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항균활성을 갖는 천연물 개발과 화장품 응용에 관한 연구

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Development of Antimicrobial Plant Extracts and its Application to Cosmetics

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요 약: 본 연구는 항균 활성을 지니는 식물추출물을 찾고 화장품에서 방부제로서 적용하기 위해 진행되었다. 디스크 확산법(disk diffusion method)을 통해 3가지 식물 추출물, 함박꽃나무(천녀목란, *Magnolia sieboldii*), 오배자(*Rhus chinensis*), 메타세콰이어(*Metasequioa glyptostroboides*)가 항균활성을 지니고 있음을 확인하였다. 세 물질의 최소저해농도(MIC)를 측정한 결과 메타세콰이어는 0.3 ~ 0.35 %, 함박꽃나무는 0.35 ~ 0.4 % 농도에서 곰팡이의 생장을 억제하고 오배자는 0.45 ~ 0.5 % 농도에서 세균의 생장을 억제함을 확인하였다. 또한 추출물 내의 항균 활성을 지니는 성분을 분리하여 분석한 결과 메타세콰이어에서 분리한 caryophyllene oxide와 caryophyllene, 함박꽃나무에서 분리한 costunolide와 dehydrocostus lactone, 오배자에서 분리한 ethyl gallate, ethyl-3-gallate 등이 항균활성을 지닌 물질임을 확인하였다. O/W 에멀션 제형에서 식물 추출물을 넣고 방부력을 확인한 결과 혼합사용 시 세균과 곰팡이 모두에 대한 방부효과가 있음을 확인하였다. 따라서 함박꽃나무, 오배자, 메타세콰이어 추출물의 혼합물은 기존의 화학 방부제를 대체할 천연 방부제로서 화장품에서 응용할 수 있을 것으로 기대된다.

Abstract: This study is focused on finding new natural materials that have antimicrobial activity. We found that 3 plants extracts, *Magnolia sieboldii K. Koch (M. sieboldii K. Koch)*, *Rhus chinensis (R. chinensis)* and *Metasequioa glyptostroboides (M. glyptostroboides)*, have antimicrobial activities by disk diffusion method. We determined the Minimum Inhibitory Concentation (MIC) of each and found that 0.3 ~ 0.35 % of *M. glyptostroboides* essential oil and 0.35 ~ 0.4 % of *M. sieboldii K. Koch* extracts inhibited fungal growth and 0.45 ~ 0.5 % of *R. chinensis* extracts inhibited bacterial growth. We isolated compounds from extracts and verified what have antimicrobial activity. As a result we found that caryophyllene oxide and caryophyllene isolated from *M. glyptostroboides*, costunolide and dehydrocostus lactone from *M. sieboldii K. Koch* and ethyl gallate, ethyl-3-gallate from *R. chinensis* have antimicrobial activities. In accordance with antimicrobial activity, O/W cosmetic emulsion containing mixture of 3 plants extracts showed preservative efficacy against both bacteria and fungi. Based on the above data we suggest that extracts from *M. siebodii K. Koch*, *R. chinensis*, and *M. glyptostroboides*, replace chemical synthetic preservatives and be applied as a natural preservative.

Keywords: *natural preservatives, plant extracts, Magnolia sieboldii, Rhus chinensis, Metasequioa glyptostroboides*

1. Introduction

Control of microbial growth in cosmetics, pharmaceuticals and foods is very important in keeping quality and safety. Cosmetics include large amount of water, nutrients supplying carbon and nitrogen sources and natural materials derived from plants or microorganisms which can be used by microorganisms. Therefore,

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cosmetics are susceptible to microbial contamination and it can cause some problems. First, microbial contamination in cosmetics reduces product's quality by changing odors, colors, viscosity or even worse destruction of emulsion. Second, it can affect consumer's health. If a cosmetic contaminated by microorganism is applied to wounded skin, it can cause infection or inflammation.

To protect cosmetics from microbial contamination while consumers are using, cosmetics are preserved with appropriate chemical preservatives such as *p*-hydroxy benzoic acid, phenoxyethanol and imidazolidinyl urea. However, some preservatives are reported to cause skin irritation, erythema, contact allergy, contact sensitization and contact dermatitis[1]. Especially parabens, one of the most widely used preservatives, are reported to increase female breast cancer incidence and influence development of malignant melanoma[2]. In addition, formaldehyde-releasing preservatives such as imidazolidinyl urea and diazolidinyl urea are thought to cause skin reaction in sensitive individuals and allergy to isothiazolinones has been reported in many publications[3-5].

Recently to resolve safety problems of chemical preservatives and meet the consumer's needs for safety, many researchers are trying to find safe and effective alternative preservatives. It is known that some plant extracts and essential oil from herbs have antimicrobial activity[5-7]. For example, the essential oils of *Rosmarinus officinalis* and *Cinnamomum zeylanicum* have been used for their antimicrobial properties[8-10]. Thyme oil, the essential oil of *Thymus vulgaris*, is often incorporated into hygiene and skin care products because it possesses antimicrobial properties that have been attributed mainly to the phenolic component, thymol and carvacrol[11].

In this paper we demonstrated that extracts of *Magnolia sieboldii K. Koch* and *Rhus chinensis* and essential oil of *Metasequioa glyptostroboides* have antimicrobial effects. *Metasequoia* is a fast-growing, deciduous tree and one of three species of conifers known as redwoods. Although it is the least tall of the redwoods it grows to at least 60 m height. We verified that *M. glyptostroboides* helps reduce wrinkle formation by inhibiting MMP1 synthesis and obtained a patent on its inhibitory effect of wrinkle formation.

M. sieboldii is a species of *Magnolia* native in Korea and China. *M. sieboldii* is a large shrub and small tree 5 ~ 10 m height. The leaves are elliptical to ovate-oblong 9 ~ 16 cm long and 4 ~ 10 cm broad with a 1.5 ~ 4.5 cm petiole. We verified that *M. sieboldii* helps whiten skin color by inhibiting α-MSH induced melanogenesis and obtained a patent on its whitening effects.

R. chinensis, the Chinese sumac or nutgall tree is a plant species in the genus *Rhus*. The species is used to produce galls called *Chinese gall* or *Galla Chinensis* which are rich in gallotannins[12], a type of hydrolysable tannins. The infestation by Chinese sumac aphids (*Melaphis chinensis* Bell) can lead to a gall which is valued as a commercial product. Chinese galls are used in Chinese medicine to treat coughs, diarrhea, night sweats, dysentery and to stop intestinal and uterine bleeding. The gall of *R. chinensis* has long been considered to possess many medicinal properties[13].

In this study, we tried to demonstrate the possibility of those plant materials as an alternative preservative in cosmetic formulations by applying them in cosmetic emulsions. Therefore we suggest that they be used as effective alternative preservatives in cosmetics.

2. Materials and Methods

2.1. Reagent

Silica gel 60 (Merk, 70 ~ 230 mash), Sephadex LH-20 (Pharmacia, USA) and silica gel F_{254} plates (Merk, USA) were used. ^{1}H and ^{13}C NMR spectra were decoded on a varian UNMR-400 spectrophotometer operating at 400 MHz for proton and 100 MHz for carbon respectively. Other chemicals were purchased from Sigma Aldrich chemical company. For microbial tests, tryptic soy agar (Acumedia, USA) and potato dextrose agar (Acumedia, USA) and D/E neutralizing broth (Difco, USA) were used. *M. sieboldii*, *R. chinensis* and *M. glyptostroboides* were purchased from Kyeong-Dong Market in Korea and *M. sieboldii* and *R. chinensis* were extracted in 70 % EtOH at room

temperature and *M. glyptostroboides* was extracted by hydrodistillation method to yield essential oil.

2.2. Test Organism and Medium

For antimicrobial test and preservative efficacy test, following microorganisms were used : *Escherichia coli (E. coli)* (ATCC8545), *Pseudomonas aeruginosa (P. aeruginosa)* (ATCC9027), *Staphylococcus aureus (S. aureus)* (ATCC6538), *Candida albicans (C. albicans)* (ATCC 10231), *Aspergillus niger (A. niger)* (ATCC 9642). As a media for bacterial growth, tryptic soy agar (Acumedia, USA) was used and for fungal growth, potato dextrose agar (Acumedia, USA) was used.

2.3. Screening for Plants with Antimicrobial Activity

To test antimicrobial activity of plant extracts, disk diffusion method was used[14]. To use as inoculum, bacteria were cultured on tryptic soy agar (Acumedia, USA) at 37 ℃ for 24 h and *C. albicans* and *A. niger* were grown on potato dextrose agar (Acumedia, USA) at 28 ℃ for 48 h and 5 days respectively. Bacterial and fungal inocula were prepared by harvesting in 0.1 % peptone water containing 0.05 % tween-80 (Junsei, Japan), agitating gently and adjusting the number of cell approximately 10^6 CFU/mL and 10^5 spores/mL respectively. Microorganisms were loaded on the solid medium and the disk loaded with each plant extracts or essential oil was put on the solid medium. After bacteria were incubated at 37 ℃ for 48 h and Fungi were incubated 30 ℃ for 72 h antimicrobial activity is determined by the size of inhibition zone around the disk.

2.4. Measurement of Antimicrobial Activity

To measure antimicrobial activity of plant extracts or essential oils, MIC (minimum inhibitory concentration) was determined through microbroth dilution method [15]. Each microbe was incubated in 4 mL broth with serial diluted plant extracts or essential oils for 48 ~ 72 h and checked which concentration inhibits microbial growth.

2.5. Preparation of Formulation for Challenge Test

To confirm preservative efficacy in cosmetics, we prepared O/W emulsion and added the mixture of extracts of *M. sieboldii* and *R. chinensis* and essential oil of *M. glyptostroboides* to emulsion.

2.6. Challenge Test

The preservative efficacy test in a cosmetic emulsion was performed following the method and criteria proposed by Cosmetic, Toiletry, and Fragrance Association (CTFA) Microbiology Guideline[16]. A cosmetic emulsion of 50 g was put into sterile bottle and bacterial and fungal suspensions were inoculated to give a final level of approximately 10^6 CFU/g and 10^5 CFU/g respectively. After a contact time of 0, 3, 7, 14, 21 and 28 days at 25 ℃, 1 g of the sample was removed from test bottle and diluted with 9 mL of D/E neutralizing broth (Difco, USA). Cell viability was determined by Aerobic plate count method in TSA or PDA and CFU(colony forming unit) was counted after 36 ~ 48 h incubation at 37 ℃ for bacteria and 5 days incubation at 25 ℃ for fungi.

3. Results

3.1. Screening for Antimicrobial Plant materials

As a result of screening for plant extracts with antimicrobial activity by disk diffusion method we found 3 plants extracts, *M. glyptostroboides*, *M. sieboldii K. Koch* and *R. chinensis*. Essential oil of *M. glyptostroboides* and extracts of *M. sieboldii K. Koch* were found to have antimicrobial activity against fungi such as *A. niger* and *C. albicans*. Extracts of *R. chinensis* was found to have antimicrobial activity against gram positive and negative bacteria such as *E. coli*, *P. aeruginosa* and *S. aureus* but not against fungi (Table 1).

3.2. Evaluation of Antimicrobial Activity (MIC)

The antimicrobial activity of these materials was further evaluated by determining the minimum inhibitory concentration (MIC), which is the lowest concentration inhibiting microbial growth. The MIC was determined using a microbroth dilution method. As a result, *R. chinensis* inhibited the growth of bacteria

Table 1. Antimicrobial Activity of *M. glyptostroboides*, *R. chinensis* and *M. sieboldii K. Koch* Against Bacteria and Fungi by Disk Diffusion Method

clear zone size unit : mm

Name of plant	Conc. (%)	Bacteria			Fungi	
		E. coli	*P. aeruginosa*	*S. aureus*	*A. niger*	*C. albicans*
R. chinensis	1	10	12	11	-[a]	-[a]
M. glyptostroboides	1	-[a]	-[a]	-[a]	24	20
M. sieboldii	1	-[a]	-[a]	-[a]	20	18

[a] No antimicrobial activity

Table 2. Minimum Inhibitory Concentration (MIC) of *M. glyptostroboides*, *R. chinensis* and *M. sieboldii K. Koch* against Bacteria and Fungi

Microorganism	MIC (%)		
	R. chinensis	*M. glyptostroboides*	*M. sieboldii K. Koch*
E. coli	0.5	-[a]	-[a]
P. aeruginosa	0.45	-[a]	-[a]
S. aureus	0.5	-[a]	-[a]
C. albicans	-[a]	0.35	0.4
A. niger	-[a]	0.3	0.35

[a] No antimicrobial activity

such as *E. coli*, *P. aeruginosa* and *S. aureus* in a range of 0.45 ~ 0.5 %. *M. glyptostroboides* and *M. sieboldii K. Koch* inhibited the growth of fungi such as *A. niger* and *C. albicans* in a range of 0.3 ~ 0.35 % and 0.35 ~ 0.4 % respectively (Table 2).

3.3. Identification of Active Compounds

To study further which compounds within extracts have antimicrobial activity we isolated compounds and verified by ^{1}H-^{13}C NMR and mass spectrometry. They were extracted in 70 % ethanol under reflux for 4 h. Crude extracts yield about 7.5 % and 15.4 % after evaporation under reduced pressure. It was suspended in 20 % ethanol and subsequently partitioned with n-hexane, CH_2Cl_2, ethyl acetate (EA) and butyl alcohol. EA fraction of *M. sieboldii* crude extracts was subjected to silica gel eluting with gradient system of n-hexane : EA (5 : 1 to 1 : 1), followed by Sephadex LH-20 eluting with CH_2Cl_2 : Methanol (1 : 1) to yield costunolide (Figure 1(a)) and dehydrocostus lactone (Figure 1(b)) as white powder.

CH_2Cl_2 fraction of *R. chinensis* extracts was chromatographed on a silica gel column eluting with n-hexane : EA (3 : 1) to give ethyl gallate (3,4,5-trihydroxynezoic acid ethyl) (Figure 1(c)) and ethyl-3-digallate (3-(3′,4′,5′-trihydorxy benzoic acid ether 4,5-dihydroxy benzoic acid ethyl)) (Figure 1(d)) as brown powder. Also EA partition of *R. chinensis* was separated and isolated by silica gel column chromatography eluting n-hexane : EA (1 : 1) to give ethyl *p*-digallate (4-(3′,4′,5′-trihydroxy benzoic acid ether)-3,5-dihydroxy benzoic acid ethyl) (Figure 1(e)) and tri gallic ester-o-glucose (tri-*o*-galloyl glucose) (Figure 1(f)) as brown powder.

Finally, *M. glyptostroboides* was extracted by hydrodistillation method. Caryophyllene oxide (Figure 1(g)) and caryophyllene (Figure 1(h)) were identified as antimicrobial compounds using gas chromatography analysis method.

Costunolide (compound a)-EI MS : M+= 232, m/z= 217, 123, 109; ^{1}H-NMR (CDCl3, 300 MHz) δ : 6.36 (1H, d, 3.4), 5.52 (1H, d, 3.4), 4.85 (1H, m), 4.72 (1H, d, 9.8), 4.55 (1H, dd, 9.8, 8.0), 1.71 (3H, d, 1.3), 1.43(3H, br s); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ : 170.3, 141.3, 136.9, 127.4, 127.1, 119.5, 81.9, 50.5, 41.1, 39.5, 28.2, 26.2, 17.3, 16.1

Dehydrocostus lactone (compound b)-EI MS : M+ = 231, ^{1}H-NMR (CDCl3, 300 MHz), 6.21 (1H, d, J = 3.2 Hz, H-13a), 5.48 (1H, d, J = 3.2 Hz, H-13b), 5.26 (1H, br. s, H-15a), 5.06 (1H, br. s, H-15b), 4.89 (1H, br. s, H-14a), 4.80 (1H, br. s, H-14b), 3.96 (1H, t, J = 9.2Hz, H-5) ; ^{13}C-NMR ($CDCl_3$, 125 MHz) 170.54, 151.46, 149.44, 139.96, 120.62, 112.81, 109.99, 85.99, 52.45, 48.04, 45.55, 36.64, 32.84, 31.33, 30.53.

Ethyl gallate (compound c)-white crystall EI MS :

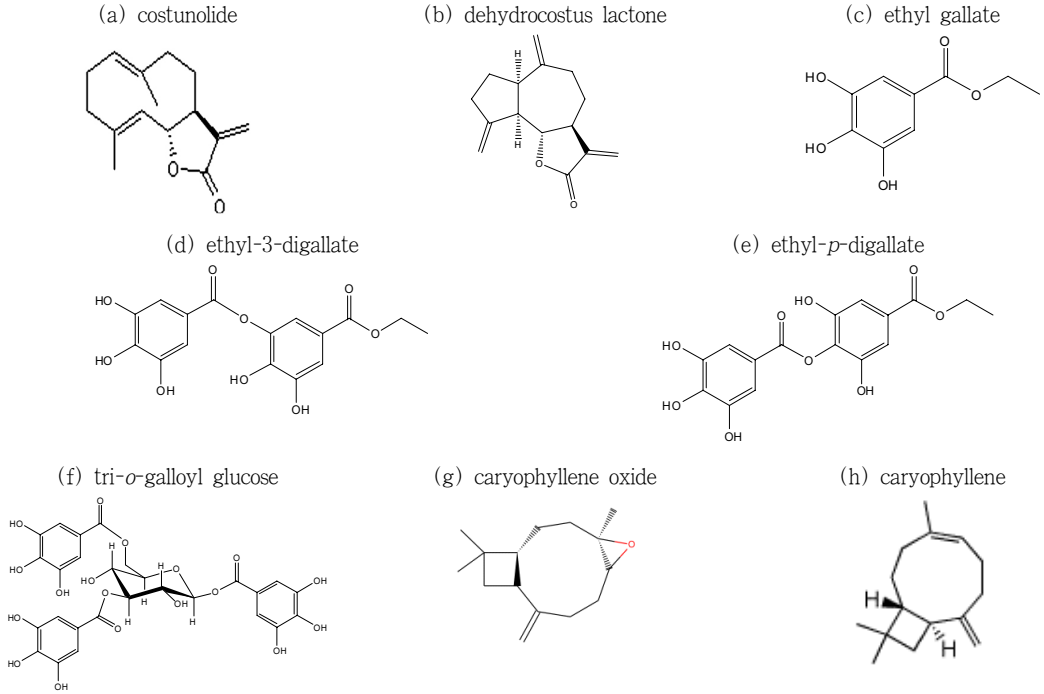


Figure 1. The structures of active compounds within plants extracts or essential oil. (a, b) compounds separated from *M. sieboldii K. Koch*, (c, d, e, f) from *R. chinensis*, and (g, h) from *M. glyptostroboides*.

m/z 199 [M+] ^{1}H-NMR (acetone-d6, 500 MHz) : 7.11 (2H, s, galloyl-2, 6), 4.23 (2H, q, J = 7.3 Hz, OCH2), 1.30 (3H, t, J = 7.3 Hz, CH3); ^{13}C-NMR (acetone-d6, 125 MHz) : 121.3 (C-1), 108.9 (C-2, 6), 145.2 (C-3,5), 137.8 (C-4), 60.0 (OCH2), 13.8 (CH3), 165.9 (C=O).

Ethyl-3-digallate (ethyl-*m*-digallate) (compound d)-white cystal : ESI-MS m/z 349 [M-H]; ^{1}H-NMR (acetone-d 6,500 MHz) 7.44 (1H, d, J =1.8 Hz, galloyl-2), 7.33 (1H, d, J = 1.8 Hz, galloyl-6), 7.26 (2H, s, galloyl-2′, 6′), 4.27 (2H, q, J = 7.3 Hz, OCH2), 1.31 (3H, t, J = 7.3 Hz, CH3); ^{13}C-NMR (acetone-d6, 125 MHz) : 121.1 (C-1), 113.6 (C-2), 146.1 (C-3), 142.5 (C-4), 138.5 (C-5), 116.4 (C-6), 165.3 (C=O), 120.0 (C-1′), 109.9 (C-2′, 6′), 145.2 (C-3′, 5′), 138.7 (C-4′), 60.6 (OCH2), 13.7 (CH3), 163.4 (C′=O).

Ethyl-*p*-digallate (compound e)-white cystal : ESI-MS m/z 349 [M-H]; ^{1}H-NMR (acetone-d6, 500 MHz): 7.25 (2H, s, galloyl-2′, 6′), 7.17 (2H, s, galloyl-2, 6), 4.29 (2H, q, J = 7.3 Hz, OCH2), 1.33 (3H, t, J = 7.3 Hz, CH3); ^{13}C-NMR (acetone-d6, 125 MHz) : 128.3 (C-1), 108.8 (C-2, 6), 150.4 (C-3,5), 131.5 (C-4), 165.5 (C=O), 119.9 (C-1′), 109.8 (C-2′, 6′), 145.3 (C-3′, 5′), 139.0 (C-4′), 60.4 (OCH2), 13.8 (CH3), 164.1 (C′=O);

Tri-O-galloyl glucose (compoud f)-white cystal EI-MS m/z 637 [M+] ^{1}H-NMR (CD_3OD, 500 MHz) : 7.10, 7.04, 6.97 (each 2H, s, galloyl-2, 6), 6.23 (1H, d, J = 8.2 Hz, H-1), 5.90 (1H, t, J = 9.6 Hz, H-3), 5.61 (1H, t, J = 9.6 Hz, H-4), 5.58 (1H, t, J = 9.6 Hz, H-2), 4.35 - 4.51 (3H, m, H-5, 6); ^{13}C-NMR(CD_3OD, 125 MHz): 92.5 (C-1), 70.8 (C-2), 72.7 (C-3), 68.4 (C-4), 73.1 (C-5), 61.8 (C-6), 118.4, 118.8, 118.9 (galloyl-1), 109.0, 109.1, 109.3 (galloy-2, 6), 144.8, 144.9, 145.0, 145.1, 145.2 (galloy-3, 5), 138.7, 139.0, 139.1 (galloy-4), 164.9, 165.6, 165.9 (C=O).

Caryophyllene oxide (compound g)-EI MS : M+ = 220; ^{1}H-NMR ($CDCl_3$-d4, 500 MHz) δ : 0.96 (6H, s, C(Me)2), 1.71 (3H, s, C(-O-)CMe-), 4.85, 4.95 (2H, m, = CH2).

Caryophyllene (compound h)-EI MS : M+ = 204; ^{1}H-NMR ($CDCl_3$-d4, 500 MHz) δ : 0.97, 1.00 (ea. 3H, s), 1.61 (3H, s), 1.4 ~ 2.6 (m), 4.82 ~ 4.94 (ea. 1H, s), 5.31 (1H, br dd, 9.9, 4.3); ^{13}C-NMR ($CDCl_3$) δ : 154.3 (C3), 134.9 (C7), 124.4 (C6), 117.7 (C12), 53.7,

Table 3. Antimicrobial Activity of Compounds Separated from *R. chinensis* and *M. glyptostoboides*

clear zone size unit : mm

Compound	Conc. (%)	Bacteria			Fungi	
		E. coli	*P. aeruginosa*	*S. aureus*	*A. niger*	*C. albicans*
Ethyl gallate	1	13	11	19	-[a]	-[a]
Ethyl 3-digallate	1	10	9	18	-[a]	-[a]
Ethyl *p*-digallate	1	10	9	12	-[a]	-[a]
Tri-*o*-galloyl glucose	1	11	9	14	-[a]	-[a]
Caryophyllene oxide	1	-[a]	-[a]	-[a]	22	20

[a] No antimicrobial activity

48.5, 40.5, 40.0, 34.8, 32.9 (C11), 30.0, 29.4, 28.4, 16.2 (C13).

3.4. Antimicrobial Test of Active Compounds

Antimicrobial activity of separated active compounds was tested by a disk diffusion method. In accordance with above data separated compounds also have antimicrobial activity (Table 3). Ethyl gallate, ethyl-3-digallate, ethyl *p*-digallate and tri-*o*-galloyl glucose separated from *R. chinensis* were effective against bacteria such as *E. coli*, *P. aeruginosa* and *S.aureus* and caryophyllene oxide from *M. glyptostroboides* was effective against fungi such as *A. niger* and *C. albicans*.

3.5. Preservative Efficacy Test in a Cosmetic Emulsion

Even though antimicrobial activity was identified, preservative efficacy should be confirmed in cosmetic formulation by preservative efficacy test to check whether it is compatible with other components in formulation. We tested preservative efficacy of extracts of *M. sieboldii K. Koch* and *R. chinensis* and essential oil of *M. glyptostroboides* in cosmetic emulsion according to the CTFA microbiology guideline. First, we confirmed preservative efficacy of each extracts respectively in emulsion. As a result, in accordance with the results of disk diffusion method and MIC test, *M. glyptostroboides* and *M. sieboldii K. Koch* showed preserving effect just against fungi such as *A. niger* and *C. albicans* (Figure 2). *R. chinensis* showed preserving effect just against bacteria such as *E. coli*, *P. aerugionosa* and *S. aureus* (Figure 3). Second, compared to the formulation containing no preservatives showing bacterial and fungal survival

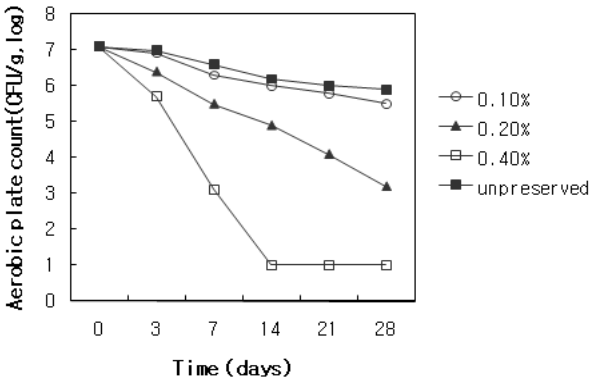
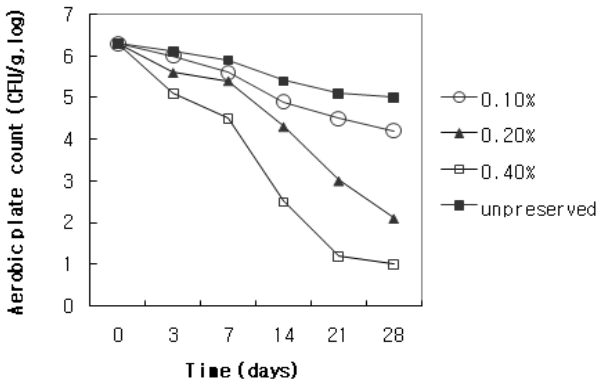


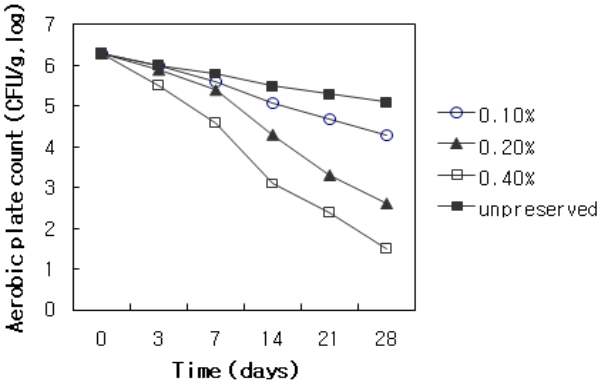
Figure 2. Preservative efficacy against fungi such as *A. niger* and *C. albicans* in O/W cosmetic emulsion. (a) essential oil of *M. glyptostroboides* and (b) extracts of *M. sieboldii K. Koch* ○ : 0.1 %, ▲ : 0.2 %, □ : 0.4 % of samples and ■ : unpreserved.

after 7 days, formulations containing extracts of *M. sieboldii K. Koch* or essential oil of *M. glyptostroboides* showed remarkable reduction of fungi such as *A. niger* and *C. albicans* after 7 days and *R. chinensis* showed remarkable reduction of bacteria such as *E. coli*, *P. aerugionosa* and *S. aureus* after 7 days. Their microbial death rate at minimum inhibitory concentration in cosmetic emulsion is satisfying the Cosmetic, Toiletry and Fragrance Association (CTFA) criteria, which is judged effective in the examined product if inoculated viable bacteria showed a 3log10 CFU/g reduction and fungi showed a 1 log 10 reduction by 7^{th} day and no growth until 28^{th} day.

Although it was confirmed that essential oil of *M. glyptostroboides* and extracts of *M. sieboldii K. Koch* have preserving effect against fungi such as *A. niger*



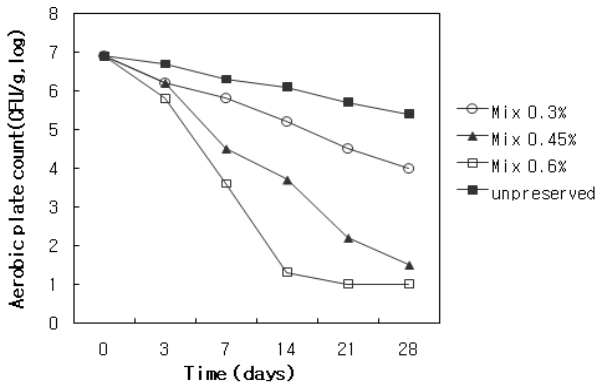
(a)



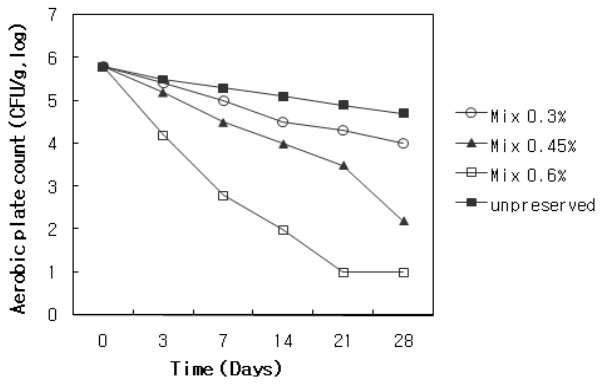
(b)

Figure 3. Preservative efficacy of extracts of *R. chinensis* against bacteria such as *E. coli*, *P. aerugionosa* and *S. aureus* in O/W cosmetic emulsion. ○ : 0.1 %, ▲ : 0.2 %, □ : 0.4 % of extracts of *R. chinensis* and ■ : unpreserved.

and *C. albicans* and extracts of *R. chinensis* against bacteria such as *E. coli*, *P. aerugionosa* and *S. aureus* respectively, it is insufficient to use just one kind of extracts or essential oil for preserving cosmetics. Therefore we checked whether they show synergistic effect when they are mixed together. Extracts of *M. sieboldii K. Koch* and *R. chinensis* and essential oil of *M. glyptostroboides* were mixed altogether in a ratio of 1 : 2 : 1 and its preservative efficacy was confirmed in cosmetic emulsion. As a result mixture showed synergistic preserving effect against both bacteria such as *E. coli*, *P. aerugionosa* and *S. aureus* and fungi such as *A. niger* and *C. albicans* at 0.6 % of mixture and it was maintained after 28 days from inoculation (Figure 4).



(a)



(b)

Figure 4. Preservative efficacy of mixture of *M. glyptostroboides*, *R. chinensis*, and *M. sieboldii K. Koch* (a) against bacteria such as *E. coli*, *P. aerugionosa* and *S. aureus* and (b) against fungi such as *A. niger* and *C. albicans*. ○ : 0.3 %, ▲ : 0.45 %, □ : 0.6 % of mixture and ■ : unpreserved.

4. Conclusion

We studied the antimicrobial effect of 3 plants, *M. glyptostroboides*, *R. chinensis* and *M. sieboldii K. Koch*. Essential oil of *M. glyptostroboides* and extracts of *M. sieboldii K. Koch* were found to have antifungal activity and extracts of *R. chinensis* have antibacterial activity. The mixture of *M. glyptostroboides*, *R. chinensis* and *M. sieboldii K. Koch* was shown to have synergistic preservative efficacy satisfying the Cosmetic, Toiletry and Fragrance Association (CTFA) criteria. Based on the above data we suggest that extracts of *M. glyptostroboides*, *R. chinensis* and *M. siebodii K.*

Koch have antimicrobial activity and replace chemical synthetic preservatives. Beside anti-microbial activity, *M. glyptostroboides* and *M. siebodii K. Koch* are known to help reduce wrinkle formation by inhibiting MMP1 synthesis and whiten skin color by inhibiting α-MSH induced melanogenesis respectively. Putting all data together, we suggest extracts from *M. glyptostroboides*, *R. chinensis* and *M. siebodii K. Koch* be not only natural alternative preservatives but also effective composites for cosmetics. Later we are planning to study each active compounds have preservative efficacy in cosmetic formulation to evaluate its possibility as an alternative preservative.

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