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Comparison of Biological Activities on Extracts and Fractions in *Compositae* Plants

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국화류 추출물 및 분획물의 생리활성 비교연구

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Abstract This study was conducted to investigate the anti-oxidative, anti-oxidative and tyrosinase inhibitory effects of MeOH 80% extract and hexane, chloroform, ethyl acetate, butanol and aqueous fraction on three kinds of *compositae* plants in Korea. In the antimicrobial effect, the extract and chloroform fraction of *Eclipta prostrata* and hexane fraction of *Carpesium abrotanoides L.* and chloroform fraction of *Siegesbeckia glabrescens* exhibited significant inhibition. The antioxidant activity of ethyl acetate and butanol fractions was more than 90% in all three plants. In case of tyrosinase activity, showed a potent inhibition ethlyacetate fraction of *Siegesbeckia glabrescens* and *Carpesium abrotanoides L,* which were higher than control group. In MeOH 80% extracts, there was not found to have antimicrobial, anti-oxidant and tyrosinase inhibitory activity, however there was ethylacetate fraction of *Siegesbeckia glabrescens* to show effectss commonly in the three assay system.

Key Words : Anti-microbial, Anti-oxidation, *Compositae*, Tyrosinase inhibitory activity, Cosmetic

요 약 본 연구는 우리나라 전역에 자생하는 국화류3종 학슬, 희렴, 한련초를 대상으로 MeOH 80% 추출물 및 헥산, 클로로포름, 에틸아세테이트, 부탄올 및 물분획을 이용한 항균활성, 항산화 및 tyrosinase 저해활성효과에 대한 연구를 통해 기능성 화장품소재로서의 이용가능성을 확인하고자 하였다. 연구결과 항균활성의 경우 MeOH 80% 추출물에서는 한련초와 용매분획 중에서는 학슬의 헥산분획, 희렴의 클로로포름분획, 에틸아세테이트 분획, 한련초의 클로로포름분획을 중심으로 항균력을 나타내었다. 항산화 활성의 경우 3종 모두 용매분획 중 에틸아세테이트, 부탄올 분획에서 90%이상의 효과를 나타내었다. Tyrosinase 저해활성의 경우 희렴과 학슬의 에틸아세테이트 분획물에서 우수한 활성을 나타내었다. 이상의 국화류3종 MeOH 80%추출물 및 분획물에서 항산화 및 미백작용이 우수하므로 기능성화장품소재 개발 활용이 가능할 것으로 생각된다.

주제어 : 항균, 항산화, 국화류, 미백활성, 화장품

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1. Introduction

Reactive oxygen species (ROS) generated during the oxidation process for energy production in vivo lead to irreversible damage of major substances in the body such as lipids, proteins, and nucleic acids, and cells are continuously exposed to excessive oxidative stress Is not only a cell death but also a cause of various diseases if the balance with the antioxidant defense system is broken[1,2]. The skin is composed of the dermis and the epidermis, and the epidermis protects and protects against various physical, chemical and mechanical stimuli and excessive divergence of body moisture.[3,4]. Damage to the skin is exacerbated by environmental factors such as ultraviolet rays and dry climate, as well as by stresses and diseases[5]. The skin is protected against skin cell damage due to ultraviolet light photoaging, and melanin pigment is formed do. Melanin is a black polymeric material that is produced and synthesized by various causes, but its production is promoted by ultraviolet rays, and it moves to and accumulates in the skin layer, causing pigmentation of the skin. This overproduction and accumulation of melanin is involved in causing freckles, spots, erythema, aging and skin cancer[6,7]. Active oxygen species in the skin accelerate aging of the skin along with wrinkle formation by collagen and elastin deformation and promotion of melanin production[8,9]. Promoting melanogenesis is regulated by tyrosinase, tyrosinase-related protein 1 (TRP-1) and tyrosinase-related protein 2 (TRP-2). Tyrosinase catalyzes the hydroxylation reaction to convert tyrosine to 3,4-dihydroxyphenylalanine (DOPA), and DOPA is metabolized to DOPA-quinone[10]. The melanin thus produced primarily removes active oxygen and free radicals generated from the skin and protects the skin by absorbing and blocking ultraviolet rays. However, melanin itself generates active oxygen and accelerates the progress of skin aging[11]. Therefore, by inhibiting the activity of tyrosinase, which is the main cause of skin aging, skin photoaging can be inhibited. In order to suppress skin photo-aging, it has been reported that the raw materials for the whitening function and the other functional ingredients are used as raw materials for skin whitening. However, Korea is highly dependent on imports of natural and other raw materials[12]. Therefore, research effort is needed to mass - produce raw materials that can be obtained in Korea. The *Compositae* plants are one of the most widely distributed and most evolved plant classifications in the world among the modern plants, and has traditionally been used as a medicinal plant, and about 300 species are known to exist in Korea. *Compositae* plants, which live in all parts of Korea and can be edible, have been used for a long time due to their advantages in large quantities and the pharmacological effects of antipyretic, detoxification, analgesic, headache, diuretic, infectious infection, cholanitis, antifungal activity, antioxidant activity, anti-inflammatory activity, and sedation in oriental medicine[13,14]. In this study, three kinds of chrysanthemum plants were selected from 80% extracts of MeOH and extracts from three kinds of chrysanthemums, which were naturally grown in Korea. In this study, hexane, chloroform, ethyl acetate, Butanol and water fraction were investigated for the antimicrobial activity, antioxidant activity and tyrosinase inhibitory activity.

2. Materials and methods

2.1 Production of experimental material

The materials used in the experiment were three kinds of *Compositae* plants which were planted in the ground part of *Carlsium abrotanoides L.,* the whole plant of *Siegesbeckia glabrescens* and *Eclipta prostrata,* After thoroughly drying in a drier, 100 g of the plant was ground and pulverized and stored at 4 ℃ at low temperature.

2.2 Preparation of Extracts and Fractions

The extracts and fractions of the samples are shown in Fig. 100 g of the dried plant was crushed and mixed with 800 ml of a mixture of methanol and water (8:2), heated at 65 ℃ for 24 hours, extracted again for 24 hours, Extracted, concentrated under reduced pressure, and then extracted with a solvent having different polarity, that is, *n*-hexane fraction, chloroform fraction, ethyl acetate fraction, *n*-butylalcohol fraction and aqueous fraction into respective fractions. Total extract 80% MeOH extract and solvent extract fraction were subjected to vacuum distillation to remove the solvent, and the solids were weighed. Then, methanol was added to the solids to give a solid concentration of 10 mg / ml. All subsequent experiments were performed.

2.3 Antimicrobial activity of extracts and fractions against Staphylococcus aureus

The strains used in the experiment were ATCC 6538, a standard strain of *S. aureus*, which is a causative organism of food poisoning, because it is widely distributed in the natural environment such as air and soil. Nutrient medium was used for S. aureus culture. Culture of *S. aureus* was carried out in a BOD incubator at 30 ℃ for 24 to 72 hours on Tryptic soy agar.

2.4 Antimicrobial activity of extracts and fractions against Pseudomonas aeruginosa

Pseudomonas aeruginosa strain ATCC9027 was used as an opportunistic infectious bacterium that is widely distributed in the environment and human body. *P. aeruginosa* cultures were incubated for 24 to 72 hours in a 30 ℃ BOD incubator on Tryptic soy agar.

2.5 Antimicrobial activity of extracts and fractions against Escherichia coli

As a pathogenic *Escherichia coli* strain, ATCC 8739, a standard strain, was used for the experiment. Nutrient medium was used for *E. coli* culture. Culture of *E. coli* was carried out in a BOD incubator at 30 ℃ for 24 to 72 hours on Tryptic soy agar.

2.6 Antimicrobial activity of extracts and fractions against Aspergillus niger

The strains used in the experiment were A. niger, one of the major sources of cosmetics, ATCC 8642, a standard strain. Nutrient medium was used for *A. niger* culture. *A. niger* cultures were prepared by inoculating a single colony of each strain into a new medium, cultivating it for 3 days, sprouting on a potato dextrose agar medium using the spores obtained, placing a sterilized 10 mm filter paper disc on a fancy medium, 50 μl (500 μg) of each fraction was absorbed and placed on the medium. After incubation at 30 ℃ for 3 days, growth inhibition rings around the paper disc were observed.

2.7 Antioxidative activity by DPPH assay

0.5 ml of a sample (extract fraction, solid content concentration 10 mg / ml) dissolved in methanol was added to 1 ml of a 200 μM DPPH ethanol solution by DPPH free radical scavenging method and left at 24 ℃ for 30 minutes The absorbance at 517 nm was measured to determine the concentration of eroded DPPH[15]. The free radical scavenging activity in the antioxidant activity test by DPPH free radical scavenging method was calculated by the following equation. As a positive control sample, vitamin C prepared at the same concentration was used. A is the absorbance after the reaction without addition of the sample, B is the absorbance after the addition and reaction of the sample, and C is replaced with distilled water instead of DPPH.

Scavenging(%)=[1- (B-C)/A] x 100 (%)

2.8. Inhibition of tyrosinase activity of extracts and fractions

Tyrosinase activity inhibition assay was performed by dissolving the sample in ethanol or a suitable solvent, diluting it with a buffer such as 0.1 M phosphate buffer (pH 7.0) and diluting it to a suitable concentration range to inhibit the activity against DOPA oxidation reaction. Respectively. 850 ㎕ of 0.1 M phosphate buffer (pH 7.0), 50 ㎕ of sample solution and 50 ㎕ of mushroom tyrosinase (1500 ~ 2000 U / ml) were added to the test tube in order and reacted at 37 ℃ for 6 minutes. To this solution, 50 ㎕ of 0.06 ㎛M L-DOPA (L-3,4-dihydroxyphenylalanine) was added and reacted at 37 ℃ for 1 minute. Absorbance was measured at 475 nm using a microplate reader. For 1 min and absorbance was measured at 475 nm using a microplate reader. A 0.1M phosphate buffer solution (pH 7.0) was used instead of the sample solution. For the positive control sample, arbutin, which had already been commercially available as a whitening active agent, was used at the same concentration. The inhibition rate of DOPA oxidation activity was calculated as 100 - reaction absorbance of each sample liquid / reaction absorbance of the blank sample liquid x 100 (%).

3. Results and Discussion

3.1 Antimicrobial activity of extracts and fractions

Table 1 shows the results of the antibacterial tests of MeOH 80% extracts and organic solvent fractions of three kinds of *Compositae* plant. In the case of MeOH 80% extract and organic solvent fraction antibacterial test, *S. aureus* 80% extract and solvent fraction The antimicrobial activity was shown in chloroform, butanol fraction and water fraction. In the case of *E. coli,* hexane fraction and water fraction in the solvent fraction showed antibacterial activity in the hexane fraction, chloroform fraction and butanol fraction in P. aeruginosa.

In the case of *S. aureus,* 80% extract of MeOH and 80% of organic solvent fraction showed the antibacterial activity against chloroform fraction and water fraction of 80% extract and solvent fraction of MeOH, 80% extract and solvent fraction of MeOH for E. coli, Chloroform fraction, butanol fraction, and water fraction. In *P. aeruginosa,* only the chloroform fraction showed antibacterial activity. As a result of observing the growth inhibition rings caused by the extracts and solvent fractions of methanol of the three kinds of the above three kinds of *Compositae* plan the clear zone of the growth inhibition was maintained for the *E. coli* and *P. aeruginosa* strains continuously And it was confirmed that it could be used as an antimicrobial active material having a sterilizing effect as well as a growth inhibiting effect.

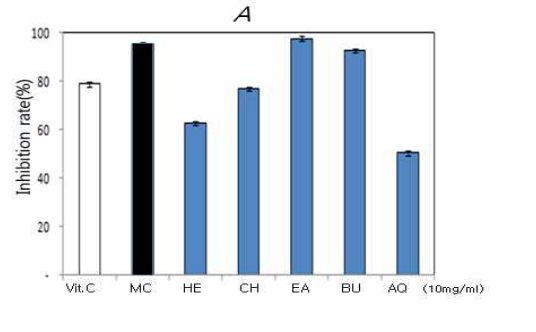
Table 1. Antimicrobial effect of each plants extracts on various microbial strains

Sample / Fraction strain		Clear zone on plate					
		MC	HE	CH	EA	BU	AQ
Carpesium abrotanoides L.	*S. aureus*	+	−	++	−	+	+
	E. coli	−	++	−	−	−	+
	P. aeruginose	−	++	+	−	+	−
	A. niger	−	−	−	−	−	−
Siegesbeckia glabrescens	*S. aureus*	−	−	++	+	−	+
	E. coli	−	+	+	++	−	−
	P. aeruginose	+	+	+	++	+	−
	A. niger	−	−	−	−	−	−
Eclipta prostrata	*S. aureus*	+	−	+	−	−	+
	E. coli	+	−	+	−	+	+
	P. aeruginose	−	−	+	−	−	−
	A. niger	−	−	−	−	−	−

Symbols: +++, very strong; ++, medium; +, weak; −, none. methanol 80% extract (MC), *n*-hexane fraction (HE), chloroform fraction (CH), ethylacetate fraction (EA), *n*-butylalcohol fraction (BU) and aqueous fraction (AQ).

3.2 Antioxidative effect of extracts and fractions

Fig. 1 shows the antioxidant activity of hexane, chloroform, ethyl acetate, butanol and water fraction of DPPH from the extracts and extracts of MeOH 80% Vitamin C as a control sample showed about 79% of activity. *Carpesium abrotanoides L.* and *Siegesbeckia glabrescens* showed more than 90% antioxidative activity in 80% MeOH extract. Ethyl acetate fraction and butanol fraction of *Siegesbeckia glabrescens* showed more than 90% activity in hexane fraction, chloroform fraction, ethyl acetate fraction and butanol fraction. The ethyl acetate fraction and butanol fraction of *Eclipta prostrata* showed more than 90% antioxidant activity. Among the three plants, *Carpesium abrotanoides L.* showed excellent antioxidant activity in all fractions compared to other plants. All three water fractions showed lower activity than vitamin C, the control sample. In this experiment, it is highly possible that the flavonoid components contained in the plant tops and the upper parts of the used plants were significantly extracted from the polar solvent, which showed higher activity than the vitamin C in the ethyl acetate and butanol fractions. These antioxidant enzymes are likely to be able to remove free radicals from the body, so they can be developed as natural products for functional foods and functional cosmetics. It is highly likely to lead to the development of more stable natural antioxidants because it is obtained from natural products.



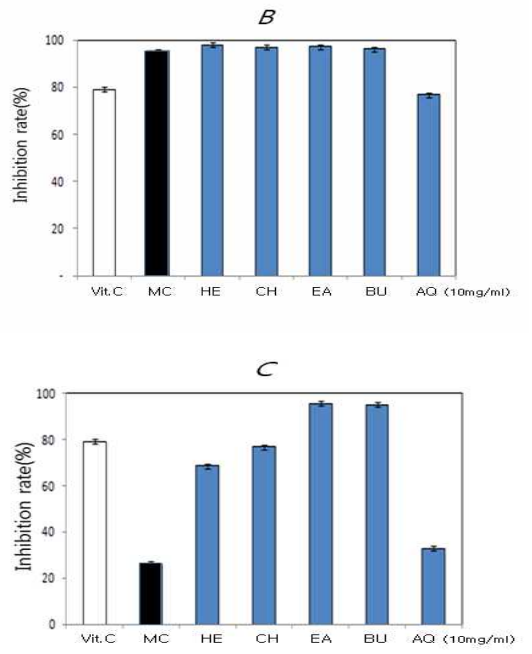


Fig. 1. DPPH radical-scavenging activities of each plants. methanol 80% extract (MC), *n*-hexane fraction (HE), chloroform fraction (CH), ethylacetate fraction (EA), *n*-butylalcohol fraction (BU) and aqueous fraction (AQ). *A : Carpesium abrotanoides L.; B : Siegesbeckia glabrescens; C : Eclipta prostrata*

3.3 tyrosinase inhibitory effect of extracts and fractions

Fig. 2 showed inhibition of tyrosinase activity by inhibition of DOPA oxidase activity against hexane, chloroform, ethyl acetate, butanol and water fraction from extracts and extracts of MeOH 80% of three kinds of *Compositae* plant. Arbutin used as a positive control showed inhibitory activity of about 22%. In all three kinds, only ethyl acetate fraction was 39.7% and 40.5%, respectively. 18.7%, which is higher than or similar to that of arbutin. In this experiment, phenolic compounds extracted from polar and non-polar solvents among the three active components of plants were thought to inhibit tyrosinase relatively strongly. Each of these solvent fractions contains a mixture of various substances, but arbutin is a pure single substance. Thus, when

separating each fraction into a single substance, the whitening activity effect appears stronger than that of the solvent fraction.

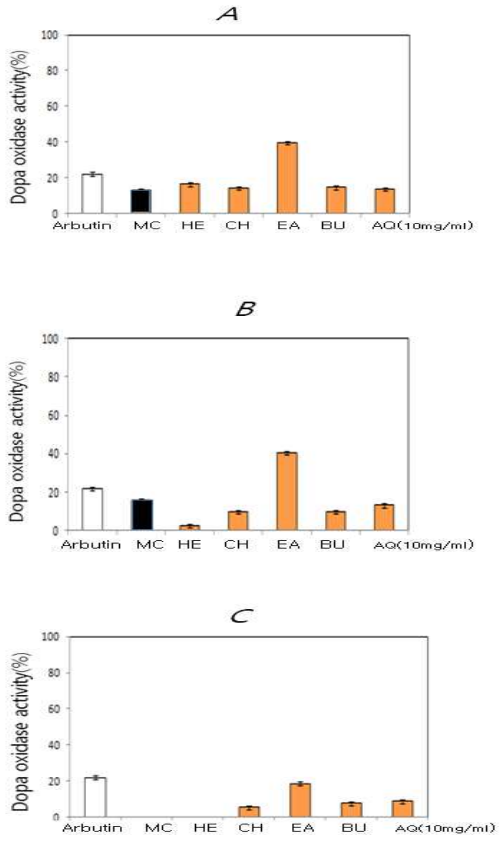


Fig. 2. Effect of fraction tyrosinase inhibition activity from each plants.methanol 80% extract (MC), *n*-hexane fraction (HE), chloroform fraction (CH), ethylacetate fraction (EA), *n*-butylalcohol fraction (BU) and aqueous fraction (AQ). *A : Carpesium abrotanoides L.; B : Siegesbeckia glabrescens; C : Eclipta prostrata*

4. Conclusion

This study was conducted to investigate the antimicrobial activity, antioxidant activity and tyrosinase inhibitory activity of 80% MeOH extracts and extracts of *Carpesium abrotanoides L.*, *Siegesbeckia glabrescens* and *Eclipta prostrata*, which are native to Korea, using hexane, chloroform, ethyl acetate, butanol and water fraction The purpose of this study was to investigate the possibility of using as a raw material for functional cosmetics related to anti - aging and whitening. The results of the study are as follows. The results of the study are as follows. Antimicrobial activity of Eclipta prostrata and 80% MeOH extracts showed antimicrobial activity against hexane fraction of *Carpesium abrotanoides L.*, chloroform fraction and ethyl acetate fraction of *Eclipta prostrata*, and chloroform fraction of *Siegesbeckia glabrescens*. In the case of the oxidative activity, the antioxidative activity of ethyl acetate and butanol fraction of the three solvent fractions was almost 90%. Tyrosinase inhibitory activity of ethyl acetate fraction of *Siegesbeckia glabrescens* and *Carpesium abrotanoides L.* showed better activity than arbutin. There were no extracts of the three kinds of *Compositae* plants MeOH 80% extracts, and only the ethyl acetate fraction of *Siegesbeckia glabrescens* showed uniform activity against antibiotic, antioxidant and tyrosinase inhibitory activities.

These results suggest that the extracts and fractions of MeOH 80% extracts and fractions of 3 kinds of *Compositae* plants can be used effectively in the development of functional cosmetic materials because of their excellent antioxidant and whitening action.

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