UV B induced dermal wound healing efficiency of aloin isolated from *Aloe Vera* gel with reference to mice model

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Abstract: This work aimed at investigating the active principles present in the leaf and the phyto-chemicals were extracted using cold extraction method and screened qualitatively. Results showed that aloin which is naturally anthraquinones are compounds possess a broad spectrum bioactivities suggesting possible clinical application in many diseases. Aloin was isolated and identified on thin layer chromatography moved as yellow spot which has confirmed as aloin when compared with standard aloin run along with *Aloe Vera* sample for separation of component. UV- B exposure induced skin pigmentation on melanocyte in the experimental animals. Mice exposed to UVB were subjected to *Aloe vera*gel (10mg) application as treatment for skin damage. The healing efficiency has been analysed by western blotting experiment in order to confirm dermal cell expression. The results showed damaged tissue protein intensity higher to explain its healing power in the tropical application of *Aloe Vera*gel on UV- B induced skin damage in mice when compared to control animal. Therapeutic potential of Aloin in the extract of the *Aloe Vera* has increased due to purified bioactive material used for dermal application of mice artificially exposed to radiation.

Key Words: *Aloe Vera,* Aloin, western blotting, animal, UV- B.

1. INTRODUCTION:

Herbal medicine has become a popular form of healthcare; even though several differences exist between herbal and conventional pharmacological treatments, herbal medicine needs to be tested for efficacy using conventional trial methodology and several specific herbal extracts have been demonstrated to be efficacious for specific conditions. Herbal medicine is the use of medicinal plants for prevention and treatment of diseases: it ranges from traditional and popular medicines of every country to the use of standardized methods for d herbal extracts.

Ultra Violet radiation (UV-R) is a non ionizing ray as part of the electromagnetic spectrum. UV-R has greatest energy of all types of optimal radiation and UV-R can be subdivided into three categories depending on wavelength as UV-A, UV-B and UV-C rays can be reflected, scattered, transmitted or absorbed. UVA-Radiation ranges between 320- 400 are *effective to induce increased pigmentation and cause indirect DNA damage.* UVB-Radiation ranges between 290-320 causes direct DNA damage and mutation and sunburns and vitamin D biosynthesis. UVC-Radiation (200-290) radiations are filtered by stratospheric ozone layers so less effective and hazardous[1].

Ultraviolet radiation is a main environmental factor of skin injury. Its effect on the skin's biology and immune system plays a major role in photo-aging, inflammation, keratinocyte proliferation, epidermal hyperplasia, and carcinogenesis. UVB irradiation increased infiltration of dermal inflammatory cells in the exposure group due to the effect of chronic UVB irradiation. The inflammatory cells are the potential mediating agents of UV-induced cytokines that leads to epidermal proliferation and hyperplastic lesion in keratinocyte. UVB irradiation increased infiltration of dermal inflammatory cells in the exposure group due to the effect of chronic UVB irradiation.

Herbal Plants produce a diverse range of bioactive molecules, thereby making a rich source of different types of medicines. Natural products play an important role in drug development programmes in the pharmaceutical industries. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. Herbal drugs are used to prevent and treat diseases, and some of the herbal plant products are widely used as a vegetable. The different parts of a plant are known to possess multifarious medicinal properties. The consumption of extracts of the seeds, leaves, roots and combination of different portions reduces the risk of various illnesses. Most of the drugs today are obtained from natural sources or semi-synthetic derivatives of natural products used in the traditional systems of medicine.

*Aloe vera*is an important medicinal plant and is a perennial, drought resisting, succulent plant belonging to the Asphodelaceae family. It has a vast traditional role in indigenous system of medicine like Ayurveda, Siddha, Unani and Homoeopathy. *Aloe vera* is a nutraceutical having loads of nutrients has a beneficial effect on human health and can cure many diseases. *Aloe vera* boosts immune function and destroys tumours [2]. Another beneficial property

of *Aloe vera* is chemopreventive activity against tumor enlargement and it has been proved with the help of an animal model[3]. The *Aloe vera* plant has been known and used for centuries for its health, beauty, medicinal and skin care properties.The present study demonstrates the efficacy of natural active compound from *Aloe vera* plant and the purified phyto-substances can be used as sources formulated for herbal medicine to treat radiation induced skin infection in animal model. The objective of the present study was to identify the novel bioactive compound by classical biochemical methods and to determine the therapeutic efficiency of Aloin as cosmetic skin protective medicine.

2. MATERIALS AND METHODS:

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.

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 [4].

Thin layer chromatography (TLC):

TLC is performed using silica gel 60 F254 percolated on alumna sheets. The metabolites were applied point wise as different spots on TLC plates and must be eluted with different solvent system. The plate was viewed under ultra violet (UV) lamp at 254nm. A solution of Cerisulphate (1.6g) and Ammonium molybdate (21.6g) Conc. Sulphuric acid (50ml) in 450ml of water and the spraying the reagent on TLC plate followed by drying at 130°C in a hot air oven. The mixture was heated to 40ºC in a water bath and subjected to ultrasound for 15 min. It was then centrifuged for 5 min at 10000 g and the supernatant was analyzed by TLC[5].

Animal Model:

After obtaining the approval from the ethical committee/College of thirty adult albino mice, BALB/c strain (15 males and 15 females) were used in this experiment, each of which weighing 25–35 g. The animals were fed with standard pellet diet (Pico Lab) and provided with water ad libitum. Animals were housed in the animal house in Department of Biology, Madurai Kamaraj university ,Madurai, under a controlled room temperature about 25°C and photo-periodicity of 12 hours light/dark system. Animals were assigned into three groups: **Batch 1**: Control group (C), which were not exposed to UVB and not treated with Aloin;
Batch 2 animals were exposed to UVB for 15 min / alternate days / for a week.
Batch 3 animals were exposed to UVB for 25 min / alternate days / for a week.
Batch 4 animals were exposed to UVB for 50 min / alternate days / for a week.

UVB Irradiation:

The source of irradiation was a lamp of 312 nm wavelength, 15 watts; VILBER-LOURMAT-FRANCE. Mice from both groups (exposure and treatment groups) were exposed to UVB light as per experimental design .This was done after making a window by shaving the mouse's back skin (cm).

Treatment of Mice with Aloin:

Mice were treated with *Aloe vera*gel (10mg) as treatment for skin damage. Treatment was started from 8th day continued for 10 days (18th day of the experiment) for the batch 1 served as normal/ control. Body weight of the animal was noted daily in all groups during treatment period.

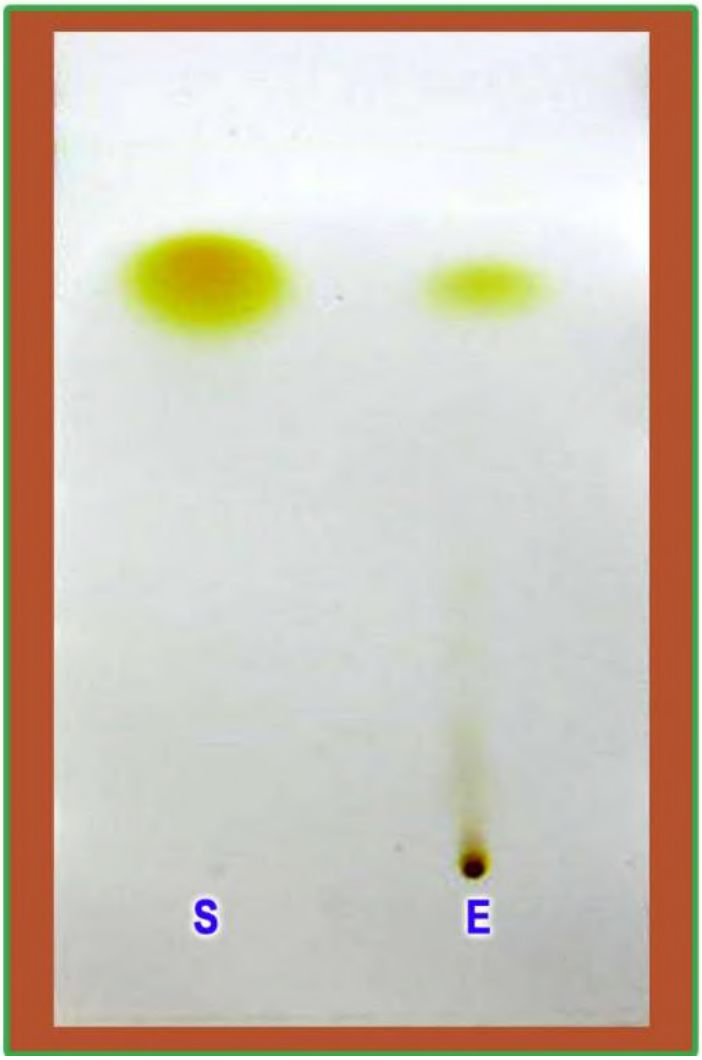
Western blotting: Skinr tissues crude extract (40µgprotein per lane) were analyzed by 15% SDS-PAGE. Proteins were transferred electrophoretically to nitrocellulose filters (for 3 h at1A) using an immunoblot transfer apparatus. After transfer, the nitrocellulose was incubated for 1 h at room temperature in 3% (w/v) BSA in Tris-buffered saline (TBS; 500mM NaCl and 20mM Tris-HCl pH 7.5) to block non specific binding. The blot was incubated overnight at 4ºC with 3% (w/v) BSA in TBS containing antiserum at a dilution of 1:500. After three 15 min washes with TBS containing 0.1% BSA and 0.2% Nonidet P40, the blot was incubated for 1 h at room temperature with peroxidase-

conjugated goat anti (mouse immunoglobulin) diluted at 1:1000 in 3% BSA in TBS. The blot was again washed three times with TBS containing 0.1% BSA and 0.2%Nonidet P40. Antibodies were visualized using a chem.-illuminescence detection system.

3. RESULTS AND DISCUSSION:

The botanical name of *Aloe vera*is Aloe barbadensis miller. It belongs to Asphodelaceae (Liliaceae) family, and is a shrubby or arbores cent, perennial, xerophytes, succulent, pea green color plant. 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 [6].Aloin an active compound obtained from the *Aloe Vera* species is already confirmed to exhibit an anti-inflammatory, anticancer effect. Aloin showed laxative action in animals also used as a stimulant – laxative, treating constipation by inducing bowel movements. Animal studies showed significantly proven information available are for the in vivo supplements of aloin based drugs Jawade*et al* (2011).

Figure 1: Separation of Aloin by Thin Layer Chromatography



S -indicates Standard Aloin; E – indicates *Aloe Vera* Extracts.

Legends: Results confirmed the presence of Aloin in *Aloe Vera* Extracts as compared with Standard Aloin which was run along with sample on TLC plate. S - Indicates Standard Aloin E – indicates *Aloe Vera* Extracts. Standard aloin sample was obtained from S.D. Fine Chemicals, India.

The present results revealed 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 [7]. Aloe Vera showed laxative effect due to presence of anthraquinone Anthraquinones were identified by thin layer chromatography (TLC) in silica G F250 using aloin as a standard and determined by mixing 100mg of the sample with 100µl solvent.

Figure: 2 Preparation of homogenized gel sample as an end product

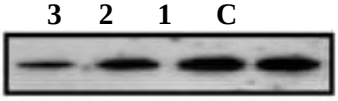
Legend: Cosmetics and some medicinal products are made from the mucilaginous tissue at the center of the Aloe vera leaf and are called Aloe Vera gel. This gel is a clear, tasteless, thin, jelly like material as Purified end product of *Aloe Vera* gel which posses profiles of aloe constituents and determination of aloin in gel.

The human body maintains a temperature of around 98.2°F. Sebaceous glands and sweat glands are heat sensitive and rapidly produce secretions. Thus, persistent rise in temperature results in an increased activity of the sebaceous glands and overproduction of sebum. Overproduction of sebum admixed with shed keratinocytes clogs the hair follicles, precipitating acne.

Tanning refers to the practice of darkening one's skin pigmentation through natural sunlight and artificial ultraviolet (UV) exposure. Studies have explored the molecular mechanism of darkening one's skin pigmentation through exposure to UV radiation [8], and an increasing amount of data supports the role of UV exposure in the development of both melanoma and non-melanoma skin cancers, reporting the effects of UV exposure on melanocyte, skin pigmentation, and melanoma.

The *Aloe vera* exudates are transparent slippery mucilage contains numerous 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.

Figure 3 Western blot analysis shows the expression of keratinocytes



Legend: Dorsal Epidermal cell expression from mice after different treatments indicated. C-control mice; 1- UVB induced aloin treated mice batch 2 experimental set up; 2- UVB induced aloin treated mice batch 3 experimental set up ; 3- UVB induced aloin treated mice batch 4 experimental set up

Experimental scheduled for UVB treatment:
Batch 1 animals were not exposed to UVB and received normal water were used as control (n=5).
Batch 2 animals were exposed to UVB for 15 min / alternate days / for a week.
Batch 3 animals were exposed to UVB for 25 min / alternate days / for a week.
Batch 4 animals were exposed to UVB for 50 min / alternate days / for a week.

Mice exposed to UVB were subjected to *Aloe vera* gel (10mg) application as treatment for skin damage. Treatment was started from 8th day continued for 10 days (18th day of the experiment) for the batch 1 served as normal/ control. *Aloe vera* gel treated mice were not undergoing apoptosis at final stage. Samples used for experiments were taken from UVB induced skin damaged bearing mice 10 days after initiation of treatment. Western blot result shows that keratin expression enhanced by aloin treated UV-B damaged mice. As increasing the duration of UV- B frequency might lead it higher damage which could be compensate by tropical application of aloin gel. The efficiency of aloin is treat damaged skin could be confirmed based on western boltting results. It can be concluded that the therapeutic superiority of an extract over a single isolated constituent thereof, and of an extract combination is caused by additive or potentiated effects of the mixtures of constituents in the extract [9]. It must be emphasized that in the comparative studies with synthetic drugs, the concentrations of all bioactive molecules of an extract together, applied per day, were much lower than the applied dosages of the synthetic drugs [10].Aloin formulated gel products

can be used to treat main constituent in sun care products, and clarifying lotions because of its ability to help heal minor wounds and promote healthy skin.

4. CONCLUSION:

The objective of the research was to isolate and characterize the novel component present in the *Aloe Vera* gel which has been prepared by using classical biochemical and Ultrasonic assisted methods of extractions. The extracted gel was subjected to analyze phytochemical, macromolecules, minerals, enzymes and other novel molecules specifically Aloin from *Aloe Vera* gel. Aloin was identified by TLC separation method and moved yellow spot confirmed as aloin when compared with standard aloin run along with *Aloe Vera* sample for separation of component. The natural product-derived agents would exhibit photo-protective efficacy on a UVB-damaged skin due to their diverse bioactive compounds its extraction efficiency and extraction rate, which if realized on industrial scale would represent economic gains for pharmaceutical and food industry.

Aloe Vera gel has been used in medicines and cosmetics, Gel preparations have a high potential due to their antioxidant activity which plays role in fighting against free radical species that are the main cause of numerous negative skin changes. It can be concluded that the therapeutic superiority of an extract over a single isolated constituent thereof, and of an extract combination over one extract of the extract combination, is caused by additive or potentiated effects of the mixtures of constituents in the extract. It must be emphasized that in the comparative studies with synthetic drugs, the concentrations of all bioactive substances of an extract together, applied per day, were much lower than the applied dosages of the synthetic drugs.

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