# Linking chemical composition of an Aloe vera extract to efficacious biomolecular mechanisms on skin

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## Introduction:

The topical use of the evergreen perirenal succulent, aloe vera, in traditional medicine has been documented as early as 16th century BCE and has been commonly used over the year for antioxidant, anti-inflammatory, sunburn relief, immune boosting , and anti-ageing purposes [1].
The extracts of aloe vera have been characterized and shown to contain a multitude of ingredients including amino acids, sugars, enzymes, vitamins, minerals, saponins, anthraquinones, lignin and salicylic acid. For an aqueous extract, the polysaccharides, in particular aloverose, as well as the saponins and flavonoids have been ascribed biological activity. In this work, we describe state of the art methods to achieve quantitative characterization of these compounds. Here we use a colorimetric assay for the quantification of the total saponins in the extract [2]. The saccharides are first identified by thin layer chromatography and then a quantitative 1H NMR method is used to define the aloverose and glucose components of the extract [3].
Although, aloe vera has been popularly use in traditional medicine, the molecular mechanism underlying its beneficial effects on skin remains poorly understood [1]. We aimed to determine the mechanism of action by which these effects are produced in skin fibroblasts for its development in skin care applications. Autophagy is a process by which long lived cell in the skin maintain homeostasis. It is also involved in the keratinocyte differentiation process and helps maintains the skin barrier. Recent research has also linked deficiency in autophagy to premature skin aging [4]. This work investigates the ability of aloe vera to modulate this crucial cellular process along with its ability to modulate expression of a major component of the dermis, Procollagen I [5]. Furthermore, the soothing properties ascribed to aloe vera are also investigated at a molecular level and are found to be related to the ability of this extract to inhibit the TRPV1 receptor, responsible for sensing environmental irritants [6-8].

## Results & Discussion

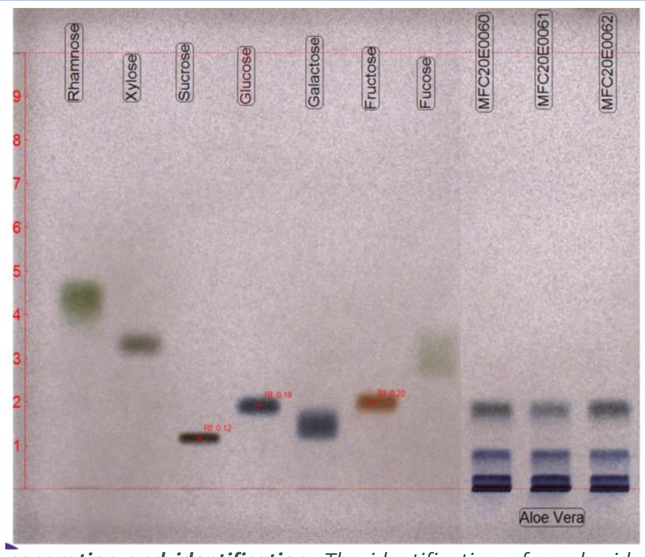


*Figure 1: **Chromatographic separation and identification:** The identification of saccharides in the aloe vera sample was carried out via TLC using a ethyl acetate–pyridine–water–acetic acid, 6:3:1:0.5 (v/v/v/v) as mobile phase. By this method, glucose could be clearly identified in the sample, showing a comparable retention factor as the standard. The TLC plate also indicated further saccharides in the sample which could not be matched to a standard.*

The chemical characterization of an aloe vera extract provides a detailed overview on the diverse chemical species present in an aqueous extract of this plant. The identification of saccharides in the aloe vera sample was carried out via TLC with silica gel as the stationary phase and using a ethyl acetate–pyridine–water–acetic acid, 6:3:1:0.5 (v/v/v/v) as mobile phase. By this method, glucose could be clearly identified in the sample, showing a comparable retention factor as that of the standard (Fig. 1). The TLC plate also indicated further saccharides in the sample which could not be matched to a standard. To identify these saccharides in the sample, quantitative 1H NMR was carried out using nicotinic acid amid as an internal standard [3]. The aloe vera sample analyzed was found to contain 6.3% aloeverose (Fig. 2). Glucose (6.6%), was reconfirmed in the sample by this method. To quantify total saponins in the aqueously extracted aloe vera sample an adapted method of Oludemi et al was used [2]. By this method a concentration of 1.8% total saponins in the aloe vera sample analyzed (Fig. 3). Taken together, the aqueous aloe vera extract was well characterized for its molecular content, helping to identify the compounds present in it that would endow it with the ascribed biological activity.

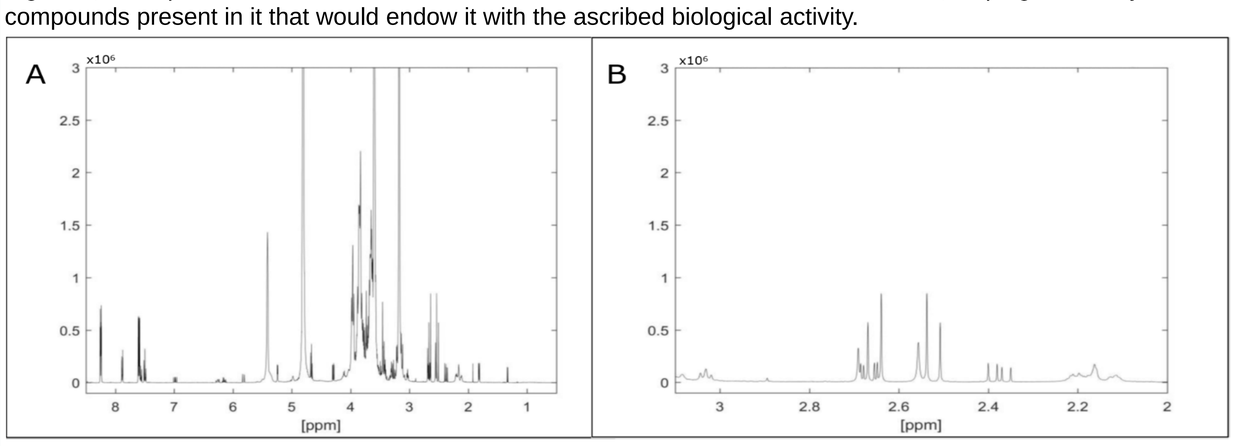


*Figure 2: **Identification and quantitation by $^1$H NMR:** The aloe vera sample analyzed was found to contain 6.3% aloeverose which could be identified and quantitated by $^1$H NMR using nicotinic acid amid as a standard. Glucose (6.6%), was reconfirmed in the sample by this method.*

## Results & Discussion:

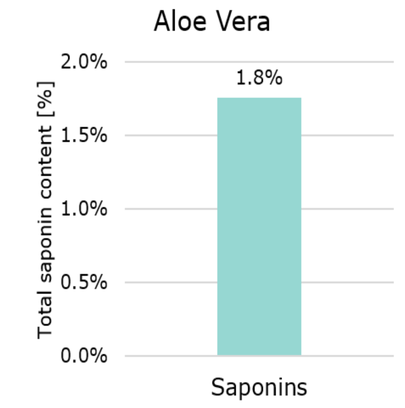


*Figure 3: **Quantification of total saponin content:** The colorimetric quantification of total saponins by an adapted method of Oludemi et al reveal a concentration of 1.8% total saponins in the aloe vera sample analyzed.*

For the investigation of the biological activity of the aqueous aloe vera extract, the influence of this extract on the expression of type I procollagen was undertaken. This collagen constitutes 70% of the dermis layer of the skin [5]. The tested extract was able to induce a 372% increase in its production (Fig. 4A). Thus, the anti-aging activity of this extract could be linked to the elucidated procollagen I stimulating activity. Furthermore, the autophagic flux in skin fibroblasts was also observed to be significantly increased by 469% after treatment with the aloe vera extract. Autophagy is known to be critically involved in maintaining skin homeostasis, including differentiation, cornification, and barrier formation as well as retarding premature skin aging. Recently the interplay of autophagy and senescence is also being clarified [4]. Consequently, further anti-aging properties can be ascribed to the aloe vera extract.
Recent advance in understanding the biology of skin describe the important role of the TRPV1 receptor [6-8]. The presence of this receptor has been corelated to sensitive skin [8]. Additionally, increase in the expression of this receptor has been reporter in aged skin and can be linked to senile pruritus and neurogenic skin inflammation [6]. Inhibition of this receptor has also been shown to aid skin barrier recovery, suppressing atopic dermatitis like symptoms [7]. Since the aloe vera extract was capable of inhibiting the TRPV1 receptor by 33.04%, it is possible that this extract is capable of not only soothing skin, but also accelerating the recovery of skin while mitigating age related pathologies.

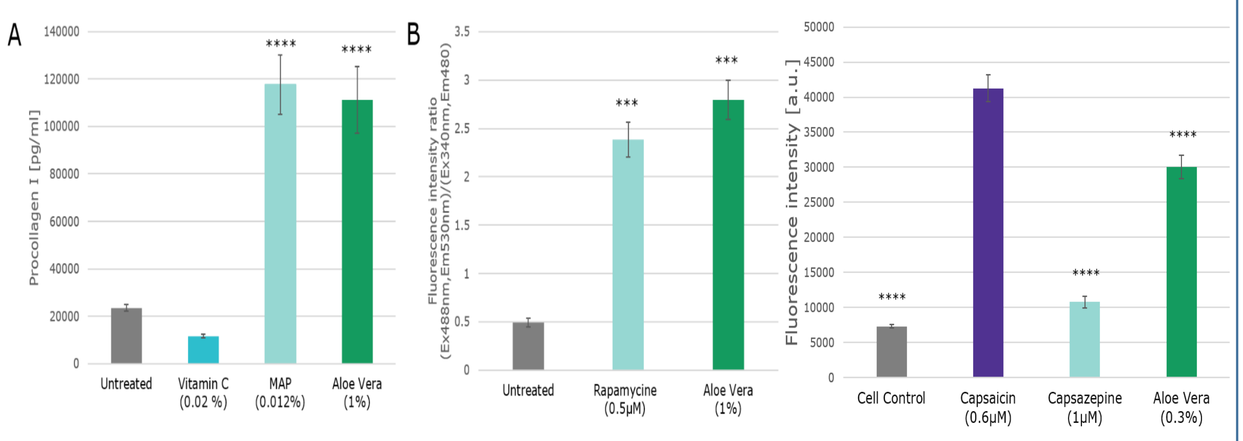


*Figure 4: **Influence of the aloe vera extract on (A) Procollagen I synthesis and (B) Mitochondrial membrane potential in WS1 skin fibroblasts: (A)** The tested aloe vera sample at 1% was found to significantly increase the protein level synthesis of Procollagen I by 372% compared to the untreated control. The positive control magnesium ascorbyl phosphate (MAP) at 0.012% was found to bring about a similar increase of 399%. **(B)** A significant increase by 469% in the autophagic flux was observed after treatment of WS1 cells with 1% aloe vera. The positive control, 0.5µM Rapamycin was able to increase the autophagic flux by 385%. Significance was analyzed by one-way ANOVA with Dunnetts multiple comparison test; ***, p<0.01, ****, p<0.001*

*Figure 5: **Inhibition of TRPV1 activity**: The ion channel receptor TRPV1 was found to be significantly inhibited by 33.04% using 0.3% aloe vera. The activation of the receptor was achieved by using 0.6µM Capsaicin and 1µM Capsazepine was used a control inhibitor, reducing activation of the receptor by 89.85%. Significance was analyzed by one-way ANOVA with Dunnetts multiple comparison test; ***, p<0.01, ****, p<0.001*

Characterizing the chemical species present in such an aqueous aloe vera extract would help refine cultivation, extraction, and enrichment processes when developing extracts for cosmetic application. Although the use of aloe vera is abundant in topical application and traditional medicine, this study helps offer an explanation for the skin matrix improvement and soothing properties ascribed to aloe vera. Understanding the biomolecular changes brought about by traditionally used ingredients like this aloe vera extract to elicit phenotypic modulations of skin tissue will help in the identification and development further potent natural skin care ingredients.

## Aknowledgments:

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