CHAPTER II

REVIEWS OF RELATED LITERATURE AND RESEARCH

The Skin Biology

The skin represents the largest organ of the human body and therefore it suits as a potential anatomical site for application of drugs and cosmetic products. The skin has many functions, it not only protects the body against mechanical, thermal and chemical influences, but it is also a highly sensitive organ for communication. Although the skin has only a thickness of a few millimeters with a surface of 1.8 m^2, it is the biggest organs of the human body. Furthermore, the skin possesses a crucial function in heat exchange and protection from loss of water. This transepidermal water loss is about 0.2 to 0.4 $mg/cm^2/h$ at 30°C comparing to the evaporation rate of water from free uncovered water surface that is about 35 $mg/cm^2/h$ at 30°C. The skin also has important endocrine functions such as the synthesis of vitamin D3, sex hormones and pheromones and it provides also immunological defenses.The skin is divided in three layers: the epidermis, dermis, and subcutaneous. The structure of the skin is shown in Figure 1.

1. The Epidermis

Human epidermis undergoes subtle but significant structural alterations during aging. The epidermis is the outer skin region that consists of several cell strata at varying levels of differentiation as shown in Figure 2. There is overall thin of unexposed epidermis by 10-50% between the age of 30 and 80 (Wulf et al., 2004. p. 186). Viable epidermis is composed of the stratum lucidum, stratum granulosum, stratum spinosum and stratum basale. The uppermost part of the epidermis, the stratum corneum is non-viable layer that is approximately 10-20 μm thick (Walters & Roberts, 2002. p. 4). It is main barrier in which a number of processes need to be taken into account including partition and diffusion within and through layers, metabolic processing and the systemic circulation (Ponec, 2002. p. 19). The main cells of the epidermis are called keratinocytes.

Beside the keratinocytes there are lymphocytes, Langerhans cells, melanocytes and Merkel cells interspersed among them. The stratum corneum is made of flattened epithelial cells arranged in multiple layers. More than 90% of all cells are corneocytes which are terminally differentiated, microscopically flat, and tightly packed in stacks that run perpendicular to the skin surface (Cevc et al., 2003. p. 677). These are called keratinized layers because of the synthesis of the protein keratin in those cells. Keratin is a protein structure that is specific to the skin, hair and this layer of skin is for the most part dead. Acidic and basic keratins make up about 80% of the dry mass of the corneocytes (Ponec, 2002. p. 20). It takes about one month from the time a basal cell leaves the bottom layer until it is desquamated.

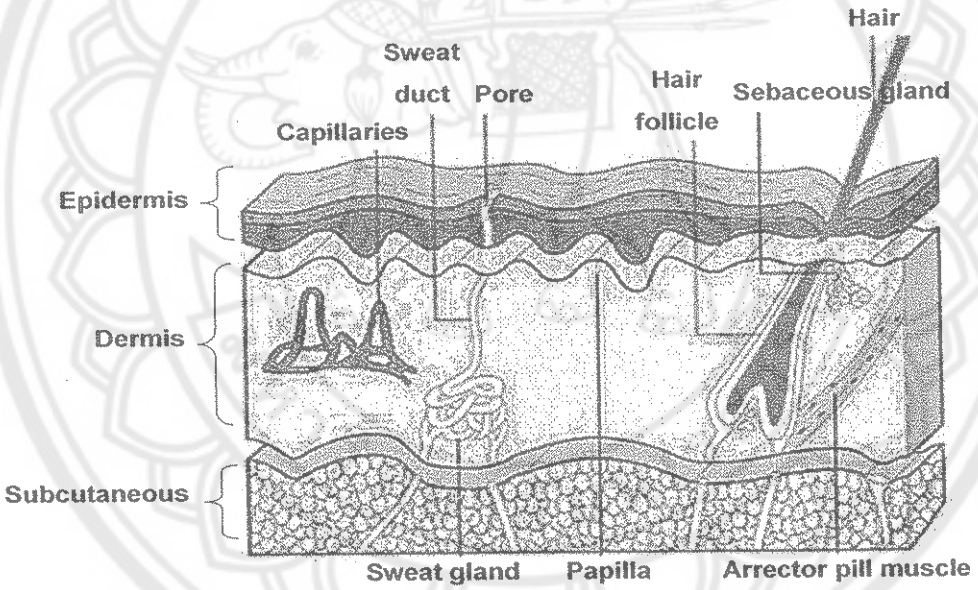


Figure 1 The Diagrammatic Illustration of the Skin

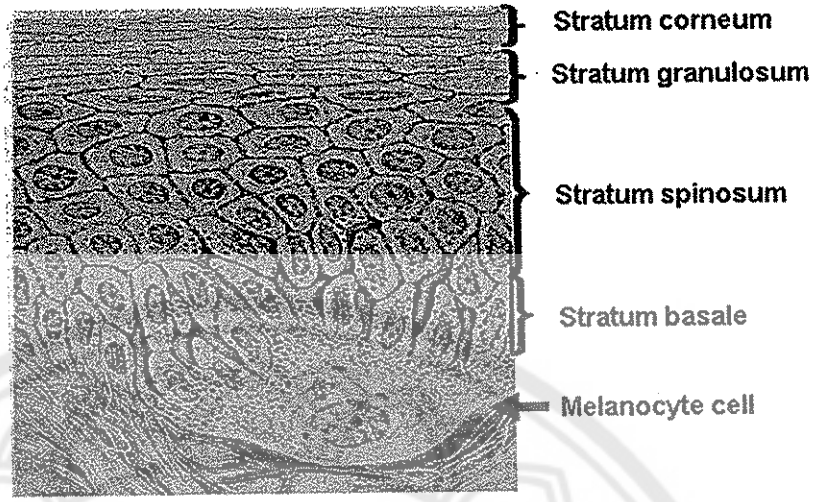


Figure 2 The Structure of Epidermis

2. The Dermis

The dermis is about 250-4,000 μm thick inner skin region that consists of collagen fibers, elastic connective tissues, nervous cells, bloods and lymphatic vessels. The collagenous fibers (70%) provide a scaffold of support and cushioning while elastin fibers provide elasticity. The main cells present are the fibroblasts and melanocytes. The dermis dose not only provides the nutritive, immune and support systems for epidermis, but also plays a role in temperature, pressure and pain regulation.

3. The Subcutaneous

The subcutaneous (hypodermis) is the deepest layer of the skin. This layer is a network of fat cells arranging in lobules and linking to the dermis by interconnecting collagen and elastin fibers. The other main cells in the hypodermis are fibroblasts and macrophages. The major role of the hypodermis is to carry the vascular and neural systems for the skin (Walters & Roberts, 2002. pp. 11-12).

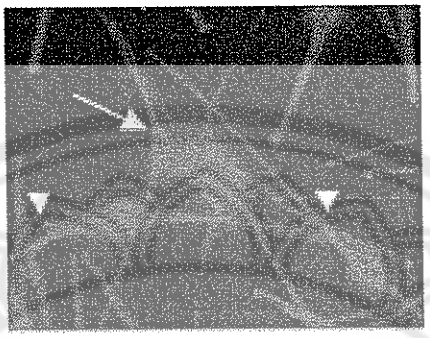
The Skin Pigmentation

Pigmentation is one of most obvious phenotypical characteristics in the natural world. It is regulated by complicated processes which results from the synthesis and distribution of melanin. These pigments play an important role in the absorption of free radical generated in cytoplasm and shielding the ionizing radiation, including the UV-light (Mallick et al., 2002. p. 243). The skin pigmentation involves the co-operation of melanocytes and kerationcytes to produce melanosomes and then transfer them to keratinocytes, which then distribute them in various fashion routes to the surface of the skin. Recently, fibroblasts have also been shown to participate in the regulation of melanocyte growth and differentiation. Therefore, skin colors between race and even on various areas of a single individual reflect the interactions of many epidermal and dermal components (Hearing, 2005. p. 4). However, the most important of the skin color is the activity of melanocyte: the quantity and quality of pigment production, not the density of melanocytes (Bolognia & Orlow, 1999. p. 936). The key of the pigmentary systems are as follows:

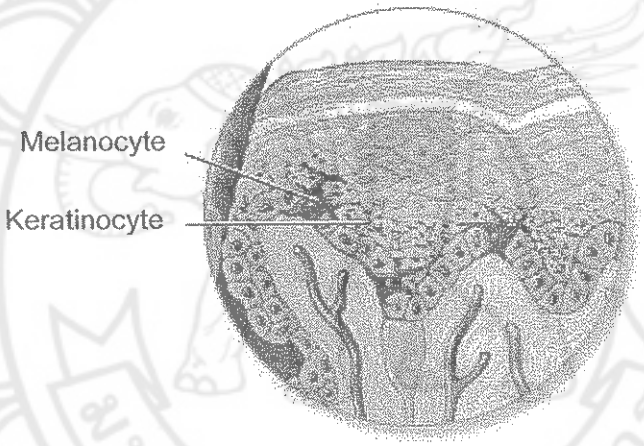
1. Melanocyte Biology

Melanocytes are highly dendritic cells that migrate from the neural crest during development until they reach the basal layer of the epidermis where they remain (Virador et al., 2001. p. 105). Although melanocytes comprise only a small proportion of the cells present in the epidermis of mammals, they are responsible for the production of the pigment melanin which accounts for virtually all of the visible pigmentation in their skin, hair and eyes. Melanocyte contains the melanosome which is the site of melanin biosynthesis. Melanocyte dendrite is to provide a conduit for melanosome trafficking and transfer to kerationocytes as shown in Figure 3. The association of the melanocyte and its surrounding keratinocytes has often been defined as "epidermal melanin unit" where one melanocyte is normally associated with 36 keratinocytes (Freedberg & Fitzpatrick, 1993. p. 265). Per 1 mm^2 of skin there are 1,100-1,500 melanocytes and that number is almost the same regardless of the skin type (Stenojevic et al., 2004. p. 204). It is logical to assume that dendrites are necessary component of melanosome transfer because

melanocytes represent a minority population in the epidermis and must therefore contact multiple keratinocytes (Scott, 2002. p. 322 citing Fitzpatrick, 1967. unpaged).



(A)



(B)

Figure 3 The Illustration of the Human Melanocyte Cells (A) and the Diagram of Melanocytes-Keratinocytes Cooperation (B)

2. The Melanin Biosynthesis

Melanin is the pigment of skin color which is synthesized in the melanosomes of melanocyte. Melanin synthesis is regulated by melanogenic enzymes such as tyrosinase, tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2). The starting material for the production of melanin, both the brown-black eumelanin and the yellow-red pheomelanin, is the amino acid tyrosine. The level and type of melanin production relate to the activity of the various enzymes as well as MSH (α-melanocyte stimulating hormone), agouti signaling protein, basic fibroblast growth factor (bFGF),

endothelin-1 and ultraviolet light (Bolognia & Orlow, 1999. p. 940). The melanin biosynthesis pathway is shown in Figure 4.

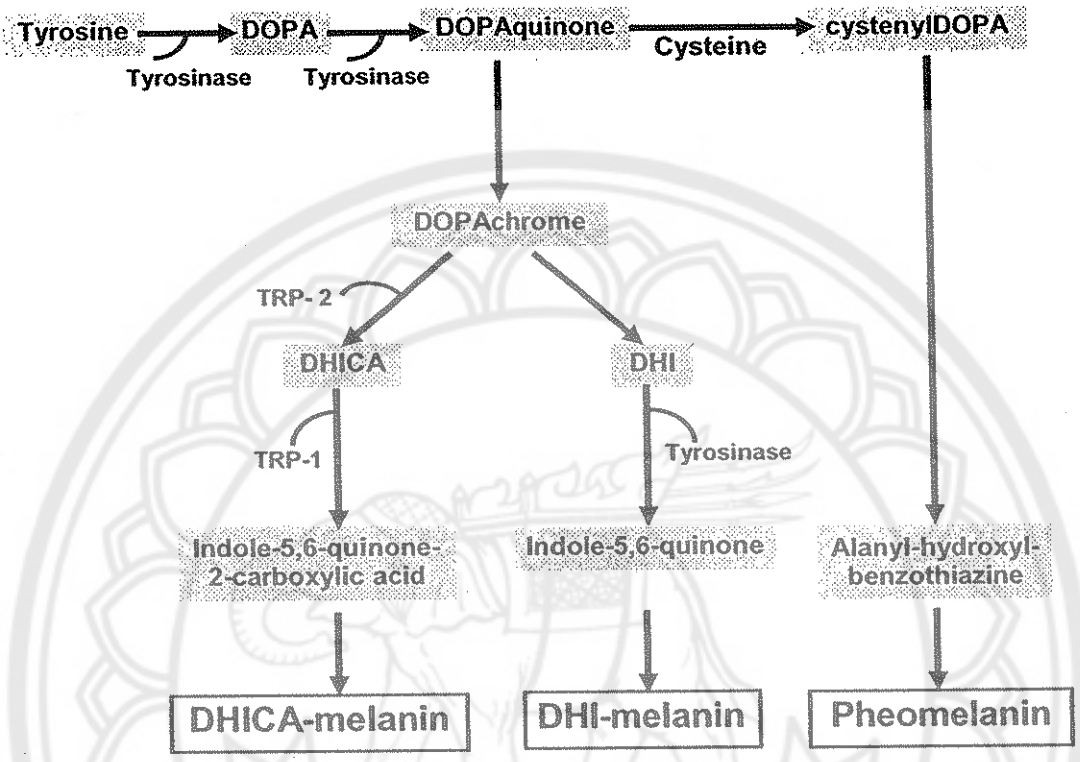


Figure 4 The Melanin Synthesis Pathway

The initial steps in the synthesis of eumelanin and pheomelanin are controlled by the enzyme tyrosinase which oxidizes the amino acid tyrosine to 3,4-dihydroxyphenyl-alanine (DOPA). DOPA spontaneously autooxidize to DOPAquinone without tyrosinase, but at slower rates than in presence of the enzyme. DOPAquinone is an extremely reactive compound that in the absence of thiols in the reaction medium, undergoes intramolecular cyclization leading to leukodopachrome and then to DOPAchrome. DOPAchrome decarboxylates spontaneously to dihydroxyindole (DHI). In the presence of divalent cations and the enzyme DOPAchrome tautomerase, also called tyrosinase related protein 2 (TRP-2), the intermediate 5,6-dihydroxyindole-2-carboxylic acid (DHICA) will result. DHI is oxidized to indole-5,6-quinone while DHICA is oxidized to Indole-5,6-quinone-carboxylic acid. It is speculated that the oxidation of DHICA is

catalyzed by an enzyme called DHICA oxidase which is synonymous with tyrosinase related protein 1 (TRP-1) and the oxidation of DHI by tyrosinase. The quinones are thought to build melanin by oxidative polymerization. Whether this polymerization step is under enzymatic control is not yet clear.

Melanins generated from DHICA are brown polymer, poorly soluble and of intermediate weight, whereas those generated from DHI are black, totally insoluble and of high molecular weight. These melanins are termed eumelanins. Eumelanins are a mixture of DHI- and DHICA melanins and the chemistry of these pigments may vary to a considerable extent (Freedberg & Fitzpatrick, 1993. pp. 270-274). Increased eumelanin content of human skin is stimulated by α-MSH, is known to control the rapid mobilization of pigment, result in changing morphological color within pigment cells (Eves, MacNeil & Haycock, 2006. p. 445). In the presence of sulfhydryl donors, probably cysteine, DOPAquinone is converted to cysteinyl DOPA. Further oxidation, cyclization and polymerization leads to the formation of pheomelanin. Pheomelanins have a yellowish-red color, are soluble in alkali and have a low molecular weight. It is synthesized in smaller amount compared to eumelanin (Stenojevic et al., 2004. p. 204). These different types of melanin are responsible for the differences in hair color in mammals and in man. Yellow to bright red hair result from the production of pheomelanin, whereas brownish to black hair have their origin in eumelanin production.

Black phenotype originated by eumelanin production, is elicited at conditions under which there is an overstimulation of the MSH receptor, whereas in contrast, conditions under which the function of the MSH receptor is abrogated or blocked by ASP result in the production of pheomelanin leading to a yellowish phenotype. Skin phototypes of cultured melanocytes revealed the total concentrations were 4.9 times higher in darker skin phototype IV-VI, when compared to lighter skin phototypes I-III. Also pheomelanin content were 2.5 times higher in the skin phototype IV-VI, which implies that the cells from light skin types contain less melanin, but relatively high proportion of pheomelanin (de Leeuw et al., 2001. pp. 110-111).

Every type of melanin has its own physical and biological characteristics. DHI melanin is very good in photoabsorption, shows no phototoxicity, but is highly cytotoxic whereas DHICA-melanins have reduced photabsorption, no phototoxicity and are less cytotoxic. In contrast, pheomelanin provides only little photoabsorption and has high phototoxic potential, but low cytotoxicity. The optimum type of melanin must be compromise between photoprotection while minimizing cyto- and phototoxicity.

3. The Enzymatic Control of Pigmentation

The melanogenic enzymes consist of tyrosinase, TRP-1 and TRP-2. They are glycoproteins embedded in the melanosome membrane that share 70-80% nucleotide sequence homology with 30-40% amino acid identity, and share common functional motifs such as epidermal growth factor receptor and copper binding sites (Jung, 2001. p. 134 citing de Marmal & Beerman, 1999. unpaged). Among these enzymes, tyrosinase is considered to be the rate-limiting enzyme and represents the major regulatory step in melanogenesis.

Tyrosinase is a multifunctional copper-containing glycoprotein with a molecular weight de novo of 65 kDa and about 75 kDa when glycosylated. This enzyme can catalyze three different reactions in the biosynthesis pathway of melanin; (i) the hydroxylation of tyrosine to DOPA (monophenolase activity), (ii) the oxidation of DOPA to DOPAquinone (diphenolase activity), and (iii) the oxidation of 5,6-dihydroxyindole (DHI) to indole-quinone (Sanchez-Ferrer et al.,1995; Hearing & Tsukamoto, 1991).

Tyrosinase is an extremely stable protein that is highly resistant to heat or proteases. It also has an unusually long biological half-life up. Tyrosinase can be divided into three domains: an inner domain that resides inside the melanosome, transmembrane domain and cytoplasmatic domain. The biggest part of the enzyme resides inside the melanosome and only 10% or 30 amino acids constitute to the cytoplasmatic domain.

Two other enzymes, TRP-1 and TRP-2, which also have catalytic functions that can modify the types of melanin produced. In the presence of TRP-2 which functions as DOPAchrome tautomerase (DCT), the carboxylic acid group of DOPAchrome, which would be spontaneously lost, is maintained, and carboxylated derivative (DHICA) is

generated rather than DHI (Hearing, 2005. p. 5). This leads to a more soluble and lighter colored melanin known as DHICA-melanin. In the part of TRP-1, the function of which remain unclear. The modulation steps in melanogenesis such as those involving cofactor of tyrosinase, tyrosinase hydroxylase activity, catalase-like activity, DCT activity, DHI oxidase activity, and DHICA oxidase activity (Maeda, 1997. p. 200 citing Jimenez, 1999. unpaged).

4. The Melanosomes Transfer and Distribution

Melanosomes are specialized members of the lysosomal lineage organelles. Melanosomes originate from the smooth endoplasmatic reticulum as a cytoplasmatic vesicle with an amorphous interior (Orlow, 1995. p. 3). As mature melanosomes arrive at the end of the melanocyte dendrite, they are secreted in areas where the melanocytes intercalate with keratinocytes. The actual transfer of melanosomes into keratinocytes and the keratinocyte-melanocyte interactions during the transfer are not well characterized. Early light and electron microscopy studies suggested numerous possible mechanisms for melanosome transfer (Seiberg, 2001. pp. 236-240). These include the release of melanosomes into the extracellular space followed by endocytosis, direct inoculation (injection), keratinocyte-melanocyte membrane fusion and phagocytosis. Recent studies show that the PAR-2, and the keratinocyte receptor PAR-2 play an important role in melanosome transfer (Seiberg et al., 2000. p. 28). Melanosomes are transferred to keratinocytes, as keratinocytes ascend to the epidermal surface from the basal and suprabasal layers where melanosome transfer takes place, melanosomes also ascend and are retained in the horny-layer cells for approximately two weeks. The phagocytic process of melanosome is increased by exposure of keratinocytes to UV-radiation or to α-MSH (Virador et al., 2002. p. 106). Recently, it has been reported that UV-induced changing of pH melanosome result in fast and dynamic regulatory mechanism for melanogenesis (Ancans & Thody, 2002. p. 60).

The distribution of melanosomes in human depends on the skin phototype. They occur singly in darker skin (phototype V and VI) and clusters in lighter skin (phototype I and II) (Virador, 2002. p.105 citing Ortonne, 1990. unpaged). The smaller

melanosomes of lightly pigmented skin are clustered in groups of two to ten within secondary lysosomes in the keratinocytes and are degraded by the mid stratum spinosum. In darkly pigmented skin, the melanosomes are larger and singly dispersed within lysosomes of the keratinocytes; they are degrade more slowly, such that melanin granules can still be found in the stratum corneum (Bolognia & Orlow, 1999. p.939).

The Classification of Depigmenting Activity and Skin Whitening Agents

1. Depigmentation

Increased production and accumalation of melanins characterize at large number of skin diseases, which include hyperpigmentation such as melanoma, post-inflammatory melanoderma, solar lentigo, etc. Several modalities of treatment for these problems are available including chemical agents or physical therapies.

Depigmenting compounds should act selectively on hyperactivated melanocytes and without short- or long-term side effect and induce permanent removable of undesired pigment. Since 1961 hydroquinone, a tyrosinase inhibitor, has been introduced and then other whitening agents specially acting on tyrosinase by different mechanisms have been proposed. Compounds with depigmenting activity have been classified by the based on their mechanism of interference with melanin synthesis as shown in Table 1.

Depigmentation can be achieved by regulating (i) the transcription and activity of tyrosinase, tyrosinase related protein-1 (TRP-1), tyrosinase related protein-2 (TRP-2), and/or peroxidase; (ii) the uptake and distribution of melanosomes in recipient keratinocytes and (iii) melanin and melanosome degradation and turnover of pigmented keratinocytes. However, as a result of the key role played by tyrosinase in the melanin biosynthesis, most whitening agents acts specially to reduce the function of enzyme by mean of several mechanisms; (i) interference with its transcription and/or glycosylation, (ii) inhibition by different modalities, (iii) reduction of by-products, and (iv) control of post–transcriptional (Briganti et al., 2003. p. 102).

Table 1 Mechanism of Melanogenesis Inhibitors

Mechanism	Whitening agent doses
1. Tyrosinase inhibition	(1-4%) Kojic acid, (3-7%) Arbutin
2. Melanin reduction and ROS scavengers	(1-3%) Magnesium-L-ascorbyl-2-phosphate, (2%) Vitamin E acetate
3. Cytotoxicity to melanocytes	(2%) Hydroquinone
4. Melanosome transfer inhibition	(2-5%) Niacinamide

The inhibition of tyrosinase function during melanin synthesis generally purposes for cosmetic whitening agents. Many of the traditionally used skin whitening products such as hydroquinone, hydroquinone derivatives, and mercury containing products are still used in many countries.

2. Whitening Agents

Hydroquinone (HQ) is a hydroxyphenolic chemical compound that inhibits the conversion of DOPA to melanin by inhibiting tyrosinase enzyme. It may also function by interfering with the formation or degradetion of melanosomes and by inhibiting the synthesis of DNA and RNA within melanocytes. HQ is a most widely used depigmenting agent at present, but HQ is considered to be highly cytotoxic to melanocytes and potentiality mutagenic to mammalian cells (Curto et al., 1999. pp. 666-668).

Monobenzyl ether of hydroquinone (MBEH) has similar mechanism of action to HQ on pigmented cells. Moreover, MBEH is subjected to select uptake by melanocytes and is capable of permanently destroying melanocytes. Therefore, both of HQ and MBEH cause permanent depigmentation, even after discontinuation of its use (Katsambas & Stratigos, 2001. p. 484).

These agents are serious health concerns, including irreversible cutaneous damages, accumulation of mercury in the body and poison. The adverse effects have lead to the search for safer and natural-based skin whitening products. Therefore, the

ideal agent for whitening products is one that inhibits melanogenesis without cytotoxicity, preferably by tyrosinase inhibition, reduces pigmentation in cells and is of "Natural" or "Plant" origin. The most of currently available whitening agents are shown in Figure 5.

Kojic acid is a fungal metabolic product that is a potent tyrosinase inhibitor and functions by chelating copper at the active site of the enzyme. Safety studies on the dermatological use of kojic acid in human showed that chronic treatment of up to 14 years resulted in no adverse local or systemic effects (Nohynek, 2004. p. 94 citing Yamamoto et al. 1998. unpaged).

L-ascorbic acid and magnesium L-ascorbyl-2-phosphate interferes with pigment production at various oxidative steps of melanin synthesis. They have the reducing effect on o-quinone and oxidized melanin, and can alter melanin from jet black to light tan (Katsambas & Stratigos, 2001. p. 485)

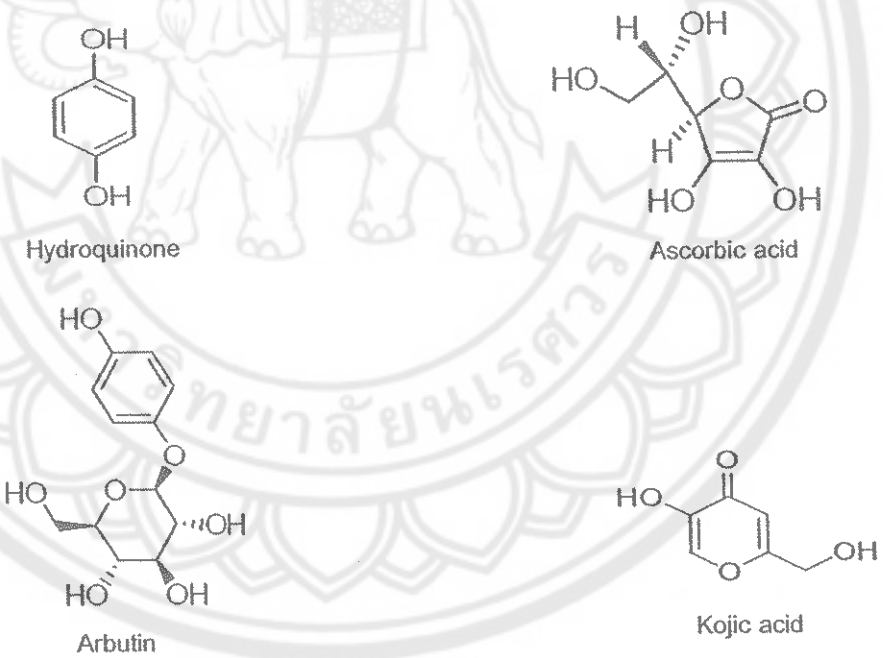


Figure 5 Chemical Structures of Whitening Agents

α-arbutin (4-hydroxyphenyl-α -D-glucopyranoside) is enzymatically synthesized from hydroquinone and saccharides. Arbutin has been widely use as a depigmenting agent in cosmetics. Its inhibitory activity against tyrosinase from

mushroom, B16 mouse melanoma and HMV-II human melanoma cells has been examined (Kubo et al., 2004; Sugimoto et al., 2004).

Licorice extract composes of glabrene and isoliquiritigenin which affect on tyrosinase activity and correlate to their ability to inhibit melanin formation in melanocyte cells (Nerya et al., 2003. p. 1205). These compound derivatives are synthesized for used as whitening agents in the cosmetic products.

Oxyresveratrol is the principle compound of mulberry (*Morus alba* L.) extract. It shows 32-fold stronger inhibitory effect on mushroom tyrosinase than kojic acid (Kim et al., 2002. p. 16342).

Methyl ester of gentisic acid (MG), is a natural product from the root of the genus Gentiana. It is effective skin lightening agent by inhibiting melanin synthesis with non-mutagenic in V79 Chinese hamster cells (Briganti et al., 2003; Curto et al., 1999).

Gallocatechin-3-o- gallate (GCG) is the most potent inhibitory of green tea component. GCG acts as a competitive inhibitor of tyrosinase enzyme result in suppress melanin formation (No et al., 1999. pp. 243-246).

The Melanogenesis Inhibitory Activity Tests

Nowadays, several methods have been used to screen depigmenting effect of the compounds. There are shown in Table 2. However, *in vitro* assay of malanogenesis inhibition is the most commonly used as the following:

1. Mushroom Tyrosinase Assay

Mushroom tyrosinase assay is the determination of dopachrome content occurred due to the reaction of DOPA substrate and tyrosinase enzyme as shown in Figure 6. When some chemical is added to this reaction mixture and the dopachrome color disappears, it mean that the added substance could successfully block the activity of tyrosinase enzyme (Elsner & Mailbach, 2002. pp. 129-134).

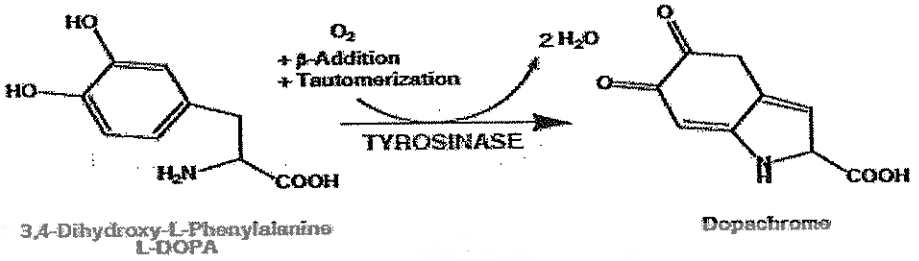


Figure 6 The DOPAchrome Formation

2. Melanin Content Assay

Melanin content assay is the determination of the melanin amount in melanocyte cells which are treated with the whitening agents. Cultured B16 melanoma cells have been used in the melanogenesis studies and are useful in demonstrating several new mechanisms of melanogenesis inhibition. The cell concentration, cell morphology and the extracts of melanin pigment in cultured cell indicate the potent of tested whitening agents. Whole melanin was determined by using two condition assays. For extracellular melanin content, the secretion of melanin in the medium was estimated whereas the intracellular melanin was quantified in pelleted cells.

Table 2 *In vitro* and Animal Assays for Whitening Agents

Melanogenesis inhibition assay	Examples
1. Tyrosinase inhibition	Kojic acid, Arbutin
2. Melanin reduction of B-16 melanoma cells	Hydroquinone,
3. Reduction of melanin pigment	Ascorbic acid

Antioxidant Properties as Potent Tyrosinase Inhibitors

1. Reactive Oxygen Species (ROS)

Nowadays, it has been reported that the reactive oxygen species (ROS) are implicated in a wide range of human diseases including skin problems. ROS are an inherent part of the anabolism and catabolism of living organisms (Pinnell, 2003. p. 2) ROS can be classified into oxygen-centered radicals and oxygent-centered nonradicals. Oxygen-centered radicals are superoxide ($\bullet O_2$-), hydroxyl radical ($\bullet$OH), alkoxyl radical (RO$\bullet$), and peroxyl radical (ROO$\bullet$). Oxygen-centered nonradicals are hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Other reactive species are nitrogen species such as nitric oxide (NO$\bullet$), nitric dioxide ($NO_2\bullet$), and peroxynitrite (OONO-) (Lee, Koo & Min, 2003. p. 22). These molecules are extremely chemically reactive and short-lived, they react at the place where they are created. Especially, $\bullet O_2$, $\bullet$OH and H_2O_2, they are dangerous to attack biological molecules, leading to cell of tissue injury associated with degenerative disease (Amarowicz et al., 2004. p. 522). Moreover, ROS enhance melanin biosynthesis, damaging DNA, and may induce proliferation of melanocytes (Wang et al. 2006. unpaged).

2. Antioxidant and Skin Whitening Effect

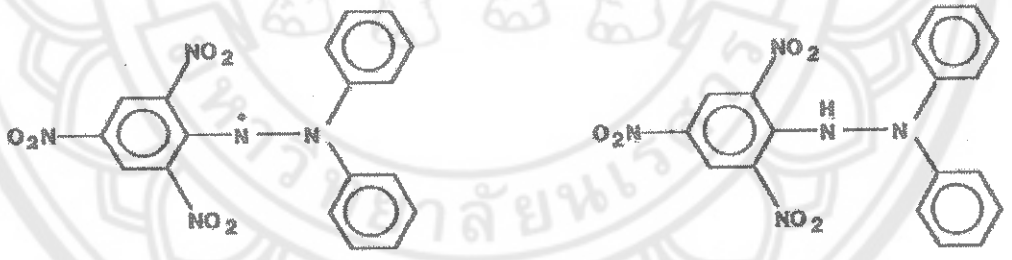
Antioxidant agents are used in pharmaceutical and cosmetic formulations mostly to prevent autooxidative deterioration of lipid raw materials. Antioxidants are also introduced as primary ingredients in cosmetics to scavenge free radical produced by UV light and environmental pollutants and involved in skin aging process (Juliano et al., 2005. p. 146 citing Lupo, 2001. unpaged). It is known that ROS scavenger or inhibitors such as antioxidant may reduce hyperpigmentation (Wang et al., 2006. citing Ma et al., 2001 unpaged).

The natural sources such as plants produce a variety of antioxidants against molecular damage from ROS, and phenolic compounds are the major class of plant-derived antioxidants. On the basis of Huckel theory calculation for tyrosinase active site model, it has been shown that ionization of hydroxyl group of phenolic compound is a

crucial step in its interaction with positively charged copper of the active site in mono phenolase reaction (Kubo et al., 2004. p. 353). Among the various phenolic compounds, the flavonoids are perhaps the most important group, according to they may exert antioxidant effects as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers and metal ions chelators (Okawa et al., 2001. p. 1202). Many tyrosinase inhibitors are phenolic derivatives of flavonoids such as artocarbene, chlorophorin, and norartocarpanone. These inhibitors are usually 4-substituted resorcinol moiety and catechol structure which inhibit and may behave as a chelator to the copper ions in the tyrosinase. In addition, these compounds can prevent pigmentation resulting from auto-oxidation processes (Khatib et al., 2005. p. 434).

3. DPPH assay

For the antioxidant activity test, diphenylpicrylhydrazyl (DPPH) method is currently popular. The DPPH assay is originally developed by Blois, is a free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule as shown in Figure 7



(A) Diphenylpicrylhydrazyl (free radical) (B) Diphenylpicrylhydrazine (nonradical)

Figure 7 (A) Free Radical Form of DPPH and (B) Reduced Form of DPPH after accepted an Electron or Hydrogen Atom

DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition, brought about by various additives (Amarowicz et al., 2004. p. 550). When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form

with the loss of the violet color. The degree of decoloration of this solution indicates the scavenging efficiency of the sample compounds. This assay can accommodate a large number of samples within a short period, and is sensitive enough to detect low concentrations of the principles (Mambro & Fonseca, 2005; Molyneux, 2004).

Artocarpus incisus Extract

Artocarpus incisus (breadfruit) belongs to the Moraceae family. This evergreen tree called "Sa-ke" in Thai is found throughout the tropical area including Thailand. Breadfruit is propagated with the root cuttings and produces its fruit up to 2-3 times in a year. This fruit is aromatic, rich in latex and can weight 1-4 kg. Maturing at 15-20 meters tall or greater can produce fruits for 50 years or more. The massive trunk may attain 2-3 meter girth and depending on the variety, it either slightly flares at the base or forms narrow buttresses. The illustration of breadfruit is shown in Figure 8. The pulps contain high carbohydrate content of 76.7% which has been used as an important source of energy over the years (Adebowale et al., 2004. p. 57). They are employed in food because their good thickening and gelling properties. They are also a good texture stabilizer and regulator in food systems. The chemical, physical and enzymatic method has been employed for modification of starches. (Amusa et al., 2002. pp. 343-344).

The heartwood of *A. incisus* has been reported that their retained components exhibit the strongly inhibit the tyrosinase activity and melanin biosynthesis. The methanol extract shows potent inhibitory of melanin biosynthesis and significant color whitening by using B16 mouse melanoma cells. In addition, the mother liquor by crystallization of *A. incisus* ether extract also shows potent inhibitory activity by using brown guinea pig brown without skin irritation.

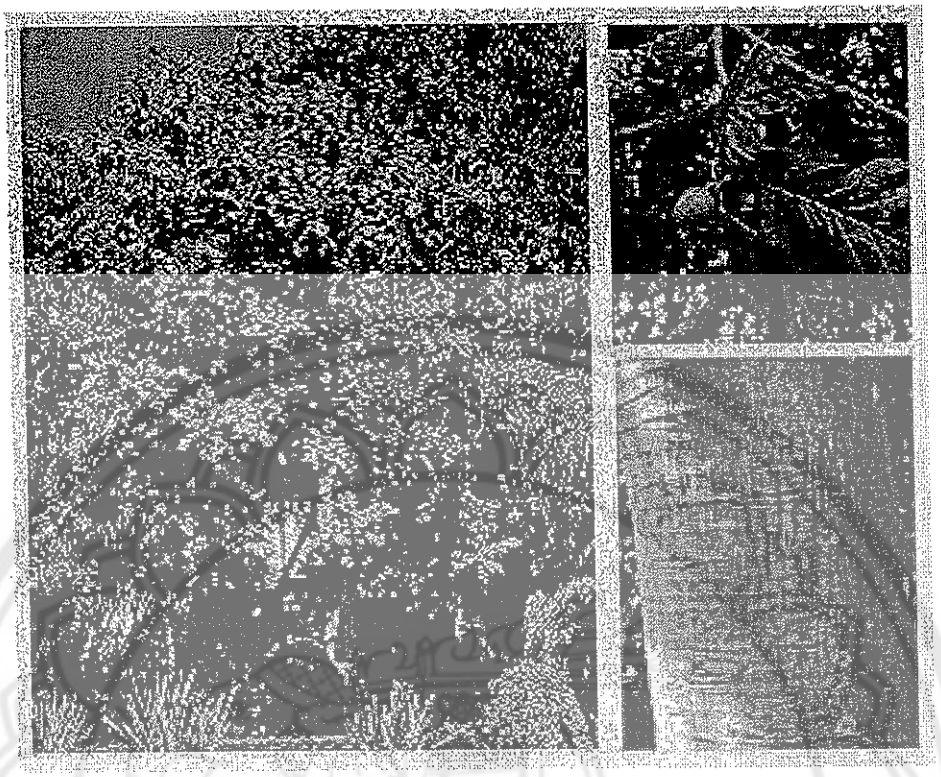


Fig 8 The Illustration of *Artocarpus incisus* (breadfruit)

The heartwood extract of *A.incisus* consists of several flavonoids, for examples artocarpin, (+)-norartocarpin, artocarpesin and cycloartocarpin. Among of these compounds, chlorophorin, (+)-norartocapanone, 4-prenyloxyreseratrol and artocarbene show much higher tyrosinase inhibitory activity than kojic acid (Shimizu et al., 1998. pp. 410-412). The chemical structures from *A. incisus* are shown in Figure 9. Furthermore, a major component of the extracts, artocarpin [6(3-methyl-1-butenyl-5,2'4'-trihydroxy-3-isoprenyl-7-methoxyflavone] shows distinct skin lightening effect on the UVB-induced hyperpigmented dorsal skin of brownish guinea pigs (Shimizu et al., 2002. p. 80). These findings have interested for application of the extracts in the skin whitening products.

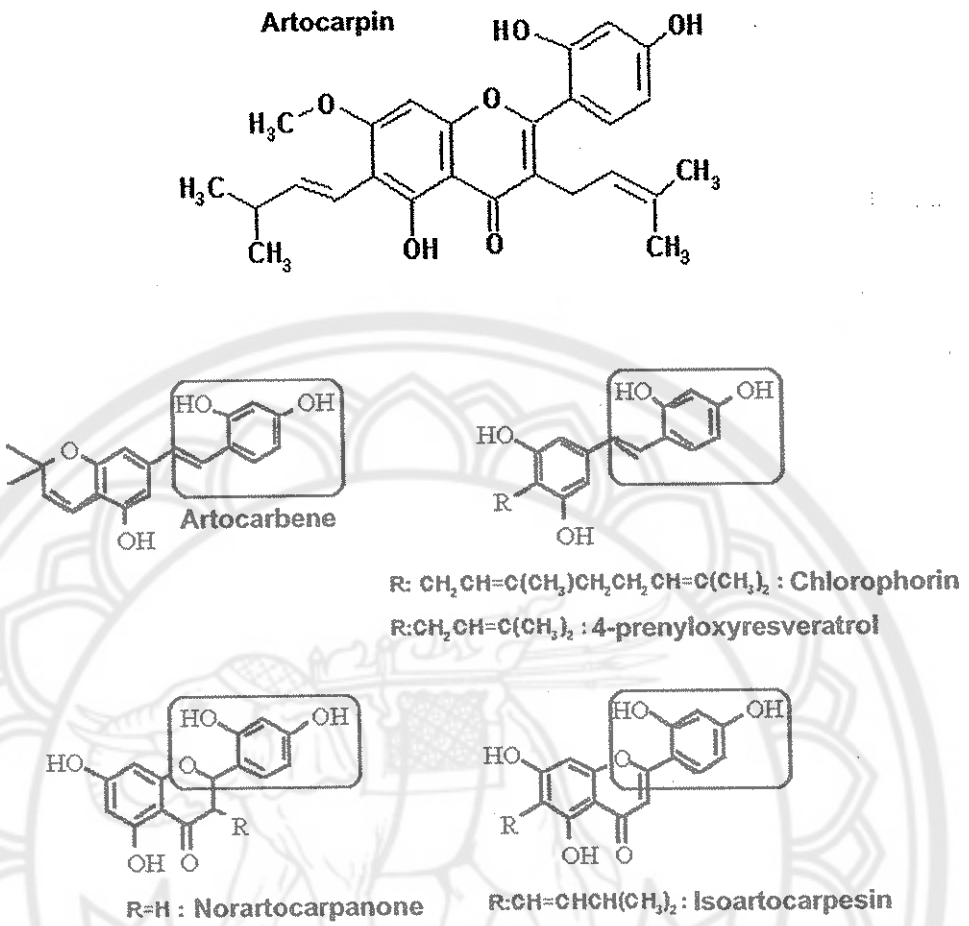


Figure 9 The Chemical Structures Isolated from *A.incisus* Extract