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Review Article

Perspective approaches on melanogenesis inhibition

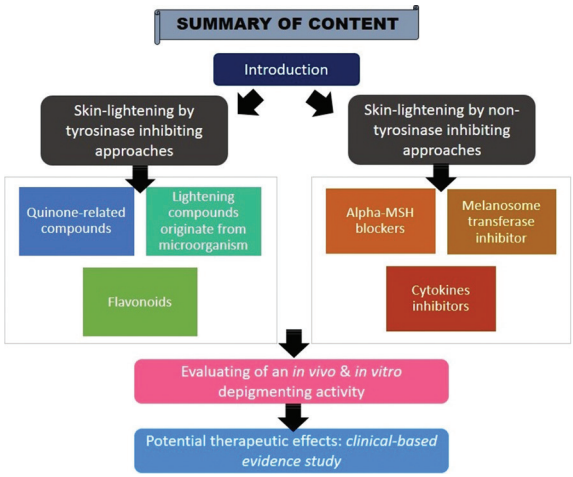
Aimi Syamima Abdul Manap[1], Yin Kei Lum[2], Lei Hui Ong[1], Yin-Quan Tang[1], Lai Ti Gew[2], Adeline Yoke Yin Chia[1]*

[1]School of Biosciences, Faculty of Medical and Health Sciences, Taylor's University, Subang Jaya, Selangor Darul Ehsan, Malaysia,
[2]Department of Biological Sciences, School of Science and Technology, Sunway University, Petaling Jaya, Selangor, Malaysia

Abstract

Melanogenesis is a melanin-forming process responsible for protecting the skin against ultraviolet radiation damage. An excess production of melanin, however, may result in hyperpigmentation (darkening of the skin) to adverse dermatological effects (freckles, solar lentigines, and melasma) and skin cancer. These hyperpigmentary skin disorders may also have a major effect on a person's appearance and could even result in emotional and mental distress, as well as a diminished quality of life. A large number of melanogenesis inhibitors have been discovered, but most of them appeared to have undesirable side effects. Therefore, in order to better understand the mechanisms of hyperpigmentary skin disorders and to establish effective and safe melanogenesis inhibitors, more fundamental research is needed. Apart from tyrosinase blockers, there are also alternative approaches that involve the manipulation of melanogenesis regulatory pathway such as α-melanocyte-stimulating hormone blockers, melanosome transferase inhibitors, and cytokines. This review abridges data on the different melanogenesis inhibitors and depigmentation agents from both natural and synthetic agents from the last few years.

Key words: Inhibiting agents, melanogenesis, skin lightening, tyrosinase inhibitor



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Introduction

Melanogenesis is defined as a series of process leading to the formation of melanin (dark-brown pigment) by melanocytes,[1] which is found in the basal layer of the interfollicular epidermis.[2] While melanin serves as an antioxidant to protect the skin against harmful ultraviolet (UV) radiation-induced generation of reactive oxygen species (ROS), abnormally high melanin formation and accumulation can lead to hyperpigmentation disorders in the skin.[3] Although hyperpigmentation of the skin is

Address for correspondence: Dr. Adeline Yoke Yin Chia, School of Biosciences, Faculty of Health and Sciences, Taylor's University, 1 Jalan Taylor's, Subang Jaya 47500, Selangor, Malaysia.
E-mail: yokeyin.chia@taylors.edu.my

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usually harmless, excessive pigmentation, particularly on the face, such as melasma, solar lentigines, and freckles, poses a significant cosmetic nuisance and can cause distress to the person affected.[4,5] One of the most common hyperpigmentation diseases is melasma,[6] which is an acquired macular brown pigmentation [Figure 1].

Melanogenesis begins with tyrosinase to catalyze in tyrosine oxidation to produce dopaquinone. It is also the rate-limiting step in melanin synthesis as the downstream processes can occur spontaneously at a physiological pH value.[8] Next, when dopaquinone is converted to dopa and dopachrome, auto-oxidation takes place (Dopa may again be oxidized by tyrosinase to dopaquinone). Subsequently, a series of oxidation reaction occurs from eumelanogenesis where dihydroxyindole (DHI) and DHI-2-carboxylic acid are transformed into eumelanin. In the presence of cysteine or glutathione, dopaquinone is converted to cysteinyldopa or glutathionyldopa before the formation of pheomelanin. In addition to eumelanin and pheomelanin, another "melanin" relying on phenolic monomers different from tyrosine is termed allomelanin.[9,10] Figure 2 shows the biosynthetic pathway of melanin.

Hyperpigmentation disorders are currently treated with a broad range of topical hypopigmenting or skin-lightening agents, chemical peels, laser therapy, cryotherapy, and superficial dermabrasion.[5] The preferred mode of treatment for managing these conditions is combination therapy, which allows synergism and reduces the likelihood of inappropriate implications. For example, one of the gold-standard melasma treatments is known to be the most common triple combination, comprising hydroquinone (HQ), tretinoin, and fluocinolone acetonide. This triple combination was also found to significantly reduce melanin levels and the amount of lentigines (marked by the appearance of a small brown patch, a benign lesion often occurring in areas exposed to sunlight).[5] Nonetheless, these treatment modalities do not completely eradicate skin lesions and cause adverse effects. Thus, alternative, more specific, safe, and more effective therapeutic options are increasingly needed. The quest for an effective melanogenesis inhibitor has led to the discovery of hundreds of natural substances with potential anti-melanogenic activity.[12] A better understanding of regulatory pathways to melanogenesis may help to identify certain specific targets for existing or new therapies that could be used to regulate these pathways and control hyperpigmentation disorders.

Skin Lightening

Skin lightening refers to the application of natural or synthetic substances to lighten the skin tone or to provide better complexion by reducing the level of melanin in the skin.[13] The application of the whitening agents can be prompted by dermatological needs in patients with dermatological disorders associated with excessive accumulation of melanin (e.g., melasma and senile lentigo)[14] or merely by culture-specific

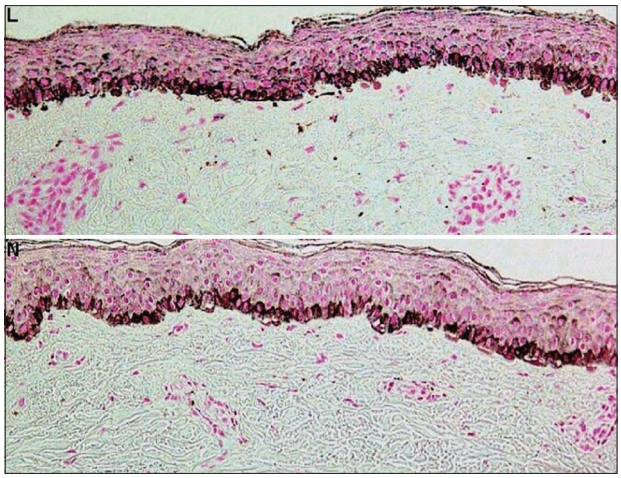

Figure 1: Increased epidermal pigmentation is the hallmark of melasma. Fontana-Masson staining shows more pronounced epidermal hyperpigmentation in lesion (L) compared to perilesional normal skin (N). Kang and Ortonne (2010)[7].

beauty preferences. Several chemical substances have already been demonstrated to be effective skin whiteners, and some even show beneficial effects (antioxidants,[15,16] antiproliferative activity,[17,18] protection of macromolecules such as collagen against harmful radiation,[19] etc.). However, certain safety issues have recently increased, resulting in the prohibition of these chemical substances in some countries.[20,21] The intense use of whitening agents, therefore, poses a real public health risk and can result in serious pathologies including burns, acne, stretch marks, hypopigmentation, and even cancer.[22,23] It is important to note that these whitening treatments are often very long term and that their application over weeks or months produces results that are not necessarily definite.[22] The demand for fairness has led in recent years to the discovery of a variety of whiteners with little to no side effects deriving from diverse biological resources. Yet, there is still a long way to go from identifying an active ingredient to incorporating it into cosmetics. Table 1 lists some examples of skin commercial lighting products and their active biological ingredients.[24]

Skin Lightening by Tyrosinase-Inhibiting Approaches

Since tyrosinase is the preliminary enzyme for melanogenesis, the strategy of tyrosinase inhibition to prevent dark spot formation is the most extensively studied approach. A reducing agent such as ascorbic acid can lead to a chemical reduction in dopaquinone due to its ability to reduce o-dopaquinone back to dopa and would then prevent dopachrome and melanin from forming. Many thiol-containing compounds such as o-dopaquinone scavenge are a melanogenesis inhibitor that reacts with dopamine to form colorless products. Thus, the melanogenesis is slowed down until the scavengers are completely consumed. In addition, acids or bases known as nonspecific enzyme inactivators may inhibit melanogenesis activity by denaturing

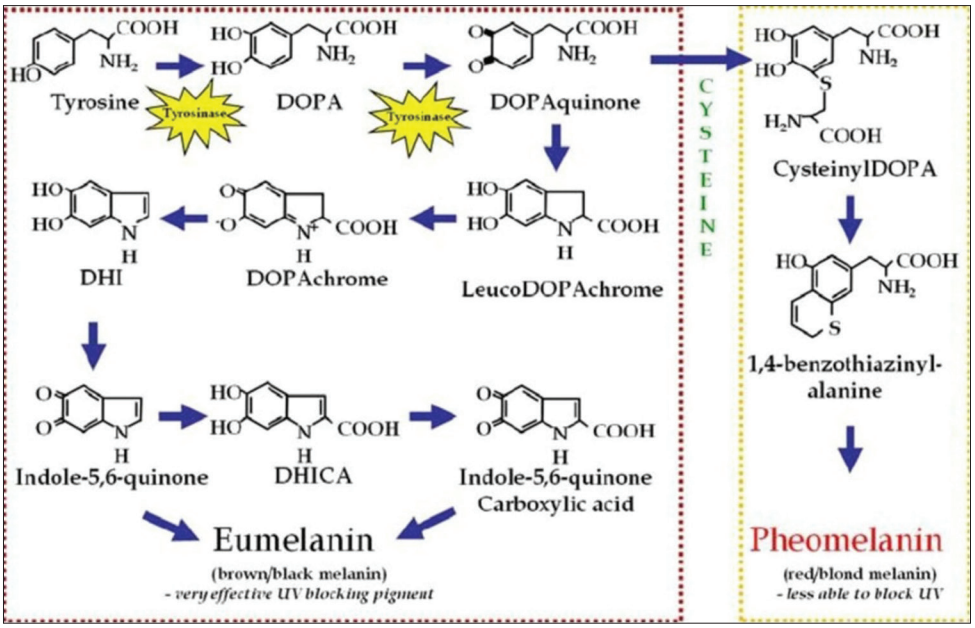

Figure 2: Biosynthetic pathway of melanin. Melanin is present in two main forms: (1) the highly ultraviolet-protective brown/black "eumelanin" pigment, and (2) the ultraviolet-permeable red/blonde "pheomelanin." Eumelanin and pheomelanin are both synthesized from tyrosine, an amino acid. Tyrosinase, the enzyme that catalyzes the synthetic rate-limiting reaction for both types of melanin, is the deficient enzyme in the most common type of albinism. The incorporation of a cysteine into pheomelanin leads to the retention of a moiety of sulfur into the pigment, which can contribute to oxidative injury caused by ultraviolet. D'Orazio *et al*.[11] (DOPA: 3,4-dihydroxyphenylalanine, DHI: 5,6-dihydroxyindole, DHICA: 5,6-dihydroxyindole-2-carboxylic acid).

Table 1: A list of some commercial skin-lightening products

Trade name	Actives
Depiwhite (ACM)	Kojic acid
Revitalift Laser x 3 lotion (L'O réal)	Glycolic acid
White objective (Bioderma)	Niacinamide, Glycyrrhiza glabra root extract
D-pigment (Avéne)	Retinaldehyde
Effaclar AI (La Roche Posay)	Niacinamide
Aqua Lotion (Amarte)	Arbutin
Whitelan (Dermica)	Morus alba root extract, Kojic acid, Glycyrrhiza glabra root extract
Hydra system (Institut Estherderm)	Salicylic acid, Morus alba leaf extract, Niacinamide
Depiderm (Uriage)	Ascorbic acid
Vinoperfect (Caudalie)	Tocopheryl acetate
Prescription anti-taches intensif (Liérac)	Glycolic acid

the enzyme which is responsible for melanogenesis. Besides that, suicide inactivators or mechanism-based inhibitors may form a covalent bond with the enzyme that has been catalyzed by tyrosinase. This can contribute to tyrosinase inhibition, and this is known as the "suicide reaction."[25]

Quinone-Related Compounds

There are three common quinone-related compounds widely used in skincare products: HQ (1,4-dihydroxybenzene), arbutin, and deoxyarbutin (dA).[26] HQ works by binding histidine at the active sites of tyrosinase, therefore, inhibiting the action of tyrosinase.[27] A previous study showed that HQ caused decreased melanosome development, marked changes in the internal structure of melanosomes, increased melanosome degradation, and eventually, destruction of the melanocytes' membranous organelles.[28] Nevertheless, although HQ remains the gold standard for depigmentation agents, the compound has been prohibited by the European Committee (24th Dir 2000/6/EC) since 2000 for general cosmetic purposes and formulation with this compound is only authorized by a doctor or dermatologist prescription.[26] This is because HQ application was shown to induce the generation of ROS and cause the oxidative damage to the membrane lipid, protein, and enzyme such as tyrosinase.[29] HQ was considered as toxic compounds[30] and may cause permanent loss of melanocytes, potentially mutagenic to mammalian cells,[31] and causes skin irritation.[32] Arbutin is a naturally occurring derivative of HQ used extensively for the prevention of dark spots.[33] It is originally developed by Shiseido Company and has been reported to exhibit less melanocyte cytotoxicity compared to HQ. It competitively and reversibly binds to tyrosinase without influencing the mRNA transcription of tyrosinase. A similar effect has been achieved by the synthetically produced arbutin derivative, the dA, which was reported to be effective and safer as a skin-lightening agent.[26]

Lightening Compounds Originate from Microorganisms

Kojic acid produced from several species of fungi (*Aspergillus sp*. and *Penicillium sp*.) is one of the naturally occurring compounds used for anti-dark spot formation. It chelates copper atoms in

the tyrosinase active sites.[10] However, it only shows moderate effectiveness in the clinical trials and possible to cause contact dermatitis, sensitization, and erythema.[34] Another popular compound is azelaic acid which is a saturated dicarboxylic acid found naturally in wheat, rye, and barley. Besides being used as treatments for acne, rosacea, and skin pigmentation, azelaic acid can also prevent the formation of dark spots by binding to the amino group and carboxyl group, thus preventing tyrosine from interacting in the active tyrosinase site.[35]

Flavonoids

Flavonoids are a large group of polyphenolic compounds, which can be derived from herbs and vegetables. Flavonoids are categorized into five major groups with the shared core backbone structure of flavan: flavones, flavonols (3-hydroxyflavone), flavanols, isoflavones, and anthocyanidines[36] [Figure 3]. The effects of flavonoids in melanogenesis have been studied extensively, and to date, there are more than 8000 flavonoids identified.[37] The main function of this pigment-reducing compound is that it acts as a ROS scavenger to interact with free radicals generated at the active site of tyrosinase. In addition, they also work as metal chelators to the copper, thus forming the copper-flavonoid complex and rendering them inactive to participate in free radical generating reactions.[38,39] According to previous studies, flavonoid compounds may have a stimulating and inhibitory effect on melanogenesis.[40,41] In a recent study conduced by Promden *et al*., of the 27 types of flavonoids tested for inhibitory activity and melanin synthesis in melanocytes, only cajanin and (6aR, 11aR) 3,8 dihydroxy 9 methoxy pterocarpan were shown to inhibit murine tyrosinase activity.

Various studies revealed that flavonoids that stimulate

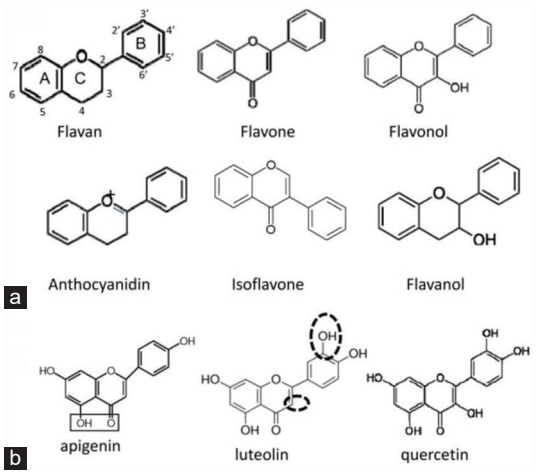

Figure 3: (a) Six main types of flavonoid which have been tested in melanoma models and their major dietary sources. (b) Structure comparison of luteolin, apigenin, and quercetin. The different side groups are circled in luteolin structure. The boxed side groups in apigenin show a typical structure that is able to bind metal ions. Comparing these three popular compounds which sometimes show opposite effects on melanogenesis may provide some hints on how each side group functions biologically.

melanogenic effects are cyanidin, hesperetin, apigenin, and fisetin. In addition, flavonoids that exhibit anti-melanogenic effects include epigallocatechin gallate (EGCG), hesperidin, luteolin, baicalein, and kaempferol. Cyanidin exhibits melanogenic effects by induces B16 differentiation by upregulating cAMP, expression of tyrosinase, and differentiation marker MART-1. Hesperetin will enhance the accumulation of melanocyte-inducing transcription factor (MITF) and lead to melanogenesis. Apigenin induces melanogenic effect by targeting tyrosinase-related protein-2/dopachrome tautomerase (TYRP-2/DCT) and TYRP-1 by p38 mitogen-activating protein kinase (PK). Fisetin plays a role in anti-melanogenesis by inhibiting melanoma cell invasion via inhibition of epithelial-to-mesenchymal transition in a three-dimensional skin model and in a xenografted mouse model. On the other hand, MITF protein accumulation can be inhibited by hesperidin and catechins, including EGCG, as well as inhibit tyrosinase accumulation. Baicalein, which is an anti-melanogenesis agent, plays a role in inhibiting accumulation of MITF via ERK1/2-phosphorylation-mediated degradation. Luteolin and kaempferol will inhibit melanogenesis by targeting tyrosinase directly or indirectly.[40]

Another well-known flavonoid till date is licorice roots (*Glycyrrhiza glabra*), which have been found to play a role in skin whitening treatment.[42] A previous study has shown that glabridin, a chemical compound that is found in the root extract of licorice, acts as a tyrosinase inhibitor which inhibits melanogenesis.[43] Moreover, 70% of skin-whitening cosmetic products containing glabridin molecules.[44] A previous study which investigated the inhibitory effect of melanogenesis and inflammation by glabridin using B16 murine melanoma cells and guinea pig skins had shown that glabridin successfully inhibited the enzyme tyrosinase activity at the concentration of 0.1–1.0 μg/ml.[45] In addition, the study also showed that it successfully prevented UVB-induced pigmentation and erythema when 0.5% glabridin was added topically to the skin of the guinea pig. However, glabridin has a limited skin-whitening efficacy as well as the instability in formulation.[45]

In order to encounter the limitation of glabridin application, the effort of discovering new compound for anti-melanogenesis continues. Another compound, glabrene was then isolated by Nerya *et al.* which has two hydroxyl groups at the 2' and 7' position with a 2,2-dimethyl-ç-pyran ring connected to the B ring as well as a double bond between carbon atoms 3 and 4 in the C ring. This configuration provides the maximum conjugation of the double bonds on the glabrene molecule. This study showed that both glabrene and another compound, isoliquiritigenin, can inhibit mushroom tyrosinase by inhibiting this enzyme's mono- and diphenolase activities. Moreover, both of these compounds are proven to be able to inhibit the biosynthesis of melanin in melanocytes. Apart from that, another report postulated that glabrene and isoliquiritigenin are the inhibitors of tyrosinase instead of its inactivators. This is because they demonstrated that preincubation of tyrosinase with both of the compounds in the absence of substrate did not

show a reduction in tyrosinase enzyme activity significantly.[46] In 2005, glycyrrhisoflavone and glyasperin C were reported as two new potential tyrosinase inhibitors from the root extract of licorice with glyasperin C exhibits a higher tyrosinase inhibitory activity than the well-known glabridin.[47]

Another work carried out where the total content of the licorice plant was isolated was found to contain more flavonoids than its root component in the licorice leaves. The most abundant flavonoid in the liquorice leaves is pinocembrin, with strong antioxidant activity and nitrile scavenging potential and mild ability to inhibit mushroom tyrosinase. Besides, the main flavonoid compound found in licorice root is the liquiritin which exhibits a strong inhibitory effect on mushroom tyrosinase.[48] This finding, however, contradicts another study reported in which four separate flavonoid compounds, namely liquiritin, licuraside, isoliquiritin, and licochalcone A, are isolated from the root of licorice. The result showed that liquiritin displayed no enzyme-inhibiting activity in mushroom tyrosinase relative to licuraside, isoliquiritin, and licochalcone A with IC50 values of 0.072, 0.038, and 0.0258 mM respectively for monophenolase activity.[49]

Skin Lightening by Nontyrosinase-Inhibiting Approaches

While most studies focus on compounds' ability to inhibit tyrosinase, other mechanisms are also available to inhibit melanogenesis, as tyrosinase development or melanogenesis initiation is also regulated by many other components including hormones and cytokines. Therefore, it is important to identify the melanogenesis regulatory pathway. Figure 4 shows the selected pathways for melanogenesis regulation.

The binding of alpha-melanocyte-stimulating hormone (α-MSH) to melanocortin 1 receptor (MC1R) will lead to activation of adenylate cyclase which in turn increases cAMP levels and activates PKA. This will then induce gene transcription of MITF and cause the production of melanogenic enzyme tyrosinase. Besides, the releases of diacylglycerol (DAG) from the cell membrane due to UV radiation will activate PKC-β, which then activates tyrosinase enzyme through phosphorylation at the serine residue on enzyme. In addition, DAG also phosphorylates the diacylglycerol kinase-ζ, which regulates tyrosinase degradation. The UV radiation also damages cellular DNA, initiating a cascade of DNA damage responses including p53 activation, and leading to increased tyrosinase transcription. UV irradiation, by decreasing the level of bone morphogenetic protein (BMP) receptors, prevents BMP-4-mediated inhibition on melanogenesis.[51]

Alpha-Melanocyte-Stimulating Hormone Blockers

α-MSH is an endogenous tridecapeptide neurohormone originating from proopiomelanocortin (POMC) that modulates inflammatory cutaneous and immune responses in normal human keratinocytes, Langerhans, melanocytes, and dermal

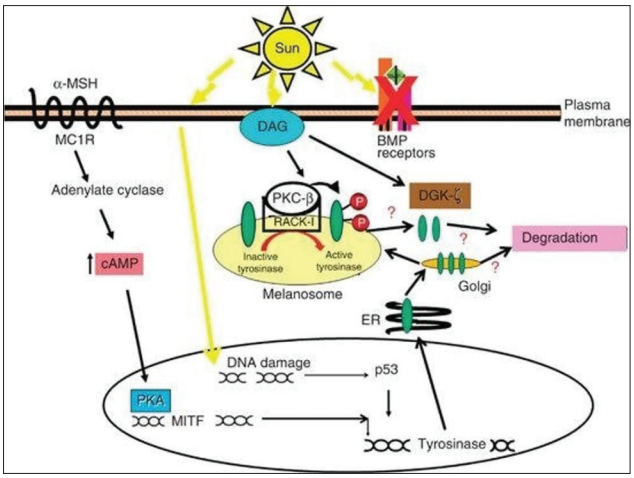

Figure 4: Selected signaling pathways regulating melanogenesis. Park *et al*.[50] ER: endoplasmic reticulum; P: Phosphate group; RACK-I: Receptors for activated C-kinase.

fibroblasts. It is the most important hormone which stimulates melanocytes in melanogenesis. α-MSH binds to the MC1R, a particular G-coupled protein receptor that induces adenylyl cyclase activation, accompanied by the increase of intracellular cAMP.[52] A previous study showed that Piperlongumine from *Piper longum L.* successfully inhibited melanogenesis although it does not have the ability to inhibit tyrosinase. Its anti-melanogenesis is achieved by inhibiting the α-MSH-induced melanogenesis where α-MSH acts via cAMP to cAMP response element-binding (CREB), which in turn regulates the expression of MITF and tyrosinase.[34] Another report showed that sophoraflavanone G is also able to inhibit α-MSH-induced melanogenesis despite its capability in tyrosinase inhibition.[34]

Melanosome Transferase Inhibitor

Soybean extract was found to contain small serine proteases such as Bowman–Birk inhibitor and soybean trypsin inhibitor which could inhibit the protease-activated receptor-2 pathway expressed on keratinocytes. Subsequently, it induced the depigmentation of skin as the phagocytosis of melanosomes by keratinocyte has been reduced.[53] Apart from that, a flowering plant, yarrow, was found to contain a flavonoid glucoside, centaureidin, which reduced in melanosome transfer and melanocyte dendrites outgrowth.[54] Melanogenesis can also be blocked by preventing the transfer of melanosomes from melanocytes to keratinocytes by niacinamide (from Vitamin B3), despite simultaneously being a tyrosinase inhibitor. Ginsenoside F1 could also disrupt the synthesis of melanin in melanocytes by inhibiting melanin transfer via dendrite retraction of melanocytes in the basal layer.[55]

Cytokines Inhibitors

Another approach reported to perform anti-melanogenesis effect is by inhibiting the cytokines. A previous study demonstrated that UV-induced skin pigmentation in brown guinea pigs and human melanoma cell cultures is reduced, likely through

downregulation of the keratinocyte-associated MITF mediated by interleukin (IL)-6.[56] Pax 3 gene, a transcription factor that regulated MITF, thus induces melanogenic activity. IL-6-mediated signaling can suppress the production of Pax 3 gene. Suppression of Pax 3 gene can lead to loss of MITF and tyrosinase expression and thus decrease in melanogenesis.[56] On the other hand, IL-4 can affect melanogenesis in epidermal melanocytes and various functions of epidermal keratinocytes, dermal fibroblasts, dendritic cells, and other pro-inflammatory infiltrating lymphocytes. IL-4 induces JAK2-STAT6 signaling, which inhibits melanogenic activity by decreasing protein expression of MITF, TRP-1, and DCT1. These genes are melanogenesis-associated genes that play an important role in skin pigmentation.[57]

Evaluation of *in vivo* and *in vitro* Depigmenting Activity

Research on the inhibition of melanogenesis by natural compounds as a potent inhibitor of tyrosinase has been widely studied by using an *in vivo* and *in vitro*. A recent study by Hseu *et al.* found that Coenzyme CoQ10 (CoQ10), a ubiquinone compound, is capable to inhibit tyrosinase activity and melanin production through suppression on p53/POMC, α-MSH production as well as ROS generation in UVA-irradiated keratinocyte HaCaT cells. In addition, CoQ10 downregulated the melanin synthesis in α-MSH-stimulated murine B16-F10 cells by suppressing the MITF expression by downregulating the cAMP-mediated CREB protein signaling cascades. With these results, CoQ10 is believed to be a promising depigmentation or skin-whitening agent and could be used in cosmetics for topical application.[58]

Novel amide derivatives (3a-e and 5a-e) as new potent tyrosinase inhibitors were identified. From *in vitro* study, 15 μg/ml of 5c is able to attenuate 36% tyrosinase and 24% reduction in melanin content of B16F10 cells without significant cytotoxicity. Furthermore, 5c effectively reduces melanogenesis without perceptible toxicity in zebrafish. These observations are believed due to interaction of 5c with copper ions and multiple amino acids in the active site of tyrosinase with the strongest glide score (−5.387 kcal/mol) via computational docking study. Based on their valuable results, 5c has been proposed as a new potent candidate to inhibit tyrosinase in hyperpigmentation.[59]

The anti-melanogenic properties of the rhizoma of *Ligustrum sinense*, a Chinese medicinal plant, have gained an intense interest recently. Twenty-four compounds from the ethyl acetate surface of *L. sinense* methanolic extracts were isolated and identified.[60] All the pure isolates from *L. sinense* were subjected to anti-melanogenesis assay using murine melanoma B16-F10 cells. Their results demonstrated that the compound isolates, 5-[3-(4-hydroxy-3-methoxyphenyl) allyl] ferulic acid and (3S,3aR)-neocnidilide, displayed anti-melanogenesis activities with IC50 values of 78.9 and 31.1 μM, respectively, without cytotoxicity. They investigated further using zebrafish embryo and found (3S,3aR)-neocnidilide at 10–20 μM and also demonstrated significant anti-pigmentation activity on zebrafish embryos compared to arbutin (20 μM). They suggested that (3S,3aR)-neocnidilide (8) is a potent anti-melanogenic and noncytotoxic natural compound and may be developed potentially as a skin-whitening agent for cosmetic uses.[60]

A bioactive compound, T1, bis (4-hydroxybenzyl) sulfide, isolated from the Chinese herbal plant, *Gastrodia elata*, has been demonstrated as a strong competitive inhibitor against mushroom tyrosinase. When the melanocyte cell lines were treated with 50 μM of T1, bis sulfide, a 20% reduction in melanin content without significant cell toxicity was noticed. Moreover, through the zebrafish model, T1, bis sulfide also inhibits melanogenesis effectively without any toxicity observed. Interestingly, computational molecular modeling indicated that coordination of the sulfur atom of T1, bis sulfide with the copper ions in the active site of tyrosinase is crucial for mushroom tyrosinase inhibition and the ability of lessening the synthesis of melanin in human.[61]

A study by Lin *et al*. using Raspberry Ketone (RK) as a melanogenesis inhibitor found that RK inhibited melanin formation through reduction of tyrosinase activity in zebrafish. They showed that in B16 melanoma cells, all RK treatments significantly decreased the melanin content of the treated cells compared with that of the isobutylmethylxanthine-stimulated cells. RK also inhibited melanogenesis by reduction of tyrosinase activity in zebrafish. While in mice, application of 0.2% or 2% gel preparation of RK to mice skin significantly raised the degree of skin whitening within 7 days of treatment. They suggested that RK would appear to have high potential for utilization in the cosmetic industry.[62]

The study conducted by Boissy *et al.* using *in vivo* pigmented guinea pig model demonstrated that the newly developed tyrosinase inhibitor, dA, was able to demonstrate a rapid and sustained skin lightening that was completely reversible within 2 months of cessation for topical application. In contrast, HQ has developed a short but impersistence skin-lightening effect, whereas kojic acid and arbutin have no skin-lightening effect. They also performed a human clinical trial, where the panel of safety test results supported the overall establishment of dA as a tyrosinase inhibitor. It showed that there was a significant reduction in overall skin lightness as well as improvement of solar lentigines in population of light skin or dark skin individuals, respectively, after topical treatment of dA for 3 months.[63]

Potential Therapeutic Effects: Clinical-based Evidence Study

As aforementioned in the previous section, skin-whitening agents from natural ingredients derived from natural compounds exhibit capability as potent tyrosinase and nontyrosinase inhibitors by modulating various pathways in melanogenesis. These botanical or natural compounds provide an alternative to the current gold standard, HQ. Nevertheless, how safe and effective of the natural compounds

Table 2: Summary of clinical trials for synthetic and natural compounds

Chemicals	Type	Study Intervention/ Year	Condition	Comparison	Status	Study Results	Location
Salicyclic acid and Hydroquinone (HQ)[64]	Synthetic	Double Blind Randomized Clinical Trial (14 weeks), 2008	Melasma	20-30% Salicylic Acid Peels Combined With 4% Hydroquinone Cream vs. 4% HQ (topical)	Completed Phase 4	Improvement of melasma based on MASI scores, melasma severity assessment, and physician and patient global improvement compared with the opposite side.	USA
Tri-Luma® Cream [64]	Synthetic	Randomized Controlled Split-face Clinical Trial (10 weeks), 2008	Melasma	Sequential Treatment With Tri-Luma® Cream (fluocinolone acetonide 0.01%, hydroquinone 4%, tretinoin 0.05%) With Intense Pulsed Light (IPL) vs. a Mild Inactive Control Cream (Cetaphil) With Intense Pulsed Light (IPL) in Subjects With Melasma	Terminated	No results available	USA
Arbutin, Triamcinolone and Tretinoin [64]	Natural (arbutin) and Synthetic	Randomized Double Blind Clinical Trial, 2008	Melasma	Tretinoin + arbutin + triamcinolone) vs. Triluma® (hydroquinone + fluocinolone + tretinoin)	Suspended	No results available	Brazil
Hydroquinone (HQ)[64]	Synthetic	Randomized Controlled Split-face Clinical Trial (8 weeks), 2014	Facial Melasma	4% HQ (topical) vs. placebo (topical)	Completed Phase 3	No results available	Spain
	Synthetic	Randomized Double Blind Clinical Trial (12 weeks), 2015	Melasma	4% HQ (topical) vs. 2% Miconazole (topical)	Completed Phase 4	No results available	Mexico
Lytera 2.0 and Hydroquinone (HQ)[64]	Synthetic	Randomized Controlled Split-face Clinical Trial (12 weeks), 2017 [64]	Facial Melasma	Lytera 2.0 vs. 4% HQ (topical)	Completed	Number of participants by responses for self-assessment questionnaire: overall improvement in skin condition	USA
Glutathione [64]	Synthetic	Randomized Double Blind Clinical Trial (12 weeks)/2019 [64]	Spot UV, spot polarization, and skin tone	Oral glutathione capsules (500 mg) vs. oral placebo tablet	Completed Phase 1	No results available	Indonesia
Tranexamic acid and Hydroquinone (HQ)[64]	Synthetic	Three-arm Randomized, Double-blinded Clinical Trial (12 weeks), 2019 [64]	Melasma	Oral and 5% Tranexamic (topical) Acid in Monotherapy vs. 4% HQ (topical)	On-going (Estimate completion: June 2020)	No results available	Mexico
Azelaic acid (AZA)[65]	Natural	Randomized controlled, open-label trial, 2011	Melasma	20% AzA vs. 4% HQ (topical)	Completed		Iran
	Natural	Controlled Clinical Trial, 2016	Melasma	Glycolic acid peel with twice daily 20% AzA cream vs. 20% AzA cream	Completed	At 12 weeks, AzA/ glycolic acid combination has a statistically significant decrease in MASI score compared with AzA alone	India

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Table 2: Contd...

Aloesin[65]	Natural	Controlled Clinical Trial, 2002	UVR-induced hyperpigmentation	Aloesin vs. Arbutin vs. Aloesin/Arbutin	Completed	Dose-dependent suppression in pigmentation with application of aloesin; synergism between arbutin and aloesin	Korea
Mulberry	Natural	Randomized Controlled Clinical Trial, 2011	Melasma	75% mulberry Extract vs. placebo	Completed	Compared to placebo, 75% mulberry extract showed significant improvement in MASI score, average Mexamater measurements, and MelasQoL scores	Philippines
Licorice Extracts [65]	Natural	Split-face 17 controlled clinical trial, 2000	Melasma	None	Completed	Sixteen out of 20 patients had an "excellent response" to 20% liquitin cream applied BID for four weeks. Glabridin was more efficacious compared to HQ	Egypt
	Natural	Controlled Clinical Trial, 2013	UVR-induced hyperpigmentation	None	Completed	Skin brightener containing glabridin was shown to be clinically efficacious	USA
	Natural	Randomized Controlled Clinical Trial, 2010	Melasma	Cream with belides, emblica, and licorice vs. 2% HQ	Completed	Although depigmentation was seen in both groups, no statistical difference in efficacy	Brazil
	Natural	Randomized Controlled Clinical Trial, 2016	Melasma	4% liquiritin vs. 2% liquiritin and HQ	Completed	4% liquiritin significantly more effective than combination group	Pakistan
Kojic acid (KA)[65]	Natural	Prospective controlled study, 2013	Melasma	0.75% KA with 2.5% Vitamin C vs. 4% HQ	Completed	Patients responded faster and better to HQ	India
	Natural	Randomized Controlled Clinical Trial, 2010	Facial dyschromia	Compound (KA, emblica extract, glycolic acid) vs. 4% HQ	Completed	Both treatment equally efficacious	USA
	Natural	Randomized Controlled Clinical Trial, 2013	Melasma	1% KA vs. KA with 2% HQ vs, KA with 0.1% betamethasone vs. combination of products	Completed	KA with HQ was most effective combination	India

Condt....

Table 2: Contd...

Niacinamide [65]	Natural	Randomized Controlled Clinical Trial, 2014	Irregular facial hyperpigmentation	Cream containing 2% niacinamide with 2% tranexamic acid vs. vehicle control	Completed	Niacinamide with TXA combination product showed efficacy	Korea
	Natural	Randomized Controlled Clinical Trial, 2013	Axillary hyperpigmentation	Niacinamide 4% vs. desonide 0.05% vs. control	Completed	4% Niacinamide with 0.05% desonide emulsion showed significant colorimetric improvement, though desonide alone was more effective	Mexico
	Natural	Open-label controlled trial, 2016	Post-inflammatory hyperpigmentation	None	Completed	Skin brightening compound containing retinol 0.5%, niacinamide 4.4%, resveratrol 1%, and hexylresorcinol 1.1% improved hyperpigmentation	USA
Ellagic acid [65]	Natural	Randomized Controlled Clinical Trial, 2008	Melasma	1% arbutin vs. synthetic 1% ellagic acid vs. synthetic 1% ellagic acid with plant extracts containing natural ellagic acid	Completed	All three treatments show efficacy	Turkey
	Natural	Randomized Controlled Clinical Trial, 2013	Hyperpigmentation and dark spots	0.5% ellagic acid combined with 0.1% salicylic acid vs. 4% HQ	Completed	Based on clinical grading, physical measurement of spot size by Chroma Meter, and patient questionnaire analysis, the compound had comparable efficacy to HQ but better aesthetics	USA
Arbutin [65]	Natural	Randomized Controlled Clinical Trial, 2008	Melasma	1% arbutin vs. synthetic synthetic 1% ellagic acid with plant extracts	Completed	All three treatments show efficacy	Turkey
	Natural	Single-group efficacy trial, 2010	Melasma	None	Completed	7% alpha arbutin in conjunction with the MedLite C6 Q-switched Nd: YAG laser showed favorable results	Thailand
Green Tea [65]	Natural	Randomized Controlled Clinical Trial, 2009	Melasma	2% analogue of green tea extract vs. placebo control	Completed		USA
Turmeric [65]	Natural	Randomized Controlled Clinical Trial, 2010	Facial hyperpigmentation	Turmeric extract cream formulation vs. unknown control	Completed	Formulation improved areas of hyperpigmentation by 14.16% (P<.0001) at four weeks	USA

Contd...

Table 2: Contd...

Soy [65]	Natural	Controlled Clinical Trial, 2001	Melasma	None; authors compared affected vs. unaffected areas	Completed	Application of soy extract to melasma lesions once daily for 3 months led to an average reduction of hyperpigmentation of 12%	USA
Ascorbic acid [65]	Natural	Single group Efficacy Trial, 2013	Severe melisma	Topical AA with trichloroacetic acid peel vs. trichloroacetic acid peel	Completed	Melasma peel (alpha-hydroxy acid, AA, and oxygen) showed improvement in 95 percent of patients at eight weeks	Korea
	Natural	Controlled Clinical Trial, 2007	Bilateral epidermal melisma	Combined trichloroacetic acid peel and topical ascorbic acid vs. trichloroacetic acid peel	Completed	According to digital photography and MASI score, combination product showed greater improvement	Eqypt
	Natural	Randomized Controlled Clinical Trial/ Split-face, 2003	Melasma	Vitamin C vs. distilled water	Completed	After 12 weeks of vitamin C iontophoresis treatment, the colorimeter recorded a clinically significant reduction in luminance value on the treated side	Korea
	Natural	Single group Efficacy Trial, 2013	Melasma and post-inflammatory hyperpigmentation	None	Completed	Novel full-face iontophoresis mask and ascorbyl glucoside preparation over a 1 to 2 month period showed clinical efficacy.	USA

in the management of hyperpigmentation? Here, we provide information on evidence-based clinical studies involving wide range of synthetic[64] as well as natural compounds such as azelaic acid, aloesin, mulberry, licorice extracts, lignin peroxidase, kojic acid, niacinamide, ellagic acid, arbutin, green tea, turmeric, soy, and ascorbic acid, extracted from systematic research on 30 chosen clinical trials.[65] Unlike some of the synthetic chemicals such as HQ, and tretinoin that has been suspended and terminated in clinical trials, natural ingredients from natural sources have demonstrated potential therapeutic effects and may provide clinicians and researchers a better insight on clinical practice in the future. The summary of clinical trials done previously is shown in Table 2.

Conclusions

Despite more serious clinical disorders like melasma, excessive melanogenesis will cause problems such as darker, uneven skin tone, hyperpigmentation, and other facial implications. Among the studies, it was shown that tyrosinase is the key and preliminary enzyme of melanogenesis, and therefore, most of the skin lightening approaches target the tyrosinase inhibition mechanism to downregulate melanogenesis. Both synthetic and natural compounds are found to be applied in the skin lightening products such as HQ, kojic acid, and flavonoid compounds. It is noted that synthetic compounds such as HQ are effective tyrosinase inhibitors, but they can cause serious adverse effects such as permanent melanosome loss. Kojic acid was shown to only portrait moderate effectiveness while bringing the threat of dermatitis and other complications. Up to now, the use of flavonoid-derived compounds such as glabridin has been shown to be very effective as a skin-lightening agent and relatively safer for human skin application. Nevertheless, more studies needed to be carried out to overcome the drawback of the flavonoid compounds such as its low stability when formulated in topical application cream.

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Conflicts of interest

There are no conflicts of interest.

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