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Formulation of Moisturizer Aloin from Aloe Vera Plant as Cosmetic Skin Protective Medicine

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Abstract: Aloe vera is an ornamental and medicinal plant is being used therapeutically and showed efficacy for the laxative effects of Aloe vera latex which contains several potentially active bioactive compounds like minerals, various enzymes and aloin compound which has been separated by SDS PAGE. The isolated molecule size confirmed as 14KDa compared with known standard molecule marker and identified as Aloin. The present research focused to identify the therapeutic compounds present in Aloe vera. The fresh solvent extracts of Aloe vera were found to be rich in total Aloin content. Results revealed that amount of macromolecules such as carbohydrate contents of 29mg, Protein 15.1mg and lipid content 2.8mg /100 g of f wt. The concentrations of mineral were estimated also various enzymes were showed in highest activity. In addition Aloe vera contains superoxide dismutase with antioxidant activity having topical application of Aloe vera gel is likely safe and demonstrates overall efficacy in healing or lowering skin infection including burn wounds. The therapeutic claims for Aloe vera cover a broad range of conditions. It is commonly used topically in the treatment of dermatological and wound healing conditions.
***Keywords: Aloe vera,* Aloin, Mineral, Enzymes, SDS PAGE**

I. INTRODUCTION

Aloe barbadensis miller, commonly referred to as Aloe vera, is one of more than 400 species of Aloe belonging to family Liliaceae that originated in South Africa, but have been indigenous to dry subtropical and tropical climates, including the southern USA [1]. Aloe vera is a perennial succulent xerophyte; it has elongated and pointed leaves that are joined at the stem in a rosette pattern and that grow to about 30–50 cm in length and 10 cm in breadth at the base in the adult plant [2]. The leaf is protected by a thick, green epidermis layer (skin or rind), which surrounds the mesophyll. Its contains vitamin (A, C, E), minerals, amino acids, enzyme, polysaccharide, palmitic acid, oleic acid, caprylic acid, stearic acid, β sitosterol [3]. There is broad list of the therapeutic claims of different parts of Aloe vera due to its Pharmacological activities which are employed in traditional management of diverse veterinary and human diseases. The herb is used internally to combat most digestive problems, including constipation, poor appetite, colitis, irritable bowel syndrome as well as, asthma, diabetes, immune system enhancement, peptic ulcers. Aloe vera has a long history of popular and traditional use in Indian medicine for constipation, colic, skin diseases, worm infestation, and infections [4], for hypertension [5] and for the treatment of type 2 diabetes mellitus [6] also recommended in the treatment of fungal diseases also used in the cosmetic, pharmaceutical and food industries. Aloe vera is used on facial tissues to act as moisturizers, soaps, sunscreens, and suntan agent. The topical application of Aloe vera prevents radiation induced skin damage. Evidence tends to support that Aloe vera products should be an effective interventions used in burn and wound healing. Aloe is also an immune enhancer because of its high level of antioxidants, which help to react with unstable compound known as free radicals which shows positive action against UV absorption. UV radiations shows damaging and harmful effects on skin, UV-A and UV-B rays causes skin melanoma, sun burn, photo ageing, skin pigmentation and various painful effects. Sun rays reaching to the surface of the earth have basically three types of radiations such as visible rays (400-700nm), UV rays with shorter wavelength (200-400nm) and infrared radiations with longer wavelength (760-5100nm)]. UV radiations particularly below 320nm are responsible for most damaging detrimental effects depending on length and frequency of exposure. Aloe vera has been used as a popular herbal medicine since ancient times for many conditions including burns. Much evidence has reported the efficacy of topical Aloe vera gel in the treatment of thermal burns through its different pharmacological actions. The mechanisms behind therapeutic properties of Aloe vera on thermal burns may include anti-inflammation, antimicrobials, wound healing promotion, and biological/immunological modulation. Aloe vera has been used externally to treat various skin conditions such as cuts, burns and eczema. It is alleged that sap from Aloe vera eases pain and reduces inflammation. It has antiseptic and antibiotic properties which make it highly valuable in

treating cuts and abrasions. The present research focused to prepare Aloin from Aloe vera extracts by using classified biochemical methods and to analyze its efficiency to protect various skin infection. It may consider as a topical product that absorbs or reflects some of the ultraviolet radiations on the skin exposed to sunlight and thus shows protection against sunburn and other skin infection.

II. MATERIALS AND METHODS

A. Plant Material

*Aloe barbadensis m*iller plants were collected from in and around cumbum belonging to the district Theni of Tamil Nadu state. The plant was identified and authenticated by Medicinal Plants Survey and Collection Unit, Ootacamund, Tamil Nadu, India.

B. Sample Extraction

Aloe vera (*Aloe barbadensis m*iller*)* leaves were collected and washed then peeled thick green epidermis layer to expose white pulp cut into small pieces weighed for about 100g extracted as follows: 100g of sample (Pulp) was homogenized with electric blender in 10mg calcium bicarbonate and 70-80% warm ethanol and filtered using whatmann no.1 filter paper and the recovered extracts were centrifuged at 5000 rpm for 10 minutes and the supernatant was discarded and the residues were preserved in refrigerated condition till further uses. Crude samples were subjected for phytochemical screening according to the standard methods as described by Trease and Evans**.**

C. Analysis of Minerals

Extracts were subjected for triple acid digestion to release minerals and the digested samples were analyzed by Atomic absorption Spectrophotometry. Extracts of samples were digested with triple mixture of nitric acid: sulphuric acid: perchloric acid [11:6:3] until a clear solution was obtained when dissolved in HCL. This solution was made up to 20ml with double distilled and de ionized water and the digested samples were used for estimation of minerals by AAS [7]. Macromolecules such as carbohydrate was analysed by Anthrone method [8], protein was measured by lowrey's method [9] and fat content was estimated by Soxhlet method [10]. The micro nutrients such as iron, phosphorus, calcium, magnesium and niacin were analyzed by standarad methods of analysis of AOAC [11] association of analytical chemicals.

D. Enzyme Activities

SOD was assayed according to Beauchamp and Fridovich [12] with slight modification. The reaction was performed at 25°C in a total volume of 1 ml in 50 mM potassium phosphate buffer (pH 7·8), 0·1 mM EDTA, 0.01 mM cytochrome C (Cyto C), 0·1 mM xanthine and xanthine oxidase (5 to 10µg), which was suspended in 2 M ammonium sulphate containing 1 mM EDTA adjusted to pH 8·0. The reaction was started by the addition of xanthine oxidase. One unit of SOD was defined as the amount which inhibited the reduction rate of Cyto C by 50% under the assay condition .*Aloe vera* soluble extracts used as sample source for other enzymes activities, Oxidase was measured by the method of [13], Similarly Catalase according to Beers and Sizer [14] Amylase was determined by the procedure of [15] and Peroxidase [16] Lipase assays also carried out by the method of Akhlaq *et al* [17].

E. Preparation Of Protein From Extract

The fresh leaves mass were homogenized with electric blender then carried out centrifugation at 12000 rpm for 5 minutes. The upper liquid content was discarded further residue has washed and re-suspension in 10mM Tris buffer (pH-8.0) resulting in crude extract. This extract was further centrifuged (10,000 for 10 min) to generating the soluble extract.

F. Sephadex G 25 chromatography

A glass column tube was packed to a bed size of 2.5 by 18 cm with Sephadex G 25 and was equilibrated with 0.1M-phosphate buffer (pH-7.0). The sample was applied on the column subsequently the elution was done with same buffer. The fractions were monitored for the protein estimation and the fractions corresponding to proteins were concentrated then the pooled fractions used for ammonium sulphate precipitation and were used for next purification step. In the same way soluble extract was loaded and the fractions were eluted by phosphate buffer (0.1M, pH 7.0) by using sephdex G50 column Chromatography.

In the first step of purification, solid ammonium sulfate was added to the extract at various concentrations and the mixture was stirred for 2 h and centrifuged at 10,000 rpm for 30 min. The fraction with the highest activity was dialyzed against Tris buffer (pH 7.0) and applied to an anion-exchange chromatography column (1.7by 7.0cm) containing DEAE Sepharose CL-6B equilibrated with buffer of the same composition. Protein was eluted at a flow rate of 30ml /h. Selected soluble protein fractions were separated on a

12% SDS –PAGE (Laemmli 1970) and stained with coo massive brilliant blue. The protein concentration was measured by Lowry *et al.*, (1951), using bovine serum albumin as the standard. Aloin was quantified in lyophilized gel, in pressed cake. The standard concentration of the solution was 1000 ppm (1mg/1mL distilled water). Aloin was determined in both freeze dried gel and cake. Before determinations, Aloin samples were filtered using 1.2 µm mesh glass fiber paper.

III. RESULTS AND DISCUSSION

Aloe vera (Aloe Barbandensis miller) is a tropical succulent plant shown in Figure 1 contains biologically active substances grouped as nutrient, macromolecules, mineral etc, anthraquinone, The parenchyma tissue produces the gel contains 99% water and active compounds.

Figure 1: Morphological View of Aloe Barbandensis miller

Macro and microelements present in Aloe vera plant leaves were analyzed by Atomic Absorbance Spectrophotometer (Table 1) revealed essential minerals were found in high level particularly K, Ca, Na and trace elements also showed its presence of mucilaginous substances which act as moisturizer.

TABLE1: Determination Of Minerals In Aloe Vera Extracts By AAS

S.No	Minerals	Concentration mg /g.f.wt
1.	Sodium	5.6
2.	Calcium	4.3
3.	Potassium	6.4
4.	Magnesium	2.0
5.	Copper	0.90
6.	Iron	0.30
7.	Phosphorus	0.18
8.	Zinc	0.25
9.	Nitrogen	1.89
10.	Manganese	1.90

The healthy plants leaves has been selected for extraction of *Aloe vera* gel preparation which has used for characterization of bioactive material and experimental confirmation for its therapeutic potential against skin infection. *Aloe vera* gel is used both topically (treatment of wounds, burns, and skin irritations) and internally to treat constipation, ulcers, diabetes, headache, arthritis, immune system deficiency. *Aloe vera* contains polysaccharides which increase the insulin level and show hypoglycemic properties. Enzymes activities were measured and results given in [figure 2] revealed superoxide dismutase [SOD] was found to be highest activities and amylase, oxidase, catalase, peroxidase and lipase enzymes. The increase in the SOD activity can be regarded as an adaptive response to increased levels of potentially destructive oxygen species. Toxic radicals accumulated /increased in any tissues either due to infection or diseases condition could be scavenged to protect the cells from infection by means of increased activities of SOD which explained protective action of enzymes found in *Aloe vera* extracts.

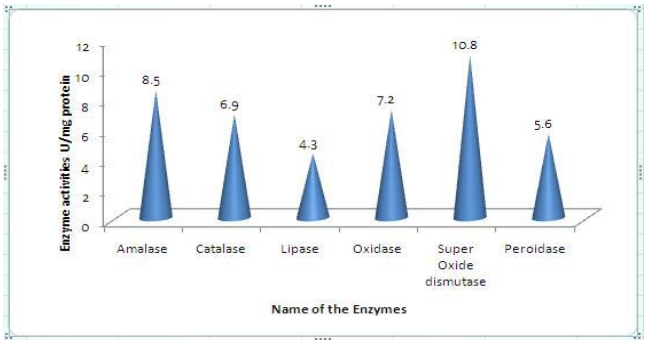

Figure 2: Estimation of various Enzyme activities of *Aloe vera* extracts

1) *Legend:* Enzyme activities are expressed as U/mg protein. Each value was represents with 5 average experimental results of *Aloe vera* extracts. Each cone represents value of one specific enzyme for comparative analysis of activities. Based on experimental results [Figure 3] the carbohydrates were high as 29gm than protein 15.1gm when compared to other macromolecules like lipid. Hence the *Aloe vera* extracts could serve as functional food with vital nutritional and biological value.

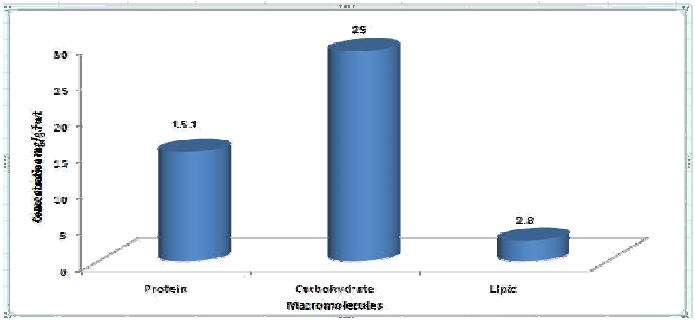

Figure 3: Determination of macromolecules of *Aloe vera*

2) *Legend:* Macromolecules estimations are expressed as mg /g f.wt of sample. Each value was represents with 5 average experimental results of *Aloe vera* extracts. Aloin is an anthraquinone glycoside used as laxative agent to maintain digestion system treating constipation by inducing bowel movements. There is a significant effect of ultrasonic waves on extraction of aloin over stirred which helps to reduce the extraction time and increased the maximum possible recovery of aloin. The rapid compression and ultrasonic waves improved cell disruption, penetration of solvent into gel matrix and improved the extraction of aloin bioactive compounds from Aloe vera used in various in pharmaceutical and food industry. Figure 4 showed separation of Aloin protein having molecular wt 14 kDa on SDS PAGE. Total soluble fraction prepared from Aloe vera leaf gel was isolated by an ion exchange chromatography using DEAE-cellulose and CM-cellulose column. The purified Aloin compound exhibited a potent anti-fungal activity also protected skins against various infection. This Aloe protein is a novel protein possessing antifungal and anti-inflammatory properties and thus sets a platform to be used as a medicinal plant product.

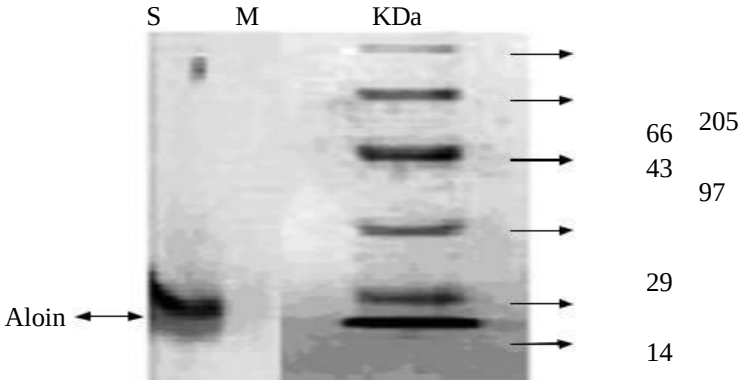

Figure 4: SDS –PAGE shows the presences of Aloin

3) *Legend:* showed the various fractions collected from chromatography separations of *Aloe vera* extract and samples were pooled then purified protein compounds separated on SDS PAGE. The data confirmed the presence of novel molecules which has size as14KDa protein and its potential properties analyzed The results clearly show that the study *Aloe vera* plants are rich sources of mineral elements that are highly essential for cosmetics and skin protection application**.** Aloin and its gel are used as skin tonic for pimples. *Aloe vera* is also used for soothing the skin and keeping the skin moist to help avoid flaky scalp and skin in harsh and dry weather. *Aloe vera* show laxative effect due to presence of anthraquinone. It has also been reported to have moisturizing and anti-aging effect along with anti-septic and anti-diabetic effects. Aloe latex contains a series of glycosides known as anthraquinones, the most prominent being aloin. The bitter aloes (dried yellow exudates) consists of free anthraquinones and their derivatives such as glycosides and chromones.

IV. CONCLUSION

The *Aloe vera* exudates are transparent slippery mucilage contains Aloin bioactive compounds which are used as antiseptic, antibacterial, antioxidant and anti-tumor agents and also effective in treating skin ailments, radiation injury, wound healing, burns. The moisture content present in human skin makes it look young and the use of moisturizer results in fastening the moisture with a surface film of oil. The present study is focused on the use of herbs as moisturizer for acne treatment. Cosmetics and skin protection application: Aloin and its gel are used as skin tonic for pimples. Aloe vera is also used for soothing the skin and keeping the skin moist to help avoid flaky scalp and skin in harsh and dry weather. Aloe vera showed laxative effect due to presence of anthraquinone. It also acts as a, beauty enhancer, hair fall, and white hair. It is known to help slow down the appearance of wrinkles and actively repair the damaged skin cells that cause the visible signs of aging and moisturizing effects whereas some experimental evidence suggests that the use of the gel may have beneficial effects in lowering inflammation and digesting dead tissue and moisturizing tissues instrumental in increasing circulation to the area also powerful in penetrating tissues, relieving pain associated with muscles. The skin absorbs *Aloe vera* up to four times faster than water, it appears to help pores of the skin open and receive moisture and nutrient is said to be a natural cleaner.

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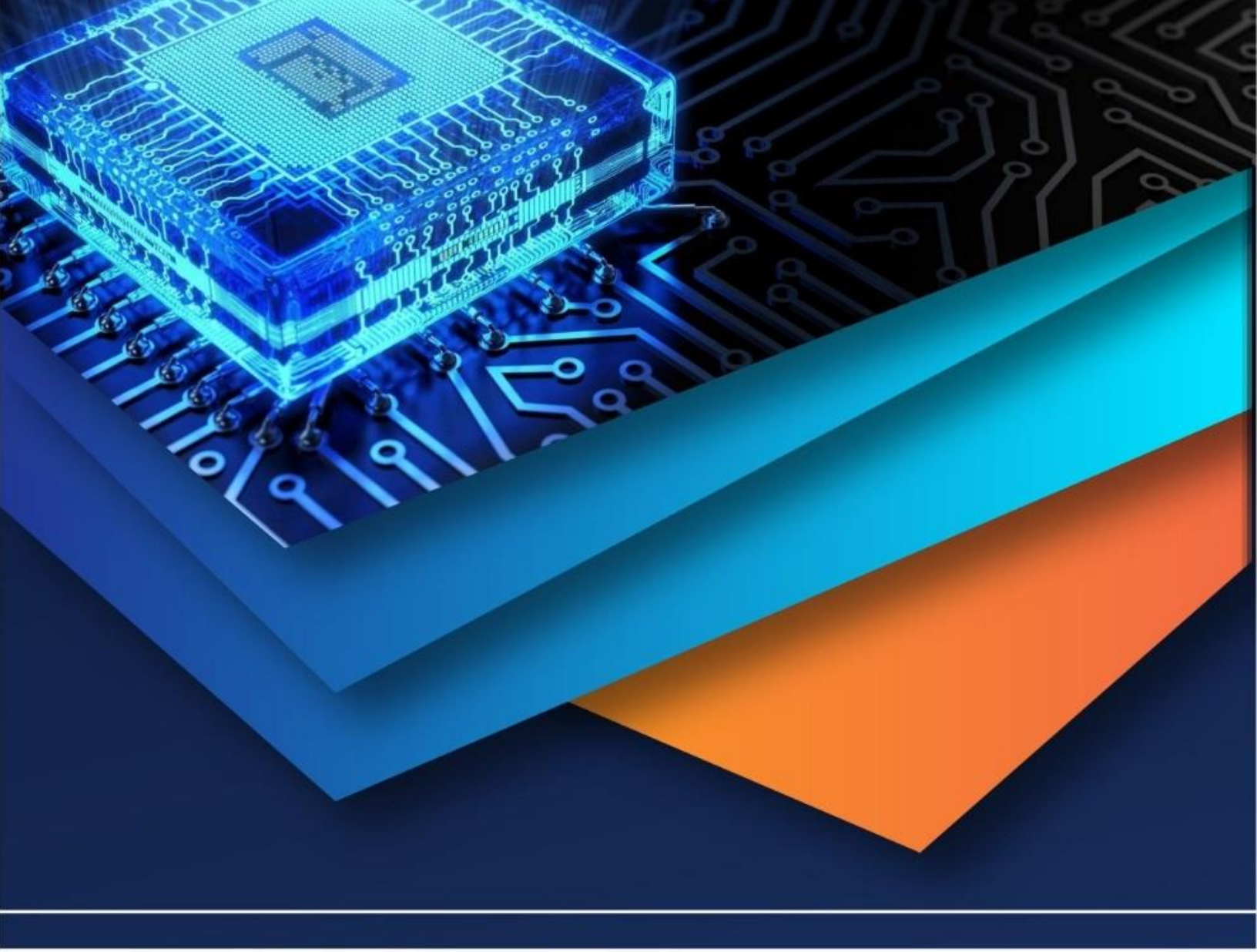

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