Efficacy of novel product for the treatment of UVB induced skin damage in animal model

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***Abstract*: The present research focused to formulate the therapeutic product to treat skin damage induced due to UVB exposure. The fresh solvent extracts of *Aloe barbadensis* were found to be rich in inner gel used as moisturizer, total Aloin content and other micro molecules for skin treatment. To evaluate the possibility of using the various pigment contents present in extract of *Camellia sinensis* contains the antioxidant, catechin and other molecules has the ability to stimulate hair growth also help in encourage hair regrowth. Previous experimental evidence tends to support that gel 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. Topical application of *Aloe vera* gel paste mixed with catechin isolated from *Camellia sinensis* extracts prevented UVB induced suppression of contact hypersensitivity response by possible restoration of the immune response. Plants constituents appear to be of biological importance in curing diseases. The supplements should be reformulated or upgraded owing to the research confirming the benefits of specific plants constituents like pigments poly phenols (catechin) and aloin contents were analysis from extracts of both plant materials and the efficient component mixed in appropriate concentration to formulate novel product which has been topically applied to UVB exposed mice animals. Western blot analysis revealed efficiency of keratin expression besides inhibiting the growth of cancer cells &destroy cancer tissues without harming healthy tissues in experimental mice.**

***Index* Terms: Aloe *barbadensis*, catechin, *Camellia sinensis*, UVB exposure, skin infections.**

I. INTRODUCTION

India is one of the oldest civilizations which are known for rich repository of medicinal plants. Plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. *Camellia sinensis* is the species of plant whose leaves and leaf buds are used to produce green tea. It is of the genus Camellia, a genus of flowering plants in the family Theaceae. The economic and social interest of tea is clear and its consumption as a therapeutic aid in many illnesses. Tea, a product made up from leaf and bud of the plant *Camellia sinensis*, is the second most consumed beverage in the world, although health benefits have been attributed to green tea consumption since the beginning of its history, scientific investigations on this beverage and its constituents have been underway for less than three decades [1]. *In vitro* and animal studies, and clinical trials employing putative intermediary indicators of disease, particularly biomarkers of oxidative stress status, provide strong evidence that green tea polyphenols (GTP) may play a role in the risk and pathogenesis of several chronic diseases, especially cardiovascular disease and cancer, and related pathologies [2].

Green tea contains numerous components with antioxidant activity: polyphenols (especially catechins), minerals, vitamins. The strong antioxidant potential of catechins, and especially EGCG (epigallocatechin -3 gallate), are widely demonstrated *in vitro* and in animal studies. In addition, catechins possess antimutagenic, antidiabetic, anti-inflammatory, antibacterial and antiviral properties. Recent human studies suggest that green tea may contribute to reduce the risk of cardiovascular disease and cancer, and has another beneficial effect on health. Green tea has a reputed role in cancer prevention as tea catechins have been shown to inhibit tumour cell proliferation as well as destruction of leukaemia cells [3]. Laboratory studies on cultures of tumour cells and mice given carcinogenic chemicals, showed green tea's potential to inhibit cancer hemorrhage [4]. In addition, increased consumption was associated with a significant delay in onset of cancer.

Green tea and black tea inhibit cell growth and induce apoptosis of cervical cancer cells [5]. Plant materials derived from the Aloe plant are used as cosmetic ingredients, including *Aloe barbadensis* leaf extract, Leaf Juice. These ingredients function primarily as skin-conditioning agents and are included in cosmetics only at low concentrations. Aloe-derived ingredients are used in a wide variety of cosmetic product types at concentrations of raw material that are 0.1% or less, although can be as high as 20%.Case reports include acute eczema, and dermatitis in individuals who applied Aloe-derived ingredients topically [6].

UV exposure induces immune suppression in skin cells, thereby blocking the normal function of protection from infection and removal of damaged cells. The depletion of the ozone layer allows easier penetration of *UV radiation* into the earth which subsequently increases the level of skin cancer among people. Sun screen are widely used to protect skin from UV [7]. The major components of interest are the polyphenols which are responsible for the antioxidant and other health benefits of green tea. Aloe vera also appears to function as an antioxidant through free radical- and superoxide radical-scavenging activities and anti-inflammatory activities.

The present work focused to study combination of two different bioactive substance formulations as a topical product in order to confirm its efficiency to heal/protect various skin infections in animal model due to exposed to ultraviolet radiations. Research of

green tea and *Aloe vera* are very promising, future studies considering dietetic, environmental and life style factors, are necessary to fully understand its contribution to human health

II. MATERIALS AND METHODS

Plant material

Aloe barbadensis miller 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. *Camellia sinensis* is an evergreen shrub, the green tea plant belongs to the Theaceae family. Green tea was purchased from AVT Carady Goody Estate, Karadikuzhy, Idukki District.

Sample extraction

Aloe vera (*Aloe barbadensis* miller*) 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. *Camellia sinensis* dried leaves were subjected for the application of heat during extraction process also causes the oxidation of water-soluble flavonols, which contribute to the yellow color of green tea infusions showed influence taste and aroma profiles. These extracts were used for phyto chemicals analysis by the method of Trease and Evans.

Estimation of pigments

Analysis of various pigments of *Camellia sinensis* tea leaves were carried out with reference to chlorophyll pigment, Total chlorophyll was estimated according to the method of Arnon [8]. Carotenoid was analyzed following the methods outlined by the method of Lia kusmita *et al* [9] polyphenoles [10].

Thin layer chromatography (TLC)

TLC is performed using silica gel 60 F254 percolated on alumni 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 [11].

Animal Mode

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 four groups:

III. EXPERIMENTAL SCHEDULED FOR UVB TREATMENT

Batch 1 animals were exposed to UVB and not treated with formulated product

Batch 2 animals were not exposed to UVB and not treated with formulated product used as control (n=5).

Batch 3 animals were exposed to UVB for 15 min / alternate days / for 15days and treated with formulated product

Batch 4 animals were exposed to UVB for 30 min / alternate days / for15days and treated with formulated product

Batch 5 animals were exposed to UVB for 45 min / alternate days / for 15days and treated with formulated product

Batch 6 animals were exposed to UVB for 60 min / alternate days / for 15 days and treated with formulated product

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).UV exposed experimental animals were treated with formulated product [combination of *Aloe vera* gel and green tea paste (10mg).Treatment was started from 8th day continued for another 10 days (18th day of the experiment) for the batch 2, 3&4 subsequently maintained batch 1 as normal/ control. Body weight of the animal was noted daily in all groups during treatment period. Animal samples were collected and used for further analysis after completion of treatment period as per experimental scheduled.

Western blots

For western blots, keratinocytes (5×10^6/10 ml) total cell lysates were prepared, and 15 µg of lysate aliquots were analyzed by Western blotting as described previously [12].Skin tissues crude extract (40µg protein 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 chemilluminescence detection system. Antibodies directed against the NH_2 terminus of Pten and antiactin were from GeNei™ Mumbai, India.

Results and Discussion

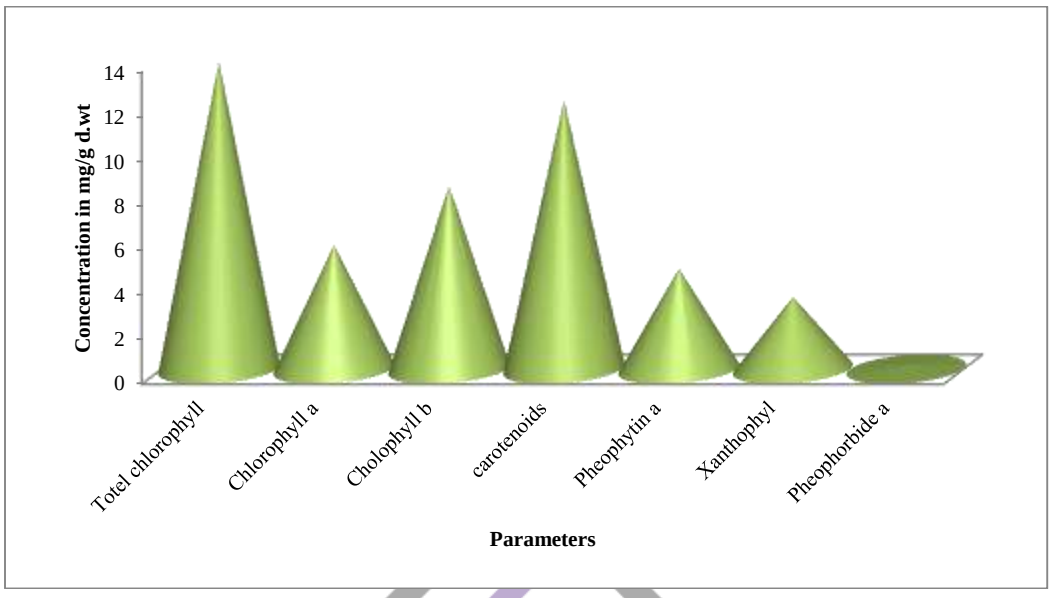
Present results focused on potential benefits of herbal extracts and bioactive compounds for human health care, provides a comparative phytoconstituent analysis of selected medicinal plants. **Table 1** showed the phytochemical characterization of *Aloe barbadensis* and *Camellia sinensis* plant extract which revealed the presences and absences of various secondary metabolites synthesis in fruits development stages. Results were explained phytochemical substances found in selected experimental samples of grape fruit varieties, almost all samples revealed the presence of compound similarly on comparison of four different varieties. Fresh fruit extracts has screened to identify compound of therapeutic potential source for the drug against human illness. Consumption of tea, wine, and cocoa, which also are plant based (tea comes from the dried leaves of the *Camellia sinensis* bush, wine from grapes, and cocoa from the dried and fermented seed of the *Theobroma cacao* tree), has been associated with reduced risk of these diseases as well. A large volume of laboratory and animal research showing preventive effects of tea polyphenols against cancer of the skin, lung, oral cavity, esophagus, stomach, liver, pancreas, small intestine, colon, bladder, prostate, and mammary gland. Tea polyphenols have been shown to affect tumor suppression and cancer cell replication and also alter gene regulation [13].

Table: 1 Comparative screening of phytoconstitutents *in Aloe barbadensis* and *Camellia sinensis*

S.No	Parameters	*C. sinensis*	*A.barbadensis*
1	*Alkaloids*	+	+
2	*Terpenoids*	+	-
3	*steroids*	+	-
4	*coumarins*	-	+
5	*Tannis*	+	+
6	*sponins*	+	-
7	*Flavonoids*	+	+
8	*Quinones*	+	-
9	*Anthroquinones*	+	-
10	*Phenols*	+	+
11	*Aromatic acid*	-	-
12	*Protein*	+	-
13	*Lipids*	-	-
14	*Carbohytrates*	-	+
15	*Glycosides*	+	+

Legends: + Positive symbol indicates the presence of the compound; - Negative symbol indicates the absence of the compound;

Figure1 Estimation of pigments in Camellia sinensis



***Legends**:* X axis indicates various pigments parameter; Y axis indicates concentration of pigment content of *Camellia sinensis* .Valves are means of three replicates.

Natural pigments are synthesized by plants which not only play a key role in photosynthesis but are also responsible for the bright colors of various plants, fruits, flowers, and vegetables. Anthocyanins are powerful antioxidants also possess anti inflammatory, antimicrobial, and anticancer properties.

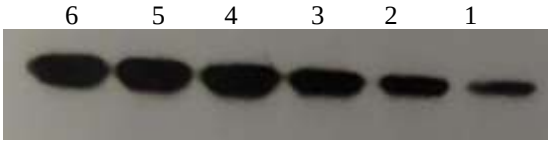
Figure: 2 Separation of Catechin by Thin Layer Chromatography

S -indicates Standard Catechin; E – indicates Camellia sinensis extracts.

***Legends**:* Results confirmed the presence of catechin in *Camellia sinensis* extracts as compared with Standard catechin which was run along with sample on TLC plate. S - Indicates catechin standard; E – indicates *Camellia sinensis e*xtracts. Standard catechin sample was obtained from S.D. Fine Chemicals, India.

Figure 2 results revealed presence of catechin isolated from *Camellia sinensis* extracts were identified by thin layer chromatography (TLC) in silica G F250 using catechin as a standard and determined by mixing 100mg of the sample with 100µl solvent. The active compound of catechins is rich in green tea, it has been effective in inhibiting the growth of tumour cells without harming healthy tissue particularly inhibition of proliferation and migration of smooth muscle cells. Products derived from plants are commonly used in primary health treatment, also play a pivotal role in the treatment of diseases via modulation of biochemical and molecular pathways.

Figure 3 Western blot analysis shows the expression of keratinocytes



Legend: Dorsal Epidermal cell expression from mice after different treatments indicated. 1-UVB induced not treated mice; batch 2 experimental set up used as control mice; 3- UVB induced [15min] mice treated with formulated product; batch 4 UVB induced [30min] mice treated; batch 5 -UVB induced [45min] mice treated ; batch 6 -UVB induced [60min] mice treated as mention in material and methods of experimental design.

UVB induced skin damaged in mice 10 days after initiation of treatment. Western blot result shows that keratin expression enhanced by formulated products 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 formulated products. The efficiency of *Aloe vera* gel and green tea pastes are used to treat damaged skin recovered by applying formulated product resulted in keratin synthesis which could be confirmed based on western blotting results.

Laboratory studies shown that topical treatment with application of formulated product [combination of *Aloe vera* gel and green tea pastes (dose 20mg/animal /day) inhibits UV B radiation induced skin tumourigensis in different batch of experimental animals. *Aloe vera*, produces gel plays a therapeutic role in health management through antioxidant, antitumor and antiinflammatory activities, and also offers a suitable alternative approach for the treatment of various types of skin diseases. Skin Tumor development and progressions constitute a multistep process including genetic and epigenetic changes [14]. *Aloe vera* and its constituents have a vital effect on the control of tumor development, through the modulation of genetic pathways. An important study was performed to investigate the antitumor activity of *Aloe vera* against stage‑2 skin carcinogenesis induced by chemical. Renuga [15] examined on the use of herbs as moisturizer for acne treatment. Cosmetics and skin protection application of 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. The author concluded that the aloe products appeared to retard exuberant granulation tissue and that pain and itching were relieved promptly. No toxic reactions or other adverse effect were seen in any animal.An earlier study showed the anti‑aging effect of *Aloe vera* to be tied to the production of collagen and elastin fibers, which make the skin more elastic and less wrinkled.
In this study, plant based natural products are formulated from the combination of both *Camellia sinensis* and *Aloe vera* constituents for its therapeutic implications in health maintenance through the modulation of various biological activities specifically keratin expression can be altered through epigenetic modification during malignant transformation. Understanding keratin functions and related regulatory mechanisms will help to design new therapeutic interventions for keratin-related skin diseases. The epidermis of the skin is a keratinized and stratified squamous epithelium composed mainly of keratinocytes, cells whose proliferation and differentiation must be tightly regulated and coordinated. Hair follicle morphogenesis and hair remodeling also depend on a balance of proliferative and apoptotic events. However, the molecular mechanisms underlying hair morphogenesis are not well understood.

IV. CONCLUSION

Epidemiological, clinical and biological studies have shown that solar ultraviolet (UV) light is a complete carcinogen and repeated exposure can lead to the development of various skin disorders including melanoma and non-melanoma skin cancers. Green tea poly phenols may play a role in the risk and pathogenesis of several chronic diseases, especially cardiovascular disease and cancer, and related pathologies. In addition, several studies suggest a beneficial impact of green tea intake on bone density, cognitive function, dental caries and kidney stones, among other effects [16]. Over the last years, numerous epidemiological and clinical studies have revealed several physiological responses to green tea which may be relevant to the promotion of health and the prevention or treatment of some chronic diseases. Present results revealed that catechins also favour action indicated that topical treatment inhibits UV radiation-induced skin carcinogenesis in different laboratory animal models. Topical treatment of *Aloe vera* gel paste mixed with catechin resulted in prevention of UVB-induced inflammatory responses, immune suppression and oxidative stress well as systemic immune suppression in laboratory animals. This fact was associated with the inhibition of UVB-induced infiltration of inflammatory leukocytes. The *in vitro* animal studies have suggested that *Aloe vera* gel paste mixed with catechin formulates new products are photo-protective in nature, and can be used as pharmacological agents for the prevention of solar UVB light-induced skin disorders. The formulated product is worth monitoring and in future vigilant research will develop new plant based cream in pharmaceutical industry.

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