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ORIGINAL ARTICLE

Antibacterial Activity of Naphthalin and Its Derivatives against Oral Bacteria

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Abstract Naphthalin derived from the essential oil of *Magnolia liliflora* was tested for antibacterial activity against oral bacteria. Naphthalin showed strong growth inhibitory activity against *Actinomyces viscosus* and *Streptococcus salivarius*, and exhibited moderate or weak growth inhibitory activity against *Porphyromonas* spp. and *Streptococcus* spp. at 2.0 mg/disc, whereas *Lactobacillus rhamnosus* showed no growth inhibition. Therefore, *M. liliflora* could be useful as a natural preventive agent.

Keywords antibacterial activity · derivatives · naphthalin · oral bacteria

Introduction

Dental caries and inflammatory periodontal diseases are considered the most prevalent oral health problems caused by oral bacteria (Petersen and Lennon, 2004; Bajpai et al., 2008; Yim et al., 2010). Dental caries are caused by the accumulation and colonization of Gram-positive bacteria (*Streptococcus mutans* and *Streptococcus sobrinus*) on tooth surfaces. Furthermore, Gram-negative bacteria, such as *Porphyromonas asaccharolytica*, *Porphyromonas gingivalis*, and *Actinomyces viscosus,* are related to the formation and development of periodontal disease resulting in tooth loss (Sato et al., 1996; Takarada et al., 2004). In addition, oral health is influenced by environmental factors such as the components and biochemical activities of bacterial biofilms (plaques), pH, and availability of nutrients. These factors can result in more serious oral cavity diseases (Burne and Marquis, 2000). Therefore, a removal method for oral bacteria should be developed.

Mechanical plaque control therapy includes brushing, flossing, scaling, and root planning, and the use of chemical agents such as chlorhexidine, delmopinol hydrochloride, fluoride, and vancomycin reduces oral plaque-associated diseases (Baehni and Takeuchi, 2003; Gomi et al., 2007). However, the use of mechanical and chemical control methods is accompanied by inherent negative consequences including their inability to provide treatment beyond the tooth surfaces (molar and premolar regions), the promotion of antibiotic-resistant oral bacteria, and tooth discoloration if these antibiotics are received for a prolonged period (Axelsson et al, 2000; Kim et al., 2003; Kim et al., 2008). Therefore, alternative natural antibacterial agents safe for human consumption, provide selective growth inhibition of oral bacteria, and help maintain oral health (Park et al., 2006). Recently, several studies have focused on the use of plant extracts and phytochemicals against oral bacteria, which can be attributed to their attractiveness as natural substances, as they constitute a rich source of bioactive materials such as phenolic compounds, terpenoids, and alkaloids (Sato et al., 1996; Lee and Ahn, 1998; Kim and Lee, 2009).

Magnolia liliflora (Magnoliaceae) is a plant used in Chinese medicine. This plant has been used for the treatment of clinical diseases including cold, headache, blood impediment, and muscle death (Wang et al., 2004). However, relatively few studies concerning the antibacterial activity of *M. liliflora* have been conducted (Bajpai et al., 2008). Therefore, the aim of the present study was to examine antibacterial activity of materials derived from *M. liliflora* against oral bacteria.

Materials and Methods

Chemicals. Eudesmol was purchased from Sigma Chemical Co. (St. Louis, MO). 1,3-Dihydroxynaphthalin, 1,5-dihydroxynaphthalin,

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1,6-dihydroxynaphthalin, 1,7-dihydroxynaphthalin, 2,3-dihydroxynaphthalin, 2,6-dihydroxynaphthalin, 1,2-dimethylnaphthalin, 2,6-dimethylnaphthalin, 1-hydroxynaphthalin, 2-hydroxynaphthalin, limonene, and β-pinene were purchased from Fluka Chemical Co. (Buchs, Switzerland). β-caryophyllene, caryophyllene oxide, camphor, 1,8-cineol 1,4-dihydroxynaphthalin, 2,7-dihydroxynaphthalin, naphthalin, α-pinene, and δ-terpineol were purchased from Aldrich Chemical Co. (Milwaukee, WI). All chemicals were reagent grade.

Plant material. *M. liliflora* flora parts were collected from a local market in Jeonju, Korea and extracted by a steam-distillation extraction method. Anhydrous magnesium sulfate was added to the oils, and were concentrated by rotary evaporation (EYELA autojack NAJ-100, Tokyo, Japan) at 30°C. The essential oil was stored at 4°C until gas chromatography-mass spectrometry (GC-MS) analyses.

GC-MS. The essential oil of *M. liliflora* was analyzed on a GC (HP6890, Agilent)-MS (5973IV, Agilent) (GC-MS). Separation was performed on a DB-5 (0.25 mm film) fused silica capillary column (30 m × 0.25 mm ID; J&W Scientific, Folsom, CA). The GC-MS instrument conditions were as follows: injector temperature, 210°C; column temperature, 50°C for 15 min, after which the oven temperature was programmed to increase to 200°C at 2°C/min, then maintained isothermally for 15 min; ion source temperature, 230°C. Helium was used as a carrier gas (0.8 mL/min). EI-MS spectra were recorded in electron ionization (EI) mode. The ionization energy was 70 eV. The analyzer of sector mass was set to scan from 50 to 600 amu for 2 s. Compounds were compared according to their retention times against the mass spectra obtained when authentic standards were analyzed using the GC-MS system. When an authentic sample was not available, mass spectra library data was used to identify the experimentally obtained mass spectra.

Bacterial strains and culture conditions. The bacterial strains used in this study were *A. viscosus* ATCC 15987, *Lactobacillus rhamnosus* KCCM 11320, *P. asaccharolytica* ATCC 25260, *P. gingivalis* ATCC 33277, *S. mutans* ATCC 25175, *Streptococcus salivarius* ATCC 13419, and *S. sobrinus* ATCC 27607 from the Korean Culture Center of Microorganisms (Seoul, Korea). The cultures of these bacterial strains were kept on an Eggerth-Gagnon (EG) liver extract-Field's slant at –80°C and incubated at 37°C for 48 h under anaerobic conditions (80% N_2, 15% CO_2, and 5% H_2). Oral bacteria, with the exception of *P. gingivalis* ATCC 33277 (gram-negative bacteria), were cultured in brain-heart infusion (BHI) and de Man, Rogosa, Sharpe (MRS) broth. *P. gingivalis* was cultured in *P. gingivalis* broth (PGB; 1.85% BHI, 0.5% yeast extract, 0.05% L-cysteine, 1% hemin solution, and 0.1% menadione solution).

Growth-inhibitory assay. The growth inhibiting activities of the essential oil derived from *M. liliflora*, its active compound, and its derivatives were tested using the paper disc agar diffusion bioassay against oral bacteria using a slightly modified method of Kim et al. (2009). A 0.1-mL aliquot of the bacterial suspension was seeded on EG agar plate, and 0.1 mL of the solution was pipetted onto paper discs (Advantec, 8 mm in diameter and 1 mm thick; Toyo Roshi, Tokyo, Japan) using a Drummond glass microcapillary. The solvents were dried and placed on the surface of the inoculated plates. The plates were incubated under anaerobic conditions at 37°C for 48 h. Methanol was used as a negative control, which showed no antibacterial activity against the oral bacteria. All experiments were performed in triplicate. The growth inhibition zone was measured and classified as described previously by Jeon et al. (2009): potent response (++++), zone diameter >30 mm; strong response (+++), zone diameter 21–30 mm; moderate response (++), zone diameter 16-20 mm; weak response (+), zone diameter 10–15 mm; and little or no response (-), zone diameter <10 mm.

Results and Discussion

The effects of the essential oil extracted from *M. liliflora* against oral bacteria were examined using the paper disc method and compared with the main component and its derivatives. The essential oil of *M. liliflora* at 5.0 mg/disc exerted weak (+) or no (-) antibacterial activity against *P. asaccharolytica, P. gingivalis, S. mutans, S. sobrinus,* and *L. rhamnosus.* Against *A. viscosus* and *S. salivarius,* the essential oil showed strong (+++) and moderate (++) activities, respectively (Table 2).

Due to the antibacterial activity of the *M. liliflora* oil, the bioactive components of *M. liliflora* oil were analyzed by GC-MS

Table 1 Chemical composition of volatile compounds from *M. liliflora*

Peak number	Compound	Retention time (min)	RI[a]	Chemical composition (%)
1	α-Pinene	3.58	1026	5.85
2	Benzene	5.13	1174	1.68
3	Limonene	5.22	1202	3.44
4	Camphene	5.59	1050	3.50
5	α-Terpineol	7.95	1189	3.15
6	δ-Terpineol	8.87	1654	4.72
7	1,8-Cineol	8.95	1203	5.33
8	Myrcene	9.98	993	6.76
9	(-)-Caryophyllene	11.32	1572	4.29
10	Longifolene	12.02	1398	3.03
11	Naphthalin	12.17	1183	28.66
12	α-Selinene	12.28	1496	4.49
13	Calamenene	12.60	1526	2.29
14	Farnesol	13.30	1720	3.02
15	Caryophyllene oxide	13.36	1586	6.15
16	β-Eudesmol	14.15	2158	0.52
17	Patchouli alcohol	14.28	1655	2.11
18	β-Humulene	16.29	1443	3.23
19	Farnesene	16.50	1486	5.45

[a]RI: Kovats Retention Index

Table 2 Growth-inhibiting activities of *M. liliflora* essential oil, active material, and commercially available compounds against oral bacteria

Compound[a)]	Dose (mg/disc)	Bacterial strains[b)]						
		P. asaccharolytica	*P. gingivalis*	*S. mutans*	*S. sobrinus*	*A. viscosus*	*S. salivarius*	*L. rhamnosus*
Essential oil	5	+[c)]	+	+	+	+++	++	-
Naphthalin	2	+	+	++	++	+++	+++	-
	1	-	-	-	-	++	+	-
Camphene	2	-	-	-	-	-	-	-
Camphor	2	-	-	-	-	-	-	-
β-Caryophyllene	2	-	-	-	-	-	-	-
Caryophyllene oxide	2	-	-	-	-	-	-	-
1,8-Cineol	2	-	-	-	-	-	-	-
Eudesmol	2	-	-	-	-	-	-	-
Limonene	2	-	-	-	-	-	-	-
α-Pinene	2	-	-	-	-	-	-	-
β-Pinene	2	-	-	-	-	-	-	-
δ-Terpineol	2	-	-	-	-	-	-	-

[a)]Each assay was performed in triplicate.
[b)]Cultured on Eggerth-Gagnon agar at 37°C for 48 h in an atmosphere of 80% N_2, 15% CO_2, and 5% H_2.
[c)]Inhibitory zone diameter >30 mm, ++++; 21−30 mm, +++; 16−20 mm, ++; 10−15 mm, +; <10 mm, -.

(Table 1). The main compound was characterized as naphthalin. Our findings were similar to that of Azuma et al. (1996), who also assessed the chemical compositions of the *M. liliflora* oil by GC-MS. They found that the volatile compounds in the *M. liliflora* extract were benzeneoids, aldehydes, monoterpenoids, and sesquiterpenoids. Additionally, Bajpai et al. (2008) reported that the essential oil of *M. liliflora* contained mainly camphene, camphor, β-caryophyllene, caryophyllene oxide, 1,8-cineol, eudesmol, limonene, naphthalin, α-pinene, β-pinene, and terpineol.

Based on these findings, we tested the antibacterial activities of the essential oil of *M. liliflora* and the commercially available components (camphene, camphor, β-caryophyllene, caryophyllene oxide, 1,8-cineol, eudesmol, limonene, naphthalin, α-pinene, β-pinene, and terpineol) against *P. asaccharolytica, P. gingivalis, S. mutans, S. sobrinus, A. viscosus, S. salivarius,* and *L. rhamnosus* (Table 2). Naphthalin had weak (+) or no (-) growth-inhibiting activity against *P. asaccharolytica, P. gingivalis,* and *L. rhamnosus* at 2.0 mg/disc. Moderate (++) and strong (+++) effects of naphthalin were observed against *S. mutans, S. sobrinus, A. viscosus,* and *S. salivarius.* None of the other components were active against the seven species of oral bacteria. These findings indicate that naphthalin selectively inhibits the growth of *A. viscosus,* and *S. salivarius*. At 1.0 mg/disc, naphthalin derivatives (1-hydroxy-naphthalin, 1,3-dihydroxynaphthalin, 1,4-dihydroxynaphthalin, 1,6-dihydroxynaphthalin, 1,7-dihydroxynaphthalin, 2-dihydroxy-naphthalin, 2,3-dihydroxynaphthalin, 2,6-dihydroxynaphthalin, and 2,7-dihydroxynaphthalin) exhibited weak (+) or no activity against *P. asaccharolytica, P. gingivalis, S. mutans, S. sobrinus,* and *L. rhamnosus*. Specifically, they selectively inhibited the growth of *A. viscosus* and *S. salivarius* at the same concentration. However, 1,5-dihydroxynaphthalin, 1,2-dimethylnaphthalin, and 2,6-dimethylnaphthalin did not exert antibacterial activity against the seven oral bacteria (Table 3).

To determine structure-activity relationships, the antibacterial activity of naphthalin against seven oral bacteria was examined and compared with 12 derivatives containing hydroxyl (-OH) and methyl (-CH_3) functional groups (Table 4). The antibacterial activity of naphthalin derivatives including a hydroxyl group had a higher activity than that of naphthalin against the seven oral pathogens. The antibacterial activities of naphthalin derivatives indicate that the C-1 position of the hydroxyl group (1-hydroxynaphthalin, 1,3-dihydroxynaphthalin, 1,6-dihydroxy-naphthalin, and 1,7-dihydroxynaphthalin) was more effective in inhibiting the growth of oral bacteria than the C-2 position (2-hydroxynaphthalin, 2,3-dihydroxynaphthalin, 2,6-dihydroxy-naphthalin, and 2,7-dihydroxynaphthalin). In addition, naphthalin derivatives (containing a hydroxyl functional group) showed relatively weak (+) and moderate (++) activity against *P. asaccharolytica*, *P. gingivalis*, *S. mutans*, and *S. sobrinus*. Furthermore, naphthalin derivatives containing a hydroxyl functional group significantly inhibited the growth of *A. viscosus* and *S. salivarius*. However, naphthalin derivatives including a methyl group (1,2-dimethylnaphthalin and 2,6-dimehtylnaphthalin) did not show the same antibacterial activity against the seven oral bacteria (Table 3). In a previous study, Park et al. (2006) reported that 1,4-naphthoquinone analogs containing a methyl group had strong (+++) antibacterial activity, and the addition of a hydroxyl group from 1,4-naphthoquinone had weak (+) activity. These results indicate that the antibacterial activities of the 1,4-naphthoquinone analogs depend on the functional group and substitution position. In addition, Benzic et al. (2003) and Bajpai et al. (2008) reported that the essential oil had a slight influence on growth inhibition of Gram-positive and -negative bacteria. Our results suggest that the introduction of a hydroxyl functional group in naphthalin at the C-1 position results in a more selective antibacterial effect against the seven oral bacteria than substitution

Table 3 Growth-inhibiting activities of the active compound and its derivatives against oral bacteria

Compound[a)]	Dose (mg/disc)	Bacterial strains[b)]						
		P. asaccharolytica	*P. gingivalis*	*S. mutans*	*S. sobrinus*	*A. viscosus*	*S. salivarius*	*L. rhamnosus*
Naphthalin	2	+	+	++	++	+++	+++	-
	1	-	-	-	-	++	+	-
1-Hydroxynaphthalin	2	+	+	++	+	+++	+++	++
	1	+	+	++	+	++	+++	+
	0.5	+	+	+	+	++	++	+
1,3-Dihydroxynaphthalin	2	+	+	+	+++	++++	+++	++
	1	+	+	+	++	+++	++	+
	0.5	-	-	+	+	+++	++	+
1,4-Dihydroxynaphthalin	2	+	+	++	+	+++	++++	++
	1	+	+	+	+	+++	++++	++
	0.5	+	+	+	+	+++	++++	+
1,5-Dihydroxynaphthalin	2	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-
1,6-Dihydroxynaphthalin	2	++	+	++	+	+++	+++	-
	1	+	+	+	+	++	++	-
	0.5	-	-	-	-	++	++	-
1,7-Dihydroxynaphthalin	2	+	+	++	+	+++	+++	-
	1	+	+	+	+	++	++	-
	0.5	-	-	-	-	+	++	-
2-Hydroxynaphthalin	2	+	+	++	+	++	++	+
	1	+	+	+	+	++	++	+
	0.5	-	+	+	-	+	+	-
2,3-Dihydroxynaphthalin	2	+	+	+	+	+++	++	+++
	1	+	-	+	+	++	+	++
	0.5	-	-	-	-	+	+	+
2,6-Dihydroxynaphthalin	2	-	-	+	+	-	+	-
	1	-	-	-	+	-	-	-
2,7-Dihydroxynaphthalin	2	+	+	++	++	+++	++	-
	1	+	+	+	+	+	+	-
1,2-Dimethylnaphthalin	2	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-
2,6-Dimethylnaphthalin	2	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-

[a)]Each assay was performed in triplicate.
[b)]Cultured on Eggerth-Gagnon agar at 37°C for 48 h in an atmosphere of 80% N_2, 15% CO_2, and 5% H_2.
[c)]Inhibitory zone diameter >30 mm, ++++; 21–30 mm, +++; 16–20 mm, ++; 10–15 mm, +; <10 mm, -.

at the C-2 position.

According to the Science Lab Material safety data sheet (2010), the oral LD_{50} values of naphthalin are 490, 533, and 1200 mg/kg in rats, mice, and guinea pigs, respectively. The dermal LD_{50} value was reported to be 20,001 mg/kg in rabbits. Therefore, we can infer that naphthalin has a relatively low acute toxicity in mammals.

In conclusion, results of the present study suggest that naphthalin derived from *M. liliflora* and its derivatives may provide useful antibacterial activity against seven oral pathogens. Further research should be conducted to determine the effects of plant-derived materials in terms of human safety, the antibacterial mode of action, and an effective formulation.

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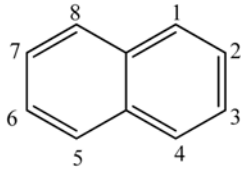
Table 4 Structure-activity relationship of the naphthalin derivatives



Naphthalin

Compound No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
Naphthalin	H	H	H	H	H	H	H	H
1-Hydroxy-naphthlin	**OH**	H	H	H	H	H	H	H
1,3-Dihydroxy-naphthalin	**OH**	H	**OH**	H	H	H	H	H
1,4-Dihydroxy-naphthalin	**OH**	H	H	**OH**	H	H	H	H
1,5-Dihydroxy-naphthalin	**OH**	H	H	H	**OH**	H	H	H
1,6-Dihydroxy-naphthalin	**OH**	H	H	H	H	**OH**	H	H
1,7-Dihydroxy-naphthalin	**OH**	H	H	H	H	H	**OH**	H
2-Hydroxy-naphthalin	H	OH	H	H	H	H	H	H
2,3-Dihydroxy-naphthalin	H	**OH**	**OH**	H	H	H	H	H
2,6-Dihydroxy-naphthalin	H	**OH**	H	H	H	**OH**	H	H
2,7-Dihydroxy-naphthalin	H	OH	H	H	H	H	OH	H
1,2-Dimethyl-naphthalin	**CH_3**	**CH_3**	H	H	H	H	H	H
2,6-Dimethyl-naphthalin	H	**CH_3**	H	H	H	**CH_3**	H	H

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