บทความรับเชิญ

Thai Breadfruit's Heartwood Extract: A New Approach to Skin Whitening

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Introduction

In recent years, skin whitening (also called "skin lightening") products have become and continue to be the best selling skin care products in Asia. Whitening products are supposed to either lighten skin or depigment skin. Several materials such as arbutin, kojic acid, and its derivatives were developed for treatment of hyperpigmentary disorders. However, the variation in clinical effect of these materials is usually found. The hydroquinone group compounds have been used as effective depigmenting agents but they exhibits cell toxicity. In this reason, new whitening agents with free from harmful side-effects are large demand in the market.

Recently, safe and effective tyrosinase inhibitors extracted from the natural sources have been reported for their potential applications in improving hyperpigmented disorders. For examples, the extracts from *Glycyrrihiza glabra* (licorice), *Morus alba* L. (white mulberry), *Carthanus tinctorius* L. (safflower), *Arctostaphylos uva-ursi* (bearberry) and *Oryza sativa* (rice bran) have been used as skin whitening agents. These materials are mostly free from harmful side-effects. For this reason, there is an increasing interest in finding natural tyrosinase inhibitors from natural sources. Additionally, an attempt to search for substitute materials with multi-functional activities in melanogenic inhibition is warranted since the synergistic effects of each activity may be taking place.

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Artocarpus incisus (breadfruit) belongs to the Moraceae family. This evergreen tree called "Sa-Ke" in Thai is found throughout the tropical. Because its pulp contains high content of carbohydrate at the amount of 76.7%, it has been used as important source of energy over the years. There has been reported that the extract of the heartwood of *A. incisus* grown in Okinawa, Japan strongly inhibits tyrosinase activity [1]. However, as far as we known, there has never been report about the effects of the extract of *A. incisus's* heartwood on tyrosinase enzyme, melanogenesis or oxidation activities. This review concludes recent study correlating the melanogenesis-inhibitory and antioxidant activity of Thai breadfruit's heartwood extract as approach to apply in skin whitening. In addition, to make easier to understand the possible action of the extract on melanogenesis inhibition, backgrounds involving the skin pigmentation and the depigmenting mechanisms are also reviewed.

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 melanins. These pigments play an important role in the absorption of free radical generated in cytoplasm and shielding the ionizing radiation, including the UV-light [2]. The skin pigmentation involves the co-operation of melanocytes and kerationcytes to produce melanins 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 [3]. 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 [4]. The key of the pigmentary systems are as follows:

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 [5]. 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 the 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 1. 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 [6]. 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 [7]. 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.

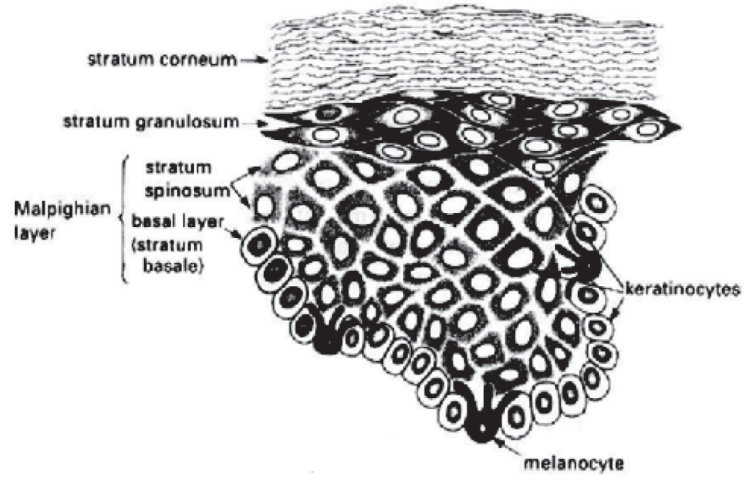

Figure 1 Diagram of melanocytes-keratinocytes cooperation

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 [4]. The melanin biosynthesis pathway is shown in Figure 2.

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 autooxidizes 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 spontaneously decarboxylates 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.

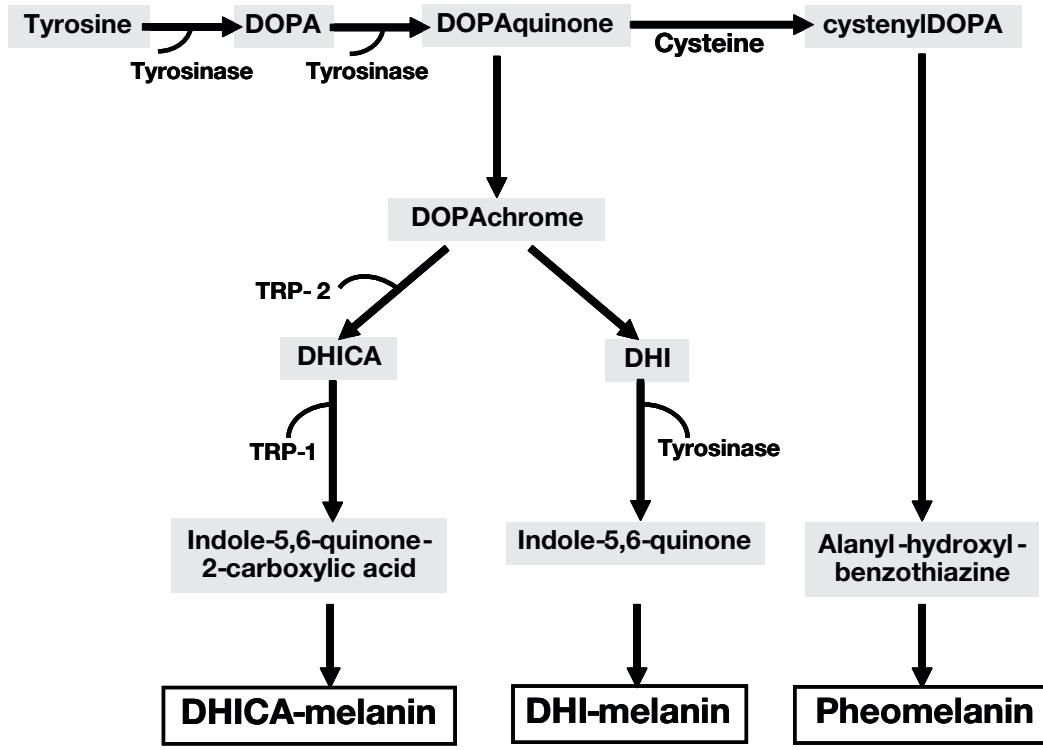

Figure 2 The melanin synthesis pathway

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 [8]. 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 about 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 [9, 10].

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 [3]. 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 include those involving cofactor of tyrosinase, tyrosinase hydroxylase activity, catalase-like activity, DCT activity, DHI oxidase activity, and DHICA oxidase activity [11].

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. 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 [12]. These include the release of melanosomes into the extracellular space followed by endocytosis, direct

inoculation (injection), keratinocyte-melanocyte membrane fusion and phagocytosis. Recent study shows that the PAR-2, and the keratinocyte receptor PAR-2 play an important role in melanosome transfer [13]. 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 [5].

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) [5]. 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 degraded more slowly, such that melanin granules can still be found in the stratum corneum [4].

The Classification of Depigmenting Activity and Skin Whitening Agents

Depigmenting Activity and Skin Whitening Agents

Increased production and accumulation 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 [14].

Table 1 Mechanisms of melanogenesis inhibitors

Mechanism	Whitening agent
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
5. Melanocytes-kearatinocytes interaction	(2-5%) tranexamic acid

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.

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 degradation 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 [15].

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 [16].

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.

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 study 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 [17].

L-ascorbic acid and *magnesium L-ascorbyl-2-phosphate* interfere 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 [16].

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 found [18, 19].

Licorice extract composes of glabrene and isoliquiritigenin which affect on tyrosinase activity and correlate to their ability to inhibit melanin formation in melanocyte cells [20]. 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 [21].

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 [14, 15].

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 [22].

Tranexamic acid (TA) shows clinical effect in depigmentation, but the mechanism of its action is unknown. Recently, there is report about the possible mechanism of TA in melanogenesis inhibitory activity. Such report indicates that TA inhibits melanin synthesis in melanocytes by interfering with the interaction of melanocytes and keratinocytes [23]

Antioxidant Properties as Potent Tyrosinase Inhibitors

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. 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 non-radicals 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-). 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.

Moreover, ROS enhance melanin biosynthesis, damaging DNA, and may induce proliferation of melanocytes.

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 [24]. It is known that ROS scavenger or inhibitors such as antioxidant may reduce hyperpigmentation [25, 26].

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 [18]. 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 [27]. 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 [28, 29].

Artocarpus incisus Heartwood 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 3. The pulps contain high carbohydrate content of 76.7% which has been used as an important source of energy over the years. 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.

Recently, there has been reported that the components of the heartwood of *A. incisus* grown in Okinawa, Japan can strongly inhibit tyrosinase activity [1]. The methanol extract of *A. incisus’s* heartwood shows potent inhibitory activity of tyrosinase enzyme.

Additionally, the mother liquor by crystallization of *A. incisus* ether extract also shows melanin biosynthesis inhibitory effect on brown guinea pig. The heartwood extract of *A. incisus* consists of several flavonoids (Figure 4) including artocarpin, (+)-norartocarpin, artocarpesin, (+)-dihydromorin and cycloartocarpin. Among these compounds, chlorophorin, (+)-norartocapanone, artocarbene and 4-prenyloxyresveratrol show much higher tyrosinase inhibitory activity than kojic acid whereas artocarpin does not shows tyrosinase inhibitory activity [1]. However, this compound shows skin lightening effect on the UVB-induced hyperpigmented dorsal skin of brownish guinea pigs [30]. These findings lead to the question if the crude extract containing several kinds of flavonoids, will exhibit higher melanogenesis inhibitory activity comparing to the purified artocarpin, according to the combination of the effects including tyrosinase inhibitory, antioxidant and other possible activity.

Figure 3 The illustration of *Artocarpus incisus* (breadfruit)

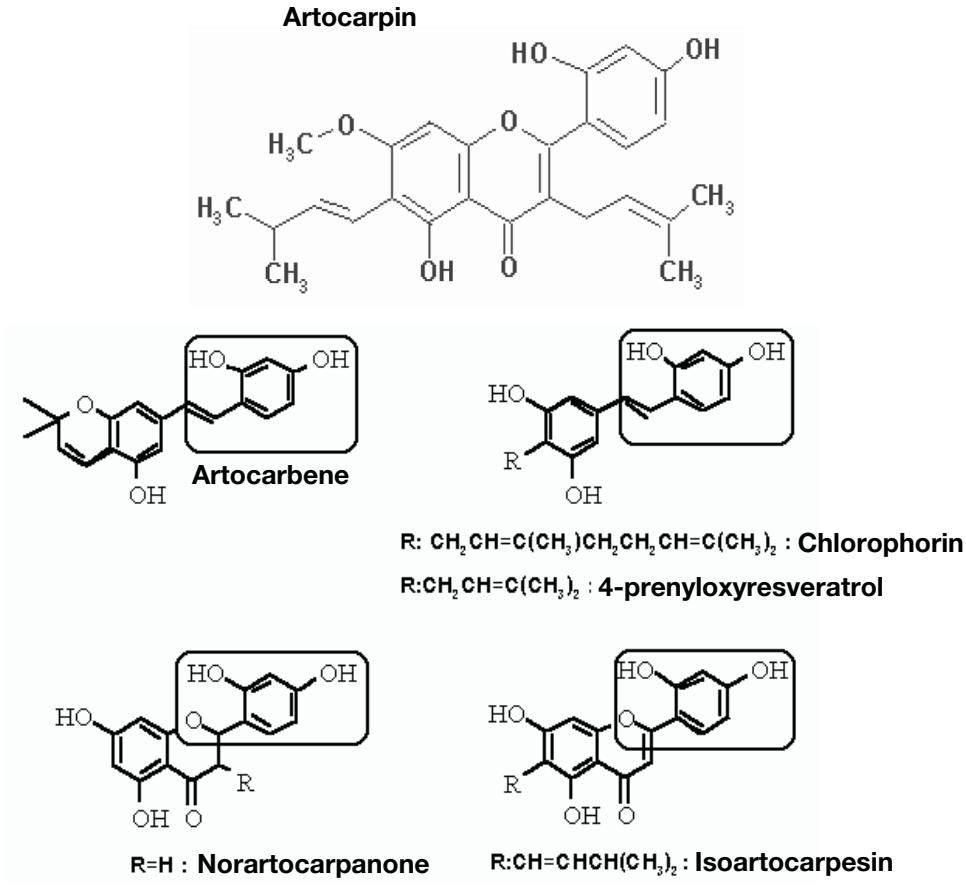

Figure 4 The chemical structures isolated from *A. incisus* extract

Therefore, to answer this question, the inhibitory effect of the extract on melanin biosynthesis is compared to that of purified artocarpin by using *in vitro* DOPAchrome assay and cell culture model [31]. The obtained results suggest that the crude extract has IC_{50} values (tyrosinase inhibition) of 10.26 ± 3.04 μg/mL. (7.89 ± 0.18 μg/mL for kojic acid). Additionally, the *A. incisus* extract at the concentration of 2 to 25 μg/mL are able to decrease the melanin production of melanocyte B16F1 cells. The obtained micrograph also confirmed that the crude extract does not change cell morphology but reduces the melanin content by inhibiting melanin synthesis, whereas the purified artocarpin at the concentration of 4.5 μg/mL causes changes in cell morphology or cell death (Figure 5). Moreover, the extract exhibits the antioxidant activity in the dose-dependent manner at the EC_{50} of 169.53 ± 9.73 μg/mL, according to DPPH assay [31].

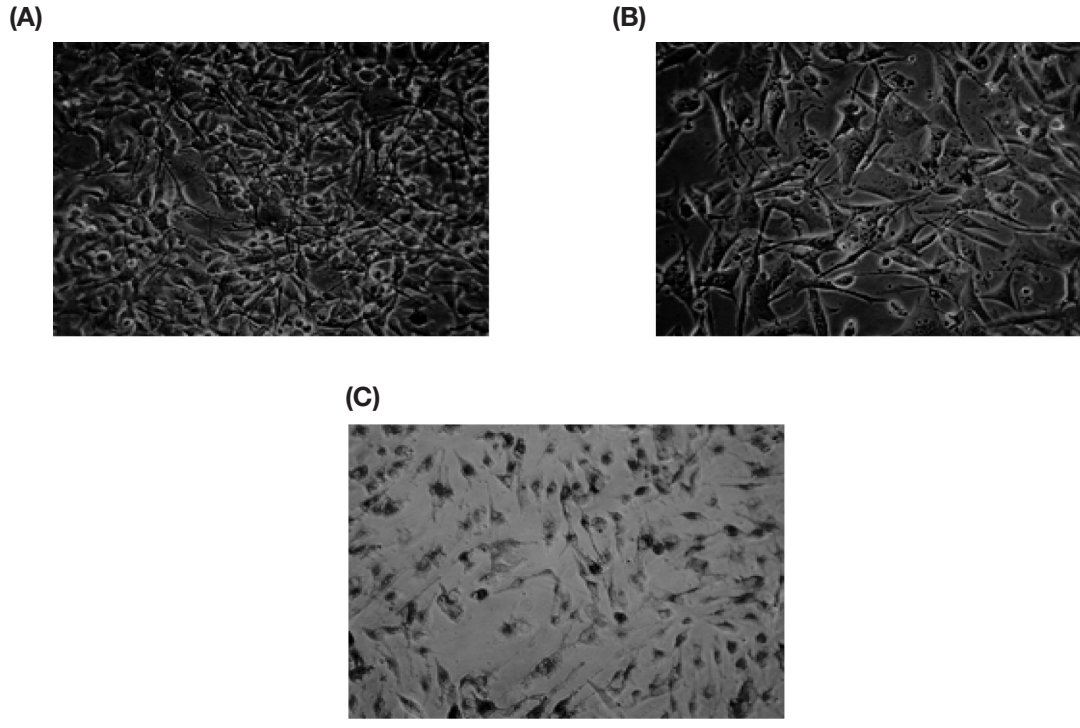

Figure 5 Morphology of melanocyte B16F1 melanoma cells treated with (A) 0.1% DMSO (control), (B) 10 μg/mL *A. incisus* crude extract and (C) 4.5 μg/mL purified artocarpin for 3 days (at magnification of 400X) [31]

Conclusion

Nowadays, the botanical extract is playing an increasingly important role in cosmetics. In the research for new natural whitening agents, several plants with the depigmenting effects have been investigated. The antioxidant activity together with tyrosinase inhibitory activity of the heartwood extract of *A. incisus* would provide much more benefit. The antioxidant property may prevent or delay pigmentation by different mechanisms, such as by scavenging ROS and reactive nitrogen species, or by reducing *o*-quinones or other intermediates in melanin biosynthesis. Therefore, the action of *A. incisus* extract on tyrosinase may be due, at least in part, to phenolic components of the extract. As comparison to *Morus alba* (mulberry extract), the *A. incisus* extract seems to be a stronger tyrosinase inhibitor. In addition, as comparing between the purified component, artocarpin (4.5 μg/mL) and the crude extract (10 μg/mL), both does not show difference in decreasing of melanin production. However, artocarpin at this concentration shows significantly decreasing in number of melanocytes. This may conclude that the crude extract of *A. incisus's* heartwood exhibit similar activity on melanogenesis

inhibition but lesser cytotoxicity comparing to the component purified from such plant. Moreover, this suggests that, as application for cosmetics, isolation and purification of the active ingredient within the crude extract are sometimes not needed because such isolation and purification may lead to a loss of the biological activity and may lead to toxicity.

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