FORMULATION AND DEVELOPMENT OF SKIN HYDRATING CREAM WITH PLEUROTUS OSTREATUS EXTRACT

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Abstract

In recent years, skincare products with natural bioactive compounds are being exploited globally. Need of skin moisturization has become potential issue worldwide due to skin exposure to environmental pollutants, UV rays and various physiological disorders. Recently mushrooms have drawn worldwide attention as the most potential natural repository of polysaccharides and numerous beneficial phytochemicals. Hence an attempt was made to formulate skin hydrating cream with oyster mushroom (*Pleurotus ostreatus*) extract as it contains high concentration of polysaccharide β-glucan, which helps in increasing skin moisturization. β-glucan was extracted by Soxhlet extraction method using 95% ethanol as a solvent and the % yield was found to be 7.2%. β-glucan presence was further verified and confirmed by HPLC method. A hydrating cream was formulated using 0.2% extract which gave comparable results with standard cream withHyaluronic acid tested using Skin moisture analyzer SK III.β-glucan acts as an incredible humectant, pulling in moisture to skin and retaining it, thereby providing good hydration, and can be used as a safe andeffective alternative to syntheticmoisturizing agent.

Key words – white-oyster mushroom (*Pleurotus ostreatus*), hydrating, moisturizing, β-glucan

1. Introduction:-

Skin represents the most superficial layer of the body, and so it is constantly exposed to different environmental stimuli[1]. Continuous exposure to these stimuli may resultin loss of moisture content. When moisture is lost from stratum corneum more rapidly than it is received from lower layers of the skin it leads to dryness of skin.

Dry skin is an extremely common problem which can be induced by complex interactions between environmental and individual factors including areas with low humidity, excessive exposure to air conditioners,aging, psychological stress, eczema and other skin diseases[2-3].Frequentuse of cleansers,detergents, and topical irritants like alcohol and hot water can also remove the lipids from the skin's surface [4]. Various skin related issues can be caused due to skin barrier disruption. Most common issue is a loss of moisture content which leads to dryness of skin such as scaling, redness, roughness, fissures and an uncomfortable sense of tightness, often with itching and stinging.

Skin hydration or moisturization is one of the important part of daily skin care routine to maintain healthy appearance of skin and outer skin barrier. The presence of proper moisture content is required for lipid synthesis and barrier restoration[5]. Application of skin hydrating cream helps to maintain skin integrity and increases stratum corneum moisture content.

Various synthetic or natural moisturizing actives are used to formulate moisturizing cream. But synthetic actives can be sometimes harmful and toxic to both human health as well as nature[6]. Also demand for natural skincare product is increasing among consumers. 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[7]. Among these ingredients, the white oyster mushroom (*Pleurotus ostreatus*) stands out for its remarkable potential advantages and bioactive components [8].It has recently been exploited for its potential components in cosmetic industry.

The major structural component of mushroom is β-1,3-D-glucan. β-glucan is extracted from fruiting body of mushroom. β-glucan acts as humectant as it attracts the water to the top layer of the skin, they lock hydration in skin and prevents moisture loss and in result soothes and soften the skin [9].*Pleurotus ostreatus* also known to possess phenolics, polyphenolics, terpenoids, selenium,vitamins and volatile organic compounds[10,11].

2. Material and Methods

2.1 Procurement ofActive: -

Pleurotus ostreatus (oyster mushroom) was procured from Sky MushroomsFarm School, Nagpur, Maharashtra.

2.2 Extraction of Active: -

The *Pleurotus ostreatus* was dried, powdered and the extraction was carried out using Soxhlet extraction method.Fifty gram of the dried powder was added with 200mL of 95% ethanol and extracted in a Soxhlet apparatus for 4h(12 cycles) and percent yield was calculated [12-13]. [%Yield =(Total weight of extract ÷ weight of material)×100]

2.3 Phytochemical Screening of Active: -

The powdered sample was subjected to phytochemicalscreening to identify bio-actives present in sample [14].

2.4 HPLC Analysis: -

The analysis of chemical constituent was done by high performanceliquid chromatography method (HPLC). The extract of *Pleurotus ostreatus* (oyster mushroom) and standard β-glucan were analyzed by HPLC supplied with S2100 quaternary gradient pump and fluorescence detector RF-20A(UV280). The condition analysis of β-glucan; mobile phase:dH_2O andorthophosphoricacid(90:10v/v);column:C18 -ODS(25cmx4.6mm);Flow rate=0.7mL/min. These separations occurred on liquid chromatography and the elutedpeaks were monitored by UV-Vis10A-SPD spectrophotometer[15].

2.5 Formulation and Development of Cream Base: -

Oil-in-water (O/W) cream was selected as a base for incorporation of *Pleurotus ostreatus* extract. Various bases of different concentrations of ingredients were formulated as shown in Table No. 1.

Ingredients	**Quantity (in %)**		
Phase A	**F1**	**F2**	**I**
Stearic Acid	4	4.5	5
Cetyl Alcohol	-	2	2
Mineral Oil	8	8	8
Propyl Paraben	0.1	0.1	0
Phase B			
Triethanolamine	0.5	1	1
Glycerin	0.5	1	1
Methyl Paraben	0.1	0.1	0
Water	q.s.	q.s	q

Table No.1 – Formulation and Development of Base

Process of Formulation: - Phase A and B were taken in separate beakers. Both the beakers were heated 75º- 80ºC temperature. Then phase A to phase Bwere mixed in a mortar and pestle and triturated well to achieve the final cream base[16].

2.6 Incorporation of Active in Selected Base:

Pleurotus ostreatus extract was added in selected base (F3) at different concentrations.

Ingredients	**Quantity (in %)**				
Phase A	**F4**	**F5**	**F6**	**F7**	**F8**
Stearic Acid	5	5	5	5	5
Cetyl Alcohol	2.5	2.5	2.5	2.5	2.5
Mineral Oil	5	5	5	5	5
Propyl Paraben	0.1	0.1	0.1	0.1	0.1
Phase B					
Triethanol -amine	1	1	1	1	1
Glycerin	0.5	0.5	0.5	0.5	0.5
Methyl Paraben	0.1	0.1	0.1	0.1	0.1
Hyaluronic Acid	0.3	-	-	-	-
Pleurotus ostreatus	-	0.1	0.2	0.3	0.4
Water	q.s.	q.s.	q.s.	q.s.	q.s.

Table No.2. Incorporationof *Pleurotus ostreatus* Extract in selected base(F3)

2.7Analysisof Finished Product Using Skin Moisture Analyzer:-

For performance evaluation of skin hydrating cream, 30 subjects were selected. Equal ratios of subjects with three different skin types i.e., dry,normal and oily skin were chosen. The creams sample F4(0.3%) Hyaluronic acid as standard cream and F5(0.1%), F6(0.2%), F7(0.3%), F8(0.4%)with *Pleurotus ostreatus*active were applied and skin moisture content was measured by skin moisture analyzer SK-III.

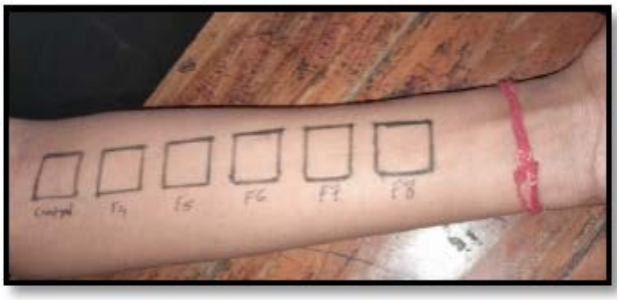

Fig.1. Patches for Analysis

Small amount of cream was given to all volunteers to carry out patch test 48 hrs. before the final analysis to confirm that volunteers are not allergic to cream.Skin of the arm of all the volunteers was disinfected and dried. The blank reading of normal skin was noted. 6 patches (2cm × 2cm) weremade with the help of marker as shown in Fig.1.Skin hydrating cream(F4 – F8) were applied and massaged gently and reading was taken after every 15 min till 120 min. After each reading the probe was cleaned,to avoid error.

2.8 Evaluation of selected finished products:-

Evaluation of the finished product was carried out by –

a) **Determination of pH:-**Using pH meter equipped with glass electrode [17].
b) **Determination of Total Fatty Matter Content:-**Theemulsionwas broken with dilute mineral acid and the fatty matter was extracted with petroleum ether. It was weighed after removal of the solvent [17].
c) **Determination of spreadability:- C**ream was spreadover the area to check its spreadability on application to skin [18].
d) **Accelerated stability testing** of selected base was carried out, in which, sample was kept at all three temperature i.e., room temperature(27±2ºC), oven temperature(45±2ºC) and fridge temperature(4ºC). It was assessedfor any changes in color, odor and pH at intervals of 72 hrs.[19].

3.Results and Discussion:-

The skin covers the outer surfaceof the body. Moisture in skin helps to repair itself constantly. Application of moisturizing cream helps to maintain skin integrity and well being by providing healthy appearance to an individual.Natural moisturizing agent is always considered safe to use as compared to synthetic moisturizing agent. One of the natural ingredient which can act as a natural moisturizing agent is*Pleurotus ostreatus* as it contain high level of β-glucan which serves the function of skin moisturization.The main aim of the study was to formulate and develop Skin Hydrating Cream with *Pleurotus ostreatus* (oyster mushroom) extract and it was further studied for its skin hydrating property.

The fresh *Pleurotus ostreatus* were dried, powdered and extracted by Soxhlet extraction method using ethanol(95%) as solvent. Extraction procedure was carried out for 4 hrs(12 cycles) and % yield was calculated and was found to be 7.2%. This extract was further subjectedto phytochemical screening and results obtained are shown in Table No.3.

Table No. 3 Phytochemicals Screening

Phytochemical Analysis	Inference
Carbohydrates	+
Alkaloids	+
Terpenoids	+
Steroids	-
Flavonoids	+
Saponins	+
Phenols	-
Tannins	-

Phytochemical screening of ethanolic extract of*Pleurotus ostreatus*showed presence of carbohydrates, alkaloids, terpenoids, flavonoids, saponins which shows that oyster mushroom is rich in various bio-actives.

The further analysis of the extract was performed by HPLC method. The separation occurred on liquid chromatography and, the eluted peaks were monitored by UV-Vis 10 A-SPD spectrophotometer. The Fig.2 revealed peak in standard β-glucan at retention time 2.94

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min and Fig.3 showed presence of β-glucan in *Pleurotus ostreatus* extract at retention time 3.16 min.This indicates the purity of extracted β-glucan and similar retention time of standard and sample indicates efficient method of extraction.

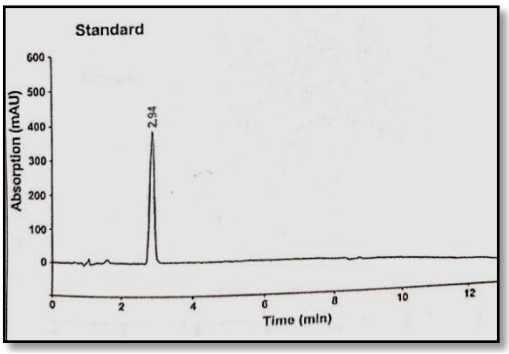

Fig. 2. Retention Time Graph of Standard β-glucan by HPLC.

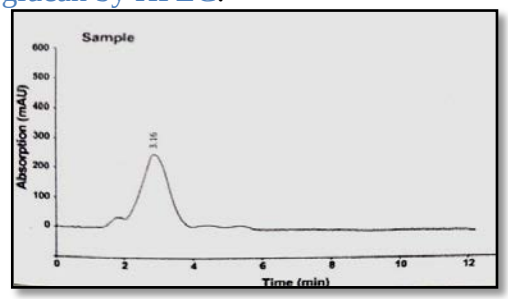

Fig. 3. Retention Time Graph of Sample β-glucan by HPLC.

For formulation of skin hydrating cream, oil-in-water (O/W) cream was selected as a base for incorporation of active because O/W type of creams are less greasy, more stable,more comfortable and consumer appealing as compared to water-in-oil creams. The base was formulated by trial and error method as shown in Table no. 1. Formulated base F1 had loose consistency,therefore,base F2 was formulated with some modifications i.e., addition of cetyl alcohol as a viscosity modifier to get desired consistency. The base F2 still found to have loose consistency hence; F3 with increased concentration ofboth, stearic acid and cetyl alcohol was formulated. The cream (F3) obtained now had acceptable consistencyin terms of aesthetics and stability and henceforth selected for incorporation of *Pleurotus ostreatus* extract.Creams sampleF5,F6, F7, F8 with various concentrations (0.1-0.4%) of*Pleurotus ostreatus*extracts were formulatedas shown in Table No.2.

To ascertain the moisturizing activity of formulated skin hydrating creams, the subjective analysiswas performed using Skin Moisture Analyzer SK III. For performance evaluation of cream samples, 30 subjects of different skin types(dry, normal and oily)were selected. The skin hydrating cream samples F5, F6, F7 and F8 were applied and results were noted using moisture meter. The efficacy of natural moisturizing agent i.e., *Pleurotus ostreatus* extract was compared with commercially proved synthetic moisturizing agent Hyaluronic acid. A cream with 0.3% Hyaluronic acid (used in range of 0.3-1%) was formulated and used as a standard for this study[20].Volunteers with dry skin reported to have initial average skin moisture content at 20.1 determined with moisture analyzer. Fifteen minutes post application of cream samples with active *Pleurotus ostreatus* extract there was a rapid rise in skin moisture content which continued upto one hour of application as shown in Fig.4.

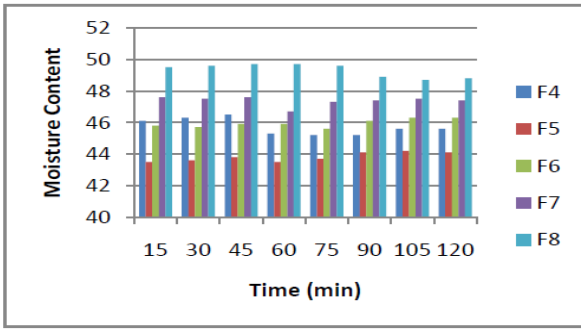

Fig.4. Moisture Content of Dry Skin

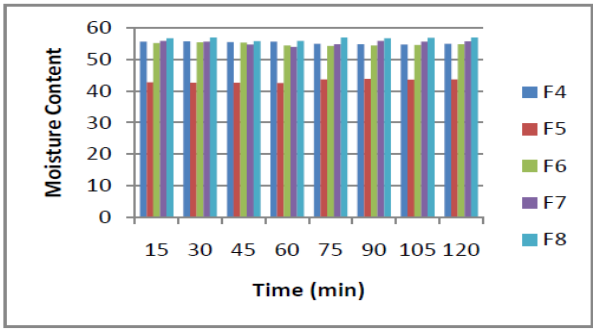

Fig.5. Moisture Content of Normal Skin

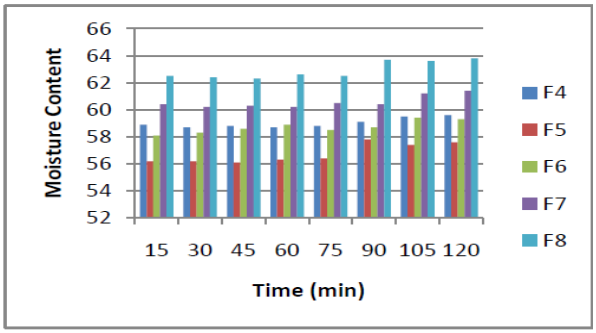

Fig.6. Moisture Contentof Oily Skin

There afterobserved a slight fall in moisture content which may be attributed to trans epidermal water loss (TEWL) due to dry nature of skin. Further it maintained the moisture level at average value of 46.7. At the end of study dry skin turned into supple and healthy skin.This result was comparable with standard Hyaluronic acid cream.

For normal skin, initial average moisture content was found to be 35.5. After application of cream samples normal skin showed sudden rise in the moisture content which was found to be maintained till the end of the study and same result were seen with standard cream (Fig. 5).

For volunteers with oily skin, the initial moisture content was high (40.3). Post application of cream samples moisture content increased gradually and maintained plateau till the end of study as shown in Fig.6.This high moisture content level is due to excess sebum secretionin oily skin. Fig.7shows the average values of moisture content of all the subjects.

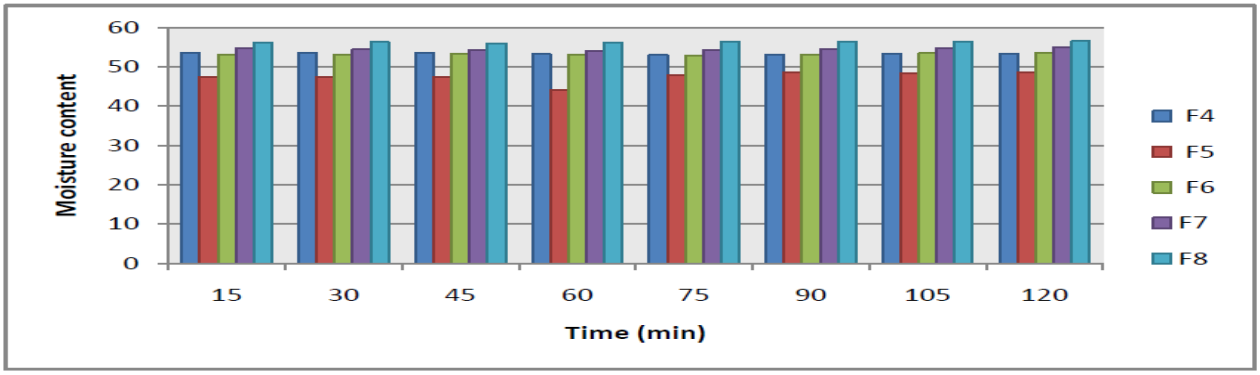

Fig.7. Graph of Moisture Content of All Subjects

The research revealed that the extract of*Pleurotus ostreatus*demonstrated significant moisturizing efficacy in dry normal and oily skin. The extract showed improved skin hydration and reduced trans epidermal water loss (TEWL) in all skin types enhancing overall skin health.The minimum concentration of active required to give moisturizing property comparable to Hyaluronic acid was found to be F6(0.2%). Hence, the skin hydrating cream(0.2%) was selected as final product and was subjected to further evaluation.

The evaluation of the skin hydrating cream F6(0.2%) with *Pleurotus ostreatus* included determination of pH, total fatty matter content spreadability as per BIS specification. All the results were obtained are shown in Table No.4.

Table No.4 Evaluation of Cream

Analysis	Standard Range	Result Obtained
pH	4.0 to 9.0	6.46
T.F.M. content	Min. 5%	15.50%
Spreadability	9.0 to 31.02g.cm/s	10.2g.cm/s

pH of the cream was found to be 6.46 and T.F.M. content of the cream was 15.50% which was obtained within the standard range as per BIS.Spreadability of the cream was 10.2g.cm/s which shows that the formulated cream have good spreading ability.

The purpose of accelerated stability studies was to ensure that the cosmetic product maintains its intended physical, chemical properties,as well as functionality and aesthetics when stored under appropriate conditions. Accelerated stability study of selected skin hydrating cream (F6) was done.

The accelerated stability testing shows that there were no significant changes in color and odor. pH determination of skin hydrating cream was done at all the three temperatures i.e., room temperature, oven temperature and fridge temperature.

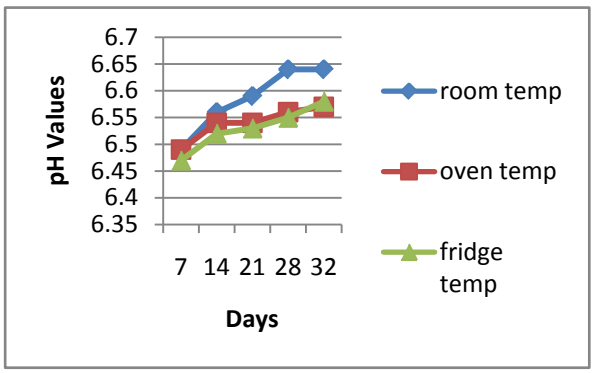

Fig.8. Changes in pH of Cream

Fig.8 shows the changes in pH of formulated cream F6 over time period of 32 days. It

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showed very little changes in pH of creamat all the three temperatures.

It was concluded that the formulated cream was skin friendly and non-irritant.TheSkin Hydrating Creamwith 0.2%*Pleurotus ostreatus* extract was found to be stable in terms of all functional parameters.

4. Conclusion:-

Pleurotus ostreatus extract contains high level ofpolysaccharide (β-glucan)which is able to deeply penetrate the skin and hydrate the skin. It boosts the natural skin barrier which protects the sensitive skin underneath from daily environmental stressors. Thus, it gives deep and lightweight skin hydration.It was observed that the skin hydrating cream with 0.2%of*Pleurotus ostreatus* extract was found to be stable in all physical parameter and had given best results in performance evaluation in terms of skin hydration. The product found to be successful in enhancing moisture level of the skin. Moisturizing efficiency of formulated Skin hydrating cream with *Pleurotus ostreatus* was found to be comparable in efficacy with synthetic moisturizing agent Hyaluronic acid which is commercially established having high consumer acceptability. Thus, *Pleurotus ostreatus* extract can be successfully projected as an effective natural alternative to synthetic hydrating agent.

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