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FORMULATION AND EVALUATION OF THE HERBAL SKIN TONER FOR DEPIGMENTATION

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ABSTRACT
The present research focuses on the formulation and evaluation of a herbal skin toner designed to reduce skin hyperpigmentation using natural, plant-based ingredients. The formulation includes extracts of Licorice (Glycyrrhiza glabra), Orange peel (Citrus sinensis), Turmeric (Curcuma longa), Green tea (Camellia sinensis), along with Aloe vera gel, Rose water, Glycerine, and Sodium benzoate as a preservative. These ingredients are known for their antioxidant, anti-inflammatory, and tyrosinase-inhibitory properties, which contribute to skin brightening and depigmentation. The prepared toner was evaluated for its organoleptic properties, pH, viscosity, stability, antioxidant activity (DPPH assay), and anti-tyrosinase activity, and was compared against a standard marketed formulation. The results demonstrated that the herbal toner showed significant potential in reducing pigmentation and improving skin tone, with favorable physicochemical stability and user acceptability. The study concludes that herbal ingredients can be effectively used in dermatological formulations to manage skin pigmentation disorders in a safe and natural manner.

KEY WORDS: *Herbal toner, Hyperpigmentation, Depigmentation, Antioxidant, Anti-tyrosinase*

INTRODUCTION
People have been enhancing their attractiveness with naturally occurring materials since ancient times. Cosmetics are known to be things that improve and provide beauty to their users. Natural components were typically employed as cosmetics in the past, but as time and science advanced, a number of chemicals that are thought to impart or enhance beauty emerged and were subsequently used as cosmetics. For a short while, using these chemical-based treatments can make us look more beautiful, but over time, they damage our skin. Since using chemical-based products has been shown to have numerous negative effects, the cosmetics business now primarily concentrates on making herbal goods. The prepared face toner is entirely chemical-free and will soothe the skin while shielding it from sunburn [1].

Herbal Cosmetics
Herbal cosmetics can also be called natural cosmetics. The lack of side effects has led to the growing popularity of herbal remedies. At the beginning of civilization, humanity made the alluring leap to use their appearance to impress others. Cosmetic procedures and pricey fairness creams were nonexistent at the time. They started with only natural knowledge acquired from the Ayurvedic method. The practice of Ayurveda involved the use of a few plants and herbs to make potent cosmetics. The use of Ayurvedic cosmetics shielded the body from external factors while also improving skin attractiveness. In the modern world, ayurvedic cosmetics, also referred to as herbal cosmetics, include the same amazing ingredients. Many traditional remedies make use of organic matter, medicinal herbs, and minerals. Many different formulations of herbal cosmetics are available, and they are often used. In addition to face wash, shampoo, and conditioner, herbal cosmetics including soaps and conditioners are immensely popular. The fact that herbal cosmetics are made only from herbs and shrubs is their best feature. Herbs' natural ingredients don't affect the body; instead, they enhance it with vitamins and other healthy minerals[1].

Advantages of Herbal Cosmetics
1. **Natural Products:** Natural and devoid of any dangerous synthetic chemicals that might potentially be fatal to the skin, herbal cosmetics are safe for the skin.
2. **Safe to use:** Natural cosmetics can be used without risk. Dermatologists have evaluated and validated their hypoallergenic properties, making them safe to use anywhere, at any time. There is no need for folks to be concerned about developing skin rashes or itching because they are composed of natural substances.
3. **No side effects:** Synthetic cosmetics have the potential to aggravate your skin and result in acne. They could cause your skin to become dry or greasy by obstructing your pores. One does not have to worry about them when using natural cosmetics. Because the natural ingredients are safe, they can be applied anywhere, at any time.
4. **Wide choice of selection:** Compared to synthetic items, these are less expensive. They are sold during sales for a low price and are provided at reasonable costs. Due to the negative consequences of modern medicine and its increasing costs, the World Health Organization estimates that 80% of the world's population relies on natural goods for their medical needs.

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5.Cosmeceuticals: The beauty industry's fastest-growing sector is cosmetics. Cosmeceuticals are pharmaceutical and cosmetic products designed to enhance the skin's health and appearance by delivering a particular outcome, such as sun protection, anti-aging, or acne treatment.[1]

Information Of Toner

The term "skin toner," or just "toner," describes a lotion or wash used on the face that is intended to cleanse the skin and minimize the appearance of pores. Moreover, it protects, hydrates, and revitalizes skin.

Types of toners

1. Skin bracers or fresheners
2. Acid toner
3. Skin tonics
4. Astringents

Toner's effect on skin

To prepare the skin for nourishing treatments, skin toner was once a common product used as a second washing agent to remove makeup residue after routine facial cleansing or to remove excess sebum secreted from the face. Toners for different skin types, such as oily, sensitive, or combination skin, can be classified as either alcohol-based or non-alcohol-based. These days, skin toners are used more often as cosmeceutical products for a variety of reasons due to the products' diversity and popularity. Skin hydration, pH balance, pore tightening, irritation relief, and antisepsis are a few examples.[2]

Skin Pigmentation

A very small group of cells called melanocytes, which are largely responsible for the production and distribution of the pigmented biopolymer melanin, are responsible for the visible pigmentation of the skin, hair, and eyes.

Melanocytes are precursor cells (also known as Melano blasts) that produce melanin throughout embryonic development and are present in human skin, hair follicles, eyes, inner ears, bones, heart, and brain. A complex web of interconnected regulatory pathways causes them to differentiate once they are born from pluripotent neural crest cells. Melanocytes produce the pigment molecules known as melanin's on their own. Melanin in skin and hair absorbs light, resulting in display coloring, camouflage, thermoregulation, photoprotection, and photoreceptor shielding. Additionally, melanin's are strong cation chelators and may have anti-free radical properties [3].

There are three main types of melanin:

1. Eumelanin: There are two types of this most prevalent form of melanin.Brown eumelanin produces lighter brown skin tones, whereas black eumelanin leads to deeper skin tones.
2. Pheomelanin: This kind of melanin gives things a reddish-yellow hue. Red and blond hair hues are also caused by it, and it is more prevalent in people with lighter complexion.
3. Neuromelanin: Found in the brain, these types of melanin doesn't contribute to skin pigmentation but is important in neurological function.

Hyperpigmentation

A prevalent dermatological condition known as hyperpigmentation causes the skin's color to generally darken. Numerous internal and external factors may contribute to these variations in skin tone. outside variables such as injury, inflammation, and hormone fluctuations, Eczema, acne, some medications, exposure to UV light, etc. The biological mechanisms that produce the skin pigment known as melanin, which is made by melanocytes in different layers of the skin, control skin pigmentation and coloration. changes in melanocyte distribution or synthesis in the skin of melanin causes problems of skin hyperpigmentation [3].

The term "hyperpigmentation" refers to a broad range of pigmentation, darkening, and skin discoloration diseases. Several hyperpigmentation conditions that are frequently observed include lentigines, ephelides, melasma, post-inflammatory hyperpigmentation, and numerous others.

Melasma is a skin disorder known as acquired hyper melanosis, wherein sun-exposed areas of the skin develop uneven patches of light to dark brown or gray-brown lesions[4]

.

A condition known as skin hyperpigmentation occurs when certain areas of skin turn darker than the surrounding skin. This happens when particular areas of the skin produce too much melanin. The process known as melanogenesis produces melanin, a crucial pigment in skin hyperpigmentation. Melanosis is the term for an increase in the melanin pigment in epithelial cells.

Dermal melanosis happens when melanin is present in the dermis between collagen bundles, while epidermal melanosis occurs when melanocytes are normal but melanin levels are elevated in hyperpigmented skin[5]. Melanocyte cells one melanocyte is encircled by roughly 36 keratinocytes produce two types of melanin pigment, pheomelanin (yellow-reddish) and eumelanin (black or brown), which give humans their skin, hair, and eyes their color. Age spot, liver spot, and melasma post-inflammatory hyperpigmentation are the three primary types of skin hyperpigmentation[6-7]. Hormonal imbalance, vitamin B12, Addison's disease, and sun exposure all contribute to skin hyperpigmentation[8-9].

Mutagen-activated protein kinase is one of the intracellular signaling pathways that are activated by reactive oxygen species (ROS) produced by UV radiation in skin cells. UV-B exposure increases the activity of p38 mitogen-activated protein kinase (MPAK), which generates pro-inflammatory cytokines such 1L-1, cyclooxygenase (cox-2), and TNF-α production in human keratinocytes [10]. Tyrosinase and dopa chrome tautomerize are the two enzymes that produce melanin. The primary enzyme involved in the formation of melanin is tyrosinase, and excessive tyrosinase activity results in hyperpigmentation[11]. The amino acid tyrosine is involved in tyrosinase, which hydroxylates it to

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L- 3,4-DOPA, which oxidizes to DOPA-quinine, which is then further oxidized by a free radical-coupling pathway to produce melanin. Dopachrome is transformed into 5,6-dihydroxyindole-2-carboxylic acid (DHICA) by the additional enzyme dopachrome tautomerize [12].

Type of Skin Hyperpigmentation:

1. Post-inflammatory hyperpigmentation:

Post-inflammatory hyperpigmentation (PIH) refers to darkened skin that develops after an inflammatory skin condition or injury, such as acne, eczema, or wound. When the skin heals from these issues, it can leave behind discolored spots or patches, typically darker than he surrounding skin. This occurs due to an overproduction of melanin.



Figure 1. Post-inflammatory hyperpigmentation

2. Melasma

Melasma is common skin condition characterized by dark, irregular patches of pigmentation that typically appear on the face. It is often found on areas that are regularly exposed to the sun, such as the cheeks, forehead, upper lip. And chin. The condition is more common in women, especially during hormonal changes such as pregnancy (often referred to as the "mask of pregnancy"), birth control use, or hormone replacement therapy [13].



Figure 2. Melasma

3. Age spot

Age spots, also known as liver spots or solar lentigines, are flat, dark spots that typically appear on areas of the skin that have been frequently exposed to the sun over time. They are common as people age, especially in those over the age of 50, although they can develop in younger people ass well, particularly if they have had a lot of sun exposure throughout their lives [14].



Figure 3. Age spot

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Causes of Hyperpigmentation

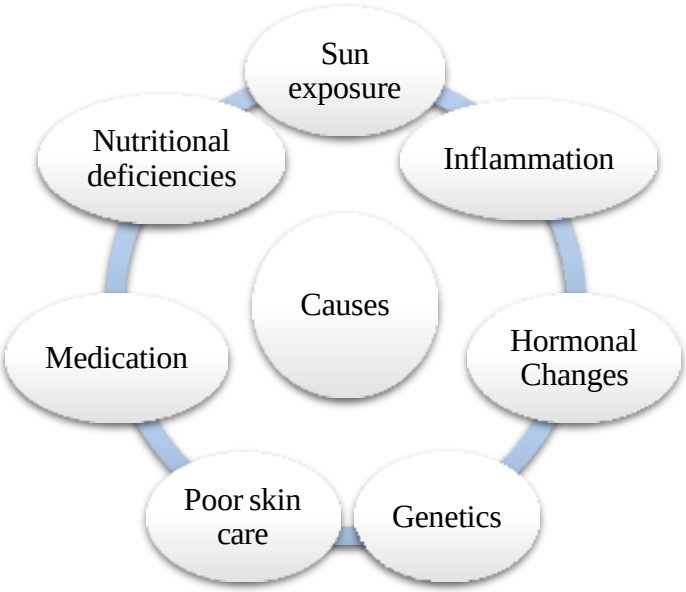


Figure 4. Causes of Hyperpigmentation

Depigmentation

The process by which the skin completely loses its color and turns white is known as depigmentation. Stated differently, it inhibits the production of melanin, which causes the skin to turn dark to fair in tone.

In order to combat skin pigmentation, we created a herbal toner with components that decrease pigmentation and make skin more radiant. Herbs and phytoconstituents are a better option for treating skin hyperpigmentation because, in addition to photo-safety, there are a number of medications and treatments that can safely and effectively treat the hyperpigmentation of darker skin patients' skin, albeit with some side effects. Some herbs with their mechanism of action for treating skin hyperpigmentation. Example Liquorice extract. Herbs may be used to treat skin hyperpigmentation through tyrosinase inhibitory, antioxidant, and skin-whitening properties, among other putative modes of action [15].

PLANT AND EXCIPIENT PROFILE

1. Licorice

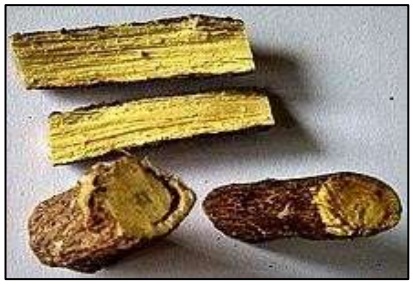


Figure 5. Licorice

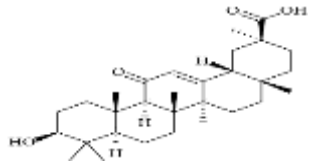
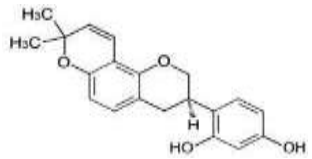
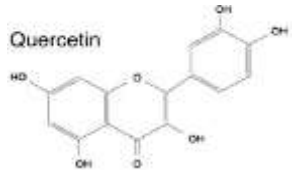
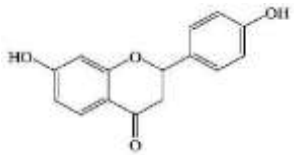
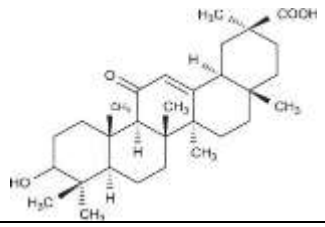
- **Biological Name:** Glycyrrhiza glabra
- **Kingdom:** Plantae
- **Order:** Fabales
- **Family:** Leguminosae
- **Subfamily:** Faboideae
- **Genus:** Glycyrrhiza
- **Species:** G.glabra
- **Biological source:** It consists of peeled and unpeeled roots of Glycyrrhiza glabra linn.
- **Distribution:** Sub-tropical and warm temperature, cultivated in many parts of India example like, Punjab, Jammu, and Kashmir and South India.
- **Major chemical compounds:** Glycyrrhizin, Glycyrrhizic acid, Glabranins, Liquiritic.

Macroscopical Characterization

- **Colour:** Externally, yellowish brown or dark brown ; and Internally, yellowish colour.
- **Odour:** Faint characteristics
- **Taste:** Sweet
- **Size:** Length is 20- 50 cm ; Diameter is 2cm.
- **Shape:** Straight and nearly cylindrical [1

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Table No. 1. Chemical Constituents of Licorice:

Compound	Structure
Glycyrrhetinic acid	
Glabridin	
Quercetin	
Liquiritigenin	
Glycyrrhizin	

Mechanism of Action of Licorice Against Hyperpigmentation

The sweet ingredient in licorice root, glycyrrhizin, is a triterpene-type saponin with antiviral, anti- inflammatory, anticancer, and antibacterial qualities. G. glabra has long been used to aid the healing of wounds. Skin protection is provided by licorice root extracts. Skin-whitening agents and oxidative stress and injuries [17-18].

Anti-Tyrosinase Activity and Hyperpigmentation:

Glycyrrhiza extracts have been found to have positive effects on skin pigmentation, which is caused by melanin production in melanocyte cells. Factors such as melanocyte size, genetic predisposition, UV light exposure, and certain illnesses like vitiligo and albinism affect melanin production and expression. High melanin levels can lead to hyperpigmentation, hypopigmentation, and unusual skin- colored patches. Tyrosinase, a copper-dependent enzyme, is responsible for melanin synthesis, which is a key target for skin-whitening products and treatments related to melanin hyperpigmentation. Flavonoids, naturally occurring phenolic substances, have been shown to inhibit tyrosinase, making them useful for treating skin pigmentation. Glycyrrhiza glabra, a species of Glycyrrhiza, has been studied for its potent anti-melanogenic properties, suppressing tyrosinase activity and inhibiting kojic acid. Secondary metabolites from G. glabra also show anti-melanogenesis action.[19]

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2. Orange peel

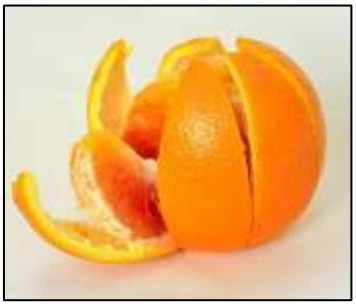


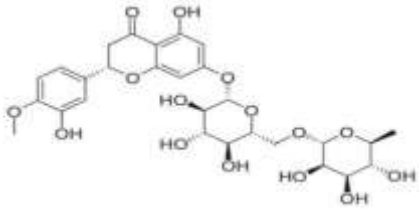
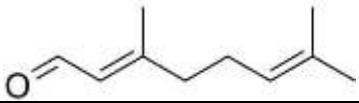
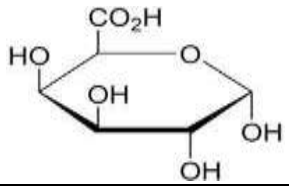
Figure 6. Orange Peel

- **Biological Name:** Citrus sinensis
- **Common Name :** Sweet orange
- **Family:** Rutaceae
- **Genus:** Citrus
- **Biological Source:** Orange peel is consisting of fresh and dried outer part of pericarp of Citrus Aurantium Linn.
- **Major Chemical Constituent:** Vitamin C, Citral, Hesperidin, Limanene, Pectin, Aurantimarin and Aurantimaric acid

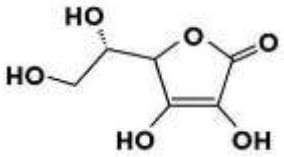
Morphology

- **Colour:** Dark orange reddish
- **Odour:** Aromatic
- **Taste:** Aromatic and bitter
- **Size:** There is Variation in the size and shape of the ribbons or strip. They are between 3 and 4 mm thick.

Table No. 2. Chemical Constituents of Orange peel:

Compound Name	Structure
Hesperidine	
Citral	
Pectin	

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Vitamin C	

Mechanism of action of Orange Peel

The antioxidant and anti-inflammatory qualities of orange peel have drawn attention to its possible health benefits. The orange peel contains a wide range of bioactive compounds, such as essential oils, flavonoids, and carotenoids. Orange peel's ability to reduce inflammation and oxidative stress is mostly due to these components. This segment delves into the intricate mechanisms that underpin these attributes, offering an insight into the interplay between substances found in orange peel and cellular functions that support health.

Antioxidant Activity

Reactive oxygen species (ROS) are highly reactive chemicals that can cause oxidative stress and damage to cells. Flavonoids and carotenoids are two compounds found in orange peels that have electron-donating qualities that allow them to successfully combat ROS. These substances quench ROS and stop chain reactions that cause oxidative damage by contributing electrons.

The chemicals found in orange peels have the capacity to chelate metals, particularly transition metals such as copper and iron. Through the Fenton and Haber-Weiss reactions, these metallic elements have the ability to catalyze the synthesis of LROS. Further reducing oxidative stress, orange peel components bind these metals to stop them from contributing to the production of ROS. Endogenous antioxidant enzymes, such as catalase and superoxide dismutase, which are necessary for neutralizing ROS and preserving cellular redox balance, can be synthesized by these substances. By increasing the activity of these enzymes, orange peel chemicals also help cells defend against oxidative stress [20].

3. Turmeric

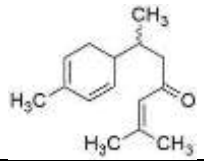
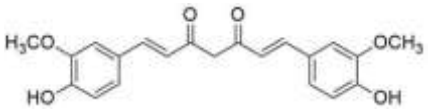
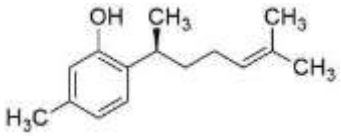
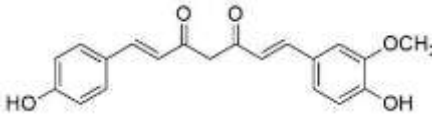
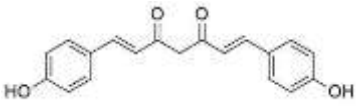


Figure 7. Turmeric

- **Biological Names:** Curcuma longa
- **Common Names:** Turmeric, Indian saffron, Jiang huang
- **Family:** Zingiberaceae
- **Genus:** Curcuma
- **Biological Source:** Turmeric is derived from the rhizome of the plant Curcuma longa
- **Major Chemical Constituents:** Turmerone, Curcumin, Curcuphenol, Desmethoxycurcumin, Bisdemethoxycurcumin.

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Table No. 3. Chemical Constituents of Turmeric

Compound Name	Structure
Turmerone	
Curcumin	
Curcuphenol	
Desmethoxycurcumin	
Bisdemethoxycurcumin	

Mechanism of action of Turmeric

Turmeric, especially its major ingredient curcumin, has anti-inflammatory and antioxidant qualities that can help lighten dark spots and decrease melanin synthesis, making it a useful treatment for hyperpigmentation.

- Anti-inflammatory and Antioxidant Properties: The antioxidant and anti-inflammatory qualities of turmeric can help calm sensitive skin and shield it from additional harm that could cause hyperpigmentation.
- Brighten Skin: Turmeric can help lighten the skin and lessen the visibility of dark spots by lowering inflammation and melanin production [21].

4. Green Tea



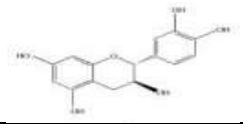
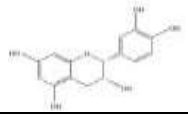
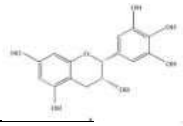
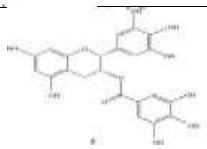
Figure 8. Green Tea

- **Biological Name:** Camellia sinensis
- **Family:** Theaceae
- **Genus:** Camellia
- **Biological Source:** Originates From the Leaves and Buds of the evergreen shrub or small tree Camellia sinensis.

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- **Major Chemical Constituents:** Catechin, Epicatechin (EC), Epigallocatechin (EGC), Epicatechin gallate (ECG), Epigallocatechin gallate (EGCG).

Table No. 4. Chemical Constituents of Green tea:

Compound Name	Structure
Catechin	
Epicatechin (EC)	
Epigallocatechin (EGC)	
Epigallocatechin gallate (EGCG)	

Mechanism of action of Green Tea

The primary bioactive components of green tea, particularly catechins like epigallocatechin gallate (EGCG), which have a number of skin-benefiting properties, are responsible for the treatment of hyperpigmentation.

- Antioxidant Activity: Free Radical Scavenging: EGCG and other green tea catechins are potent antioxidants. Free radicals, also known as reactive oxygen species (ROS), are created by environmental stressors such pollution, inflammation, and UV rays. They are neutralized by them. The oxidative stress caused by these free radicals can harm skin cells, which in turn causes the synthesis of melanin, the pigment that gives black spots their appearance. Green tea catechins inhibit oxidative stress and melanin overproduction by scavenging free radicals, which helps to diminish hyperpigmentation [22].
- Anti-inflammatory Effects: Inflammation plays a major role in the development of hyperpigmentation, especially in diseases such as post-inflammatory hyperpigmentation (PIH). " The catechins in green tea prevent the inflammatory response by blocking the synthesis of pro- inflammatory cytokines (such IL-1 and TNF-alpha) and enzymes (like COX-2, or cyclooxygenase- 2). One important regulator of inflammation, the NF-kB signaling system, is inhibited by EGCG, which is connected to its anti-inflammatory effects. Melanin synthesis is triggered by skin irritation or injury, and reducing inflammation helps avoid this.
- Tyrosinase Inhibition: The primary enzyme that involved in the making of melanin, which gives pigmentation its color, is tyrosinase, which is inhibited. By attaching to the enzyme and blocking its ability to catalyze the conversion of tyrosine into melanin, green tea catechins have been demonstrated to directly decrease tyrosinase activity. This can help lighten dark spots that already exist and stop new ones from appearing by reducing the skin's over production of pigment. Catechins, especially EGCG, bind to the active site of tyrosinase and prevent it from processing substrates into melanin. This mechanism is known as competitive inhibition [23].

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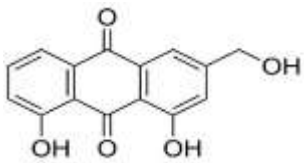
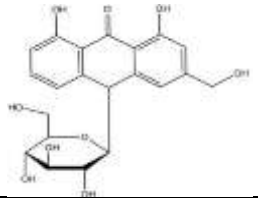
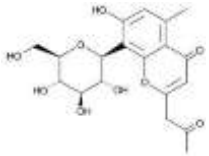
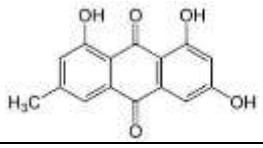
5. Aloe Vera



Figure 9. Aloe vera

- **Scientific Name :** Aloe barbadensis Miller
- **Synonyms:** Aloe indica Royle, Aloe perfoliate L. var. vera
- **Family:** Asphodelaceae
- **Biological source:** The aloes, or the dried juice, are obtained from the fleshy leaves of these plants.
- **Major Chemical Constituents:** Aloe emodin, Aloin/ Barbaloin, Aloesin, Emodin.

Table No 5. Chemical Constituents of Aloe vera

Compound	Structure
Aloe emodin	
Aloin / Barbaloin	
Aloesin	
Emodin	

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Mechanism of Aloe vera

Aloe vera is often used in skincare for its soothing and healing properties, and it may help in treating hyperpigmentation to some extent. Hyperpigmentation is the darkening of the skin due to excess melanin production, often caused by sun exposure, acne scars, or inflammation.

- Anti-inflammatory Effects: Aloe vera has anti-inflammatory properties that can help calm the skin, reduce redness, and prevent the exacerbation of dark spots. Inflammation can often worsen hyperpigmentation, so soothing it can prevent further darkening.
- Moisturizing: Aloe vera is a natural humectant, meaning it helps retain moisture in the skin. Well- hydrated skin may have an improved texture and tone, which can help diminish the appearance of hyperpigmentation over time [24].

6. Rose Water



Figure 10. Rose water

- **Scientific Name:** Rosa damascene
- **Common Name:** Damask Rose

Mechanism of action of Rose Water

Rose water has been used in skincare for centuries due to its soothing and anti-inflammatory properties. While it is not a strong treatment for hyperpigmentation, it can still play a supportive role in managing and reducing the appearance of dark spots over time

- Anti-inflammatory Properties: Rose water has anti-inflammatory effects that can help calm irritated or inflamed skin. Since inflammation is often a trigger for hyperpigmentation, reducing it may help prevent the worsening of dark spots or uneven skin tone.
- Gentle Skin Brightening: Rose water has mild astringent properties that may help in toning and slightly brightening the skin over time. It can assist in promoting a more even skin tone, which can reduce the contrast of dark spots [25].

7. Glycerine

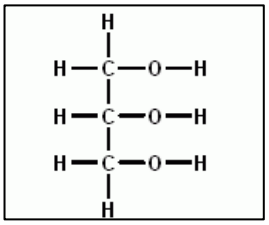


Figure 11. Structure of Glycerine

- **Chemical Name:** Glycerol,1,2,3-propanetriol
- **Chemical Formula:** $C_3H_8O_3$
- **Melting Point:** Approximately 17.8 ^{0}C (64.0 ^{0}F).
- **Boiling Point:** The boiling point of glycerine is 290 ^{0}C.
- **Density:** Density of glycerine is 1.261 g/cm^3.

Role of Glycerine

- Hydration and Moisture
- Skin barrier protection
- Soothing and calming
- Improve skin texture

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8. Sodium Benzoate

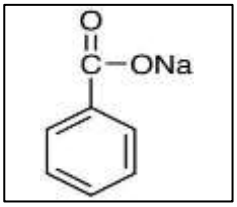


Figure12. Structure of Sodium Benzoate

- **Other Name:** Benzoate of soda
- **Chemical Formula:** C_6H_5COONa
- **Melting point:** 410 ^{0}C (770 ^{0}F)
- **Density:** 1.497 g/cm^3
- **Odor:** Odourless

Application

- Preservative in cosmetics and personal care products.
- Used as a corrosion inhibitor and fragrance ingredient.

MATERIAL AND METHODOLOGY

Material

- **Collection of Plant Material**

The root of licorice , orange peel, green tea leaves. Were collected from the college lab, botanical garden and local market area in Shrirampur.

The collect plants material was meticulously sun dried, carefully pulverized, and then stored in sealed bottles for the studies. Sun drying allows for the preservation of the plant material qualities, ensuring they remain intact for future use and research purpose.

Table No 6. List of Ingredient:

S/N	Ingredient	Source
1	Licorice	Pharmacognosy Lab P.P.C.O.P
2	Orange Peel	Local Market
3	Turmeric	Pharmacognosy Lab P.P.C.O.P
4	Green Tea	Local Market
5	Aloe vera	Botanical garden of P.P.C.O.P
6	Rose Water	Local Market
7	Glycerine	Laboratory P.P.C.O.P
8	Sodium Benzoate	Laboratory P.P.C.O.P

Table No.7. List of Glassware

S/N	Glassware
1	Conical flask
2	Funnel
3	Glass rod
4	Spatula
5	Filter paper
6	Test tube
7	Beaker

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Table No.8. List of Instrument

S/N	Instrument
1	Weighing Balance
2	pH Paper
3	Heating mental
4	Ostwald's viscometer
5	Stalagmometer

Methodology

Preparation of Extract

Extraction of an active constituent from crude drug by Decoction method. Decoction is a method of extraction by boiling herbal material to dissolve the chemicals of the material.

1. Licorice

- Measure and rinse: Measure out the dried licorice root and briefly rinse it to remove any dust.
- Simmer: Place the root in a pot with cold water. Bring to a boil, then reduce to a simmer.
- Boil gently: Simmer uncovered for 15–30 minutes. Longer simmering (up to 45 minutes) will yield a stronger decoction.
- Strain: Remove from heat and strain out the root pieces.
- Store: Pour the strained decoction into clean container. You can store it in the refrigerator for a few days, but its best to use it fresh for maximum potency [26].

2. Orange Peel

- Rinse the peel: Rinse dried orange peel to remove any dust or impurities.
- Boil with water: Place the orange peel in a non-aluminium pot and add water.
- Simmer: Bring to a boil, then reduce heat to a low simmer. Simmer for 20–30 minutes, uncovered or partially covered.
- Strain and serve: Remove from heat, strain the liquid.
- Store: Pour the strained decoction into clean container. You can store it in the refrigerator for a few days, but its best to use it fresh for maximum potency [27].

3. Turmeric

- Measure and rinse: Measure out the dried turmeric root and briefly rinse it to remove any dust.
- Simmer: Place the root in a pot with cold water. Bring to a boil, then reduce to a simmer.
- Boil gently: Simmer uncovered for 15–20 minutes. Longer simmering (up to 35 minutes) will yield a stronger decoction.
- Strain: Remove from heat and strain out the root pieces.
- Store: Pour the strained decoction into clean container. You can store it in the refrigerator for a few days, but its best to use it fresh for maximum potency [28].

4. Green tea

- Weigh the Sample: Take 10–20 grams of dried green tea leaves.
- Add Solvent: Add 100–200 mL of distilled water (ratio: about 1:10 w/v).
- Boiling: Boil gently for 15–30 minutes in a covered vessel. Maintain low to medium heat; avoid vigorous boiling to preserve heat-sensitive compounds.
- Cooling: Allow the decoction to cool to room temperature.
- Filtration: Filter the mixture using muslin cloth, Whatman filter paper, or a Büchner funnel. Collect the filtrate [29].
- Storage: Store the decoction extract in a refrigerator (4°C).

Phytochemical Test

1. Test for Alkaloid [Mayers test]:
   Take solvent extract in a test tube, then add in few drops of Mayers reagents. Positive result indicates formation of orange or brown precipitate; it indicates presence of alkaloids.[30]
2. Test for Flavonoid [Sodium hydroxide test]:
   Add a few drops of NaOH to the solvent extract. A positive result is showing yellow color indicates the presence of flavonoids.[30]
3. Test for Tannins [Ferric chloride test]:
   Add a few drops of $FeCl_3$ to the solvent extract. A positive result is blue or greenish color indicates the presence of tannins. [31-32]
4. Test for Phenols [Ferric chloride test]:
   Add ferric chloride solution to the solvent extract. A blue or greenish color indicates the presence of phenolic compounds.[33]
5. Test for Saponin [Foam test]:
   The extract was shaken vigorously with distilled water and allowed to stand for 10 minutes. Persistent formation indicated the presence of Saponins.[34]

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Table No. 9. Phytochemical Test:

S/N	Chemical Constituents	Licorice	Orange peel	Turmeric	Green tea
1	Alkaloid	Negative	Positive	Positive	Positive
2	Flavonoid	Positive	Positive	Positive	Positive
3	Tannins	Positive	Positive	Positive	Positive
4	Phenols	Positive	Positive	Positive	Positive
5	Saponin	Positive	Positive	Positive	Positive

FORMULATION AND PREPARATION

Table No.10. Formula For Herbal Toner For Depigmentation

S/N	Ingredient	F1	F2	F3	F4	F5	F6	Role
1	Licorice	5 ml	7 ml	6 ml	4 ml	7 ml	6.5 ml	Anti-inflammatory,
2	Orange peel	5 ml	7 ml	6 ml	4 ml	7 ml	6.5 ml	Antioxidant
3	Turmeric	2 ml	5 ml	3 ml	2 ml	5 ml	2 ml	Enhance skin tone
4	Green tea	5 ml	7 ml	6 ml	4 ml	7 ml	6.5 ml	Antiaging
5	Aloe vera	10 ml	10 ml	10 ml	12 ml	10 ml	8 ml	Hydration, Soothing
6	Rose water	60 ml	50 ml	55 ml	60 ml	55 ml	60 ml	Calming, Fragrance
7	Glycerine	10 ml	10 ml	10 ml	8 ml	6 ml	8 ml	Moisturizer
8	Sodium Benzoate	0.25 gm	0.25 gm	0.25 gm	0.25 gm	0.25 gm	0.25 gm	Preservative
9	Distilled Water	q.s	q.s	q.s	q.s	q.s	q.s	Vehicle

Preparation/ Procedure

1. Prepare the extract from crude drugs used like, Licorice , Orange peel, Turmeric, Green tea.
2. In clean beaker add prepared active extract and mix well.
3. Then add Aloe vera gel into the mixture of extract and continuously stirred.
4. Add Rose water which helps the balance skin pH and provide fragrance.
5. Add glycerine this will help attract moisture to the skin. Keeping it hydrated and soft.
6. Final add Sodium Benzoate as preservative for preserving formulation.
7. Then final mixing and ensure all the ingredients are well blended and evenly distributed.
8. Using a funnel transfer the toner into clean spray bottle or container.

EVALUATION TEST

The herbal toner formulated was evaluated for the following:

1. Organoleptic characteristics

- Colour: Yellow
- Odour: Fragrant
- Appearance: Goo



Figure 13. Herbal Toner

2. Homogeneity

Homogeneity was analysed by visual inspection for the appearance and existing of any clog.

3. pH:

A small strip of universal pH indicator paper was taken. A few drops of the herbal skin toner were placed on clean surface using a dropper. Then the pH paper strip was dipped into the sample. The color change on the pH paper was immediately observed and compared with the standard pH color chart.

The formulation showed a pH of 5-6 which is suitable for topical skin application.

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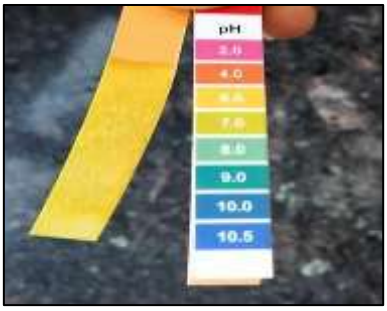


Figure 14. Determination of pH by with pH paper

4. Surface tension

The formulation was transferred in the stalagmometer and the surface tension was recorded. Surface tension is calculated based on the number of drops of liquid that fall from a stalagmometer. It is compared with a reference liquid

Observation:

Weight of empty specific gravity bottle (W1) = 23.19 g

Weight of empty specific gravity bottle with distilled water (W2) =56.93 g Weight of empty specific gravity bottle with sample formulation (W3) = 57.45 g

$$\text{Density of sample formulation } (\rho)_2 = \frac{\text{Mass of sample}}{\text{Mass of water}}$$

$$= \frac{W3-W1}{W2-W1}$$

$$= \frac{57.45-23.19}{56.93-23.19}$$

$$= \frac{34.26}{33.74}$$

$$= 1.015 \text{ g/ml}$$

Density of sample (ρ_2) = 1.015 g/ml (calculated value)

Density of water at room temperature (ρ_1) = 0.997 g/ml (standard value) Surface

Tension of water at room temperature = 72 dyne/cm

$$\text{Surface tension } (\gamma)_2 = \frac{\rho_2 \, n_1}{\rho_1 \, n_2} \times \gamma_1$$

Where,

ρ_1 = Density of water (g/ml) ρ_2=

Density of sample (g/ml)

γ_1 = Surface tension of water (dyne/cm) γ_2 =

Surface tension of sample (dyne/cm) n_1 = No.

of drops of water from A to B

n_2 = No. of drops of sample from A to B

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Samples	No of Drops			Average No. of Drops(n)	Density (g/ml)	Surface tension (dyne/cm)
	1	2	3			
Distilled water	42	41	43	42	0.997 g/ml	72
Sample	123	126	123	123	1.015 g/ml	25.02

Calculation:

Surface tension of sample formulation $(\gamma_2) = \frac{\rho 2\ n1}{\rho 1\ n2} \times \gamma_1$

$$= \frac{1.015 \times 42}{0.997 \times 123} \times 72$$

$$= \frac{42.63}{122.631} \times 72$$

$$= 0.3476 \times 72$$

$$= 25.0272$$

The surface tension of the herbal skin toner was found to be 25.02 dyne/cm at room temperature

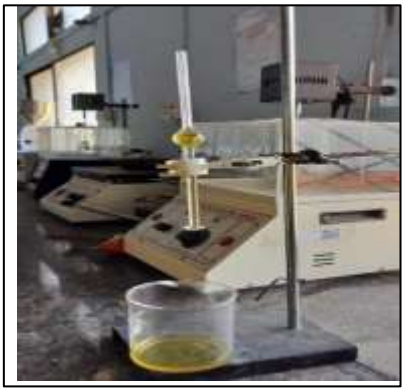


Figure 15. Determination of Surface tension by with Stalagmometer.

5. Viscosity

Ostwald's viscometer was used to measure the viscosity of the formulation. The viscosity of water and the formulation was recorded in centipoise.

Observation:

Weight of empty density bottle (W_1) = 6.06 gm Weight of

empty bottle with water (W_2) = 19.24 gm Weight of empty

bottle with sample (W_3) = 19.50 gm

Density of sample $(\rho) = \frac{\text{Mass of liquid sample}}{2}$

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$$\text{Mass of equal water} = \frac{W3-W1}{W2-W1}$$

$$= \frac{19.50-6.06}{19.24-6.06}$$

$$= \frac{13.44}{13.18}$$

$$= 1.197$$

Density of sample (ρ_2) = 1.0197 g/ml

Density of water (ρ_1) = 0.997 g/ml

Viscosity of water at room temperature = 1. 0266

$$\text{Viscosity } (n_2) = \frac{\rho 2}{\rho 1} \times \frac{t2}{t1} \times n_1$$

Where,

n_2 = Viscosity of sample n_1=

Viscosity of water ρ_2 =

Density of sample ρ_1 =

Density of water

t_2 = time taken by sample t_1 =

time taken by water

Samples	Time taken by sample			Average time(sec)	Density (g/ml)	Viscosity (c.p)
	1	2	3			
Distilled water	47	45	46	46 (sec)	0.997	1.0266 c.p
sample	132	130	128	130 (sec)	1.0197	2.96 c.p

Calculation:

$$\text{Viscosity of sample formulation } (n_2) = \frac{\rho 2}{\rho 1} \times \frac{t2}{t1} \times n_1$$

$$= \frac{1.0197}{0.997} \times \frac{130}{46} \times 1.0266$$

$$= 1.0227 \times 2.82 \times 1.0266$$

$$= 2.96$$

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So, viscosity of sample is 2.96 centipoise



Figure 16. Determination of Viscosity by with Ostwald viscometer

6. **Skin Irritation**
   After a brief application of the toner to the dorsal skin of the left hand, the skin was found to be non-irritating.



Figure 17. Before application of toner



Figure 18. After application of toner

7. **Skin Conditioning**
   The appearance of the skin after application of the toner was seen to be smooth, hydrated and supple.
8. **Temperature variation**
   To test the stability, the formulation was subjected to a range of temperatures at 45^0 C for several months.
9. **Light exposure**
   To check for formulation discoloration, the product is placed in its original packaging and subjected to direct sunlight. There was no visible discoloration.
10. **Antioxidant Activity**
    To evaluate the antioxidant potential of the herbal skin toner using the DPPH radical scavenging assay.
    Method:
    - 0.1mm DPPH solution was used
    - Various concentrations (20 µL to 100 µL) of the toner were tested.
    - Absorbance measured at 517 nm after 30 minutes.
    - % Scavenging = [(A0 - A1)/A0] × 100
    - Result:- Approx. 82% inhibition at 100 µL, showing strong antioxidant activity.[41-42]

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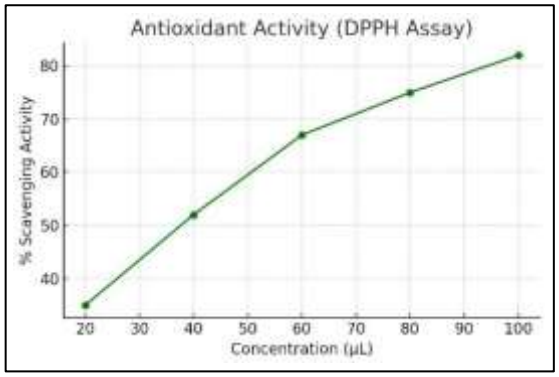


Figure 19. Plot of Antioxidant activity study (DPPH Assay)

11. Anti – Tyrosinase Activity

To assess inhibition of tyrosinase enzyme responsible for melanin synthesis. Method:

- Mushroom tyrosinase and L-DOPA used.
- Toner incubated at 37°C for 30 min.
- Absorbance at 475 nm measured.
- % Inhibition = [(A_control - A_sample)/A_control] × 100
- Result:- 65-70% tyrosinase inhibition at 100 µL.[43]

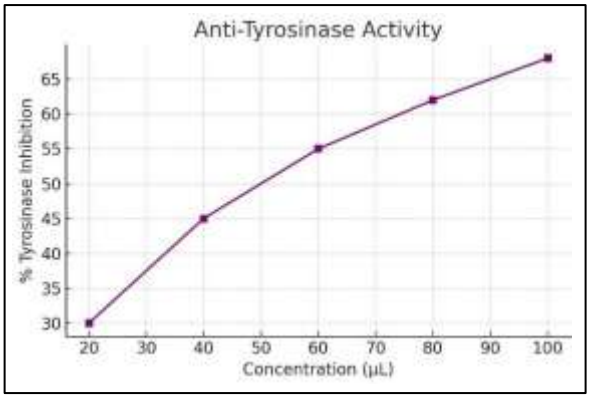


Figure 20. Plot of Anti – Tyrosinase activity

RESULTS AND DISCUSSION

Table No.11 Result of Evaluation of herbal toner

S/N	Evaluation Test	Result
1	Colour	Yellow
2	Odour	Characteristics
3	pH	5-6
4	Surface tension	25.02 dyne/cm
5	Viscosity	2.96 cp
6	Skin irritation	Non-irritable
7	Skin conditioning	The skin was moisturized, soft, and supple
8	Temperature variation	The product is stable at extreme temperature and room temperature
9	Light exposure	No discoloration or No Physical and Chemical changes seen

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Several physicochemical tests were performed on the finished product. Every test was conducted in accordance with its standard operating procedure. Every result was noted and discovered to be within the typical ranges. After careful analysis, the pH, surface tension, viscosity, and stability were all within the range. After the formulation was exposed to light, no discoloration was observed. Additionally, the mixture was non-irritating and successful in conditioning the skin. Finally, it was discovered that the toner was readily removed.

This herbal mixture, particularly in the form of a toner, was made with very natural and safe substances that are also found in daily life. Its spread ability and ease of application were the primary factors in its formulation as a toner. The purpose of creating a toner was also to see if they could get the same cleansing effect in liquid form. It turned out to be satisfactory after looking at the observations and outcomes. When applied, the composition had a very calming, cleansing, and toning impact on the skin, which was the most crucial factor. It made the skin feel tight.

COMPARATIVE ANALYSIS

Table No. 12. Comparative Analysis

S/N	Characteristics	Herbal skin toner	Marketed toner
1	Colour	Yellow	Colourless
2	Odour	Fragrant	Fragrant
3	pH	5-6	5-6
4	Surface tension	25.02 dyne/cm	27.02 dyne/cm
5	Viscosity	2.96 cp	2.6 cp
6	Skin irritation	Non-irritable	Non-irritable
7	Skin conditioning	Moisturized, soft and supple skin	Moisturized, soft and supple skin
8	Temperature variation	Stable at extreme and room temperature	Stable at extreme and room temperature
9	Light exposure	No Physiochemical Changes	No Physiochemical Changes
10	Removable	Easily removable	Easily removable

PACKAGING AND DIRECTION OF USE

Packaging

The herbal skin toner was packaged in well closed bottle with spray or flip-top cap to ensure ease of use, protection from light-induced degradation, and to maintain product hygiene.

Storage

Store the toner in cool, dry place away from direct sunlight.

Direction of use

1. Cleanse your face thoroughly with a mild herbal face wash and pat dry.
2. Shake the herbal skin toner bottle well before use
3. Moisten a cotton pad with the toner and gently apply it all over the face and neck, avoiding eye area.
4. Allow it to air dry; do not rinse.
5. Use twice daily morning and evening for best results.

CONCLUSION

The spray toner mixture yielded outstanding outcomes. The products were all affordable and useful, and they were all bought fresh from the neighbourhood market. It was decided that the cooling and toning impact the toner was supposed to have on the skin was sufficient. To make it easier to travel and apply the mixture whenever and wherever needed, it was also made in toner form. The investigated formulation was likewise satisfactory from that perspective. After application, there was a cleansing effect but no rashes or irritation. It was discovered that the developed formulation possessed the characteristics of a traditional skincare formulation from a cosmeceutical and was physiochemically stable. Since the spray formulation was able to better penetrate the skin's tiny pores by spraying tiny particles into the skin with a specific level of force, it was more effective than any other form, including gel or lotion.

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