Synthesis of Novel Kojic Acid Derivative and Its Anti-pigmentation Effect

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Abstract

A kojic acid derivative, kojic acid 7-O-β-D-tetraacetylglucopyranoside(KTG) was synthesized. Regio- and stereo-selective glycosylation at 7-postion in kojic acid with β-D-pentaacetylglucose was achieved with high yield(80%) by the use of Lewis acid and organic base in nonpolar solvent. KTG was hydrolyzed in methanol by the aid of sodium methoxide to give kojic acid 7-O-β-D-glucopyranoside(KGP). KGP is freely soluble in water and soluble in methanol and ethanol. Its structure was comfirmed by ^{1}H-NMR and ^{13}C-NMR. Tyrosinase activity inhibition of KGP was measured with mushroom tyrosinase compared with ascorbic acid, kojic acid and arbutin. KGP showed higher tyrosinase inhibition activity(IC_{50}=33.3 μg/ml) than ascorbic acid(63.2 μg/ml) and arbutin(91.8 μg/ml) but lower inhibition activity than kojic acid(8.3 μg/ml). To test free-radical scavenging activity, we used 1,1-diphenyl-2-picrylhydrazyl(DPPH) as a free-radical source. Free - radical scavenging activity of KGP was very low(SC_{50}>1000 μg/ml) compared with ascorbic acid(SC_{50}=2.68 μg/ml) and arbutin(SC_{50}=180μg/ml). Melanin formation inhibition of KGP was measured in B16 melanoma, compared with kojic acid, arbutin and Vitamin C. Inhibition activity of KGP for melanin formation was not found within test concentrations.

Introduction

Skin color is mainly determined by the amount of melanin present in the surface of skin. Melanin is synthesized in melanocytes which are normally found in the epidermal basal layer. Within the melanocytes melanin is bound to a protein matrix to form melanosomes. In the melanosomes, tyrosinase converts tyrosine to eumelanin or pheomelanin through the pathways of melanin biosynthesis in the melanocytes. By blocking at the various points of the pathways, skin depigmentation agents can inhibit melanin biosynthesis or even can also be used to treat local hyperpigmention or spots which are caused by a local increase in melanin synthesis or uneven distribution. Last decades, a number of depigmentation agents have been developed and applied to the cosmetics for external uses. Among them, kojic acid is one of popular ingedient for skin whitening agent[1]. It is known that kojic acid inactivates tyrosinase by chelating with its vital copper ion and suppressing the tautomerization from dopachrome to DHICA(5,6-dihydroxyindole-2-carboxylic acid)[2]. Even though kojic acid has been used for long time in cosmetics, its cosmetic applications have been restricted by instability against heat or light.

To overcome these drawbacks and elevate its biological activity, kojic acid derivatives such as kojic dipalmitate[3], kojic acid α-glucopyranoside[4] and amino acid derivatives[5], have been synthesized.

In this study, we synthesized a kojic acid derivative, kojic acid 7-O-β-D-tetraacetylglucopyranoside (KTG) and its hydrolysis product, kojic acid 7-O-β-D-glucopyranoside(KGP). We also tested depigmentation effect of KGP, compared with kojic acid, arbutin and ascorbic acid, by using tyrosinase activity Inhibition assay, free radical scavenging assay and melanin content assay in B16 melanoma.

Materials and methods

Chemicals

Kojic acid, BF_3 etherate, and β-D-pentaacetylglucose were purchased from Sigma-Aldrich Co. All other reagents were used directly without further purification. Mushroom tyrosinase was also perchased from Sigma Co(EC 1.14.18.1)

Equipments

Melting points were taken by MEL-TEMP^R (Laboratory Devices inc. USA). The ^1H and ^{13}C-NMR spectra were recorded by Varian-gemini 200 spectrometer and chemical shifts were referenced to tetramethylsilane(TMS)

Synthesis of kojic acid 7-O-β-D-tetraacetylglucopyranoside(KTG)

Under the stream of nitrogen, to a 300 mL round bottom flask were added kojic aicd (10 g, 0.07 mol), β-D-pentaacetylglucose (27.45 g, 0.07 mol), dried methylene chloride (50 mL) and triethylamine (14.16 g, 0.14 mol). The mixture was stirred while borontrifluoride (39.9 g, 0.28 mol)was added dropwise for 30 min. After stirring for 48 hours at 30°C. After the reaction was terminated and 70 mL of water was added and the organic layer was separated. The separated organic layer was washed with 50 mL of water, dried over anhydrous $MgSO_4$, filtered, and concentrated to yield a crude product. The crude product was recrystallized from toluene to give kojic acid 7-O-β-D-tetraacetylglucopyranoside(33 g). : Yield 80% , mp : 130°C, ^1H-NMR (200 MHz, $CDCl_3$, ppm) 2.04 (d, 6H), 2.1 (d, 6H), 3.70-3.770 (m, 1H), 4.18-4.30 (m, 2H), 4.44-4.69 (m, 3H), 5.03-5.28 (m, 3H), 6.49 (s, 1H), 7.84 (s, 1H).

Synthesis of kojic acid 7-O-β-D-glucopyranoside(KGP)

Under the stream of nitrogen, to 100 mL round bottom flask were added kojic acid 7-O-β-D-tetraacetylglucopyranoside (2 g, 4.23 mmol), dried methanol (10 mL) and sodium methoxide (0.24 g, 4.4 mmol) in methanol (5 mL) solution. The mixture was stirring for 12 hours at reflux condition. After the reaction was terminated, 0.5 g of cation exchange resin was added and stirring for 30

minutes, filtered. The solution was concentrated to yield a crude product. The product was recrystallized from ethanol to give kojic acid 7-O-β-D-glucopyranoside(0.9 g). : Yield 70 %. mp: 194-196°C. ^{1}H-NMR (200 MHz, DMSO-d6. ppm) 3.66-3.75 (m, 2H), 4.25 (d, 1H), 4.47-4.69 (m, 3H), 4.98 (d, 1H), 5.04 (d, 1H), 5.30 (d, 1H), 6.61 (s, 1H), 8.09 (s, 1H), 9.17 (s, 1H). ^{13}C-NMR (50MHz, DMSO-d6, ppm) 60.00, 64.18, 68.95, 72.33, 75.54, 75.96, 101.10, 110.32, 138.28, 144.78, 163.04, 172.79.

Inhibition of tyrosinase activity

Inhibition of tyrosinase activity is generally determined by spectrophotometry. The procedure followed that described by Vanni *et al.*[6]. The reaction mixture consisted of 0.1M phosphate buffer(pH 6.8) 1 mL, 0.3mg/mL L-tyrosine solution 1 mL and 1250U/mL mushroom tyrosinase(Sigma). A sample solution(0.2mL) was added to reaction mixture and incubated at 37°C for 10 min. The optical density at 475nm was measured by a spectrophotometer. The percentage of inhibition was calculated as :

$$\text{Inhibition (\%)} = (A-B/A) \times 100$$

where, A is the enzyme activity without sample, and B is the activity in the presence of sample.

Free radical scavenging activity

Scavenging effect against free radical generation was measured by following the procedure of Fugita *et al.*[7]. The sample solution(1mL) was added to 1mL of 60μM 1,1-diphenyl-2-picrylhydrazine(DPPH) ethanolic solution and kept at room temperature for 10 min. The absorbance was measured at 520nm.

Measurement of melanin contents in B16 melanoma

B16 melanoma cells were purchased from Korean Cell line bank and cultured in DMEM(Dulbecco's modified eagle's medium, sigma, D-2902, St. Louis, MO 63178 USA) supplemented with

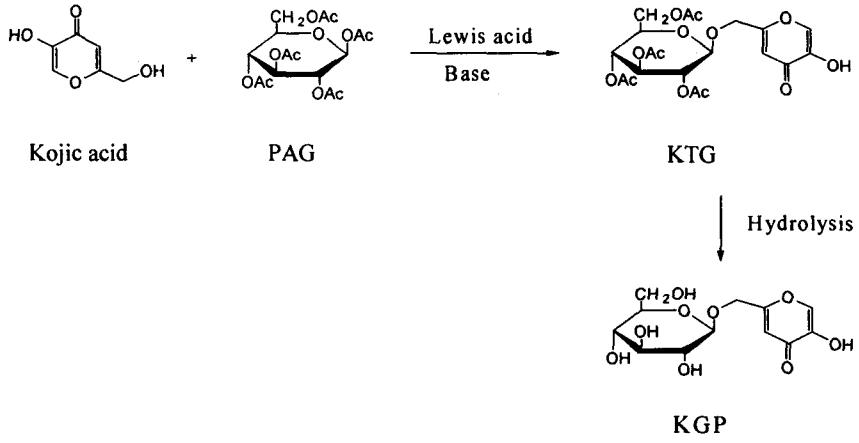
penicillin(100 U/mL), streptomycin(100 U/mL), and 10% fetal bovine serum (Fetal bovine serum, gibco, 26140-079, Invitrogen Co.) at 37 °C in an incubator flushed continuously with 5% CO_2.

B16 melanoma cells cultured above conditions were seeded at 2 x 10^5cell/mL in 12-multiwell plates(Nunc). After one day, media were changed and 0.34 g/mL of a-MSH(α-Melanocyte stimulating hormone, sigma, M-4135) was treated. The desired concentrations of samples (KGP, kojic acid, arbutin, ascorbic aicd) were added to each well. The cells were incubated at 37 °C in a humidified incubator for four days and media of each well was transferred into 15mL tube. After washing with PBS (phosphate buffered saline, 136.89 mM NaCl, 2.68mM KCl, 8.06mM Na_2HPO_4 $7H_2O$, 1.47mM KH_2PO_4, pH7.4), 100 l of Typsin-EDTA(Gibco, 25300-054) was treated in each well and collected cells were transferred into previous 15 mL tube. 500μl of PBS was treated in each well in order to collect remained cells of well. Cells and media were centrifuged at 3000rpm for 30min and supernatants were discarded. 200μl of 1N NaOH were treated and the pellet was sonicated for 20min. Freed melanin was transferred 96 wellplates(Nunc) and measured by ELISA reader(Tecan A-5082, Austria) at 470nm

Results and Discussion

Synthesis of kojic acid 7-O-β-D-tetraacetylglucopyranoside(KTG) and kojic acid 7-O-β-D-gulcopyranoside(KGP)

KTG was synthesized from the reaction of kojic acid with penta-O-acetyl-β-D-glucopyranose in the presence of BF_3 and triethylamine as cocatalysts and characterized its structure by NMR spectrometer. As we reported previously[8] it is well known that the glycosylation of phenols with penta-O-acetyl-β-D-glucopyranose gives phenyl β-glucosides as major products, due to the neighboring group effect of the 2-O-acetyl group.



Scheme 1

KTG was hydrolyzed by the aid of sodium methoxide in methanol to give **KGP**. In ^{13}C-NMR spectrum of **KGP**(Fig. 2), chemical shift of anomeric carbon in glucose residue is appeared at 101.10ppm. According to the literature[4], it is known that chemical shift of α-anomeric carbon of glucose residue is appeared at 98.5ppm. **KGP**, therefore, has β-glycoside bond. Among two kinds of hydroxyl groups of kojic acid(5- and 7-position), 7-position is expected to be more reactive to carbocation due to its higher basicity. Chemical shift of 7-position carbon in ^{13}C-NMR of **KGP** is appeared at 64.18ppm which is coincide with the chemical shift of 7-position isomer in the literature[4].

Inhibition of tyrosinase activity

Figure 3 shows inhibition activity of KGP for tyrosinase, compared with ascorbic acid, kojic acid and arbutin. KGP showed higher tyrosinase inhibition activity(IC_{50}=33.3 μg/ml) than ascorbic acid(63.2 μg/ml) and arbutin(91.8 μg/ml) but lower inhibition activity than kojic acid(8.3 μg/ml). This result can be attributed to the decrease of chelating ability of KGP for copper ion of tyrosinase due to the conjugation of glucose moiety to 7-position.

Free radical scavenging activity

To test free-radical scavenging activity, we used 1,1-diphenyl-2-picrylhydrazyl(DPPH) as a free-radical source. Free radical scavenging activity of KGP was very low(SC_{50}>3000 μg/ml) compared with ascorbic acid(SC_{50}=2.68 μg/ml), arbutin(SC_{50}=180μg/ml) and kojic acid(SC_{50}=1400μg/ml).

Inhibition of melanin formation in B16 melanoma

Inhibition activity of KGP for melanin formation in B16 melanoma was measured, compared with kojic acid, arbutin and Vitamin C. Inhibition activity of KGP was not found within ranges of test concentrations. Conjugation of glucose moiety to kojic acid increases polarity of kojic acid. Because KGP molecule with increased polarity can not easily permeate cell membrane, inhibition activity of KGP against melanin formation in B16 melanoma might be low, even though KGP showed high inhibition activity for tyrosinase. We will carry out further study with other evaluation systems to verify exact action mechanism of KGP.

Conclusions

Kojic acid 7-O-β-D-tetraacetylglucopyranoside(KTG) was synthesized with high yield(80%) by the use of Lewis acid and organic base in nonpolar solvent. KTG was hydrolyzed in methanol by the aid of sodium methoxide to give kojic acid 7-O-β-D-glucopyranoside(KGP). Their structure were comfirmed by ^{1}H-NMR and ^{13}C-NMR. KGP showed higher tyrosinase inhibition activity(IC_{50}=33.3 μg/ml) than ascorbic acid(63.2 μg/ml) and arbutin(91.8 μg/ml) but lower inhibition activity than kojic acid(8.3 μg/ml). Free -radical scavenging activity of KGP was very low(SC_{50}>1000 μg/ml) compared with ascorbic acid(SC_{50}=2.68 μg/ml) and arbutin(SC_{50}=180μg/ml). Melanin formation inhibition of KGP was measured in B16 melanoma, compared with kojic acid, arbutin and Vitamin C. Inhibition activity of KGP for melanin formation was not found within test concentrations, indicating that KGP molecule with increased polarity, compared with kojic acid, can not easily permeate cell membrane.

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Figure Captions

Fig 1. ^{1}H-NMR spectra of kojic acid 7-O-β-D-gulcopyranoside(KGP) in DMSO-d_6

Fig 2. ^{13}H-NMR spectra of kojic acid 7-O-β-D-gulcopyranoside(KGP) in DMSO-d_6

Fig 3. Comparison of tyrosinase activity inhibition; KGP(♦), Arbutin(✖), Kojic acid(■), and Ascorbic acid(▲)

Fig 4. Comparison of free radical scavenging activity ; KGP(♦), Arbutin(✖), Kojic acid(■), and Ascorbic acid(▲)

Fig 5. Comparison of melanogenesis inhibition

Fig. 1

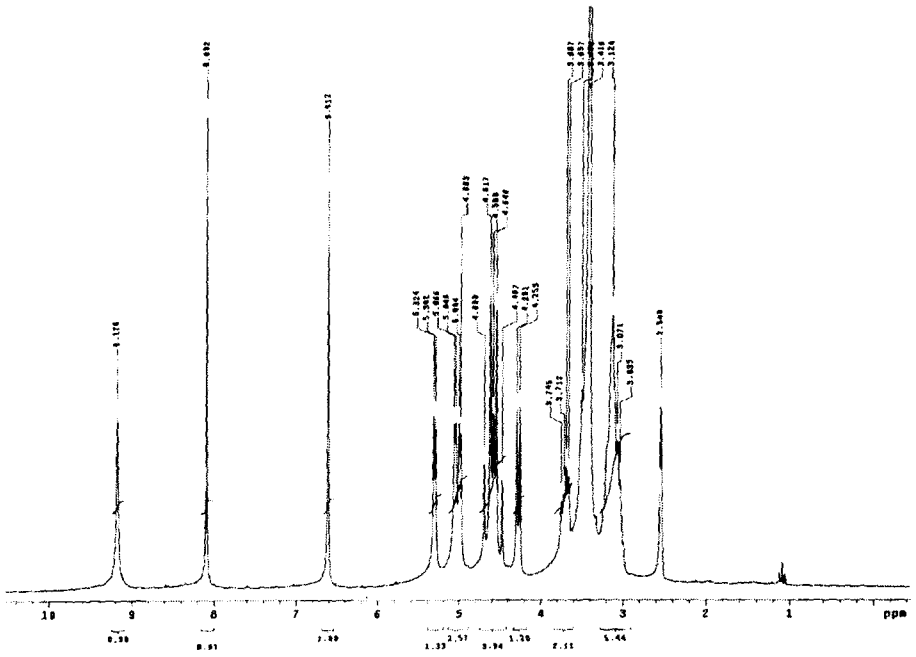
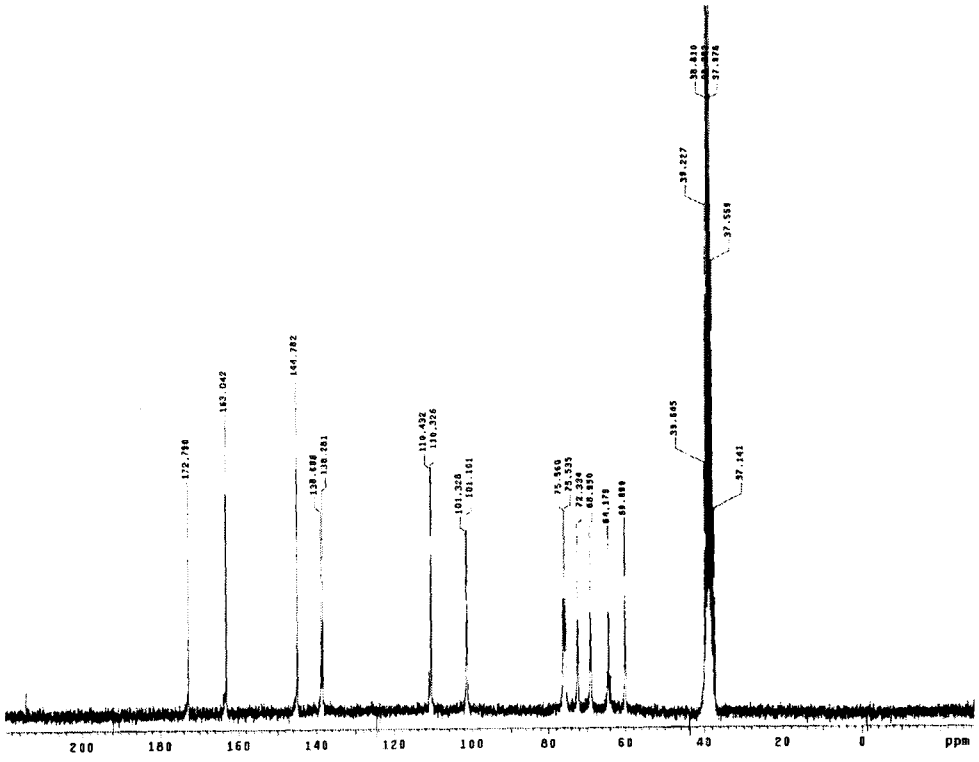


Fig. 2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64.179
59.889
39.810
39.645
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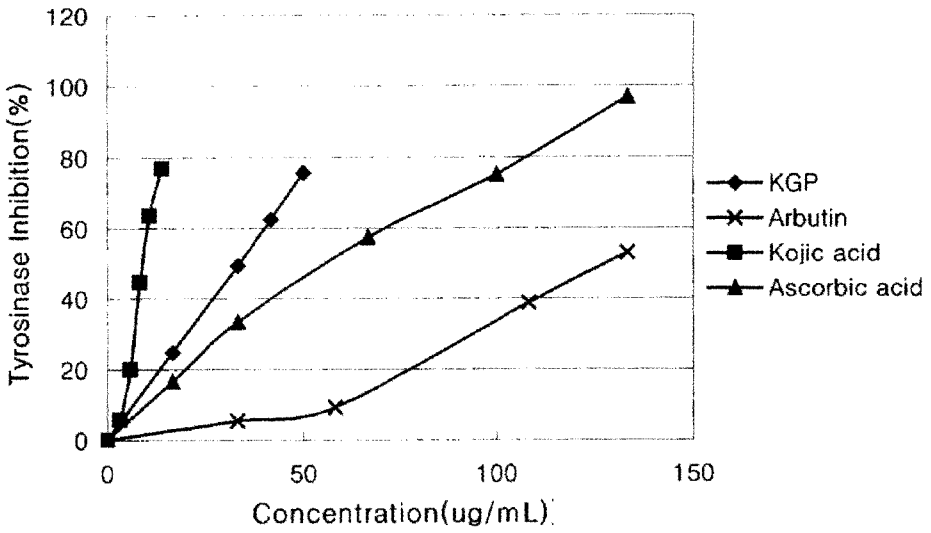
180
160
140
120
100
80
60
40
20
0
ppm

Fig. 3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100
80
60
40
20
0
Tyrosinase Inhibition(%)
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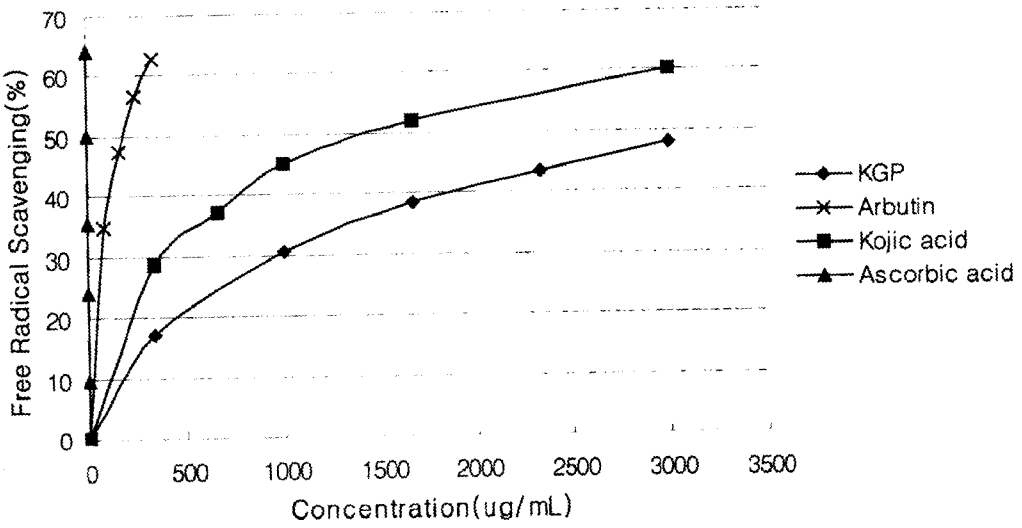
50
100
150
Concentration(ug/mL)
KGP
Arbutin
Kojic acid
Ascorbic acid

Fig. 4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60
50
40
30
20
10
0
Free Radical Scavenging(%)
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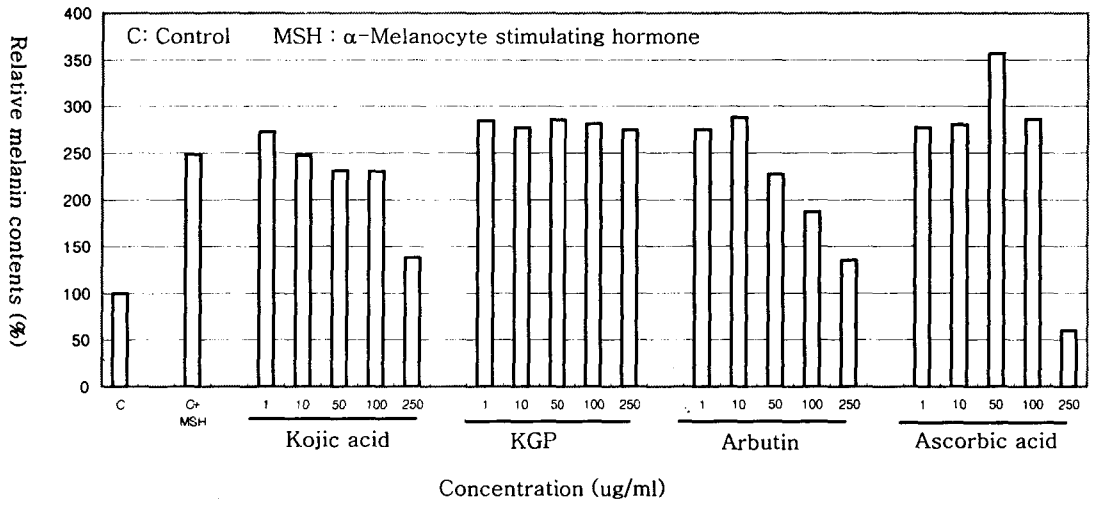
1000
1500
2000
2500
3000
3500
Concentration(ug/mL)
KGP
Arbutin
Kojic acid
Ascorbic acid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Relative melanin contents (%)
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350
300
250
200
150
100
50
0
C
C+ MSH
1 10 50 100 250
Kojic acid
1 10 50 100 250
KGP
1 10 50 100 250
Arbutin
1 10 50 100 250
Ascorbic acid
Concentration (ug/ml)