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NOTE

Antimicrobial Activities of 2,6-Dimethoxy-1,4-benzoquinone and Its Structurally Related Analogues against Seven Food-borne Bacteria

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Abstract Antimicrobial activities of 2,6-dimethoxy-1,4-benzoquinone and its structurally related analogues (2,6-dichloro-, 2,6-dimethyl-, 2,5-dichloro-, and 2,5-dimethyl-) were evaluated at 0.5 mg/disc against seven food-borne bacteria. 2,6-Dimethoxy-1,4-benzoquinone showed antimicrobial activity against *Staphylococcus intermedius* (22.0 mm), *Staphlococcus epidermidis* (19.5 mm), *Shigella sonnei* (16.0 mm), and *Listeria monocytogenes* (15.0 mm). Furthermore, these structural analogues exhibited antimicrobial activity against all tested food-borne bacteria except 2,6-dichloro-1,4-benzoquinone. In conclusion, 2,6-dimethoxy-1,4-benzoquinone and its structural analogues are useful as a source of food supplemental agents.

Keywords 2,6-dimethoxy-1,4-benzoquinone · antimicrobial activity · food-borne bacteria · structural analogues

Introduction

Food-borne illness is caused by consumption of foods contaminated with various food-borne bacteria (Murali et al., 2012). Major food-borne bacteria including *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli* O157 are responsible for a large number of food-borne illness outbreaks (Park et al., 2001; Murphy et al., 2006; Gandhi and Chikindas, 2007; Velusamy et al., 2010; Wang et al., 2011). For this reason, many attempts such as use of various synthetic chemicals have been made to reduce the incidence of food poisoning and spoilage by controlling microbial growth (Shan et al., 2007). However, food preservatives including antibiotics, sulfites, and nitrates have many side effects on human health such as cancer, headache, mental retardation, and nausea (Lee and Ahn, 1998; Gull et al., 2012). Therefore, there is a worldwide trend to explore new types of alternatives to control food-borne illness, giving priority to methods that avoid side effects on human health (Lee, 2002; Bautista-Banos et al., 2006; Nedorostova et al., 2009).

2,6-Dimethoxy-1,4-benzoquinone easily obtained from the oxidation of wood tar part is enriched in which 2,6-dimethoxy-phenol and has been also isolated from many plant species (Chen et al., 1994; Harasawa and Tagashira, 1994; Lana et al., 2006). Moreover, the antifeedant and antifungal activities of 2,6-dimethoxy-1,4-benzoquinone have been reported (Kokpol et al., 1993). However, despite these biological activities, relatively little work has been reported on the antimicrobial activities of 2,6-dimethoxy-1,4-benzoquinone and its structurally related analogues against the seven food-borne bacteria. Therefore, we evaluated the antimicrobial activities of 2,6-dimethoxy-1,4-benzoquinone and structure-activity relationships of four structurally related analogues against the seven food-borne bacteria.

Materials and Methods

Chemicals. 2,6-Dimethoxy-1,4-benzoquinone, 2,6-dichloro-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, 2,5-dichloro-1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone, and tetracycline were purchased from Sigma-Aldrich (USA).

Microorganisms and culture conditions. The food-borne bacteria including *Bacillus cereus* (ATCC14579), *Listeria monocytogenes* (ATCC 15313), *Salmonella enterica* (ATCC 43971), *Staphylococcus epidemidis* (ATCC 12228), *Staphylococcus intermedius* (ATCC 29663), *Shigella sonnei* (ATCC 25931), and *Salmonella typhimurium* (IFO 14193) used were obtained from the Korean Culture Center

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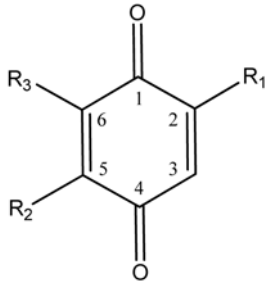
Table 1 Antimicrobial activities 2,6-dimethoxy-1,4-benzoquinone and its structurally related analogues against food-borne pathogenic bacteria, as determined by the paper disc agar diffusion method

Compounds[1)]	Clean zone (mm)[2)]						
	Microorganisms[3)]						
	Bc	Lm	Si	Se	Sae	St	Ss
2,6-Dimethoxy-1,4-benzoquinone	nd[5)]	15.0±1.2	22.0±1.5	19.5±0.9	nd	nd	16.0±1.8
2,6-Dichloro-1,4-benzoquinone	nd	nd	nd	nd	nd	nd	nd
2,6-Dimethyl-1,4-benzoquinone	17.0±1.4	15.5±0.8	19.0±1.8	18.5±2.3	14.5±1.6	13.5±1.1	16.5±1.3
2,5-Dichloro-1,4-benzoquinone	12.0±1.3	13.5±1.2	16.5±2.2	14.5±1.8	14.0±2.5	13.5±2.1	14.0±1.1
2,5-Dimethyl-1,4-benzoquinone	47.0±0.8	51.0±2.4	48.0±2.1	45.5±1.4	24.0±1.2	22.5±1.8	32.5±1.4
Tetracycline[4)]	24.0±1.2	23.5±1.4	29.4±1.5	25.0±1.1	22.3±1.1	24.5±1.2	31.1±1.7

[1)]Exposed to 0.5 mg/disk.
[2)]Values (mm) are expressed as mean±SD of three parallel measurements, $p < 0.05$.
[3)]Bc, *Bacillus cereus* ATCC14579; Lm, *Listeria monocytogenes* ATCC 15313; Si, *Staphylococcus intermedius* ATCC 29663; Se, *Staphylococcus epidermidis* ATCC 12228; Sae, *Salmonella enterica* ATCC 43971; St, *Salmonella typhimurium* IFO 14193; *Ss, Shigella sonnei* ATCC 25931.
[4)]Chloramphenicol and tetracycline served as positive control.
[5)]nd; not detected.

of Microorganisms (Korea). The microorganisms were cultured on nutrient broth (NB, Difco, USA) at 37°C for 1 day.

Antimicrobial activity. The antimicrobial activities of these organisms were assessed by the paper disc agar diffusion method. Filter paper discs (8 mm diameter; Tokyo Roshi Kaisha, Japan) containing the test sample dissolved in methanol solution (0.1 mL) were placed on the surface of agar plates of Mueller Hinton agar (MHA, difco, USA), seeded by spreading the test bacterial suspension on the plates. The test plates were incubated overnight at 37°C under aerobic condition. The control discs received 0.1 mL of methanol. All tests of microbial growth inhibition were replicated thrice. The scale of measurement was considered as potent activity, more 30 mm; strong activity, 21 to 30 mm; moderate activity, 16 to 20 mm; weak activity, 10 to 15 mm; slight activity, less than 10 mm; no activity.



Compounds	R_1	R_2	R_3
2,6-Dimethoxy-1,4-benzoquinone	**OCH_3**	H	**OCH_3**
2,6-Dichloro-1,4-benzoquinone	**Cl**	H	**Cl**
2,6-Dimethyl-1,4-benzoquinone	**CH_3**	H	**CH_3**
2,5-Dichloro-1,4-benzoquinone	Cl	Cl	H
2,5-Dimethyl-1,4-benzoquinone	**CH_3**	**CH_3**	H

Fig. 1 Structures of 2,6-dimethoxy-1,4-benzoquinone and its structurally related analogues.

Results and Discussion

In test with the seven food-borne bacteria at a dose of 0.5 mg/disc, 2,6-dimethoxy-1,4-benzoquinone was evaluated using the paper disc agar diffusion method and were then compared with that of tetracycline as a positive control (Table 1). 2,6-Dimethoxy-1,4-benzoquinone showed strong inhibition against *S. intermedius* (22.0 mm), moderate inhibition against *S. epidermidis* (19.5 mm) and *S. sonnei* (16.0 mm), weak inhibition against *L. monocytogenes* (15.0 mm), and no growth inhibition was observed against *B. cereus*, *S. enterica*, and *S. typhimurium*. According to, Lana et al. (2006), 2,6-dimethoxy-1,4-benzoquinone was particularly active against *S. aureus*. To establish the structure-activity relationships of 2,6-dimethoxy-1,4-benzoquinone analogues, its structurally related analogues (2,6-dichloro-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, 2,5-dichloro-1,4-benzoquinone, and 2,5-dimethyl-1,4-benzoquinone) were selected and evaluated using the paper disc agar diffusion method at 0.5 mg/disc (Table 1 and Fig. 1). 2,5-Dimethyl-1,4-benzoquinone exhibited potent inhibitory activity against *L. monocytogenes* (51.0 mm), *S. intermedius* (48.0 mm), *B. cereus* (47.0 mm), *S. epidermidis* (45.5 mm), and *S. sonnei* (32.5 mm), and strong inhibitory activity against *S. enterica* (24.0 mm) and *S. typhimurium* (22.5 mm). 2,6-Dimethyl-1,4-benzoquinone and 2,5-dichloro-1,4-benzoquinone exhibited moderate or weak inhibitory activity (12.0–19.0 mm) against seven food-borne bacteria. However, the treatment with 2,6-dichloro-1,4-benzoquinone showed no inhibitory activity against the seven food-borne bacteria. These results indicate that various functional groups in 1,4-benzoquinone change the growth inhibiting activities by decreasing or inducing selective activity. For instance, 2,6-dimethoxy-1,4-benzoquinone conjugated with methoxy group had no inhibitory activity against *B. cereus*, *S. enterica*, and *S. typhimurium*. However, 2,6-dimethyl-1,4-benzoquinone conjugated with methyl group exhibited inhibitory activity against *B. cereus*, *S. enterica*, and *S. typhimurium*.

Furthermore, 2,5-dimethyl-1,4-benzoquinone conjugated with methyl group at positions 2 and 5 showed significantly greater inhibitory activity than 2,6-dimethyl-1,4-benzoquinone conjugated with methyl group at positions 2 and 6. Jeong et al. (2009) reported that various functional groups and its position lead to selective inhibitory activity against intestinal bacteria. Moreover, Cho et al. (2009) reported that minimum inhibitory concentration (MIC) values of 2,6-dimethoxy-1,4-benzoquinone were 5.3 and 8.0 ppm against *S. aureus* and *S. sonnei*, respectively. The toxicities of 2,6-dimethoxy-1,4-benzoquinone and its structurally related analogues toward human and mammal have not been reported. Based on our findings, 2,6-dimethoxy-1,4-benzoquinone and its structurally related analogues are useful as a source of food supplemental agents. However, further work is necessary to determine their safetyness on humans.

References

Bautista-Banos S, Hernandez-Lauzardo AN, Velazquez-del Valle MG, Hernandez-Lopez M, Barka EA, Bosquez-Molina E, and Wilson CL (2006) Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Prot* **25**, 108–18.

Chen CC, Yu HJ, Ou, JC, and Pan TM (1994) Constituents of the heartwood of *Diospyros eriantha*. *J Chin Chem Soc* **41**, 195–8.

Cho SC, Sultan MZ, and Moon SS (2009) Anti-acne activities of pulsaquinone, hydropulsaquinone, and structurally related 1,4-quinone derivatives. *Arch Pharm Res* **32**, 489–94.

Gandhi M and Chikindas ML (2007) *Listeria*: A foodborne pathogen that knows how to survive. *Int J Food Microbiol* **113**, 1–15.

Gull I, Saeed M, Shaukat H, Aslam SM, Samra ZQ, and Athar AM (2012) Inhibitory effect of *Allum sativum* and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria. *Ann Clin Microb Anti* **11**, 8.

Harasawa A and Tagashira A (1994) Isolation of 2,6-dimethoxy 1,4-benzoquinone from Hydrangea (*Hydrangea macrophylla* Seringe var otaksa Makino) and its deodorant activity against methyl mercaptan. *Biosci Biotechnol Biochem* **58**, 2073–4.

Jeong EY, Jeon JH, Lee CH, and Lee HS (2009) Antimicrobial activity of catechol isolated from *Diospyros kaki* thunb. roots and its derivatives toward intestinal bacteria. *Food Chem* **115**, 1006–10.

Kokpol U, Chavasiri W, Chittawong V, Bruce M, Cunningham GN, and Miles DH (1993) Long chain aliphatic alcohols and saturated carboxylic acids from heartwood of *Rhizophora apiculata*. *Phytochemistry* **33**, 1129–31.

Lana EJL, Carazza F, and Takahashi JA (2006) Antibacterial evaluation of 1,4-benzoquinone derivatives. *J Agric Food Chem* **54**, 2053–6.

Lee HS (2002) Tyrosinase inhibitors of *Pulsatilla cernua* rootderived materials. *J Agric Food Chem* **50**, 14003.

Lee HS and Ahn YJ (1998) Growth-inhibiting effects of *Cinnamomum cassia* bark-devived materials on human intestinal bacteria. *J Agric Food Chem* **46**, 8–12.

Murali N, Kumar-phillips GS, Rath NC, Marcy J, and Slavik MF (2012) Antibacterial activity of plant extracts on foodborne bacterial pathogens and food spoilage bacteria. *Agri Food Anal Bacteriol* **2**, 209–21.

Murphy C, Carroll C, and Jordan KN (2006) Environmental survival mechanisms of the foodborne pathogen *Campylobacter jejuni*. *J Appl Microbiol* **100**, 623–32.

Nedorostova L, Kloucek P, Kokoska L, Stolcova M, and Pulkrabek J (2009) Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. *Food control* **20**, 157–60.

Park SS, Worobo RW, and Durst RA (2001) *Escherichia coli* O157: H7 as an emerging foodborne pathogen: a literature review. *Crit Rev Biotechnol* **21**, 27–48.

Shan B, Cai YZ, Broods JD, and Corke H (2007) Antibacterial properties and major bioactive components of Cinnamon Stick (*Cinnamomum burmannii*): activity against foodborne pathogenic bacteria. *J Agric Food Chem* **55**, 5484–90.

Velusamy V, Arshak K, Korostynska O, Oliwa K, and Adley C (2010) An overview of foodborne pathogen detection: In the perspective of biosensors. *Biotechnol Adv* **28**, 232–54.

Wang YX, Ye ZZ, Si CY, and Ying YB (2011) Subtractive inhibition assay for the detection of *E. coli* O157:H7 using surface plasmon resonance. *Sensors* **11**, 2728–39.