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FORMULATION AND EVALUATION OF WHITE-OYSTER MUSHROOM (PLEUROTUS OSTREATUS) POWDER-BASED ANTI-OXIDANT CREAM WITH NO ADDED FRAGRANCE AND ARTIFICIAL PRESERVATIVES.

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Abstract: To meet the growing demand for natural skincare products, this study introduces a novel approach to address oxidative stress and inflammation by formulating and evaluating an antioxidant cream that utilizes white-oyster mushroom (Pleurotus ostreatus) powder as the primary active ingredient. The primary objective of this research is to develop a skincare solution that is free from artificial fragrances and preservatives while harnessing the potential benefits of white-oyster mushrooms in enhancing skin health. Specifically, the study focuses on investigating the antioxidant and anti-inflammatory properties of white-oyster mushrooms in skin care applications. To gain a comprehensive understanding of the active ingredient, the study includes an extensive analysis of its chemical composition, storage requirements, and microbial assay. Additionally, phytochemical testing is conducted to evaluate the antioxidant activity of the white-oyster mushroom powder extract, using the DPPH method. The findings of this study contribute to the advancement of knowledge in the field of natural skincare solutions and provide valuable insights into the development of gentle yet effective antioxidant creams. By utilizing white-oyster mushrooms as the primary active ingredient, this research aims to meet the demand for natural skin care products that can effectively combat oxidative stress, promote skin health, and potentially alleviate conditions associated with erythema and inflammation.

Key terms: White-oyster mushroom (Pleurotus ostreatus), antioxidant, anti-inflammatory, free from artificial fragrances and preservatives.

I. INTRODUCTION

The skin is continuously exposed to oxidative stress, originating from both internal and external factors. Internally, oxidative stress occurs as a result of natural metabolic processes, such as cellular respiration, which leads to the generation of reactive oxygen species (ROS) as byproducts. **Poljšak, B., & Dahmane, R. G. (2012) [1]** Externally, factors such as UV radiation, air pollution, and cigarette smoke further contribute to oxidative stress. This oxidative stress impairs the skin's barrier function, increases vulnerability to environmental pollutants, and exacerbates various skin conditions, including acne, rosacea, and eczema. Additionally, it enhances the permeability of damaged cell and mitochondrial membranes, resulting in aggravated damage caused by oxidative stress to cells and neighboring tissues, while triggering inflammatory responses that manifest as erythema. **Xi Chen, Chunsheng Yang and Guan Jiang (2022) [2]**

Although there is limited research directly linking erythema to oxidative stress, evidence suggests the involvement of oxidative stress in the development of several skin conditions characterized by skin redness. For instance, studies have indicated its role in conditions like rosaceaSchallreuter, **K. U., Krüger, C., Würfel, B. A., & Panske, A. (2015) [3]** and sunburn. **Trémezaygues, L., Reichert, S., Zillikens, D., & Ludwig, R. J. (2018)[4]** Effectively managing and preventing erythema and inflammation requires addressing the underlying oxidative stress. Antioxidants play a crucial role in neutralizing ROS and reducing oxidative stress within the skin. By providing protection against free radicals and promoting skin healing, antioxidants help mitigate inflammation, maintain skin health, and alleviate erythema. Topical application of antioxidant-rich formulations, such as creams or serums, can effectively combat oxidative stress, reduce erythema, and offer a defense against free radicals, while promoting overall skin rejuvenation.

In summary, while erythema can have multiple causes, oxidative stress can contribute to its development by triggering inflammation and vasodilation. Managing oxidative stress and incorporating antioxidants into skincare routines can be beneficial in reducing erythema and promoting healthier skin. The need for antioxidants in skincare arises from their ability to counteract oxidative stress and have anti-inflammatory properties, promoting healthier and more balanced skin.

Skin care products have emerged as essential tools for nurturing and enhancing the health and aesthetic of our skin. **Goh, C. L., Toh, M. P. H. S., & Ng, S. K. (2018)** [5] With the rise of natural and plant-based skincare solutions, there has been a surge in interest surrounding the utilization of ingredients derived from nature. **Draelos, Z. D. (2000)** [6] Among these ingredients, the white-oyster mushroom (Pleurotus ostreatus) stands out for its remarkable potential health advantages and bioactive components. **Ganceviciene, R., Liakou, A. I., Theodoridis, A., Makrantonaki, E., & Zouboulis, C. C. (2012)** [7] Notably, this mushroom species is known to exhibit antioxidant properties that can effectively shield the skin from oxidative stress and associated damage. **Cheung, P. C. K. (2008)** [8] Oyster mushroom have also been reported to possess moisturizing properties, providing hydration to the skin and helping to maintain its natural moisture balance. **Jayakumar, T., Thomas, P. A., & Geraldine, P. (2013)** [9] P. ostreatus is known for its rich nutritional composition, including proteins, dietary fibers, vitamins, and minerals.**Chang, S.-T., & Miles, P. G. (2004)** [10] It is also a valuable source of bioactive compounds with potential medicinal properties. Studies have highlighted the presence of various bioactive components in P. ostreatus, such as polysaccharides, phenolic compounds, terpenoids, and antioxidants. **Chen, H., Liu, Y., Yang, W., Shen, Y., & Tang, Y. (2019)** [11]

The exclusion of artificial fragrances and preservatives in the formulation aligns with the increasing consumer demand for natural and organic skincare products. Such additives have been implicated in skin sensitivities and allergic reactions, highlighting the need for safer alternatives. **Kim, S. P., Kang, M. Y., Choi, H. J., & Nam, S. H. (2012)** [12] Moreover, formulating the cream without these additives offers a gentler approach to skincare, appealing to individuals seeking healthier and more sustainable options.

II. Materials and Methods

2.1 Procurement of the active

The white oyster mushroom powder was procured from Rooted Active Naturals, Gurugram.

2.2 Evaluation of the active

The powder sample was subjected to qualitative and quantitative analysis to identify the Phytochemical composition , water soluble extract value, moisture content, pH and Microbial assay.

2.3 Determination of Antioxidant analysis by DPPH method

To assess the antioxidant capacity of the Pleurotus ostreatus mushroom extract, the DPPH (2,2-diphenyl-2-picryl hydrazyl) method was employed. **Genuis, S. J., & Lipp, C. T. (2012)** [13] Test tubes were utilized to hold a freshly prepared solution of DPPH (0.004% w/v) in methanol. Different concentrations of the methanolic extract of Pleurotus ostreatus (20, 40, 60, 80, and 100 µg/ml) were added to each test tube, resulting in a final volume of 3 ml. For comparison purposes, a positive control was prepared using ascorbic acid with methanol at identical concentrations, with 0.004% (w/v) DPPH solution added. A control sample devoid of any extract was included as well. Vigorous shaking of the test tubes was followed by incubation in darkness for 30 minutes. Subsequently, the absorbance of the reaction mixtures was measured at 517 nm, which corresponds to the peak absorbance of DPPH. By graphically determining the inhibitory concentration at which the DPPH absorbance is halved (IC50), the antioxidant activity of the extract was quantified. The scavenging activity was calculated as a percentage of inhibition using Eq.1.

$$\% \text{ inhibition} = (A0 - A1) / A0 \times 100 \quad (1)$$

Here, A0 represents the absorbance of the control sample, and A1 represents the absorbance of the reaction mixture. The Jain Research and Development Lab in Jalgaon conducted the quantitative analysis to evaluate the antioxidant activity of the Pleurotus ostreatus mushroom powder extract through the DPPH assay. The specific results obtained from this analysis are detailed below.

2.4 Formulation of cream base

An oil in water cream base was formulated. Three different base formulations, F1, F2, F3 were prepared. And the final selected base formulation is shown in the table 2.4. It had good consistency, viscosity, spreadability and required sensory properties. Hence, F3 was selected as the final base.

Table 2.4: Final Base Formulation

Sr No.	Ingredients	Quantity for 100 gms
1	Aqua	Upto 100
2	EDTA	0.1
3	Glycerine	2
4	Sodium Polyacrylate	1
5	Kokum Butter	2
6	Cetosteryl Alcohol	4
7	Cetyl Alcohol	2
8	Capric Capryl Triglyceride	2
9	Almond oil	2
10	Caprylhydroxamic Acid, Phenethyl Alcohol and Glycerin	1

To the selected base F3, Oyster mushroom (Pleurotus Ostreatus) Extract powder was added at the concentrations of 0.25, 0.45 and 0.64%

2.5 Analysis of the finished product

The finished product was analysed for total fatty matter, Ph and thermal stability. **BIS (I.S No. 6608-2004) for skin cream.** [14]

2.6 Accelerated stability test

To check the stability of the product various physical parameters like change in pH, colour, odour, were checked for one month as per BIS of skin cream **Sharma P.P, Cosmetic formulation , Manufacturing and quality control, 4th ed.; Vandana Publication Pvt Ltd Delhi, pp. 765.** [15]

2.7 Instrumental analysis of the finished product

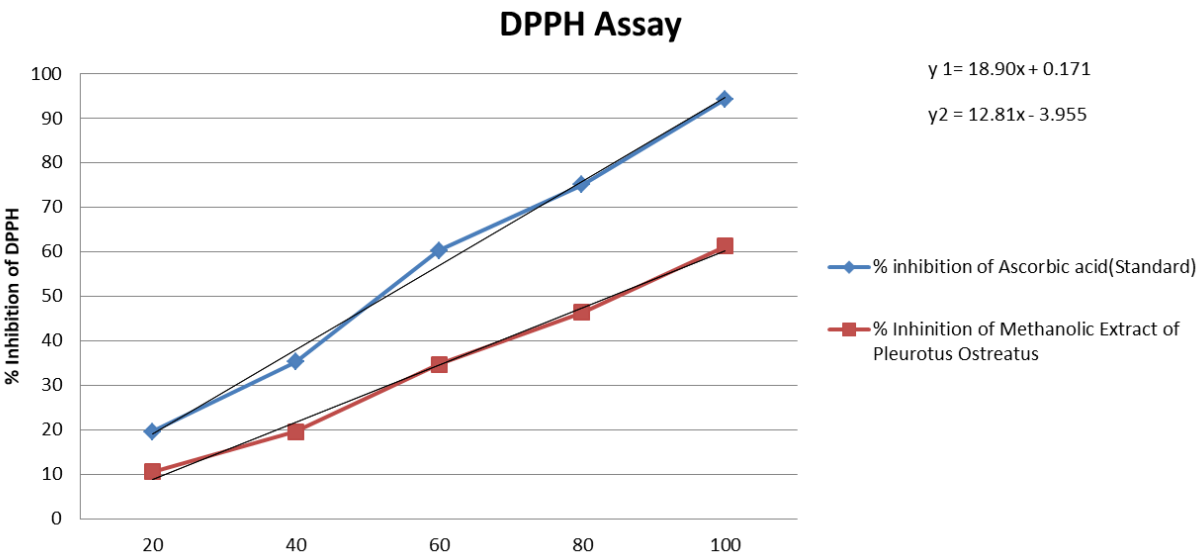
Skin was irritated with occlusive application of 3% SLS(Sodium lauryl sulphate) and perfume on flexor sides of both forearms for 24 hours in the form of patches(large Finn chambers (+12 mm) with filter disc) that were fixed to mark test sites. On the next day, 6 hours after removing the patches, 2 $mg \cdot cm^{-2}$ (40 mg) of each sample(F3,F4,F5) was applied gently at different patches until it was totally absorbed by the skin and it was left to dry down. The sample was applied twice a day for 9 consecutive days. Erythema index was measured using Mexameter MX18 (Cologne, Germany) on D1 (before treatment), D3,D5,D7 and D9. The visual erythema was investigated. During the investigation, the room temperature and humidity was maintained at 22˚C ± 1˚C and 50% ± 3% respectively.

III. Result and Discussion

3.1 Determination of the antioxidant activity of the active

The antioxidant activity was evaluated as decrease in absorbance as a result of DPPH color change from purple to yellow. The higher the sample concentration used the stronger was the free radical scavenging effect. It was found that 0.58% of ascorbic acid is equivalent to 0.75% of *pleurotous ostreatus* extract.

Figure 1: Graph showing DPPH Assay



3.2 Determination of dosage percentage of the active

Quantity of extract required for antioxidant property equivalent to ascorbic acid was calculated. In order to incorporate *pleurotous ostreatus* powder extract equivalent to 0.2-0.5% of ascorbic acid was calculated and found to be 0.25 and 0.64% respectively. Therefore, a minimum concentration ranging from 0.25-0.64% of *Pleurotus ostreatus* powder extract was found to be useful in skin care formulations.

3.3 Evaluation of the powder

Various parameters of the powder were tested. Flavanoids, terpenoids and phenols were found to be present in the phytochemical testing.

3.4 Determination of pH

The pH of the powder was found to be 4.99 and was within the standards.

3.5 Water-soluble extractive value

The water soluble extractive value for *Pleurotus osteratus powder* was found to be 85.50%

3.6 Moisture content

The moisture content for *Pleurotus osteratus* powder was noted to be 3.98%

3.7 Microbial assay

Escherichia coli, Salmonella and Total Coli forms were found to be absent in the *Pleurotus osteratus* powder.

3.8 Instrumental analysis of the final product

To the final cream base (F3) Pleurotus osteratus powder was incorporated in 0.25%, 0.45% and 0.64% as shown in the table 3.8.1.

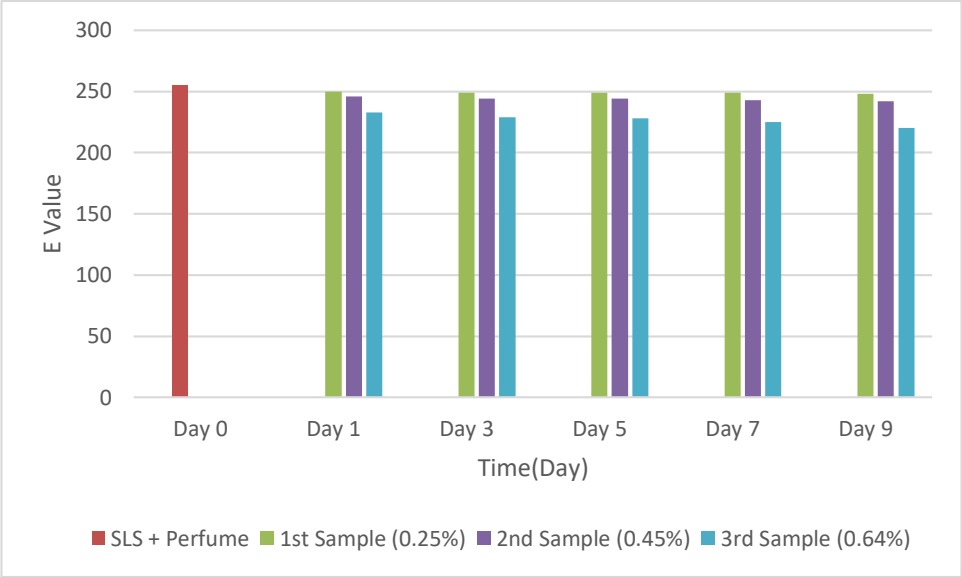
Table 3.8.1: Incorporation of active in Final base

Sr No.	Ingredients	F4 (0.25%)	F5 (0.45%)	F6 (0.64%)
1	Aqua	Upto 100	Upto 100	Upto 100
2	EDTA	0.1	0.1	0.1
3	Glycerine	2	2	2
4	Pleurotus Ostreatus Extract	0.25	0.45	0.64
5	Sodium Polyacrylate	1	1	1
6	Kokum Butter	2	2	2
7	Cetosteryl Alcohol	4	4	4
8	Cetyl Alcohol	2	2	2
9	Capric Capryl Triglyceride	2	2	2
10	Almond oil	2	2	2
11	Caprylhydroxamic Acid, Phenethyl Alcohol and Glycerin	1	1	1

Table 3.8.2: Determination of Erythema Value by Mexameter

Determination of Erythema Value by Mexameter						
Sample	**Day 0**	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**
SLS + Perfume	255	...	...	...	...	...
1st Sample (0.25%)	...	250	249	249	249	248
2nd Sample (0.45%)	...	246	244	244	243	242
3rd Sample (0.64%)	...	233	229	228	225	220

Figure 2 : Graph showing Erythema value



It was observed that skin cream with 0.64% of *Pleurotus ostreatus* powder extract showed more reduction in Erythema value in the skin as compared to other skin samples with 0.25% and 0.45% of active as shown in graph

3.9 Accelerated stability test

The accelerated stability studies of the finished product was carried out daily for a period of three month. Various physical parameters like change in colour, odour and pH were checked at oven temperature (45°C), room temperature and refrigerated temperature (7°C). It was found that there was no significant change in the colour, odour & pH. Product was found to be stable.

IV. Conclusion

It is concluded that the product was acceptable in view of colour, odour,consistency and anti-inflammatory effect. Anti-oxidant activity in powder was determined by DPPH method and studies revealed that the incorporation of *pleurotous ostreatus* powder equivalent to 0.2-0.5% of ascorbic acid is calculated and found to be 0.25 and 0.64% respectively. The *pleurotous ostreatus* powder was explored for its anti-inflammatory property on the skin. Cream with 0.64% of powder showed most reduction in Erythema. White oyster mushroom (*Pleurotous ostreatus)* has a potential to be used as Anti-oxidant and anti-inflammatory active ingredient in skin care products.

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