Colegio de Postgraduados

Nopal mucilage and its synergistic-humectant effect in combination with different polyalcohols

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ABSTRACT

Objective: This study aimed to evaluate the *in vitro* wetting capacity of cactus mucilage (*Opuntia ficus-indica*) in combination with three wetting agents through permeability and water retention capacity tests, as well as its *in vivo* effects by measuring transepidermal water loss (TEWL) and hydration levels in volunteers. The goal was to determine the efficacy of this agent and its potential synergistic effects.

Design/Methodology/Scope: Nopal mucilage films (1.5% and 3%) were formulated using glycerin, sorbitol, and propylene glycol (1.5% and 3%). To assess their performance, water retention capacity tests were conducted at relative humidities of 40%, 60%, 80%, and 90%. TEWL was measured using a Tewameter® TM210 on a 4×4 cm area of each participant's forearm. Additionally, film characterization was performed using Scanning Electron Microscopy, and water vapor permeability was determined by calculating the permeability constant.

Results: The water vapor permeability test indicated that 3% of mucilage and 3% of sorbitol combination exhibited the lowest permeability constant, making it the most effective formulation. Furthermore, TEWL and stratum corneum hydration assessments confirmed that this combination demonstrated the highest moisturizing properties, as it showed the greatest hydration value and the lowest TEWL compared to the other formulations.

Conclusions/Limitations: The application of cactus mucilage emerges as a viable strategy to enhance the moisturizing effect in cosmetic product formulations.

Keywords: mucilage, nopal, humectant.

INTRODUCTION

The skin is the largest organ of the human body, with a surface area of approximately 1.8 m^2, accounting for 16% of total body weight (Chambers & Vukmanovic-Stejic, 2020). It is composed of three primary layers: the epidermis, dermis, and subcutaneous tissue. The epidermis, the outermost layer, is responsible for maintaining skin moisture and consists of five sublayers: the *stratum basale*, *stratum spinosum*, *stratum granulosum*, *stratum lucidum*, and *stratum corneum*. Among these, the stratum corneum plays a crucial role in the skin's water barrier function. This layer, measuring approximately 10-20 μm in thickness, is primarily composed of corneocytes (dead keratinocytes devoid of nuclei) and intercellular lipids, which are organized in a "brick-and-mortar" structure that regulates and minimizes

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transepidermal water loss (TEWL) (Bouwstra *et al.*, 2021). The water content in the *stratum corneum*, commonly referred to as skin hydration, must remain above 10% for healthy skin, with an upper limit of 30% to prevent overhydration (Chambers & Vukmanovic-Stejic, 2020). One approach to maintaining optimal moisture levels in the epidermis is the use of cosmetic formulations containing moisturizing agents, which ensure an adequate balance between water and lipid content. These agents, known for their hygroscopic properties, enhance the water-retention capacity of *stratum corneum* cells (Pavlou *et al.*, 2021). Humectants are classified into three categories based on their composition: inorganic, metal-organic, and organic. Among these, organic humectants exhibit the highest moisturizing efficacy and are therefore the most widely used in cosmetic formulations. Examples include glycerol, sorbitol, and propylene glycol. Additionally, mucilages naturally occurring in various plants, seeds, and husks are known for their exceptional water-binding capacity due to their high concentration of hydroxyl groups in the polysaccharide chain (Tosif *et al.*, 2021; Prajapati *et al.*, 2013).

Nopal (*Opuntia ficus-indica*), a cactus species native to semi-arid and arid regions of Mexico, is a rich source of mucilage. The annual production of nopal reaches approximately 812 tons, with 70% consumed fresh. Nopal cladodes contain significant amounts of polysaccharides, including mucilage, cellulose, and hemicellulose, among others (García-Barradas *et al.*, 2023). This study aims to evaluate the in vitro humectant capacity of *Opuntia ficus-indica* mucilage in combination with organic humectant agents through permeability and water retention capacity tests, as well as its in vivo effects by assessing TEWL and skin hydration in volunteers. The objective is to determine the efficacy and potential synergistic effects of the formulation.

MATERIAL AND METHODS

Glycerol, sorbitol, and propylene glycol were purchased from Droguería Cosmopolita, Mexico. *Opuntia ficus-indica* mucilage was freeze-dried at the Facultad de Estudios Superiores Cuautitlán and donated by Dr. Elsa Gutiérrez Aburto. NaOH was obtained from Sigma-Aldrich Corporation, St. Louis, MO, USA.

The evaluated formulations are presented in Table 1.

Water retention assay

This assay assesses the percentage of weight gained by the formulations when exposed to different relative humidity (RH) levels. Four desiccators were conditioned at 40%, 60%, 80%, and 90% RH using glycerol and NaCl solutions. A total of 10 mL of each formulation

Table 1. Evaluated Formulations of Mucilage-Organic Humectants.

Mucilage	Humectants								
	Sorbitol			Glycerol			Propylene Glycol		
	0%	1.5%	3%	0%	1.5%	3%	0%	1.5%	3%
0%		x	x		X	x		X	x
1.5%	x	x	x		X	x		X	x
3%	x	x	x		x	x		X	x

(Table 1) was placed in a 15 mL beaker. The weight gain of each sample was recorded at 2, 4, 6, and 8 hours from the start of the test, followed by measurements every 24 hours until a constant weight was achieved. Each test was conducted four times.

Transepidermal water loss assay (TEWL)

The effects on TEWL were measured using a TEWAmeter™ TM 210 (Courage + Khazaka Electronic GmbH, Cologne, Germany). Ten volunteers aged between 20 and 50 years, with healthy skin and no application of cosmetic treatments on the tested area for one week, were selected for this evaluation. The results were expressed in $g/h/mm^2$. Two 4×4 cm areas were marked on each volunteer's arm. In quadrant 1, 5 mL of each formulation (Table 1) was applied and left to rest for 30 minutes, while quadrant 2 served as a reference for basal TEWL measurements. After the resting period, excess formulation was removed. Each test was performed in triplicate.

Skin hydration

Skin hydration measurements were conducted using a Corneometer® CM 825 (Courage + Khazaka Electronic GmbH, Cologne, Germany) mounted on a Derma Unit SSC3 (Courage + Khazaka Electronic GmbH, Cologne, Germany). This device measures the electrical capacitance of the skin surface, thereby indirectly assessing skin hydration, as electrical capacitance is dependent on water content (Vater *et al.*, 2021). All experiments were performed in triplicate.

Scanning Electron Microscopy (SEM)

The morphological analysis of the films formed by mucilage-organic humectant formulations was conducted using a JEOL scanning electron microscope (Model JSU-5600) at a resolution of 5 nm, with accelerating voltages of 10 and 15 kV, and a chamber pressure of 12-20 Pa (García-Betanzos *et al.*, 2016).

Water Vapor Permeability (WVP)

Water vapor permeability was determined at 25±2 °C following the ASTM E96/E96M-05 method (ASTM, 2005a, b) with slight modifications. Glass vials were filled with 8 mL of deionized water (100% RH), covered with the conditioned films, sealed, and placed in a chamber with water (0% RH). Weight changes in the vials were recorded three times until the samples lost 10% of their initial weight. Determinations were performed in triplicate for each batch of film.

RESULTS AND DISCUSSION

Water retention assay

The water retention capacity test, shown in figure 1a, indicates that at the lowest relative humidity levels, mucilage at 1.5% and 3% behaves similarly to glycerin (1.5% and 3%) and sorbitol (1.5%). This graph also reveals that propylene glycol exhibits the lowest wetting capacity under low relative humidity conditions. This phenomenon occurs because, in low-humidity environments, moisturizing agents can absorb water from the epidermis and dermis, leading to increased skin dryness (Wolff, 2003). At 60% RH (Figure 1b), mucilage, propylene glycol, sorbitol, and glycerin at 3% exhibit a comparable effect, with no significant differences observed, indicating that all three wetting agents and mucilage have a similar water retention capacity. However, at this relative humidity, glycerin at 1.5% demonstrates the highest water retention compared to the other humectants. In Figure 1c (80% RH), mucilage at 3% displays the lowest water retention, whereas glycerin, at both tested concentrations, exhibits the highest retention capacity. The remaining substances show no significant variations. Finally, at 90% RH (Figure 1d), glycerin at 1.5% continues to demonstrate the highest humectant capacity, while mucilage and propylene glycol at 1.5% exhibit lower water retention.

Nopal mucilage has greater moisturizing capacity at low relative humidity, explained since mucilage is a component whose main function is water retention in nopal.

Transepidermal water loss assay (TEWL)

TEWL measurement is a fundamental parameter for assessing the integrity of the stratum corneum and is considered a powerful non-invasive method to evaluate the effects of chemical compounds on the epidermal barrier function (Sotoodian & Maibach, 2012). As shown in Figure 2, the mean TEWL values for the mucilage 3% sorbitol 3% and mucilage 3% propylene glycol 3% systems exhibit statistically significant differences compared to the other formulations. This finding supports previous observations, as the mucilage 3%-propylene glycol 3% system presented the highest TEWL value, likely due to the drying effect of propylene glycol. Conversely, the mucilage 3% sorbitol 3% system demonstrated the greatest humectant effect, as evidenced by the lowest TEWL value.

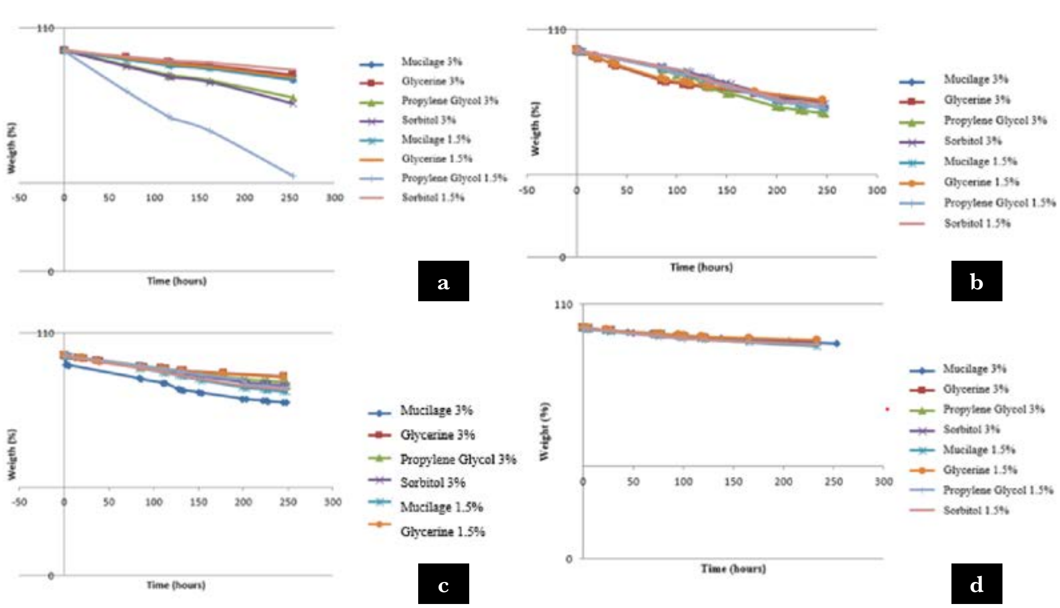

Figure 1. Water retention capacity over time for mucilage, sorbitol, glycerin, and propylene glycol at 1.5% and 3% under different relative humidity conditions: (a) 40% RH, (b) 60% RH, (c) 80% RH, and (d) 90% RH.

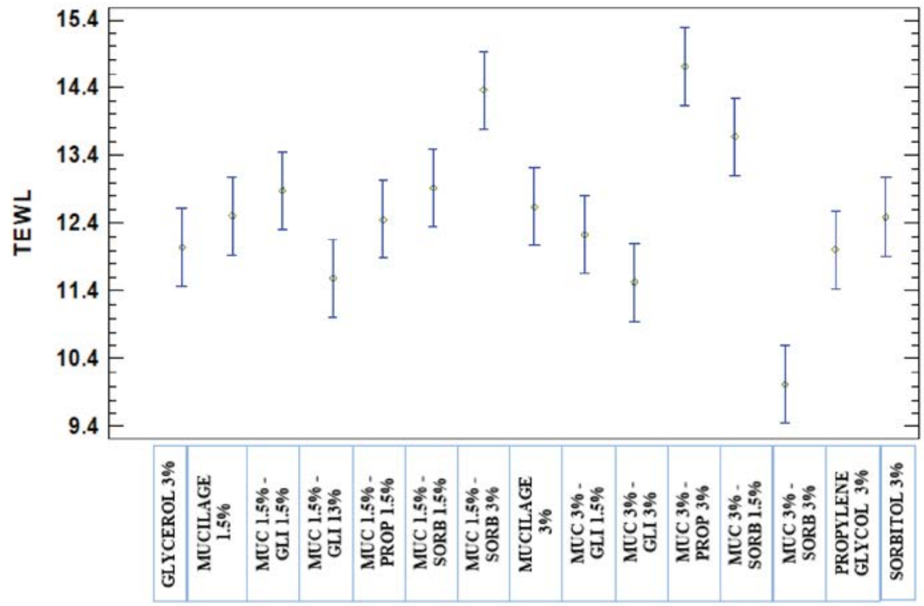

Figure 2. Least Significant Difference (LSD) plot of mucilage humectant systems for TEWL.

Skin hydration

As shown in Figure 3, the mean hydration values for the mucilage 3% sorbitol 3%, sorbitol 1.5%, and sorbitol 3% systems exhibit statistically significant differences compared to the other formulations. The mucilage 3% propylene glycol 3% and mucilage 3% propylene glycol 1.5% systems displayed the lowest hydration values, likely due to the drying effect of propylene glycol. Conversely, the mucilage 3% sorbitol 3% system demonstrated the highest hydration value, confirming its superior humectant effect. Regarding the water content in the *stratum corneum*, the mucilage 3% sorbitol 3% (Figure 3) and mucilage 3% glycerin 1.5%

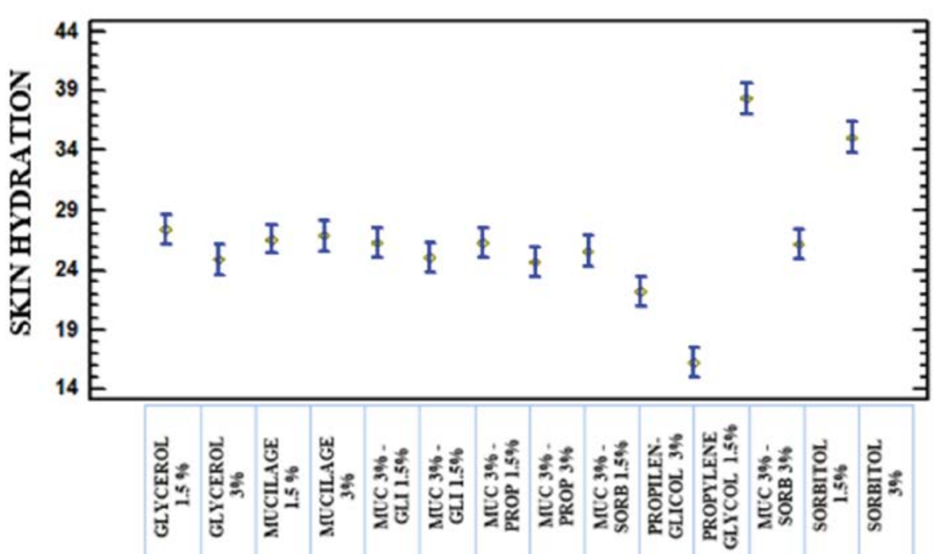

Figure 3. Least Significant Difference (LSD) graph of mucilage-humectant systems for hydration.

mixtures were the most effective, maintaining a level of hydration twice as high as that achieved by the humectants individually. The combination of nopal mucilage with sorbitol and glycerin forms an occlusive substance, leading to the formation of a film on the skin's surface that reduces transepidermal water loss and enhances *stratum corneum* hydration.

In this manner, the water retention capacity of the stratum corneum is enhanced. Additionally, it is well established that glycerol, sorbitol, mannitol, sucrose, propylene glycol, and polyethylene glycol act as plasticizers, facilitating the formation of the mucilage film in combination with the wetting agent (Olawuyi, 2021).

Scanning Electron Microscopy (SEM)

The films containing sorbitol at both concentrations (1.5% and 3%) exhibited a continuous structure, as did the film obtained with 1.5% glycerin. These films were flexible to the touch, in contrast to the one formed with 3% propylene glycol, which developed cracks and exhibited slight brittleness. Notably, the film produced with 3% glycerin was extremely thin and highly adhesive, making it difficult to handle.

Figure 4 presents micrographs of representative fields of the films formed by the mucilage-humectant mixtures. Polysaccharides, such as cactus mucilage, are effective film formers; however, they tend to be fragile and brittle unless a plasticizer is incorporated (Ghanbarzadeh, 2007). The humectants propylene glycol, sorbitol, and glycerin serve this function, as they are low-molecular-weight organic compounds that reduce intermolecular forces between the polymer chains of the mucilage. This interaction decreases tensile strength while enhancing the flexibility of the films (Pastor, 2010).

The film with the best characteristics was the 3% mucilage–3% sorbitol formulation (Figure 4d), as it exhibited the most uniform and continuous structure, followed by

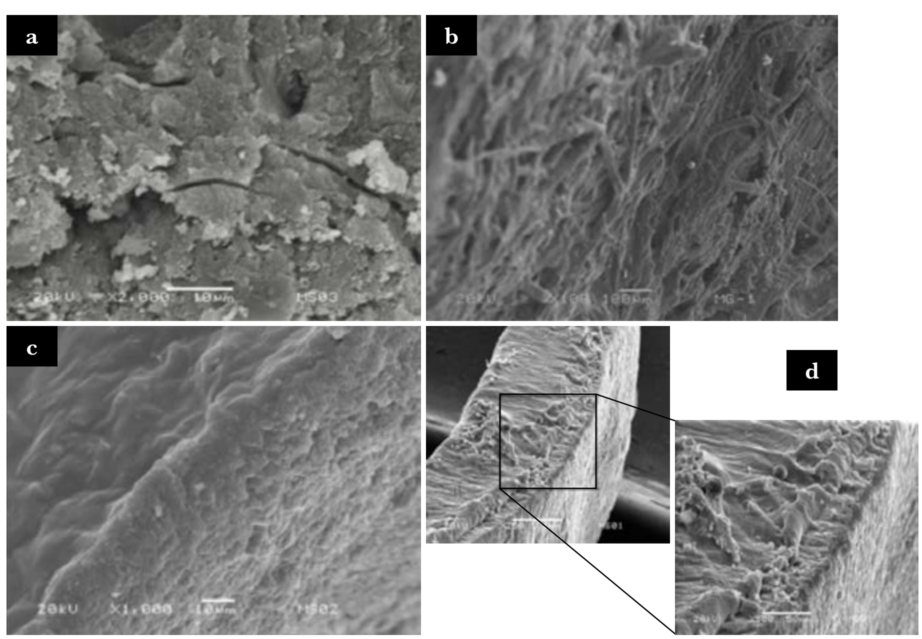

Figure 4. Micrographs of the films obtained: (a) 3% mucilage 3% propylene glycol, (b) 3% mucilage 1.5% glycerin, (c) 3% mucilage-1.5% sorbitol, (d) 3% mucilage 3% sorbitol.

the 3% mucilage-1.5% sorbitol and 3% mucilage-1.5% glycerin films (Figure 4). This evident continuity is attributed to the ability of plasticizer molecules to bind with water molecules, protecting the active centers along the polymer chains and thereby reducing intermolecular distances (Koc-Bilican, 2024). In contrast, the 3% mucilage-3% propylene glycol film presented a particular case, as its micrograph revealed structural gaps and surface particles, suggesting incomplete incorporation of the plasticizer (Ghanbarzadeh *et al.*, 2006). Consequently, this formulation failed to produce a continuous film.

Water Vapor Permeability (WVP)

The table below presents the permeability constant values obtained for the mucilage systems formulated with different humectants.

Table 2. Permeability constants for mucilage-humectant systems.

System	Water Vapor Permeability (*10^{-7}) (mg mm/mm^2 h Pa)
mucilage 3% - sorbitol 3%	2.606
mucilage 3% - sorbitol 1.5%	3.351
mucilage 3% - glycerine 3%	4.730
mucilage 3% - glycerine 1.5%	3.424
mucilage 3% - Propylene glycol 3%	3.520
Control	5.791

Since the permeability constant represents water vapor permeability, a higher constant indicates greater water loss from the system to the external environment. According to Table 2, the 3% mucilage-3% sorbitol system exhibited the lowest permeability constant, making it the most effective film. This can be attributed to the molecular structure of sorbitol, which contains a higher number of hydroxyl groups compared to glycerol and propylene glycol. This increased hydroxyl content enhances interactions with the carboxyl groups of mucilage, reducing the mobility between mucilage chains (Mannai *et al.*, 2023). As a result, a more uniform film is formed, which effectively minimizes water vapor permeability. When sorbitol is used at 1.5%, an increase in the permeability constant is observed, likely because this concentration is insufficient to produce a film with higher resistance. The 3% mucilage-1.5% glycerin and 3% mucilage-3% propylene glycol systems exhibit higher permeability constants, as these plasticizers are more hydrophilic than sorbitol, leading to greater water permeability (Koc-Bilican, 2024).

CONCLUSIONS

The water retention capacity of mucilage in combination with glycerin, sorbitol, and propylene glycol was evaluated, with the glycerin and sorbitol mixtures demonstrating the highest retention capacity. The water vapor permeability test confirmed that the 3% mucilage-3% sorbitol mixture, exhibiting the lowest permeability constant, was the most effective formulation. TEWL and stratum corneum hydration assessments further reinforced that the 3% mucilage-3% sorbitol system possesses the greatest moisturizing properties, as it exhibited the highest hydration value and the lowest TEWL compared to the other formulations. The combination of cactus mucilage with conventional moisturizing agents presents a promising strategy for developing various cosmetic and dermatological formulations, including solutions, lotions, creams, suspensions, masks, and scrubs.

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