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DEVELOPMENT AND EVALUATION OF A TOPICAL BRIGHTENING SERUM CONTAINING MUSHROOM EXTRACT FOR COSMETIC AND THERAPEUTIC BENEFITS

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ABSTRACT:

The kingdom Fungi comprises magnificent biodiversity and includes some of the most diverse species on Earth. Mushrooms possess both cosmetic and therapeutic applications, making them valuable ingredients for cosmetic products. Cosmeceuticals combine these benefits by delivering active ingredients to the skin in a biologically effective form. They have been widely used in East Asia for centuries, and their demand has recently increased due to the growing preference for safer, cost-effective, toxin-free, and organic products. This rising demand for sustainable products has encouraged extensive research into the cosmetic potential of mushrooms. Fruiting bodies of several wild and cultivable mushroom species, rich in medicinal compounds and secondary metabolites, are now being utilized in the cosmetic industry. Various bioactive compounds isolated from medicinal mushrooms, such as ceramides, omega-3, omega-6, and omega-9 fatty acids, carotenoids, lentinans, and resveratrol, exhibit remarkable antioxidant properties. Species of medicinal mushrooms widely used in cosmeceuticals include Agaricus subrufescens, Cordyceps sinensis, Ganoderma lucidum, Grifola frondosa, Inonotus obliquus, Lentinula edodes, Tremella fuciformis, and Polyporus species. The topical application of mushroom extracts offers numerous skin benefits, including the prevention of photoaging, enhanced skin hydration, and antioxidant protection against pollution and ultraviolet (UV) damage. In the past, most cosmetic formulations primarily relied on plant-based ingredients. However, recent advances in the understanding of the anti-inflammatory, antimicrobial, and free radical scavenging properties of medicinal mushrooms have revealed significant opportunities for their utilization in the cosmetic industry. By integrating these beneficial properties with modern genomics and pharmacological approaches, the development and evolution of mainstream fungal-based cosmetics can be successfully achieved.

Keywords: Anti-aging, Cosmeceutical, Fungi, Medicinal mushrooms, Skincare.

1. INTRODUCTION:

Mushrooms have been used medicinally for more than 2,000 years in Ayurveda and Traditional Chinese Medicine. The "mushroom of immortality," Ganoderma lucidum(Reishi), was utilized to promote skin health and longevity. For bright, glowing complexion, Japanese ladies have traditionally utilized extracts from_Lentinula12` edodes_ (Shiitake). Since the 1980s, research has shown that kojic acid fromAspergillus and mushroom species is a safe melanin inhibitor, which has led toitscommercial application in cosmetics.

Fig No.1: Representation of the cosmetic properties of the muschrooms

Cosmetics: is defined by the Drug and Cosmetic Act as "an item intended to be rubbed, poured, sprinkled or sprayed on, introduced into or otherwise applied to the human body for cleansing, beautifying, and promoting." The word "cosmetics" is derived from the Greek word "kosmeticos," which means an adorn. Since then, any material used to beautify or improve the appearance of external parts of the body, such as nails, skin, or hair, has been called cosmetics. attractiveness or changing one's look. [1]

Skin: With a surface area of roughly 1.5–2 m2 in adults, the skin is the biggest organ in the human body. Additionally, it makes up around 15% of an adult's entire body weight. It serves a number of purposes, such as shielding the body from the elements and preventing the body from retaining too much water.

Structure of the skin: The outer epidermis and the inner dermis are the two separate layers that make up the skin's anatomical structure. Beneath the dermis layer, which is made up of adipose and aerolar tissue, is a subcutaneous layer. [2]

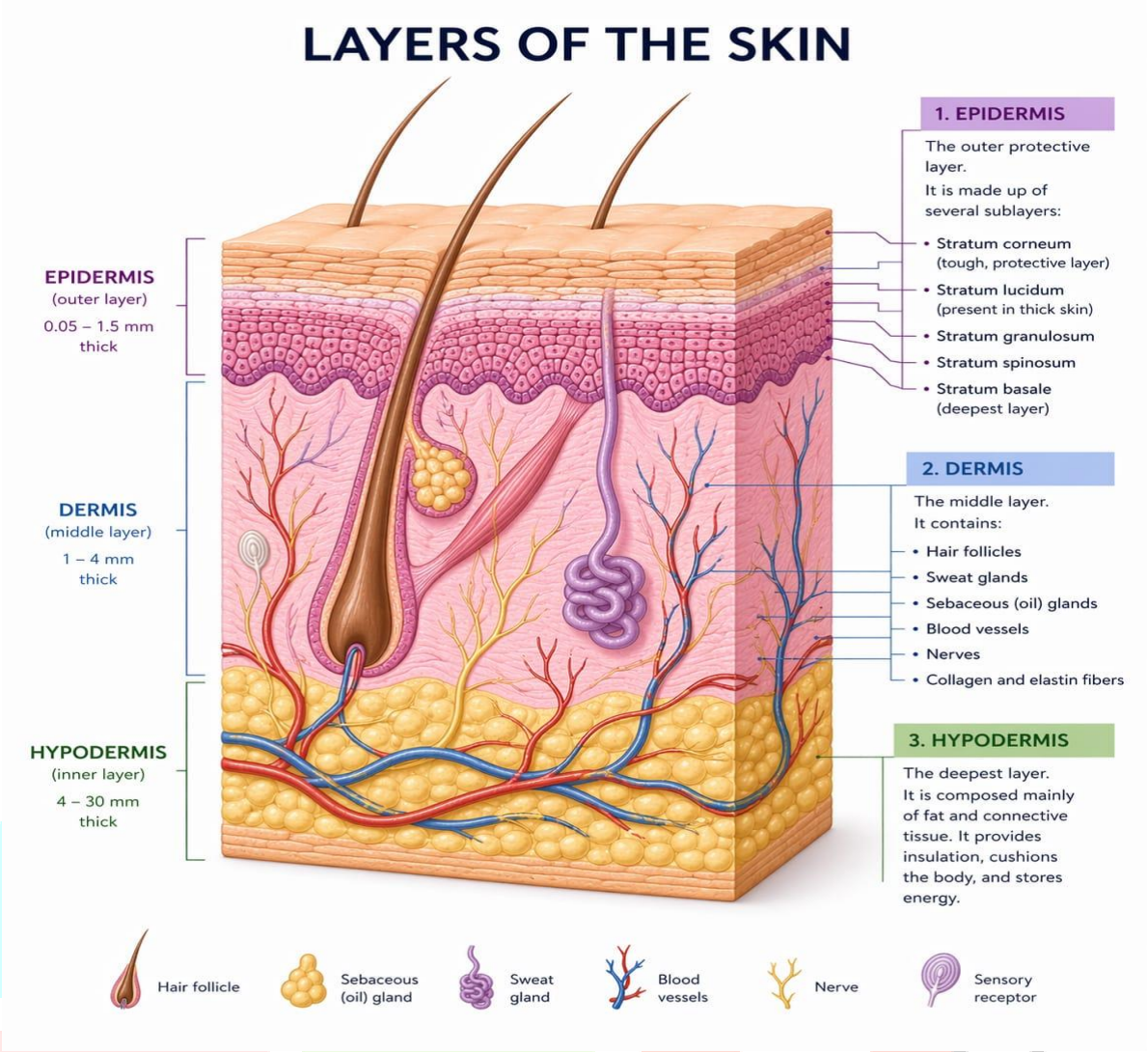


Fig No.2: Layers of the skin

A. Epidermis: The superficial, thinner portion, composed of epithelial tissue, is the epidermis

•The skin's outermost layer, known as the epidermis, is made up of stratified squamous keratinized epithelial cells. It creates a barrier that keeps germs from penetrating the body's surface. Many additional cells, including keratinocytes, melanocytes, Langerhans cells, and markel cells, make up the epidermis.

B. Dermis: The deeper, thicker connective tissue portion is the dermis.

•Under the dermis, but not part of the skin is the subcutaneous layer called as hypodermis.

•This layer consists of areolar and adipose tissues.

•The second layer of skin, directly behind the epidermis, is called the dermis. Depending on the area of the human body, the thickness of the skin ranges from 0.5 to 5 millimeters. The entire mass of the dermis may make up 15–20% of the body.

C. Hypodermis: This layer, sometimes referred to as the subcutaneous or subcutis layer, is situated beneath the dermis and is made up of loose connective tissue. The skin's structure may move freely and with suppleness thanks to the loose connective tissue. [3]

Function of the skin:

1. Protective function: The skin shields the body's organs from harmful substances, UV radiation, microbial invasion, and skin dryness.

2. Body temperature regulation: The skin controls blood flow in the dermis and uses sweet evaporation to keep the body temperature at 37 degrees Celsius.

3. Sensory organ: Nerve endings in the skin allow for the distinction of touch, warmth, and pain. The brain and spinal cord receive the sensation from that receptor.

4. Vitamin D Formation: Sunlight transforms 7-Dehydrocholesterol, a lipid-based material in the skin, into vitamin D.

5. The skin's excretory function: it is a minor excretory organ that eliminates urea, ammonia, and sodium chloride salts.

Synthesis of Skin:

1.Synthesis of vitamin D requires activation of a precursor molecule in the skin by UV rays of sunlight.

Enzymes in the liver and kidneys then modify the activated molecule finally producing calcitriol, the most active form of vitamin-D

Calcitriol is a hormone that helps in absorption of calcium in foods from GIT into the blood

Skin colour: The colour of skin depends on two important factors: pigmentation of the skin and hemoglobin in the blood. The most important factors is the pigmentation of skin. The stratum basal contains cells called Melanocytes, which are responsible for the skin pigmentation. Melanin is synthesized from tyrosine in presence of tyrosinase enzyme by melanocytes. Exposure of sunlight promotes the melanin that absorbs ultraviolet radiation, prevents DNA damage in epidermal cells and thus protects the skin from harmful UV radiation, the another factors which responsible for skin is hemoglobin in the blood. The amount and nature of blood pigments, Hb circulating in the cutaneous blood vessels play an important role in skin colouration. Light skinned individuals exhibits pink to red skin colour because of Hb in red blood cells

Skin Whitening: Skin whitening is the elimination of the melanin that act as self defense mechanism for the human skin against exposure to ultraviolet radiations. Skin whitening agent inhibits the further synthesis of melanin. Hyperpigmentation disorders in the skin common problem, this condition often does not affect patients physical health but many patients effected psychologically

Skin whitening can be achieved by

• Regulating the transcription and activity of tyrosinase.

• Regulating the uptake and distribution of melanosomes in keratinocytes.

• Interfere with melanosomes maturation and transfer.

• Block the melanin production process

• Tyrosinase inhibitors inactivate the tyrosinase by chelating with its vital copperion. [4]

Effect of Skin Whitening: Every woman aspires to have white skin because, particularly in Asia, it is a sign of prosperity and beauty. Melanin production was the primary source of skin tone and was crucial in preventing UV-induced skin irritation. Tyrosinase primarily controls the biosynthetic pathway for melanin synthesis in different bioforms through two different oxidation processes. Tyrosinase essentially catalyzes the hehydroxylation of tyrosine to dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to DOPAquinone, which, depending on various physiological conditions, is further transformed to either pheomelanin (yellow-red pigment) or eumelanin (brown-black pigment). Tyrosinase inhibition is therefore the most used method for skin lightening. [5]

Face serum: Face serums are lightweight, fast absorbing liquids used as part of a skincare routine to deliver high concentration of active ingredients directly to the skin, they target specific skin concerns, such as wrinkles, fine lines, hyperpigmentation, acne and hydration. Also the Face serum are highly concentrated emulsion which is available in water and oil hased formulations. Face serum contain biomolecules which are very small molecules that will help to penetrate deep into the skin quickly. The common ingredients in face serums include hyaluronic acids, vitamin c, retinol, niacinamide and peptides which are responsible for the hydration, brightening, antioxidants protection, antiaging, reducing redness, improving elasticity and collagen production. [1]

• suitable for : all skin types

Types Of Face Serum:

1. Oil based serum

2. Water based Serum

3. Emulsion based serum

4. Gel serum [6]

2. AIM AND OBJECTIVE

Aim: To develop and evaluate a topical brightening serum containing mushroom extract for cosmetic and therapeutic benefits

Objective:

• To develop a stable topical brightening serum formulation using mushroom extract

• To extract, identify, and standardize bioactive compounds from mushroom extract

• To perform phytochemical screening and characterization of mushroom extract

• To formulate and optimize the topical serum using suitable pharmaceutical techniques

• To evaluate the physicochemical properties of the formulated serum

• To evaluate the safety and dermal compatibility of the formulated serum

• To investigate the cosmetic and therapeutic benefits of the serum

• To compare the formulated serum with commercially available skin brightening products

• To perform stability and shelf-life studies of the formulation

3. PLANT PROFILE:

Shiitake mushroom (Lentinula edodes) belongs to the kingdom fungi and is widely used for medicinal, nutritional, and cosmetic purposes. It is mainly found in Japan and China and grows on decaying hardwood trees. It contains important bioactive compounds such as beta-glucans, lentinan, and antioxidants, which provide various health benefits. In cosmetic formulations, shiitake mushroom is used for its skin brightening, anti-aging, and anti-inflammatory properties.

Shiitake mushrooms are not only delicious but also contain beta-glucans that contribute to skin hydration and digestive health. Shiitake mushroom (Lentinula edodes) used in. cosmetics as an exfoliant, reduces inflammation, promotes skin regeneration, and maintains skin flexibility. Shiitake mushrooms hold the

antioxidant L-ergothioneine, which hampers cell damage and exfoliates the skin. They are a vital source of kojic acid and it brightens the skin and treats acne scars. Its anti-inflammatory effects help improve strength, rejuvenate skin, and enhance skin elasticity. Thus, it is often found in skin care products preferably those with anti-aging benefits. Lentinula edodes preparations contain kojic acid, a natural substitute to hydroquinone that upgrades skin appearance by lightening age spots and scars (Rahman and Choudhury, 2012)

Shiitake Mushroom (Lentinula edodes): Lentinula edodes is a widely cultivated edible fungus known for its nutritional and medicinal value. It contains proteins, dietary fibre, B vitamins (riboflavin and niacin), essential minerals like potassium and zine, and bioactive compounds such as lentinan and phenolics. As a white-rot fungus, it can degrade lignocellulosic materials, making its cultivation sustainable and environmentally friendly. In cosmeceutical applications, shiitake demonstrates strong anti-aging properties due to its antioxidant profile. It is rich in L-ergothioneine, a sulphur-containing antioxidant that neutralizes reactive oxygen species (ROS), protects skin cells from oxidative stress, and inhibits matrix metalloproteinases (MMPs), thereby preventing collagen breakdown and reducing wrinkles.

Lentinan contributes anti-inflammatory and immunomodulatory effects by reducing cytokines like IL-6 and TNF-a, helping to soothe irritation and improve skin healing Additionally, shutake contains phenolic compounds with tyrosinase inhibiting activity, which helps reduce hyperpigmentation and promote an even skin tone. It also exhibits antimicrobial activity against Staphylococcus supporting management. Overall, shiitake extracts are effective in enhancing hydration, improving elasticity, and promoting healthy, radiant skin in natural cosmetic formulations. [7]

Fig No.3: Overview of Lentinula edodes: Key Botanical Features and Health-Promoting Bioactive Constituents

Cosmetic use Shiitake mushrooms (Lentinula edodes)

Shiitake mushrooms (Lentinula edodes) are increasingly popular in cosmetics for their potent bioactive compounds like B-glucans, polysaccharides, polyphenols, and kojic acid-like substances, which deliver anti-aging, brightening, and protective benefits. Extracted from fruiting bodies or mycelium, they inhibit collagenase and elastase enzymes to prevent breakdown of skin's structural proteins, while stimulating new collagen synthesis for firmer, more elastic skin that resists wrinkles and sagging. As tyrosinase inhibitors, shiitake extracts curb melanin production, effectively fading hyperpigmentation, dark spots, melasma, and uneven tone for a brighter, radiant complexion without the irritation of synthetic whiteners. Their antioxidants-ergothioneine, selenium, and phenolics-scavenge ROS from UV exposure and pollution, shielding cells from oxidative damage and photoaging.

Polysaccharides act as humectants superior to hyaluronic acid in some formulations, bolstering the skin barrier, locking in moisture, and enhancing smoothness for plump, resilient texture. Anti-inflammatory properties soothe redness, calm sensitive or acne-prone skin, and accelerate barrier repair, making them ideal for eczema or rosacea [8]

Botanical Name: Lentinula edodes (Berk.) Pegler

Family: Marasmiaceae (formerly placed in Tricholomataceae)

Biological Source: It is the fresh or dried fruiting body of the basidiomycete fungus Lentinula edodes.

Geographical Source

Native Habitat: Shiitake is indigenous to East Asia, particularly mainland China, Japan, Korea, and Taiwan. It thrives in temperate, moist montane forests

Bioactive Properties of Mushroom:

Skin brightening: Inhibits tyrosinase and TRP-1 enzymes in melanogenesis pathway

Antioxidant: Neutralizes ROS, prevents photoaging

Anti-inflammatory: Suppresses IL-6, TNF-α, useful for acne/rosacea-prone skin

Anti-aging: Stimulates collagen synthesis, inhibits MMPs

Mulberry (Morus alba): Mulberry (Mulberry) is a fruit-bearing plant/tree from the Morus family. Its fruits are small and can be black, red, or white, and the leaves are also widely used.

Cosmetic uses of Mulberry: Mulberry is used in skincare and cosmetics because it contains antioxidants, vitamins, and natural compounds that help the skin.

MORUS RUBRA:

Kingdom: Plantae

Order: Rosales

Family: Moraceae

Genus: Mulberries

Botanical Name: Morus Rubra

Synonyms: Shahtoot, Mulberry, Morus

Medicinal properties of the Mulberry:

Rich in antioxidant

Promote collagen production

Moisturizing and Hydrating

Protect against UV damage

Reduce hyperpigmantation

Fig No.4: Mulberry Fruits

4. PLANE OF WORK

Table No.1 Ingredient Table

Chemicals	**Examples**	**Function**
Kojic acid	Mushroom extracts	Brightening ,pigmentation
Alpha Arbutin	Mulberry	Reduce dark spots
Gelatin	dairy product	Hydration, Thikness
Glycerin	_	Deep Hydration, Skin Softning
Rose water	Flowers, Patels	Facial Toner, Cleanser
Sodium hydroxide (NaOH)	_	PH Abjuster, Skin Care
Methyl paraben	Blueberries	Antiagent,
Vitamin-E	_	Antioxident Agent

1) Kojic Acid: Kojic acid is a natural organic compound produced by fungi, used in skincare and cosmetics as a skin-lightening agent to reduce pigmentation and dark spots by inhibiting melanin production.

Physical State: Off-white to pale yellow crystalline powder

Solubility: Kojic acid's solubility profile is critical for determining the solvent system in topical formulations

Uses:

• Skin Brightening

• Hyperpigmentation

• post-inflammatory

• hyperpigmentation

• Antioxidant

• Antimicrobial Agent

2) Alpha Arbutin: Alpha-Arbutin (often written as \alpha-arbutin) is a biosynthetic active ingredient widely used in skincare for its potent skin-brightening and depigmenting properties.

Physical Form: Alpha Arbutin exists as a stable, solid crystalline powder at room temperature

Nature: A glycosylated hydroquinone; it is the alpha-glucoside of hydroquinone

Uses:

• Hyperpigmentation Control

• Skin Evening Brightening

• Safe Alternative to Hydroquinone

• Synergistic Ingredient

3) Gelatin: Gelatin is a protein obtained from collagen present in animal bones, skin, and connective tissues.

Odor: Taste Nearly odorless and tasteless

• Solubility Soluble in hot water, glycerol, and acetic acid

Uses:

• Capsule Shells

• Gelling & Thickening

• Binding & CoatingWound

• Care & Hemostatics

4) Glycerin: Glycerin (also commonly referred to as glycerol) is a naturally occurring, colorless, odorless, and viscous (thick) liquid carbohydrate with a sweet taste

Physical Appearance: A clear, colorless, odorless

Solubility: Miscible with water and alcohol.

Uses:

• Sweetening Agent

• Humectant

• Pharmaceutical Solvent

5) Rose Water: In general botany and horticulture, "grows water" usually refers to plants that grow either entirely or partially submerged in water, or plants that are adapted to thrive in highly saturated aquatic environments.

Physical State: Clear, colorless to pale pink liquid with a characteristic, pleasant floral

Uses:

•Skin Hydration and Toning

•Antioxidant Protection

•Natural Fragrance

6) Sodium hydroxide (NaOH): Sodium hydroxide is a strong alkaline (basic) chemical compound with the formula NaOH, commonly called caustic soda. It is used in soap making, cleaning products, and chemical industries.

Physical state:

colour: White

order: orderless

Solubility:

• Highly soluble in water , Soluble in ethanol and methanol.

•Insoluble in non-polar solvents like ether

Uses:

•pH Adjustment

•Water Treatment

•Cleaning Agent

7) Methyl paraben: methylparaben is generally considered safe for the skin in the small, regulated concentrations used in cosmetics

Physical State:

•Colorless crystals or a white crystalline powder.

•It is odorless or has a faint characteristic odor

Uses:

•Broad-Spectrum Preservative

• Pharmaceutical Formulations

8) Vitamin E: Vitamin E is a group of essential, fat-soluble compounds that function primarily as antioxidants.

Physical State: Clear, colorless to viscous yellowish or greenish-yellow oily

Uses:

• Antioxidant Protection (Free Radical Scavenging)

• Skin Barrier Repair and Deep Moisturiza

5. METHODOLOGY:

1. Collection of Lentinula edodes:

edodes Fruiting bodies are purchased from Head of botany department ,JES college of arts, commerce and Science, Nandurbar Maharashtra and transported to the cosmetic technology laboratory.

Extraction of Lentinula edodes (maceration method):

A. Collection of Fresh Mushrooms: Select fresh, clean mushrooms (free from contamination).

B. Drying: Dry the mushrooms under shade or in a hot air oven (40–50°C) until completely moisture-free.

Fig No.5: Drying of Mushroom

C. Grinding: Grind the dried mushrooms into a fine powder using a grinder.

Fig No.6: Grinding of Dried Mushroom

D. Maceration (Soaking): Soak the powder in a suitable solvent such as ethanol, water mixture

Keep the mixture in a closed container for 24–72 hours.

Stir occasionally to enhance extraction.

Fig No.7: Maceration (Soaking) of Mushroom Powder

E. Filtration: Filter the mixture using filter paper or muslin cloth to separate the liquid extract from solid residue.

Fig No.8: Filtration of Mushroom Extract

F. Storage: Store the final mushroom extract in an airtight container at cool temperature (preferably 4°C).

Fig No.9: Storage of Mushroom Extract

2. Collection of Mulberry: Mulberry Fruiting are purchased from Head of botany department ,JES college of arts, commerce and Science, Nandurbar Maharashtra and transported to the cosmetic technology laboratory.

Fig No.10: Collection of Mulberry Fruits

Extraction of Mulberry: (maceration method): mashing the fruit and add in to suitable solvent such as ethanol, water mixture

Keep the mixture in a closed container for 24–72 hours.

Stir occasionally to enhance extraction

Fig No.11: Maceration of Mulberry Extract

Filtration Of Mulberry Extract: Filter the mixture using filter paper or muslin cloth to separate the liquid extract from solid residue.

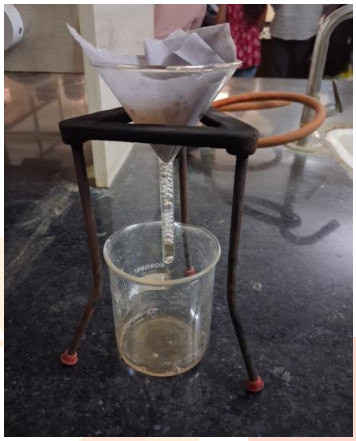


Fig No.12: Filtration of Mulberry Extract

6. FORMULATION OF SERUM :

Table No.2 FormulationTable

Ingredient	Quantity (F1)	Quantity(F2)
Rose Water	20 ml	22 ml
Glycerin	3.0 ml	2 ml
Mushroom Extract	3.5 ml	3 ml
Mulberry Extract	2 ml	1.5ml
Gelatin	0.3g (1%)	0.4g (1%)
Vitamin-E	1capsule	1capsule
Sodium Hydroxide	0.5 ml	0.5 ml
Methyl Paraben	1-2 Drops	3 Drops

Step 1: Preparation of Base (Carbopol Dispersion)

• Take a required quantity of distilled water in a clean beaker.

• Slowly add Carbopol (gelling agent) while stirring continuously.

Step 2: Addition of Humectant & Active Extract

• Add glycerin (acts as a moisturizer/humectant) to the hydrated Carbopol solution.

• Add mushroom extract (active ingredient for brightening/therapeutic effect).

• Stir gently until a uniform mixture is obtained.

Step 3: Addition of Active Ingredient

• Add Vitamin C (antioxidant and skin-brightening agent).

• Mix thoroughly to ensure proper dissolution and uniform distribution.

Step 4: pH Adjustment & Gel Formation

• Add sodium hydroxid dropwise.

• Stir continuously.

• This neutralizes Carbopol and converts the liquid into a gel-like serum.

• Adjust pH to around 5.5–6.5 (skin-friendly range).

Step 5: Addition of Preservatives & Fragrance

• Add a suitable preservative (to prevent microbial growth).

• Add fragrance if required.

• Mix gently to avoid air bubbles.

Step 6: Final Product

Check:

• pH

• Consistency

• Appearance

• Transfer the serum into a sterile container/spry bottle

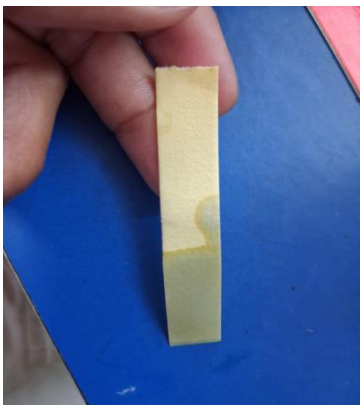
7. EVALUATION TEST:

1. Physical Parameter:

Table No.3 Physical Parameter

Sr. No	Parameters	Observation
1	State	Liquid
2	Colour	Faint Yellow, Off white
3	Odour	Pleasant
4	Texture	Smooth, Non Gritty

2. Determination of PH: A digital pH meter will be used to measure the pH test. The digital pH dipper will be inserted deeply into the serum formulation sample, and the pH value will be noted. Since the skin has an acidic pH of about 4-5.5, the formulation should have an acidic pH. This spectrum of formulations is appropriate for skin because skin has an acidic pH of 4.1-6.7. [9]



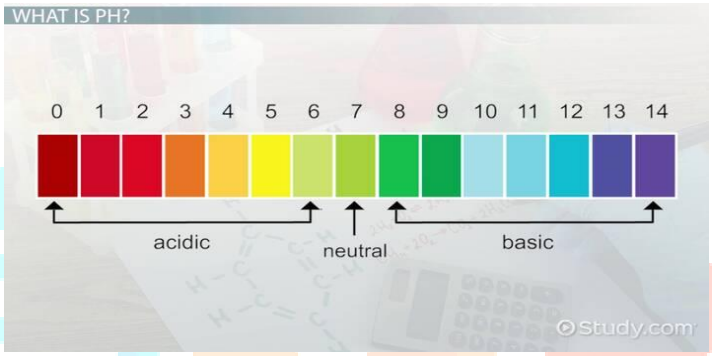


Fig No.13: Determination of pH of Serum Formulation

3. Determination of Viscosity: The Brook Field Viscometer is used to measure viscosity. Viscosity is the most important consideration when evaluating a cosmetic product. Viscosity controls a number of characteristics, such as the substance's pourability from the container. Viscosity can be affected by a number of variables, such as temperature variations, alterations to the manufacturing process, and the caliber of the raw materials. Determining the viscosity of the substance is therefore essential. Method: After measuring the serum's viscosity using Spindle No. 4 of a Brookfield viscometer, all of the operating parameters were determined. [9]

4. Determination of Spreadability: The area of the skin that is prepared to spray after applying a serum is known as Weit Spredadability. A number of filter paper sizes are selected, and each filter paper's weight (WI) and total area (A1) are measured. After selecting the formulation to be tested and drawing several milliliters into the 5 milliliter pipette, 20 drops of serum were added drop by drop to the center of the filter paper. Set a time or stopwatch to count down for precisely ten minutes after the most recent drip touches the filter paper. The liquid will cover the filter paper in a comparatively homogeneous circular pattern over the course of the 10-minute test [10]

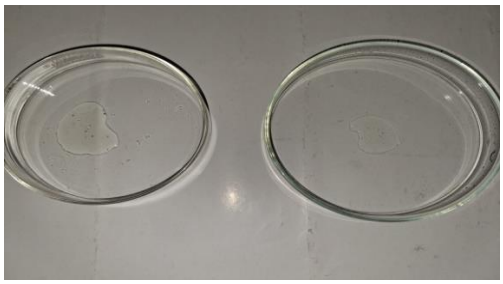


Fig No.14: Determination of Spreadability of Serum

5. Skin Irritation Test:

Fig No.15: Skin Irritation Test of Serum

8. RESULT:

1. Physical Parameter:

Table No.4

Sr. No	Parameters	Observation
1	State	Liquid
2	Colour	Faint Yellow, Off white
3	Odour	Pleasant
4	Texture	Smooth, Non Gritty

2. Determination of pH:

Table No.5

Sr No	Formulation	ph
1	F1	5.5
2	F2	6.0

3. Determination of Viscosity:

Table No.6

Sr No	Formulation	Viscocity
1	F1	1.433 pa.s
2	F2	1.334 pa.s

4. Determination of Spreadability:

Table No.7

Sr No	Formulation	Observation
1	F1	16%
2	F2	18%

5. Skin Irritation Test:

Table No.8

Sr No	Formulation	Observation
1	F1	Non irritant
2	F2	Non irritant

Fig No.16: Final Serum Formulation Samples

9. CONCLUSION:

Although mushrooms have long been utilized as a natural source of bioactive compounds, they are also being researched as potential components of cosmetics. Numerous mushrooms and their extracts have been shown to have moisturizing and skin-whitening qualities; these products have either been patented or are now in use. As mentioned before, just a small percentage of all mushroom species are now identified and utilized; more species will be discovered, verified, and cultivated. Multidisciplinary studies combining genomes, proteomics, metabolomics, and systems pharmacology will uncover the molecular mechanisms behind the therapeutic effects of mushrooms (often referred to as the "research area of molecular cosmetology"). This will make it possible to add more mushrooms to cosmetics in a number of ways.

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