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Understanding the Challenges of Melanogenesis: Key Role of Bioactive Compounds in the Treatment of Hyperpigmentory Disorders

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Abstract

Skin pigmentation is an important human phenotypic trait that gives special aesthetic hue to individuals and also protective covering against solar ultra violet radiations, but excessive pigment production and uneven distribution, appear as serious undesirable hyperpigmentory anomalies. To overcome this, various therapeutic agents and skin-lightening cosmetics are in big demand across Asia, and the quest for fairness has led to identification of many new ingredients. The mechanisms underlying pigmentation has been researched extensively and the knowledge are being updated regularly. To understand the etiology of normal and uneven skin pigmentation, first part of the review serves us basic updated information regarding the normal process of melanogenesis and strict coherent attributes that monitor the skin pigmentation control machinery. While the later part of the review focuses on some abnormal hyperpigmentory anomalies, their mode of action at the cellular level and various approaches that exploit the natural botanicals for their permanent and cost effective treatment.

Keywords: Skin pigmentation; Hyperpigmentation disorders; Melanogenesis; Natural products/botanicals

Introduction

Melanin is a prime contributor of pigmentation patterns in vertebrates including human beings and is synthetized by melanocytes which remain distributed in the basal layer of the skin epidermis. Generally two types of melanin pigment are produced within the melanocytes, i.e. black or brown eu-melanin and red or yellow pheo-melanin, which decides the colour of hair, skin or eyes [1,2].

Upon exposure to the UV radiations, melanocytes synthesise melanin in the membrane bound organelle, the melanosomes by multistep, complex process of melanogenesis, and transfer them via their dendritic process to the adjacent keratinocytes where they form perinuclear melanin cap to protect the cells from UV induced damage of nuclear DNA and also by removing the reactive oxygen species (ROS). Moreover in keratinocytes, these melanin pigments give colour to skin in combination with other pigments like carotenoid and haemoglobin derivatives [3].

Melanin synthesis is highly cooperative step carried out by tyrosinase gene family protein i.e. tyrosinase (TYR), tyrosinase related protein-1 (TRP-1) and tyrosinase related protein-2 (TRP-2). Though TYR is the key player of melanogenesis that catalyse the crucial two initial steps of melanin synthesis, TRP-1 and TRP-2 also take important position in the synthesis of melanin [4,5].

Melanogenesis is under a mosaic regulatory control comprising interplay of different factors and signaling pathways. Among them α – melanocyte stimulating hormone (α-MSH), agouti signal protein (ASP) and micropthalmia associated transcriptional factor (MITF) are the predominant regulators of melanin synthetic pathways. Conjointly, one of such regulatory factors or pathways is the discordant involvement or subsidiary projection of tyrosinase via unorthodox routes resulting into varied skin related abnormalities [6,7].

Hyper reactivity of melanocytes as well as abnormal synthesis and accumulation of melanin leads to the consequences of skin related pigmentation disorders. Looking at the human history, humans have continually tagged and categorized end another on the basis of skin complexion. In most African and Asian populations, fair complexion is labelled/tagged as beauty, grace and high social class whereas dark skin complexion is seen as being of lowest social status. This perception inspires most people of both genders for the recent craze for a fair skin and has given a forceful indulgence of their in skin care merchandise, that improve the skin complexion [8].

The present review is dedicated to the updated knowledge of melanocytes biology including (1) biogenesis of melanosomes and melanin (2) regulation of melanogenesis, (3) hyperpigmentary disorders (4) scientifically validated plants for the treatment of hyperpigmentation, and their mechanism.

Biogenesis of Melanosomes and Melanin

Melanin synthesis starts concurrently with the biosynthesis of specialized intracellular organelles, the melanosomes. Melanosomes are formed by multistep process during which immature, unpigmented melanosomes matured into developed pigmented melanosomes. Based on electron microscopic observations by Seiji, [9] melanosome biogenesis has been categorized into four stages (I-IV), based on the degree of maturation with discrete morphological and biochemical features that reveal discrete routes of the biosynthesis of structural and enzymatic proteins. Hence, melanin production occurs in these particular compartments as the biogenesis of melanosomes proceeds. Early stages (I & II) of melanosome development are referred as premelanosomes which lack pigment but possess other distinct features. Stage I premelanosomes originate from endosomes, are electron lucent and are limited by unit membrane which matures into

stage II premelanosomes. Pmel17 is a transmembrane glycoprotein that localizes in the limiting membrane of stage I premelanosomes and then enters into the matrix of stage II premelanosomes, forms the intralumenal striations here, the formation of intraluminal fibrils being at stage I which get completed in stage II [10].

When the internal striation is formed, the following transport of integral membrane proteins take place: for example enzymes TYR, TRP-1 and TRP-2 begin the production of melanin that conceals the Pmel17-interlumenal striation, ultimately melanosomal development proceeds towards stage III melanosomes which is partially pigmented [11]. TYR is the key enzyme of melanin synthesis [12] as it catalyses the initial two crucial steps of melanogenesis, i.e. the hydroxilation of L-tyrosine to 3,4-dihroxyphenylalanine (L-DOPA), and subsequent oxidation of L-DOPA to DOPA-quinone. DOPA-quinone is the branching point to form either eumelanin or pheomelanin. In the absence of thio compounds like cysteine or glutathione (GSH), DOPA-quinone gets converted into DOPA-chrome. DOPA-chrome either tautomerize into indole 5,6-quinone 2-carboxylic acid (DHICA) the presence of TRP-2 or decarboxylated into 5,6-dihydroxyindole (DHI). TRP-1 is a DHICA oxidase which catalyses the formation of carboxy group containing eumelanin. DHICA and DHI are further oxidized and polymerized into eumelanin [13].

In the presence of thiol compound, the sulfhydryl group of these compound conjugate with DOPA-quinone to form 5-S-cysteinyldopa or 5-S-glutathionyldopa (in the presence of cysteine or glutathione). Thiol-DOPA gets subsequently oxidized and is polymerised into pheomelanin [14]. Eumelanosomes are elliptical or oval in shape whereas pheomelanosomes are of spherical shape. Fully pigmented stage IV melanosomes are generated after additional synthesis of melanin. These fully melanized stage IV melanosomes are then transported from melanocytic dendrites to the keratinocytes.

Crucial Factors Regulating Melanin Synthesis

Pigmentation factors that regulate biosynthesis of melanin within melanocytes are typically situated within, on, or near the melanosomes. These factors can be divided into three types i.e. protein that regulated melanosomes development, proteins that control melanin synthesis and proteins involved in intracellular trafficking of melanosomal components as well as their transport to the adjacent keratinocytes [15].

Melanosomal structural proteins

PMEL17/GP100 and MART-1 are the important structural proteins that play principal role in biogenesis of melanosomes. PMEL17 is an integral membrane protein that endures transfer from the endoplasmic reticulum to the Golgi apparatus where it is glycosylated and eventually exported to the limiting membrane of stage I premelanosomes [16]. Here it undergoes proteolytic cleavage and forms intraluminal striation of stage II premelanosomes, melanin pigment mask these fibrils and ultimately mature stages III and IV melanosomes formed. MART-1 is considered as melanosome specific marker and is found plentiful in the early melanosomes, is obligatory for the maturation of PMEL17 [17].

Enzymes / Proteins that control melanin biosynthesis

The three important enzymes that take part in melanin synthesis belong to tyrosinase gene protein family. These are tyrosinase (TYR), tyrosinase related protein-1 (TRP-1) and tyrosinase related protein-2 or dopachrome tautomerase (TRP-2 or DCT). All the three enzymes of melanin synthesis are transcriptional products of micropthamia associated transcriptional factor (MITF) which is also known as "master regulator" of melanogenesis. Under normal conditions, after getting stimulation from UV radiation, MITF ensures the uninterrupted transcription of tyrosinase genes via cAMP signalling etc. [18]. Instead of MITF, many factors including biogenesis of lysosome-related organelles complexes (BLOC-1), ocular albinism type 1 protein (OA1), oculocutaneous albinism II (OCA2 also known as P) and SLC45A2 affect the trafficking and hence the function of these enzymes [10,19].

Receptors and proteins involved in melanosomal transport

After the deposition of melanin granules within the melanosomes, these are being migrated through microtubules (MT) from perinuclear zone to dendritic tips of melanocytes for transfer into the neighbouring keratinocytes. In addition to the MT, proteins like kinesin (prograde) and dynein (retrograde) seem to participate in trafficking of melanosomes [20].

A combination of genetic and cell biological studies have revealed that the activated small GTPase Rab27a together with its effector protein melanophilin, associates with mature melanosomes and recruits the actin motor protein, myosin VA (MYO5A) expressed in melanocytes. These proteins connected to the mature melanosomes in the order of Rab27A-melanophilin-MYO5A, this complex enables migration of melanosomes from microtubules onto the peripheral actin network of melanocytes, enabling successive transport to the adjacent keratinocytes [21-22].

Previous pioneering research work done in our laboratory, related to skin pigmentation has revealed that the major signals involved in pigment transport within the pigment producing cells are dependent on a special class of cell surface receptors called G-protein-coupled receptors (GPCRs). Many of these receptors i.e. adrenergic [23-27], cholinergic or cholinomuscarinic [28-34], histaminergic [35-39], serotoninergic [40] have been found on melanophores/melanocytes of lower vertebrate species that regulate the translocation of pigment granules from one place to another. These large numbers of studies have also demonstrated the physiological and pharmacological role of autonomic receptors from a comparative evolutionary aspect of skin pigmentation. Our data categorically point that, at the cellular and molecular level, receptors particularly those of histamine (H1, H2, H3 and H4) and serotonin (5HT1, 5HT2, 5HT3 and 5HT4) play specific role in animal pigmentation including mammalian melanocytes Figure 1.

On the other hand endothelin and melatonin receptors have also found to be present on melanocytes, regulating translocation of pigment granules leading to control of skin pigmentation or phonotypical appearance of skin [41].

Hyperpigmentation of skin

Hyperpigmentation may be defined as increased pigmentation of the skin, which is generally recognized by darkening or increase in the natural color of the skin usually due to an increased deposition of melanin pigment in the epidermis and/or dermis [42]. On the basis of location of over deposition of melanin granules, hyper pigmentation can be classified as epidermal and dermal hyper pigmentation [43]. Epidermal hyper pigmentation is because of melanin pigmentation and has a brownish hue, while, dermal pigmentation is called

'ceruloderma' or 'blue hyper pigmentation' which may either be due to melanin or due to non-melanin pigments [44].

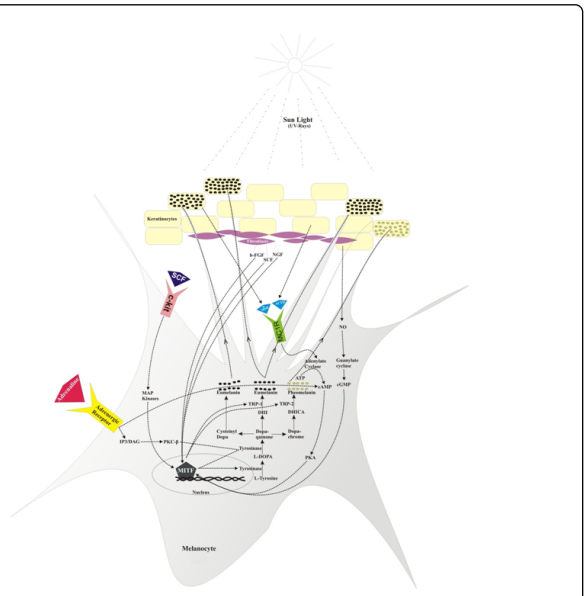

Figure 1: Schematic illustration of signalling pathway associated with UV induced melanogenesis and translocation of pigment granules from melanocytes to keratinocytes.

Hyper pigmentation also results from inflammation or other skin insults i.e. skin diseases such as acne or shingles with may leave darkened spots. Scars from skin injury or surgery also may become hyper pigmented. Cosmetic procedures including laser resurfacing, laser hair removal, chemical peels and dermabrasion also may leave the affected area darker than the normal skin color [45].

Although hyper pigmentation is not harmful, it can cause significant cosmetic disfigurement and become a persistent psychosocial burden for the patient, due to the limited efficacy of the available treatments. Apart from this, disfiguring facial lesions can significantly affect a person's overall emotional well-being and can contribute to decreases in social functioning, productivity at work or school, and self-esteem [46,47].

Mechanism of Hyperpigmentation

Abnormal pigmentation or hyper pigmentation may develop at a number of points in the biochemical cascades that regulate healthy pigmentation [48]. It typically results from increased melanin production by existing melanocytes (melanotic hyper pigmentation), which may occur in the epidermis/dermis or in both or from proliferation of active melanocytes (melanocytotic hyper pigmentation). Hyper pigmentation also may develop when epidermal cells retain too much melanin.

The main causes of skin hyper pigmentation are various: auto immune conditions, sun damage (UV radiation and ionizing radiation), drug reactions (chemicals), hormonal changes (extensive release of α-MSH), genetic factors, medications, hormonal therapy or birth control pills and also rubbing of the skin which can be the triggering factors, resulting in the hyper secretion of melanin from melanocytes causing hyper pigmentation [49-52].

Normally, the melanocytes are located in the basal layer of the epidermis and an increase in their number or activity will cause epidermal hyper pigmentation. However, formed melanin may be transferred to the dermis or, in some cases, dermal melanocytes are also present. A heightened activity or number of melanocytes in these instances will lead to dermal hyper pigmentation. Also, a combination of the above may take place, triggering mixed hyper pigmentation.

Hyperpigmentation Disorders

Hyper pigmentation disorders are characterized by darker skin appearance i.e. brown to gray-brown patches on the face melasma [45,53], dark plaques on skin, (post inflammatory hyper pigmentation) [53-55], light to dark brown spots of 1 to 20 cm ("Café-au-lait" macules)[53], pale brown to dark brown spots on the skin (agespots or solar lentigo)[53], well-circumscribed round to oval or irregular grey patches on the face, neck and trunk (Erythema dyschromicum perstans), netlike pigmentation (Prurigo pigmentosa)[56,57], dark vertical line across the abdomen in pregnant females (linea nigra) [58].

These skin pigmentary abnormalities are seen as aesthetically unfavourable and have led to the development of cosmetic and therapeutic treatment modalities of varying efficacy aimed to modulate skin pigmentation. These depigmenting agents are any ingredient or combination of ingredients that interfere one or more steps of melanogenic pathway, melanosome transfer or post-transfer pigment processing and degradation that result in lowering pigmentation on the surface of the skin [59,60].

Many modalities of treatment for acquired skin hyperpigmentation are available including chemical agents or physical therapies, but none are completely satisfactory. Traditional depigmenting agents, such as hydroquinone, corticosteroids, and kojic acid, although highly effective but can raise several safety concerns (i.e. ochronosis, atrophy, carcinogenesis, and other local or systemic side effects) with long-term exposure [61,62].

To overcome these adverse side effects, benefits of natural and botanical extracts can be used, which provides opportunities to develop new products as novel treatment for various hyperpigmentory anomalies, as medicinal plants are rich sources of bioactive chemicals, free from harmful side effects and are potentially safe and effective skin lightening agents [52,63].

Active compounds isolated from plants, such as arbutin, aloesin, gentisic acid, flavonoids, hesperidin, licorice, niacinamide, yeast derivatives and polyphenols, inhibit melanogenesis without melanocyte toxicity by different mechanisms i.e. acting on one or more steps in the melanogenic pathway, melanosome transfer, or post-transfer pigment processing and degradation.

Approaches for Treatment of Hyperpigmentation by Natural Botanicals

Inhibition of tyrosinase and related enzyme expression

The transcriptional level is the first stage by which the expression of tyrosinase and related melanogenic enzymes may be modulated [64]. To develop a new skin whitening agent for cosmetics and treatment of hyperpigmentation form natural products various phyto molecules have been screened in past few decades which exerting inhibitory effect on formation of tyrosinase, tyrosinase related protein-1 (TRP1)

and tyrosinase related protein-2 (TRP-2), enzymes that catalyses few rate limiting steps of melanogenic pathway [65].

Studies of Cho et al. [66] have demonstrated that isoimperatorin and imperatorin isolated from ethanoloic extract of Angelica daburica significantly inhibited tyrosinase synthesis in Bl6 melanoma cells by reducing the level of tyrosinase m-RNA leading to lightening of the skin (Table 1).

Inhibition of tyrosinase activity

Most botanical depigmenting agents act by interfering in the pathways leading to melanin synthesis by inhibiting the activity of tyrosinase [65]. These kinds of tyrosinase inhibitors are widespread in nature and botanical extracts are good source of naturally occurring tyrosinase inhibitors which are commonly used as traditional remedies for pigmentation disorders [67-68].

Regarding this, some earlier studies have suggested that arbutin, isolated from the fresh fruit of the California buckeye and Aesculus californica, was used to inhibit the oxidation of L-DOPA catalyzed by mushroom tyrosinase and was effective in the topical treatment of various cutaneous hyperpigmentory disorders characterized by hyperactive melanocyte function [69-71].

Green tea is also reported to be a competitive tyrosinase inhibitor based on *in vitro* studies. The gallocatechin moiety in the major catechin constituents epicatechin gallate, epigallocatechin gallate and gallocatechin gallate is reported to be responsible for this effect [72].

Studies of Picardo and Carrera, [73] have demonstrated that aloesin from Aloe vera works as a non-competitive inhibitor of tyrosinase, affecting the action of tyrosinase complex in the substratum and reducing the conversion of DOPA into melanin. Kojic acid also inhibits tyrosinase directly, while L-ascorbic acid and its derivatives are believed to act as reducing agents on intermediates in melanin biosynthesis at various points in the oxidation chain reaction from tyrosine/DOPA to melanin. Green tea is also reported to be a competitive tyrosinase inhibitor based on *in vitro* studies.

Tan et al. [74] and Cheng et al. [75] have reported that aloin isolated from leaf extract of A. vera acts as a natural skin lightener which can bind not only to the enzyme tyrosinase but also to the enzyme–substrate complex, leading to inactivation of the enzyme resulting lightening of skin.

Glycyrrhiza glabra is a commonly used plant in the skin lightening industry, which works to inhibit the enzyme tyrosinase and limit the amount of pigment produced in melanocyte cells. Previous studies of Nerya et al. [76] and Fu et al. [77] showed that glabridin and isoliquiritigenin, the main ingredients of the hydrophobic fraction of licorice extract have been also shown to inhibit tyrosinase activity in B16 murine melanoma cells. Research also indicated that skin lightening potential of glabridin is found to be greater than that of hydroquinone, the artificial depigmentation agent widely used [76, 78].

Lee et al. [79] have reported that mulberroside F, the active component of dried mulberry (Morus alba) leaves also showed inhibitory effects on tyrosinase activity and on melanin formation in melan-a cells. This compound also exhibited superoxide scavenging activity that is involved in the protection against auto-oxidation [80], suggesting a role for M. alba as a component of lightening cosmetics.

Yamakoshi et al. [81] have reported the oral administration of a proanthocyanidin rich extract from grape seeds for one year reduced effectively the hyperpigmentation of women with chloasma. Ellagic acid is a natural polyphenol that is widely found in fruits and vegetables and the main active ingredient of fruit rinds of pomegranate, showed inhibitory activity in the process of melanogenesis by inhibition of the proliferation of melanocytes and melanin synthesis by tyrosinase in melanocytes [82].

Hesperidin, a bioflavonoid existing extensively in the peel and membranes of citrus fruits also shows potent dose-dependent antityrosinase activity of hesperidin in B16 melanoma cells leading to inhibition of melanin synthesis without cytotoxicity [83].

Apart from these, there are several other plant extracts and active ingredients i.e. Greyia flanaganii leaf extract [84], ethanolic extracts of Salvia cryptantha and Salvia cyanescens [85], ethanolic extracts of grape seeds and peels [86], stem bark extract of Bauhinia rufescens [87], dried flower extract of Inula britannica [88], stems extract of Liriodendron tulipifera [89], Cotoneaster nummularia extract [90], Cocoa pod extract [91], Salacia reticulate extract [92], resveratrol and oxyresveratrol isolated from citrus fruits [93], which have been found to possess strong tyrosinase inhibitory activity as shown in various *in vivo* and *in vitro* pigment cell systems.

Recent studies of Cho et al. [94] have also demonstrated that trans-p-coumaric acid methyl ester and N-(trans-cinnamoyl) tryptamine isolated from the roots of Oryza sativa L., works as strong inhibitor of tyrosinase and exerts skin lightening effect on murine B16-F10 melanoma cells. While Han et al. [95] have also shown that there is considerable skin lightening activity in the aerial parts of Pueraria thunbergiana extract, which interrupts the maturation of tyrosinase through inhibiting a-glucosidase, consequently reducing the tyrosinase activity in the pigment cells.

Inhibition of Melanin dispersion or translocation

Several studies have focused on the identification of regulatory factors of the melanosome movement in dendrites and of the interaction between keratinocytes and melanocytes during the transfer process [96-97]. In this regard, several molecules are also known to have an effect on the transfer of melanin from melanocytes to keratinocytes, leading to lightening of the skin and can be used as therapeutic agents for hyperpigmentory skin disorders [66].

Niacinamide is a biologically active form of niacin (vitamin B3) found widely in many root vegetables and yeasts, and it is also an important precursor of NADH (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate), inhibits the transfer of melanosomes from melanocytes to keratinocytes in cocultures of human melanocytes and keratinocytes. Some clinical studies using topically applied niacinamide have also demonstrated a reversible reduction in hyperpigmented lesions and increased skin lightness [98].

Recent research work done in our laboratory has demonstrated that plant extracts of Arachis hypogaea [24], Citrus reticulata [26], Aloe vera [25], Chlorophytum tuberosum [27] Ocimum sanctum [99], G. glabra and Punica granatum (unpublished data) significantly lighten the skin via stimulation of adrenergic receptors of various subtypes in different melanophore models of animals like fishes, amphibians and reptiles. These findings have provided a strong basis to understand the receptor based mechanism of the skin lightening at the cellular level.

S. No.	Plant Name	Bioactive compound	Experimental Assay / Cell type/ Animal model	Target site/ Mode of action	Reference
1	Angelica daburica	Isoimperatorin and Imperatorin	B16 melanoma cells	Tyrosinase m-RNA level (Tyrosinase Synthesis)	Cho et al. [66]
2	Aesculus californica	Arbutin	Human melanocytes in culture	inhibition of tyrosinase activity at post transcription level, DHICA polymerase activity	Tomita et al. [69] Chakraborty et al. [70] Hori et al. [71]
3	Camellia sinensis (Green tea)	Epicatechin gallate, Epigallocatechin gallate and Gallocatechin gallate	*In vitro* mushroom Tyrosinase Assay	Inhibition of tyrosinase activity	No et al. [72]
4	Aloe vera	Aloesine and Aloin		Inhibition of tyrosinase activity, Inhibition of pigment translocation	Picardo and Carrera, [73] Ali et al. [25]
5	Glycyrrhiza glabra	glabridin and isoliquiritigenin	B16 murine melanoma cells	Inhibition of tyrosinase activity	Nerya et al. [76] Fu et al. [77] Holloway, [78]
6	Morus alba	mulberroside F	melan-a cells	Inhibition of tyrosinase activity	Lee et al. [79,80]
7	Vitis vinifera	proanthocyanidin	*In vivo* Human Females	Inhibition of tyrosinase activity	Yamakoshi et al. [81]
8	Punica granatum	Ellagic acid	UV-irradiated guinea pigs	Inhibition of tyrosinase activity	Yoshimura et al. [82]
9	Citrus Fruits	Hesperidin	B16 melanoma cells	Inhibition of tyrosinase activity	Zhang et al. [83]
10	Greyia flanaganii	Ethanolic extract of leaves	Cultured melanocytes cells	Inhibition of tyrosinase activity	Mapunya et al. [84]
11	Salvia cryptantha	Ethanol (EtOH) extracts	*In vivo* and *in vitro* models	Tyrosinase activity by ferrous ion-chelating ability, (DPPH) and superoxide radical scavenger activity	Süntar et al. [85]
12	Salvia cyanescens	Ethanol (EtOH) extracts	Rats and mice	Inhibition of tyrosinase activity	Süntar et al. [85]
13	Bauhinia rufescens	a-amyrin acetate	*In vitro* tyrosinase inhibition assay	Inhibition of tyrosinase activity	Muhammad and Sirat [87]
14	Inula britannica	1-O-acetylbritannilactone, britannilactone	Cultured B16 melanoma cells	Tyrosinase activity, Inhibition of ERK, Akt, cAMP related signalling,	Choo et al. [88]
15	Liriodendron tulipifera	Yangambin, Methyl 4-hydroxy-2-methylbenzoate, Methyl haematommate, 2,6-Dimethoxy-p-quinone	B16F10 cell line	Inhibition of tyrosinase activity	Li et al. [89]
16	Cotoneaster nummularia	ferulic acid, chlorogenic acid, epicatechin, catechin	*In vitro* Mushroom tyrosinase assay	Inhibition of tyrosinase activity	Zengin et al. [90]
17	Theobroma Cacao (Cocoa pod) extract	flavonoids	*In vitro* Mushroom tyrosinase assay	Inhibition of tyrosinase activity	Karim et al. [91]
18	Salacia reticulate	root extract	B16 melanoma cells	Inhibition of tyrosinase activity	Suwannalert et al. [92]
19	Arachis hypogaea	Resveratrol, oxyresveratrol	tyrosinase assay	Inhibition of tyrosinase activity, Inhibition of pigment translocation	Park et al. [93] Galgut and Ali, [24]

20	Oryza sativa L	trans-p-coumaric acid methyl ester and N-(trans-cinnamoyl) tryptamine	murine B16-F10 melanoma cells	Inhibition of tyrosinase activity	Cho et al. [94]
21	Pueraria thunbergiana	Aerial part extract	B16F10 melanoma cell line	Downgrading microphthalmia-associated transcription factor, interrupting maturation of tyrosinase	Han et al. [95]
22	Helianthus annuus	Niacinamide	keratinocyte/ melanocyte coculture model and pigmented reconstructed epidermis (PREP) model	Inhibition of pigment translocation from melanocytes to keratinocytes	Hakozaki et al. [98]
23	Chlorophytum tuberosum	Aqueous extract	Fish scale melanophores	Inhibition of pigment translocation	Choudhari et al. [27]
24	Ocimum sanctum	Eugenol	B16F10 Cell line, Amphibian dorsal skin melanophores	Tyrosinase activity, Inhibition of pigment translocation	Ali et al. [99]

Table 1: Summary of scientifically validated active botanicals which can be used as skin lightening agents and their possible mechanism of action.

Control of melanin degradation and its removal

Apart for above two strategies, some other substances that increase the degradation and removal of pigment granules from the skin are also commonly used to remove excessive melanin content within the skin and used as cosmetic and therapeutic agents [66].

Chemical substances have been used as exfoliantes, such as a-hydroxy acids, free fatty acids and retinoic acid, which stimulate cell renewal facilitating the removal of melanised keratinocytes, leading to melanin pigment loss. Earlier studies of Amer and Metwalli, [100] had demonstrated that liquirtin isolated from Licorice root bud extracts, exerted potent skin lightening effects in melasma patients, by removing keratinocytes (desquamation), shortening the cell cycle and facilitating rapid pigment loss.

Conclusion and Future Perspectives

It is a well known fact that melanin is very essential pigment found in melanocytes and provides a defensive mechanism against photo damage of skin cells, but excessive melanin production and uneven distribution leads to undesirable conditions specified as hyperpigmentation. Researchers have determined the chemical changes that occur at every step of the melanogenic pathway, both in healthy individuals and in those with pigmentation disorders and huge advances have been made to understand pigment biology and the processes underlying skin pigmentation in past few decades. More basic understanding of the regulation of melanogenesis could help to develop safe and natural agents for treatment of hyperpigmentation disorders.

There are several conventional chemical treatments used for treating hyperpigmentory dysfunctions but none have been found suitable because of their adverse side effects. On the other hand natural ingredients from plant origin offer safer alternatives in comparison to other hazardous chemical skin lighteners, as well as they also offer additional functionalities as sunscreen boosters, moisturizers, or "anti-aging" ingredients, thereby supporting skin health, and reducing the appearance of wrinkles. On the light of these facts it can be concluded that the natural skin whiteners from plant extracts are more effective, safer, non-toxic and cost effective compared to the chemical skin whiteners with diverse side effects.

At the end of the discussion it is important to note that the previous knowledge and current updates in melanocyte biology and the processes underlying melanin synthesis have made remarkable progresses over the last few years and have undoubtedly opened new paths in pharmacologic approaches for the treatment of hyperpigmentation. But at the same time, the topic has become more complex and the classification of the bio molecules is continually becoming more complicated to understand. *In vitro* pigmented skin substitutes can be produced by tissue engineering and *in vivo* models which can exploited as useful tools for understanding these mechanisms and developing appropriated treatments or drugs. Also, as the number of putative depigmenting agents grows there is an increased need for studies to clarify product efficacy, cytotoxicity, topical skin penetration, target oriented drug delivery, stability, safety and efficacy for *in-vivo* pigment cell system.

However it is clear that great progress has been made till now in the filed of normal and uneven skin pigmentation related to its physiology and pharmacology, but it is even more apparent that there is a great deal of work is still left to be done. There are still many questions which are unanswered, concerning the precise mechanism of action and *in-vivo* efficacy of the applied natural molecules. Several other mechanisms involved in pigmentation disorders remain unknown and need to be elucidated upon in order to give affected people a better quality of life. In the coming days we expect inter merging of several disciples to unravel the mystery of human pigmentation.

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