

Identification of Anti-Microbial Material Originated from *Opuntia ficus-indica* var. *saboten* Makino

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In order to discover physiologically active substance, we investigated a powder obtained by processing of *Opuntia ficus-indica* var. *saboten* Makino trunk. The powder was treated by sulfuric acid and then extracted by several solvents such as methanol, methylene chloride, ethanol etc. Among them, the best antimicrobial activity was showed by methylene chloride extract. To identify materials exhibiting physiological activation, the acid hydrolyzed extract was separated by 7 fractions through preparative silica gel TLC. The effective fraction exhibiting the best broad antimicrobial activity was identified, named as MBT-01108. From structural analysis of the products extracted to acid hydrolysis, a compound exhibiting the antimicrobial activities is identified to levulinic acid. Levulinic acid isolated from *Opuntia ficus-indica* var. *saboten* Makino may be applicable as a natural preservative of food or cosmetic and for prevention of bacterial diseases, an ingredient of acne, ageing and whitening cosmetics and an antimicrobial agent.

Key words – Cactus (*Opuntia ficus-indica* var. *saboten* Makino), Anti-microbial activity, Levulinic acid (4-oxopentanoic acid), MBT-01108, Physiologically active material.

Introduction

Among cactus farming or growing naturally in Jeju-island, Korea, fruits of *Opuntia littoralis* cock (California and Nevada, USA) and *Opuntia ficus-indica* var. *saboten* Makino (Mexico) including in genus *Opuntia* are edible as perennation originated from tropical region [25]. The natural growth regions of genus *Opuntia* in South Korea have been distributed at seashores of Jeju-island, Geojedo, Namhae, and Wando etc [13]. If the trunk or fruit extracts of the cactus drink at situation of an empty stomach, there are effects for constipation treatment, urination, appetite improvement, and activation of intestinal movement, which have been transmitted orally to traditional medicines. The trunk extracts of the cactus are also effects for treatment of skin diseases, rheumatis, and burn.

In oriental medicine, extracts of the cactus were utilized for nourishing tonics, febricide sedative, anti-inflammatory detoxication, treatments of neurogenic pain, acute mastitis, and dysentery [9]. The extracts have been used for hematotharsis and treatment of melena [9]. The effective com-

ponents discovered from cactus until now are anhalinin, indicaxanthin, isobetain, betain, and saponin etc [5]. Through results of *in vitro* or animal model experiments, the extracts of cactus have been reported to inhibitory effect of viral replication [1], decrease of cholesterol value [7,24], inhibitory effect against damage of mucosal membrane on stomach [18], effect as a antioxidant [11], and activation of immune system [22].

Recently, since antibiotic resistances not only were widely distributed by infection acquired in hospital, but also by infection due to social acquirement such as pneumonia and tuberculosis, a velocity transmitting antibiotic resistance is progression faster than that of antibiotic development and is difficult problems for public health. Therefore, instead of screening antibiotics from microbes, we attempt to isolate physiologically new and active materials from plants to be utilized as folk medicines or edible plants. Among them, since genus *Opuntia* is farming or growing naturally in South Korea and has been orally transmitted to pharmacological effect, we investigated antibiotic active materials from genus *Opuntia* against antibiotic resistant and other bacteria. Thus in this study, we performed extraction from *Opuntia ficus-indica* var. *saboten* Makino by various methods, investigated antibiotic activities, and obtained a new antibiotic material.

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Materials and Methods

Bacterial strains and culture condition

The bacterial strains used in this study are listed in Table 1. The media used for bacterial culture were as follows: tryptic soy broth (TSB) for *Streptococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus*; brain heart in-

fusion (BHI) for *Lactococcus garvieae*, *Edwardsiella tarda*, *Vibrio anguillarum*, *Enterococcus faecium* and tooth-decaying *Streptococcus* strains; GAM for *Propionibacterium acnes*; complex media (0.8% nutrient broth, 1% yeast extract, 2% NaCl and 0.02% dextrose) for *Bacillus cereus*, *Bacillus subtilis*, and *Escherichia coli*. All bacteria were cultured at 37°C with shaking except tooth-decaying *Streptococcus* strains and *P.*

Table 1. Bacterial strains used for this study.

Strains	References or sources
<i>Pseudomonas aeruginosa</i> KCTC 1636	Human Gene Bank, Biological Resources Center, KRIBB [*]
Methicillin-resistant <i>Staphylococcus aureus</i>	Isolation of a hospital patient in clinical Laboratory science in Pusan
Resistant- <i>Pseudomonas aeruginosa</i>	Isolation of a hospital patient in clinical Laboratory science in Pusan
Vancomycin-resistant <i>Enterococcus faecium</i>	Isolation of a hospital patient in clinical Laboratory science in Pusan
Vancomycin-resistant <i>Enterococcus faecalis</i>	Isolation of a hospital patient in clinical Laboratory science in Pusan
<i>Staphylococcus aureus</i> KCTC 1621	Human Gene Bank, Biological Resources Center, KRIBB [*]
<i>Staphylococcus epidermidis</i> KCTC 3958	Human Gene Bank, Biological Resources Center, KRIBB [*]
<i>Staphylococcus hominis</i> KCTC 3343	Human Gene Bank, Biological Resources Center, , KRIBB [*]
<i>Staphylococcus capitis</i> KCCM 41466	Korean culture center of Microorganisms, Korean Federation of culture collections
<i>Enterococcus faecium</i> KCTC 3095	Human Gene Bank, Biological Resources Center, KRIBB [*]
<i>Propionibacterium acnes</i> ATCC 3320	Human Gene Bank, Biological Resources Center, KRIBB [*]
<i>Escherichia coli</i> ATCC 10536	Human Gene Bank, Biological Resources Center, KRIBB [*]
<i>Escherichia coli</i> (strain forming ESBL)	Isolation of a hospital patient in clinical Laboratory science in Pusan
<i>Bacillus subtilis</i> IFO 3007	Institute for Fermentaio, Osaka, Japan
<i>Staphylococcus aureus</i>	Isolation strain of Institute of Health & Environment, in Pusan
<i>Escherichia coli</i> O-157	Isolation strain of Institute of Health & Environment, in Pusan
<i>Escherichia coli</i> O-157H (-)	Isolation strain of Institute of Health & Environment, in Pusan
<i>Salmonella enteritidis</i> ATCC 13076	Isolation strain of Institute of Health & Environment, in Pusan
<i>Salmonella typhimurium</i> KCTC 1925	Human Gene Bank, Biological Resources Center, KRIBB [*]
<i>Vibrio parahaemolyticus</i>	Isolation strain of Institute of Health & Environment, in Pusan
<i>Vibrio vulnificus</i>	Isolation strain of Institute of Health & Environment, in Pusan
<i>Bacillus cereus</i> ATCC 11778	Isolation strain of Institute of Health & Environment, in Pusan
<i>Shigella</i> sp.	Isolation strain of Institute of Health & Environment, in Pusan
<i>Shigella sonnei</i> ATCC 9290	Isolation strain of Institute of Health & Environment, in Pusan
<i>Shigella dysenteriae</i> ATCC 9752	Isolation strain of Institute of Health & Environment, in Pusan
<i>Streptococcus mutans</i> KCTC 3065	Human Gene Bank, Biological Resources Center, KRIBB [*]
<i>Streptococcus mutans</i> BHT	Department of Biology, Washington University : Serotype b
<i>Streptococcus mutans</i> OMZ-176	Department of Biology, Washington University : Serotype d
<i>Streptococcus mutans</i> LM-7	Department of Biology, Washington University : Serotype e
<i>Streptococcus mutans sobrinus</i> Coykendall 6715-1 ATCC 27352	Department of Biology, Washington University : Serotype g
<i>Streptococcus mutans</i> HS-6	Department of Biology, Washington University : Serotype a
<i>Streptococcus mutans</i> Ingbritt	Department of Biology, Washington University : Serotype c
<i>Streptococcus mutans</i> OMZ-175	Department of Biology, Washington University : Serotype f
<i>Streptococcus salivarius</i> ATCC 13419	Department of Biology, Washington University
<i>Streptococcus downnei</i> MFe 28	Department of Biology, Washington University : Serotype h
<i>Streptococcus mutans</i> GS-5	Department of Biology, Washington University : Serotype c
<i>Citrobacter freundii</i> ATCC 6750	Isolation strain of Institute of Health & Environment, in Pusan
<i>Lactococcus garvieae</i> YT-3	Isolation in National Fisheries Research & Development Institute
<i>Edwardsiella tarda</i> GY-01	Isolation in National Fisheries Research & Development Institute
<i>Vibrio anguillarum</i> YI-85805	Isolation in National Fisheries Research & Development Institute
<i>Salmonella typhimurium</i> KCTC 1925	Human Gene Bank, Biological Resources Center, KRIBB [*]

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acnes. Anaerobic tooth-decaying *Streptococcus* strains and *P. acnes* were incubated at 37°C with standing in an anaerobic chamber such as anaeroGen™ (Oxoid, England).

Fractionation of anti-microbial compounds from *Opuntia cactus* extract

The cactus powder prepared from *Opuntia* trunk as a form of freeze-dried was purchased from Pukjeju Agricultural Development and Technology Extension center (Jeju-Do, Korea).

In order to test preliminary, each 100 g power of genus *Opuntia* was reflexed by each 50% ethanol, butanol, hexane, ethyl acetate for 3 hr, and then the supernatants obtained by centrifugation for 20 min at 17,000 x g. The obtained supernatants were used by x 10 fold concentrations. For the acid hydrolysis, the cactus powder (400 g) was added in 1.5 liter of 2 N sulfuric acid, and incubated it for 10 hr at 110°C. For base hydrolyses, 400 g powder of cactus was reacted with 1,500 ml, 2 N sodium hydroxide for 10 hr at 110°C, and then neutralized by 2 N sulfuric acid. For extractions with α -amylase, β -amylase, glucoamylase, and cellulase, each 10 unit/ml enzyme was added with 400 g powders in 0.05 M acetic acid buffer, pH 5.0. The mixtures were reacted for 24 hr at each optimum temperature (30 and 55°C), centrifuged, and then extracted by each solvent. After complete reactions, all reacted mixtures were treated by methylene chloride and divided into two phases, methylene chloride and water. The methylene chloride fraction was collected as a test sample. The water phase sample was further extracted with the treatment of ethyl acetate, resulting in the preparation of ethyl acetate fraction. The remained water phase sample was separated to n-butanol phase and water phase, after treatment of n-butanol. The organic solvents in the all fractions obtained from sequential extraction processes were removed by concentrations at decreased pressure.

Structure determination of anti-microbial compound

The 300 g powder of genus *Opuntia* was hydrolyzed by 700 ml of 2 N sulfuric acid for 10 hr at 110°C. After hydrolysis, the reactant was cooled to room temperature, neutralized by 2 N sodium hydroxide, and then extracted 3 times with methylene chloride. The CH₂Cl₂ solution was washed and dried by sodium sulfate, and then filtered. The filtrate was concentrated by decreased pressure which was made of final 3 ml. The compounds in methyl

chloride extract were separated on preparative silica gel thin layer chromatography in the organic solvent system composed of ethylacetate: methylene chloride: hexane (1:1:1). The seven fractions were obtained based on the different rate of flow (R_f) in the condition.

In order to determine the structure of compound in the separated TLC-fraction, ¹H-Nuclear magnetic resonance spectroscopy (¹H-NMR) and Infrared absorption spectroscopy (IR-spectra) were performed with the use of Varian Gemini 500 spectrophotometer and Matton IR-1040E spectrophotometer, respectively. ¹H-NMR spectra were recorded on CDCl₃ solution unless otherwise specified and chemical shifts are reported in parts per million (ppm) relative to residual CHCl₃ at 7.24 ppm.

Measurements of clear zones and determination of anti-microbial activity

Anti-microbial activities of compounds in various fractions were observed by paper disc plate method. The 100 μ l precultured broths were put on fresh media and smeared by grass rod. 50 μ l of each fraction (300 mg/ml) was absorbed into paper disc (Φ 8 mm), the treated plates were incubated at 37°C and the diameters (mm) of clear zones were measured.

Anti-microbial activities of compounds in various fractions prepared in this study was determined by minimal inhibitory concentration (MIC). Each serially diluted (3 fold dilution in appropriate media) compounds were added in each well on 96-well plates. Pre-cultured bacterial suspension (10⁶ CFU/ml) was added in well containing diluted compounds, and followed incubation at 30°C for 12-18 hr. The bacterial growth was determined by measurement at 600 nm in wavelength. MIC of the compound against anaerobic bacteria was performed in capped tubes in anaerobic chamber.

Results

An extract from acidic hydrolysate of cactus showed anti-microbial activities by broad host ranges

In order to search new anti-microbial materials, we used the powder of cactus trunk and various extraction methods. The extraction method were simply extracted with several organic solvents or hydrolyzed with α -amylase, β -amylase, glucoamylase, cellulase, acid, and base, and then extracted with several organic solvents. Among the used methods,

extracts by methanol or methylene chloride showed anti-microbial activities against *S. aureus* KCTC 1621, MRSA [6], and *P. acnes* KCTC 3326 (Table 2). Especially, methylene chloride extract derived from acid hydrolysis revealed broad host ranges exhibiting anti-microbial activities against all the used bacterial strains. We selected extract derived from acid hydrolysis for further study due to these properties. As shown in Fig. 1, after hydrolysis with sulfuric acid from the powder of cactus trunk, the obtained products were sequentially separated to methylene chloride, ethyl acetate, n-butanol, and water phases. The fractionated amounts (yields) were showed to 5.2 g (38.8%), 0.9 g (6.7%), 6.1 g (45.5%), and 1.2 g (9.0%), respectively. Each fraction was investigated for anti-microbial activity with

each 300 mg/ml concentration (Table 2). As shown in Table 2, ethyl acetate, n-butanol, and water phases did not show almost anti-microbial activities. However, the anti-microbial activities for methylene chloride phase were exhibited by 9 mm clear zones against *P. aeruginosa* KCTC 1636 and resistant *P. aeruginosa*. Antibiotic resistant strain *S. aureus* KCTC 1621, MRSA and *P. acnes* KCTC 3326 were observed by 10 mm clear zones. Taken all together, methylene chloride extract derived from acid hydrolysis is the best extraction method among the used extraction methods.

Each fraction obtained from silica gel TLC showed anti-microbial activities against various strains

In order to analyze materials exhibiting anti-microbial

Table 2. Antimicrobial activities from materials extracted by various methods.

solvents		strains				
		Inhibitory zones (mm) ¹⁾				
		<i>S. aureus</i> KCTC 1621 ²⁾	<i>P. aeruginosa</i> KCTC 1636	MRSA	Resistant <i>P. aeruginosa</i>	<i>P. acnes</i> KCTC 3326
Methanol		9	0	0	0	9
Ethanol		0	0	0	0	0
Butanol		0	0	0	0	0
Hexane		0	0	0	0	0
Ethyl acetate		0	0	0	0	0
α -amylase	Methylene Chloride	9	0	9	0	9
	Ethanol	0	0	0	0	0
	Butanol	0	0	0	0	0
	Water	0	0	0	0	0
β -amylase	Methylene Chloride	9	0	0	0	0
	Ethanol	0	0	0	0	0
	Butanol	0	0	0	0	0
	Water	0	0	0	0	0
Gluco-amylase	Methylene Chloride	1	0	10	0	11
	Ethanol	0	0	0	0	0
	Butanol	0	0	0	0	0
	Water	0	0	0	0	0
Cellulase	Methylene Chloride	0	0	0	0	0
	Ethanol	0	0	0	0	0
	Butanol	0	0	0	0	0
	Water	0	0	0	0	0
Acid hydrolysis	Methylene Chloride	10	9	10	9	10
	Ethanol	0	0	0	0	0
	Butanol	0	0	0	0	0
	Water	0	0	0	0	0
Base hydrolysis	Methylene Chloride	9	0	9	0	9
	Ethanol	0	0	0	0	0
	Butanol	0	0	0	0	0
	Water	0	0	0	0	0

¹⁾ Each fraction (300 mg/ml) was absorbed into paper disc (Φ 8 mm), the treated plates were incubated at 37°C and the diameters (mm) of clear zone were measured. ²⁾ Final cell concentration for each bacterium was adjusted by approximately 1×10^7 CFU/ml.

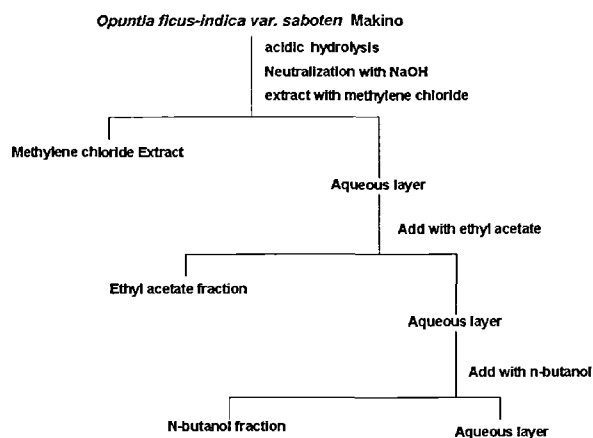


Fig. 1. Extraction and solvent fraction procedure of acid hydrolysis of *Opuntia ficus-indica* var. *saboten* Makino trunk powder. The powder was treated by acidic hydrolysis, neutralized with sodium hydroxide, and submerged with methylene chloride. The extraction of each solvent was done by filtration under decreased pressure.

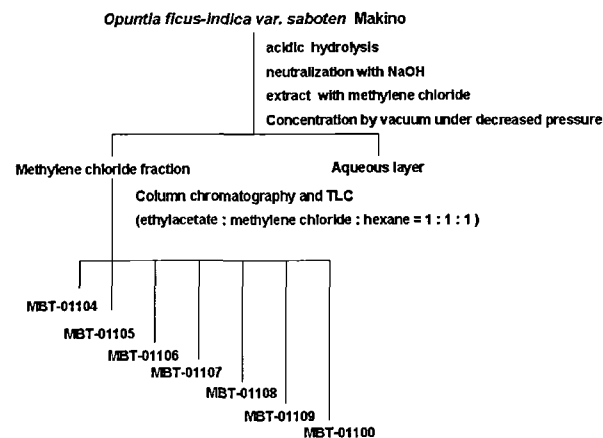


Fig. 2. Scheme for Separation and purification of products extracted from acid hydrolysate of *Opuntia ficus-indica* var. *saboten* Makino trunk powder. The powder was extracted by methylene chloride after acid hydrolysis. TLC development was performed by movement phase followed ethylacetate: methylene chloride: hexane (1:1:1). The names of MBTs were designed by orders of R_f values.

activities, methylene chloride extract of the acidic hydrolysate was separated by 7 fractions according to R_f (rate of flow) using preparative thin layer chromatography and silica gel column chromatography which were applied by organic solvent system as a movement phase with ethylacetate: methylene chloride: hexane (1:1:1) (Fig. 2). Fraction 1, R_f 0.66-0.62, was obtained to 130 mg, fraction 2, R_f 0.45-0.40, 140 mg, fraction 3, R_f 0.40-0.35, 150 mg, fraction 4, R_f 0.35-0.30, 150 mg, fraction 5, R_f 0.10-0.08, 1.5 g, fraction 6, R_f 0.08-0.05 600 mg, and fraction 7, R_f 0.00, 200 mg (Fig. 2).

Fractions 1, 3, 4, 6, and 7 (MBT 01104, 01106, 01107, 01109, and 01100, respectively) were showed by narrow spectrum of anti-microbial activities, whereas 2 and 5 (01105 and 01108, respectively) were observed to broad ranges (Table 3). Especially, MBT01100 (fraction 7) showed very narrow anti-microbial activities against various strains.

Structural analyses of major acquired products was identified as levulinic acid

Acidic hydrolysis of this cactus trunk powder gave 7 kinds

Table 3. Anti-microbial activities of each fraction of extract after acid hydrolysis.

Microorganisms ²⁾	Fractions						
	Inhibition zone (mm) ¹⁾						
	MBT 01104	MBT 01105	MBT 01106	MBT 01107	MBT 01108	MBT01109	MBT 01100
<i>S. aureus</i> KCTC 1621	9	15	16	8.5	14	13	9
<i>P. aeruginosa</i> KCTC 1636	0	11	0	0	10	0	0
MRSA	9	14	14	8.5	15	14	9
Resistant <i>P. aeruginosa</i>	0	11	9	0	11	0	0
VRE	0	24	0	0	20	0	0
<i>L. garvieae</i> YT-3	9	15	15	9	15	11	10
<i>E. tarda</i> GY-01	10	14	18	12	20	16	12
<i>V. anguillarum</i> YT-85805	12	18	20	14	16	10	11
<i>E. faecium</i> KCTC 3095	0	26	16	14	18	10	0
<i>S. mutans</i> KCTC 3065	18	22	24	18	24	22	9
<i>P. acnes</i> KCTC 3326	9	10	10	0	9	10	0
<i>E. coli</i> KCTC 1039	12	14	10	0	14	10	0
<i>E. coli</i> O157	12	14	12	0	12	10	0

¹⁾The measurements of clear zones were used by the same methods as described at the Table 2. ²⁾ Final cell concentration for each bacterium was adjusted by approximately 1×10^7 CFU/ml.

of isolating compound materials to column chromatography. Two of seven fractions (MBT-01108 and -01109) were isolated as major ones and other five compounds as minors through silica gel chromatography. In order to identify one of two major compounds (MBT-01108), we analyze structures with $^1\text{H-NMR}$ and IR-spectra through direct comparison of the isolated products with commercially available authentic samples (Fig. 3). The results of $^1\text{H-NMR}$ revealed that a methyl peak was showed by a singlet at 2.11 ppm, a methylene peak neighboring at carboxyl group was exhibited by a triplet at 2.52 ppm ($J=8.8$ Hz), a methylene peak neighboring at carbonyl group was showed by a triplet at 2.68 ppm ($J=8.8$ Hz). A singlet carboxyl proton was observed at 11.36 ppm. The results of IR-spectra revealed that carbonyl group was dictated at 1716 cm^{-1} and carboxyl group was dictated at $3000\sim 3500\text{ cm}^{-1}$. Taken together all the results, MBT-01108 was identified as levulinic acid. Since 5-(hydroxymethyl)-2-furaldehyde which was identified by the same methods as described at upper, another major compound MBT 01109, was converted to levulinic acid by the more acid hydrolysis, it was suggested that the material is intermediate product of levulinic acid during process of acid hydrolysis. Levulinic acid obtained as one of the most abundant component of extract and also exhibited the anti-microbial activity towards broad spectrum of microorganisms.

Antimicrobial activity of levulinic acid

Since levulinic acid is possible of obtaining better large

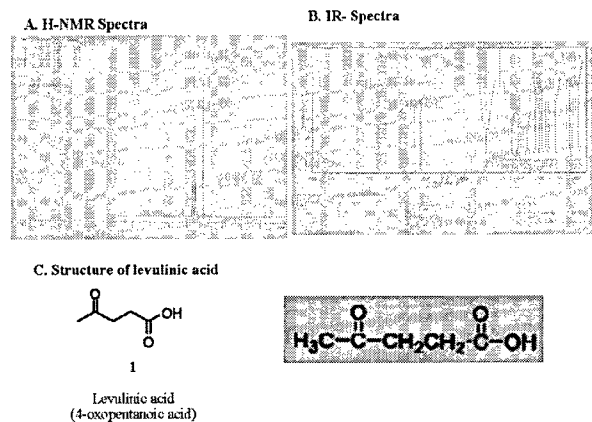


Fig. 3. Result of structural analysis for anti-microbial material. (A) $^1\text{H-NMR}$ Spectra (Nuclear magnetic resonance spectroscopy). $^1\text{H-NMR}$ was used by Varian Gemini 500 spectrophotometer. (B) IR-Spectra (Infrared absorption spectroscopy). IR-spectra was measured by Matton IR-1040E spectrophotometer. (C) Structure of levulinic acid. The levulinic acid contains carbonyl and carboxyl groups.

amount from extract of cactus trunk, we selected levulinic acid for proposes to can do economic and easily acquirement as a material for further study. MIC for levulinic acid was measured against various strains. Generally, when compared in Table 4, strains causing decay of teeth and

Table 4. Anti-microbial activities of levulinic acid against various strains

Characteristics of strains	Microbes Descriptions	MIC (mg/ml) of levulinic acid
Drug-resistance	<i>P. aeruginosa</i> KCTC 1636	1.50
	Methicillin-resistant <i>S. aureus</i>	1.56
	Resistant- <i>P. aeruginosa</i>	1.56
	Vancomycin-resistant <i>E. faecium</i>	3.00
	Vancomycin-resistant <i>E. faecalis</i>	1.56
	<i>S. aureus</i> KCTC 1621	3.50
	<i>S. aureus</i>	0.78
	<i>E. coli</i> (strain forming ESBL)	0.78
Acne & skin disease	<i>S. epidermidis</i> KCTC 3958	3.12
	<i>S. hominis</i> KCTC 3343	0.78
	<i>S. capitis</i> KCCM 41466	3.12
	<i>E. faecium</i> KCTC 3095	1.56
	<i>B. subtilis</i> IFO 3007	0.78
	<i>P. acnes</i> KCTC 3326	3.13
	<i>E. coli</i> ATCC 10536	0.78
Food intermediation	<i>S. aureus</i>	0.78
	<i>E. coli</i> O-157	0.78
	<i>E. coli</i> O-157H (-)	0.78
	<i>S. enteritidis</i> ATCC 13076	0.78
	<i>S. typhimurium</i> KCTC 1925	0.78
	<i>V. parahaemolyticus</i>	0.78
	<i>B. cereus</i> ATCC 11778	0.39
	<i>Shigella</i> sp.	0.78
	<i>S. sonnei</i> ATCC 9290	0.78
	<i>S. dysenteriae</i> ATCC 9752	0.39
<i>S. typhimurium</i> KCTC 1925	0.78	
<i>V. vulnificus</i>	0.39	
Decay of teeth	<i>S. mutans</i> KCTC 3065	4.00
	<i>S. mutans</i> BHT	4.00
	<i>S. mutans</i> OMZ-176	5.00
	<i>S. mutans</i> LM-7	4.00
	<i>S. mutans sobrinus</i> Coykendall 6715-1 ATCC 27352	4.00
	<i>S. mutans</i> HS-6	4.00
	<i>S. mutans</i> Ingbritt	4.00
	<i>S. mutans</i> OMZ-175	4.00
	<i>S. salivarius</i> ATCC 13419	4.00
	<i>S. downnei</i> MFe 28	4.00
<i>S. mutans</i> GS-5	5.00	
<i>C. freunii</i> ATCC 6750	0.78	
Disease of finny tribe	<i>L. garvieae</i> YT-3	3.50
	<i>E. tarda</i> GY-01	3.50
	<i>V. anguillarum</i> YT-85805	3.00

disease of finny tribe showed MIC at higher concentration, whereas strains causing food intermediation exhibited lower concentration. Strains included in drug resistance and acne/skin disease showed MIC of intermediate level. The lowest MIC (0.39 mg/ml) was observed from *B. cereus* ATCC 11778, *S. dysenteriae* ATCC 9752, and *V. vulnificus*. These results mean that levulinic acid can play effectively roles as an anti-microbial agent regardless of Gram (+) or (-), especially bacteria exhibiting resistances against antibiotics [6,12,17,23].

Discussion

Natural systems for organisms have been reported a lot of materials exhibiting anti-bacterial activities. These materials contain anti-bacterial functions as self defense mechanisms by various features in animal, plant and micro-organism [2,20]. The researchers have vigorously done searches of natural anti-microbial materials and applications for food from food materials or herb medicines [8,10,14,16,21]. Natural materials made of manufactured goods or exhibited anti-microbial activity until now have been reported by proteins such as lysozyme, polylysine, protamine, conalbumin, avidin etc, organic acid such as acetic, benzoic, maleic, succinic etc, and others such as pectin lysate, browning reaction material, lower fatty acid ester, and spice etc [19]. Recently, researchers of South Korea have actively progressed studies related with oil components from pepper and onion, anti-fungi components from herb medicine, and chitin/chitosan etc [3,4,14,15,18].

Since genus *Opuntia* is naturally growth at regions including in Jeju-island, Korea, and possible of farming to a large amount, thus, since the cactus is the best merit for material supply and guarantee by a large amount, we select as a major material for study. Among components extracted and isolated in methylene chloride solution from the powder of genus *Opuntia* trunk, the levulinic acid is a major component and the material was identified to physiologically active.

Since physiologically active material was identified from the powder of genus *Opuntia* trunk to have been utilized for food or crude medicine, it is expected to a new hallmark that the result leads to safe medicine development for human being. Although material exhibiting anti-microbial activity was reacted by high concentration (mg level), it is a critical value because of natural compound obtained

from edible plant. The levulinic acid isolated from *Opuntia ficus-indica* var. *saboten* Makino may be applicable as a natural preservative of food or cosmetic and for prevention of bacterial diseases, an ingredient of acne, ageing and whitening cosmetics and an antimicrobial agent.

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초록 : 손바닥 선인장 분말로부터 추출된 항균물질의 특성

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본 연구자들은 동·서양에서 오랫동안 민간요법으로 사용되어져 왔고, 문헌에도 약리적인 작용이 기록되어 있는 *Opuntia ficus-indica* var. *Saboten Makino* (손바닥 선인장, 백년초) 줄기 분말을 사용하여 항균생리활성 물질을 규명하고자 하였다. 줄기 분말을 황산으로 가수분해 처리한 후 메탄올, 메틸클로라이드, 에탄올 등의 용매로 추출하였다. 용매추출물 가운데 methylene chloride 추출물의 경우 가장 탁월한 항균활성을 나타내었다. 항균활성을 나타내는 생리활성 물질을 분리 분석하기 위하여 조제용 TLC를 이용하여 산 가수분해 추출물로부터 7개의 분획물을 분리하였다. 7개 분획물 가운데 가장 광범위한 항균활성을 나타내는 유효 분획물로 분리된 것을 MBT-01108 이라 명명하였다. 산가수분해로 추출된 분획물 MBT-01108을 구조 분석한 결과 항균 활성을 나타내는 화합물이 levulinic acid임을 밝혀냈다. *Opuntia ficus-indica* var. *Saboten Makino*로부터 분리된 levulinic acid는 식품 또는 화장품의 천연 보존제로써 사용가능하리라 판단되며, 또한, 세균성 질병예방, 여드름, 노화 예방, 미백 화장품 성분, 그리고 항생제로도 사용 가능한 물질임을 밝혀냈다.