Extraction, Isolation and Evaluation of Pitera from Fermented Rice water and its Incorporation as Active in Bi-phasic Makeup Removal

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Abstract: Rice is one of the major cereals and essential food for Indian population. It is also Asian’s best beauty secret. It is rich in minerals and vitamins that make it fantastic for both skin and hair. Rice water has many properties of cosmetic value, it’s effects are further enhanced, if left to ferment. Fermented rice water is a rice water that is left to ferment and has gone slightly sour. It is rich in antioxidants, minerals, B vitamins, vitamin E, and traces of Pitera, a substance produced during the fermentation process. It is also known as Saccharomyces Ferment Filtrate, a natural by-product of yeast fermentation containing amino acids, minerals, vitamins & organic acids. The objective of present study is to explore the properties of fermented active in skin care since, researches regarding fermented active is not overly abundant. Furthermore, fermentation process helps to preserve the product’s shelf life with anti-microbial property and also increase the nutrient density, making the product’s natural ingredients more powerful. In the present study, rice water was fermented and evaluated for the presence of Pitera and incorporated as an active in Bi-phasic Makeup Removal. The product was evaluated for its cleansing and moisturizing properties.

Keywords: Antioxidants, Saccharomyces is ferment filtrate, Minerals, Vitamins, Antibacterial

1. Introduction

India is an important centre for rice cultivation and consumption. India stands in second position after China in the production of rice. This is also staple food of the people living in the eastern and the southern part of the country, especially in Asia. Fermented foods share an integral part of age-old from ancient Indian civilization. Rice based fermented foods with different tastes and textures are prepared by rural women following village art technique since years. [1]

It is the agricultural commodity with the third-highest worldwide production. Rice grain is rich in nutrients, vitamins, and minerals and is the staple food for more than 3 billion people. [1]

Rice is grown across the world, and there are many varieties of rice and different ways of cultivating them. However, all rice plants share common features and go through three main stages of growth – vegetative, reproductive, and ripening – before the seed can be harvested. [2]

Biological Source

Rice, is the seed of grass species that belongs to the genus Oryza. The two species are Oryza sativa (Asian rice) and Oryza glaberrima (African rice). The Oryza sativa is the most commonly grown species throughout the world while Oryza glaberrima is grown only in South Africa. [3]

Taxonomical Classification [4]:

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clade</td>
<td>Angiosperms</td>
</tr>
<tr>
<td>Clade</td>
<td>Monocots</td>
</tr>
<tr>
<td>Clade</td>
<td>Commelinids</td>
</tr>
<tr>
<td>Order</td>
<td>Poales</td>
</tr>
<tr>
<td>Family</td>
<td>Gramineae</td>
</tr>
<tr>
<td>Genus</td>
<td>Oryza</td>
</tr>
<tr>
<td>Species</td>
<td>O. Sativa</td>
</tr>
</tbody>
</table>

Geographical Sources

Today, the majority of all rice produced comes from China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Pakistan, Philippines, Korea and Japan. Asian farmers still account for 87% of the world's total rice production. [5]

Benefits of Fermented Rice Water

The Yao ethnic women from the village of Huangluo in China is a testament to the tradition of using Rice Water. Female rice farmers in China, Japan, and other Southeast Asian countries used to bathe and wash in the water used for cleaning rice. With their average hair length of about 6 feet, these women made it to the Guinness Book of World Records as the “world’s longest hair village”. These women do not have grey hair till the late 80s. The Yao women believe that the fermented rice water, which they use to cleanse their hair, is what helps to keep their hair long, dark and clean. Rice water enables these women to de-tangle and manage their long hair. It is used for centuries to attain crystal clear skin and shiny hair by women. [6]

Rice water is a milky liquid left after washing rice which can be used for taking care of skin and hair. When the rice is cooked in water, due to high-temperature rice releases some of its starch in that water, the colour and turbidity of a medium is conditioned by rice starch. Rice water also has many skin benefits. Due to its cooling and soothing effects on skin. Rice water also has moisturising, antioxidant, and healing properties. All these help to improve circulation, prevent or fade age-related spots, and ease inflammation to give you soft, smooth and radiant skin. As long as it’s left on the skin, rice water is even believed to offer mild protection from the sun. [9]

The effects of rice water are further enhanced, if it is fermented. Fermented rice water is rice water that is left to ferment and has gone slightly sour. It is rich in antioxidants, minerals, B vitamins, vitamin E, and traces of Pitera, a

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substance produced during the fermentation process which is by-product of Saccharomyces fibuligera yeast. Fermented rice water can be use as a face cleanser, skin toner and to condition hair. The nutrients in the fermented rice water are believed to shrink pores, reduce fine lines, and tighten and brighten skin – this is a perfect recipe to look radiant and youthful. It also contains phenolic and flavonoid compounds, which can minimize free radical damage from environmental factors. [9]

Plain rice water’s pH is higher than skin’s pH i.e. 5.5. Fermentation brings down the pH levels of the rice water to that of skin and helps close the pores thereby protecting the skin. Fermented rice water has acidic pH which is same as that of the skin. [10]

2. Material and Methods

Collection and Authentication of Rice

Collection: The rice grains (HMT species) were collected from local market at Nagpur. The collected sample of rice was washed twice with tap water to remove any dirt or impurities and then dried.

Authentication: These rice grains were taken to the Botany Department of Rashtrasant Tukdoji Maharaj, Nagpur University, Nagpur for its authentication.

The Authentication number of Rice Grains is 10127

Method of extraction

The washed rice was placed in a bowl and covered with water. The rice was boiled for about 15 to 30 minutes. Swirled it around or lightly kneaded it until the water turned cloudy. The water was collected in the container. After collecting rice water in a container, it was left in warm place for 3 days until it turned slightly sour indicating that it has started to ferment. pH of the water was taken to compare it with skin’s pH (5.5) which indicated that rice water was fermented as required. It was kept in refrigerator for 2 hours to let it cool to stop further fermentation process and stored in a glass container. [11]

Drying of extract

The fermented water was dried by storing at 4°C in refrigerator, the dried powder extract obtained was stored in a glass container. [11]

Phytochemical analysis of the Powdered Extract

Phytochemical studies were conducted qualitatively to identify the presence of bioactive chemical constituents like alkaloids, flavonoids, glycosides, steroids, phenols, tannins, proteins. [12, 13]

Identification of the Saccharomyces Fibuligera Yeast in Dried Extract [14]

- Sterilization of glasswares and media was done in autoclave.
- 1% conc of extract was cultured on saboured dextrose agar plate and incubated at 30°C for 2days.
- A drop of lacto phenol cotton blue stain was placed on a clean slide.
- Some culture was removed from modified SDA plate and mixed with the drop of stain. The slide was heated slightly using spirit lamp.
- The cover slip was placed and observed under high dry objective lens.
- The Presence of yeast was identified by its characteristic features.

Product Formulation

Biphasic Makeup Removal [15]

This is a two-phase, or Biphasic Make-up Remover – it’s made up of two separate oil and water – based layers. When the bottle is shaken, the layers mix to create a product that easily removes all make-up, including stubborn waterproof mascara. This remover is super – gentle on the skin.

The water phase helps to soften and soothe the skin, while the upper phase contains highly purified oils to help eliminate all traces of make-up.

The idea of applying oil can seem daunting, especially for those with grease-prone skin, but this leaves little to no residue.

It’s also a great option for anyone with sensitive eyes and skin.

Formulation and Development

Selection of Base

Biphasic Makeup Removal was selected as a base for the product because it contains less amount of chemicals and maximum oils were obtained from natural source making it more natural. It also increases the aesthetic appeal of the product.

Procedure of Preparation of Solution of Dried Powder Extract of Pitera

For making 1% concentration of solution of dried powdered extract, 1gm of extract has to be dissolved in 100 ml hot distilled water [12], for experimentation 0.582 gm was dissolved in 58.2 ml of hot distilled water.
For making 2% concentration of solution of dried powdered extract, 2gm of extract has to be dissolved in 100ml of hot distilled water [12], for experimentation 1.164 gm was dissolved in 58.2 ml of hot distilled water.

Similarly, for 3% concentration of solution of dried powdered extract, 1.746 gm was dissolved in 58.2 ml of hot distilled water.

**Procedure for Formulating the Product**
1) All the apparatus required were washed and dried.
2) In 3 different beakers, all the oil phase ingredients were measured and antioxidant, Butylated Hydroxyl Toluene was added.
3) In another beaker, solutions of different concentrations of active were taken.
4) To these solutions sodium benzoate was added as a preservative and synthetic blue color for aesthetic appeal.
5) The oil phases were poured in water phase beakers of different concentrations and perfume was added.
6) The formulated product was filled in the spray container and labelled.

**Table 1: Trials Formulation of Bi-phasic Makeup Removal**

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Ingredients</th>
<th>Trials 100%</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mineral oil</td>
<td>16</td>
<td>15</td>
<td>14</td>
<td>12</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sunflower oil</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Olive oil</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Almond oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Neem oil</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Silicone oil</td>
<td>6.8</td>
<td>6.8</td>
<td>6.8</td>
<td>6.8</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Solution of dried powder of fermented rice water</td>
<td>51.2</td>
<td>51.2</td>
<td>54.2</td>
<td>58.2</td>
<td>58.2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>BHT</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Sodium Benzoate</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Perfume</td>
<td>58.2</td>
<td>58.2</td>
<td>58.2</td>
<td>58.2</td>
<td>58.2</td>
<td></td>
</tr>
</tbody>
</table>

**3. Observations of Trials**

*The ratio of oil: water 58.2:32.8 was selected as final ratio for formulating the Bi-phasic Makeup Removal.

F1 - Mineral oil : 16%, Active :51.2%, Neem oil : 6, Color : 0.01g, Perfume : 2ml
F2 - Mineral oil : 15%, Active :51.2%, Neem oil : 6, Color : 0.01g, Perfume : 2ml
F3 - Mineral oil : 14%, Active :54.2%, Neem oil : 6, Color : 0.02g, Perfume : 1ml
F4 - Mineral oil : 12%, Active :58.2%, Neem oil : 5, Color : 0.015g, Perfume : 1ml
F5 - Mineral oil : 11%, Active :58.2%, Neem oil : 4, Color : 0.015g, Perfume : 1.5ml

**Table 2: Final Formulation of Bi-phasic Makeup Removal**

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Ingredients</th>
<th>For 100%</th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mineral oil</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sunflower oil</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Olive oil</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Almond oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Neem oil</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Silicone oil</td>
<td>6.8</td>
<td>6.8</td>
<td>6.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Concentration of Powdered Extract</td>
<td>1%</td>
<td>2%</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>BHT</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Physical properties of Bi-phasic Make-up Removal**

After formulating the product its physical properties were noted down.

<table>
<thead>
<tr>
<th>Observation</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Blue and yellow</td>
<td>Blue and yellow</td>
<td>Blue and yellow</td>
</tr>
<tr>
<td>Fragrance</td>
<td>Pleasant</td>
<td>Pleasant</td>
<td>Pleasant</td>
</tr>
<tr>
<td>Appearance</td>
<td>Bi-phasic</td>
<td>Bi-phasic</td>
<td>Bi-phasic</td>
</tr>
<tr>
<td>Consistency</td>
<td>Flowable</td>
<td>Flowable</td>
<td>Flowable</td>
</tr>
<tr>
<td>Irritancy</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>7.2</td>
<td>7.3</td>
</tr>
</tbody>
</table>

**Accelerated Stability Test for Bi-phasic Makeup Removal Products**

The most important consideration with respect to cosmetic formulation is the stability of the finished products. The following stability parameters were studied for the product.

1) Color
2) Fragrance
3) pH
4) Viscosity

All the samples of Bi-phasic Makeup Removal were subjected to accelerated test conditions and stability study was performed.

Room temperature -30 ± 2°C
Oven-45 ± 2°C
Fridge-4 ± 2°C

*For further studies 2% and 3% concentration of Bi-phasic Makeup Removal were selected.

**Determination of Total Microbial Count of 2% and 3% concentration of the Product**

**4. Procedure**

**Sterilization of Apparatus:** All the apparatus were cleaned, dried and sterilized. The sterilization was done by wrapping the apparatus in the paper. The autoclaving is done in a hot air oven at 150 °C for 1 hour. [16]

**Preparation of Media:**
1) 65.0 g SDA was dissolved in 1000ml of distilled water. Similarly, 40g of SCDA was dissolved in 1000ml of distilled water
2) It was kept for 20 minutes for proper dissolution of agar by heating on the water bath.
3) The media was transferred into clean conical flask after dissolution with tight fitting cotton plug.
4) It was sterilized in an autoclave at 121°C and lbs. pressure for 20 minutes. [16]

**Method**
1) 1ml of product and 9ml of peptone water was weighed. It was transferred in sterile test tube.

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2) Similarly, 1:1000(10⁻³), 1:10000(10⁻⁴) dilutions was prepared from the above samples using same diluents.
3) 1ml of each dilution was aseptically transferred in petridish using sterile pipette for SCDA and SDA media.
4) The media was quickly poured into the petridishes to avoid solidification of the medium or formation of lumps because of rapid drop in temperature.
5) The medium was allowed to solidify and petridishes were quickly poured into the petri dishes to solidify and formation of lumps was prevented.

Procedure modified as required

Evaluation of Moisturizing Effect of Bi-phasic Makeup Removal using Corneometer

This was performed to check the moisturizing effect of formulated product i.e. Biphasic Makeup Removal with active on skin. For this method the instrument used was Corneometer. [18]

The measurement of skin moisture is based on Corneometer also known as Capacitance method. In vivo analysis was carried out on 4 subjects. In practice, the use of Corneometer, is the technique, which measures Corneum hydration before and after application of a cosmetic. It is used in industries as it is one of the easy and most effective as well as reliable method. [18]

Plate 2: Corneometer

**Principle of Corneometer:** The measuring principle of Corneometer is based on capacitance measurement of a dielectric medium. Any change in the dielectric constant due to skin surface hydration variation alters the capacitance of a precision measuring capacitor. [17]

The dielectric constant of a material is a number that reflects the electrical properties of a material by its level of hydration, the greater the water content, greater the dielectric constant. [17]

Corneometer consist of a probe which is used to measure the dielectric constant of the skin. [17]

Interpretation of Results [19]:

The following table is valid for healthy skin at normal room condition 30° and 40% air humidity.

<table>
<thead>
<tr>
<th>Type</th>
<th>Inner Forearm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Dry</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Dry</td>
<td>30-45</td>
</tr>
<tr>
<td>Sufficiently Moisturized</td>
<td>&gt;45</td>
</tr>
</tbody>
</table>

Moisture Measurement

1) 4 subjects were selected for this test, the volar forearm of subjects were cleaned and dried.
2) 2×2 cm 5blocks were drawn on the hands of subjects for blank, base, 1%, 2% and 3 % concentration of bi-phasic make-up removal.
3) The probe head was placed vertically on the skin surface on the marked sites with little pressure for one second and reading was noted displaced by Corneometer as a blank.
4) Similarly the readings were taken for the reference, 1%, 2% and 3% sample at 0 minutes, after 30 minutes and 60 minutes.
5) The average was taken for all subjects after every interval and graph was plotted.

Plate 3: Moisture Measurement using Corneometer on Volar Forearm of Subject

Table 4: Study of Skin Moisturizing Property of Bi-phasic Makeup Removal

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Concentration</th>
<th>0 min</th>
<th>30 mins</th>
<th>60 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject-1</td>
<td>Blank</td>
<td>32.2</td>
<td>37.3</td>
<td>38.5</td>
</tr>
<tr>
<td></td>
<td>Base</td>
<td>49.5</td>
<td>32.9</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>51.2</td>
<td>38.3</td>
<td>32.9</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>55.5</td>
<td>35.3</td>
<td>38.5</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>48.6</td>
<td>34.8</td>
<td>39.8</td>
</tr>
<tr>
<td>Subject-2</td>
<td>Blank</td>
<td>42.1</td>
<td>36.7</td>
<td>32.4</td>
</tr>
<tr>
<td></td>
<td>Base</td>
<td>39</td>
<td>38.8</td>
<td>48.2</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>44.3</td>
<td>46.9</td>
<td>36.6</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>53.3</td>
<td>48</td>
<td>51.3</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>34.8</td>
<td>41.1</td>
<td>49.3</td>
</tr>
<tr>
<td>Subject-3</td>
<td>Blank</td>
<td>48.7</td>
<td>40.5</td>
<td>41.8</td>
</tr>
<tr>
<td></td>
<td>Base</td>
<td>48.8</td>
<td>42.1</td>
<td>46.2</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>48.4</td>
<td>43</td>
<td>43.9</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>51.5</td>
<td>45.2</td>
<td>41.8</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>48.3</td>
<td>40.4</td>
<td>41.3</td>
</tr>
<tr>
<td>Subject-4</td>
<td>Blank</td>
<td>42.8</td>
<td>37.3</td>
<td>34.9</td>
</tr>
<tr>
<td></td>
<td>Base</td>
<td>44.9</td>
<td>41.8</td>
<td>34.6</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>52.2</td>
<td>47.4</td>
<td>41.9</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>63.7</td>
<td>51.6</td>
<td>43.7</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>65.2</td>
<td>43.4</td>
<td>40.5</td>
</tr>
</tbody>
</table>
Subjective Evaluation
Subjective evaluation was carried on 10 different subjects of different age group, skin type. For Practice purpose subjects were given numbers like S1, S2, S3, S4 etc.

Procedure:
Directions to use the product:
1) 10 subjects were selected for this test, the volar forearm of subjects were cleaned and pat dried.
2) 2x2 cm 4 blocks were drawn on the hands of subjects for base, 1%, 2% and 3 % concentration of bi - phasic make-up removal and different makeup products i.e. liner, eye-shadow, foundation and lipstick was applied.
3) The cotton wipes were kept on the patches to let cotton absorb the product.
4) The makeup was wiped-off with cotton wipe.

5. Results and Discussion

Extraction yield
The 500 gm of washed rice (HMT) was covered with 1000 ml water. The rice was boiled for about 15-30 minutes and swirled. The 1000 ml of rice water was collected in the container and left for fermentation. The fermented water was freeze dried at 4°C. The powdered extract was weighed and collected in the glass container.

<table>
<thead>
<tr>
<th>Weight of rice used</th>
<th>Volume of water taken</th>
<th>Volume of extract obtained</th>
<th>Yield of Powdered extract(g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500gm</td>
<td>1000ml</td>
<td>700ml</td>
<td>8.9gm</td>
<td>1.27%</td>
</tr>
</tbody>
</table>

Phytochemical Analysis [13]

<table>
<thead>
<tr>
<th>Group of phytochemicals</th>
<th>Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alkaline reagent test</td>
<td>++</td>
</tr>
<tr>
<td>Phenols</td>
<td>Phenol test</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Keller Kiliani test</td>
<td>++</td>
</tr>
<tr>
<td>Proteins</td>
<td>Biuret test</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>Salkowski test</td>
<td>-</td>
</tr>
</tbody>
</table>

The Phytochemical studies were conducted based on qualitative analysis to identify the presence of bioactive chemical constituents. From the observation table, we can say that alkaloids, flavonoids, Glycosides and proteins were present where phenols, tannins and steroids were absent.

Identification of Saccharomycopsis Febuligera Yeast
The microbial study done to identify the presence of Saccharomycopsis Ferment Yeast by culturing the 1% concentration of extract on Sabouraud dextrose agar plate and incubating at 30°C for 2 days and then mixing some culture with lactophenol cotton blue stain and observing under high objective lens (10x). [20]

The presence of Saccharomycopsis Ferment Yeast was identified by observing its characteristic features. The culture creates budding yeasts cells, which are short oval or oval long branched. The yeast cell range from 5 to 8 µm in diameter. [21, 22]

Plate 5: Microscopic view of Saccharomycopsis Febuligera Yeast

Accelerated Stability Study Results
The following changes in Stability Parameters were observed:-

Color Change: Appearance of color of the product was observed visually with naked eye. The samples were kept at different temperature changes i.e. room temperature, oven and fridge the change in color if any was noted down.

No color change was observed in finished product of 1, 2, 3% concentration kept at different temperatures.

Fragrance Change: Change in its fragrance was observed by smelling the products kept at different temperature conditions and any change was noted down.

pH Change: pH changes were determined using pH meter of all the samples kept at different temperature conditions and changes were noted down. Slight change in pH was observed which ranged from 6.6 to 7.4 in finished product of 1, 2, 3% concentration kept at different temperatures.

Viscosity Change: Change in the viscosity at different temperatures of all the samples was observed by using Brook field Viscometer and changes were noted.

No viscosity change was observed in finished product of 1, 2, 3% concentration kept at different temperatures.

Total Microbial Count of 2% and 3% Concentration of Product
Counted the number of colonies in the plates, multiplied by the dilution factor to obtain the number of bacteria and fungi.
in each sample which is expressed as cfu/g or/ml of the sample. [16]

Total microbial count was obtained by adding the bacterial count and fungal count. Microbial limits for cosmetic finished product as recommended by CTFA/BIS guideline is 1000 \((10^3)\) Max. [16]

**Calculation:**

**Formula to Calculate TMC [16] -**

1) **Bacterial Count** = No. of bacterial count \times Dilution factor

2) **Fungal Count** = No. of fungal count \times Dilution factor

**Total Microbial Count** = bacterial count + fungal count = cfu/g or/ml

Colony counting was done for 3rd dilution since in 1st and 2nd dilution colonies were merged.

**Observation Table of TMC of Bi-phasic Makeup Removal**

<table>
<thead>
<tr>
<th>Concentration of Product</th>
<th>Bacterial Count (SCDA)</th>
<th>Fungal Count (SDA)</th>
<th>Total Microbial Count (TMC) cfu/g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>2%</td>
<td>7000</td>
<td>3000</td>
<td>10000</td>
</tr>
<tr>
<td>3%</td>
<td>3000</td>
<td>1000</td>
<td>4000</td>
</tr>
</tbody>
</table>

**For 2% biphasic make-up removal**

**For 3% biphasic make-up removal**

2% concentration of product showed 3 colonies in SDA plate with total fungal count 3000 whereas 7 colonies in SCDA plate with total bacterial count 7000.

3% concentration of product showed 1 colony in SDA plate with total fungal count 1000 whereas showing 3 colonies in SCDA plate with total bacterial count 3000.

**Evaluation of Moisture measurement using Corneometer**
6. Result Interpretation

1) From the graph 1 it can be stated that the increase in skin moisturization for 2% Product was 56 greater than 1% and 3%conc which were 45.55 and 49.22 when compared to control at 0 minute.

2) After 30 minute interval the skin moisturization in 2% Product was 45.02 which was greater than 1% and 3% Product when compared with control.

3) After 1 hour application of the product the skin moisturization for 2% Product was 43.82 which was same as 1% Product but was greater than 3% Product 42.72 when compared to control.

Subjective Evaluation

The products were evaluated for the following parameters and the results are summarized in the following table

Table 7: Subjective evaluation of Bi-phasic Makeup Removal

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Parameters</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S8</th>
<th>S9</th>
<th>S10</th>
<th>Av</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Color</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>Fragrance</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Irritancy</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>4</td>
<td>Tackiness</td>
<td>^^</td>
<td>^^</td>
<td>^</td>
<td>^</td>
<td>^</td>
<td>^</td>
<td>^</td>
<td>^</td>
<td>^</td>
<td>^</td>
<td>^</td>
</tr>
<tr>
<td>5</td>
<td>Cleansing Action</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>Moisturizing power</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>Ideal concentration</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Abbreviations : Excellent (+++), Very Good (++), Good (+), Irritancy (*), Non-Irritancy (**), Tackiness(^^), NonTackiness (^), Poor (#), Very Bad (##), Av (average)

Plate 10: Before application of Bi-phasic Makeup Removal

Plate 11: After Application of Bi-phasic Makeup Removal
7. Conclusion

The Phytochemical studies were conducted based on qualitative analysis, the phytochemical screening demonstrated the presence of different types of phytochemical constituents in fermented rice water extract including phenolic compounds, flavonoids, alkaloids which would be responsible for the biological activity and the presence of Saccharomyces ferment filtrate (Pitera), the active ingredient which enhances the benefits of the rice water.

Microbial evaluation was done to identify the presence of Pitera which is by-product of Saccharomyces Fehuligera yeast, & its characteristic feature was identified and confirmed using microscope.

Total five formulations were prepared, to formulate optimized Bi-phasic Makeup Removal. As mentioned in formulation and development table the ratio 58.2 to 41.8 of oil/water was selected as final ratio for the product on the trial basis.

Accelerated stability test was performed on all the concentrations of Bi-Phasic Makeup removal i.e. 1%, 2% and 3%. All the concentrations of product was found to be stable with respect to its color, fragrance, viscosity and pH. Total Microbial Count of 2% and 3% concentration of Bi-Phasic Makeup Removal was performed. It was observed that the products of both the concentration were not within the microbial limits as recommended by CTFA/BIS guidelines.

Since, many makeup removal products wipes-out the natural moisture from the layers of the skin making the skin dry and therefore, skin hydration test was performed for all concentration of Bi-phasic Makeup Removal, by using sophisticated instrument i.e. Corneometer. It was observed that the product containing 2% extract gave more skin hydration property as compared to 1%, 3% and base.

Subjective evaluation was also performed using all the concentrations of Bi-phasic Makeup Removal and the parameters such as color, odor, irritancy, tackiness, cleansing action, moisturizing action and ideal concentration of Bi-phasic Makeup Removal was evaluated, the results found were satisfactory.

After conducting all the tests and interpreting the results it was found that 2% Bi-phasic makeup removal was giving better cleansing action and also moisturizing . Thus this concentration was selected as a final concentration.

Since, Fermented Rice Water has many benefits and also the results obtained for cleansing and moisturizing property were satisfactory, we can conclude that it can be used in variety of products as active for its cosmetic value making it Asia’s best beauty secret.

8. Future Scope

This study supports further research:

1) To isolate the active constituents and elucidate antimicrobial property from different species of rice
2) Comparison of actives present in rice grains to evaluate properties of cosmetic value and their antimicrobial activity.
3) Supports to evaluate other skin and hair care properties like anti-ageing, closing pores, tightening skin, softening skin, maintaining healthy and shiny hair etc. present in Fermented Rice water. [10]
4) Since it has many skin & hair care benefits, it can be incorporated as active in variety of products like shampoos, serums, facial and hair oils, anti ageing products etc.

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