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Natural Products with Skin – Whitening Effects

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ABSTRACT

UV radiation is widely considered as a major cause of skin pigmentation. Upon exposure to UV radiation, the melanocytes increase the production of intracellular nitric oxide, which triggers signal transduction cascades to initiate melanogenesis by tyrosinase. UV radiation also influences melanogenesis through a paracrine regulation process involving the keratinocytes. Although a number of hypopigmenting products have been developed, those from natural sources are preferred and will predominate in the cosmetics market. More active compounds such as phenols, flavonoids, coumarins and other derivatives have been identified from natural sources. This article summarized natural skin whitening products involving tyrosinase blockers like phenols and polyphenols, and non-tyrosinase blockers like α-MSH, melanosome transferase and cytokine inhibitors. In addition, the skin-whitening effects and the mechanisms of these natural products were also reviewed. Most of the compounds achieve the hypopigmenting effect by inhibiting tyrosinase; however, some interesting compounds blocking the upstream regulation points of melanogenesis are extraordinarily promising for developing novel whitening agents.

Key words: natural products, whitening, melanogenesis, tyrosinase

INTRODUCTION

Long ago in the West, white skin was desirable because it signified wealth. In Asia, women were deeply influenced by the concept that a white complexion is powerful enough to hide a number of faults. Melanin synthesis was the major source of skin color and played an important role in protection of UV-induced dermal irritation; however, overproduction of melanin posed not only an esthetic problem but also a dermatological issue. The processes of cellular melanogenesis and response of the pigment-producing cells to UV radiation and initiated melanogenesis have been well studied and instrumental in promoting the development of depigmenting agents. Ingredients such as hydroquinone, ascorbic acid and retinoic acid were used as whitening agents to lighten the skin. Despite their benefits, these whitening agents would cause some harmful side effects resulting in the limited application. The "return to nature" trend of recent years had been accompanied by a booming interest in whitening agents from natural products[1]. This paper will review the process of melanin synthesis and signal transduction related to connection between keratinocytes and melanocytes; moreover, some natural skin-depigmenting products and how they affect the pigmentation will be summarized here.

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MELANOGENESIS

Dermal pigmentation is either dependent on the number, size, composition and distribution of melanocytes or activity of melanogenic enzymes. Furthermore, cutaneous pigmentation is resulted from melanin synthesis by melanocytes and transfer of melanosome to keratinocytes. UV radiation from the sun stimulated melanin synthesis on the skin. Upon exposure to UV radiation, the cytokines, growth factors and other inflammatory factors would be released by fibroblasts to stimulate melanin production. The melanocytes increased the production of intracellular nitric oxide (NO), which triggered signal transduction cascades to initiate melanogenesis[2,3] through the enzyme tyrosinase, a glycosylated copper-containing oxidase. Tyrosinase was synthesized by melanosomal ribosomes found on the rough endoplasmic reticulum[4], glycosylated *en route* to and within the golgi, and subsequently delivered to melanosomes via coated vesicles[4,5]. The biosynthetic pathway for melanin formation in various bioforms had largely been elucidated[6-10] (Figure 1). Tyrosinase catalyzed two distinct oxidation reactions. First, tyrosinase catalyzed the oxidation of monophenol (L-tyrosine) to *o*-diphenol (3,4-dihydroxyphenylalanine, L-DOPA). Second, L-DOPA was oxidized to *o*-quinone (dopaquinone). From dopaquinone, the melanin synthesis pathways diverged. The tyrosinase-related protein 1 (TRP1) and DOPAchrome tautomerase (DCT, also knows as TRP2) subsequently metabolited dopaquinone into eumelanin. In

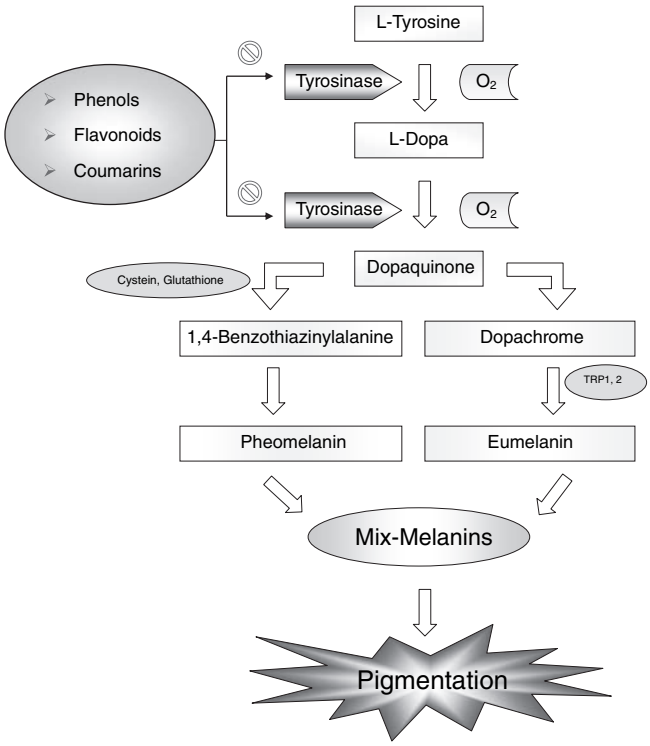


Figure 1. Scheme of melanogenesis. ⃠: inhibition.

the other, dopaquinones conjugated with thiol-containing cysteine or glutathione to form pheomelanin. Afterwards, melanin-containing melanosomes were transferred to the neighboring keratinocytes. In the human epidermis, melanocytes worked in close harmony with their neighboring cells such as keratinocytes via their dendrites.

UV radiation could also influence the melanogenesis of melanocytes through a paracrine regulation process involving keratinocytes[10,11] (Figure 2). UV stimulated the secretion of prostaglandin E_2 (PGE_2), α-melanocyte stimulating hormone (α-MSH), adrenocorticotropin melanocyte stimulating hormone (ACTH), endothelin-1, ß-fibroblast growth factor (ß-FGF), NO, nerve growth factor (NGF), hepatocyte growth factor, granulocyte-macrophage colony-stimulating factors, leukemia inhibitory factor, *p*-locus and stem-cell factor from keratinocytes and then induced the melanogenesis of melanocytes[10,12-15]. In addition, α-MSH and ACTH stimulated melanogenesis in human epidermal melanocytes[16]. They bond to melanocortin receptor-1 (MC-1R) on the melanocytes[17], activated intracellular adenylate cyclase through G proteins, and then elevate cyclic AMP (cAMP) concentration from adenosine triphosphate[18]. Cyclic AMP exerts its function through protein kinase A (PKA)[19]. The PKA phosphorylated and activated the cAMP-response element binding protein (CREB) that bond to cAMP response element (CRE) presenting in the M promoter of the microphthalmia-associated transcription factor (MITF) gene[20,21]. Finally, the transient increase of MITF would lead to the up-regulation of tyrosinase as well as TRP-1 and TRP-2. α-MSH also stimulated p38 mitogen-activated protein kinase (MAPK), which would phosphorylate upstream transcription factor that would bind to the tyrosinase promoter[10].

Although lots of mechanistic points can be targeted, tyrosinase inhibition is still the most common approach to achieve skin whitening. Tyrosinase inhibitors have been reviewed by Seo *et al.*[22]. Several hypopigmenting products have been developed; however, those from natural sources are still considered more desirable and will predominate in the cosmetics market. Growing active compounds such as phenols, flavonoids, coumarins and other derivatives have been identified from natural sources and have recently been reviewed by Solano *et al.*[23] and Parvez *et al.*[24]. Natural whitening products inhibiting tyrosinase can be roughly divided into two categories, simple phenols and polyphenols. In addition, some depigmenting agents blocking the upstream of melanogenesis were discussed.

NATURAL WHITENING PRODUCTS BLOCKING TYROSINASE

I. *Phenols*

(I) *Arbutin and Derivatives*

Arbutin (hydroquinone-*O*-beta-D-glucopyranoside (Figure 3) isolated from the fresh fruit of the California buckeye[25], *Aesculus californica*, was reported by various researchers to inhibit the oxidation of L-DOPA catalyzed by mushroom tyrosinase and was effective in

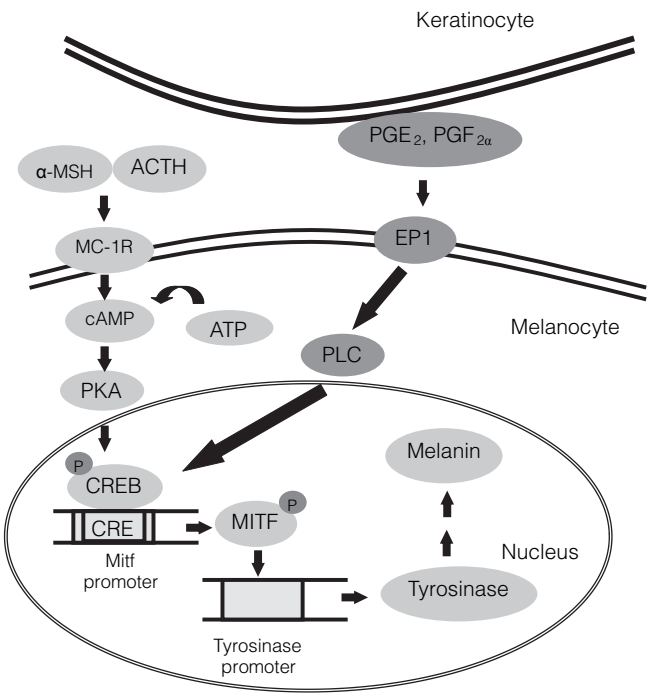


Figure 2. The signaling pathways within epidermal melanin.

the topical treatment of various cutaneous hyperpigmentations characterized by hyperactive melanocyte function[26-28]. Structurally related to hydroquinone, arbutin inhibited tyrosinase activity by interacting with copper at the active site. Arbutin exerted its effect through a controlled release of hydroquinone by the *in vivo* hydrolysis of the glycosidic bond. To increase the efficiency, α-glucosides of arbutin had been chemically synthesized because they hydrolyze more easily to release hydroquinone by α-glycosidases in cells[29]. Despited the safety of arbutin as an agent to lighten skin, some reports failed to confirm its effect in clinical trials[30]. Recently, deoxyarbutin, synthesized by removing every hydroxyl group of arbutin, had been identified as an excellent tyrosinase inhibitor due to its increased skin penetration and binding affinity to tyrosinase[31].

(II) *Kojic Acid and Derivatives*

Kojic acid (5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one) (Figure 3) was a tyrosinase inhibitor derived from various fungal species such as *Aspergillus* and *Penicillium*[32,33]. Its function was chelating copper at the active site of the tyrosinase and scavenging free radicals[34]. However, kojic acid had been found to cause allergic reactions[35], and it showed only modest effectiveness in clinic trials[36]. Recently, some stable kojic acid derivatives had been synthesized for better penetration through the skin. The most important ones were those synthesized by joining two pyrone rings through an ethylene linkage[37], and kojyl-APPA (5-(3-aminopropyl)-phosphino-oxy-2-(hydroxymethyl)-4*H*-1-pyran-4-one) was tested in melanoma cells and normal human melanocytes[38].

(III) *Gentisic Acid and Derivatives*

Gentisic acid (2,5-dihydrobenzoic acid) (Figure 3), found in gentian roots, seemed to be a good inhibitor of melanogenesis[39]. Its alkyl esters were investigated as tyrosinase inhibitors *in vitro* and in cell cultures[30]. Methyl gentisate appeared to be more efficient than the free acid form as well as other well-known hypopigmenting agents, such as hydroquinone, kojic acid, arbutin, and magnesium ascorbyl phosphate. Furthermore, the methyl gentisate was also less cytotoxic and less mutagenic than hydroquinone, although some side effects were reported.

(IV) *Hydroxycinnamic Acid Derivatives*

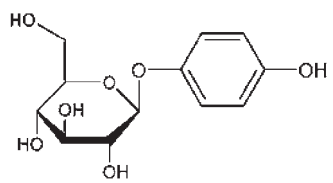
Some hydroxylated cinnamic derivatives (Figure 3) also inhibited melanogenesis. It was observed that *p*-coumaric acid from ginseng leaves inhibited tyrosinase on its monophenolase and diphenolase activities[40]. Ferulic acid exhibited a non-competitive inhibition for L-DOPA oxidation by mushroom tyrosinase[41]. N-feruloyserotonin and *N*-(*p*-coumaryl)serotonin from safflower (*Carthamus tinctorius L.*) seeds were strongly inhibited the melanin production[42]. Methylation and hydroxylation of cinnamic acid derivatives may play an important role in tyrosinase inhibition[43].

II. *Polyphenols*

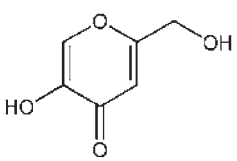
Polyphenols are a group of chemical compounds that are widely distributed in nature and are also known as tannins because they are responsible for the colors of many flowers.

(I) *Flavonoids*

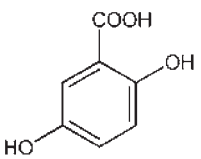
Flavonoids belonged to the best studied group of plant polyphenols. They all had phenolic and pyrane rings and were classified into six major groups, flavanols, flavones, flavonols, flavanones, isoflavones and anthocyanidins. These groups differed in the conjugation of rings and the position of hydroxyl, methoxy and glycosidic groups[44]. It had been reported that flavonoids inhibit enzymes due to their abilities to chelate copper at



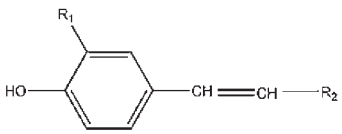
1. Arbutin



2. Kojic acid



3. Gentisic acid



4. Hydroxycinnamic acid	R_1	R_2
p-Coumaric acid	H	COOH
Ferulic acid	OMe	COOH
N-feruloylserotonin	OMe	A
N-(*p*-coumaroyl)serotonin	H	A

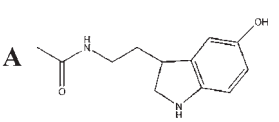


Figure 3. Chemical structures of phenols with tyrosinase inhibition. 1. arbutin; 2. kojic acid; 3. gentistic aid; 4. hydroxycinnamic acid.

the active site[45]. A recent fluorescence quenching study demonstrated that dihydroxy substitutions in both the A and B rings of flavonoids are crucial for tyrosinase inhibitory activity[46]. Flavonoids and flavonoid-like agents with hypopigmenting properties were discussed.

Some flavonols (Figure 4) were competitive inhibitors of mushroom tyrosinase. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) was present as a glycosylated derivative in onions and the flowers of plants such as Mexican Heteroteca inuloides[47]. Quercetin was suggested to be more effective than its analogues kaempferol and morin[48]. Kaempferol was found in the petals of *Crocus sativus* (saffron), and its 3-*O*-glucoside did not inhibit tyrosinase[49,50]. In addition, mulberroside F (moracine M-6) was obtained from the *Morus alba* leaves, and it greatly decreased melanin formation in normal melanocytes[51]. Ethanolic extracted from the leaves of *Myrica rubra*, which contained quercetin, myricetin and some 3-*O*-ramnosides derivatives, showed good depigmenting effects *in vitro*[52]. Furthermore, galangin, luteolin, chrysin and baicalein would inhibit tyrosine activity[45]. Galangin showed competitive inhibition of tyrosinase[53]. Kurarinone, kuraridin, kushnol F, kurariol and Sophoraflavanone G, prenylated flavonoids from *Sophora flavescens*, were also found to have an potent inhibitory effect on tyrosinase[54-56].

Flavanones, also named chalcones, were featured with their double bond at the 2–3 position of the pyrone ring. Isoliquiritigenin (2′,4′,4-trihydroxychalcone) in licorice extract was claimed to inhibit both the monophenolase and the diphenolase activities of tyrosinase[57] (Figure 4). A study on the influence of number and position of hydroxyl groups on the basic ring of chalcones indicated the importance of position 4 on the ring B[58]. Another study reported that the 2,4-resorcinol subunit on the ring B is very important for tyrosinase inhibition[59]. Nevertheless, all these studies were conducted only on mushroom tyrosinase.

(II) *Aloesin*

Aloesin (2-acetonyl-8-D-glucopyranosyl-7-hydroxy-5-methylchromone) was a glycosylated chromone isolated from the aloe plant[60] and its structure was rather similar to flavonols (Figure 4). It modulated melanogenesis via competitive inhibition of tyrosinase[61]. Combined treatment of aloesin and arbutin seemed to show synergistic effects by respectively non-competitive and competitive tyrosinase inhibition[62].

(III) *Gallic Acid and Derivatives*

Various gallic acid derivatives of hydroxyflavanols (Figure 4) had been isolated from green tea and *Galla rhois*, and some of them were identified as strong tyrosinase inhibitors[63-65]. Gallic acid and its short alkyl (<C10) chain esters were oxidized by tyrosinase as substrates, yielding yellow oxidation products, but the long alkyl (>C10) chain esters inhibited the enzyme without producing the pigmented products, indicating that the carbon chain length was related to their tyrosinase inhibitory activity[64,66-68]. The most abundant hydroxyflavanols in green tea included ECG [(-)epicatechin-3-*O*-gallate], GCG [(-)gallocatechin-3-*O*-gallate], EGCG [(-)epigallocatechin-3-*O*-gallate], and EGC [(-)epigallocatechin]. It was reported that EGCG and hinokitiol (structurally not related to hydroxyflavanols) were not only tyrosinase inhibitors, but also agents that decreased MITF production in cells[69].

(IV) *Procyanidins*

Procyanidins, polymers of catechins found in tea and fruits such as apples and grapes had been recently introduced as inhibitors of melanogenesis[70,71]. Procyanidin-rich extract would reduce DOPA-postive melanocytes and 8-hydroxy-2′-deoxyguanosine positive melanin containing cells, and this effect may related to inhibition of tyrosinase and ROS-induced melanocytes proliferation[70].

(V) *Hydroxystilbene Derivatives*

Hydroxystilbene derivatives such as resveratrol and oxyresveratrol were effective whitening agents probably due to their high affinity to tyrosinase[72] (Figure 4). Some data indicated that resveratrol was not simply a tyrosinase inhibitor; it also reduced MITF in B16 mouse melanoma cells[73]. However, Kim *et al*.[72] reported that resveratrol only inhibited tyrosinase activity without effecting on tyrosinase expression. On the other hand, oxyresveratrol showed potent tyrosinase inhibitory activity[74] and inhibited tyrosinase more efficiently than resveratrol[75]. It had also been investigated that melanogenesis was inhibited by the *Ramulus mori* extract that contains 2-oxyresveratrol[76].

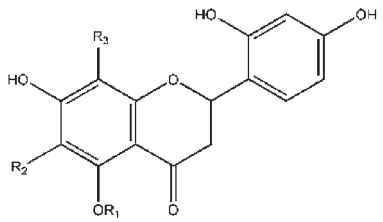
(VI) *Ellagic Acid*

Ellagic acids (EA) (Figure 4) was a polyphenol found in berries, green tea and pomegranate with strong antioxidative properties and tyrosinase inhibition[68]. The skin lightening effects of EA may due to chelating copper at the active site of tyrosinase to reduce its activity[67] and inhibition of proliferation of melanocytes and melanin synthesis[68]. In addition, the antioxidative and ROS-scavenging activities of EA may contribute to its skin-whitening effect.

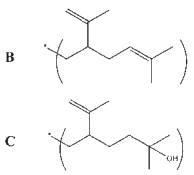
III. *Other Compounds*

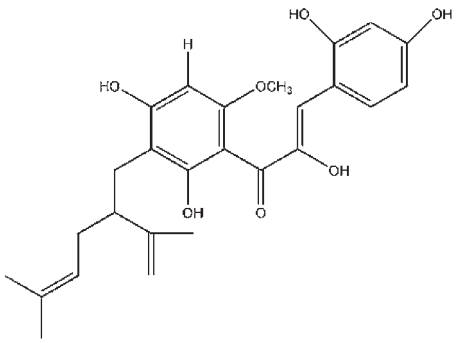
Other active compounds such as isoimperatorin and imperatorin (Figure 4) had been identified from *Angelica dahurica* plant and have been shown to had a strong inhibition effect on tyrosinase synthesis[77].

Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008

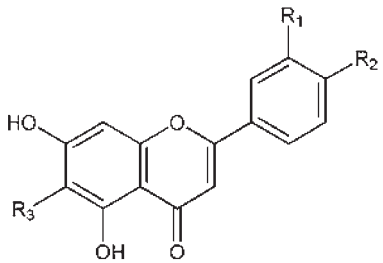


1. Flavanone	R_1	R_2	R_3
Kurarinol	Me	H	C
Kurarinone	Me	H	B
Kushnol F	H	B	H
Sophoraflavanone G	H	H	B

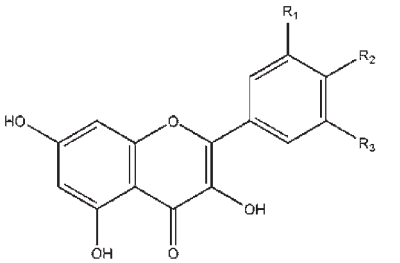




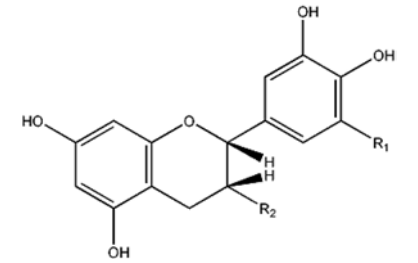
2. Kuraridin



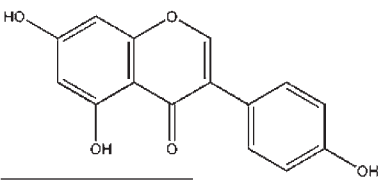
3. Flavone	R_1	R_2	R_3
Chrysin	H	H	H
Luteolin	OH	OH	H
Baicalein	H	H	OH



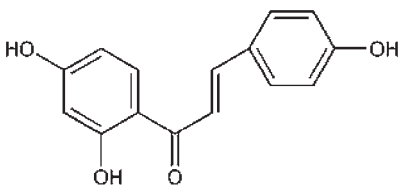
4. Flavonol	R_1	R_2	R_3
Galangin	H	H	H
Quercetin	OH	OH	H
Kaempferol	H	OH	H
Myricetin	OH	OH	OH



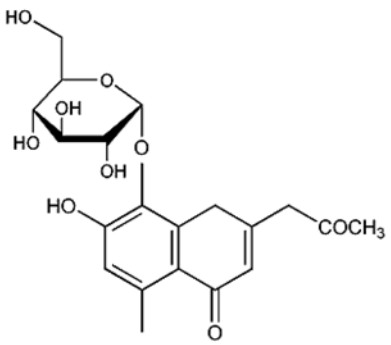
5. Flavanol	R_1	R_2
Catechin	H	OH
Epicatechin	H	OH
EGCG	OH	Gallate



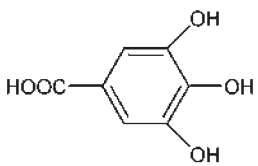
6. Isoflavone
Genistein



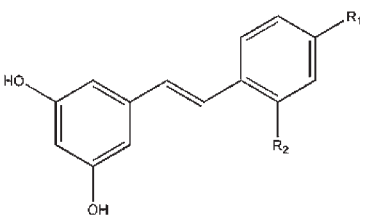
7. Isoliquiritigenin



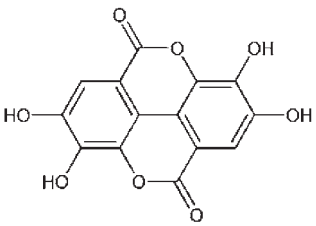
8. Aloesin



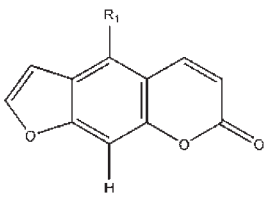
9. Gallic acid



10. Hydroxystilbene	R_1	R_2
Resveratrol	OH	H
Oxyresveratrol	OH	OH



11. Ellagic acid



12. Others	R_1
Imperatorin	H
Isoimperatorin	E

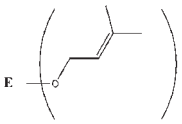


Figure 4. Chemical structures of polyphenols with tyrosinase inhibition. 1. flavanone; 2. kuraridin; 3. flavone; 4. flavonol; 5. flavanol; 6. isoflavone; 7. isoliquiritigenin; 8. aloesin; 9. gallic acid; 10. hydroxystilbene; 11. ellagic acid; 12. others.

Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008

AGENTS BLOCK MELANOGENESIS EXCEPT TYROSINASE INHIBITION

In addition to inhibition of tyrosinase, blocking other sites of melanogenesis by natural products was observed.

I. *α-MSH Blockers*

(I) *Sophoraflavanone G*

Sophoraflavanone G was also found to have an inhibitory effect on α-MSH-stimulated melanogenesis[78] except tyrosinase inhibition[54].

(II) *Piperlonguminine*

Piperlonguminine (Figure 5) from *Piper longum* inhibited the α-MSH-induced melanogenesis with no effect on cell-free tyrosinase[79]. Piperlonguminine inhibited the α-MSH-induced signaling that functioned through cAMP to the CREB that in turn regulates MITF and tyrosinase expression[80].

II. *Melanosome Transferase Inhibitor*

(I) *Soybean Extract*

Soybean contained small serine proteases such as Bowman Birk inhibitor (BBI) and soybean trypsin inhibitor (STI) that inhibited the protease-activated receptor-2 (PAR-2) pathway expressed on keratinocytes. Interference with the PAR-2 pathway was shown to induce depigmentation by reducing the phagocytosis of melanosomes by keratinocytes, to diminish melanin transfer[81,82].

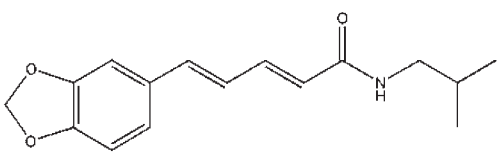


Figure 5. Chemical structure of piperlonguminine.

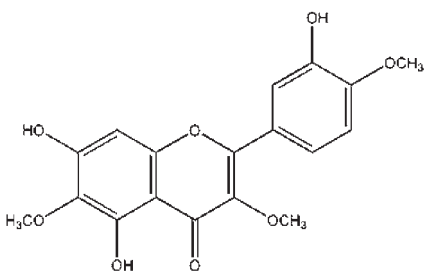


Figure 6. Chemical structure of centaureidin.

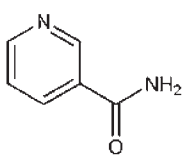


Figure 7. Chemical structure of niacinamide.

(II) *Centaureidin*

Centaureidin (5,7,3'-trihydroxy-3,6,4'-trimethoxyflavone) (Figure 6), a flavonoid glucoside from yarrow, had been shown to reduce melanosome transfer and melanocyte dentrites outgrowth[83,84] that was required for melanosome transfer. It would directly or indirectly activate Rho leading dendrite retraction to block melanocytes trafficking of melanin to keratinocytes[84], but did not inhibit melanin synthesis or protein expression.

(III) *Niacinamide*

Niacinamide (nicotinamide; 3-pyridinecarboxamide) (Figure 7) is the amide form of vitamin B_3 and had been shown to down regulate melanogenesis via inhibiting the transfer of melanosomes from melanocytes to keratinocytes[85,86]. In addition, niacinamide is also a tyrosinase inhibitors[87,88].

III. *Cytokines Inhibitors*

The extract of *Lepidium apetalum* had been reported to reduce UV-induced skin pigmentation in brown guinea pigs and cultures of human melanoma cells[89]. Although the active compound(s) had not yet been identified, the effect was probably due to an IL-6-mediated downregulation of MITF by the keratinocytes.

CONCLUSIONS

Some natural whitening ingredients and their mechanisms are listed in Table 1[23,24]. More natural compounds inhibit melanogenesis without direct effect on the cell-free tyrosinase activity have been reported[77,79,80,89-91]. It is also noteworthy that lots of active compounds with whitening mechanisms not directly related to tyrosinase are extraordinarily important. These compounds, blocking the upstream regulation points of melanogenesis, are not only interesting but also extremely promising for developing the next generation of whitening products.

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Table 1. Some skin-whitening agents and their effects on melanogenesis

Analogues	Mechanism	Reference
Aloesin	Tyrosinase inhibition (noncompetitive)	60-62
Arbutin	Oxidation of L-DOPA inhibition	25-28
Centaureidin	Melanosome transferase inhibition Rho activation	83,84
ECG	Tyrosinase inhibition (competitive)	69
EECG	Tyrosinase inhibition (competitive) Decrease MITF production	69
Ellagic acids	Tyrosinase inhibition	67,68
Ferulic acid	Tyrosinase inhibition (noncompetitive)	41
Galangin	Tyrosinase inhibition (competitive)	45,53
Gallic acid	Tyrosinase inhibition (competitive)	63-65
Imperatorin	Tyrosinase inhibition	77
Isoimperatorin	Tyrosinase inhibition	77
Isoliquiritigenin	Monophenolase and diphenolase inhibition	57,58
Kaempferol	Tyrosinase inhibition (competitive)	48-50
Kojic acid	Chelating copper at the active site of the tyrosinase	32-36
Kurarinone	Tyrosinase inhibition	54-56
Niacinamide	Melanosome transferase inhibition	86-88
Oxyresveratrol	Tyrosinase inhibition (noncompetitive)	74,75
p-Coumaric acid	Tyrosinase inhibition (competitive and noncompetitive)	40
Piperlonguminine	α-MSH blockers	79,80
Procyanidins	Tyrosinase inhibition Inhibition of ROS-induced melanocytes proliferation	70,71
Quercetin	Tyrosinase inhibition	47,48
Resveratrol	Tyrosinase inhibition Reduces MITF	72,73
Sophoraflavanone G	Tyrosinase inhibition α-MSH blockers	54,78

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