A Lighter Skin Tone and More…
With Natural Actives

presented by

SABINSA CORPORATION

Authors:

Muhammed Majeed, Ph.D. & Lakshmi Prakash, Ph.D.

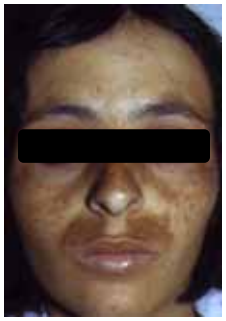
info@sabinsa.com www.sabinsa.com www.sabinsacosmetics.com

INTRODUCTION

Skin lightening products form a major segment of cosmetic products worldwide and carry with them the promise of flawless skin free from age spots, blemishes and scars. Whatever the color of the skin, it is susceptible to damage due to environmental agents, physiological changes and psychological factors. The demand for "skin fairness products" is rooted in the need to eliminate localized hyperpigmentation as well as to lighten the general skin tone.

Motives behind the use of skin lightening products vary considerably between cultures. In Western countries, people wish to eliminate or inhibit the development of irregular pigmentation including melasma (chloasma or localized discoloration), age spots (*Lentigo senilis*) or liver spots (associated with sun damage or aging sometimes appearing as raised spots or *Seborrheic keratoses*) and freckles (*Lentigo aestiva*). In Asia, a lighter skin color is associated with beauty and aristocracy. Therefore, in Asian countries, skin lightening products are used with the intent to lighten and brighten the skin tone.

Skin tones



Melasma

Freckles

FACTORS THAT INFLUENCE SKIN COLOR

Skin pigmentation (Lee, O-S, et al., 1995; Masuda, M et al, 1996; Blume, G et al., 2001) is influenced by several factors, including hemoglobin in the blood vessels, carotenoids in the dermis and, particularly, the dark pigment, melanin in the epidermis (Figure 1). Two forms of melanin are produced in the epidermis: pheomelanin,

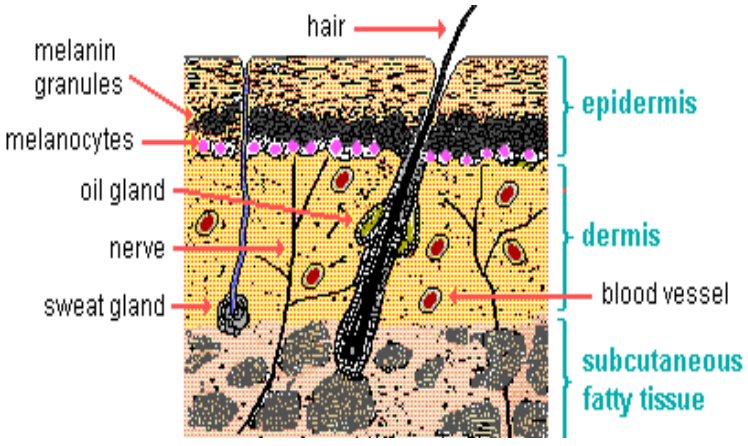


Figure 1: Skin structure and melanin

which is red to yellow in color, and eumelanin which is dark brown to black. The relative proportions of these also influence skin color. In addition, individuals differ in the number and size of melanin particles.

Melanin biosynthesis (melanogenesis) is influenced by genetics, environmental factors, diet and medication. The production of melanin by specialized cells called melanocytes (in the basal layer of the epidermis in light skinned people and in the basal as well as horny layer in dark skinned people) occurs through the action of the enzyme tyrosinase. The rate-limiting step in melanogenesis is the conversion of L-tyrosinase to melanin, through the action of tyrosinase. Copper and oxygen act as catalysts. Other enzymes also control melanin production, particularly in the presence of sulfur. These include the following:

- ✓ Dopachrome oxidoreductase which controls melanogenesis in the absence of tyrosinase. It helps to convert dopachrome into 5,6-dihydroxyindole
- ✓ alpha.-glutamyl transpepsidase which helps to maintain the balance in the biosynthesis of eumelanin and pheomelanin

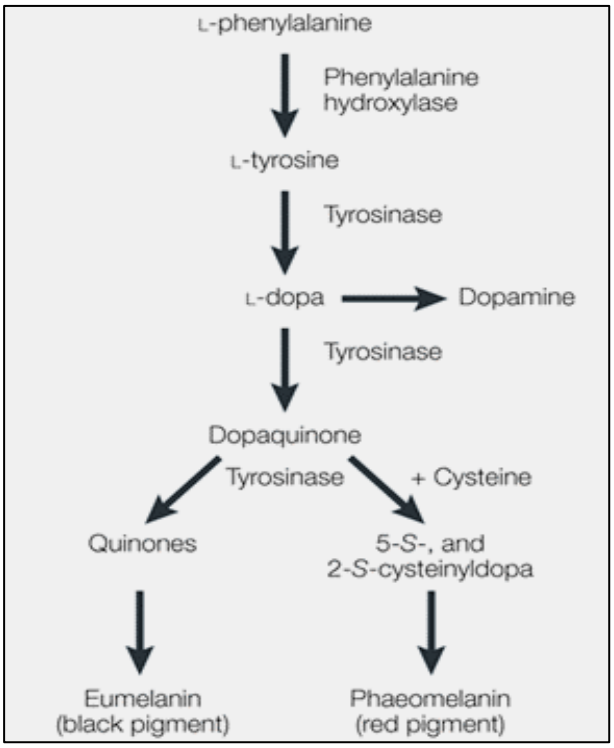


Figure 2: Formation of Melanin

The currently accepted scheme for melanin biosynthesis is shown in Figure 2. Variation in skin pigmentation is attributed to the levels of melanin produced and the number of melanocytes present. Although light skinned and dark skinned people may have the same number of melanocytes present, the rate of melanin production is greater in darker skin tones. Additionally, the melanin present in the epidermal layers of darker skins is resistant to enzymatic degradation. Increased production of melanin on one side of the skin and dramatically reduced decomposition of melanin on the other side results in darker skin tones, in light skinned people.

Melanin granules synthesized in the melanocytes are then transferred from the cytoplasm of the melanocytes to the basal cytoplasm of the keratinocytes. They thus form a protective covering in the inner layers of the epidermis, absorbing UV rays and inhibiting their penetration.

Various types of inflammatory mediators such as leukotrienes and prostaglandins, cytokines and growth factors may influence melanin synthesis by affecting the proliferation and functioning of melanocytes. This explains why inflammatory diseases often induce hypopigmentation or hyperpigmentation. The enzyme, protein kinase C that phosphorylates proteins may also influence the growth and differentiation of melanocytes. Cytokines such as endothelins (also known as vasoconstrictive peptides) are also reported to accelerate melanogenesis.

Thus tyrosinase inhibitors, agents that increase keratinocyte turnover, agents that inhibit the hormone melanotropin, physical sunscreens, reducing agents that convert dopaquinone to DOPA, indole-blockers that inhibit the formation of intermediates in melanin biosynthesis, antioxidants that chelate metal ions (which catalyze tyrosinase activity), cytokine regulators and genetic manipulation would all be beneficial in controlling melanin synthesis.

SKIN LIGHTENING PRODUCTS: A HISTORICAL PERSPECTIVE

Ancient cultures used botanicals and mineral compositions of various kinds to facilitate skin lightening. Several of these materials, researched in recent years, have been found to contain natural enzyme/hormone inhibitors, antioxidants and sunscreens. Commercial skin bleaching products in the earlier part of the last century were based on phenolic derivatives such as hydroquinone/resorcinol and peroxygenated mercury derivatives.

The efficacy of hydroquinone as a skin bleaching agent was discovered accidentally during World War II, when African-American workers in rubber manufacturing factories (where hydroquinone (monobenzone) was a process chemical) complained of discolored areas on their hands and forearms.

The inherent toxicity of hydroquinone and mercury triggered research into safer botanicals and natural/nature identical isolates that would achieve similar functional effects. Hydroquinone is known to produce serious side effects if used over a long period of time. This has lead to regulations or ban on its use in several countries. For instance, in France, hydroquinone usage was first restricted to 5% and then to 2% and current European legislation prohibits its use completely, in cosmetics. The USFDA has classified hydroquinone as a drug and it is no longer approved for use in cosmetics. Hydroquinone use is also reported to increase the risk of developing leukemia, liver cancer, skin irritation, irreversible hyperpigmentation and reproductive damage. The permanent depigmentation produced by hydroquinone photosensitizes the skin and makes it vulnerable to damage by UV-rays thereby increasing the risk of development of skin cancer.

Most skin lighteners currently in use are of botanical or natural origin. Placental proteins and estrogen were used earlier as depigmenting agents, but are rarely used nowadays. Ascorbic acid derivatives such as ascorbyl acetate and ascorbyl palmitate have been used for over 25 years as depigmenting agents in concentrations of 2-3%. These are now replaced by the more stable derivative magnesium ascorbyl phosphate in several formulations (Lee, O-S, et al., 1995).

SKIN LIGHTENING & NATURAL COSMECEUTICALS

The toxicity associated with hydroquinone use, induced researchers to identify less dangerous botanicals with comparable activity. In addition to lightening, these botanical extracts offer multifunctional skin health benefits. The general modes of action include inhibition of the formation of melanosomes, inhibition of tyrosinase biosynthesis, inhibition of melanin biosynthesis and interference of the transfer of melanosomes into the keratinocytes. Some agents also have a chemical effect on melanin with an increase in the degradation of melanosomes in the keratinocytes. Antioxidants such as ascorbic acid and others help to decompose preformed melanin. Hyperpigmentation due to UVA and UVB damage may

also be addressed by preventive measures using antioxidant compounds with sunscreen effect and free radical scavenging action.

Research efforts are generally aimed at achieving one or more of the following effects (Masuda, M et al, 1996):

- ✓ Regulation/inhibition of tyrosinase, dopachrome oxidoreductase and dopachrome tautomerase involved in Melanogenesis
- ✓ Regulation of the cytokine network including endothelin
- ✓ Regulation of genes related to melanogenesis
- ✓ Combinations of the above approaches

Tyrosinase inhibitors such as arbutin (from the leaves of the common bearberry, (*Arctophylos urva ursi* and other plants), glabridin from licorice (*Glycyrrhiza glabra* roots), ascorbic acid and its derivatives, kojic acid (a bacterial carbohydrate metabolite) are better tolerated than hydroquinone. Aloesin from Aloe is reported to be a non-competitive inhibitor of tyrosinase., affecting the action of tyrosinase complex in the substratum and reducing the conversion of DOPA into melanin. Arbutin and kojic acid inhibit 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 (Lee, O-S, et al., 1995; Masuda, M et al, 1996; Blume, G et al., 2001).

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 (No, JK, et al., 1999). Additionally, the antioxidant, anti-inflammatory and UV protectant effects of green tea catechins are well-documented.

Paper mulberry extract (from the root bark of *Broussonetia kazinoki* x *B. papyrifera*) also contains active depigmenting agents, which were shown to be more efficacious than hydroquinone (IC_{50} of 2.5 mg/ml aagainst 5.5 mg/ml for hydroquinone) (Jang DI, et al, 1997). Soy extract is also reported to have moderate skin lightening action in solar lentigenes (hyperpigmentation due to sun exposure) (Hermanns, J.F. et al., 2002).

An extract from matricaria (*Chamomilla recutita*) is reported to contain an endothelin inhibitor. Endothelin inhibitors are reported to work faster than tyrosinase inhibitors on account of the fact that

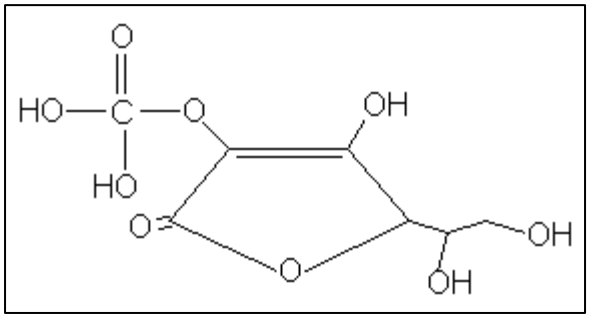
their mechanism of action is outside the melanocyte cell membrane. Tyrosinase inhibitors on the other hand have to cross four barriers – the stratum corneum (outer epidermal layer), deeper epidermal layers, the melanocyte membrane and the melanosome membrane (Masuda, M et al, 1996).

Flavanone derivatives from the roots of Sophora flavescens, were found to show significant inhibitory activity on tyrosinase and melanin production (Hyun, HK, et al, 2008). *Sophora flavescens* (Leguminosae) is Chinese medicinal herb whose leaves and roots have been applied in folk medicine as antipyretic,analgesic, anthelmintic, and a stomachic.

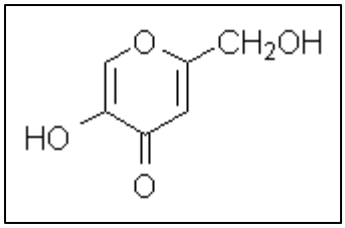
The mechanism of action of some skin lightening agents is summarized in Table 1, and the chemical structures of some of the active ingredients are indicated in Figure 3.

Table 1: Mechanism of action of depigmenting agents (Lee, O-S et al, 1995; Briganti, S et al., 2003):

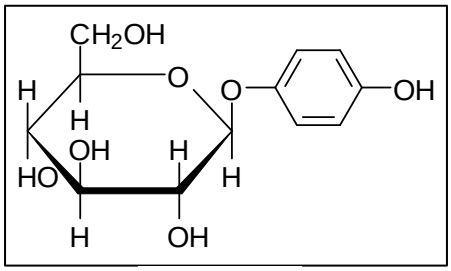
Mechanism	**Examples of active agents**	**Comments**
UV absorber	Sunscreens such as titanium dioxide, oxybenzone	Does not directly affect pigmentation, only blocks UV B light
Free radical scavenging	Antioxidants	Lower whitening effect (indirect mechanism) perhaps through inhibiting polymerization of melanin
Tyrosinase synthesis inhibitor	Glucosamine, galactosamine, manosamine, and others	No specificity to tyrosinase, have cytotoxic effect
Tyrosinase inhibitor	Hydroquinone, ascorbic acid derivatives, kojic acid, arbutin, glutathione, mulberry extract, licorice extract	Stability, availability and safety issues need to be established
Interrupts intermediates in melanin biosynthesis	Kojic acid	Chelation of Copper ions from active site of the enzyme
Cytotoxic effects to melanocytes	Hydroquinone	High dermal toxicity
Reduce preformed melanin	Tocopherol, vitamin C derivatives	Inhibit polymerization of melanin
Endothelin -1 (ET-1) inhibitor	*Chamomilla recutita* Extract	Inhibit ET-1-induced DNA synthesis, but not the IL-a induced ET-1 production and tyrosinase activation



L-Ascorbic acid derivative



Kojic acid



Arbutin

Figure 3: Skin lightening compounds - chemical structure

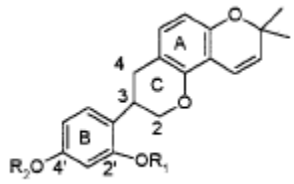
Arbutin is a glycosylated hydroquinone (beta-D-glucopyranoside) effective in the topical treatment of various skin hyperpigmentations characterized by hyperactive melanocyte function. It is found that several plants including Arctostaphylos Uva-Ursi (bearberry), leaves of pear trees and certain herbs. In *in vitro* studies, it was determined that arbutin inhibited tyrosinase activity of cultured human melanocytes at noncytotoxic concentrations, unlike hydroquinone (Sakuma, K. et al.,1999). Melanin production was significantly inhibited by competitive inhibition of tyrosinase. Additionally, arbutin-rich extracts from several *Arctophylos* species have been shown to exhibit superoxide-dismutase-like activity and moderate absorbance in the UV-B area.

An *in vitro* evaluation of the depigmenting action is reported (Sugai, T et al, 1992). L-tyrosine solution (0.5 ml) at a concentration of 0.10, 0.25 or 0.50 mg/ml was mixed with 0.5 ml tyrosinase (0.1 mg/ml) in 1/15M Sorensen phosphate buffer (pH 6.8), and the mixture was incubated for 5 minutes at 37±1° C. Absorbance at 475 nm of the resulting solution was measured. The 50% inhibitory concentration (IC_{50}) was used as the concentration of the inhibitors to express 50% inhibition of tyrosinase activity. The IC_{50} for arbutin was found to be 7.48×10^{-3}M.

Melasma is a benign hyperpigmentary disorder affecting sun-exposed areas of the face and is most commonly encountered in darker-skinned women. Factors implicated include exposure to UV light, pregnancy, the use of oral contraceptives and racial predisposition (Sanchez, NP et al., 1981). In a clinical trial performed with 28 Japanese women with melasma, it was found that a 3% arbutin-containing skin lotion, milky lotion or cream applied twice daily for three months was effective in reducing melasma intensity and lesion size (good-to-excellent clinical response in 71.4% of the patients). A significant lightening of pigmented macules with a simultaneous reduction in lesional size was observed (Sugai, T et al, 1992).

Glabridin, from Glycyrrhiza glabra (Licorice) was shown to inhibit both melanogenesis and inflammation (Yokota, T et al, 1998). It was found to have tyrosinase inhibitory activity and demonstrated efficacy in reducing UV radiation induced inflammation in animal models. An in vitro study revealed that glabridin inhibits tyrosinase activity in cultured B16 murine melanoma cells at concentrations of 0.1 to 1 mcg/ml, with no detectable effect on their DNA synthesis. Glabridin was also found to inhibit the superoxide anion production and cyclooxygenase activities, in vitro.

In animal model studies, topical application of 0.5% glabridin was shown to inhibit UVB induced skin pigmentation and inflammation in guinea pig skin. Through testing synthesized derivatives of glabridin for anti-inflammatory activity, the authors of this study concluded that the hydroxyl groups in the molecule are important in the inhibition of melanin synthesis, more specifically, the hydroxyl group in the 4’ position (Yokota, T et al, 1998, Briganti S et al, 2003).



Glabridin and its derivatives

1. Glabridin	R1=R2=H
2. 2'-O-Methylglabridin	R1=Me R2=H
3. 4'-O-Methylglabridin	R1=H R2=Me
4. 2',4'-O-Methylglabridin	R1=R2=Me

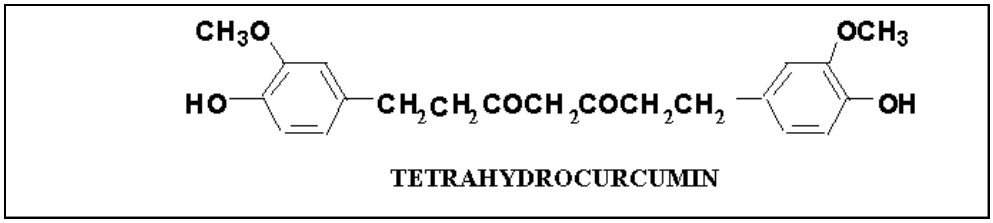
Kojic acid is reported to inactivate tyrosinase by chelating the copper ions that are essential for its activity and suppressing the conversion of dopachrome to 5,6-dihydroxyindole-2-carboxylic acid in the melanin biosynthesis pathway.

A 14-month clinical trial revealed that a cream containing 1% kojic acid produced significant improvement in various types of pigmentary disorders, including chloasma, *Lentigo senilis* and postinflammatory pigmentation, with over 75% of the female patients tested showing marked to moderate improvement in chloasma and postinflammatory pigmentation, and 63% showing marked to moderate improvement in *Lentigo senilis* (Lee, O-S, et al., 1995; Masuda, M et al, 1996).

TETRAHYDROCURCUMIN: MULTIFUNCTIONAL NATURAL SKIN LIGHTENING/BRIGHTENING

When natural yellow curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin) from *Curcuma longa* (Turmeric) roots are hydrogenated a color free mixture of Tetrahydrocurcuminoids is obtained. This natural blend is valued as a topical antioxidant and antinflammatory agent, with superior free radical scavenging and lipid peroxidation inhibition efficacy as compared to vitamin E. Studies indicate that Tetrahydrocurcuminoids[*], particularly ultrapure Tetrahydrocurcumin (trademarked SabiWhite®)[†] efficiently inhibit tyrosinase. The parent compound curcumin Curcumin is a potent inhibitor of protein kinase C, EGF-receptor tyrosine kinase and IkappaB kinase (Li JK, et al., 2001).

Laboratory studies revealed that SabiWhite® is an effective skin lightening agent with multifunctional topical benefits. The extract is safe for topical use with no irritant or sensitization side effects. (Research Reports, 2000, 2002, 2003). Ingested curcumin is metabolized into tetrahydrocurcumin *in vivo*. Thus tetrahydrocurcumin is a natural biotransformation product of curcumin (Pan, MH et al, 1999).



TETRAHYDROCURCUMIN

Multifunctionality:

1. Antioxidant action:

SabiWhite® offers effective topical antioxidant protection. Its antioxidant action is of a comprehensive "bioprotectant" nature, efficiently preventing the formation of free radicals, while quenching pre-formed ones as well. This dual action protects the skin cells from damage by UV radiation and the resultant

[*] **US Patent #6,653,327, EP1171144 and International Patents**
[†] **A trademark of Sabinsa Corporation, patents pending**

inflammation and injury with far reaching beneficial effects on overall health and well being. The free radical scavenging activity of SabiWhite® was found to be superior to that of the synthetic vitamin E analog, Trolox (Research report, 1998).

Curcuma longa (turmeric) Roots

Curcuminoids are reported to protect normal human keratinocytes from hypoxanthine/ xanthine oxidase injury in in-vitro studies. This study suggests that curcuminoids and therefore SabiWhite® offer protection to the skin and could be included in as functional antioxidants in topical preparations (Bont'e F et al., 1997).

In vitro data reveal that SabiWhite® efficiently scavenges free radicals (Research Report, 2007):

1. **Reactive oxygen species scavenging in Swiss 3T3 fibroblast cell line** –
   IC_{50} is 1.44μg/ml

2. **Oxygen Radical Absorbance Capacity (ORAC)** –
   10,815 μ Mol trolox equivalents/gram.

3. **Hydroxyl Radical Averting capacity (HORAC)** –
   3,152 μ Mol gallic acid equivalents/gram.

4. **Lipid peroxidation inhibition** –
   IC_{50} is 13.7 μg/ml

5. **DPPH scavenging**
   IC_{50} is 0.93 μg/ml

2. Luminosity Booster and Powerful Tyrosinase Inhibitor

In vitro studies indicate that SabiWhite® efficiently inhibits tyrosinase, the rate limiting enzyme in the synthesis of melanin. Its efficacy is superior to that of commonly used natural skin lightening agents such as kojic acid, and of related compounds (Table 1, Figure 4).

Table 1:Tyrosinase activity of single entity Tetrahydrocurcumin and analogs.

Compounds	Inhibitory concent. IC_{50}(mcg/ml)	(as % of THC)
Tetrahydrocurcumin (THC)	0.000492	100
Tetrahydrocurcuminoids	0.0493	0.99
Curcuminoids	0.102	0.482
Tetrahydro-demethoxycurcumin	0.0693	0.709
Tetrahydro-bisdemethoxy curcumin	0.2386	0.206
Curcumin	0.730	0.067
De-methoxycurcumin	1.196	0.041
Bis-demethoxycurcumin	0.93	0.052
Glabridin 40%	0.045	1.09
Kojic acid	9.14	0.0054
Vitamin C	12.2	0.0040

(Research Report, 2003)

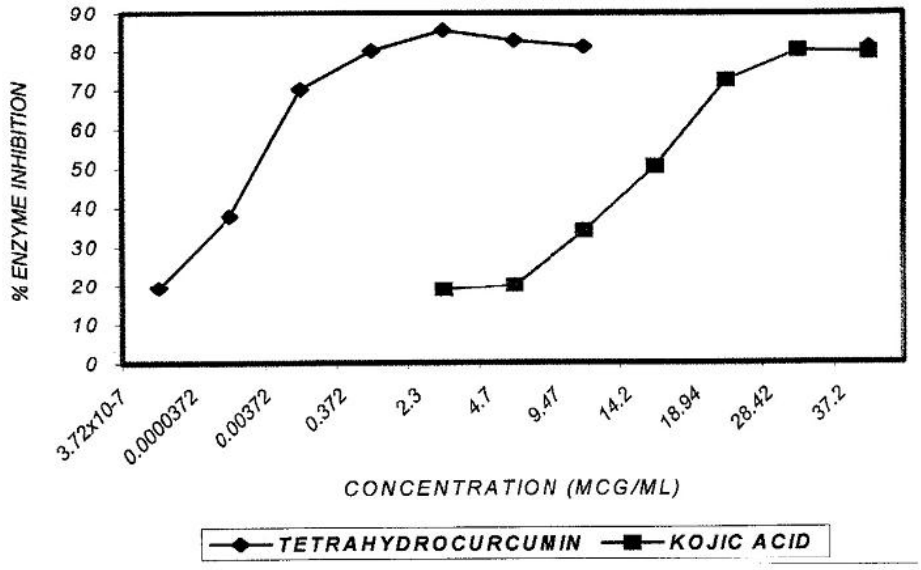


Figure 4
Comparative Luminosity Boosting Property of SabiWhite and Kojic Acid (Research Report, 2003)

3. Anti-inflammatory and UV protectant effects

Laboratory studies revealed that SabiWhite® offers topical protection against UVB induced inflammation and the resultant damage to the skin. These properties are particularly useful in antiaging, skin lightening, sun care and after sun care formulations.

Free radical chain reactions are implicated in most degenerative biological reactions. Free radicals on the surface of the skin, generated through exposure to ultraviolet radiation, chemicals or other environmental stress factors catalyze aging of the skin. SabiWhite® scavenges free radicals, and prevents their formation. The anti-inflammatory effect of SabiWhite® combined with the efficient antioxidant action is useful in anti-aging formulations and in topical formulations designed to maintain general skin health and integrity. The powerful tyrosinase inhibitory activity of SabiWhite® could also slow down melanogenesis, thereby lightening the skin tone. Use levels range from 0.05 to 2% w/w.

4. Formulating with SabiWhite®

SabiWhite® is color free, as reflected by absorbance in the visible range (400-800nm, Figure 5) and can be conveniently dispersed into cosmetic formulations.

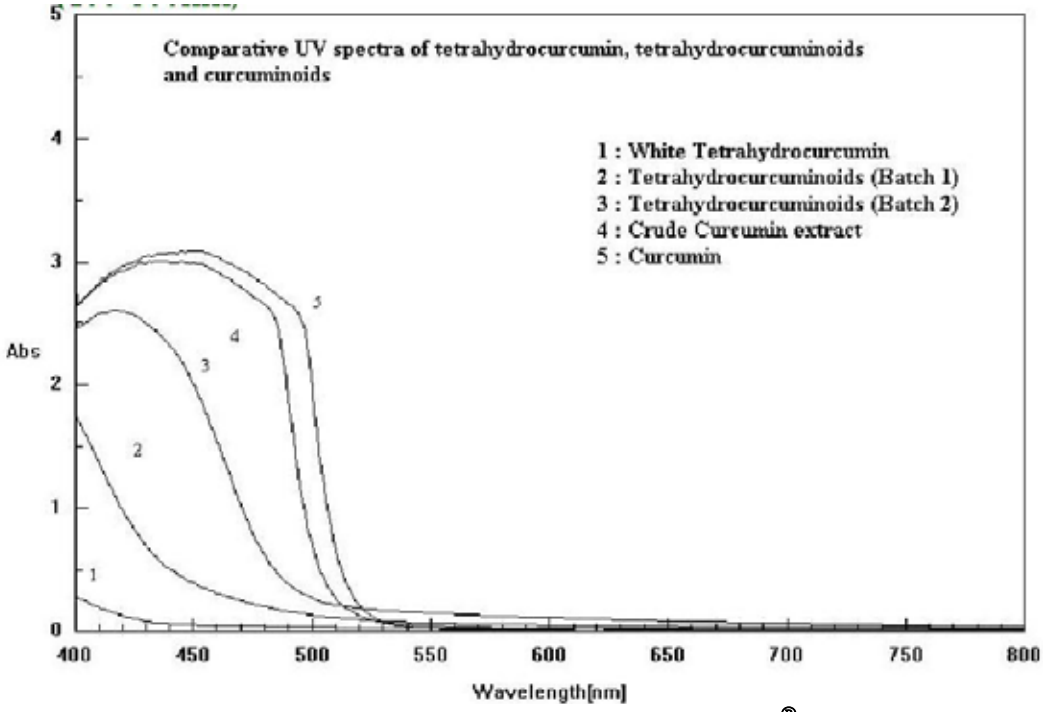


Figure 5: Comparative Absorbance of Curcuminoids and SabiWhite® in the Visible Spectral Range

Sample Formulation with SabiWhite® (Skin Lightening Cream)

	Ingredients	%w/w	Function
A	Isopropyl palmitate	4.0	Emollient
	Caprylic/Capric triglycerides	5.0	Emollient
	Glyceryl stearate	1.0	Emollient
	Cetearyl octanoate	3.0	Emollient
	Dioctyl adipate	1.0	Emollient
	Dimethicone	1.0	Slip aid
	Steraeth 21 (BRIJ 721)	1.0	Emulsifier
	Steareth 2 (BRIJ 72)	0.5	Emulsifier
B	Glycerin	3.0	Humectant
	Tetrasodium EDTA	0.02	Chelating agent
	Imidurea	0.15	Preservative
	Sodium methylparaben	0.2	Preservative
	Sodium propylparaben	0.02	Preservative
	Demineralized water	Qs	Diluent
	Citric acid(10%)	Qs	Acidulant
C	SabiWhite™*	0.25	Active
	Cosmoperine®*	0.05	Permeation enhancer
	Ethanol	2	Solvent
D	FLOCARE ET 30	5.0	Viscosity modifier

* Trademarks of Sabinsa Corporation

Combine Part A ingredients and heat it to 70-75° C. Combine Part B ingredients in a separate vessel and heat it to 70-75° C. Add part A to Part B with continuous agitation. When the temperature is 45°C add Part C. Add Part D and mix to form a homogenous mixture.

CONCLUSIONS

Tyrosinase inhibitors and other agents that affect the melanin biosynthesis pathway are widely distributed in plant materials. These natural ingredients offer safer alternatives to hydroquinone, for use in topical skin lightening compositions. Such actives would offer additional functionalities as sunscreen boosters, moisturizers, or “anti-aging” ingredients, thereby supporting skin health, and reducing the appearance of wrinkles. Contact Sabinsa Corporation for further information on innovative skin tone lightening natural actives.

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