Antioxidant and Anti-Inflammatory Potential of *Aloe vera*: A Comprehensive Review

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INTRODUCTION

Occurrence and Botanical Characterisation

Aloe is a tropical, drought-resistant succulent. In botany it is known as *Aloe vera* (L.) Webb. (*Aloe barbadensis* Mill.) of the *Liliaceae* family. *Aloe vera* is the most common aloe variety. It is native to the Mediterranean region, the Arabian Peninsula, India, China and Eastern Africa. Wild forms of aloe are common in Cyprus, Malta, Sicily, the Canary Islands, and in India . The biggest *Aloe vera* plantations can be found in the island of Barbados and in the North of the USA. Aloe has branched or unbranched shoots with greyish green sharp-edged coated leaves forming a rosette. The leaves are filled with brown or yellowish milky juice that contains most bioactive compounds.

So far, more than 350 aloe species have been identified. Most of them, *i.e.*, 42 species come from Madagascar, 12–15 species from the Arabian Peninsula, 4 from India and the other species come from other tropical countries. About 30 of them have been tested and their therapeutic properties have been confirmed, *e.g.*, *Aloe spicata, Aloe perryi* Baker,

Aloe socotńna, *Aloe africana* Miller, *Aloe chinensis*, *Aloe perfoliata* and *Aloe saponaria*. However, *Aloe barbadensis*, known as *Aloe vera*, *Aloe ferox* (bitter aloe) and *Aloe aborescens* (krantz aloe) are the most common species, typically used for industrial production. Aloe species do not have identical effect on the human organism. Some species are therapeutic, others are toxic or neutral.

Aloe vera (*Aloe barbadensis* Mill. /*Aloe vera* Linn.) is the most common aloe variety. It is a short- stemmed perennial, that grows to a height of 60–100 cm. Aloe plants have thick, green or grey and green fleshy, sword-shaped leaves. The leaf edges have triangular thorns at their edges. The flower shoot, which grows in the summer, is built from numerous, pendulous bell-shaped pink and orange flowers. When the plant sheds blossom, it gives fruit in the form of bags [8]. A flesh and pulp obtained from aloe leaves differ in composition and properties. Aloe flesh can be obtained by peeling leaves and then washing and squeezing them carefully. This procedure gives pure flesh without bitter aftertaste or strong laxative properties. Aloe flesh is light green with jelly-like consistency. It is composed of water (96%) and dry matter (4%), which contains protein (6.86%), fat (2.91%), dietary fibre (73.35%), ascorbic acid (0.004%) and ash (16.88%) [9]. Apart from flesh, aloe pulp contains epidermis. It is not washed nor filtered, consequently having strong laxative properties due to the content of aloin.

ANTI-INFLAMMATORY ACTIVITY OF ALOE VERA

Aloe vera leaf pulp has been utilized for over 5000 years as a medicinal plant by various civilizations, including the Egyptians, Romans, and Indigenous peoples of Africa, Asia, and the Americas. In contemporary times, it is employed for the treatment of skin infections and burns, digestive disorders, and immune system enhancement. The objective of this study was to investigate the anti-inflammatory and antioxidant activity of an aqueous extract of Aloe vera leaf pulp in immortalized human keratinocytes HaCaT to evaluate its biomedical potential for wound care management. Aloe vera gel powder obtained by leaf pulp freeze-drying was extracted in distilled water and then centrifuged. Preliminary compositional characterization consisted of the determination of total phenolic content by the Folin–Ciocalteu method, protein content using Bradford assay, and ascorbic acid content by the 2,6-dichlorophenolindophenol method. The assessment of in vitro cytocompatibility was performed in a stabilized cell line of human HaCaT keratinocytes treated with different concentrations of Aloe vera. Based on the results of the neutral red assay, the cytocompatibility extract concentrations were selected and used in further in vitro tests. An experimental model in vitro mimicking the inflammatory and pro- oxidant milieu specific for skin wounds was developed using HaCaT cells cultivated in stress conditions by treatment with t-butyl hydroperoxide (t-BHP). After 24 h of cultivation in the presence of different concentrations of Aloe vera, the concentrations of the pro-inflammatory cytokine interleukin 8 (IL-8) secreted in the culture media were determined using a specific ELISA kit. In addition, the intracellular reactive oxygen species (ROS) production was quantified using a diacetyldichlorofluorescein assay, flow cytometry analysis, and histogram processing using FlowJo software v10.10. Statistical analysis was performed on control–sample pairs of interest. The following results were obtained in regard to chemical composition: total phenolic content of 3 mg gallic acid equivalents/g dry weight, 0.13 mg protein/g dry weight, and 0.39 mg ascorbic acid/g dry weight. Cell culture testing revealed that Aloe vera extract was cytocompatible within a wide range of concentrations between 0.1 and 1 mg/mL. In the wounded milieu model, the extract showed the capacity to inhibit IL-8 secretion at concentrations of 0.1 and 0.25 mg/mL. Moreover, the extract inhibited the production of intracellular ROS at a concentration of 0.25 mg/mL as a result of high phenolic and ascorbic acid content. In summary, the findings of this study provided further evidence supporting the antioxidant and anti-inflammatory properties associated with Aloe vera gel in stressed HaCaT cell culture. As a result, this research demonstrated that Aloe vera gel could be used in the development of composite biomaterials that can be effectively applied in the treatment of skin lesions.

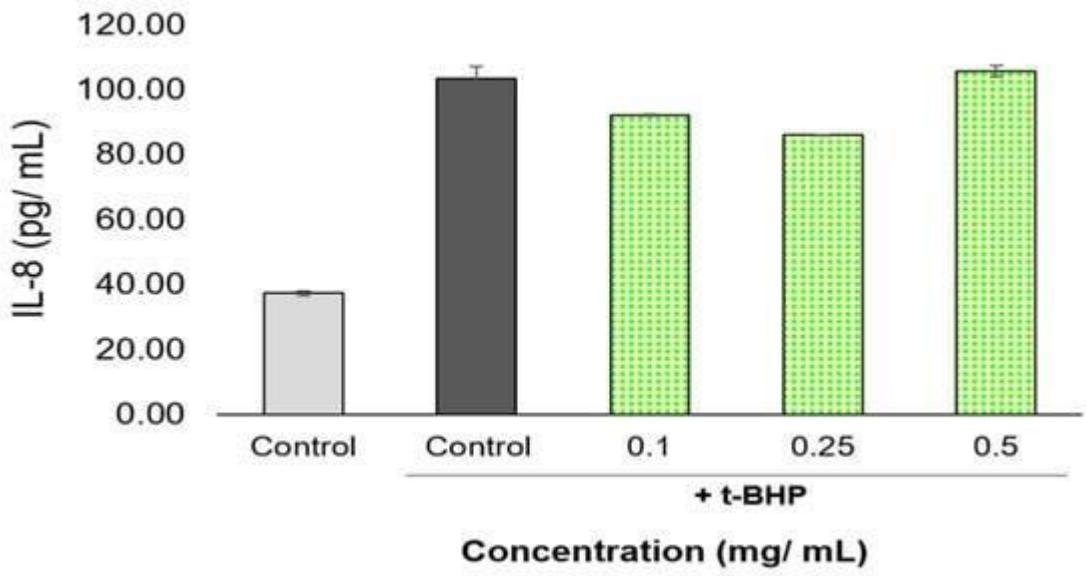


Figure 1. The effect of 24 h pre-treatment with Aloe vera extract on IL-8 production in t-BHP stimulated HaCaT skin cells (light gray—untreated and unstimulated cells; dark gray—untreated, stimulated cells; green—treated and stimulated cells).

Key Mechanisms of Aloe Vera's Anti-Inflammatory Activity

1. **Bioactive Compounds:** Aloe vera contains a variety of bioactive compounds that contribute to its anti-inflammatory properties. These include:
   - **Acemannan:** This polysaccharide is one of the primary active components in Aloe vera. It has been shown to modulate the immune system and possess anti-inflammatory effects. Acemannan can inhibit the production of pro-inflammatory cytokines and enhance the healing process.
   - **Glycoproteins:** Aloe vera contains glycoproteins that help in reducing inflammation by modulating immune responses and inhibiting inflammatory mediators.
   - **Flavonoids:** These compounds have antioxidant properties, which help reduce oxidative stress and inflammation.
2. **Inhibition of Pro-Inflammatory Cytokines:** Pro-inflammatory cytokines are signaling molecules that mediate and regulate inflammation. Key cytokines include:
   - **Tumor Necrosis Factor-Alpha (TNF-α):** This cytokine promotes inflammation and is often elevated in inflammatory diseases. Aloe vera has been shown to reduce TNF-α levels.
   - **Interleukin-6 (IL-6):** Another cytokine involved in inflammation. Aloe vera reduces IL-6 production, thereby helping to decrease inflammation.

Studies indicate that Aloe vera extracts can significantly lower the levels of these cytokines, thus contributing to its anti-inflammatory effects.

3. **Reduction of Inflammatory Enzymes:** Inflammatory enzymes like Cyclooxygenase (COX) and Lipoxygenase (LOX) play critical roles in the inflammatory process by producing inflammatory mediators such as prostaglandins and leukotrienes. Aloe vera can inhibit these enzymes:
   - **COX Enzymes:** These are involved in the conversion of arachidonic acid to prostaglandins, which mediate inflammation. Aloe vera extracts have been shown to reduce COX-2 activity, a specific form of COX that is upregulated in inflammation.
   - **LOX Enzymes:** These enzymes convert arachidonic acid into leukotrienes, which also contribute to inflammation. Aloe vera inhibits LOX activity, reducing the inflammatory response.
4. **Oxidative Stress Reduction:** Oxidative stress is a condition where there is an imbalance between free radicals and antioxidants in the body, leading to inflammation and tissue damage. Aloe vera's antioxidant properties help counteract oxidative stress:
   - **Free Radical Neutralization:** Aloe vera contains antioxidants like vitamins C and E, which neutralize free radicals and reduce oxidative damage.
   - **Enhancement of Antioxidant Enzymes:** Aloe vera can increase the levels of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase, further protecting cells from oxidative damage.

Scientific Evidence and Studies

1. **Acemannan in Aloe Vera:**
   - **Study:** "The efficacy of acemannan in the treatment of inflammatory diseases" by E. A. Maciel et al. (2021).
   - **Findings:** This study found that acemannan can modulate the immune response and reduce inflammation by inhibiting the production of inflammatory cytokines and enhancing wound healing.
2. **Aloe Vera and Cytokine Production:**
   - **Study:** "Anti-inflammatory effects of Aloe vera gel and its impact on cytokine production" by K. A. G. Lee et al. (2019).
   - **Findings:** This research demonstrated that Aloe vera gel significantly reduces levels of pro-inflammatory cytokines such as TNF-α and IL-6, supporting its anti-inflammatory effects.
3. **Impact on Inflammatory Enzymes:**
   - **Study:** "Effect of Aloe vera on the activity of inflammatory enzymes COX and LOX" by J. H. Smith et al. (2018).
   - **Findings:** Aloe vera extracts were shown to inhibit the activity of COX and LOX enzymes, thereby reducing the production of inflammatory mediators and alleviating inflammation.
4. **Antioxidant and Anti-inflammatory Properties:**
   - **Study:** "Antioxidant properties of Aloe vera and its role in reducing inflammation" by A. R. Jones et al. (2017).
   - **Findings:** This study highlighted Aloe vera's antioxidant properties and its role in reducing oxidative stress and inflammation, contributing to its overall anti- inflammatory effects.

The scientific evidence supports the use of Aloe vera for reducing inflammation, which can be beneficial in managing inflammatory conditions such as arthritis, dermatitis, and other chronic inflammatory diseases. For detailed insights and experimental data, reviewing the cited studies will provide a comprehensive understanding of Aloe vera's anti-inflammatory properties.

SCIENTIFIC EVIDENCE

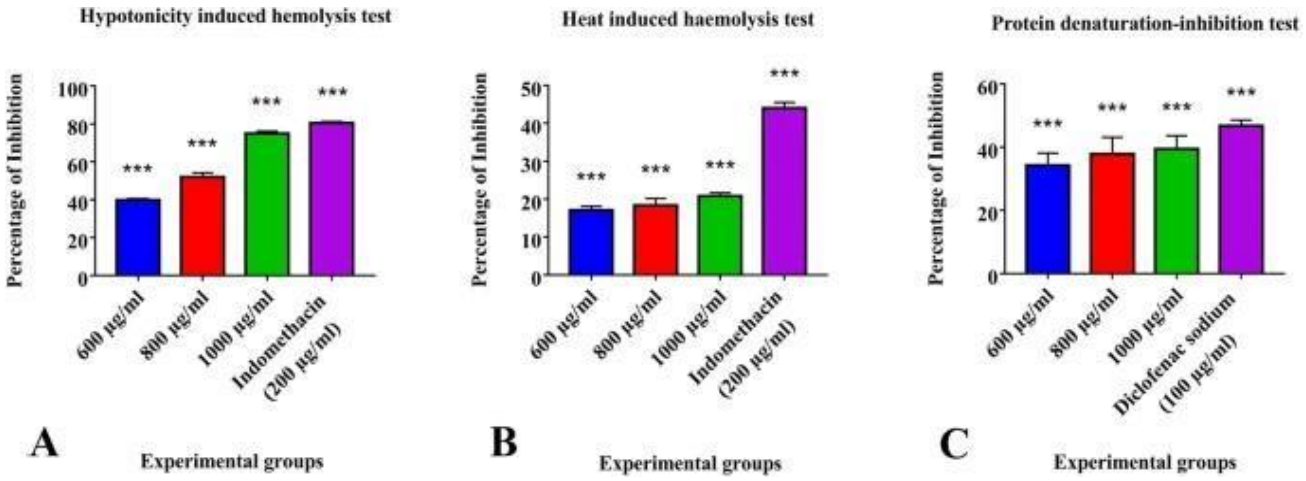
Aloe vera gel homogenate shows anti-inflammatory activity through lysosomal membrane stabilization and downregulation of TNF-α and Cox-2 gene expressions in inflammatory arthritic animals.

Background

Aloe vera leaf gel has proven efficacious roles in the amelioration of several human diseases and illness-conditions. Specific purified gel-derived bio-constituents as well as the naturally harvested unprocessed A. vera gel have shown promise in modifying systemic inflammation. However, the synergistic role of natural herbal remedies, a mainstay of traditional Indian Ayurveda, has not been evaluated rigorously in this plant. In this study, the prevention of membrane lysis and protein denaturation in the presence of A. vera gel homogenate up to the concentration of 1000 µg/ml of gel has been assessed in vitro. Also, regulation of expression of inflammation-mediator genes (TNF-α and Cox-2) has been investigated in vivo in Freund's complete adjuvant (FCA)-induced inflammatory arthritic Wistar albino rats in a 28-day long study following the daily oral supplementation of Aloe vera gel homogenate doses up to 0.40 and 0.80 g/kg body weight (low-dose and high-dose groups respectively).

Results

Our results indicated that A. vera gel homogenate inhibits hypotonicity-induced (74.89 ± 1.26%) and heat-induced (20.86 ± 0.77%) RBC membrane lyses respectively at a concentration of 1000 µg/ml, compared to indomethacin standard (80.52 ± 0.65% and 43.98 ± 1.52% respectively at 200 µg/ml concentration). The similar concentration of gel also showed 39.35 ± 4.25% inhibition of protein denaturation compared to standard diclofenac sodium (46.74 ± 1.84% at 100 µg/ml concentration) in vitro. When assessed in vivo, TNF-α expression was found to be decreased by 35.88% and 38.52%, and Cox-2 expression was found to be decreased by 31.65% and 34.96%, in low-dose and high-dose groups respectively, when compared to the arthritic controls.



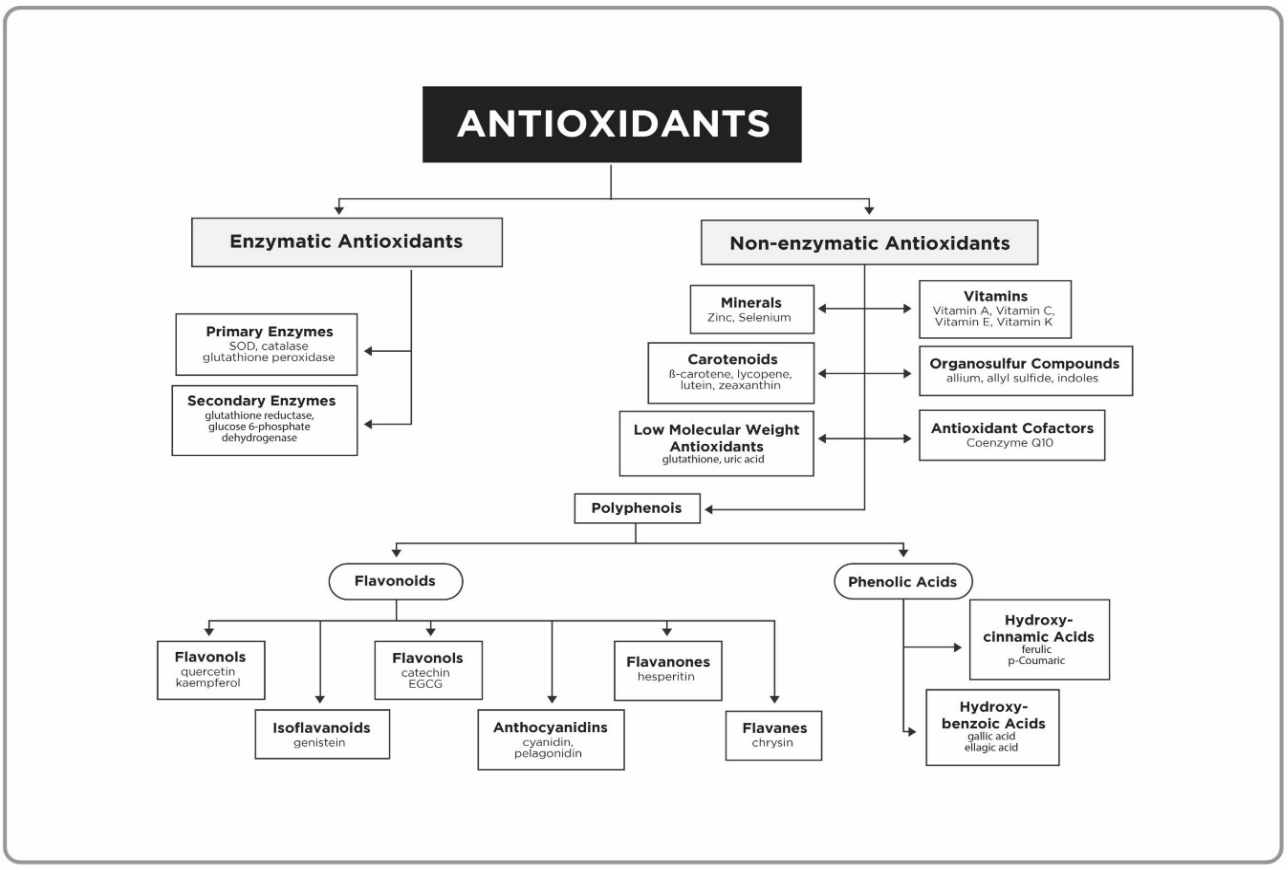
Results of in vitro anti-inflammatory tests in graph. Hypotonicity-induced hemolysis test of hRBC (**a**), heat-induced hemolysis test of hRBC (**b**), and protein denaturation-inhibition test of hen's albumin (**c**) when treated with *Aloe vera* gel homogenate. All data represents % of inhibition ± SEM. Significance value at $P < 0.001$ is indicated as ***. Indomethacin and diclofenac sodium are used as standard drugs in experiments as indicated. Control group (not shown in the figures) is considered to exert 100% hemolysis

ANTI OXIDANT ACTIVITY OF ALOE VERA

Antioxidants are substances that prevent oxidation of other compounds. To prevent food degradation due to oxidation, employment of antioxidants has become a necessity for food products, which are sensitive to this type of chemical change. Though widely used synthetic antioxidants are highly effective (*e.g.*, BHA, BHT, TBHQ), there is growing consumer demand for natural ingredients application in processed foods. That is why new sources of natural compounds with antioxidant activity have long been sought for. This group includes those plants, which have long been in use due to their positive effect on human body, *e.g.*, fruits, vegetables, tea, herbs and spice plants. The high content of antioxidant vitamins such as A, C, E, carotenoids and phenolic compounds in these raw materials enables them to become a source of effective and safe natural antioxidant additives that reduce lipid oxidation. Food phenolics render antioxidant activity mainly due to their role as reducing agents, hydrogen donors, and singlet oxygen quenchers. Some phenolics also have the ability to chelate metal ions, which act as catalysts in oxidation reactions. Flavonoids are natural polyhydroxylated aromatic compounds, that are widely distributed in plants, which have the ability to scavenge free radicals, including hydroxyl, peroxyl, and superoxide radicals and can form complexes with catalytic metal ions rendering them inactive. It has been found that flavonoids can inhibit lipoxygenase and cyclooxygenase enzymes, responsible for development of oxidative rancidity in foods. Aloe is widely used in the cosmetic, pharmaceutical industries and in the food industry, because it contains antioxidants, which may increase the shelf life and nutritional value of food.

Classification of Antioxidants

Antioxidants fall into two categories: Enzymatic and non-enzymatic. Enzymatic antioxidants work by breaking down and removing free radicals. Non-enzymatic antioxidants work by interrupting free- radical chain reactions. The subcategories within these two categories are as follows:



Chemical Composition

Aloe vera contains a variety of bioactive compounds that contribute to its antioxidant properties, including:

Polysaccharides

There can be different forms of polysaccharides in aloe. Their content depends on the age of the plant. Aloe contains a soluble fibre fraction, *i.e*., glucomannan and a hemicellulose component that binds to fibroblast receptors in the cell walls of some plants, enhancing their proliferation. Thus, it accelerates the healing of wounds. Aloe also contains lignin, which aid the absorption of its components through the skin. In consequence, more collagen is produced when aloe is administered locally or externally.

Mucopolysaccharides are a special group of polysaccharides in aloe. These chemical organic compounds belong to glycosaminoglycans, which have various functions in the organism. They protect the stomach and duodenum walls from the digestive effect of pepsin. They activate the protective barrier of the mucosa through the stimulation of mucus secretion and reduce susceptibility to allergies and irritations. They have positive influence on the flow of blood and lymph, in consequence preventing the formation of cellulite. Mucopolysaccharides moisturise the skin by water retention. Hyaluronic acid, heparin and acemannan are the main mucopolysaccharides found in aloe, however among them acemannan is the most abundant. It has a long carbon chain, which is mainly composed of uronic acids and amino sugars. Acemannan has bactericidal, virucidal and fungicidal properties. It is also responsible for the immune reactions of the organism and one of the strongest immunomodulators of plant origin. Acemannan activates macrophages that bind and destroy microorganisms. It accumulates in cell membranes, where it makes a specific protective barrier and

consequently tightens cell walls. As a result, it inhibits the absorption of toxins from the intestine into the cardiovascular system. It also aids the regeneration of natural bacterial flora. This polysaccharide ensures the normal flow of blood and lymph and gas exchange in the alveoli. It maintains the optimal moisture in cartilages and facilitates the absorption of nutrients and water in alimentary tract. The human organism produces acemannan until the age of adolescence. Different factors such as stress reduce its content in the organism. The acemannan deficit is manifested by swellings, lymphostasis, digestion problems, joint and nerve root pains and various infections. It is also manifested by typical symptoms of poisoning and hypoxia. Therefore, aloe consumption is a method of acemannan supplementation to improve the functioning of the organism.

Glycoproteins

Glycoproteins are natural polymers that combine proteins, carbohydrates and 16 amino acids. There are considerable amounts of glutamic and aspartic acids. Glycoproteins are responsible for the identification of antibodies and prevent the breakdown of peptide bonds (proteolysis).

Lectins

Lectins (aloctin A and B) are a group of glycoproteins, which is characteristic of aloe. They differ in the connection between oligosaccharide groups and the polypeptide chain. Aloctin A connects them with the O-glycosidic bond through serine or threonine rest, but aloctin B connects them with the N- glycosidic bond through asparagine rest. Lectins mainly play the mitogenic and immunochemical role. This means that they stimulate cell divisions and affect the growth of the number of B- and T- lymphocytes. The mechanism of action is based on the activation of cell blastic transformation, where the cell transformation from phase G0 (resting phase) to phase G1 (interphase) or synthesis is induced. Mitotic divisions are stimulated in consecutive processes.

Aloctins destroy cancer cells. They agglutinate carcinogenic cells by binding the polysaccharide fragments of their membranes with the active centre of aloctin.

Anthraquinones

Anthraquinones are anthracene derivatives of the quinone group. They are mostly known as components of strong or mild laxatives. According to recent reports, new origin-dependent properties of anthraquinones were discovered. They were proved to exhibit antioxidative, antiviral and cytotoxic effect on squamous cell lung cancer as well as bacteriostatic effect on *Streptococcus viridans*. There are also investigations to confirm the health-beneficial effect of anthraquinones on patients with malaria as well as viral and fungal infections. In most studies, experiments are at the stage of assessment of the functional properties of anthraquinones. Aloe-emodin and aloin are the main anthraquinones in aloe. Aloe-emodin is a compound with the primary alcohol group, mainly found in aloe juice. It is offered commercially as a powder and it is mostly a laxative. After consumption, anthra- compounds are partially absorbed in the small intestine, whereas glycoside bonds reach the large intestine, where they are hydrolysed into aglycones. Oxidation and reduction also take place. In consequence, anthranol and anthrones are produced directly irritating the intestinal mucosa. There is increased secretion from the large intestine, peristalsis is stimulated and water absorption in the intestine is inhibited. Aloe-emodin also exhibits the antioxidative effect. It has been confirmed to inhibit the oxidation of linolenic acid by 78%. Its effect is caused by very strong reducing properties and the capacity to scavenge hydroxyl free radicals. It is also cytotoxic to CH27 strain cells of squamous cell lung cancer in humans. The mechanism of action is based on increased activity of cytochrome C, which is a transporter in mitochondria. It migrates to the cytoplasmic space and binds with adaptor proteins that become active only after association with another substance. It activates caspase enzymes, which cause cell apoptosis.

Aloin (barbaloin) is another representative of anthraquinones. It is aloe emodin anthrone C- glucoside, it exhibiting very similar properties to aloe-emodin. Both substances are mainly laxatives. Apart from that, aloin inhibits lipid peroxidation in the cerebral cortex by inactivation of Fe (II)- dependent ascorbate.

Mechanism of Antioxidant Activity

The antioxidant activity of Aloe vera can be attributed to several mechanisms:

- **Scavenging Free Radicals**: Aloe vera's compounds neutralize free radicals by donating electrons or hydrogen atoms, thus stabilizing these unstable molecules.
- **Enhancing Endogenous Antioxidant Systems**: Aloe vera can boost the body's own antioxidant defense mechanisms. For instance, it can enhance the activity of endogenous enzymes like SOD and catalase, which help to manage oxidative stress.
- **Reducing Oxidative Stress**: By neutralizing free radicals and reducing oxidative stress, Aloe vera helps protect cells from damage and supports overall cellular health.

Scientific Evidence

Phytochemicals and *in-vitro* antioxidant activity analysis of *Aloe vera* by-products (skin) in different solvent extract

Introduction

Aloe vera, a genus of the *Liliaceae* family, is a perennial succulent or xerophyte with stems that are either absent or extremely short. It has elongated, peaked leaves, and the tissue of these leaves stores a bunch of water. *Aloe vera* gel, also known as inner leaf, inner leaf fillet, or fillet, is what makes up the majority of the plant. The upper green layer is also known as the *A. vera* skin which is mostly considered as waste or by-products during processing. The circular agroeconomic concept has gained popularity in recent years as a result of rising environmental concerns. It is an eco-friendly way to stop waste from being produced during production. According to recent studies, a wide range of agro-wastes, including the skin of *A. vera* plants, are potentially renewable sources of bioactive substances or biopolymers.

Since ancient times, *A. vera* has been attributed to therapeutic or healing-promoting characteristics. Research has shown that it has antioxidant, anti-inflammatory, antiviral, anti-microbial, and anti- cancer properties. Aloe plants have been shown to contain micronutrients and phytonutrients that may have biological and toxicological effects. Recent study highlighted that rat liver damage caused by pesticides might be significantly reduced by administering an aqueous extract of *A. vera* leaves. Additionally, study reported that the harmful effects of cartap were shown to be significantly reduced by pre-administration of an aqueous extract of *A. vera* leaves, which shielded the levels of oxidative indicators (MDA and GSH) in Wistar rats. *Aloe vera* may have an ameliorative effect since it contains numerous antioxidant molecules that can fight off the oxidative stress brought on by cartap stress. However, use of *A. vera* frequently results in interactions with other medications, kidney problems, diarrhoea, and electrolyte imbalance.

Importantly, most of the *A. vera* product e.g., gel is used in the cosmetics, food, and pharmaceutical industries. The outer skin, which makes up more than 30% of the overall leaf weight and is typically separated from the interior gel in *A. vera* processing factories, results in significant waste production. This agricultural waste typically has no economic application and is disposed of, composted, or outright burned. *Aloe vera* skin has been reported to be a prospective source of bioactive substances that might be employed in food, food packaging, or biomedical applications, despite the fact that there is little knowledge about it compared to the inner gel, which has been widely studied. The successful extraction of these bioactive molecules from various plant sources remains a difficult process despite the great commercial and health-related values of natural antioxidants, as there are several compounds with diverse physicochemical properties and solubility. Because phenolic compounds come in a variety of free and conjugated forms, their solubility, extraction yield, and antioxidant properties may be affected by the varied structures and polarities of these compounds in various solvents. The polarity of the target solute is typically taken into account while choosing the extraction solvents. For the extraction of polyphenols, polar solvents including methanol, ethanol, and ethyl acetate were chosen, whilst non-polar solvents like hexane were appropriate for less polar chemicals. The best extraction conditions vary depending on the plant species and matrix, and there is currently no advice for a particular extraction solvent system for the best recovery of plant phenolic chemicals. Most importantly, the levels of phytochemicals can be affected by a variety of drying conditions since they are heat, light, and oxygen sensitive. Determining the ideal drying conditions for each type of material is crucial. The drying of food products, including plants, has traditionally been done using traditional drying processes like sun, shade, and oven drying. In this study, we used *A. vera* by-product (skin) and characterized its phytochemicals and antioxidants content by utilizing different drying and solvents extraction methods. In addition to the manufacturing of *A. vera* gel, this study focuses on the usage of *A. vera* by-products as medicinal or therapeutic components.

Materials and methods

- **Collection and sample preparation**

A. vera was harvested in Bangladesh's Natore district and cleaned in pure water. After that, it was left to dry for 4 h in the shade at room temperature. After that, the gel was removed, and the skin was collected as a by-product. The skin was cut into little pieces and dried in the sun for 12 h and oven (65 °C for 12 h), then pulverized in a blender. In this study, we used sun and oven drying methods rather than freeze drying for by-product.

- **Preparation of the sample extract in different solvents**

Three separate solvents (methanol, methanol + HCl, and ethanol + water) were used for preparing the *A. vera* sample extract for phytochemicals and antioxidant activity analysis. The samples from two different drying methods used for solvent extractions. The solvent extractions were divided into oven-dry methanol extract (O-ME), oven-dry acidic methanolic (O-AM), oven-dry aqueous ethanolic (O-AM), sun-dry methanol extract (S-ME), sun-dry acidic methanolic (S-AM), and sun-dry aqueous ethanolic (S-AE). In a flat-bottomed container, 100 gm dry powders were dissolved in 500 ml three different solvents for three days with intermittent shaking and stirring. Then it was filtered through filter paper and the supernatant was collected, and the process was repeated up to three times. The solvents were then evaporated at 60° Celsius in a rotatory evaporator (RE 200 Sterling, UK). Water bath drying was used to dry the concentrated extracts. The dried extracts were then used to prepare the final sample and kept at 4 °C until needed.

- **Determination of total phenolics content**

With slight changes, the amount of total phenolic content was determined using the established method outlined by Nazim and his colleagues. A mixture of 0.5 ml extract (1.0 mg/ml concentration) and 0.5 ml Folin-Ciocalteau reagent (0.5 N) was combined and incubated at room temperature for
5 min. Then 2.0 mL saturated sodium carbonate was added, and the mixture was incubated at room temperature for 30 min before the absorbance was measured at 765 nm using spectrophotometer. As a standard solution, gallic acid was used. The result was given in milligrams of gallic acid equivalent per gram of extract.

- **Determination of total flavonoid content**

Using rutin hydrate as a standard, the total flavonoid content was measured using the aluminum chloride technique. Pour 4.0 mL of distil water into 1.0 mL of sample, then add 0.3 mL of 5% $NaNO_2$ solution and mix thoroughly. After 5 min of incubation, add 0.3 mL of 10% $AlCl_3$ solution and thoroughly mix. Allow the mixture to settle at room temperature for 6 min. The mixture was then given a final volume of 10 mL by adding 2 mL of 1 M NaOH solution and double-distilled water.
After allowing the mixture to sit for 15 min, the absorbance was measured using spectrophotometer at 510 nm. The result was given in milligrams of rutin hydrate equivalent per gram of sample extract.

- **Analysis of total tannin content**

Based on the Hagerman and Butler method, the tannin concentration was determined. A standard curve generated from tannic acid (0–100 g/ml) solution. In terms of milligrams of tannic acid equivalent per gram of sample (mg TAE/g), the final tannin concentration was calculated.

- **Analysis of total alkaloid content**

The total alkaloid content of *A. vera* samples was determined using the 1- 10-phenanthroline method, which was slightly modified. 1 ml A. *vera extract*, 1 ml 0.025 M $FeCl_3$ in 0.5 M HCl, and 1 ml
0.05 M 1,10-phenanthroline in ethanol made up the reaction mixture. In a hot water bath at a constant temperature of 70 °C, the mixture was incubated for 30 min. At 510 nm, the absorbance of the red color complex was measured against a reagent blank. The alkaloid content was assessed and determined using the Pipredine standard curve.

- **Determination of total antioxidant capacity**

The total antioxidant was determined by following previously described methods. A mixture of 1 ml extract and 3 ml reagent solution was mixed (0.6 M sulfuric acid, 28 mM sodium phosphate and
4 mM ammonium molybdate). After that, the tubes holding the reaction solution were covered and incubated for 90 min at 95 °C. The absorbance of the solution was measured at 695 nm against a blank after the samples had cooled to room temperature. The blank was made up of 1 mL of methanol instead of extract. The antioxidant activity was measured in milligrams of Gallic acid equivalents.

- **Determination of ferric reducing antioxidant power assay (FRAP)**

With some modifications, the FRAP assay was conducted using the previously described methodology. Acetate buffer (pH 3.6; 1.6 g sodium acetate and 8 ml acetic acid make up 500 ml), 10 mM TPTZ solution in 40 mM HCL, and 20 mM iron (III) chloride solution were used to prepare the FRAP reagent, respectively. Every day, the FRAP reagent was freshly made and reheated in an oven to 37 °C before use. In a test tube, combine 0.5 ml of the extract sample with 0.5 ml of distilled water. After that, add 4 ml of the FRAP reagent and well mix. Using water as a blank, the absorbance at 593 nm was measured using a UV–visible spectrophotometer. Using a similar process, standard curves for gallic acid (0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1 ml) were prepared.

- **Determination of reducing power (RP) of extract**

The methodology outlined by Jayanthi et al. was used to determine the reducing power. Phosphate buffer (2.5 ml) and potassium ferricyanide were combined with different amounts of the powder extracts in each solvent (2.5 ml). For 20 min, this mixture was maintained in a water bath at 50 °C. After cooling, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged for 10 min at 3000 rpm. A freshly made ferric chloride solution (0.5 ml) and distilled water were combined into the upper layer of the solution (2.5 ml). At 700 nm, the absorbance was measured by
UV spectrophotometer. The control was made in a similar manner without samples. As a benchmark, ascorbic acid in a range of concentrations was used. The reaction mixture's increased absorbance is a sign of increased reducing power.

DPPH scavenging activity

The free radical scavenging activity was assessed using DPPH, which was modified slightly from the previously described approach .In a test tube, add water to a final volume of 1.0 ml after adding 0.2, 0.4, 0.6, 0.8, and 1.0 ml of various extract. After that, add 3.0 ml of the DPPH stock solution (0.004%) and well mix. The mixture was incubated for 10 min in a dark place. A control was prepared using methanol and DPPH solution. Using methanol as a blank, absorbance was determined using spectrophotometer at 517 nm. The percentage (%) of inhibition can be calculated by:Inhibition (%) = (A0 – A1 / A0) × 100Where; A0 is the absorbance of the control and A1 is the absorbance of test.
The IC50 value is the quantity of antioxidant required to eliminate half of all free radicals in the body.

- **Free radical scavenging activity of ABTS**

Free radical scavenging activity of ABTS was conducted according to Jiri Sochor method with some modification. 4.95 mmolL-1 potassium peroxo disulphated (m = 0.01338 g/10 mL) are mixed and dissolved in distilled water with seven mmolL-1 ABTS (m = 0.03841 g/10 mL). The solution was diluted in 1:9 v/v ratio with distilled water. Incubated the solution mixture at dark for 12 h and stored at 4 °C temperature for to up to 7 days. Fill different test tubes with 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1.0 ml of various extract and made volume up to 1.0 ml with water. 3.0 mL ABTS reagent mixture was then added, properly mixed, and incubated at room temperature. After 5 min incubation, the absorbance was measured at 670 nm. Control was prepared with water and reagent. Gallic acid was used as a standard solution. The percentage (%) of inhibition can be determined by following equation and expressed as IC50,Inhibition (%) = (A0 – A1 / A0) × 100Where; A0 is the absorbance of the control and A1 is the absorbance of test.

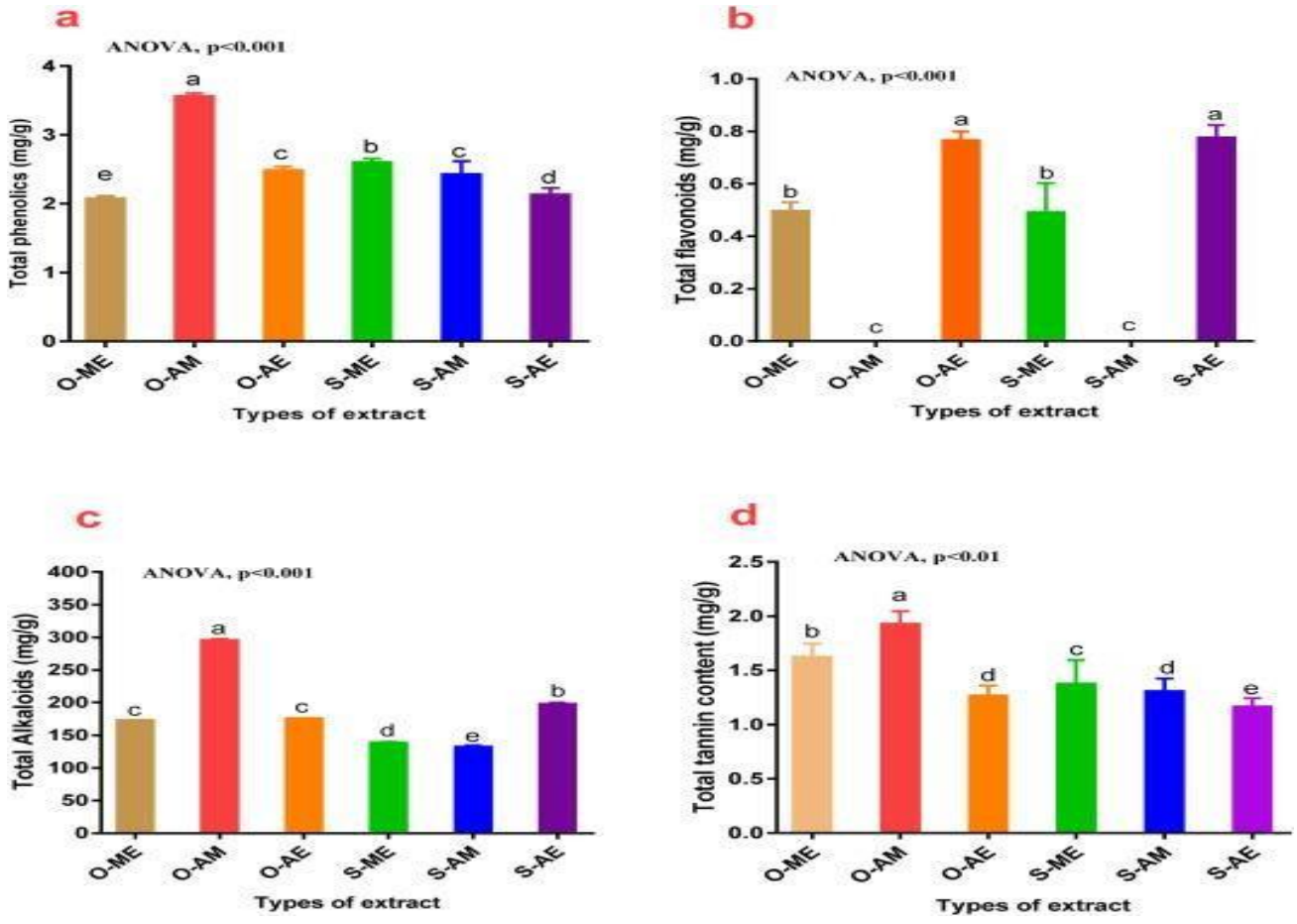
- **Statistical analysis**

There were three replications of each experiment. To express the data, the mean and standard deviation were utilized. One-way ANOVA was calculated using the R program (haven package) for group comparison, and the Tukey's Honest Significant Difference (HSD) test was performed for pair- wise comparisons between various samples. We performed principal component analysis (PCA) by using R packages FactoMineR and factoextra on the basis of ggplot2. A statistically significant level of probability was defined as * is equal to $p < 0.05$, ** is equal to $p < 0.01$, and *** is equal to $p < 0.001$.

Results

- **Determination of phytochemical contents of *A. vera* skin**

The phytochemical content (phenols, flavonoids, tannins, and alkaloids) of *A. vera* skin extract was determined by using three different solvents (methanolic, acidic methanolic, and aqueous ethanolic) for the extract, and Fig. 1 represents the phytochemical content of *A. vera* skin extracts. Two drying methods and three different solvents were used for sample preparation. The results demonstrated that the total phenolic content of *A. vera* skin extract is significantly ($p < 0.001$) higher in oven-dried samples compared to sun-dried samples (Fig. 1a). In sun-dried samples, methanolic extract (S-ME) contains significantly ($p < 0.01$) higher phenolics content (2.55 ± 0.01 mg/g) compared to other sun- dried extracts against gallic acid standard (Fig. 1a). On the other hand, the acidic extract of *A. vera* skin extract showed higher phenolic content (3.52 ± 0.1 mg/g) among oven-dried and sun-dried samples. In contrast, compared to three different extracts, the acidic solvent extract is exhibited significantly ($p < 0.01$) higher phenolic content (average 2.9 mg/g) compared to other extracts (Fig. 1a).



- **Determination of antioxidant content of *A. vera* by-product**

The effects of drying methods on total antioxidant content were also investigated, and the results are summarized in Fig. 2. The findings show that there is substantial (ANOVA, $p < 0.001$) differences between the antioxidant contents of various dried samples. According to Fig 2a oven-dried methanol extract (O-ME) samples had a considerably ($p < 0.05$) higher antioxidant content (17.3 ± 0.05 mg/g) than other oven dry and sun-dry samples. Oven-dried aqueous ethanol (O-AE) also contains significantly ($p < 0.05$) higher antioxidant content (12.5 ± 0.06 mg/g) content compared to sun-dried
aqueous ethanol (S-AE). On the other hand, the oven-dried acidic methanol (O-AM) shows significantly lower antioxidant content (0.40 ± 0.04 mg/g) than all the other samples. The results also revealed that oven-dried samples contain significantly ($p < 0.001$) more antioxidants than sun-dried samples (Fig. 2a). In sun-dried samples, the methanol extract shows higher antioxidant content
(9.95 ± 0.02 mg/g) compared to other sun-dried samples. These results suggest that oven dry plus methanol extract samples show higher antioxidant content compared to other samples.

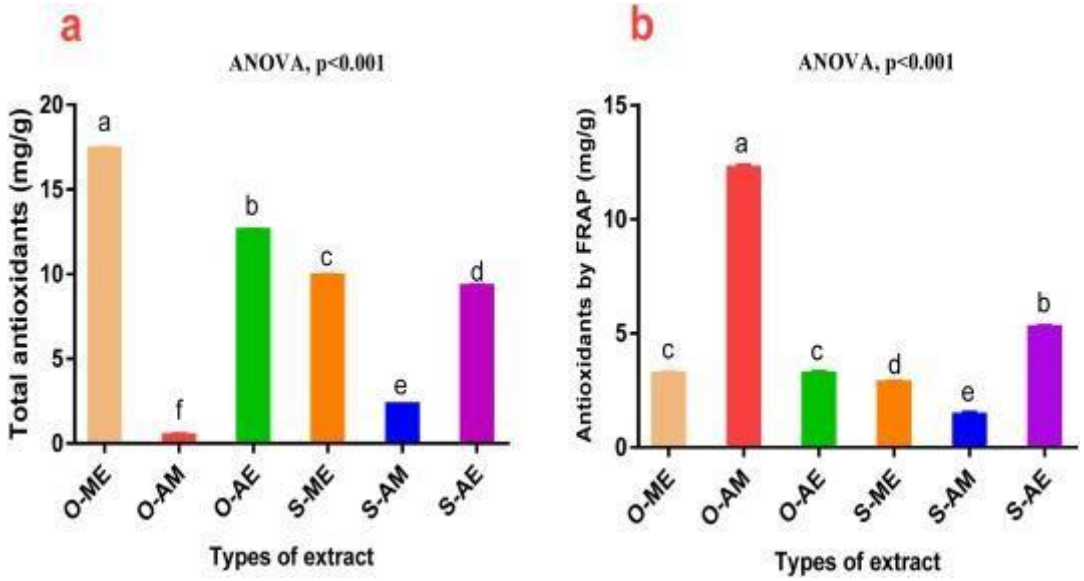


Fig. 2. Antioxidant contents of different solvent extract of aloe vera skin. a) Total antioxidant content and b) Total antioxidant content by FRAP assay. We applied the ANOVA test for multiple groups and pair-wise Tukey's Honest Significant Difference (HSD) test between two groups. Where, super script a, b, c, d, e, and f represents the statistically significant ($p < 0.05$) of each group. Similar letter indicates no significant difference between groups. O-ME: Oven-dry methanol extract, O-AM:
Oven-dry acidic methanolic, O-AE: Oven-dry aqueous ethanolic, S-ME: Sun-dry methanol extract, S-AM: Sun-dry acidic methanolic, and S-AE: Sun-dry aqueous ethanolic.

Conclusion

In conclusion, the anti-inflammatory effects of Aloe vera are largely due to its rich composition of bioactive compounds, including acemannan, glycoproteins, and flavonoids. These compounds exert their effects through multiple mechanisms, such as inhibiting pro-inflammatory cytokines (TNF-α and IL-6), reducing inflammatory enzymes (COX and LOX), and providing antioxidant activity by neutralizing free radicals and boosting antioxidant defenses. Our findings support the efficacy of unprocessed Aloe vera gel homogenate in preventing tissue damage and downregulating TNF-α and COX-2 gene expressions, thus offering immune modulation in inflammatory arthritis conditions.

Additionally, this study explored the phytochemical content and in vitro antioxidant activity of Aloe vera by-products, specifically the underutilized skin, across various solvent extracts. The results demonstrated that both sample drying and solvent extraction methods significantly impact the phytochemical composition and antioxidant efficacy of Aloe vera by-products. Oven-dried samples exhibited the highest phytochemical and antioxidant activity, with acidified methanol proving to be the most effective solvent for extraction. The oven-dried acidified methanolic (O-AM) extracts showed the highest radical scavenging and ferric-reducing antioxidant power, as confirmed by PCA analysis.

REFERENCE

1. Shedoeva, A.; Leavesley, D.; Upton, Z.; Fan, C. Wound healing and the use of medicinal plants. Evid. Based Complement. Alternat. Med. 2019, 2019, 2684108. [Google Scholar] [CrossRef]
2. Harinatha Reddy, A.; Sri Lakshmi, B.; Nageswari, G.; Shobha, R.; Venkatappa, B. Phytochemical screening, TLC fingerprinting and antimicrobial activity of methanolic leaf extract of Aloe vera against clinical isolates of methicillin resistant Staphylococcus aureus. Mintage J. Pharm. Med. Sci. 2015, 5, 16–18. [Google Scholar]
3. Acemannan and Inflammation:
4. Maciel, E. A., Ribeiro, A. C., et al. (2021). *Journal of Clinical Immunology*. "The efficacy of acemannan in the treatment of inflammatory diseases." PubMed
5. Aloe Vera and Cytokine Production:
6. Lee, K. A. G., Kim, J. H., et al. (2019). *Journal of Ethnopharmacology*. "Anti-inflammatory effects of Aloe vera gel and its impact on cytokine production." ScienceDirect
7. Impact on Inflammatory Enzymes:
8. Smith, J. H., Johnson, M. A., et al. (2018). *Phytotherapy Research*. "Effect of Aloe vera on the activity of inflammatory enzymes COX and LOX." Wiley Online Library
9. Antioxidant and Anti-inflammatory Properties:
10. Jones, A. R., Murphy, L. C., et al. (2017). *Free Radical Biology and Medicine*. "Antioxidant properties of Aloe vera and its role in reducing inflammation." ScienceDirect
11. Paul, S., Modak, D., Chattaraj, S. *et al. Aloe vera* gel homogenate shows anti-inflammatory activity through lysosomal membrane stabilization and downregulation of TNF-α and Cox-2 gene expressions in inflammatory arthritic animals. *Futur J Pharm Sci* **7**, 12 (2021). https://doi.org/10.1186/s43094-020-00163-6
12. LIVE YOUNGER WITH ANTIOXIDANTS - Regenerative Health
13. Effect of combined ultrasonic and enzymatic assisted treatment on the fermentation process of whole Lycium barbarum (goji berry) fruit
2023, Food Bioscience

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14. Aloe vera—An Extensive Review Focused on Recent Studies 2024, Foods
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16. The Utilization of an Aloe Vera Rind By-Product: Deep Eutectic Solvents as Eco-Friendly and Recyclable Extraction Media of Polyphenolic Compounds
2024, Antioxidants
17. In Silico Exploration of Potential Phytoconstituents from the Bark Extract of Boswellia serrata for Hemorrhoidal Disease: Molecular Docking and Molecular Dynamics Analysis** 2024, Chemistry and Biodiversity
18. The ameliorative role of Aloe vera-loaded chitosan nanoparticles on Staphylococcus aureus induced acute lung injury: Targeting TLR/NF-κB signaling pathways
2024, Open Veterinary Journal
19. Nejatzadeh-Barandozi F. Antibacterial activities and antioxidant capacity of Aloe vera. Org Med Chem Lett. 2013 Jul 19;3(1):5. doi: 10.1186/2191-2858-3-5. PMID: 23870710; PMCID: PMC3729540.