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BOTANICAL STUDY OF SKIN LIGHTENING AGENTS

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ABSTRACT: Both physicians and dermatology patients are searching for long term skin care solutions to address problems presented by skin hyperpigmentation. Traditional depigmenting agents such as hydroquinone, corticosteroids, kojic acid, although highly effective, can raise several safety concerns (for example, ochronosis, atrophy, carcinogenesis, and other local and systemic side effects) with long term exposure. An understanding of the benefits of natural and botanical extracts provides opportunities to develop new products to address pigmentation problems. This study presents an overview of trends in the application of plant extracts for the treatments of hyperpigmentation disorders. It highlights some of the potent natural products, their specific components, mode of action and optimum doses.

INTRODUCTION: Hyperpigmentation disorders of the skin are common and can be the source of significant psychosocial distress for patients. The most common is the melasma. Melasma is a common acquired hyperpigmentary disorder that occurs mainly in women in their 30s or older (more than 90% of cases) of all racial and ethnic groups, but about 10% of cases do occur in men ¹. It particularly affects those with Fitzpatrick skin types IV-VI. Multiple etiological factors include thyroid dysfunction cosmetics, phototoxic and anti-seizure drugs, ovarian dysfunction, hepatic dysfunction, nutritional deficiency, endocrinopathies, emotional factors, anti-convulsive drugs, genetics, *etc.* have been implicated in melasma.

Melasma presents as brown to grey macules and patches, with serrated, irregular, and geographic borders. The pigmented patches are usually sharply demarcated and symmetrical ². Melasma has a predilection for sun-exposed areas. The three major patterns of distribution are centrofacial (cheeks, forehead, upper lip, nose, and chin) (66% of cases), malar (cheeks and nose) (20% of cases) and mandibular (rami of the mandible) (15% of cases). Based on Wood's examination of the skin, melasma can be classified into four major clinical types and patterns, with good correlation with histology (by the depth of melanin pigment) ³: epidermal, dermal, mixed and indeterminate.

Regardless of the various types and patterns, melasma is characterized by a slight increase in the number of melanocytes and an increase in their function. The treatment of melasma is one of the most challenging from a dermatologist. As it is a common condition, it is of broad interest for control. Hypothetically, the condition is self-limiting.

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However, spontaneous resolution is time-consuming and unpredictable, and it may take months to years to resolve normal pigmentation. The major problems in treating chloasma are the prolonged time to response, the inconsistency of response to treatments, and the unpredictability regarding the result after any procedure and the substantial relapse rate when the therapy is discontinued. Treating this dyschromia is also challenging due to the feared post-inflammatory hyperpigmentation after inflammation inducing therapies.

METHODS: Melasma is commonly resistant to all treatments, and is therefore very frustrating to the patient and clinician. Topical therapy can yield

some improvement but rarely does it cure this condition permanently. This study is an endeavor to pile up all the traditional trends for the treatment of melasma including remedies used, type of formulation used by vaishyas and tribal people, active components of the plants, their role in the treatment of melasma and amount to be given as therapeutic against melasma. All the plants are collected and kept in herbarium for further studies. The herbarium is authenticated from the botanist of Central Ayurvedic Research Institute, Gwalior, Madhya Pradesh.

RESULTS: The results are concluded in **Table 1** as:

TABLE 1: LIST OF SOME OF THE POTENT NATURAL PRODUCTS, THEIR SPECIFIC COMPONENTS, MODE OF ACTION AND OPTIMUM DOSES

Common Name	Botanical name	Family	Parts	Components	Depigmenting Mechanism	Amount
Aloe	<i>Aloe barbedensis</i>	Liliaceae	Leaves	Aloesin	↓Tyrosinase competitively, ↓DOPA polymerase	125 -500 mg/kg
Apricot	<i>Prunus armeniaca</i>	Rosaceae	Seed	3,4-Dihydroxy benzoic acid, quercetin	Antioxidant activity	-
Banyan	<i>Ficus benghalensis</i>	Moraceae	Bark, fruits	Flavonols, triterpene,	Free radical scavenger	3-5 gm / kg
Barberry	<i>Berberis aristata</i>	Barberideae	Rhizome	Berberine	Tyrosinase inhibitor	13-25 centigrams
Bearberry	<i>Arctostaphylos uva-ursi</i>	Ericaceae	Leaf extract	Polyphenolic compounds, arbutin	↓ Tyrosinase, ↓ DHICA polymerase	100 mg / kg
Bilberry	<i>Vaccinium cyanoococcus</i>	Ericaceae	Leaf, fruits	Arbutin, anthocyanin, flavonols	↓ Tyrosinase, ↓ DHICA polymerase	100 mg / kg
Blueberry	<i>Vaccinium myrtillus</i>	Ericaceae	Fruits	Anthocyanoside, tannins, hydroxyl benzoic acids, flavonol glycosides	Antioxidative effects	4-8 gm
Bitter orange	<i>Citrus aurantium</i>	Rutaceae	Peel	Polymethoxy flavonoids	Antioxidant activity	1 ml
Castor	<i>Ricinus communis</i>	Euphorbiaceae	Seed oil, leaves	Ricinoleic acid	Free radical scavenger	5-20 ml
Catechu	<i>Acacia catechu</i>	Fabaceae (Mimosaceae)	Heartwood leaves	Phenolic compounds, flavonoids	Antioxidant activity	20-30 gm
Chamomile	<i>Matricaria camomilla</i>	Asteraceae	Flower, Oil	Flavonoids, Luteolin	Free radical scavenger	2-3 gm
Comfrey	<i>Symphytum officinale</i>	Boraginaceae	roots	Allantoin, poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene	Free radical scavenger	Topical
Cucumber	<i>Cucumis</i>	Cucurbitaceae	Seed, Fruit	Rutin, ascorbic	Free radical	3-6 gm,

	<i>sativus</i>		juice	acid oxidase, cucurbit-aside	scavenger	25-50 ml
Cumin	<i>Cuminum cyminum</i>	Apiaceae	Seed	The flavonoid, <i>p</i> -cymene cuminaldehyde.	Free radical scavenger	1-3 g powder.
Daisy	<i>Bellis perennials</i>	Asteraceae	Seed Extract	Anthocyanin	Antioxidant activity	100 µg /ml
Evening primrose	<i>Oenothera biennis</i>	Onagraceae	Seed oil	γ – Linolenic acid	↓mRNA level of Tyr. Related Protein 1 & 2.	4-8 gm
Flame of the forest	<i>Butea monosperma</i>	Fabaceae	Flower, fruits	Flavonoids, steroids	Free radical scavenger	100-400 mg/kg
Geranium	<i>Geranium nepalese</i>	Geraniaceae	Leaves, oil	Geraniin, kaempferol, flavonoid	antioxidative effects, inhibitory effects on elastase and tyrosinase	15 µg/ml
Gooseberry	<i>Phyllanthus emblica</i>	Euphorbiaceae	Extract, fruit	Vitamin C, Superoxide dismutase	↓Tyrosinase, the antioxidant of collagen	10-20 gm
Grapes	<i>Vitis vinifera</i>	Vitaceae	Fruits, Seeds	Flavonoids, Tannin	Tyrosinase inhibitor	5-10 gm
Greenleaf manjanita	<i>Aractostaphylos patula</i>	Ericaceae	Leaves	Polyphenolic compounds	Tyrosinase inhibitor, exhibit Superoxide dismutase	-
Horse-radish	<i>Armoracia lapathifolia</i>	Brassicaceae	Roots	Phenolic compounds	Free radical scavenger	3-5 gm
Indian ginseng	<i>Panax ginseng</i>	Araliaceae	Roots	<i>p</i> -Coumaric acid	↓ L-Tyrosinase oxidation	200-500 mg/kg
Indian Sarsaparilla	<i>Hemidesmus indicus</i>	Asclepiadaceae	Roots, Bark	Coumarin-lignoids, hemidesmine	Free radical scavenger	20-30 gm
Kuhseng	<i>Sopohora flavescens</i>	Fabaceae	Fruits	Sophocarpine	↓ Tyrosinase activity	-
Lemon	<i>Citrus limon linn</i>	Rutaceae	Peel	Hesperidin, Ascorbic Acid	↓Tyrosinase, the antioxidant of collagen	5-10 ml./kg
Lodh tree	<i>Symplocos racemosa</i>	Symplocaceae	Bark	Alkaloids (lot urine, loturidine,)	Antioxidant activity, lipid peroxidation inhibition	3-5 gm/kg
Lotus	<i>Nelumbo nucifera</i>	Nelum-bonaceae	Leaf, seed, Rhizome	Luteolin/luteolin -7-glucoside, flavonoids	Free radical scavenger	10-20 ml
Maidenhair tree	<i>Ginkgo biloba</i>	Ginkgoaceae	Leaves, root bark	Flavonoid glycosides, terpenoids	Neutralize free radicals	200 mg per day
Mangostin	<i>Garcinia mangostana</i>	Guttiferae	Peri carp	γ – mangostin, tannin	Free radical scavenger	10-60 grains
Manjishtha	<i>Rubia cordiolia</i>	Rubiaceae	Root	Rubiadin	Free radical scavenger	200-400 mg
Marigold	<i>Calundula officinalis</i>	Asteraceae	Flower	Coumarins, flavonoids	Free radical scavenger	0.20 µg/ml
Milfoil	<i>Achillea millefolllum</i>	Asteraceae	Extract, oil	Eucalyptol, camphor, α terpineol, β-pinene, borneol	Free radical scavenger, effects on lipid peroxidation	0.25-7.5 mg/ml

Mulberry	<i>Morus alba</i>	Moraceae	Leaves	Flavonoids, mulberroside F	Tyrosinase inhibitor, a Superoxide scavenger	5-10 gm
Mulethi/ Licorice	<i>Glycyrrhiza glabra</i>	Fabaceae	Roots	Glabridin, glycyrrhizin	Free radical scavenger	2-4 gm/kg
Onion	<i>Allium cepa</i>	Liliaceae	Bulbs	Quercetin	Antioxidant activity	10-20 ml
Papaya	<i>Carica papaya</i>	Caricaceae	Latex	Papain, chymopapain, carpaine, pseudocarpaine	Free radical scavenger	40-50 ml
Peanut	<i>Arachis hypogaea</i>	Fabaceae	Seed	Vit E, p-coumaric acid, flavonoids	Antioxidant activity	-
Pear	<i>Pyrus communis</i>	Rosaceae	Leaves	Arbutin	↓ Tyrosinase, ↓DHICA polymerase	100 mg per kg
Saffron	<i>Crocus sativus</i>	Iridaceae	Stigma	Carotenoids, Crocetin	Free radical scavenger	50-100 mg
Sandal	<i>Santalum album</i>	Santalaceae	Heartwood powder, Oil	Alpha-and beta-santalol.	↓ DNA damage, ↓ Tyrosinase	Sandal
Siris , benth	<i>Albizia lebback</i>	Fabaceae	Bark, leaves	Flavonoids, saponins	Free radical scavenger	3-6 ml/day
Soybean	<i>Glycine max</i>	Fabaceae	Whole plant	Vitamin C, B, soy protein, Isoflavone	Inhibit protease-activated receptor 2 pathway	-
Strawberry	<i>Physalis alkekengi</i>	Solanaceae	Fruits	Flavonoids, luteolin-7-glucoside and asteroids	Free radical scavenger, lipid peroxidation inhibiton	-
Sunflower	<i>Helianthus annuus</i>	Asteraceae	Seed oil	Vitamin E, helianol	Anti-inflammatory	30 µg/ml
Sweet Flag	<i>Acorus calamus</i>	Acoraceae	Rhizome, oil	Asarone	Antioxidant activity	400 mg/kg
Sweet Marjoram.	<i>Origanum majorana</i>	Labiatae	Oil	Hydroxyquinone , flavonoid	Superoxide anion radical scavenger	1.44 µg/ml
Turmeric	<i>Curcuma longa</i>	zingiberaceae	Rhizome	Curcumin	Free radical scavenger	1-3 gm
Watercress	<i>Enhydra fluctuans</i>	Compositae	Leaf	Beta carotene	Antioxidant activity	3.6-4.2 mg/100g

In the present study, we conclude that about 49 plants of 31 genera have potential activity against melasma. The plants are listed by their botanical names, recommended doses, depigmenting mechanism, chemical constituents, and their other functions also. These plants are used in the form of therapeutics and topical, on the affected skin. The parts of the plant most used for medicinal purposes are leaves, root, stem, fruits, the complete aerial parts, the whole plant, barks (root and stem) and flowers (including the flowering heads) in decreasing order. Juice (almost mix with water and goat's or cow's milk) and paste are the main

methods of preparation, either for oral or for external administration. For topical use, the most important methods used are a direct application of the paste or ointment (with oil). The Ayurvedic clinicians medicate these remedies because Ayurveda is the ancient medicinal custom of India. Ayurveda is a good supplement of regional medicinal values. Tribes are using these remedies very frequently, and 76% of patients even concern the allopathic clinicians.

DISCUSSION: In the search for novel depigmenting agents, the investigation of natural

plant extracts has led to the identification of many potentially active compounds. Many plant extracts are potent inhibitors of melanin formation and not associated with cytotoxicity or mutagenicity of melanocytes⁴. These plants work against melasma because they have multiple chemical components like arbutin, aloesin, flavonoids, niacinamide, vitamins, etc. which inhibit the melanin formation.

Arbutin: Arbutin, a naturally occurring β -D-glucopyranoside derivative of hydroquinone, exists in the dried leaves of certain plant species, such as bearberry. The mode of action appears to be by inhibition of melanosomal tyrosinase and DHICA (5, 6- dihydroxyindole-2-carboxylic acid) polymerase activities at noncytotoxic concentrations rather than by suppression of the synthesis and expression of this enzyme^{5, 6}. Studies have shown that α -arbutin (4-hydroxyphenyl α -glucopyranoside) demonstrates an even stronger inhibitory effect on human tyrosinase activity than arbutin itself. Deoxyarbutin (dA, 4-[tetrahydrofuran-2-yl-oxy]-phenol) has also demonstrated effective inhibition of mushroom tyrosinase *in-vitro*⁷.

Aloesin: Aloesin, a compound isolated from the aloe plant, has been proven to competitively inhibit tyrosinase from human, mushroom, and murine sources. Studies have shown that tyrosine hydroxylase and DOPA (3, 4-dihydroxy phenylalanine) oxidase activities (of tyrosinase from normal human melanocyte cell lysates) are inhibited by aloesin in a dose-dependent manner². The topical application of aloesin on UV-irradiated (210 mJ) human volar forearm (four times a day for 15 days) showed pigmentation suppression in a dose-dependent manner³. Aloesin, along with arbutin, was observed to synergistically inhibit melanin production by combined mechanisms of noncompetitive and competitive inhibitions of tyrosinase activity⁸.

Flavonoids: Flavonoids are polyphenolic compounds that are ubiquitous and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins, and chalcones. The effects of many flavonoids on the oxidation of L-DOPA have been studied. Isoflavones, including glycitein, daidzein, and genistein, showed little antityrosinase activity, but 6, 7, 4'-trihydroxyisoflavone has been

identified as a potent tyrosinase inhibitor stronger than kojic acid. Flavanones, such as hesperidin, eriodictyol, and naringenin, have a structure that is similar to that of hydroquinone⁹. Dr. Buhler and Miranda reported that flavonoids might be potentially useful in the prevention of human diseases attributed to free radical damage. The observation that prenyl groups are important in conferring antioxidant activity to certain flavonoids may lead to the discovery or synthesis of novel prenylated flavonoids as preventive or therapeutic agents against human diseases associated with free radicals.

Hesperidin: Hesperidin is a bioflavonoid existing extensively in the peel and membranes of citrus fruits. Studies by Zhu and colleagues have demonstrated hesperidin's potent ability to inhibit melanin synthesis without cytotoxicity. Also, hesperidin was found to protect against UVA-induced damage of fibroblasts and oxidative damage of collagen¹⁰. Thus, hesperidin offers potential skin-lightening benefits, including improved overall skin tone and antiyellowing effects.

Niacinamide: 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). A large number of cellular enzyme reactions in which these cofactors participate may be the basis for the variety of cosmetic benefits, including barrier enhancement observed from the topical use of niacinamide¹¹.

Using cocultures of human melanocytes and keratinocytes, investigators have shown that niacinamide inhibits the transfer of melanosomes from melanocytes to keratinocytes. Results of clinical studies using topically applied niacinamide have demonstrated a reversible reduction in hyperpigmented lesions and increased skin lightness compared with vehicle alone after 4 weeks of use. In a separate clinical study, topical niacinamide was also shown to decrease collagen oxidation products and improve aging-induced yellowing or sallowness¹².

Glabridin: Glabridin, the main ingredient in the hydrophobic fraction of licorice extract, inhibits tyrosinase activity, without affecting DNA synthesis. Other active compounds, such as glabrene, isoliquiritigenin licuraside, isoliquiritin, and licochalcone A, isolated from licorice extracts, were also shown to inhibit tyrosinase activity^{13, 14}. Liquiritin does not affect tyrosinase; however, it causes depigmentation by other mechanisms, and studies demonstrate that a 20% liquiritin cream applied at 1 g /day for 4 weeks is therapeutically effective in melasma¹⁵.

Polyphenols: Polyphenols are a class of compound that has the antioxidant capacity and are found widely within plants. The inhibition of melanogenesis has been observed with many types of polyphenol plant extracts. Proanthocyanidins or procyanidins, classified as polyphenols, exist in red wine and cranberry juice; grape seeds are another especially rich source. The antioxidative activities of proanthocyanidins were found to be much stronger than the activity of vitamin C or E in aqueous systems. Ellagic acid is another natural polyphenol that is widely found in fruits and vegetables. The extract of the rinds of pomegranate contained 90% ellagic acid and showed inhibitory activity. The mechanism of action may be inhibition of the proliferation of melanocytes and melanin synthesis by tyrosinase in melanocytes¹⁶.

P-Coumaric Acid: P-coumaric acid, extracted from the fresh leaves of Panax ginseng, was shown to inhibit the oxidation of L-tyrosine more strongly than the inhibition of tyrosinase demonstrated by L-DOPA¹⁷. Treatment with ginseng in the presence of various concentrations of Radix trichosanthin suppressed tyrosinase activity and melanin content but increased cell proliferation slightly in melanoma cells, raising the possibility that this combination may be effective as a skin-lightening agent¹⁸.

CONCLUSION: Melasma poses a substantial emotional and psychosocial burden on patients. Many undergo multiple therapies, from cosmetic treatments to ineffective or even aggressive medical treatments that do not solve their problem or even make it worse. Some patients spend a fortune on treatments over the years. Others hide away, feeling ashamed and stigmatized. Even

though melasma is a benign and easily diagnosed disease, clinicians must rule out melanoma and its precursors and must be able to distinguish and diagnose skin manifestations of systemic diseases.

During the past decades, thousands of plant extracts have been screened, and hundreds of compounds were identified as potential skin-lightening ingredients. It is clear that natural sources and extracts represent a repository of ingredients that can be used in topical treatments to achieve improvement of hyperpigmentation and the overall appearance of skin. These ingredients may also provide additional potential for protective cosmeceutical use, through antioxidant efficacy and protection of macromolecules, such as collagen from UV irradiation. With natural sources offering a multitude of different extracts and isolated compounds, it is apparent that we are only beginning to realize the potential of natural extracts for skin lightening applications.

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