

자생식물을 이용한 미백화장품 개발

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기능성 화장품

- 피부에 생리 화학적인 변화를 줄 수 있는 화장품
- 미백화장품, 육모제, 양모제, 노화억제제 등
- 2000년 7월부터 법제화

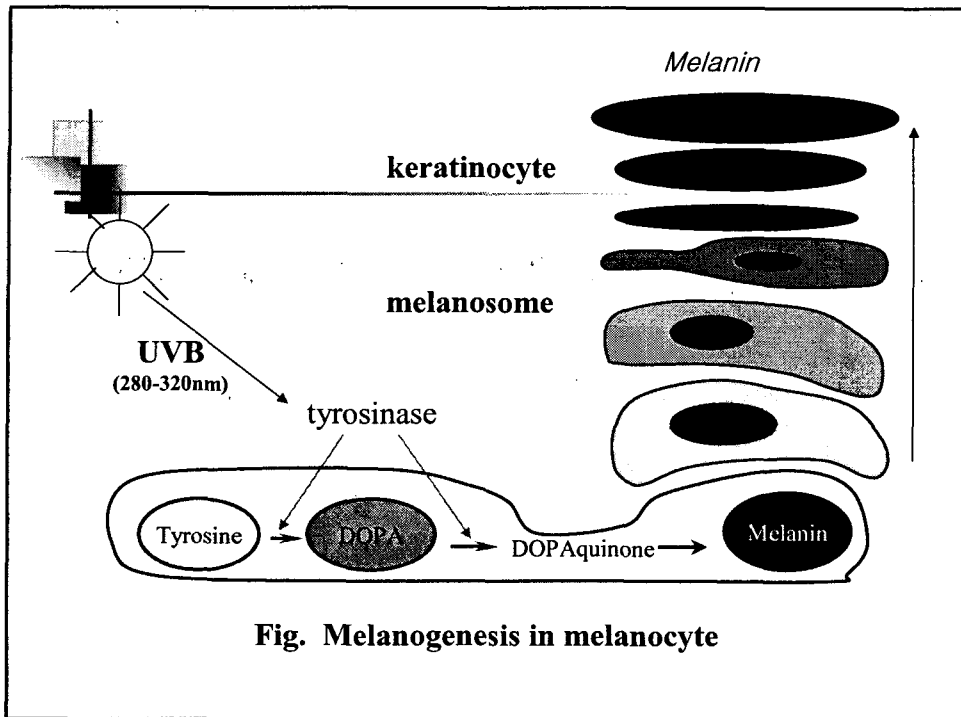


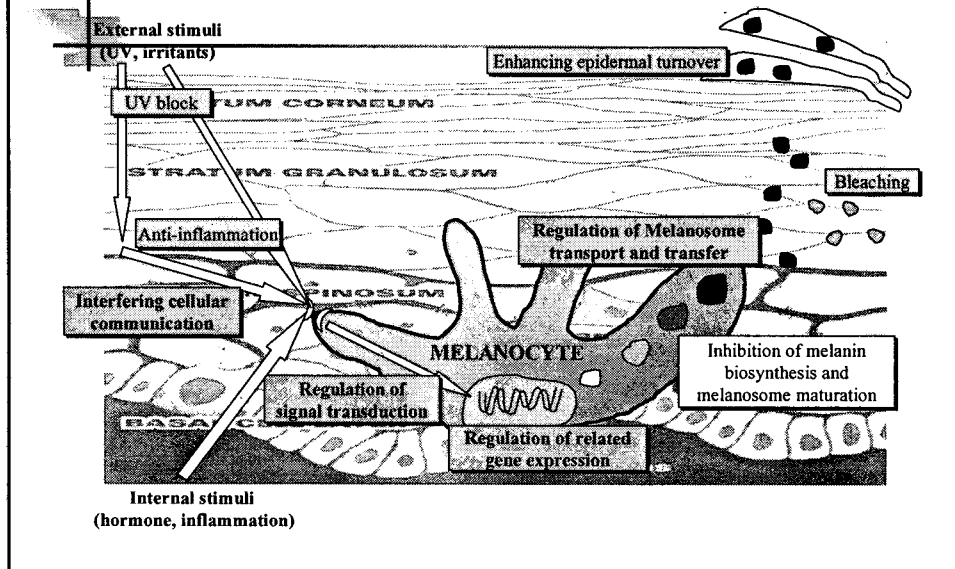
Fig. Melanogenesis in melanocyte

- 피부에 있어서의 melanin의 역할

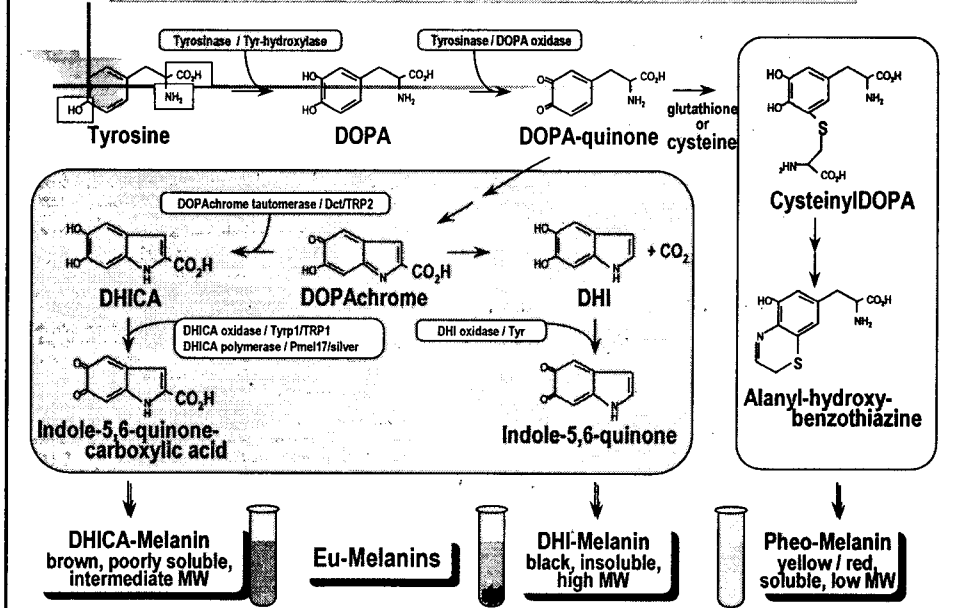
일광의 자외선을 직접 흡수하여 피부를 자외선 장애로부터 보호.

- 피부 그을음
- 홍반
- 수포형성
- 광노화
- 피부면역계 이상
- 피부암

Biological Target Mechanisms



Melanin synthesis in melanocyte



Melanin 생성 억제제 개발 전략

1. Melanocyte의 세포독성

- hydroquinone monobenzyl ether, isopropyl catechol
- 백반증 등 부작용 수반

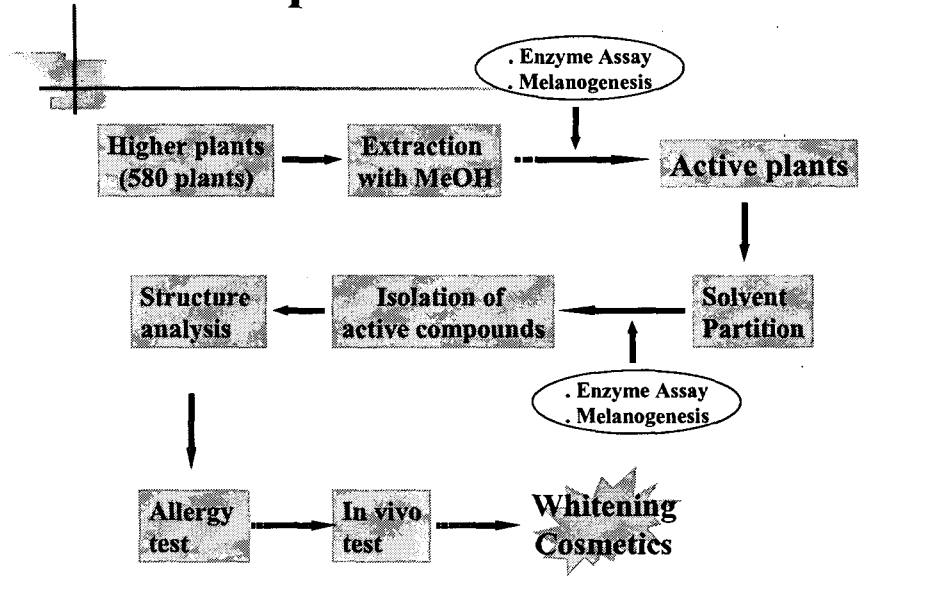
2. Melanocyte 내에서의 melanin 생성 억제

- tyrosinase 활성 저해제 – kojic acid, arbutin
- total inhibitor – 감초, 고삼, 상백피 추출물

Bioassay

- *In vitro* screening
 - Physicochemical evaluations: free radical scavenging, UV absorption/blocking
 - Enzymes: tyrosinase
 - Cell cultures: melanoma cells, melanocytes, melanocyte/keratinocyte co-culture
 - 3D skin cultures: reconstituted epidermis, Dermal and Skin equivalents
- *In vivo* efficacy evaluations
 - Brown guinea pig, hairless mouse
 - Human

Experimental Scheme



Isolation of Inhibitory Compounds
on Tyrosinase Activity from the
Rhizomes of *Curcuma longa*

Procedure of tyrosinase activity test

Reagent preparation

Na-Phosphate buffer: pH 6.8 50mM
Tyrosine: 1.5mM in 50mM Na-phosphate buffer
Dopa: 0.6mM in 50mM Na-phosphate buffer
Tyrosinase: 60U/ml in 50mM Na-phosphate buffer

Sample solution preparation

Taking sample with 10mg/ml
Serial dilution with water
1mg/ml, 0.1mg/ml

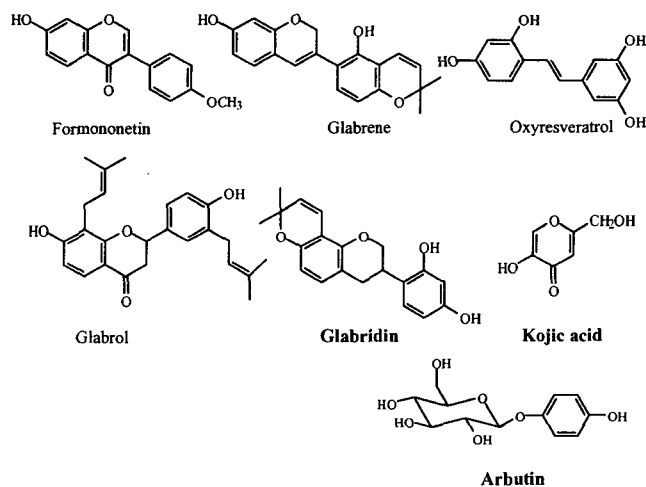
Reading O.D value at 475nm

Measuring Dopachrome

Reaction mixture

Tyrosinase 900ul
Dopa 100ul
Buffer + sample soln 900ul
Incubation for 10min.

Tyrosinase inhibitors isolated from natural sources



[Preparation of tested samples]

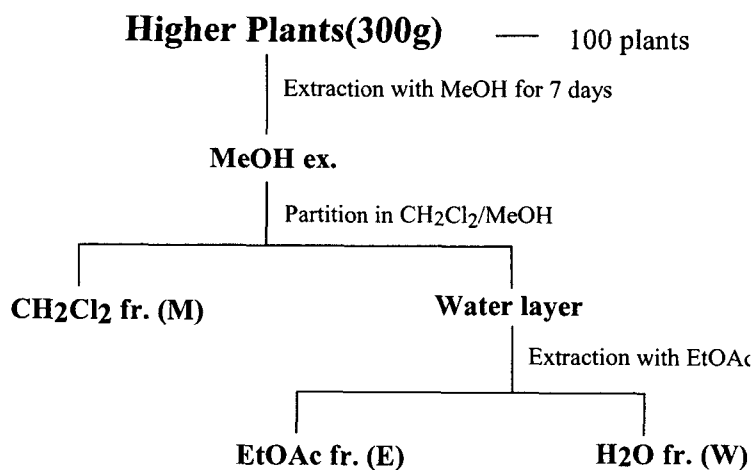
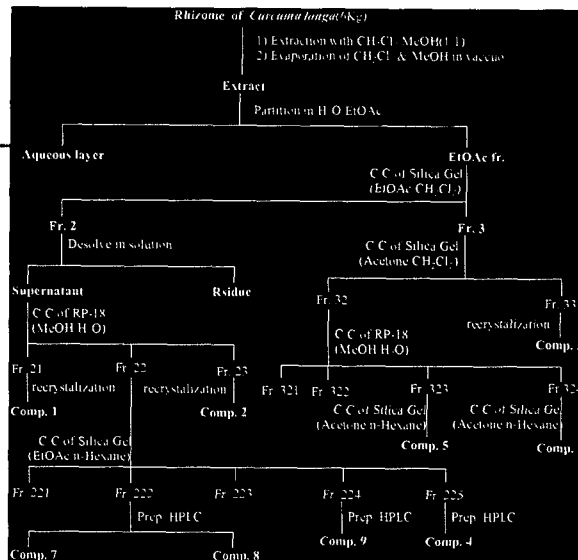


Table Inhibitory effects of MeOH extracts of some higher plants on the tyrosinase activity *in vitro*

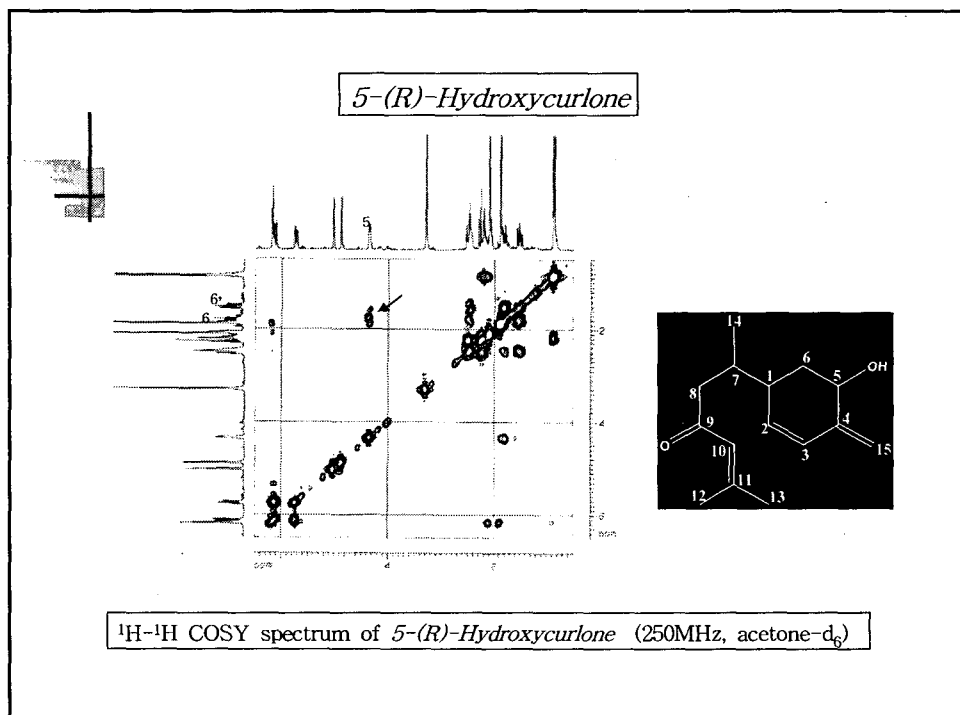
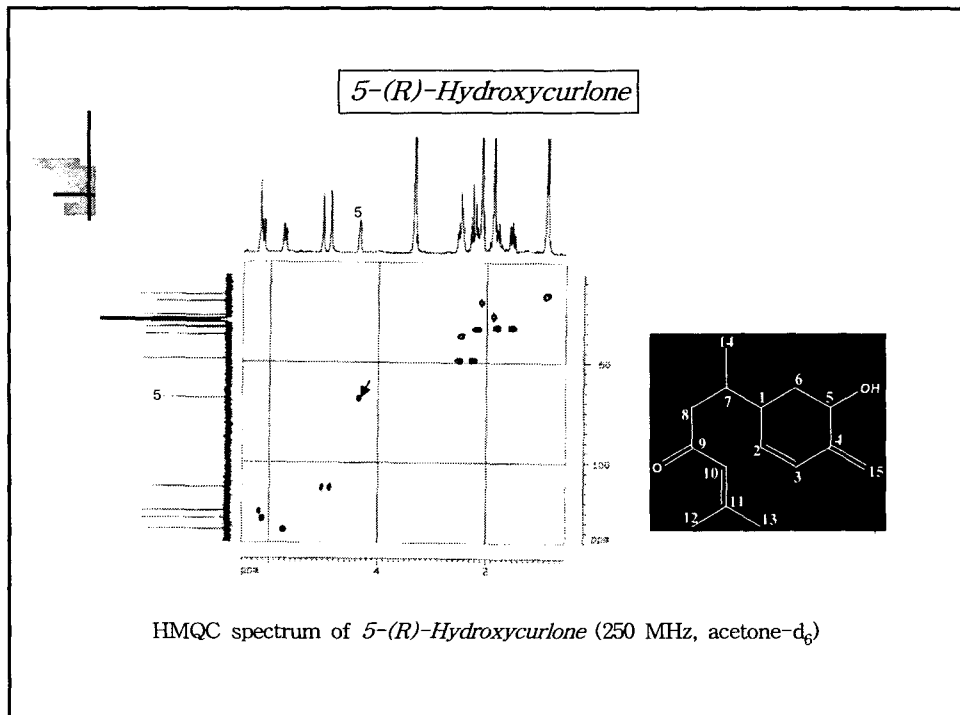
Plant (part of use)	% of inhibition	
<i>Acalypha australis</i> (whole plant)	21	개췌
<i>Acer ginnala</i> (aerial part)	29	신나무
<i>Agrostis clavata</i> var. <i>nukabo</i> (whole plant)	21	거이삭
<i>Alangium platanifolium</i> var. <i>macrophyllum</i> (aerial part)	18	단풍박귀
<i>Artemisia princeps</i> var. <i>orientalis</i> (aerial part)	20	쑥
<i>Arthraxon hispidus</i> (whole plant)	22	조개풀
<i>Asiasarum mandshuricum</i> var. <i>heterotropoides</i> (radix)	15	세신
<i>Aster scaber</i> (whole plant)	29	참취
<i>Athyrium yokoscense</i> (aerial part)	16	밭풀고사
<i>Boehmeria nivea</i> (aerial part)	18	모시풀
<i>Bulbostylis barbata</i> (whole plant)	16	모기풀
<i>Campanula takesimana</i> (whole plant)	15	섬초롱꽃
<i>Carex humilis</i> (underground part)	24	산거울
<i>Carex kobomugi</i> (whole plant)	19	통보리사
<i>Carex siderosticta</i> (whole plant)	16	대사초
<i>Cersis chinensis</i> (aerial part)	22	박태기나
<i>Citrus aurantus</i> var. <i>tachibana</i> (fructus)	15	광귤
<i>Curcuma longa</i> (rhizoma)	44	강황

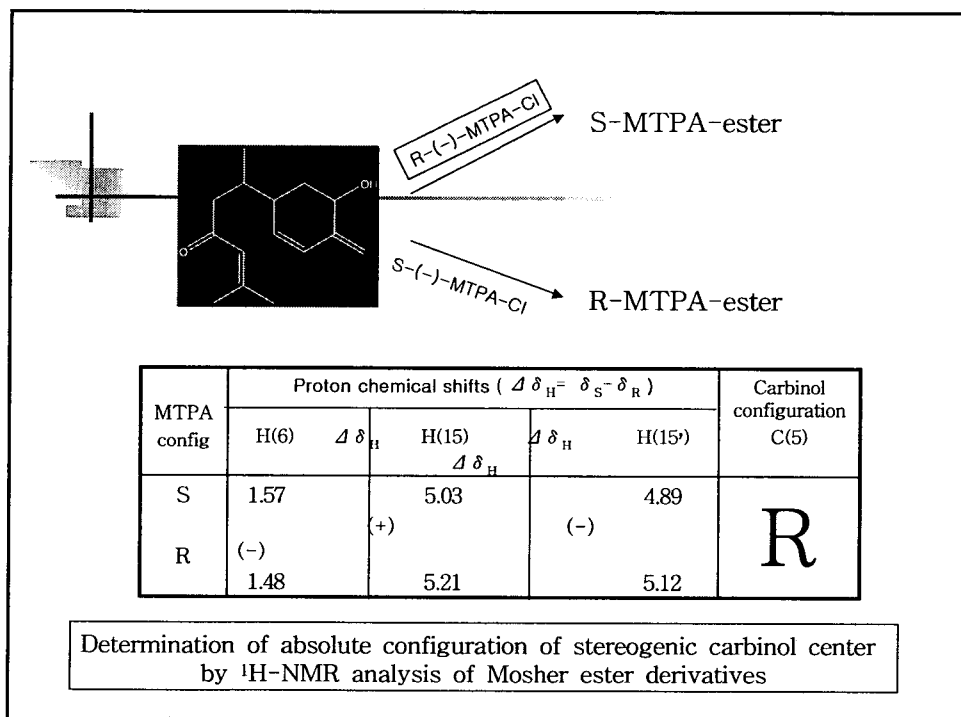
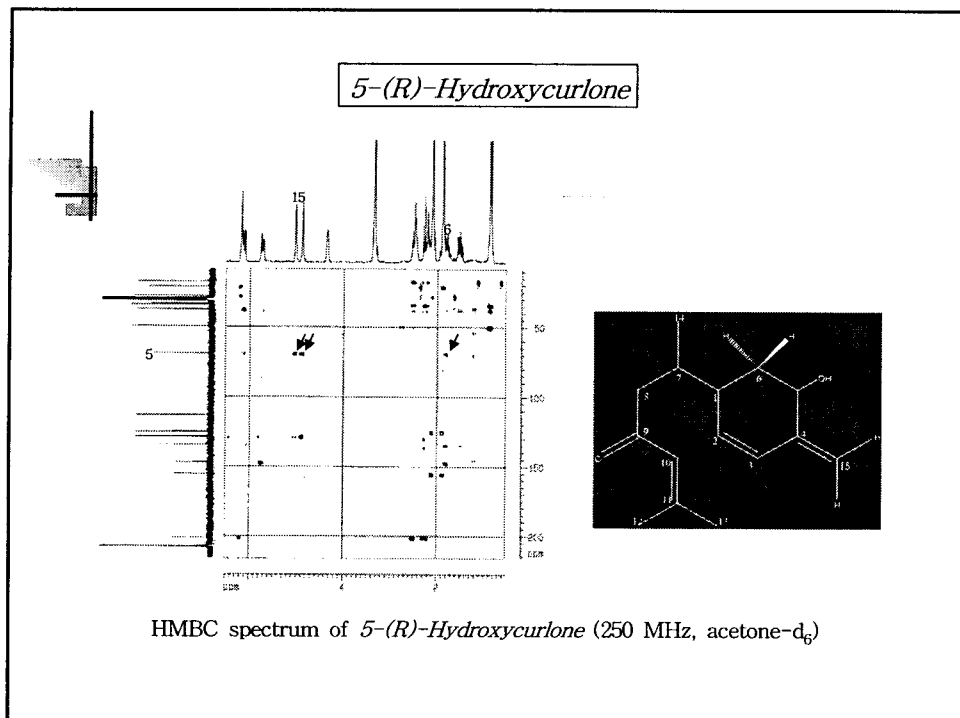
Plant (part of use)	% of inhibition	강 황 (<i>Curcuma longa</i>)	
9. <i>Acer ginnala</i> (aerial part)	29		
37. <i>Aster scaber</i> (whole plant)	29	Fraction	IC ₅₀ value (μ g/ml)
102. <i>Carex humilis</i> (underground part)	24	CH ₂ Cl ₂ fr.	100<
198. <i>Curcuma longa</i> (rhizoma)	44		
226. <i>Desmodium oldhami</i> (whole plant)	23		
283. <i>Dryopteris crassirhizoma</i> (whole plant)	23		
312. <i>Isodon japonicus</i> (aerial part)	29	EtOAc fr.	10 ~ 20
469. <i>Morus alba</i> (radicis cortex)	81		
545. <i>Potentilla paradoxa</i> (aerial part)	25	H ₂ O fr.	200<

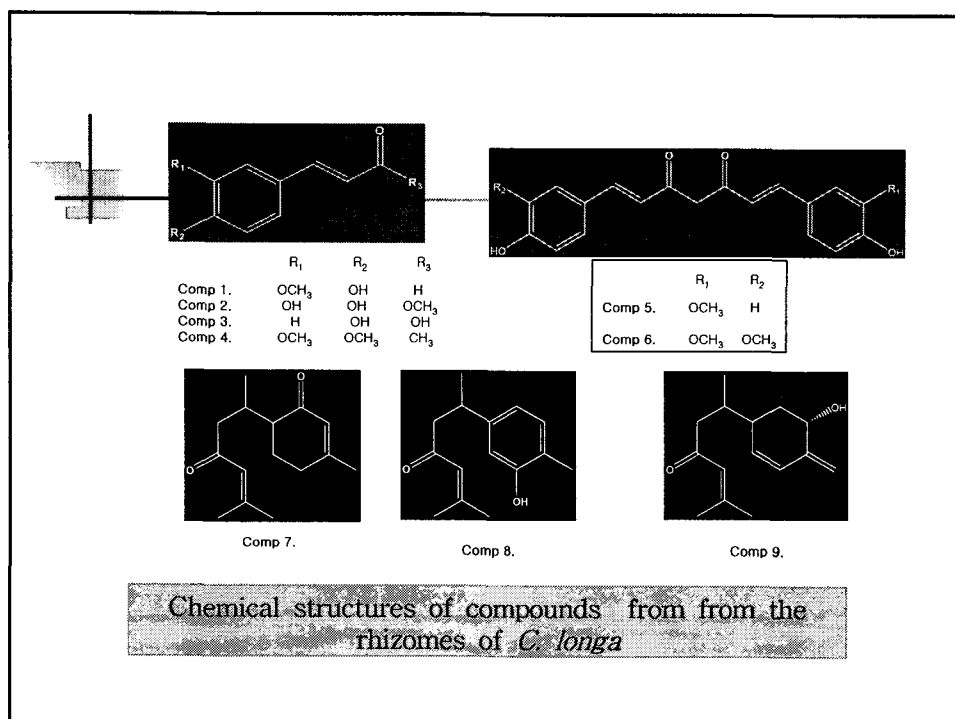
Inhibitory effect of MeOH extract of some higher plants on the tyrosinase activity *in vitro* (150 μ g/ml)



Isolation of the tyrosinase inhibitory components from the rhizomes of *C. longa*



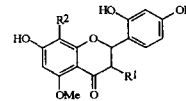
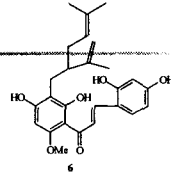
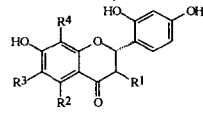




Inhibitory effect of isolated compounds from the rhizomes of *C. longa* on the mushroom tyrosinase activity *in vitro*

Comp. No	Comp. Name	IC ₅₀ values (μM)
Comp. 1	<i>Coniferaldehyde</i>	28.1
Comp. 2	<i>Methyl-3,4-dihydroxycinnamate</i>	20.6
Comp. 3	<i>p-Hydroxycinnamic acid</i>	100<
Comp. 4	<i>4-(3',4'-Dimethoxyphenyl)-3-buten-2-one</i>	200<
Comp. 5	<i>Demethoxycurcumin</i>	11.3
Comp. 6	<i>Curcumin</i>	10.9
Comp. 7	<i>2,10-Bisaboladien-1,9-dione</i>	-
Comp. 8	<i>Turmerol A</i>	-
Comp. 9	<i>5-(R)-hydroxycurlone</i>	-
control	<i>Kojic acid</i>	8.9

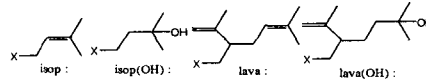
The Roots of *Sophora flavescens*



	R ¹	R ²	R ³	R ⁴	IC ₅₀ (μM)
1	H	OH	isop	isop	55.4
2	H	OH	isop	lava	38.3
3	H	OH	H	lava	44.7
4	β(OH)	OH	isop	isop	43.3
5	β(OH)	OH	isop	lava	37.5
7	H	OMe	H	lava	1.3
9	β(OH)	OH	isop(OH)	lava	36.9
10	H	OH	H	Lava(OH)	2.1
12	α(OH)	OMe	H	Lava(OH)	40.0

IC₅₀(μM): 1.1

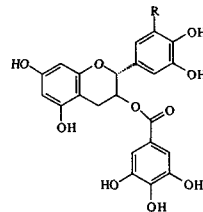
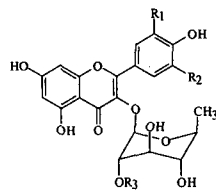
	R ¹	R ²	IC ₅₀ (μM)
8	OH	lava	21.0
11	H	Lava(OH)	10.6



IC₅₀(μM) of kojic acid: 11.3

Lee et al: *Planta Medica*, accepted (2003)

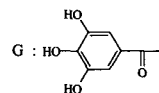
The Leaves of *Cercis chinensis*



	R ₁	R ₂	R ₃	IC ₅₀ (μM)
CE 1	H	H	H	450<
CE 2	OH	H	H	67
CE 3	OH	OH	H	150
CE 4	OH	OH	G	63

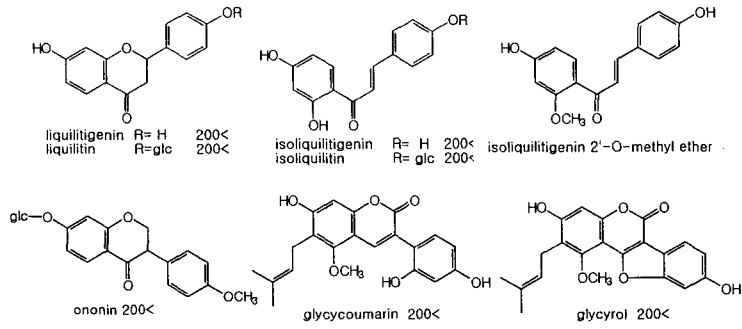
	R	IC ₅₀ (μM)
CE 5	H	92
CE 6	OH	69

IC₅₀(μM) of Kojic acid : 9

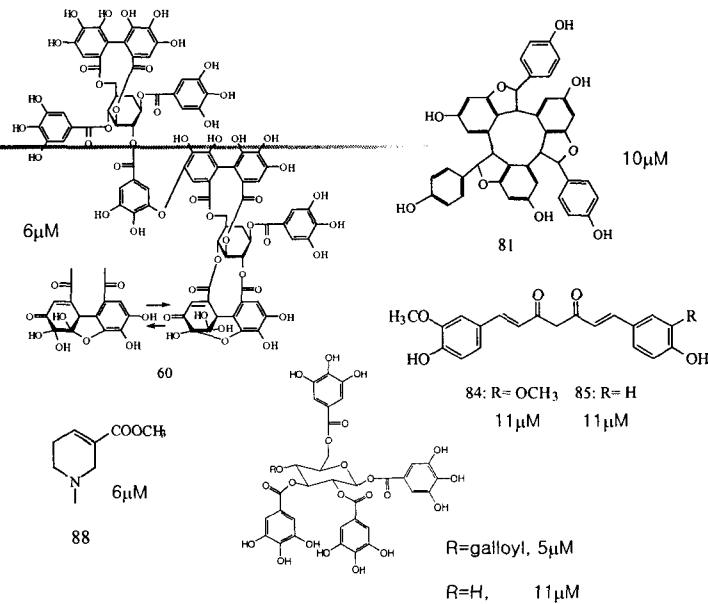


Lee et al: *생약학회지* 30(4) 1999

The Roots of *Glycyrrhiza glabra*



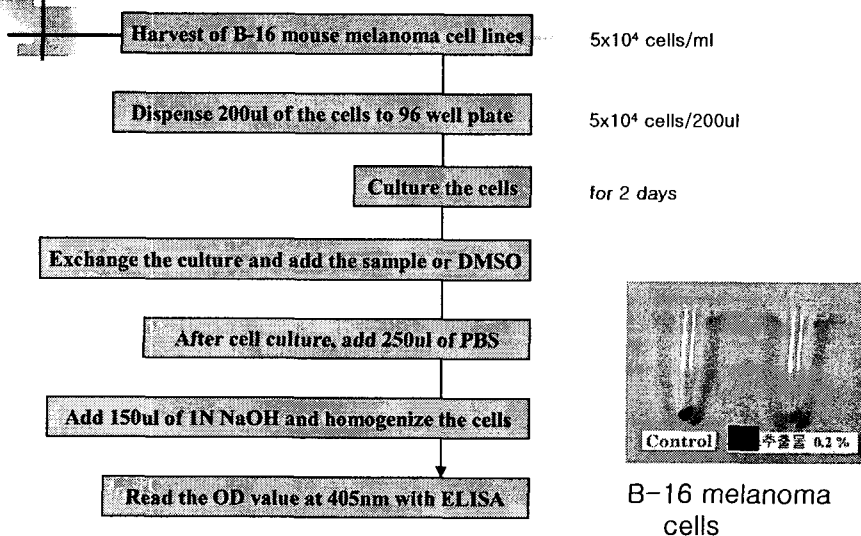
Lee et al : 생약학회지, accepted (2003)



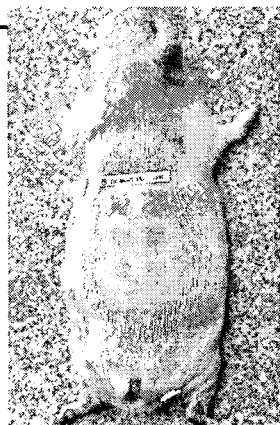
Inhibitory effect of some natural products on melanogenesis in B-16 mouse melanoma cell lines

Protocol of melanogenesis test in B-16 mouse melanoma cell lines

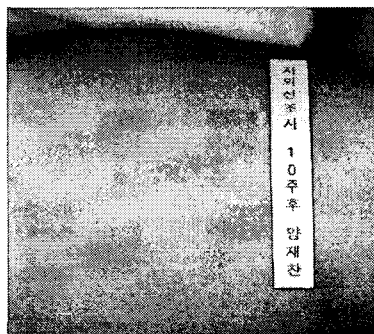
Natural Product
Chemistry Lab.



In vivo bioassay



Brown guinea pig



in vivo Human test

Inhibitory effects of solvent fraction of natural resources on melanogenesis in the B-16 mouse melanoma cell lines_{cytotoxicity}

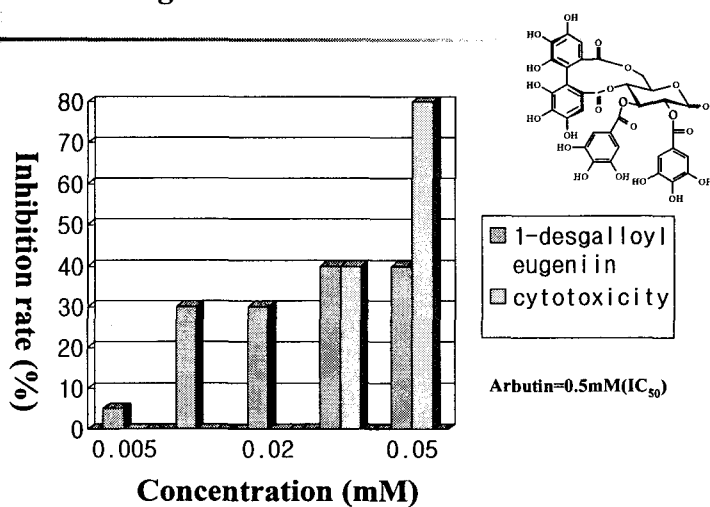
No.	Scientific name	Inhibition ratio(%) at the concentration of 250µg/ml			Kojic acid
		C	E	W	
1	<i>Carex humilis</i> , Cyperaceae	-14.902	-54.985 *	-25.694	5.139
2	<i>Evodia officinalis</i> , Rutaceae	-379.239	-386.948	-90.442	
3	<i>Pueraria thunbergiana</i> , Leguminosae	-14.83	-19.42	-1.35	
4	<i>Trifolium pratense</i> , Leguminosae	-138.746	-147.482	-25.694	
5	<i>Acer tegmentosum</i> , Aceraceae	-373.073	-445.015	-152.621	
6	<i>Artemisia scoparia</i> , Compositae	-195.272	-616.650	-176.259	
7	<i>Gentiana scabra</i> , Gentianaceae	-162.384	-130.10	-25.180	
8	<i>Smilax sieboldii</i> , Lilaceae	-214.800	-280.576	-75.540	
9	<i>Filipendula glaberrima</i> , Rosaceae		-129.469	-84.789	
10	<i>Morus alba</i> , Moraceae	-453.237	-905.447	-915.725	
11	<i>Aconogonum divaricatum</i> , Polygonaceae	-234.964	-284.687	-100.206	
12	<i>Acer ukurunduense</i> , Aceraceae	-351.490	-343.268	-218.911	
13	<i>Ulmus davidiana</i> , Ulmaceae	-117.163	-779.034 *	-227.873	
14	<i>Crisium maackii</i> , Compositae	-94.719	-245.599	-14.669	
15	<i>Paederia scandens</i> , Rutaceae	-79.631	-897.594	-18.860	
16	<i>Clinopodium chinense</i> , Labiatae	-10.059	-227.158	-67.896	8.382
17	<i>Sapium japonicum</i> , Euphobiaceae	-7.963	0.419 *	23.051	
18	<i>Clerodendron lichotomum</i> , Verbenaceae	-111.484	-391.031	-34.786	
19	<i>Cnidium officinale</i> , Umbelliferae	45.683 *	38.977	16.345	

Continued.

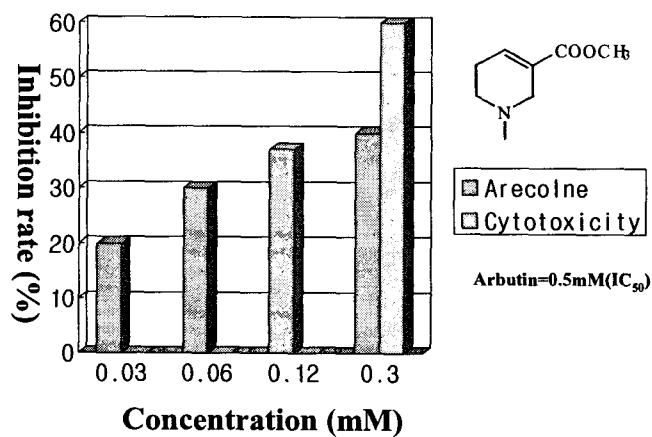
(* cytotoxicity)

No.	Scientific name	Inhibition ratio(%) at the concentration of 250µg/ml			Kojic acid
		C	E	W	
20	<i>Magnolia kobus</i> , Magnoliaceae	80.889	-37.301		8.382
21	<i>Gaedenia jasminoides</i> , Rutaceae	-36.463	-746.018	-658.843	
22	<i>Astragalus membranaceus</i> , Leguminosae	-41.911	9.220	25.147	
23	<i>Atractylodes japonica</i> , Compositae	21.794	21.985	61.190	
24	<i>Cyperus rotundus</i> , Cyperaceae	45.683	20.956	40.235	
25	<i>Liriope platyphylla</i> , Liliaceae	20.117		24.728	
26	<i>Geranium thurgelii</i> , Geraniaceae	-143.336	-198.240	-25.566	
27	<i>Phellodendron amurense</i> , Rutaceae	-18.3	-14.6	-11.6	
28	<i>Corydalis ternata</i> , Papaveraceae	-143.2	-89.8	-6.20	
29	<i>Paeonia suffruticosa</i> , Paeoniaceae	-5.39	-56.09	-1.89	
30	<i>Scutellaria baicalensis</i> , Scrophulariaceae	-87.92	-7.28	-21.31	
31	<i>Magnolia officinalis</i> , Magnoliaceae	-14.83*	-335.22	-3.51	
32	<i>Epimedium koreanum</i> , Berberidaceae	-125.94	-92.23	-5.12	
33	<i>Anemarrhena ashpodeloides</i> , Liliaceae	9.44	8.63	11.60	
34	<i>Gentiana scabra</i> , Gentianaceae	12.68	14.29	18.88	
35	<i>Gastrodia elata</i> , Orchidaceae	43.15	24.00	7.82	
36	<i>Crataegus pinnatifida</i> , Rosaceae	1.62	-110.57	13.21	
37	<i>Sophora flavescens</i> , Leguminosae	-7.55	-115.70	-5.12	
38	<i>Akebia quinata</i> , Lardizabalaceae	-18.88	-122.71	19.69	
39	<i>Cornus officinalis</i> , Comaceae	-5.12	-86.84	4.85	

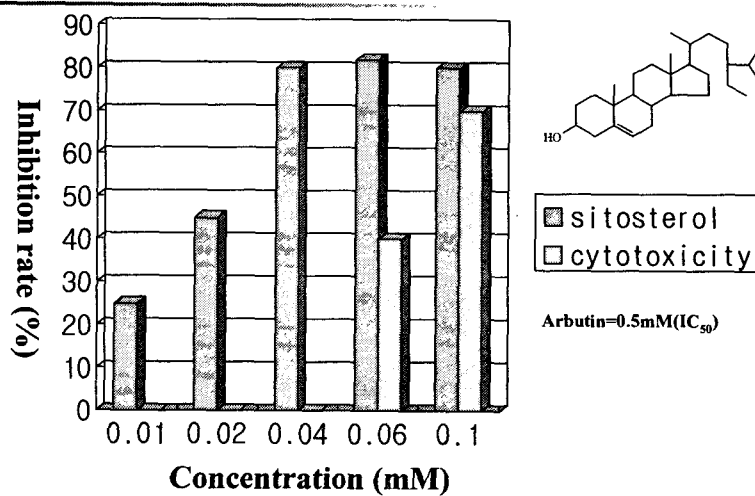
Inhibitory effect of 1-desgalloyl eugenin on melanogenesis in B-16 mouse melanoma cell lines



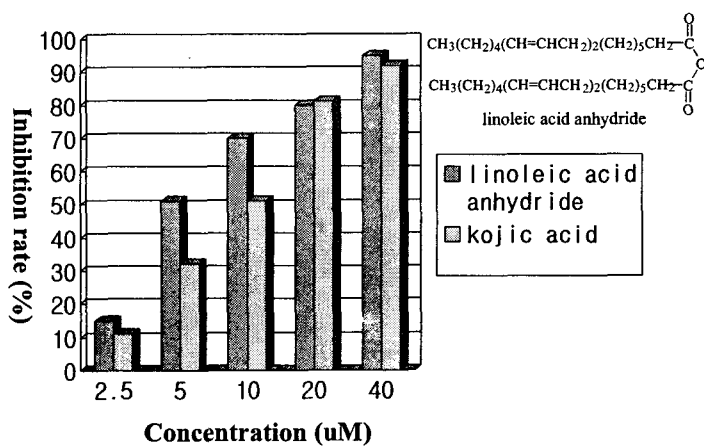
Inhibitory effect of arecoline on melanogenesis in B-16 mouse melanoma cell lines



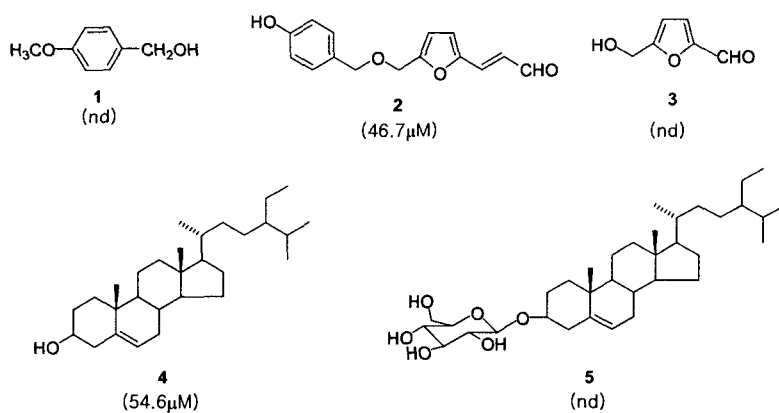
Inhibitory effect of compound sitosterol on melanogenesis in B-16 mouse melanoma cell lines



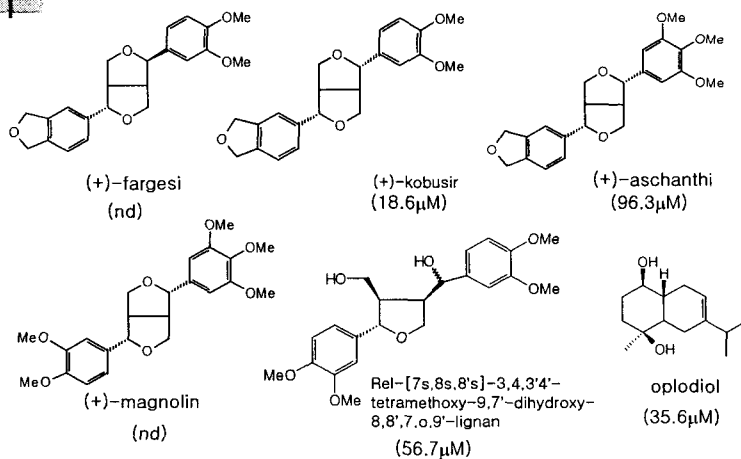
Inhibitory effect of linoleic acid anhydride on melanogenesis in B-16 mouse melanoma cell lines



Rhizomes of *Gastrodia elata*



Flowers of *Magnolia denudata*



Machilus thunbergii at 25 μ g/ml.

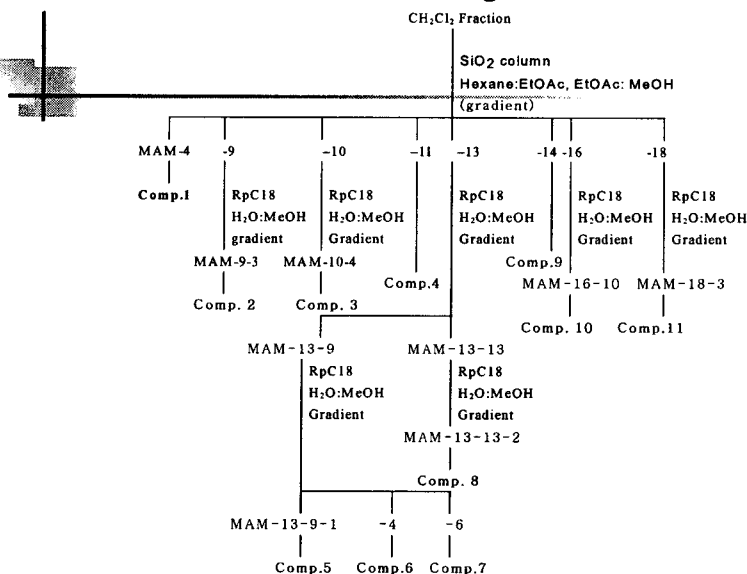
	Inhibition ratio (%)
CH ₂ Cl ₂ extract	42.9 \pm 5.2 ^a
EtOAc extract	2.45 \pm 4.2
H ₂ O extract	3.12 \pm 4.7
Kojic acid ^b	22.0 \pm 3.8 ^{a,c}

^a Significant difference from the control is $p < 0.01$.

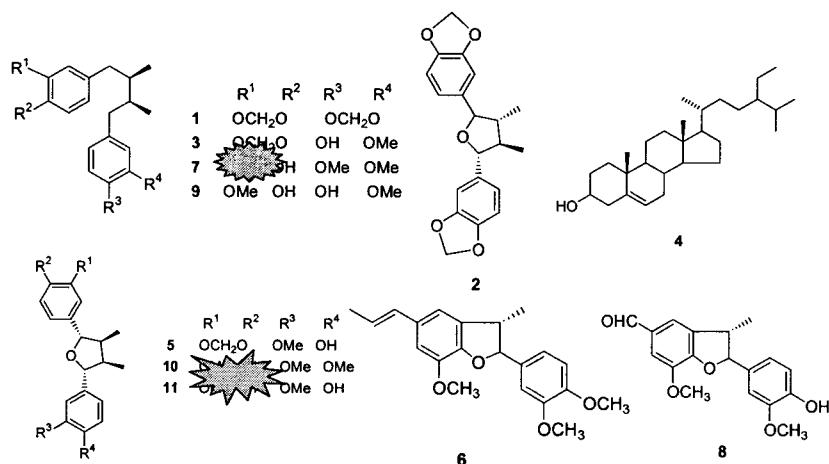
^b positive control

^c at 150 μ g/ml

Isolation of Compounds from Methylene Chloride Fraction of *Machilus thunbergii*



Compounds isolated from the bark of *Machilus thunbergii*.



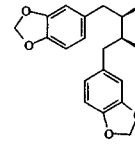
Inhibitory effect on melanin biosynthesis and dose for cytotoxicity initiated in B-16 mouse melanoma cell lines

compound	Melanin synthesis (IC ₅₀ μM)	Dose of cytotoxicity initiated (μM)
1	39.9	75.0
2	NA	37.5
3	NA	18.8
4	NA	18.8
5	NA	37.5
6	NA	18.8
7	15.1	37.5
8	NA	18.8
9	NA	18.8
10	19.4	75.0
11	37.8	75.0
Kojic acid	1700	NA

Melanogenesis and cytotoxicities of compounds 1, 7, 10 and 11 in B-16 mouse melanoma cell lines

Compounds tested	Melanin synthesis IC ₅₀ (μM)	Cytotoxicities LD ₅₀ (μM)
1	39.9	62.8
7	15.1	>150.0
10	19.4	70.6
11	37.8	140.9
Arbutin	109.5	>150

Machilin A



machilin A (1)

- IC₅₀ values in B-16 mouse melanoma cell : 10ppm
- Human melanocyte : 20% inhibition at 1ppm, toxic at 2ppm
- Allergy test : safe
- Genomics/proteomics with human melanocyte :
Inhibition of enzyme revelation
- 현재 임상실험중
- Human patch 실험, 외부독성실험, 경피흡수실험

연구성과물

1. Inhibitory effects of herbal extracts on dopa oxidase activity of tyrosinase, *Natural Product Science* (1997)
2. 고등식물로부터 피부멜라닌 생성에 관여하는 티로시나제 활성억제 물질의 탐색, 약학회지 (1997)
3. 목단피로부터 멜라닌 생성 억제성분의 분리, 약학회지 (1998)
4. Inhibitory effects of a-viniferin and resveratrol on the L-dopa oxidase activity of tyrosinase, *Planta Medica* (1998)
5. 박태기나무 잎으로부터 피부멜라닌 색소생성 억제성분의 분리, 생약학회지 (1999)
6. 대극과식물 등대풍로부터 단리한 가수분해형 탄닌의 tyrosinase 활성 억제효과, 약학회지 (2001)
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11. 아레콜린을 함유하는 피부미백용 조성물(특허)
12. 시토스테롤을 함유하는 피부미백용 조성물(특허)
13. 1-테스갈로일유게닌을 함유하는 피부미백용 조성물(특허)
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