

Study of Different Properties of Kalanchoe Pinnata

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Abstract- Human skin is considered the largest organ of the body. Skin is also prone to many problems like dryness, aging, clogging, dullness etc due to environmental factors. Although natural ingredients have been traditionally used from centuries for skin care purposes. The use of bioactive extracts from variety of botanicals in cosmetics accomplishes two functions that are care of the body and as ingredient to influence the biological function of the skin providing the nutrient for healthy skin. The study was planned to exploit the properties of Kalanchoe pinnata leaves as it can be used for emollient, antioxidant, antimicrobial, astringent components which may be potentially utilized in the cosmetic formulations.

Keywords- Skin, Emollient, Antioxidant, Antimicrobial, Astringent, Poly Acrylic Acid, Kalanchoe Pinnata, Cosmetic.

I. INTRODUCTION

Skin is one of the important and largest organ of human body. The surface area of the skin on an average adult is 1.8 m² and represent 16% of the total body weight. The thickness of the skin varies throughout the body. The skin is a multifunctional organ.³ It is divided into two main layers: Epidermis- It is the outermost layer of the skin. This layer consists of many special cells including keratinocytes and melanocytes.

Dermis- It is the second major layer of the skin and dermis is made up of strong connective tissues.

The skin has very important vital function in keeping the physiological and biochemical conditions of the body in optimum state.⁴ As the skin plays such a important role taking care of it is very important. Skin caring includes the range of products that support skin integrity, enhance its appearance and relieve skin conditions. This can include nutrition, avoidance of excessive sun exposure, appropriate use of emollients, antioxidant, antimicrobial, astringent etc.

Emollients are fattening agents added to the cosmetic formulations to provide the skin moisturizer that may be required by the skin almost daily. They increase the moisture

content of the skin by reducing evaporation, providing a soft smooth and non greasy feel to the skin.⁵

Antioxidants play an important role in facial creams and skin aging products. They are natural substance made up of vitamins and minerals.⁶ It helps in reducing the free radicals that damage DNA, lipids and proteins because damage skin cells can leads to aging with wrinkles, dry skin, dark under eye circles, decrease elasticity and pliability.⁷

Antimicrobial agents are materials that protect against the growth of microorganisms on skin as well as personal care products that include bacteria, viruses, fungi etc.⁸

Astringent are chemical compounds that contract the tissues. In skin care astringent are used to tone the skin and make it firm by constricting the pores. It forms a protective layer between the underlying layers of the skin with the external elements.⁹

As skin becomes the barrier for the many external elements and protect the body from its harmful effects while doing that it face some problems like dryness, dullness etc. Above mentioned ingredients help in maintaining healthy skin. Knowing the significance of the problem study is planned to explore the properties of Kalanchoe pinnata leaves as it was observed during the literature study that Kalanchoe pinnata leaves can acts as emollient, antioxidant, antimicrobial agent and astringent. Considering these facts it is thought to study these properties by incorporating in cosmetic formulation.

Kalanchoe Pinnata is a succulent plant that grows 3-5 feet tall. It is commonly has tall hollow stems, fleshy dark green leaves and bell like pendulous flowers. Kalanchoe pinnata leaf, stem and root portions have high index in therapeutic values.¹⁰

Taxonomy of Kalanchoe Pinnata ¹¹

Table No -1

| Taxonomy | |
|----------------|-------------------|
| Kingdom | Plantae |
| Subkingdom | Tracheobionta |
| Super division | Spermatophyta |
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Subclass | Rosidae |
| Order | Saxifragales |
| Family | Crassulaceae |
| Genus | Kalanchoe |
| Species | Kalanchoe pinnata |

Chemical constituents

Kalanchoe pinnata is rich in alkaloids, terpenes, glycosides, flavonoids, cardenolides, steroids, bufadienolides and lipids. The leaves contain a group of chemicals called bufadienolides. It also contains various groups of phenols. ¹²

II. MATERIALS AND METHODS

Preparation of Active

Literature survey was carried out by referring books, journals, abstracts and research papers after that kalanchoe pinnata leaves were collected, with the help of reflux extraction process the active constituents that were tannins, flavonoids, phenolic compounds and fatty acids were got from the kalanchoe pinnata leaves. Test were performed for the identification of the different components in the extract we got from the leaves.

Saponification test was done to find the fatty acids. ¹³ Secondly extract was tested with lead acetate formation of precipitation indicates the presences of tannins after that flavonoids test was performed by treating the extract with NaOH as a result yellow color was obtained. Phenolic compounds test also confirms the presences of it in the extract. ¹⁴

Antibacterial activity evaluation was based on zone of inhibition process. In this micro organisms used were staphylococcus aureus and escherichia coli. Media used were nutrient agar. The test samples having antibacterial activity, they inhibited the growth of the bacteria and a distinct zone of inhibition was visualized surrounding the media. The antibacterial activity of the test samples were determined by measuring the zone inhibition expressed in millimeters.

Zone of Inhibition of Different Concentration of Extract

Table No- 2

| Sr. No. | Organisms | 0.1% | | 0.3% | | 0.5% | |
|---------|-----------|---------|---------|---------|---------|---------|---------|
| | | Control | Extract | Control | Extract | Control | Extract |
| 1 | S.aureus | 10 mm | N Z I | 13 mm | 8 mm | 16 mm | 11 mm |
| 2 | E.coli | 7 mm | N Z I | 9 mm | 5 mm | 12 mm | 8 mm |

N Z I – No Zone of inhibition

Spectrophotometric analysis was done for the determination of the antioxidant activity of substances by measuring their capacity to absorb light of various wavelengths. This test is used to find out absorbance of active at their specific wavelength range according to the Ruch et al method ¹⁵ for that kalanchoe pinnate extract and ascorbic acid solution was taken in testing cell simultaneously along with 2 ml of phosphate buffer, 0.6 ml of 40 M hydrogen peroxide and 1 ml distilled water.

The percentage of scavenged hydrogen peroxide of extract was calculated using the following formula

$$\% \text{ Scavenged } H_2O_2 = [Ac - Ae / Ac] \times 100$$

Ac= absorbance of ascorbic acid solution

Ae= absorbance of extract ¹⁶

Percentage of Scavenged H₂O₂ by Ascorbic Acid and Extract

Table No-3

| Concentration (µl) | Percentage of H ₂ O ₂ by Ascorbic Acid (%) | Percentage of H ₂ O ₂ by Extract (%) |
|--------------------|--|--|
| 20 | 44.6 | 21.9 |
| 40 | 48.0 | 27.4 |
| 60 | 54.3 | 35.3 |
| 80 | 59.4 | 46.1 |
| 100 | 67.1 | 52.6 |

Preparation of Formulation

Gel is composed of polyacrylic acid polymer dispersed in water along with neutralizer, preservative and active ingredient. The percentage of the chemicals was adjusted to get the required formulation of the gel. The raw materials, which were used in formulation, are carbomer, TEA, sodium benzoate, glycerin, extract, water, alcohol. Considering all above properties and uses of active, formulation of gel was selected. After various trial and errors methods formulation as per Table no 4 was selected as final formulation.

Table No -4

| Sr. No. | Ingredients | Function | Quantity (%) |
|---------|--------------------------|-------------------|--------------|
| 1 | Carbopol 934 | Rheology Modifier | 0.7 |
| 2 | Triethanolamine | Neutralizer | 0.5 |
| 3 | Glycerin | Humectant | 7.0 |
| 4 | Water | Solvent | Up to 100 |
| 5 | Sodium benzoate | Preservative | 0.5 |
| 6 | Alcohol | Solvent | 5.0 |
| 7 | <i>K.pinnata</i> extract | Active | 5.0 |

Evaluation of Stability Parameters of Active in Gel Formulation

The stability of final formulations was observed at two different temperatures that is $45 \pm 2^\circ\text{C}$ and $10 \pm 2^\circ\text{C}$. Various parameters such as pH, color and odor was observed. There was no significant change observed in above mentioned parameters of the product at $45 \pm 2^\circ\text{C}$ and $10 \pm 2^\circ\text{C}$ temperatures. Accelerated stability studies showed that formulation with active was stable during the period of study.

Subjective Evaluation

Moisturizers have been used to alleviate dry skin they reduced trans epidermal water loss (TEWL) by promoting barrier repair, smooth exposed dermal nerve ending by creating temporary barrier and restore skin smoothness.¹⁷ Moisturizers are designed to make stratum corneum soft and more pliant by increasing its hydration.

Moisturizer has multifunctional effect and also contain required amount of humectant in it. It includes reduction of dryness, roughness, scaling, decrease in perceived feeling of tightness and itching.¹⁸ During the high exposure of sun radiation or sun burn the skin become red and eventually shed or peel. Humectant help in reducing the inflammation, repair sun damage and prevent wrinkle formation by balancing the water content of the skin. The moisturizing efficiency was measured in vitro by the Trans epidermal water loss measurement. Loss of water in the skin to the outside that is TEWL is measured using a device cutometer.

The Cutometer is designed to measure elasticity of the upper skin layer using negative pressure which deforms the skin mechanically. The measuring principle is based on the suction method. Negative pressure is created in the device and the skin is drawn into the aperture of the probe and after a defined time released again. Inside the probe, the penetration depth is determined by a non-contact optical measuring

system. This optical measuring system consists of a light source and a light receptor, as well as two prisms facing each other, which project the light from transmitter to receptor. The light intensity varies due to the penetration depth of the skin. The resistance of the skin to the negative pressure (firmness) and its ability to return into its original position (elasticity) are displayed as curves (penetration depth in mm/time) in real time during the measurement. This measurement principle allows getting information about the elastic and mechanical properties of skin surface.¹⁹

Procedure

Six volunteers in the age group of 20-25 years were selected for the study. The volunteers were allowed to rest for at least 10-20 minutes before study, so that their blood circulation can regain a normal level. The volunteers were asked not to wash the forearm at least four hours prior to the study and not to apply any cosmetic or soap to the inner forearm during protocol.

Prior to the trials, the baseline values of the volunteers were taken using 2 x 2 cm test areas (2 cm apart) on the inner forearm. Each designated areas were then treated with standard and test formulation (dose 2 mg/cm²) along with blank that is nothing was applied. The TEWL values were measured immediately at 0 minutes and then after every 30 minutes using cutometer.

The subjective evaluation was carrying out for a week at the same time of the day. All the values of TEWL during 6 days study were noted down. Mean of these values was calculated and plotted against time.

III. RESULTS

Accelerated stability studies showed that formulation with active was stable in respect to pH, color, odor and separation. Subjective evaluations also indicated that volunteers were satisfied with the TEWL capacity of the active. There was no significant change observed in previously mentioned parameters for the final formulations at $45 \pm 2^\circ\text{C}$ and $10 \pm 2^\circ\text{C}$ temperatures. Obtained results were tabulated and are mentioned below.

Ph

pH values determines the potential of hydrogen ions in the formulations.

pH Values of Gel Formulations having Active kept at $45 \pm 2^\circ\text{C}$ in Table no 5

Table no 5

| No. of Days | k. pinnata |
|-------------|------------|
| 0 | 6.27 |
| 16 | 6.27 |
| 30 | 6.34 |
| 45 | 6.35 |
| 60 | 6.35 |
| 90 | 6.36 |
| 120 | 6.36 |

pH Values of Gel Formulations having Active kept at $10 \pm 2^\circ\text{C}$ in Table no 6

Table no 6

| No. of Days | k. pinnata |
|-------------|------------|
| 0 | 6.27 |
| 16 | 6.27 |
| 30 | 6.33 |
| 45 | 6.33 |
| 60 | 6.33 |
| 90 | 6.35 |
| 120 | 6.35 |

Colour of the Gel Formulation Having Active

Color change of gel were observed visually

Gel Formulations having Active kept at $45 \pm 2^\circ\text{C}$ in Table no 7

Table no 7

| No. of Days | k. pinnata |
|-------------|------------|
| 0 | OC |
| 16 | NC |
| 30 | NC |
| 45 | NC |
| 60 | NC |
| 90 | NC |
| 120 | S D O C |

Gel Formulations having Active kept at $10 \pm 2^\circ\text{C}$ in Table no 8

Table no 8

| No. of Days | k. pinnata |
|-------------|------------|
| 0 | OC |
| 16 | NC |
| 30 | NC |
| 45 | NC |
| 60 | NC |
| 90 | NC |
| 120 | S D O C |

OC – Original Color, N C- No Change, S D O C- Slightly Dark of Original Color

Odor of the Gel Formulation Having Active

Odor change of gel were noted by smelling the product

Gel Formulations having Active kept at $45 \pm 2^\circ\text{C}$ in Table no 9

Table no 9

| No. of Days | k. pinnata |
|-------------|------------|
| 0 | OC |
| 16 | NC |
| 30 | NC |
| 45 | NC |
| 60 | NC |
| 90 | NC |
| 120 | S D O C |

Gel Formulations having Active kept at $10 \pm 2^\circ\text{C}$ in Table no 10

Table no 10

| No. of Days | k. pinnata |
|-------------|------------|
| 0 | OC |
| 16 | NC |
| 30 | NC |
| 45 | NC |
| 60 | NC |
| 90 | NC |
| 120 | S D O C |

OC – Original Color, N C- No Change, S D O C- Slightly Dark of Original Color

Subjective Evaluation

The subjective evaluation was done on the group of 6 subjects. It was found that 97% of volunteers were showing that they were satisfied with the TEWL property of the product. The volunteers were comfortable throughout the study. Readings obtained from subjective evaluations shows the positive results.

Table No -11

| No. of Subjective | 0 min | 30 min | 60 min |
|-------------------|-------|--------|--------|
| 1 | 81 | 83.7 | 85.2 |
| 2 | 72.3 | 74.8 | 77.6 |
| 3 | 65.6 | 68.1 | 71.3 |
| 4 | 63.1 | 64.8 | 68.6 |
| 5 | 73.5 | 77.2 | 79.6 |
| 6 | 51.3 | 54.7 | 59.1 |

IV. DISCUSSION AND CONCLUSION

Skin is prone to problems like dryness, aging, clogging, dullness due to environmental factors.

Due to this reason the study was planned to exploit the properties of *K. pinnata*. It was observed during the literature survey that *K. pinnata* can act as emollient, anti oxidant, anti bacterial, and astringent agent. Considering these facts it was thought to study these properties by incorporating in formulations.

Extraction of *K.pinnata* was carried out by reflux method. In phytochemical analysis it was observed that tannins were present in water extract, phenol as well as flavonoids were present in ethanol extract and fatty acids were present in the petroleum extract.

Antibacterial agents prevent the growth of bacteria in the product and increase their shelf life. They also help in killing the bacteria on the skin surface ensuring maximum personal hygiene. The extract was tested for antibacterial activity against *E. coli* and *S. aureus* by well diffusion method and the results obtained were positive.

Antioxidants help inhibit free radical production and thus protects the cell from damage preventing the signs of aging. Antioxidant activity was determined using UV Spectrophotometer. The extract at the concentrations of 20 μ l – 100 μ l were subjected to hydrogen peroxide radical scavenging assay by Ruch et al method. Results indicates the positive antioxidants property.

Emollients help in keeping the skin moist and supple by reducing TEWL from the epidermis. The emolliency of extract was examined by incorporating it in the gel formulation and TEWL was calculated with help of cutometer. The gel formulation was succeed in preventing the TEWL and make the skin hydrated effectively.

Astringent can help improve skin appearance by minimizing pores and drying up oily skin. *K. pinnata* leaf extract shows the astringent property during the analysis.

The formulation was subjected to stability assessment studies. The colour, odor, texture, pH etc were observed through out the duration of the study. No significant change was observed and hence it can be concluded that the product was stable. The antibacterial, astringent and emollient properties are equivalent to the standards which were compared during the study whereas its antioxidant activity is

better than ascorbic acid which was used as standard for comparison.

Hence it can be concluded that *Kalanchoe pinnata* leaf extract is used as antioxidant, antibacterial, emollient and astringent in cosmetic formulations.

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