

PREPARATION AND EVALUATION OF NOVEL *ALOE VERA* GEL BEADS

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Abstract

Aloe vera has long been regarded as “nature’s gift” for burns and wounds, and its soothing/moisturizing properties have afforded aloe a leading role in many cosmetic products. Now, as science has begun to elucidate the many biological actions of aloe, evidence supports topical use of aloe for treatment of burns, wound healing, inflammation, and the role of the immune system in skin health. *Aloe vera* has got different activities such as Antifungal, Antimicrobial, Antineoplastic, Antidiabetic, Immunomodulatory etc., so it can be used as a better dermal preparation for treating various skin diseases. This work has been regulated regarding preparation and evaluation of Aloe Gel Beads including the morphological and rheological properties. Hence, an attempt was made to prepare and evaluate the dermal formulation of Aloe Gel Beads. Such dermal preparation may provide potential beneficial effects as compared to conventional available dermal preparation with less or no side effect.

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INTRODUCTION

The semi-tropical plant, *Aloe vera*, has a long and illustrious history dating from biblical times. It has been mentioned throughout recorded history and given a high ranking as an all-purpose herbal plant. *Aloe vera* has been consumed by millions of people without incident and has been used in cosmetic products more than any other herb. These days it is easy to go to a local pharmacy store and find a product that contains *Aloe vera*. It can be found in a range of health care and cosmetic products such as hand lotions, shampoos, cosmetics, beverages and dietary supplements. *Aloe vera* gel is used for its emollient and wound healing activities. The untreated gel is also a therapeutic agent and is available in the market in various concentrated, diluted and otherwise modified products. A number of reviews have been written covering the uses of the gel. But in the recent years there has been considerable effort towards definition of active constituents so that they can be accurately used in formulations. This review aims to emphasize on the research work being carried out on the constituents and varied biological activities of the constituents of *Aloe vera* with special emphasis on the research work on the *Aloe vera* leaf gel. (R.M Shelton; 1991, J. Haller; 1990, P. Atherton; 1998, D. Grindlay and T. Reynolds; 1986). The aloe leaf is chemically complex, which affects the pharmacological activity of processed *Aloe vera*. There are various methods for processing and stabilizing aloe gel. As a result, there is great variation in the levels of acetylated polysaccharides and other bioactive constituents in commercial aloe products. This poses a challenge for manufacturers to control ingredient and product quality and has contributed to some of the inconsistent reports seen in the literature on aloe’s efficacy. By far the bulk of research intended to elucidate the immunomodulatory; wound healing and anti-inflammatory activities of aloe gel have focused on its polysaccharides. Several biological activities have been reported in the literature on aloe polysaccharides, including antiviral, antibacterial, anti-tumor, wound healing and, above all, immunomodulatory activities involving diverse mechanisms, including T-cell activation, phagocyte stimulation and induction of cytokines. Essential research has elucidated the structure of aloe’s

acetylated polysaccharides, while other findings suggest polysaccharide molecular weight can enhance its immune modulating properties. Investigations of the effects of aloe gel on wound healing in animal models has generated an abundance of evidence indicating it can accelerate wound healing and affect every stage of the wound-healing process. It is clear; aloe can influence multiple variables important in the wound healing process. Antimicrobial activity, stimulation of angiogenesis in the latter stages of healing and activation of macrophage activity all appear to play a role; but, the key mechanism appears to be the stimulation of fibroblast activity and the regeneration of collagen fibers. Clinicians have recognized for decades, based on case observations, that aloe gel in burn treatment has anesthetic, antiseptic and anti-thromboxane activities. In 2007, a systematic meta-analysis of clinical studies on the efficacy of *Aloe vera* treatment in burn wound healing was published (Burns. 2007 Sep; 33(6):713- 8). The authors searched several databases for information on placebo-controlled clinical trials of aloe in burn wound healing, evaluating study design, patient characteristics, intervention and outcome measure. Four studies comprising a total of 371 patients were included in the meta-analysis. Using duration of wound healing as the outcome measure, the weighted mean duration for aloe-treated groups was 8.79 days shorter than the untreated groups, a significant difference. While the researchers noted differences in product types, interventions and outcome measures rendered specific conclusions difficult. They stated the cumulative evidence indicated that aloe gel was an effective treatment in first- and second-degree burn treatments. Aloe is also well known among consumers for its ability to help heal the skin after overexposure to the sun. The primary protective effect of aloe for UV damage to skin is its ability to reverse UV-induced immune suppression. Research shows application of aloe gel within 48 hours of UV exposure can restore the function of epithelial immune factors involved in the healing response, including stimulation of collagen synthesis. UV-B irradiation suppresses the accessory function of Langerhans cells, measured by their ability to support anti-CD3 monoclonal antibody-primed T-cell mitogenesis. An addition of partially purified aloe gel to UV-exposed cultures of epithelial cells restored this accessory function. Skin exposure to UV radiation suppresses induction of T cell mediated responses such as contact and delayed type hypersensitivity (DTH), while crude extracts of aloe gel can prevent this photo suppression. Chronic exposure to UV radiation can cause skin cancer; and suppression of T-cell mediated immune response by UVB radiation has been suggested as one of the mechanisms responsible for antigenic skin cancer. Previous studies with aloe indicate cutaneous application of aloe gel can mitigate contact hypersensitivity and delayed type hypersensitivity responses in animal models from suppression by UVB irradiation. However, these studies have shown inconsistent results thought to be associated with manufacturing process variations. Research now shows the immunoprotective activity of aloe gel is dependent on polysaccharide molecular weight. Application of purified aloe polysaccharides to UVB irradiated mice restored contact hypersensitivity immune response. Fractionation of purified polysaccharide in this model further showed the optimal molecular weight range of aloe polysaccharides to be 50kDa to 200kDa (kilodaltons)

CHEMICAL COMPOSITION

The gel or mucilage obtained from the flesh of the leaf contains quite different compounds from the bitter latex extracted from the leaf lining. Aloe gel is 99% water with a pH of 4.5 and is a common ingredient in many non-prescription skin salves. The remaining solid material contains over 75 different ingredients. The gel contains an emollient polysaccharide, glucomannan. It is a good moisturizer, which accounts for its use in many cosmetics. Acemannan, the major carbohydrate fraction in the gel, is a water-soluble long chain mannose polymer which accelerates wound healing, modulates immune function (particularly macrophage activation and production of cytokines) and demonstrates antineoplastic and antiviral effects. The gel contains enzymes such as bradykinase, cellulase, carboxypeptidase, catalase, amylase and an oxidase. Magnesium lactate, which helps prevent itching, and salicylic acid and other antiprostaglandin compounds which relieve inflammation have also been reported in the gel.

Vitamins reported from the gel include the important antioxidant vitamins A, C, E, B₁ (thiamine), B₃ (niacin), B₂ (riboflavin), as well as choline and folic acid. Some researchers suggest that there is also a trace of vitamin B₁₂, which is normally only available from an animal source. The gel also contains saponins (about 3%), this may contribute cleansing and antiseptic properties to the gel. The leaf lining (latex, resin or sap) contains anthraquinone glycosides (aloin, aloe-emodin and barbaloin) that are

potent stimulant laxatives. These water soluble glycosides are split by intestinal bacteria into aglycones which effect the laxative action. In small quantities when they do not exert their purgative effect, they aid absorption from the gastrointestinal tract and have antimicrobial and pain killing effects. (D.Urch et al; 1999, M.Afzal et al; 1991, M.I. Ali et al; 1999)

POTENTIAL CLINICAL BENEFITS

1. wound Healing

Clinical studies have shown that the gel preparations accelerate wound healing. In vivo studies have demonstrated that the gel promotes wound healing by directly stimulating the activity of macrophages and fibroblasts. Fibroblast activation by *Aloe vera* gel has been reported to increase collagen and proteoglycan synthesis, thereby promoting tissue repair. Some of the active principles appear to be polysaccharides composed of several monosaccharides, predominantly mannose. It has been suggested that mannose 6-phosphate, the principal sugar component of *Aloe vera* gel, may be partly responsible for the wound healing properties of the gel. Mannose 6-phosphate can bind to the growth factor receptors on the surface of the fibroblasts and thereby enhance their activity. Furthermore, acemannan, a complex carbohydrate isolated from Aloe leaves, has been shown to accelerate wound healing and reduce radiation induced skin reactions. The mechanism of action of acemannan appears to be twofold. First, acemannan is a potent macrophage-activating agent and therefore may stimulate the release of fibrogenic cytokines. Second, growth factors may directly bind to acemannan, promoting their stability and prolonging their stimulation of granulation tissue. (A.U.Tizard; 1995). The therapeutic effects of the gel also include prevention of progressive dermal ischaemia caused by burns, frostbite, electrical injury and intraarterial drug abuse. In vivo analysis of these injuries demonstrates that the gel acts as an inhibitor of thromboxane A₂, a mediator of progressive tissue damage. Several other mechanisms have been proposed to explain the activity of the gel, including stimulation of the complement linked to polysaccharides, as well as the hydrating, insulating, and protective properties of the gel. Because many of the active ingredients appear to deteriorate on storage, the use of fresh gel is recommended. Studies of the growth of normal human cells in vitro demonstrated that cell growth and attachment were promoted by exposure to fresh *Aloe vera* leaves. (D.B.Roberts and EL Travis ;1995, K.Karaca et al ; 1976, W.D.Winters; 1991).

2 Anti-inflammatory

The anti-inflammatory activity of *Aloe vera* gel has been revealed by a number of in vitro and in vivo studies. Fresh gel significantly reduced acute inflammation in rats (carrageenin-induced paw oedema), although no effect on chronic inflammation was observed. The gel appears to exert its anti-inflammatory activity through bradykinase activity and thromboxane B₂ and prostaglandin F₂ inhibition. Furthermore, three plant sterols in *Aloe vera* gel reduced inflammation by up to 37% in croton oil-induced oedema in mice. Lupeol, one of the sterol compounds found in *Aloe vera*, was the most active and reduced inflammation in a dose dependent manner. These data suggest that specific plant sterols may also contribute to the anti-inflammatory activity of *Aloe vera* gel. (R.H.Davis et al; 1995)

3. Burn treatment

Aloe vera gel has been used for the treatment of radiation burns. Healing of radiation ulcers was observed in two patients treated with *Aloe vera* cream, although the fresh gel was more effective than the cream. Complete healing was observed, after treatment with fresh gel, in two patients with radiation burns. Twenty-seven patients with partial thickness burns were treated with the gel in a placebo-controlled study. The gel-treated lesions healed faster (11.8 days) than the burns treated with petroleum jelly gauze (18.2 days), a difference that is statistically significant (t-test, P = 0.002). (C.Collin Roentgen; 1935, C.S.Wright; 1936, H.Rattner; 1936, A.B.Loveman; 1937)

4 Antibacterial

Aloe vera gel is bacteriostatic or bactericidal against a variety of common wound-infecting bacteria in vitro: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E.coli*, *Salmonella typhosa* and *Mycobacterium tuberculosis*. Aloe-emodin

also inhibits the growth of *Helicobacter pylori* in a dose-dependent fashion.(H.Wang et al; 1998, L.Lorenzetti et al; 1964)

5. Antiviral

Recent studies indicate that *Aloe vera* can be used in the treatment of HIV-AIDS. This is attributed to the anti-viral and immuno-modulating properties of acemannan. Acemannan has direct effects on the cells of the immune system, activating and stimulating macrophages, monocytes, antibodies, and T-cells. The acemannan acts as a bridge between foreign proteins, such as virus particles macrophages, and facilitating phagocytosis this is a key component in boosting cell-mediated immunity, which is deficient in HIV infection. Acemannan hydro gel (trade name is Carrisyn) is currently under investigation as a treatment for persons infected with HIV; doses are up to 250 milligrams QID (about one quart of raw Aloe gel daily).

In a randomized, controlled double blind clinical trial of 60 men suffering from an initial episode of Herpes simplex infection, those assigned to treatment with an *Aloe vera* extract (0.5%) in a hydrophilic cream had a significantly faster healing time and a higher number of healed lesions than the placebo comparison group (J.S. Montaner et al; 1996, J. Singer et al; 1993, T. Syedet al; 1997).

6. Antifungal

Antifungal activity in the gel has received little attention. Growth of a yeast *Candida albicans* is somewhat inhibited by a processed *Aloe vera* gel preparation. Aloe extract treatment of guinea pig feet that had been infected with *Trichophyton mentagrophytes* resulted in a 70% growth inhibition compared with untreated animals.(K. Kawai et al; 1998).

7 Antineoplastic

Lecitin like substances from the leaves of *Aloe vera* and *A.saponaria* and a commercial Aloe-gel were shown to have haemoagglutinating properties and fresh preparations also promoted growth of normal human cells in culture but inhibited tumor cell growth. In a group of laboratory mice implanted with malignant sarcoma cells who were treated with intraperitoneal injections of acemannan, all the mice in the control group developed malignant tumors and died within seven weeks, but 40% of the treated mice survived and showed signs of tumor necrosis and regression. Based on findings from animal studies, aloe research in human cancer patients is currently in progress. At the University of Texas-Houston Medical School and Herman Hospital, a Phase I study with injectable aloe for cancer patients is being conducted. In a preliminary study of 50 patients suffering from lung cancer, gastrointestinal tract tumors, brain stem gliomas or breast cancer who were treated with melatonin alone or melatonin plus aloe, those in the combination therapy group had significantly better one-year survival.(S.Y. Peng et al; 1991)

8. Gastrointestinal function and ulcers

Aloe vera gel is being used commercially for oral consumption and many claims are made for benefits in various internal inflammatory conditions. Effect of aloctin A, glycoprotein isolated from leaves of *Aloe arborescens*, on gastric secretion and on acute gastric lesions in rats was examined. Aloctin A given intravenously dose dependently inhibited the volume of gastric juice, acid and pepsin output in pylorusligated rats. Aloctin A given intravenously significantly inhibited the development of Shay ulcers and indomethacin induced gastric lesions in rats. It also inhibited water immersion stress lesions induced in pylorusligated rats. Studies and case reports provide support for the use of *Aloe vera* in the treatment of radiation ulcers and stasis ulcers in man. The anti-inflammatory actions of gel in vitro provide support for the proposal that it may have a therapeutic effect in inflammatory bowel disease. Acemannan is under consideration as an experimental remedy for inflammatory bowel disease.(N. Parmar; 1986, W.Suivitay et al; 1997, R.Teradaira et al; 1993)

9. Anti diabetes

Evidence for anti-glycation properties of *Aloe vera* can be found in studies with diabetics, both animal and human. Diabetes mellitus, marked by insulin deficiencies and resultant inability to properly utilize glucose in the blood, has been demonstrated to benefit from *Aloe vera*'s hypoglycemic effects. *Aloe vera* assists in reducing blood glucose to more normal levels specifically in type I and type II diabetics, not in non-diabetics. This indicates that the activity of *Aloe vera* is condition specific.

Nearly half of diabetic patients surveyed in Texas reported using *Aloe vera* or other herbal remedies as complementary therapies for their diabetes. Aloe gel appeared to enhance the hypoglycemic effect of glibenclamide when given orally to diabetic patients in doses of 1 – 2 tablespoons twice daily. There are no reported randomized controlled trials comparing aloe to any oral hypoglycemic agent or insulin in treating human diabetics. (H. Beppu et al; 1993, P. Bwitti and C. Musabayane; 1997, M. Koo; 1994, P. Noel et al; 1997)

10. Other Activities

Aloe vera gel provides powerful anti-oxidant action, due to - amongst other properties - its vitamin content, especially vitamins A, E, and C. In addition to its innate anti-oxidant properties and constituents, *Aloe vera* have the ability to stimulate the body's own anti-oxidant activities. This results in reduced oxidative stress, which has been shown to "play an important role in age related diseases". *Aloe vera* appears to enhance immunity on many levels. Recent studies, identified specific fractions that enhance anti-tumor activity as well as a second molecular trigger for fibroblast activation. It further elucidated the structure of modified aloe polysaccharide (MAP) and illustrated that this promising nutraceutical can prevent UVB radiation damage to the skin even when it is applied post exposure. The gel has been used in the field of dentistry. It is extremely helpful in the treatment of gum disease; it reduces the bleeding of the gums; it is powerfully antiseptic in gum pockets and its antifungal properties help greatly in the problem of denture stomatitis.

(Kojo Eshun and Qian He; 2004, A. Valerie et al; 2003, Rajasekaran. Subbiah et al; 2005)

ALOE VERA IN COSMETICS

Aloe vera has been added to many cosmetic products for many years because of its known rejuvenating action. It achieves this in several different ways. Firstly the polysaccharides act as moisturizers, hydrating the skin. Secondly, aloe is absorbed into the skin and stimulates the fibroblasts to replicate themselves faster and it is these cells that produce the collagen and elastin fibres, so the skin becomes more elastic and less wrinkled. Aloe also makes the surface of the skin smoother because of its cohesive effect on the superficial flaking epidermal cells by sticking them together. It also possesses the ability to interfere with the enzyme that produces melanin deposits in the skin, preventing the formation of 'liver spots' which tend to form in ageing skin. (I.E danhof and B.H Mcanally; 1983)

GEL STABILISATION

Upon cutting, there is a maximum of six hours to stabilize the active ingredients within the gel and leaf. When exposed to air the gel rapidly oxidizes, decomposes and loses much of its biological activities. Various patented methods are known for stabilization of the gel. In the heat treatment processing, sterilization is achieved by subjecting the aloe liquid obtained from the activated carbon treatment to pasteurization at high temperature. Aloecorp has reported that the biological activity of *A. vera* gel essentially remains intact when the gel is heated at 65°C for periods less than 15 minutes. Extended heating of *Aloe vera* places great stress on its constituents and compromise their structure and activity. Heating denatures the amino acids, deactivates the enzymes and breaks the lengths of the polysaccharide chains (the longer the polysaccharide chain, the greater its ability to stimulate the immune system). For these reasons, cold processing is the superior method to ensure and protect the activity and integrity of *Aloe vera*'s constituents. In this method there is no application of heat and enzymes (glucose oxidase and catalase) are used to inhibit growth of aerobic organisms within the gel. Unfortunately, many of the *Aloe vera* formulas on the market today are the result of poor technology in stabilizing the aloe gel. Additionally, these formulas are processed with excessive heat and filtration, destroying the valuable active polysaccharide fraction. In throwing away the rind or outer portions of the *Aloe vera* leaf and using only the gel or matrix, which is standard practice in the industry, the vast majority of active constituents of *Aloe vera* are also thrown away. It is estimated that 80 – 85% of the actives in *Aloe vera* are found just underneath the surface of the rind. (I.E Danhof, B.H. McAnally; 1983, B.C Coats; 1994, H.H Cobble; 1975, Homecare Lberica S.A; 1983, R.G. Maugan; 1984, Cerqueria; 1999)

PREPARATION OF GEL BEADS:

Aloe gel was collected by making cut at mid of the leave. In 300 mg of sodium alginate solution in 10 ml of distilled water add 2gm of gel, mixed at 1000 rpm using magnetic stirrer (remi India) for 15

min. This was then extruded via syringe (no-18) into 5% calcium chloride solution with gentle agitation at 37° C. The formed beads were allowed to stand for 5 min in the solution , separated by filtration through wattman filter paper (size 0.45mm). (S. Kapoor, S. Saraf* et al; 2008)

Preparation of lotion containing beads and aloe gel:

Novel lotion was prepared by suspending equivalent to 2gm aloe gel beads in 15ml of calamine lotion BP. The conventional lotion was prepared by incorporating the 2gm aloe gel directly into 15ml of calamine lotion BP.



Figure 1. Prepared Aloe Gel Beads

EVALUATION OF GEL BEADS

- 1 Morphology of gel beads: The mean diameter dried beads were performed using optical microscopy.
- 2 Crushing property: 50gm beads of aloe gel were pressed over butter paper to observe the crushing of beads to access its handling during application of lotion.
- 3 pH: Ph of 15ml of lotion was determined by using pH meter (181remi, India) and colour is observed with naked eye to observe the compatibility with skin.
- 4.Reological studies: Both lotion were allowed to stand for 24hrs and sedimentation ratio was calculated by using standard formula . redispersability of lotion(15 ml) observed after 24hrs by its flocculation . Viscosity of both lotions was measured by Brookfield viscometer. Cracking property for 5ml of each lotion was determined by keeping them at refrigerated temperature (-4 degree centigrade) for 24hrs and separation of solid and liquid phase were observed.
5. Stability study: Stability study of both lotions is carried out in terms of physical evaluation by naked eye at room temperature.
6. Sensitivity of lotion: Six volunteers (25-30 yrs), after applying the both lotions on forearm for 20 min, observed for any irritation or edema formation (S. Kapoor, S. Saraf* et al; 2008)

RESULT AND DISSCUSION

The prepared Aloe beads were evaluated for following parameters:-

1. Morphology of Beads: Beads formed are in spherical shape and have diameter $0.206\text{mm} \pm 0.03$.(Table 2)
2. When beads pressed over butter paper , Aloe gel exudes out easily showing easy to squeezing of gel from bead at the time of application.
3. Rheological studies:
 - Sedimentation volume: Sedimentation for novel is 0.893 ± 0.01 and for conventional lotion is 0.489 ± 0.03 . Novel lotion is more stable as higher volume of sedimentation ration indicating higher suspendability.

- The Rheological property i.e redispersability, viscosity and cracking property show the good stability characteristics of novel lotion at room temperature compared to conventional one.(Table 3)
- The pH of both lotion compliance with skin pH, the pH of the novel lotion was found to be 5.5 ± 0.02 as compared to conventional lotion $pH 4.0 \pm 0.03$, which shows that it is more compatible with the skin.

Table No.2 Morphology of Aloe gel beads

Evaluation parameter	Result
a) Shape	Spherical
b) Diameter	0.206 ± 0.03 SD
c) Crushing strength	Exudes out

SD = Standard deviation

Table No.3 Evaluated parameters of two lotions

Evaluation parameters	Novel lotion	Conventional lotion
1) Physical evaluation		
a. pH	5.5 ± 0.02 SD	4.0 ± 0.03 SD
b. Colour	Pink	Pinkish green
Pinkish green		
2) Rheological studies		
a. Sedimentation volume	0.869 ± 0.01 SD	0.489 ± 0.03 SD
b. Resdispersability	Easily dispersible	Not uniform
c. Viscosity	$1700cp \pm 1$ SD	$1000cp \pm 1$ SD
d. Cracking property	Flocculated	Deflocculated

CONCLUSION

The list of the beneficial uses of *Aloe vera* continues to grow, as more scientific research is performed. There is growing experimental evidence for its use as a moisturizing agent. Given the exponentially growing demand for it in the international market, *Aloe vera* presents the finest commercial opportunity among the various medicinal plants. Utilization of proper manufacturing techniques and development of appropriate analytical tests are essential requisites. More extensive studies on the active constituents of *Aloe vera* still need to be carried out to market this product. We have formulated *Aloe vera* beads and all the rheological and the stability studies shows that the formulated product is stable and hence we conclude that it may be given as an effective dermal preparation for treating various skin diseases and abnormal skin conditions. Overall study revealed that as compared to conventional lotion, the prepared novel lotion is more stable and effective

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