Anti-Pigmentary Natural Compounds and Their Mode of Action

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Abstract: Hyper-activated melanocytes are the major cause of skin hyper-pigmentary disorders, such as freckles and melasma. Increasing efforts have been made to search for materials with depigmenting activity to develop functional cosmetics. As a result, numerous materials have been reported to have depigmenting activity but some of them are known to cause unwanted side effects. Consequently, anti-pigmentary natural compounds without concern of toxicity are in great demand. Virtually all sorts of natural sources have been investigated to find anti-pigmentary natural compounds. This review summarizes recently reported anti-pigmentary natural compounds and their mode of action from the ocean, plants, and bacteria.

Keywords: skin pigmentation; melanogenesis; Tyrosinase; natural compound

1. Melanogenesis

Melanin plays a pivotal role in protecting the skin against UV radiation and oxidative stress from various pollutants, but its overproduction is the main cause of human skin hyperpigmentation [1]. Melanin is synthesized in the epidermal melanocytes by a process called melanogenesis. Each melanocyte in the basal layer of the skin epidermis is surrounded by approximately 36 keratinocytes [2]. During melanogenesis, melanin is stored inside the melanocytes in subcellular lysosome-like organelles called melanosomes, and transferred to the surrounding keratinocytes [3,4]. Melanogenesis happens with the activation of several melanogenesis-related proteins, including Tyrosinase (TYR), Tyrosinase-related protein-1 (TRP-1), and Tyrosinase-related protein-2 (TRP-2), under the orchestration of the principal melanogenesis regulating factor, Microphthalmia-associated transcription factor (MITF) [5].

There are two kinds of melanin pigment: brownish-black eumelanin and reddish-yellow pheomelanin [6]. The synthesis of melanin starts with the oxidation of L-tyrosine to Dopquinone (DQ) and/or L-dihydroxyphenylalanine (L-DOPA) to DQ by Tyrosinase activation. DQ reacts with cysteine to form 5- or 3-cysteinyl DOPAs, which then oxidize and polymerize to ultimately make pheomelanin. Alternatively, DQ cyclizes to form a DOPAchrome, which is spontaneously decarboxylated to 5, 6-dihydroxyindole (DHI). DHI rapidly oxidizes and polymerizes to yield DH1-melanin. In case TRP-2 is available, DOPAchrome tautomerizes to form DHI-2-carboxylic acid (DHICA), which can be oxidized and polymerized by TRP-1 to yield DHICA-melanin. The availability of substrates and enzymes decides the type(s) of melanin produced [3].

MITF is a crucial transcription factor stimulating the expression of Tyrosinase, TRP-1, and TRP-2. Multiple signaling pathways are involved in MITF regulation. UV exposure induces the activation of p53 and results in increased expression of proopiomelanocortin (POMC), which is then cleaved into small peptides including α-melanocyte-stimulating hormone (α-MSH). The POMC-derived α-MSH binds to the Melanocortin-1 receptor (MC1R) on the surface of melanocytes, leading to the production of cAMP through the
activation of adenyl cyclase. cAMP activates the Protein kinase A (PKA), which translocates to the nucleus and phosphorylates the cAMP response element-binding protein (CREB), causing the upregulation of MITF [7,8].

Additionally, MITF is controlled by the Mitogen-activated protein kinases (MAPKs), which are composed of three subtypes: Extracellularly responsive kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 [9]. The phosphorylation of p38 activates the expression of MITF and eventually stimulates melanogenesis. On the contrary, JNK or ERK activation induces MITF degradation and downregulates melanin synthesis [8]. MITF is also activated by the Wnt/β-catenin pathway. Wnt is a cysteine-rich glycoprotein that couples with the G protein-coupled receptor to inhibit the glycogen synthase kinase-3β (GSK-3β). It causes the accumulation of β-catenin in the cytosol, increasing the upregulation of MITF [7].

When melanin production is completed, melanin contained in the melanosome is transferred to keratinocyte through melanocyte dendrites. Transfer of melanin is an important process in skin pigmentation, although much about the process is yet to be understood [10].

2. Skin-Whitening Agents Derived from Plants

Efforts to discover new plant extracts with skin-whitening efficacy have been made using various kinds of melanoma cells. In vivo evaluations using animal models such as mice or zebrafish have also been performed to confirm skin-whitening efficacy. Various parts of ginseng including the roots, seeds, and fruit have been widely used in both food and pharmaceutical products in East Asia for over 1000 years. Recently, a lot of research has been conducted to identify the skin-whitening ingredient from ginseng. Korean Red Ginseng (KRG) extract was verified as possessing inhibitory effects on melanin production in B16F10 cells through the suppression of Tyrosinase activity [11].

Panax ginseng calyx is the peduncle between the berry and root of ginseng. This study showed Panax ginseng calyx ethanol extract (Pg-C-EE) suppressed TRP1, TRP-2, Tyrosinase, MITF, p38, ERK, and CREB levels dose-dependently in B16F10 [12]. Ethanol extract of Panax ginseng seed was non-cytotoxic to Melan-A cells at a concentration of 100 ppm, at which it significantly (p < 0.01) suppressed cellular melanin production by inhibiting Tyrosinase activity [13]. Floralginsenoside A (FGA), ginsenoside Rd (GRD), and ginsenoside Re (GRE) purified from the P. ginseng berry also inhibited melanin production and MITF protein expression in melan-A cells [14]. Additionally, ginsenoside F1 (GF1), the major active component of P. ginseng, inhibited the melanin transfer on MNT-1/HaCaT coculture system and three-dimensional (3-D) human skin equivalent [15].

The extract from Aster spathulifolius extract (ASE) inhibited melanin production at concentrations of 50, 100, and 200 μM in B16F10 cells. Western blot analysis revealed that ASE extract activated both MAPK/ERK and Akt/GSK3β phosphorylation, promoting the ubiquitin-dependent degradation of MITF. The whitening effect of ASE was also observed on C57BL/6J mice, in which hyper-pigmentation was induced by 3, 6, and 9 weeks of UV irradiation. A significant reduction in melanin content was identified in the epidermis at a dose of 140 mg/kg [16].

Sweroside was isolated from Lonicera japonica and, upon testing, inhibited melanogenesis in Melan-A cells at 300 μM without cytotoxicity through decreasing Tyrosinase, TRP-1, and TRP-2 expression. It has been shown that this effect occurred in a dose-dependent manner by promoting the Akt and ERK signaling pathways. In addition, sweroside was proven to be effective in vivo by reducing melanin spots in zebrafish embryos [17]. Isoliquiritigenin (ISL) is the hydrolysis product from licorice root. ISL inhibited melanin production through the degradation of MITF by activating the ERK signaling pathways, decreasing the expression of Tyrosinase, TRP-1, and dopachrome tautomerase (DCT) in SK-MEL-2 cells. Furthermore, ISL inhibited the melanocyte dendricity and melanin transport in cocultured SK-MEL-2 cells and HaCaT by suppressing the expression of Rab27a and Cdc42 proteins [18]. The ethanol extract of Pueraria thunbergiana significantly
inhibited melanogenesis in B16F10 cells via anti-Tyrosinase activity. Phosphorylation of GSK-3β was decreased by P. thunbergiana in a dose-dependent manner, but the amount of phospho-ERK had no change, suggesting that Pueraria thunbergiana inhibits MITF through the Akt/GSK-3β pathway [19]. Leaf and root extracts from Juglans mandshurica (1–10 μM) suppressed Tyrosinase and MITF in B16F10 cells and primary human epidermal melanocytes (PHEMs). The extract was proved to be non-toxic up to 10 μM. The mechanism of suppression was revealed as a result of markedly enhanced phospho-ERK [20].

The ethanol extracts of Sophora flavescens root inhibited the transfer of melanin to keratinocytes via regulating both RAC1 in SK-MEL-2 cells and PAR2 in HaCaT cells. RAC1 promotes dendrite and lamellipodium formation in melanocytes. PAR2 stimulates the phagocytosis rate of keratinocytes and increases melanin transfer. The levels of RAC1, PAR2, and MITF mRNAs were suppressed following the treatment with S. flavescens extract [21]. A 70% ethanol extract of Morus alba leaves has an anti-melanogenesis effect via inhibiting Tyrosinase activity in α-MSH-activated B16-F10 cells in a dose-dependent manner. It inhibited MITF, Tyrosinase, and TRP-1 by downregulating CREB and p38 signaling pathways [5]. Studies on the whitening effects of black tea extract (BT) have been conducted in several melanoma cells. Black tea water extract (BT) suppressed MITF in Mel-Ab cells [22]. Camellia oleifera extract was shown to inhibit melanin synthesis in B16F10 by suppressing Tyrosinase and TRP-2 production [23]. Camellia sinensis water extracts also significantly (p < 0.001) decreased Tyrosinase protein expression in a concentration-dependent manner [24]. Eupafolin (0.1–10 μM) isolated from Phyla nodiflora reduced cellular melanin content and Tyrosinase activity in B16F10 cells and had no cytotoxic effects. The mechanism was associated with the downregulation of CREB and MITF. It also induced the phosphorylation of ERK1/2 and p38 MAPK [25]. The following is a table listing plant extracts with skin-whitening effects and their mechanisms (Table 1).

Table 1. List of skin-whitening agents derived from plants.

| Name                               | Mode of action                                      |
|------------------------------------|------------------------------------------------------|
| Korean Red Ginseng (KRG)           | Tyrosinase inhibition activity                      |
| Panax ginseng calyx ethanol extract (Pg-C-EE) | Inhibition of melanin production by suppressing MITF, p38, ERK, and CREB |
| The seed of Panax ginseng           | Tyrosinase inhibition activity                      |
| Floralginsenoside A (FGA), Ginsenoside Rd (GRD), Ginsenoside Re (GRE) | Inhibition of melanin production by suppressing MITF |
| Ginsenoside F1 (GF1)               | Inhibition of melanosome transport                  |
| Aster spathulifolius extract (ASE)  | Tyrosinase, TRP-1 and MITF suppressions by activating MAPK/ERK pathway. |
| Sweroside (Lonicera japonica)      | TRP-1, TRP-2, and MITF suppressions                 |
| Isoliquiritigenin (ISL), licorice root | Tryosinase and TRP-1 suppressions; induction of melanin degradation; reduction in the number of dendrites and melanin transport |
| Pueraria thunbergiana              | Tyrosinase, TRP-1, MITF, and GSK-3β suppressions    |
| Juglans mandshurica               | Tyrosinase and MITF suppressions                    |
cofuroeckol A and Per404 of the extracts have more significant effects on dephenolase competitively. IC50 values for diphenolase are less than those for monophenolase for rosine inhibition activity measured on Turbinaria conoides noncompetitive inhibitor of the competitive Ishige okamurae pounds were noncompetitive inhibitors phloroglucinol and eckstolonol were competitive inhibitors, and the remaining three competitive inhibitors showed inhibitory effect used.

and 90.75% temperature Schizymenia dubyi, Ecklonia cava of melanogenesis.

Table listing marine microorganisms with skin organisms have already been investigated as from the cosmetic industry protective, anti-aging, antioxidant, and moisturizing activities, attracting great attention from the cosmetic industry [26]. Besides, numerous compounds derived from marine organisms have already been investigated as Tyrosinase inhibitors [27]. The following is a table listing marine microorganisms with skin-whitening effects and their mechanisms (Table 2). These compounds show skin-whitening effects by regulating one or more steps of melanogenesis.

The Tyrosinase inhibitory activity of four types of extract, Endarachne binghamiae, Schizymenia dubyi, Ecklonia cava (EC), and Sargassum silquastrum (SS), was measured at two temperatures. Schizymenia dubyi (SD) and Sargassum silquastrum (SS) extracts showed 90.75% and 70.75% inhibitory activity at 20 °C, respectively. Endarachne binghamiae (EB) and Ecklonia cava (EC) extracts showed 81.26% and 73.22% inhibitory activity at 70 °C, respectively. To investigate the inhibitory activities in vivo model, a zebrafish embryo was used. Ecklonia cava (EC) and Sargassum silquastrum (SS) extracts showed 48% and 50% inhibitory effects, respectively [28]. Fucoidan extracted from marine brown seaweed inhibited Tyrosinase catalytic activity competitively. [29]. Five compounds derived from Ecklonia stolonifera, OKAMURA, phloroglucinol, eckstolonol, eckol, and eckol were treated to mushroom Tyrosinase. The inhibitory kinetics showed that phloroglucinol and eckstonol were competitive inhibitors, and the remaining three compounds were noncompetitive inhibitors [30]. Diphlorethohydroxycarmalol (DPhC) from Ishige okamurae showed a 78.73% inhibition against mushroom Tyrosinase. It binds non-competitively to the enzyme and changes the shape of the enzyme, which leads to inhibit the binding of substrate to the active site [31]. Fucofuroeckol-A from Eisenia bicylis is a noncompetitive inhibitor of Tyrosinase and blocks IBMX-induced melanin formation [32]. Turbinaria conoides is a brown alga that produces zinc oxide. Inhibition activities were measured on Tyrosinases from potato and L-TYrosine. The combination of crude extracts of Turbinaria conoides and zinc oxide nanoparticles as a comparator showed 75% and 56% inhibition activity [33]. Methanol extracts of Sargassum plagophyllum and Eucheuma cottonii were treated with L-tyrosine substrate and L-DOPA substrate. IC50 values for L-tyrosine substrate (monophenolase) were 2691.478 μg/mL and 2195.206 μg/mL. IC50 values for L-DOPA substrate (diphenolase) were 2631.648 μg/mL and 1769.336 μg/mL, respectively. IC50 values for diphenolase are less than those for monophenolase, which means the extracts have more significant effects on dephenolase [34]. Bromophenol obtained from Symphyochaeta latiuscula exerts a strong hydrogen bond interaction with Arg268 and Per404 of Tyrosinase. Bromophenol only binds to free enzymes and works as a competitive inhibitor. Furthermore, it significantly reduced Tyrosinase expression levels [35]. Phlorofucofuroeckol A and 2-O-(2, 4, 6-trihydroxy phenyl)-6,6′-bieckol from Ecklonia cava are slow-

| Sophora flavescens | Inhibition of melanin transport |
|-------------------|---------------------------------|
| Morus alba        | Tyrosinase, TRP-1, and MITF suppressions by downregulating CREB and p38 signaling pathways |
| Black tea extract (BT) | Tyrosinase, TRP-2, and MITF suppressions |
| Camellia oleifera | Tyrosinase and TRP-2 suppressions |
| Camellia sinensis | Tyrosinase suppression |
| Eupafolin, Phyla nodiflora | Inhibition of Akt and activation of phospho-ERK or p38 MAPK |

3. Skin-Whitening Agents Derived from Ocean

The ocean has enormous biodiversity, with more than 250,000 species listed and many other species yet to be discovered. Marine resources have the properties of photoprotective, anti-aging, antioxidant, and moisturizing activities, attracting great attention from the cosmetic industry [26]. Besides, numerous compounds derived from marine organisms have already been investigated as Tyrosinase inhibitors [27]. The following is a table listing marine microorganisms with skin-whitening effects and their mechanisms (Table 2). These compounds show skin-whitening effects by regulating one or more steps of melanogenesis.

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binding inhibitors. Both compounds interacted with His85 and Asn260 on the Tyrosinase active site, but each had different binding mechanisms. The former slowly binds to the active site following a single step-binding mechanism. The latter binds to the enzyme and induces a new conformational state of enzyme that inhibits substrate to make a complex with Tyrosinase [36]. Octaphloethol A (OPA) from Ishige foliaceae inhibited melanin synthesis and Tyrosinase activity through the ERK pathway-mediated suppression of MITF, Tyrosinase, TRP-1, and TRP-2 in α-MSH-stimulated B16F10 cells [37].

The skin-whitening effects of a marine-derived mixture from sea squirt skin were investigated. The mixture included a fucoidan-rich extract of Undaria pinnatifida (UEP), a phlorotannin-rich extract of Ecklonia cava (ECE), and glycosaminoglycans (GAGs). Each component alone inhibited mushroom Tyrosinase in α-MSH-stimulated B16F10 cells but was cytotoxic. However, the combination of three components showed a stronger whitening effect without cytotoxicity. A mixture of compounds in a ratio of 4:5:1 (UEG-451) showed 71.1% whitening activity with the highest cell viability. Additionally, a UEG-451 combination downregulated melanogenesis-related proteins, including Tyrosinase, TRP-1, and TRP-2, in α-MSH-stimulated B16F10 cell by regulating the expression of MITF [38]. Stichopus japonicus extracts were evaluated by measuring the inhibition of mushroom Tyrosinase and melanogenesis in B16F10 melanoma cells. The results indicated that the extracts markedly inhibited Tyrosinase activity and melanin synthesis. MITF expression was also suppressed by the phosphorylation of ERK, leading to reduced melanogenesis-related gene expression [39]. Among Streptomyces extracts, Trichostatin A (TSA) showed the strongest Tyrosinase inhibition activity. TSA is a mixed-type inhibitor that can bind to the enzyme with or without substrates being bound [40]. Jeju magma-seawater (JMS) was pumped up from a depth of 130 m below sea level, and was found to have significant anti-melanogenesis activity. Since 100% of JMS showed cytotoxicity due to osmotic shock, research was undertaken at 25% and 50% concentrations. JMS activated CaMKβ (calcium/calcmodulin-dependent protein kinase), which then activated AMPK (5’ adenosine monophosphate-activated protein kinase). AMPK blocks α-MSH-induced MAPKs (mitogen-activated protein kinase) and PKA (Protein kinase A) signaling pathways, resulting in the inhibition of Tyrosinase activity, melanin secretion, and melanogenesis-related gene expression [41]. Pandina boryana extracts regulated the phosphorylation of ERK and inhibited the expression of melanogenesis-related protein via the MAPK pathway [42]. Investigation with Sargassum siliquosum extracts with mushroom Tyrosinase revealed that 125 μg/mL of the extracts show 73% inhibition activity. The extracts have a phenol group; thus, the hydroxyl group of phenol makes a hydrogen bond with the active site of Tyrosinase that interrupts substrate binding with the enzyme and causes a conformational change in the enzyme [43]. One of the Sargassum serratifolium extracts, Sargaquinoic acid (SQA), was investigated with mouse melanoma cells. SQA reduced cAMP by hydrophobic interaction with the binding site between CAM and PKA. Reduced cAMP causes PKA inactivation, leading to the inhibition of CREB and MITF signals. Besides, the downregulation of MITF led the inhibition of melanogenesis-related protein expressions including TRP-1, TRP-2, and Tyrosinase. Additionally, SQA inhibited Tyrosinase by MITF degradation through the phosphorylation of ERK1/2 [44]. Chitosan is natural marine product that inhibits the synthesis of melanogenesis-related proteins and melanosome transfer. Chitosan was evaluated for its melanosomal inhibition with human melanocyte cells and human keratinocyte cells. The results verified that the inhibitory activity of chitosan in melanosomal transfer was mediated by PAR-2, which is related to melanosomal transfer from melanocyte to keratinocyte. Chitosan inhibited melanosomal release from melanocyte and melanosomal uptake by keratinocyte [45]. 4-(phenylsulfonyl) butan-2-one from Cladiella australis (coral) exhibited a skin-whitening effect in B16-F10 cells (in vivo). MITF, Tyrosinase, TRP-1, TRP-2, and Gp100 were significantly reduced by Cladiella australis-4-(phenylsulfonyl) butan-2-one compared to the control group. Since there were no cytotoxic, carcinogenic, or teratogenic properties, the research suggested that 4-phenylsulfonyl-butan-2-one could be a safe and effective skin-whitening agent [46].
Table 2. List of skin-whitening agents derived from ocean.

| Name                                | Mode of action                                                                 |
|-------------------------------------|-------------------------------------------------------------------------------|
| Endarachne binghamiae               | Tyrosinase inhibition activity                                                 |
| Schizymenia dabyi                   |                                                                               |
| Ecklonia cava (EC)                  |                                                                               |
| Sargassum silquastrum (SS)          |                                                                               |
| Fucoidan                            | Competitive inhibition of Tyrosinase activity                                  |
| Ecklonia stolonifera OKAMURA        | Noncompetitive and competitive inhibition of Tyrosinase activity               |
| Diphlorethohydroxycarmalol (DPHC)   | Noncompetitive inhibition of Tyrosinase activity                               |
| Fucofuroeckol-A                     | Noncompetitive inhibition of Tyrosinase activity                               |
| Turbinaria conoides                 | Tyrosinase inhibition activity                                                 |
| Sargassum plagyophyllum Eucheuma cottonii |                                             |
| Symphyocladia latiuscalas          | Tyrosinase inhibition activity                                                 |
| Phlorofucofuroeckol A and 2-O-(2, 4, 6-trihydroxy phenyl)-6, 6′-bieckol from Ecklonia cava | |
| Octaphlorethol A(OPA) from Ishige foliaceae | Tyrosinase inhibition activity                                                 |
| Combination of Undaria pinnatifida (UPEF), Ecklonia cava (E), glycosaminoglycans (GAGs) | Tyrosinase inhibition activity; MITF suppression                                |
| Stichopus japonicus                 | Tyrosinase inhibition activity                                                 |
| Trichostatin A (TSA) from Stichopus japonicus | Tyrosinase inhibition activity                                                 |
| JMS (Jeju magma-seawater)           | Inhibition of Tyrosinase activity; melanin secretion; melanogenic gene expression |
| Pandina boryana                     | Regulated phosphorylation of ERK                                               |
| Sargassum siliquosum                | Tyrosinase inhibition activity; conformational change of the enzyme            |
| Sargaquinoic acid (SQA) from Sargassum serratifolius | TYR, TRP-1, and TRP-2 suppression                                              |
4. Skin-Whitening Agents Derived from Bacteria or Fungi

5-hydroxy-2-hydroxymethyl-4-pyrene, also known as Kojic acid, is naturally derived from fungal metabolites produced by species of Acetobacter, Aspergillus, and Penicillium [47]. Kojic acid has been used in concentrations of 2–4% either alone or in combination with 2% hydroquinone, as reported by Monteiro et al. According to the study, 0.75% Kojic acid with 2.5% vitamin C was shown to be a highly effective topical hypopigmenting agent in the treatment of facial melasma. Besides, the combination of glycolic acid with 5% Kojic acid showed better efficacy (28%) compared to glycolic acid with 5% hydroquinone (21%) [48]. Furthermore, Kojic acid is effective in regulating melanogenesis by inhibiting the catecholase activity of Tyrosinase in a non-classical manner [49]. Thus, kojic acid is widely used in cosmetics for its excellent whitening effect. Saccharomyces cerevisiae extract had inhibitory effects on melanogenesis and melanosome transfer.

Tyrosinase activity was blocked by the extract without cytotoxicity in B16F10 cells. However, the extracts had no effects on the expression of the MITF protein, which means they may indirectly inhibit Tyrosinase activity, but through post-transcriptional control. In addition, they inhibited PAR-2, melanosome transfer-related protein, so that melanosome transportation would be suppressed [50]. The effects of Bifidobacterium adolescentis on melanin contents and Tyrosinase activity were measured. Melanin content of a B16F10 melanoma cell treated with 7.5% B. adolescentis was 55.1% (% of control), and that of the positive controls was 86.4% (kojic acid) and 70.7% (arbutin). Tyrosinase activity of mushroom Tyrosinase treated with 7.5% B. adolescentis was 19.1%, and that of kojic acid and arbutin was 93.6% and 89.4%, respectively. These results indicated that B. adolescentis has a potent anti-melanogenesis activity superior to that of the positive controls, kojic acid, and arbutin [51]. Angelica dahurica root (ADR) was fermented with four different bacteria: Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus, and Lactobacillus brevis. IC50 for the Tyrosinase inhibition of extracts of ADR fermented by these four bacteria was 0.57, 2.80, 0.07, and 0.52 mg/mL. Among the four bacteria, L. acidophilus-fermented ADR showed the highest activity. The IC50 of non-fermented ADR extract was 8.20 mg/mL, meaning that Tyrosinase inhibition activity of ADR has increased after fermentation [52]. Another study investigated the bioactive properties of Lactobacillus rhamnosus spent culture supernatant (Lr-SCS) for cosmetic applications. The Tyrosinase inhibitory activity of 100% Lr-SCS was 71.3% compared to 43.6% and 83.6% of 2 mM and 10 mM kojic acid. In this study, the lactic acid in Lr-SCS appeared to suppress tyrosine activity in a dose-dependent manner [53]. Lipoteichoic acid (LTA) is one of the cell wall components isolated from Lactobacillus plantarum (pLTA). pLTA decreased melanin synthesis by 57.9% compared to the control group. While pLTA did not inhibit Tyrosinase directly, it decreased intracellular activity of Tyrosinase and suppressed Tyrosinase-related proteins TRP-1 and TRP-2. Furthermore, pLTA induced the phosphorylation of ERK and AKT, leading to MITF degradation [54]. Lactobacillus acidophilus (AL) is a popular probiotic strain, and the study investigated the effects of tyndallized lactobacillus acidophilus on melanogenesis. AL significantly inhibited melanin secretion and intracellular melanin content in α-MSH-treated melanocytes. Specifically, in α-MSH activated melanoma cells, AL inhibited the phosphorylation of PKA and CREB and suppressed the expression of Tyrosinase and MITF. However, when AL was treated on mushroom TYRosine to investigate Tyrosinase inhibition activity, there was no observed effect. Instead, AL reduced the mRNA expression of Tyrosinase, TRP-1, and TRP-2. Therefore, AL does not inhibit Tyrosinase directly but it can modulate melanogenesis through the regulation of the cAMP signaling pathway [55]. The extract of Rhodobacter sphaeroides (lycogen) was treated to B16F10 melanoma cell to evaluate anti-melanogenesis
activity in vitro. The relative melanin content of lycogen-treated cells was significantly lower than the control group. To identify the mode of action of lycogen, the expressions of Tyrosinase and MITF were examined. Lycogen increased the ERK signaling pathway, which degraded MITF, leading to an anti-melanogenesis effect [56].

The anti-Tyrosinase activity of three Bifidobacterium bifidum-fermented Chinese herb extracts—walnut, Moutan cortex radices, and asparagus root—was investigated. B. bifidum-fermented extracts showed higher Tyrosinase inhibition activity than nonfermented extracts, suggesting that fermentation improved some biochemical activities of the three herb extracts. As a result, the highest Tyrosinase inhibition activities of the three fermented extracts were 58.4% (walnut), 66.5% (moutan cortex), and 92.8% (asparagus root) [57]. Urolithin A (UA) and Urolithin B (UB) are major metabolites found in human plasma. They can be produced from ellagic acid by Gordonivater urolithinaciens and Gordonivater pamelae, the urolithin-producing bacteria. Cytotoxicity and Tyrosinase inhibitory effects of UA and UB were evaluated in B16F10 melanoma cells. Cell viability was not affected by up to 10 μM of concentration. The melanin content of B16F10 cells treated with UA10 μM was 56.5%, which is comparable to that of the positive control (kojic acid). The result showed that, while neither Urolithin A (UA) or Urolithin B (UB) affected the mRNA expression of Tyrosinase, both competitively inhibited the enzymatic activity of Tyrosinase [58]. Tyrosinase inhibition activity of nonfermented Rhodiola rosea and Lonicera japonica extracts was 10 mg/mL and 6.93 mg/mL, respectively. However, the Tyrosinase inhibition activity of Alcaligenes piechaudii-fermented extracts was observed to be 0.78 mg/mL and 4.07 mg/mL, suggesting that the whitening effects of fermented R. rosea increased about 12 times, and that of L. japonica increased 70% compared to nonfermented extracts [59]. Lactobacillus helveticus NS8-fermented milk (NS8-FS) was examined for hypopigmentation. By measuring the melanin content and Tyrosinase activities in B16F10 cells, the whitening activity of NS8-FS was investigated. The color of the B16F10 cells was lightened and melanin content was reduced by nearly 40%. Tyrosinase activity was inhibited, and the gene expression of TYR osine-related protein was also downregulated. Therefore, NS8-FS could be both the enzymatic inhibitor and the gene expression regulator of Tyrosinase [60]. The anti-melanogenic activity of Magnolia officinalis bark (MOB) extracts after Aspergillus niger fermentation was investigated. The highest anti-Tyrosinase activity was shown with 3 days fermentation, which increased from 52.8% (before fermentation) to 93.6% (after fermentation). Additionally, the melanin content of human epidermal melanocytes was reduced. Melanin content was almost completely depleted at 200 μg/mL of fermented extracts [61]. Asparagus cochinchenensis extract fermented with Aspergillus oryzae was evaluated for its effects on cell viability and Tyrosinase inhibition. Cytotoxicity effects on HEMs, HaCaT, and A375.S2 cells were not significant. Anti-Tyrosinase activity of both fermented and unfermented extracts was evaluated in mushroom Tyrosinase and human epidermal melanocytes. Anti-Tyrosinase activity of the fermented extract was significantly lower in HEMs than in mushroom Tyrosinase, indicating some discrepancy in Tyrosinase inhibitory activity between mushroom Tyrosinase and human melanocytes. Additionally, with the fermented extract, the inhibition of melanin production was higher than Tyrosinase inhibition activity, suggesting that the fermented extract may downregulate not only Tyrosinase but also the Tyrosinase-related protein MITF. In contrast, the unfermented extract directly inhibited Tyrosinase activity [47,49,62]. The following table is a table listing skin-whitening agents derived from bacteria or fungi (Table 3).

Table 3. List of skin-whitening agents derived from bacteria or fungi.

| Name                                      | Mode of action                                      |
|-------------------------------------------|-----------------------------------------------------|
| Acetobacter, Aspergillus and Penicillium   | Inhibition of Tyrosinase activity and melanosome transfer |
| Natural Compound                                                      | Action                                                                                     |
|---------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Saccharomyces cerevisiae yeast extract                               | Inhibition of Tyrosinase activity and melanosome transfer                                 |
| Bifidobacterium adolescentis                                        | Inhibition of Tyrosinase activity and melanin production                                  |
| Bifidobacterium bifidum, Bifidobacterium lactis,                    | Inhibition of Tyrosinase activity; fermented extracts showed higher anti-TYRosine activity than nonfermented extracts |
| Lactobacillus acidophilus, Lactobacillus brevis                      | Inhibition of Tyrosinase activity                                                          |
| Lactobacillus rhamnosus                                              | Inhibition of Tyrosinase activity                                                          |
| Lipoteichoic Acid (LTA) from Lactobacillus plantarum                | Inhibition of Tyrosinase activity; inhibition of melanogenesis-related enzymes             |
| Lactobacillus acidophilus (AL)                                       | Inhibition of the mRNA expression of melanogenesis-related genes                          |
| Rhodobacter sphaeroides (lycogen)                                   | Inhibition of melanogenesis-related protein                                                 |
| Bifidobacterium bifidum-fermented Chinese herb extracts              | Anti-Tyrosinase activity; fermented extracts showed higher anti-TYRosine activity than nonfermented extracts |
| Urolithin A (UA) and Urolithin B (UB) from urolithin-producing bacteria | Inhibition of Tyrosinase activity                                                          |
| Nonfermented Rhodiola rosea and Lonicera japonica extracts          | Inhibition of Tyrosinase activity; fermented extracts showed higher anti-TYRosine activity than nonfermented extracts |
| Alcaligenes piechaudii-Fermented extract                            | Inhibition of Tyrosinase activity; regulation of melanogenesis-related gene expression     |
| Lactobacillus helveticus NS8-fermented milk (NS8-FS)                | Inhibition of Tyrosinase activity                                                          |
| Magnolia officinalis bark (MOB) extracts by Aspergillus niger fermentation | Inhibition of Tyrosinase activity                                                         |
| Asparagus cochinchinensis extract fermented with Aspergillus oryzae  | Inhibition of Tyrosinase activity; regulation of melanogenesis-related protein; expression; non-fermented extract directly inhibited Tyrosinase activity |

5. Conclusions

In this review, we investigated plant-, ocean-, and bacteria-derived natural compounds with whitening effects and their modes of action. Most plant-derived skin-whitening agents suppressed melanin production by modulating the expression of melanogenesis-related proteins, such as MITF, Tyrosinase, TRP-1, and TRP-1. A few plant-derived skin-whitening agents promoted ERK signaling pathways, resulting in accelerated MITF degradation and regulating the length of melanocyte dendrites, which may block melanin transportation. Ocean-derived agents mostly suppressed melanin production by inhibiting the enzymatic activity of Tyrosinase, either competitively or noncompetitively.
Bacteria-derived skin-whitening agents suppressed melanin production by regulating Tyrosinase activity and melanogenic proteins. Interestingly, fermented extracts showed higher inhibitory activity than non-fermented extracts, which may be owed, at least in part, to the combined effects of plant- and bacteria-derived active ingredients. Of note, most natural skin-whitening agents exhibited lower toxicity than existing skin-whitening agents, including arbutin and kojic acid. Since melanogenesis is complex and a variety of factors and pathways are involved, further research on the whitening activity of natural compounds is needed. Furthermore, although the whitening activity of these natural compounds has been well established in vitro, it is difficult to ensure efficacy in humans, since there are many confounding factors for manifesting whitening effects in human skin [63,64]. Thus, further investigation of these natural compounds’ whitening activity in vivo, in artificial skin models, or in clinical trials would be required to apply them to whitening cosmetics [65].

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Abbreviations

| Abbreviation | Definition                                           |
|--------------|------------------------------------------------------|
| TYR          | Tyrosinase                                           |
| TRP-1        | Tyrosinase-related protein-1                         |
| TRP-2        | Tyrosinase-related protein-2                         |
| MITF         | Microphthalmia-associated transcription factor       |
| DQ           | Dopaquinone                                          |
| L-DOPA       | L-dihydroxyphenylalanine                            |
| DHI          | 5, 6-dihydroxyindole                                |
| α-MSH        | α-melanocyte stimulating hormone                     |
| MC1R         | Melanocortin-1 receptor                              |
| PKA          | Protein kinase A                                     |
| CREB         | cAMP response element binding protein                |
| MAPKs        | Mitogen-activated protein kinases                    |
| ERK          | Extracellularly responsive kinase                    |
| JNK          | c-Jun N-terminal kinase                              |
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