to make concentrations ranging from 125,000 to 61 ppm. Briefly, 96-well plates were prepared by dispensing 180 μ l of the diluted inocula into each well. Aliquots of the stock solution of essential oil (20 μ l) were added to the first series of wells. Each serial dilution (100 μ l) was then dispensed into 6 consecutive wells. The final volume in each well was 100 μ l. The contents of each well were mixed on a plate shaker at 300 rpm for 20 s, and then incubated at the appropriate temperature for 24 h. The MIC value was defined as the

lowest concentration of the essential oil to inhibit the growth of microorganisms.

3 RESULTS AND DISCUSSION

3.1 Aromatic composition of wild and cultivated *M. gale* var. *tomentosa*

The components of the essential oils prepared from the

leaves of wild *M. gale* var. *tomentosa* collected in Hokkaido, Japan, are shown in **Table 1**. According to the area percentage of the peaks in the chromatogram, mono- and sesquiterpenes were major components of the essential oil, and the content of these terpenes was more than 75%. The major compounds found in the oil from wild leaves were β -elemenone, selina-3,7(11)-diene, cymene, limonene, 1,8-cineole, and β -pinene (13.3%, 11.0%, 10.6%, 9.8%, 7.7%, and 5.0%, respectively). γ -elemene, α -terpinyl

Table 1 Comparisons of chemical composition of the leaf essential oils from wild and cultivated *M. gale* var. *tomentosa*.

Components	RI	GC peak area (%)	
		Wild	Cultivated
α -pinene	1023	1.92	3.84
β -pinene	1114	4.97	5.83
α -phellandrene	1169	1.25	0.12
α -terpinene	1183	0.61	—
limonene	1206	9.81	10.96
cineole	1219	7.73	15.94
γ -terpinene	1249	0.46	—
cymene	1276	10.6	13.12
isoamyl isovalerate	1299	0.42	0.57
ylangene	1490	0.22	—
α -cubebene	1499	0.22	—
benzaldehyde	1527	—	0.33
linalool	1549	0.97	1.45
β -elemene	1596	0.55	0.39
caryophyllene	1606	0.42	0.16
terpinen-4-ol	1610	0.80	1.56
β -guaiene	1614	0.59	—
2,6-dimethyl-1,3,5,7-octatetraene	1626	—	0.18
γ -elemene	1644	1.85	0.85
γ -muurolene	1696	0.69	0.54
α -terpinyl acetate	1703	2.68	—
β -selinene	1730	—	0.95
α -selinene	1734	1.21	0.60
eremophilene	1744	1.74	0.78
lavandulol,acetate	1761	—	1.67
nerol	1762	2.72	—
selina-3,7(11)-dien	1791	11.00	3.73
calamenene	1841	—	0.23
benzyl isovalerate	1898	2.16	1.17
nerolidol	2046	1.73	1.78
β -elemenone	2104	13.27	16.12
3,7-dimethyl-10-(1-methylethyidene), 3,7-cyclodecadien-1-one	2177	1.14	1.49
unidentified		20.16	14.51
Total		99.73	99.06

^a Retention indices (RI) were calculated from retention times in relation to those of a series of C8-C30 n-alkanes on a polar DB-WAX column.

^b Not detected.

acetate, α -pinene, nerol, benzyl isovalerate, and nerolidol were also identified. Among the sesquiterpenes, gerumacran and eleman were the most dominant monocyclic sesquiterpenes, whereas caryophyllene, guajan, cadinan, eudesman, and eremophilan were the most dominant type of bicyclic sesquiterpenes. The levels of eleman and eudesman were particularly higher. The components, for which standard samples were available, were quantitatively analyzed by GC/MS, and the results are shown in **Table 2**. No difference was observed in the contents of the components between wild and cultivated samples.

There have been many reports on the volatile oil composition of sweet gale (*M. gale*) obtained from different countries^{2, 4-8}. The major components of Canadian sweet gale were myrcene, limonene, α -phellandrene, and β -caryophyllene⁸. α -Pinene and 1,8-cineole were the major components in the oils collected from Scotland, Finland, and the Netherlands, whereas the contents of myrcene, γ -terpinene, and *p*-cymene were very low. These results suggest that the composition of volatile components in the essential oil of Japanese *M. gale* var. *tomentosa* is different from those in other countries.

It is well known that the composition and the amount of plant volatile compounds varies depending on climatic, geographic, and growing conditions¹⁴. Therefore, the essential oil compositions of wild and cultivated plants propagated via shoot tip cultures were compared. The compositions of major compounds of cultivated plants were quite similar to those of wild plants, but the composition ratio of limonene and 1,8-cineole were different (**Table 1**). In wild *M. gale* var. *tomentosa* plants, the limonene content (9.81%) was higher than 1,8-cineole (7.73%). In contrast, the contents of cultivated plants was two-fold higher than that of wild plants. In addition, β -selinene, benzaldehyde, and calamenene were identified only in the essential oil derived from cultivated plants, whereas γ -terpinene, ylangene, α -cubebene, and nerol were not.

Table 2 The contents of the components of essential oil from cultivated *M. gale* var. *tomentosa* by GC/MS.

Components	Wild (mg/g)	Cultivated (mg/g)
α -pinene	0.22	0.48
β -pinene	0.03	0.04
limonene	1.07	1.33
cineole	0.18	0.42
cymene	1.19	1.63
isoamyl isovalerate	0.56	0.85
linalool	0.44	0.73
terpinen-4-ol	1.66	3.60
nerolidol	0.79	0.90

These results suggest that the composition of volatile components in cultivated *M. gale* var. *tomentosa* is similar to that of wild plants, even though their aromatic properties may differ from each other.

3.2 Determination of aroma-impact compounds of cultivated *M. gale* var. *tomentosa*

The aroma-impact compounds from the essential oil of cultivated *M. gale* var. *tomentosa* was then investigated by GC/MS-O analysis and AEDA. Eight aroma-impact compounds were identified from the essential oil (**Fig. 1** and **Table 3**). The strongest aromatic note was given by linalool (FD 128); this was followed by limonene (64), 1,8-cineole (64), and β -elemenone (64). Among these compounds, β -elemenone had a unique aromatic note that invoked medicines, which suggests that it plays an important role in the characteristic aroma of cultivated *M. gale* var. *tomentosa*.

3.3 Antioxidant activity of cultivated *M. gale* var. *tomentosa*

The antioxidant activities of the essential oil and solvent extracts from the leaves of cultivated *M. gale* var. *tomentosa* were evaluated using DPPH assays, SOD like activity assays, and β -carotene bleaching assays.

The extracts of EtOH, BG, and PD showed DPPH radical scavenging activity, with IC₅₀ values of 134.0 $\pm$ 2.7 μ g/ml, 17.2 $\pm$ 0.1 μ l/ml, and 13.5 $\pm$ 0.1 μ l/ml, respectively (**Table 4**). SOD-like activities of these extracts showed similar tendency to DPPH radical scavenging activity. The IC₅₀ values were 171.9 $\pm$ 2.2 μ g/ml, 36.3 $\pm$ 3.8 μ l/ml, and 22.6 $\pm$ 1.3 μ l/ml, respectively. These results indicate that the EtOH, BG, and PD extracts of the leaves of *M. gale* var. *tomentosa* can scavenge the hydroxyl and superoxide radicals in the aqueous phase. However, little antioxidant activity was shown in the essential oils.

The β -carotene bleaching inhibitory activities of the EtOH, BG, and PD extracts are summarized in **Table 4**. The percentage of β -carotene bleaching inhibitory activities of the EtOH, BG, and PD extracts were 58.1% $\pm$ 2.4%, 85.8% $\pm$ 4.8%, and 72.05 $\pm$ 3.2%, respectively. These results revealed that EtOH, BG, and PD extracts from *M. gale* var. *tomentosa* leaves have not only the hydroxyl and superoxide radicals scavenging activities but also inhibitory effects of lipid peroxidation.

Total phenolic content of the EtOH, BG, and PD extracts were 2.4 $\pm$ 0.1 mg/g, 11.8 $\pm$ 0.8 μ g/ml, and 18.8 $\pm$ 2.6 μ g/ml, respectively (**Table 4**). Phenolic compounds are known to have antioxidant effects on plants; therefore, phenolics must be responsible for the antioxidant activity of these extracts.

3.4 Antimicrobial activity of cultivated *M. gale* var. *tomentosa*

The antimicrobial activities of essential oil and solvent

Fig. 1 GC/MS chromatograms (A) and the results of AEDA analysis (B) of the essential oil from cultivated *M. gale* var. *tomentosa* leaves.

Table 3 Aroma impact components of the essential oil from cultivated *M. gale* var. *tomentosa*.

Components	RI	Aroma quality	FD-factor
β -pinene	1114	green	1
limonene	1206	citrus	32
cineole	1219	light, woody	32
cymene	1276	citrus	1
linalool	1549	citrus, floral, light	64
terpinen-4-ol	1610	floral	2
β -selinene	1730	green cut grass	16
β -elemenone	2107	medicine like	32

^a Odor quality perceived at the sniffing port. Flavour dilution factor obtained by AEDA.

extracts prepared from leaves of cultivated *M. gale* var. *tomentosa* against *Staphylococcus aureus*, *Escherichia coli*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, and *Candida albicans* are shown in **Table 5**. The essential oil showed antibacterial activity against *S. aureus*, *B. subtilis*, *S. cerevisiae*, and *C. albicans*, exhibiting a 3.0-mm inhibition zone in the paper disc assay (data not shown). EtOH, BG, and PD extracts showed no inhibition. The MIC against bacteria exerted by the essential oil was measured using a liquid medium assay. The MIC value against *B. subtilis* was the lowest (61 ppm), whereas other bacteria had values over 1,000 ppm. From these results, we concluded that the

essential oil had antibacterial activities against *S. aureus*, *B. subtilis*, *S. cerevisiae*, and *C. albicans*, and in particular, strong activity was observed against *B. subtilis*.

There have been many reports on the antimicrobial properties of plant volatile oils and their constituents^{15, 16}. Dorman & Deans¹⁷ summarized the antibacterial activity of volatile components of plants, and a wide activity spectrum was observed by thymol, carvacrol, α -terpineol, terpinen-4-ol, eugenol, linalool, thujone, δ -3-carene, cis-hex-3-en-1-ol, geranyl acetate, citral, nerol, geraniol, menthone, β -pinene, α -terpinene, borneol, sadinene, γ -terpinene, 1,8-cineole, α -phellandrene and cymene. It has been suggested that the antimicrobial activity of cultivated *M. gale* var. *tomentosa* is attributed to the complex effects of components of its essential oil such as β -pinene, 1,8-cineole, linalool, and terpinen-4-ol. The EtOH, BG and PD extracts showed no inhibitory activity against the 4 bacterial strains although these extracts contained monoterpenes such as β -pinene, α -terpineol, terpinen-4-ol, γ -terpinene, and cymene (data not shown). Further studies are needed to clarify this phenomenon.

4 CONCLUSION

The comparison of aromatic components from the leaves of wild and cultivated *M. gale* var. *tomentosa* indicated that although there was no significant difference in the components, their content ratio was significantly different.

Table 4 Antioxidant activities of extracts from leaves of cultivated *M. gale* var. *tomentosa*.

Extract	DPPH IC ₅₀ (μl/ml)	SOD IC ₅₀ (μl/ml)	Inhibition of lipid peroxidation (%)	Phenol (μg/ml)
Oil	>1000	>1000	—	—
BG	17.2 ± 0.1	36.3 ± 3.8	85.8 ± 4.8	11.8 ± 0.8
1,3-PD	13.5 ± 0.1	22.6 ± 1.3	72.0 ± 3.2	18.8 ± 2.6

Data represent the mean ± S.E. of three separate experiments.

^a Oil : Essential oil, BG : 1,3-butylen glycol, PD : 1,3-propanediol.

^b Not tested.

^c Data represent the μg/ml.

^d Date represent the mg/g..

Table 5 Antimicrobial activities of extracts from leaves of cultivated *M. gale* var. *tomentosa*.

Extract	MIC (ppm)				
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. cerevisiae</i>	<i>C. albicans</i>
Oil	>1500	—	61	>1500	>1500
1,3-BG	—	—	—	—	—
1,3-PD	—	—	—	—	—
Kanamycin	25	50	50	—	—
Aureobasidin A	—	—	—	16	16

^a Oil : Essential oil, BG : 1,3-butylen glycol, PD : 1,3-propanediol.

^b MIC : minimal inhibitory concentration, *S. aureus* : *Staphylococcus aureus*, *E. coli* : *Escherichia coli*, *B. subtilis* : *Bacillus subtilis*, *S. cerevisiae* : *Saccharomyces cerevisiae*, *C. albicans* : *Candida albicans*.

^c No inhibition.

^d Kanamycin was used for positive control of *S. aureus*, *E. coli* and *B. subtilis*.

^e Aureobasidin A was used for *S. cerevisiae* and *C. albicans*.

This is the first study to examine the aromatic components in the essential oil of cultivated *M. gale* plant. The aroma-impact components of the essential oil were also surveyed, and 8 compounds, including linalool, limonene, and 1,8-cineole, were shown to contribute to the aromatic properties in cultivated *M. gale* var. *tomentosa*. Moreover, the essential oil and extracts of leaves showed scavenging activities against hydroxyl and superoxide radicals as well as inhibitory effects on lipid peroxidation. The essential oil also showed antimicrobial activities against *B. subtilis*.

We have already developed a mass propagation system for *M. gale* var. *tomentosa* using a tissue culture technique. The results of this study provide further evidence that the essential oil and extracts of leaves from cultivated *M. gale* var. *tomentosa* have aromatic and medicinal characteristics and may have a potential use as food or cosmetic ingredients.

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