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FORMULATION AND EVALUATION OF SKIN WHITENING FACE SERUM CONTAININIG AGARICUS BISPORUS EXTRACT

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ABSTRACT

Skin whiteners are kind of therapy methods that can be used for the treatment of hyperpigmentation. They inhibit the biosynthesis of melanin using tyrosinase inhibitors. Nowadays, lightening the skin tone is one of the most common procedure. Agaricus bisporus extract is used as a skin whiteining agent. The Agaricus bisporus bodies extraction was carried out by using soxhlet apparatus by using ethanol as solvent. The Percentage of yield was about 31%. Preliminary phytochemical test for extract was performed and showed positive results for tannins, phenol, terpenoids, saponin and alkaloids. Phytochemicals like phenol, tannins and terpenoids are for antityrosinase activity. A three formulation of skin whitening serum containing agaricus bisporus extract was prepared and evaluation parameters such as physical parameters, pH, viscosity, spredability and Antityrosinase activity was performed. Percentage of Tyrosinase inhibition of F3 formulation is highest as compare to the F1 and F2.

KEYWORDS: Skin whitening, Agaricus bisporus, Tyrosinase inhibition, Phytochemicals.

INTRODUCTION

Cosmetics: The word ‘cosmetics’ is derived from Greek word ‘kosmeticos’ which means a adorn. Since that time any of the material used for beautification or improvement of appearance of external parts of the body, i.e., nails, skin, hair is known as cosmetics. Drug and cosmetic act define the cosmetic as – “it is an item intended to be rubbed, poured, sprinkled or sprayed on, introduced into or otherwise applied to the human body for cleansing, beautifying, promoting, attractiveness or altering the appearance.

Skin: The skin is the largest organ of the human body, skin cover surface area of about 1.5-2 m2 in adults. Also it is about 15% of the total adult body weight. It perform various functions, including protection from external environment and prevention of excess water from body.[1]

Structure of the skin: The skin is anatomically divided into the two distinct layers: the outer epidermis and the inner is dermis. A subcutaneous layer lies in beneath the dermis layer, which consists of aerolar and adipose tissue.[1-2]

A. Epidermis: Epidermis is a outermost layer of the skin which is composed of stratified squamous keratinized epithelial cells. It forms a protective barrier over the body surface and preventing from entering of pathogens.The epidermis consists a number of other cells such as keratinocytes, melanocytes, Langerhans cells and markel cells.

B. Dermis: The dermis is the second layer of the skin located right below the epidermis.Thedermis is approximately 0.5 to 5 millimeters thick, depending on the human body site. The whole mass of the dermis may constitute 15-20 % of the total body.[5-8]

C. Hypodermis: This layer is located below the dermis which consists of loose connective tissue known as subcutaneous or subcutis layer. The loose connective tissue helps to flexibility and free movement of the skin structure.[1]

Function of the skin

1. **Protective function:** The skin protect the body organs from microbial invasion, toxic chemicals, UV rays and dehydration of the skin.
2. **Temperature regulation of the body:** Skin regulate the body temperature through sweet evaporation and adjust the flow of blood in dermis which maintain the temperature at 37 c.
3. **Sensory organ:** Skin contains nerve ending that enable discrimination of pain, temperature and touch. That receptor convey the sensation to brain and spinal cord.
4. **Formation of Vitamin D:** 7-Dehydrocholesterol is a lipid based substance in the skin which is converted to vitamin D by sunlight.
5. **Excretory function of the skin:** it is a minor excretory organ which excrete the sodium chloride salts, ammonia and urea.[7]

- **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.[20]

The term skin whitening also called as skin lightening, brightening and skin bleaching. This covers the variety of cosmetic markets used in an attempt to whiten the skin. Skin whitening is a term used for lightening the skin tone or provide an even complexion by reducing the melanin concentration in the skin. The various chemical substances are proven as effective skin whitener but recently raised safety concerns, leading to their ban in some countries. the ingredients which are banned such as mercury, monobenzone, corticosteroids and glutathione. The natural whitening compounds are become more prevalent nowadays in cosmetic formulation due to consumers concern about synthetic ingredients. Various natural skin lighters are kojic acid, licorice, mulberry extract, alpha hydroxy acids, retinol etc.Types of skin whitening products containing sunscreen, and ingredients that produces chemical change on skin[16]
Mechanism of skin whitening:

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.

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 based 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.

METHOD

1. Collection of Agaricus bisporus

Agaricus bisporus Fruiting bodies are purchased from local super market,Solapur, Maharashtra and transported to the cosmetic technology laboratory.

2. Extraction of Agaricus bisporus

Agaricus bisporus bodies are cleaned and shade dried in order to avoid degradation of chemical constituents. These bodies are then grinded into a coarse powdered subjected to extraction.[23]

3. Procedure of extraction

Dried powdered bodies was subjected to soxhlet extraction (Continueous hot extraction) using Ethanol solvent. After extraction solvent was evaporated extract was stored in airtight container till use.

4. Preliminary phytochemical test

- **Test for Tannins-** The extract (50 mg) was dissolved in 5 ml of distilled water for the ferric chloride test. A few drops of 10% lead acetate solution were then added to this. Tannins are indicated by a bulky white precipitate...
- **Test for saponins-** 2ml of extract was shaken vigorously with 5 ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.
- **Test for phenols-** To Iml extract, add distilled water followed by few drops of 10% Ferric chloride. The formation of green colour indicates the presence of phenolic groups.
- **Test for terpenoids:** Extract (5 ml) was mixed with chloroform (2 ml), and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.
- **Test for alkaloids:** 2 ml of extract, add waganers reagent. Reddish brown precipitate indicate presence of alkaloids. (26)

5. Tyrosinase Inhibition test

Tyrosinase inhibition activity was determined according To the modified method. Extract was dissolved in dimethyl sulfoxide (DMSO) to a Concentration of 10,000 ppm and then diluted in potassium Phosphate buffer (50 mM, pH 6.8) to 0.05 ppm. All steps were conducted at room temperature. extract (70 µl) was incubated with 30 µl of the Tyrosinase enzyme (333 U/ml in phosphate buffer, pH 6.8) For 5 min, followed by the addition of 2 mM of the Substrate, L-tyrosine (110 µl) and further incubated for 30 Min at RT, ensuring that effect of light is limited. Kojic Acid was used as positive control. The mixture of sample and other components, except L-tyrosine, was used as a Blank. Absorbance reading was performed at 475 nm. The Percentage of tyrosinase inhibition was calculated using formula.

$$\% \, inhibition = \frac{A_{Control} - A_{Sample}}{A_{Control}} * 100$$

Where, % inhibition is the tyrosinase inhibition percentage. Acontrol is the absorbance of the control solution and Asample is the absorbance of the sample solution. For each Sample concentration, the absorbance was recorded by spectrophotometer.[27]

❖ **FORMULATION AND EVALUATION**

FORMULATION

Formulation table

Sr no	Ingredients	F1 Quantity taken (60gm)	F2 Quantity taken (60gm)	F3 Quantity taken (60gm)
1	Xanthan gum	0.18 gm	0.18 gm	0.18 gm
2	Triethanolamine	0.18 ml	0.18 ml	0.18 ml
3	Glycerin	4.2 ml	4.2 ml	4.2 ml
4	Tween 80	1.2 ml	1.2 ml	1.2 ml
5	Almond oil	0.6 ml	0.6 ml	0.6 ml

6	Sodium benzoate	0.06 gm	0.06 gm	0.06 gm
7	Lemongrass oil	0.18 ml	0.18 ml	0.18 ml
8	Citric acid	0.3 gm	0.3 gm	0.3 gm
9	Distilled water	53.4 ml	53.4 ml	53.4 ml
10	Extract	0.25 gm	0.5 gm	1 gm

1.2 Procedure: Accurately weighed quantity of Xanthan gum was dispersed in water. Triethanolamine was added at last for desired consistency after adding remaining ingredients given in the table one bye one.[30]

- **EVALUATION OF FORMULATION**

1. Physiochemical parameters of serum

1. **Homogenisity:** The formulation was tested for homogenisity by visual appearance and touch.
2. **Appearance:** The appearance of the serum was judged by the color, pearl essence and roughness

2 Determination of pH

The pH test will be determined by using Digital pH meter. Dipper of digital pH will be deep into the sample of serum formulation and the pH value will be recorded. The pH of the formulation should having acidic pH as the skin is having an acidic pH of around 4-5.5. As the skin having an acidic pH around 4.1-6.7, this range of formulation isuitable for skin.[29]

3. Determination Spredability

Spredadability is defined as the area on the skin to which on application of serum is ready to spreds. Some size of filter paper are choose and each filter paper is measure the total area of filter paper (A1) and weighing of each filter paper (WI). Choose the formulation to be tested and drawn several ml into the 5ml of pipette then 20 drop of serum was put drop by drop in center of filter paper. When latest drop hits the filter paper, start a time or stopwatch to count down for exactly 10 minutes. During the 10 minute test, the liquid will spread in a relatively uniform circular pattern over the filter paper. After 10 minutes, exactly underline saturated spread and write the paper by using cutter. Weight the remaining dry (unsaturated) filter paper. Record this weight as W2. Measure diameter of the saturated portion of filter paper. If the spread was not a perfect circle then take several diameter reduce around a spread area and determine average diameter. Record this measurement as A2.[31]

$$\%\text{Spread by Area} = (A1/A2) * 100$$

Where, A1 = Total area of filter paper

A2 = Average diameter

4. Determination of viscosity

Viscosity is measured in Brook Field Viscometer, Principle: The most crucial factor in a Cosmetic product's assessment is viscosity. Many Characteristics, including s and pourability of the substance from the container, are governed by Viscosity. As a variety of factors, including changes in Temperature, modifications to the production process, and the quality of the raw materials, can impact viscosity. Thus, it is crucial to determine the product's viscosity. Procedure: Spindle No. 4 of a brook field viscometer was used to measure the viscosity of the serum, and all of the working parameters were then established.[29]

5. Tyrosinase Inhibition test

Tyrosinase inhibition activity was determined according To the modified method. Extract was dissolved in potassium Phosphate buffer (50 mM, pH 6.8) to 0.05 ppm. All steps were conducted at room temperature. extract (70 µl) was incubated with 30 µl of the Tyrosinase enzyme (333 U/ml in phosphate buffer, pH 6.8) For 5 min, followed by the addition of 2 mM of the Substrate, L-tyrosine (110 µl) and further incubated for 30 Min at RT, ensuring that effect of light is limited. Kojic Acid was used as positive control. The mixture of sample and other components, except L-tyrosine, was used as a Blank. Absorbance reading was performed at 475 nm. The Percentage of tyrosinase inhibition was calculated using

$$\% \; inhibition = \frac{A_{Control} - A_{Sample}}{A_{Control}} * 100$$

Where, % inhibition is the tyrosinase inhibition percentage. Acontrol is the absorbance of the control solution and Asample is the absorbance of the sample solution. For each Sample concentration, the absorbance was recorded by spectrophotometer[27]

6. Stability testing

Long term stability

Testing frequencies for product which proposed shelf life of at least 12 months, should normally be every three months over first year; every 6 months over the second year and annually there after throughout the proposed shelf life.

Study	Storage condition	
	Temperature	Relative Humidity
Long term	25°C ± 2 °C or 30°C± 2	60% ±5% or 65% +5

❖ **RESULTS**

2. Method

2.2 Extraction of Agaricus bisporus

Fig. Before drying.

Fig. After drying.

Fig. Soxhlet extraction.

Percentage of yield

The extract of agaricus bisporus in ethanol weighted and percentage yield was determined.

Sample used	Solvent used	Amount of sample (gm)	Amount of extract (gm)	Percentage yield (%)
Agaricus bisporus	Ethanol	10	3.157	31.57%

2.4 Preliminary phytochemical analysis

Phytochemical test	Agaricus bisporus Extract
Tannins	+
Saponin	+
Terpenoids	+
Phenol	+
Alkaloids	+

2.5 Tyrosinase inhibition activity

The tyrosinase inhibition activity of extract is checked using spectrophotometer absorbance at 475nm.

Sr no.	Absorbance at 475nm	% of Tyrosinase inhibition
Control	0.1174	-
Extract	0.0310	73.59%

Evaluation parameter of the formulation

Physical properties

Sr no	Parameters	F1	F2	F3
1	Appearance	Opaque	Opaque	Opaque
2	Colour	cream	Dark cream	Light brown
3	Homogenisity	Homogeneous	Homogeneous	Homogeneous

Determination of pH

Sr no	F1	F2	F3
pH	5.60	5.51	5.40

Determination of Viscosity

Sr. no	Viscosity
F1	1.533 pa.s
F2	1.433 pa.s
F3	1.500 pa.s

Determination of Spredability

Sr no.	Observation
F1	19%
F2	22%
F3	21%

Tyrosinase inhibition activity

Sr.no	Absorbance at 475 nm of Control	Absorbance at 475 nm of Sample	% of tyrosinase inhibition
F1	0.1193	0.0626	47.52%
F2	0.0862	0.0287	66.70%
F3	0.0807	0.0226	71.99%

Stability testing

Long term stability testing for three formulation as per ICH and product was found be stable.

IX. SUMMARY AND CONCLUSION

The aim of the present study is to formulate the skin whitening face serum containing agaricus bisporus extract to overcome the above condition.

The agaricus bisporus bodies extraction was carried out by using soxhlet apparatus by using ethanol as solvent. Percentage of yield was about 31%. Preliminary phytochemical test for extract was performed and showed positive results for tannins, phenol, terpenoids, saponin and alkaloids. Phytochemicals like phenol, tannins and terpenoids are for antityrosinase activity. Antityrosinase activity for extract was performed which was about 73%.

Evaluation parameters of all three formulation are carried. Physical parameter, pH, Spredability, Viscosity, and Antityrosinase activity. Tyrosinase inhibition of formulation were found increase as the amount of extract in the formulation was varied. The tyrosinase inhibition of all three formulation are F3 71.99%, F2 66.70%, F1 47.52%. it concluded that highest percentage of tyrosinase inhibition in Agaricus bisporus than the other species of mushroom.

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