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Antibacterial, whitening, and anti-wrinkling effects of essential oil from *Curcuma aromatica* leaves

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ABSTRACT

In this study, the chemical compositions of steam-distilled essential oil from the leaves of Curcuma aromatica, as well as its antibacterial, anti-elastase, and anti-melanogenic activities, were investigated for the first time. The chemical constituents of the essential oil were further analyzed by GC–MS and were found to be α-terpinolene (44.81%), 1,8-cineole (13.88%), and bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene- (11.76%). The antibacterial activities of C. aromatica oil (CAO) against drug-susceptible and drug-resistant skin pathogens were also examined. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values indicated that CAO has excellent antibacterial activities. The MIC of CAO against drug-susceptible and drug-resistant skin pathogens ranged from 70 to 400 μg/mL. In addition, the capacity of the CAO to inhibit elastase, a key enzyme known to be involved in skin wrinkle formation, was investigated. CAO contained moderate anti-elastase activity (IC_{50}: 667.88 μg/mL). Furthermore, CAO reduced α-melanocyte stimulating hormone (α-MSH)-induced melanin production in B16/F10 murine melanoma cells, indicating that it has anti-melanogenic effects. These findings demonstrate that CAO has great potential for use in promoting human skin health.

Keywords: Chemical composition, *Curcuma aromatica*, Elastase, Essential oil, Melanogenesis

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INTRODUCTION

Essential oils are volatile, natural, and complex compounds characterized by a strong odor, and are produced by aromatic plants as secondary metabolites. Structurally, the oils contain hydrocarbons such as terpenes and sesquiterpenes and oxygenated compounds such as alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, and phenol ethers. Currently, approximately 3500 essential oils are known, 350 of which are commercially important, especially as ingredients in cosmetic and sanitary products and food preservatives and additives and as natural remedies. For example, d-limonene, citronellal, geraniol, eugenol, and citral are employed in perfumes, creams, and soaps; flavor additives for food; fragrances for household cleaning products; and industrial solvents. Therefore, both industrial and academic research fields are increasingly focusing on aromatic plants with biological properties that can be used to improve skin health [1-4].

Curcuma aromatica Salisb., a traditional and perennial herb belonging to the Zingiberaceae family, is of great medicinal importance in Korea [5]. Studies have demonstrated that *C. aromatica* oil (CAO), a volatile oil extracted from this herb, can reduce total collagen in rats with renal fibrosis, ameliorate symptoms of diabetic nephropathy in rats, and protect against renal fibrosis through the up-regulation of heme oxygenase and through the Nrf2-Keap1 signal transduction pathway [6-9]. However, to the best of our knowledge, no prior studies have reported on the essential oil extracted from *C. aromatica* leaves. Therefore, in the present study, the possibility of its effectiveness as

cosmetic ingredients has been investigated. chemical compositions of steam-distilled essential oil obtained from *C. aromatica* leaves were analyzed by gas chromatography–mass spectrometry (GC–MS), and the effects of essential oil on the growth of skin pathogen and elastase inhibition were studied. In addition, the effects of COA on melanin production in α-MSH-activated B16/F10 murine melanoma cells were also examined.

MATERIALS AND METHODS

Plant

An ethnobotanical survey was carried out in Gujwa-eup area of Jeju Island in August 2015. Voucher specimens were identified by Dr. Minkyung Cho and deposited in the herbarium of Cosmetic Science Center of Jeju National University.

Extraction and Analysis of Essential Oil

Essential oil was extracted from *C. aromatica* leaves by steam distillation as described by Kim *et al* [10]. In brief, approximately 700 g of fresh *C. aromatica* leaves were immersed in 2 L of distilled water in a 10-L three-neck flask. Steam distillation was carried out for 4 h at atmospheric pressure. The essential oil was diluted with ethanol to a final concentration ranging from 70 to 2000 μg/mL. Quantitative and qualitative data were determined by GC and GC-MS, respectively. The oil was injected the Agilent 7890A/5975C XL MSD (Agilent Technologies, USA) operating under electron-impact (70 eV, *m*/*z* 40–450) mode, equipped with a split/splitless injector. The capillary column used was a DB-1HT(0.1 μm × 30 m × 0.32 mm), operated with the following oven temperature program: 40 °C, held for 5 min, rising at 5 °C/min to 100 °C by 17 min, and then rising at 5 °C/min to 230 °C by 43 min; injection temperature and volume were 230 °C and 1 μL, respectively; injection mode, split; split ratio, 10:1; carrier gas, helium at 1.5 mL/min; detector temperature, 230 °C. The oil was identified by comparing its mass spectra with that of the oils from the data bank (Wiley 138 database).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Eight isolates of *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, all of which are known to cause pimples and inflammation on the skin, were obtained from the Culture Collection of Antimicrobial Resistant Microbes (CCARM, Seoul, Korea) and used in the experiments. The growth of bacteria was examined at 37 °C in 0.20 mL of medium (*P. acnes*, *S. aureus*, and *S. epidermidis* were cultured in GAM, LB and TSB, respectively) containing various concentrations of CAO. These tubes were inoculated with 1×10^6 colony-forming units (CFU)/mL and cultured overnight at 37 °C (except for *P. acnes*, which was cultured under anaerobic conditions). After 24 h of incubation, the absorbance was measured spectrophotometrically at 600 nm. The MIC was recorded as the lowest concentration of test samples resulting in the complete inhibition of visible growth. The MBC was determined based on the lowest concentration of extracts required to kill 99.9% of bacteria from the initial inoculum as determined by plating on agar.

Elastase Inhibition Assay

The anti-wrinkle effect was measured by the porcine pancreatic elastase (PPE) inhibition assay [11]. In this assay, N-Succ-$(Ala)_3$-ρ-nitroanilide (SANA) was used as the substrate. In brief, PPE (0.1 mg/mL, 0.013 mL) and SANA (6.25 mM, 0.010 mL) were mixed with tris-hydrochloride (HCl) buffer (0.2 M, pH 8, 0.157 mL) containing the CAO samples (0.020 mL). The test mixture (0.2 mL) was allowed to react for 15 min at 25°C, and the absorbance from the formation of ρ-nitroaniline was measured at 405 nm using an ELISA multiplate reader (Sunrise, Tecan). A similar mixture without CAO and a solution of oleanolic acid were used as negative and positive controls, respectively. Each treatment was carried out three times, and the inhibition activity (%) of elastase was calculated using the following equation:

$$\text{Inhibition (\%)} = [1 - (Abs_{sample} - Abs_{blank})/Abs_{control}] \times 100$$

Cell Culture

The murine B16/F10 melanoma cells were purchased from the Korean Cell Line Bank (KCLB; Seoul, Korea). B16/F10 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (GIBCO, Inc., NY, USA) and 1% penicillin-streptomycin. The cells were maintained in a humid atmosphere of 5% CO_2 at 37°C and were subcultured every 3 days.

Cytotoxicity and Cell Viability

B16/F10 murine melanoma cells were treated with various concentrations (13, 25, 50 μM) of CAO. Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cell viability assay. In brief, cells were seeded into 24-well plates at a density of 2×10^4 cells per well in the presence or absence of CAO or α-MSH. After incubation for 24 h, the cells were treated with various concentrations (12.5, 25, 50, and 100 μg/mL) of

CAO for 72 h. Thereafter, the medium was removed and 200 μL of MTT reagent (2 mg/mL) was added. Absorbance was measured at 540 nm by using microplate reader (Sunrise, Tecan). The effect of CAO or α-MSH on cell viability was evaluated as the relative absorbance when compared with that of control cultures.

Determination of Melanin Content

The melanogenesis inhibitory activity was measured as previously described with some modifications [12]. B16F10 murine melanoma cells were plated at a density of 2×10^4 cells/well and allowed to attach for 24 h in a 5% CO_2 humidified atmosphere incubator at 37°C. The cells were then incubated in fresh medium containing various concentrations (25, 50, and 100 μg/mL) of CAO and α-MSH at 37°C for 72 h. Thereafter, the remaining medium was removed and the plate was washed with ice-cold phosphate-buffered saline (PBS) twice. Cells were harvested by centrifugation, and the cell pellet was resuspended in 100 μL of 1 N NaOH/10% DMSO and incubated at 80°C for 60 min to solubilize the melanin. The relative quantity of intracellular melanin content was estimated by measuring the absorbance at 405 nm using ELISA multiplate reader (Sunrise, Tecan). Arbutin was used as positive control.

Statistical Analysis

All data were presented as mean ± S.D. or relative percentage. Statistical comparisons between groups were performed using Student's t test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSTION

The essential oil from *Curcuma aromatica* (CAO) was analyzed to determine the chemical components. CAO was also tested for antibacterial activity against antibiotic-resistant skin pathogens such as *P. acnes*, *S. aureus*, and *S. epidermidis*. In addition, CAO was evaluated for its anti-elastase and anti-melanogenic activities as well as cytotoxicity towards B16/F10 melanoma cells for development as a potential ingredient in cosmetic products. Steam distillation of *C. aromatica* yielded 0.11% (w/w) essential oil according to dry weight. Thirteen compounds (Table 1) were identified in CAO, representing 92.01% of the total essential oil. Based on GC–MS and GC–FID analyses, the main constituents in CAO were determined to be α-terpinolene (44.81%), 1,8-cineole (13.88%), and bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene- (11.76%).

The *in vitro* antibacterial activity of CAO against drug-resistant *P. acnes*, *S. aureus*, and *S. epidermidis* was assessed by the MIC and MBC values. The MIC was recorded as the lowest concentration (highest dilution) of CAO that inhibited visible growth (no turbidity). The MIC of CAO was determined using a two-fold serial dilution method. As shown in Table 2, CAO exhibited notable antibacterial activity against drug-susceptible and drug-resistant skin pathogens. This oil was most active against antibiotic-resistant *S. epidermidis* CCARM 3711 (MIC value of 70 μg/mL). Meanwhile, the two antibiotic-susceptible strains, *S. aureus* CCARM 0027 and *P. acnes* CCARM 0081, showed less susceptibility to the essential oil (MIC values of 400 μg/mL). These activities can be attributed to the presence of α-terpinolene, 1,8-cineole, bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, α-pinene, limonene, and β-caryophyllene. However, it is difficult to attribute the activity of a complex mixture to a single constituent. Major or trace compounds might give rise to the antibacterial activity exhibited by CAO. Possible synergistic and antagonistic effects of the compounds in CAO should also be considered [13]. To our knowledge, this is the first study to provide data that CAO possess antibacterial activities against drug-resistant skin pathogens. Elastase is a proteolytic enzyme involved in degradation of the extracellular matrix (ECM) that includes elastin. Loss of elastin is a major cause of skin wrinkles. In terms of anti-wrinkle, elastase inhibitors can therefore be useful in preventing loss of skin elasticity and thus skin ageing [14]. As shown in Fig. 1, CAO decreased the activity of elastase in a concentration-dependent manner with an IC_{50} value of 667.99 μg/mL. To investigate the effect of CAO on melanin production, we measured the accumulation of melanin in B16F10 murine melanoma cell line stimulated α-MSH in the presence or absence of CAO for 24 h. Melanin content in α-MSH-stimulated cells increased significantly compared to control. As shown in Fig. 2, CAO at concentrations of 25, 50, and 100 μg/mL inhibited α-MSH-induced melanin production by B16F10 cells by 19.2%, 15.9%, and 36.1%, respectively. The inhibitory activity of CAO was higher than that of the positive control arbutin, which is a known whitening agent. The number of viable activated B16F10 cells was not altered by CAO as determined by MTT assay, indicating that the inhibition of melanin synthesis was not due solely to the cytotoxic effects of CAO. Therefore, CAO may be employed as an effective whitening agent to ameliorate skin stains and freckles.

Table 1. Chemical composition of the essential oils from *Curcuma aromatica* leaves

Peak #	RT (min)	Area (%)	Name	Quality (%)
1	9.623	3.8	α-pinene	96
2	13.337	11.76	bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-	97
3	14.315	1.85	β-myrcene	96
4	15.271	4.33	1-phellandrene	94
5	16.135	3.79	α-terpinene	98
6	16.718	2.37	dl-limonene	99
7	18.853	13.88	1,8-cineole	99
8	22.709	44.81	α-terpinolene	98
9	23.854	1.09	linalool	96
10	25.010	0.91	benzen, 1-methyl-4-(1-methylethenyl)-	96
11	33.106	1.15	β-fenchyl alcohol	91
12	35.286	1.04	benzene, 1-methyl-4-(1-methylethenyl)-	92
13	41.466	1.23	caryophyllene	99
Total		92.01		

Table 2. Antimicrobial activity of the essential oils from *Curcuma aromatica* leaves

	Skin pathogens	MIC (μg/mL)	MBC (μg/mL)
P. acnes	CCARM0081	400	1,000
	CCARM9009	300	1,000
	CCARM9010	400	900
S. aureus	CCARM0027	600	900
	CCARM3708	200	>2,000
S. epidermidis	CCARM3709	200	900
	CCARM3710	200	800
	CCARM3711	70	>2,000

Fig.1. Effects of CAO on elastase activity. Data are expressed as a percentage of control and are mean ± SEM of triplicated experiments

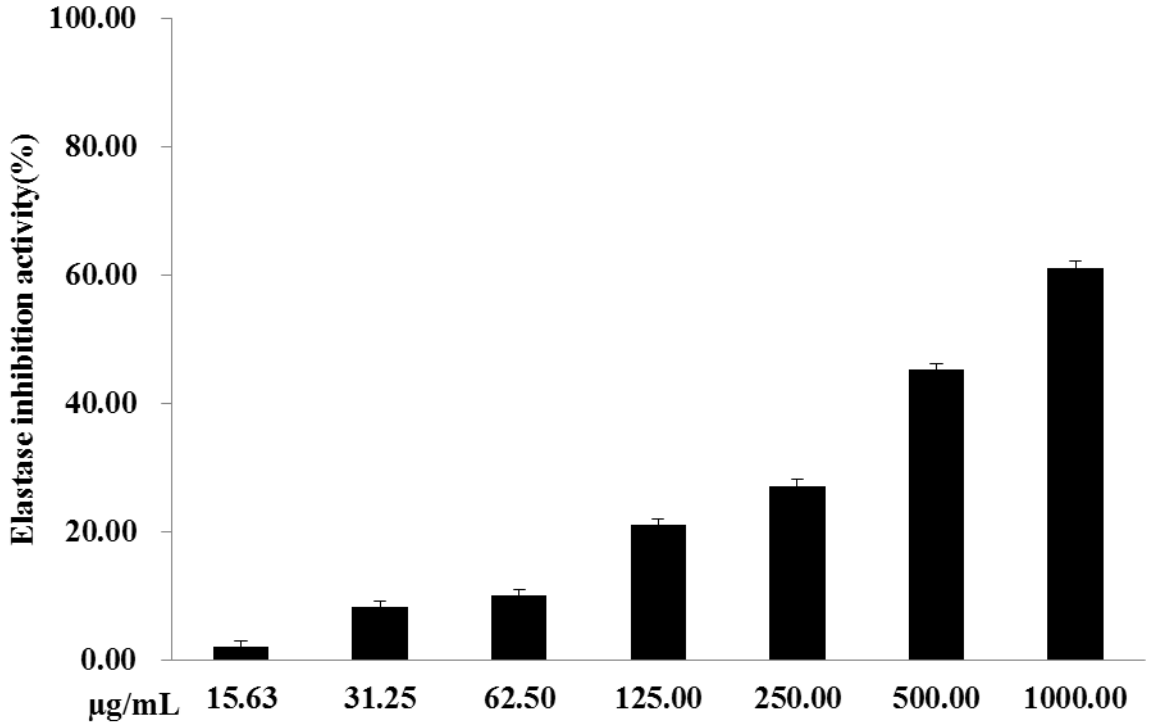
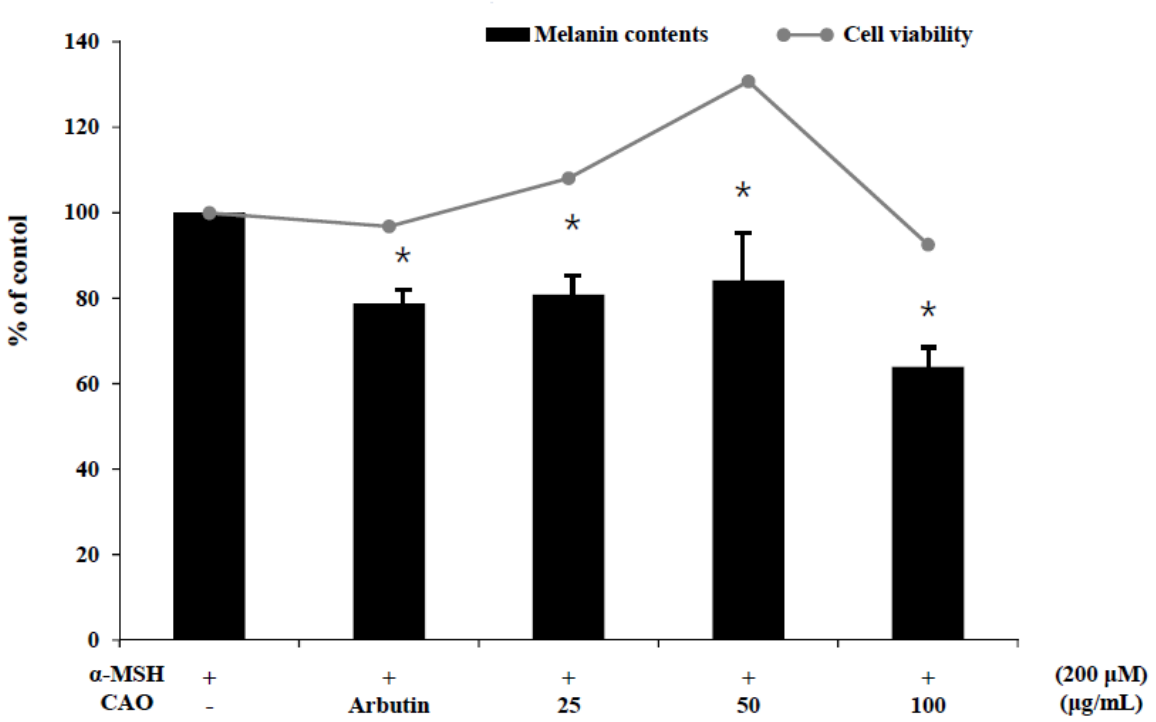


Fig. 2. Effects of CAO on melanin contents and cytotoxicity. After incubation of B16F10 murine melanoma cells with various concentrations of CAO for 72 h, melanin contents and cytotoxicities are determined. Data are expressed as a percentage of control and are mean ± SEM of triplicated experiments



In conclusion, we have demonstrated that CAO actively suppressed the growth of skin pathogens, elastase activity, and melanin production, all of which are implicated in poor skin health. Therefore, we suggest that CAO may be an effective therapeutic agent to promote skin health.

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