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Antimicrobial Activities of (-)Epicatechin from *Ulmus davidiana* var. *japonica* Cortex

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Abstract

The extract of *Ulmus davidiana* var. *japonica* cortex has known as natural anti-inflammatory substance in East Asia. For the identification of antimicrobial substance, it was extracted by using methanol and fractionated by using different organic solvents. The fraction of butanol was represented the highest antimicrobial activities. Therefore, the butanol fraction was purified and identified the chemical structure by ^{1}H and ^{13}C-NMR spectra, FT-IR and EI/MS spectroscopies. The isolated antimicrobial substance was identified as cis-2-[3,4-dihydroxy phenyl]-3,4-dihydro-2H-1-benzopyran-3,5,7-triol, which has commonly known as (-)epicatechin. Its minimum inhibitory concentrations (MICs) against *Staphylococcus aureus* and *Listeria monocytogenes* were shown as 100 μg/mL and 500 μg/mL, respectively.

Key words: *Ulmus davidiana* var. *japonica*, antimicrobial substance, (-)epicatechin

INTRODUCTION

The discovery of more effective and less toxic antibiotics has relied primarily on the isolation from natural resources. The major advantage of this approach is the likelihood of identifying new prototype drugs with different chemical structures and hence, possible new mechanism and less likelihood of similar toxicities and cross resistance. Although in the past microorganisms have been the primary source of new antibiotics, higher plants are now recognized as important sources of new antimicrobial agents. As recent efforts to discover new prototype antibiotics with potential utility, the initial *in vitro* evaluation of higher plant extracts for antimicrobial activity is followed by fractionation and purification of active extracts using a bioassay-directed scheme (1). Therefore, many investigators had studied for natural origin antimicrobial substances such as spices, herbs and medicinal fruits. Oregano and thyme were highly toxic to *Vibrio parahaemolyticus* when present in growth media at a concentration of 0.5% (2). Rosemary and sage inhibited gram-positive bacteria to a greater extent than gram-negative bacteria (3). Tetrastilbene from the subterranean parts of *Carex pumila* (4), kobophenol a from subterranean parts of *Carex kobomugi* (5), cercidin from leaves of *Cercidiphyllum japonicum* (6), quercitrin and vincetoxicoside B from *Hypericum japonicum* (7), plicatin B from the leaves and steams of *Psoralea juncea* (8) and monoterpene glycosides from *Erigeron linifolius* (9) have been focused as antimicrobial active substances. 1-Allyl-2,6-dimethoxy-3,4-methylenedioxy benzene, 1-allyl-2,4,5-trimethoxybenzene, 1-(2-E-propenyl)-2,4,5-trimethoxybenzene, and 1-allyl-2-methoxy-4,5-methy lenedioxybenzene from the leaves of *Piper sarmentosum* were shown antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* (10). Most of the green tea volatiles, such as nerolidol, linalool, indole, delta-cadinene, beta-caryophyllene inhibited the growth of one of the most important carcinogenic bacteria, *Streptococcus mutans* (11).

In the focus of antimicrobial substance isolation from nature, the antimicrobial activity of the extracts of *Ulmus davidiana* var. *japonica* cortex, which has been used for edema, articular rheumatism and acne as an ethnic treatment medicine in Asia, was investigated in this study. The methanol extract of *Ulmus davidiana* var. *japonica* cortex showed significant anti-inflammatory action and inhibitory effect of leukocyte emigration in rats and the growth inhibition of *Staphylococcus aureus*, *Streptococcus faecalis*, *Psuedomonas aeruginosa* and *Bacillus* sp. (12) and triterpenoids such as friedelin, epifriedelanol and taraxerol from *Ulmus davidiana* var. *japonica* cortex were identified as anti-inflammatory substances (13). Also, authors (14) previously demonstrated that the methanol extracts of *Ulmus davidiana* var. *japonica* cortex had shown the antimicrobial activity. In this study, the antimicrobial active substance was identified by high pressure liquid chromatography (HPLC) and the structure was configured by nuclear magnetic resonance (NMR), electron impact mass spectrum (EI/MS), UV-visible spectrum and infrared spectrum (IR) result.

MATERIALS AND METHODS

Materials

The dried Ulmi cortex was collected from Ye-cheon (Kyungpook province, Korea) in September 1998, ground and passed

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through 15~20 size mesh sieve. The reagent grade solvents were purchased from Fisher (Fair Lawn, NJ, USA). (-) Epicatechin was obtained from Sigma Chemical Co. (St. Louis, MO., USA). Silica gel for column chromatography purchased from Merck (Darmstadt, Germany).

Extraction and fractionation

One Hundred grams of the dried Ulmi cortex powder were extracted by 1000 mL acetone, ethyl acetate, ethanol, methanol, chloroform and tap water at 25°C for 24 hrs with shaking occasionally and extracted by boiling tab water at 100°C for 1 hr (14). The water extract was centrifuged at 10,000 rpm for 30 min for removing muco compound and the supernatant was collected. Extraction was performed twice as same method as first extraction. The each collected solvent layers were filtrated through filter paper No. 2 (Whatman Ltd., Madiston, UK). The filtrates were concentrated by using rotary vacuum evaporator (Model Eyela A-3S; Tokyo Rikakikai, Tokyo, Japan) at 60°C and freeze dried. The freeze dried extract powder were reconstituted in 100 mL sterilized distilled water and measured the antimicrobial activities. And the powder obtained from methanol extract was fractionated by using hexane, chloroform, ethyl acetate and butanol, orderly. The butanol fraction was represented the highest antimicrobial activity. Then the butanol fraction was concentrated and freeze dried for purification.

Silica-gel column chromatography

The freeze dried butanol fraction was reconstituted with methanol. The reconstituted solution was loaded and passed through silica gel (70~230 mesh silica 60 powder, Merck, Germany) column (600 mm length × 40 mm I.D) chromatography. The elution was performed with a mixture of acetone-methanol with stepwise increases in ratio of acetone from 0 to 100 ratio. The eluted solvents were collected each 100 mL by fraction collector (Model Retriver 500; ISCO Co., USA). And the antimicrobial active fraction were collected and concentrated in vacuum.

High pressure liquid chromatography (HPLC)

Further purification of concentrates was carried out by preparative HPLC, using an μ-bondapack C_{18} column (7.8 × 300 mm, Waters Co., USA). The elution solvent was 7/3 ratio of water/acetonitrile at flow rate 2 mL/min. Each purified peaks were used for screening of the antimicrobial activity.

Measurement of antimicrobial activity

The used microbials such as *Bacillus cereus* ATCC 11778, *B. subtilis* ATCC 6633, *S. aureus* ATCC 13301, *Streptococcus faecalis* ATCC 13301, *Salmonella typhimurium* ATCC 14028, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 10536, *E. coli* O157 : H7 ATCC 43894 were obtained from Gene bank of Biotechnology Research Center in Korea Institution Science and Technology (KIST). For screening of antimicrobial activity during fractionation procedures, the disc diffusion method in agar was used. Overnight cultures of microbials were inoculated onto respective agar media and incubated for 24 hrs at 37°C. Sterile paper discs (8 mm Dia., Whatman, Japan) containing reconstituted fractions in H_2O were deposited onto the preinoculated agar surface and incubated for 24 hrs at 37°C. Results were recorded as average diameter of the clear zone in mm.

Instruments for structure elucidation

The FT-IR spectra were obtained by Bio-Rad Win IR (FTS-175C century series). ^1H and ^{13}C-NMR spectra were obtained by using JEOL JMN-EX 300 spectrometer. Chemical shifts of NMR were recorded in δ values using tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained by JOEL SX-102A. The mass spectra were represented as *m/z* ratio and electron impact (EI) type ionic source was used.

Determination of MIC of (-)epicatechin

The MICs of microbials were measured by agar diffusion method. The growth medium contained the (-)epicatechin 100 μg/mL, 500 μg/mL, 1 mg/mL, 10 mg/mL and 20 mg/mL, respectively. The controls included growth medium without (-)epicatechin only. All plates were incubated at 37°C under appropriate atmospheric conditions for growth and estimated after 24 hrs. The MICs were defined as the minimum concentration of (-)epicatechin when microbial growth was not detected on eyes.

RESULTS AND DISCUSSION

Isolation and purification of antimicrobial substances

The yields of ethyl acetate, chloroform, water and boiling water extracts were shown blow 3% after vacuum concentration. Those of acetone, ethanol and methanol were shown 4.7%, 8.9% and 9.3%, respectively. The antimicrobial activities of each extracts were shown as Table 1. The methanol and ethanol extracts have had antimicrobial effect to tested microbial organisms. The methanol extract, especially, was shown the highest clear zone diameter and yields. Therefore, the methanol extract was chosen for the next continuous step.

Fractionation of methanol extract

The concentrated methanol extract was fractionated by using hexane, chloroform, ethyl acetate and butanol, orderly. The hexane fraction was not shown antimicrobial activity. The chloroform fraction showed antimicrobial activity against *E. coli*. The ethyl acetate fraction has antimicrobial activities against some organisms. The butanol fraction showed the highest clear zone against microbial organisms. Therefore, the butanol fraction was vacuum dried for next step.

Silica gel column chromatography and preparative liquid chromatography

The antimicrobial activities were shown the highest activity at acetone/methanol mixing ratio 2/8 through silica gel column chromatography. The fraction of acetone/methanol mixing ratio 2/8 was vacuum dried and then reconstituted in methanol. The sample was injected to preparative liquid chromatography and seven peaks were obtained. The HPLC chroma

Table 1. Antimicrobial activities of *Ulmus davidiana* var. *japonica* cortex extracts socked by different solvents against various microorganisms (Inhibition zone diameter: mm)

Strains \ Solvents[1)]	S1	S2	S3	S4	S5	S6	S7
Bacillus cereus ATCC 11778	11	11	17	15	-	13	11
Bacillus subtilis ATCC 6633	-	-	15	14	-	10	11
Staphylococcus aureus ATCC 13301	10	10	16	15	-	13	12
Strepcoccus faecalis ATCC 13301	-	-	15	13	-	11	-
Salmonella typhimurium ATCC 14028	10	11	15	14	-	10	10
Listeria monocytogenes ATCC 7644	10	11	14	14	-	10	-
Escherichia coli ATCC 10536	10	10	14	13	12	11	11
Escherichia coli O157:H7 ATCC 43894	-	11	14	13	10	11	11

[1)]S1: Boiling water, S2: Water extract, S3: Methanol extract, S4: Ethanol extract, S5: Chloroform extract, S6: Acetone extract, S7: Ethyl acetate extract.

togram was shown at Fig. 1. Each seven peaks were collect separately and vacuum dried for next purification. Peak number five of them was shown the highest antimicrobial activity. Then it was injected again at preparative liquid chromatography whether this peak was only peak. Therefore, one peak was obtained and this peak was chosen as one of the important antimicrobial substance of *Ulmus davidiana* var. *japonica* cortex and used for structure elucidation.

Structure identification of isolated antimicrobial substance

Results of FT-IR spectrum

The spectrum was shown at Fig. 2. The most wide and strong absorbance was shown at 3384.7 cm^{-1}. The other absorbances were shown at 1605.5, 1534.5 and 1435.7 cm^{-1} (aromatic C = C bond) and at 1042 cm^{-1} (C-O bond).

Results of ^{1}H, ^{13}C-NMR spectrum

The spectrum was shown at Fig. 3. The peaks of ^{1}H-NMR (600 MHz, D_2O) spectrum were represented at δ 6.79 (H-2′), δ 6.72 (H-5′), δ 6.65 (H-6′), δ 6.02 (H-8), δ 5.97 (H-6), δ 5.25 (H-2), δ 4.78 (H-4), ^{13}C-NMR (600 MHz, D_2O) δ 156.2 (C-5), δ 155.6 (C-7), δ 155.2 (C-9), δ 144.8 (C-4′), δ 130.4 (C-3′), δ 118.3(C-1′), δ 115.1 (C-6′), δ 114.3 (C-5′), δ 106.9 (C-2′), δ 98.3 (C-10), δ 96.1 (C-6), δ 95.0 (C-8), δ 78.1 (C-2), δ 69.1 (C-3), δ 27.4 (C-4).

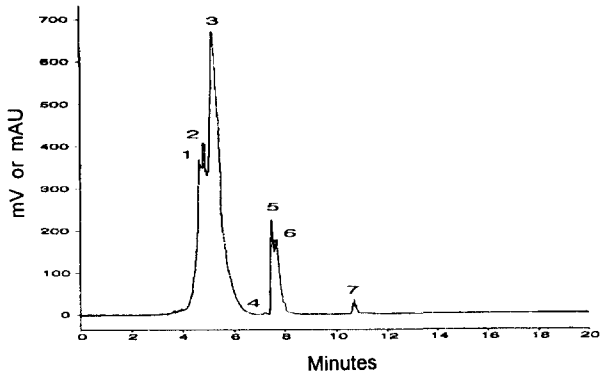

Fig. 1. The HPLC chromatogram of antimicrobial substance. Column: μ-Bondapak C_{18} (7.9×300 mm), mobile phase: water/ACN (7 : 3, v/v), flow rate: 2 mL/min, detector: UV 254 nm, injection vol. : 100 μL.

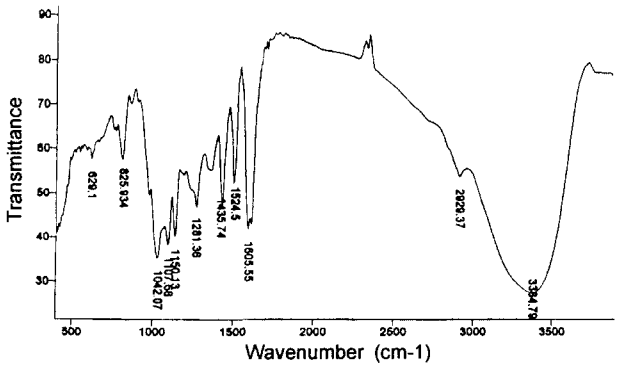

Fig. 2. FT-IR spectrum of the isolated antimicrobial substance of *Ulmus davidiana* var. *japonica* cortex.

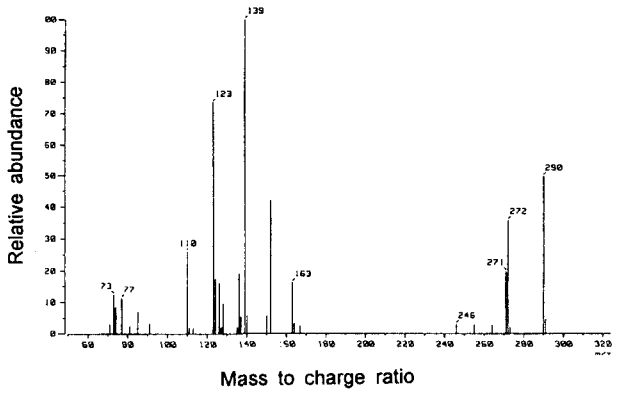

Fig. 3. EI/MS spectrum of the isolated antimicrobial substance of *Ulmus davidiana* var. *japonica* cortex.

Results of EI/MS spectrum

The spectrum was shown at Fig. 4. The most abundance mass to charge ratio (*m/z*) was 139 and the highest mass to charge ratio (*m/z*) was 290.

Results of UV-visible spectrum

The spectrum was shown at Fig. 5. The most wide and strong absorbance was shown at 209, 212, 280 and 365 nm.

Results of identified structure

The identified structure was shown at Fig. 6. The structure of isolated antimicrobial substance was confirmed as cis-

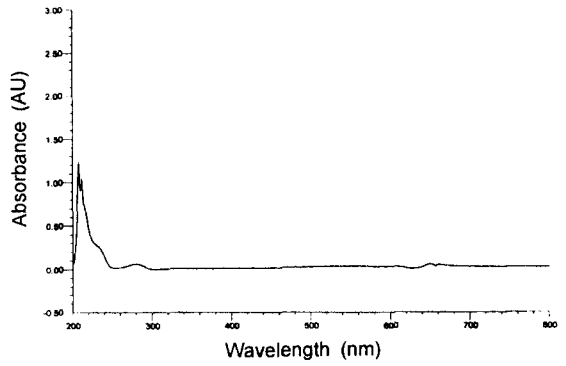

Fig. 4. UV-visible spectrum of the isolated antimicrobial substance of *Ulmus davidiana* var. *japonica* cortex.

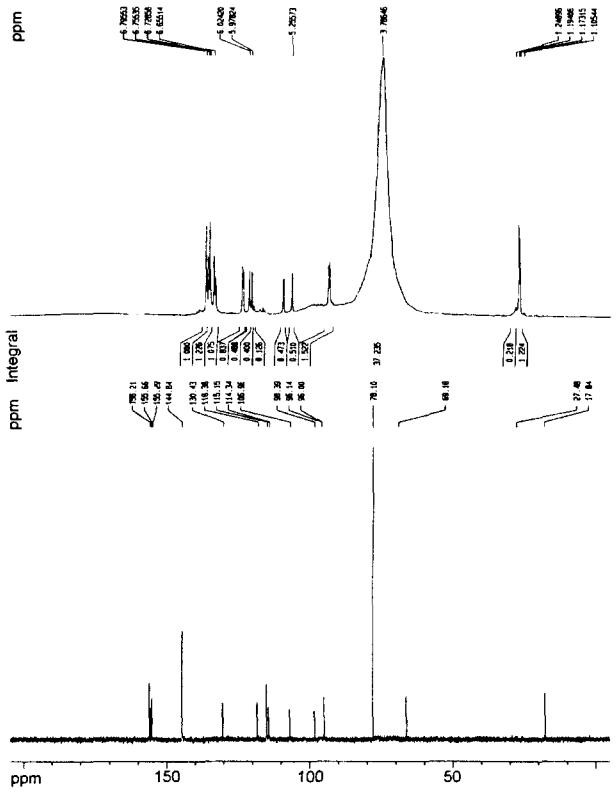

Fig. 5. ^{1}H-NMR, ^{13}C-NMR spectrum of the isolated antimicrobial substance of *Ulmus davidiana* var. *japonica* cortex.

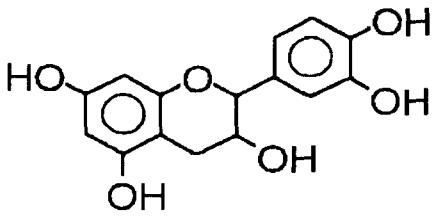

Fig. 6. Identified chemical structure of isolated antimicrobial substance of *Ulmus davidiana* var. *japonica* cortex.

2-[3,4-Dihydroxy phenyl]-3,4-dihydro-2H-1-benzopyran-3,5, 7-triol, which was called as (-)epicatechin, by FT-IR spectrum, ^{1}H,^{13}C-NMR spectrum, GC/MS (EI) spectrum and UV visible spectrum. This elucidated result was same as (-)epicatechin in the roots of *Robus parvifolius* (15).

MICs of (-)epicatechin

The isolated and identified antimicrobial substance from *Ulmus davidiana* var. *japonica* cortex was (-)epicatechin. Therefore, the standard of (-)epicatechin was purchased for measuring the antimicrobial activity and the MICs against the microbials were shown at Table 2. The MICs against *B. subtilis, S. faecalis* and *E. coli* were 10 mg/mL each and *B. cereus, S. typhimurium,* and *E. coli* O157 : H7 were represented 20 mg/mL each. The MICs against *S. aureus,* which has known as an origin of flammatory, were 100 μg/mL. That of *L. monocytogenes,* which had known as an origin of food borne pathogens, were 500 μg/mL. This level was shown as similar antimicrobial activities with coumaric acid, ferulic acid, caffeic acid and gallic acid (16). Roedig-Penmean and Gordon (17) had studied the antioxidative activity of (-)epicatechin in green tea. In this study, it also had antimicrobial activity against S. *aureus* and *L. monocytogenes* as less than 1 mg/mL concentration. These results will be implied that antimicrobial effect substance from Ulmi cortex might be used as a natural antimicrobial source.

REFERENCES

1. Clark, A.M. and Hufford, C.D. : The series analytic : Human medicinal agents from plants. In "*Discovery and development of novel prototype antibiotics for opportunistic infections related to acquired immunodeficiency syndrome*" Kinghorn, A.D. and Balandrin, M.F. (eds.), *ACS symposium series,* American Chemical

Table 2. Minimum inhibitory concentrations of (-)epicatechin against microorgarnisms

Strains	100 μg	500 μg	1 mg	5 mg	10 mg	20 mg
Bacillus cereus	-[1)]	-	-	-	-	+
Bacillus subtilis	-	-	-	-	±	+
Staphylococcus aureus	±	+	++	+++	+++	+++
Streptococcus faecalis	-	-	-	-	±	+
Salmonella typhimurium	-	-	-	-	-	±
Listeria monocytogenes	-	±	+	++	+++	+++
Escherichia coli	-	-	-	-	±	+
Escherichia coli O157:H7	-	-	-	-	-	+

[1)]-: no inhibition, ±: very slight inhibition (clear zone diameter: 8 ~ 9 mm), +: slight inhibition (9 ~ 11 mm), ++: moderate inhibition (11 ~ 13 mm), +++: heavy inhibition (> 13 mm).

Society, Washington D.C., Vol. 534, p.228 (1993)
2. Beuchat, L.R. : Sensitivity of *Vibrio parahaemolyticus* to spices and organic acids. *J. Food Sci.*, **41**, 899 (1979)
3. Shelef, L.A., Naglik, O.A. and Bogen, D.W. : Sensitivity of some common food borne bacteria to the spices sage, rosemary and allspice. *J. Food Sci.*, **45**, 1042 (1980)
4. Kawabada, J., Mishima, M., Kurihara, H. and Mizutani, J. : Kobophenol B, a tetrastilbene form *Carex kobomugi*. *Phytochem.*, **30**, 645 (1991)
5. Kurihara, H., Kawabada, J., Ichkawa, S., Mishima, M. and Mizutani, J. : Oligostilbenes from *Carex kobomugi*. *Phytochem.*, **30**, 649 (1991)
6. Tada, M. and Sakurai, K. : Antimicrobial compound from *Cercidiphyllum japonicum*. *Phytochem.*, **30**, 1119 (1991)
7. Ishiguro, Y., Nagata, S., Fukumoto, H., Yamaki, M., Takagi, S. and Isoi, K. : A flavanol rhamnoside from *Hypericum japonicum*. *Phytochem.*, **30**, 3152 (1991)
8. Schmitt, A., Telikepalli, H. and Mitscher, L.A. : Plicatin B, the antimicrobial principle of *Psoralea juncea*. *Phytochem.*, **30**, 3569 (1991)
9. Ragasa, Consolacion, Y., Rideout, John A., Sy, Jennifer O. : Bioactive monoterpene glycoside from *Erigeron linifolius*. *Phytochem.*, **30**, 3569 (1991)
10. Masuda, T., Inazumi, A., Yamada, Y., Padolina, W.G., Kikuzaki, H. and Nakatani, N. : Antimicrobial phenylpropanoids from Piper sarmentosum. *Phytochem.*, **30**, 3227 (1991)
11. Kubo, I. : Bioactive volatile compounds from plants. In "*Antimicrobial activity of green tea flavor components. Effectiveness against Streptococcus mutans.*" Teranishi, R., Buttery, R.G. and Sugisawa, H. (eds.), *ACS symposium series*, American Chemical Society, Washington, D.C., Vol. 525, p.57 (1993)
12. Hong, N.D., Rho, Y.S., Kim, N.J. and Kim, J.S. : A study on efficacy of Ulmi cortex. *Kor. J. Pharmacogn.*, **21**, 217 (1990)
13. Hong, N.D., Rho, Y.S., Kim, N.J. and Kim, J.S. : Studies on the constituents of Ulmi cortex. *Kor. J. Pharmacogn.*, **21**, 201 (1990)
14. Park, J.S., Shim, C.J., Jeong, J.H., Lee, G.H., Sung, C.K. and Oh, M.J. : Antimicrobial activity of Ulmi cortex extract. *J. Korean Soc. Food Sci. Nutr.*, **28**, 1022 (1999)
15. Do, J.C., Son, K.H. and Kang, S.S. : Studies on the constitutions of the roots of *Rubus parvifolius* (I). *Kor. J. Pharmacogn.*, **19**, 170 (1988)
16. Payne, K.D., Rico-munoz, E. and Davidson, P.M. : The antimicrobial activity of phenolic compounds against *Listeria monocytogenes* and their effectiveness in a model milk system. *J. Food Prot.*, **52**, 151 (1989)
17. Roedig-Penman, A. and Gordon, M.H. : Antioxidant properties of catechins and green tea extracts on model food emulsions. *J. Agric. Food Chem.*, **45**, 4267 (1997)

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