

The Mechanism and Potential of Palm Vitamin E as a Skin-lightening Agent

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INTRODUCTION

A light skin tone is considered a superior trait in most races, particularly among Asian and African women. Realising the demand for fair skin, many cosmetic companies are developing different molecules for use as skin-lightening products. There are many commercial skin-lightening products readily available over-the-counter which use depigmenting agents such as hydroquinone, azelaic acid, kojic acid, vitamin C, niacinamide, corticosteroids, licorice extract, glutathione, glycolic acid and gentsic acid (Sonthalia *et al.*, 2016). However, several skin-lightening agents such as hydroquinone and kojic acid have been banned in cosmetic products due to their side effects on the skin, and this has led to the search for safer plant-based skin-lightening materials. The wide range and variety of available biomass is presumed to be an attractive bioresource for screening for inhibitors of melanin synthesis (Lin *et al.*, 2007; Thongchai *et al.*, 2007).

The main skin-lightening mechanism reported is the direct inhibition of tyrosinase enzyme; this is followed by other methods such as microphthalmia transcription factor inhibition, down-regulation of melanocortin-1-receptor (MC1R) activity, interference with melanosomal transfer, and loss of melanocytes (Kamakshi, 2012).

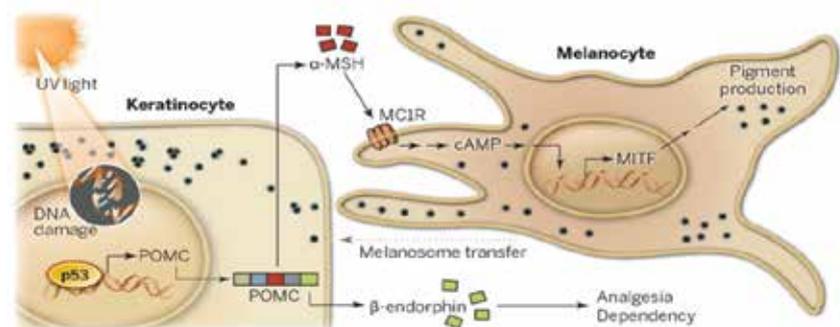
ULTRAVIOLET-INDUCED SKIN PIGMENTATION

Melanogenesis or the skin pigmentation process is initiated by the enzyme tyrosinase upon exposure to ultraviolet (UV) radiation, resulting in darkening

of the skin (Balakrishnan *et al.*, 2011). Melanogenesis is a complex pathway which occurs in highly specialised cells called melanocytes. This process produces the melanin pigment and is increased during skin aging (Tobin, 2006).

When our skin is exposed to sunlight, some damage can be caused in the upper layers of the skin. This activates DNA damage repair and also induces signalling towards the melanocytes. Signalling involves the excretion of signalling molecules (mostly alpha-melanocyte stimulating hormone, α -MSH) which bind to the melanocortin 1 receptor (MC1R) on the surface of the melanocyte (the cell which produces the pigment) and induce (or increase) the production of melanin. The melanin in the melanosomes is then transferred to the adjacent keratinocytes, which protect the skin from sun's UV radiation (Lo and Fisher, 2014).

Tanning as a result of UV radiation is an example of acquired



Note: POMC – pro-opiomelanocortin; MITF – microphthalmia-associated transcription factor; cAMP – cyclic adenosine monophosphate; MC1R – melanocortin 1 receptor; and α -MSH – alpha melanocyte stimulating hormone.

Source: Lo and Fisher (2014).

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Figure 1. Cutaneous response to UV radiation.

pigmentation. The consequences of UV radiation are marked by the signs of 'sunburn' and/or 'suntan' to the naked eye (Lin and Fisher, 2007). Tanning involves p53 activation in keratinocyte-induced DNA damage, leading to p53-mediated upregulation of pro-opiomelanocortin (POMC) (Lo and Fisher, 2014). The central regulatory element in the processes that control the effects of DNA damage in the vertebrate cell is the tumour-suppressor gene p53 (Farmer *et al.*, 1992). p53 protein exhibits sequence-specific DNA-binding activity and can transactivate reporter genes. Post-translational cleavage of POMC produces β -endorphin and α -MSH. MC1R in neighbouring melanocytes is stimulated by the secreted α -MSH, resulting in melanin synthesis and transfer of melanin-containing vesicles (melanosomes) to keratinocytes as shown in *Figure 1* (Lo and Fisher, 2014).

About 90%-95% of ultraviolet A (UVA) and 5%-10% of ultraviolet B (UVB) from solar radiation can reach the human skin. UVA can penetrate deeper into the dermis, with about 20%-50% of solar UVA reaching the depth of the melanocytes. In contrast, only 9%-15% of solar UVB reaches the melanocytes in the skin (*Figure 2*). Therefore, melanin pigmentation is

stimulated by UVA, but the resultant tan appears to be less protective against UV-induced injury and is more transient than a tan induced after UVB exposure. UVA has a 1000-fold less erythema-producing effects than UVB even though UVA reaching the Earth's surface is in several orders of magnitude greater than the amount of UVB (Costin and Hearing, 2007).

MELANOSOMES AND MELANOGENESIS

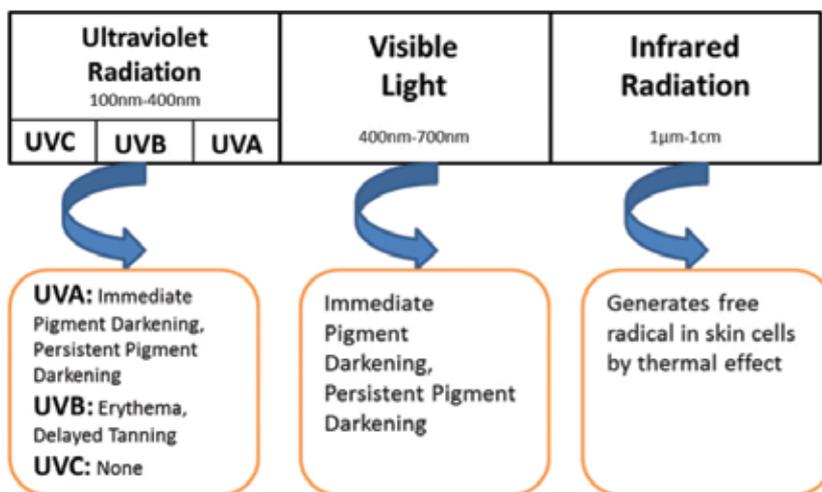
The epidermis is the outermost layer of the skin, where melanocytes (pigment-producing cells) are localised for melanin production which determines human skin colour (D'Ischia *et al.*, 2013). Melanogenesis is a biochemical process for melanin production in the skin (Kamakshi, 2012).

Melanin pigments are transferred into the basal layers of the epidermis by the melanocytes. Melanocytes are derived from fibroblasts and keratinocytes in the dermis and epidermis (basal and super basal keratinocytes), respectively (Ma and Sun, 2012). As an important pigment in the skin, melanin absorbs sunlight and removes reactive oxygen species (ROS), thus protecting the skin from UV radiation. Melanin is produced by approximately 10% of

the skin cells in the innermost layer of the epidermis (Balakrishnan *et al.*, 2011). Melanosomes are mostly circulated in the keratinocytes which absorb radiation that causes the dark colour of the skin (Tadokoro *et al.*, 2003). The Raper-Mason pathway of melanogenesis (*Figure 3*) shows that melanin in human skin is a polymer of various indole compounds synthesised from L-tyrosine with tyrosinase being the rate-limiting enzyme.

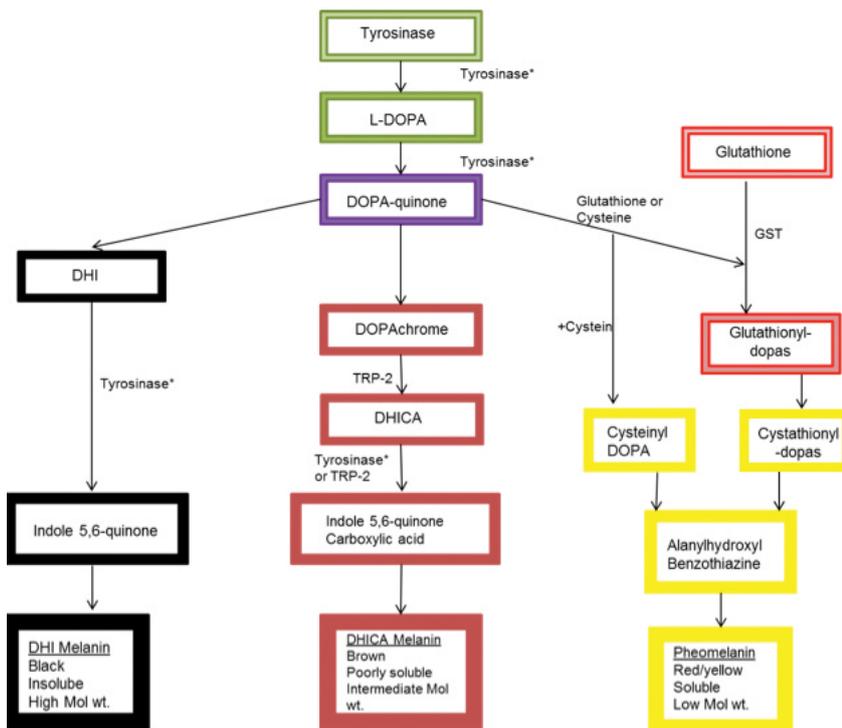
The black-brown coloured eumelanin and yellow-red pheomelanin are two types of melanin found in the skin. Skin colour is determined by the ratio of the two melanins (Nordlund and Boissy, 2001). The amount of melanocytes in the skin does not establish the difference in skin colour between fair and dark people. This difference is due to the level of activity of the melanocytes (quantity and relative amounts of eumelanin and pheomelanin).

Melanins are derived from a common tyrosinase-dependent pathway with the same precursor, tyrosine. Tyrosinase is hydroxylated to dopaquinone during which L-dihydroxyphenyl-alanine (L-DOPA) is also formed as a side product. Then, the eumelanin and pheomelanin pathways diverge from dopaquinone. Tyrosinase-related proteins (TRP1 and TRP2) are crucial enzymes to eumelanogenesis. Pheomelanin is derived from conjugation by thiol-containing cysteine and glutathione (Land and Riley, 2000). Both TRP1 and TRP2 are involved in the downstream reactions of the melanogenic pathway. Microphthalmia-associated transcription factor is potentially activated to increase the expression of tyrosinase and the TRP1 and TRP2 melanogenic enzymes when α -MSH binds and acts on G-protein-coupled MC1R. Melanin synthesis is induced by the increased expression of tyrosinase and both the melanogenic enzymes (Costin and Hearing, 2007).



Source: Maddodi *et al.* (2012).

Figure 2. Effects of solar radiation on skin pigmentation.



Note: DHI – 5,6-dihydroxyindole; DHICA – 5,6-dihydroxyindole-2-carboxylic acid; DOPA – 3,4-dihydroxyphenylalanine; GGT – Gamma-glutanyl transpeptidase; GST – Glutathione-S-transferase; L-DOPA – Levo-DOPA; and TRP-2 – Tyrosinase-related protein 2.

Source: Sonthalia *et al.* (2016).

Figure 3. The Raper-Mason pathway depicting the steps in melanin synthesis. The presence of glutathione/cysteine can induce a switch towards higher pheomelanin production as compared with eumelanin.

PALM VITAMIN E AND MELANOGENESIS

Palm oil contains a number of phytonutrients. To date, most studies using palm phytonutrients have focused on palm vitamin E. Tocopherols (TP) and tocotrienols (T3), belong to the group of palm vitamin E, and play a pivotal role as essential, fat-soluble nutrients that function as antioxidants in the human body. Both TP and T3 have isomers, designated as alpha (α -), beta (β -), gamma (γ -) and delta (δ -), which differ by the number and position of the methyl groups on the chromanol ring (Schneider, 2005). Safety evaluation of palm tocotrienol-rich fraction (TRF) for topical application has been reported. Palm TRF at 50% concentration did not induce any cutaneous irritation, or cause

sensitisation of the skin at 1%, 2.5% and 5%. Thus, it is considered safe to be used as an active ingredient for topical applications such as in cosmetics (Zafarizal *et al.*, 2008).

In recent years, specific vitamin E isomers were studied on the inhibition of melanogenesis for treatment of skin pigmentation. In mouse B16 melanoma cells, γ -TP inhibited up to 39% of melanin synthesis and 45% of tyrosinase activity. In fact, δ -T3 inhibited melanin synthesis significantly in B16 melanoma cells (Yap *et al.*, 2010).

The activity of tyrosinase in B16 melanoma cells treated with δ -tocotrienol, γ -tocotrienol, sodium lactate, kojic acid, α -tocopherol and palm tocotrienol-rich fraction (palm TRF) respectively beyond 24

and 48 hr was determined using the Western Blot analysis. After 48 hr of incubation, the results show the enhancement of tyrosinase suppression by γ -T3 and δ -T3 in B16 cells. In contrast, the anti-tyrosinase activity of sodium lactate and kojic acid diminished after 48 hr. Palm TRF showed lower anti-tyrosinase activity at 20 μ M than γ -T3 and δ -T3. α -TP had no effect on the suppression of tyrosinase (Yap *et al.*, 2010).

A study to examine xenografted solid tumours in immunocompromised mice was conducted by Yap *et al.* (2010). The results show that pigmentation of solid tumours was lighter in colour than the control after 14 days of δ -T3 supplementation. In fact, the size of the tumours was also significantly smaller for the mice supplemented with γ -T3 and δ -T3. γ -T3-treated B16 solid tumours had lower tyrosinase protein expression based on immunoblots of tyrosinase in solid tumours.

A study done by Ng *et al.* (2014) showed that the inhibitory effects of δ -T3 on melanogenesis was mediated by the activation of extracellular signal-regulated kinase signalling, thus resulting in downstream suppression of melanogenesis-related proteins and melanin production. The strong antioxidant property of δ -T3 decreased the melanin levels in murine B16 melanocyte cells by preventing the oxidative reactions of tyrosinase (Michihara *et al.*, 2010; Yap *et al.*, 2010; Michihara *et al.*, 2009). To date, none of the studies have reported inhibitory effects of a mixture of tocopherol and tocotrienols on melanogenesis. Inhibition of the UVA and UVB cell damage pathway is a better strategy to prevent UV-induced melanoma.

Exposure to UV radiation is a well-known external risk factor for developing melanoma (Zhang and Rosdahl, 2003). Studies have found that UVA exerted a strong

influence on skin melanogenesis via activation of the oxidative stress pathway involving generation of ROS, in particular, hydrogen peroxide. Thus, improving the capacity of the antioxidant defence system in the cells could be beneficial in preventing melanogenesis mediated by UVA irradiation. Exposure to UVA resulted in a decrease in the number of melanocytes, and this effect was due to inhibition of melanocytes proliferation (Makpol *et al.*, 2014; Mengeaud and Ortonne, 1996).

Improvement of undesirable skin pigmentation such as skin spots and freckles caused by UV exposure could be achieved by developing cosmetics with the individual tocopherol or tocotrienol isomers. However, the high cost of each isomer may render it economically non-viable to develop skin-whitening products. Palm TRF which has both tocopherol and tocotrienol isomers may offer a better alternative for developing feasible cosmetics for the treatment of skin pigmentation.

CONCLUSION

There have been many studies on the individual isomers of palm vitamin E as potential skin-lightening agents. However, development of skin-whitening products using palm TRF may offer better synergistic effects as a natural skin-lightening agent.

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