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Original Article

Influence of herbal extracts in physicochemical properties and stability of antibacterial gels

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ABSTRACT

The use of plants to treat diseases and heal wounds is a custom that dates back thousands of years and is a legacy of ancient civilizations. Although a significant proportion of the planet's plant biodiversity is found on the American continent, there are very few pharmaceutical products developed from it. This work aimed to develop and characterize topical formulations (gels and emulgels), including a combination of plant extracts with recognized antibacterial activity. Hydroalcoholic extracts of *Lippia turbinata* Griseb. and *Lippia alba* (Mill.) N. E. Brown were obtained by leaching. The excipients used were Carbopol® 934 and 940, Sepigel® 305, sodium carboxymethylcellulose, methylcellulose, propylene glycol, and ethanol. The finished product was characterized by properties: organoleptic characteristics, extensibility, pH, texture profile, permeation performance, and microbiological quality. Then, they were subjected to stability studies in different conditions of temperature and humidity. They had a characteristic smell of plant species, color brown, without the presence of lumps, and with good extensibility. The gels had an in vitro permeation of porcine skin of up to 30% and low retention in the epithelium (<15%). They did not present microbial contamination and were stable for six months. Of the gels formulated, the gel with Sepigel® 4% (w/w) presented a better appearance. These results demonstrate the feasibility of transporting non-hydro soluble extracts in a gel formulation. All formulations are appropriate to preserve the antibacterial effect of original extracts. They maintain stability over time without the use of antimicrobial preservatives.

Keywords: Herbal gel, Natural product, Topical formulation, Antibacterial formulation

Introduction

Communities in the most disadvantaged sectors and rural areas frequently use medicinal plants to cure their illnesses. The Verbenaceae family is an important element in the flora of South America [1] and its members have medicinal uses closely related to bacterial infections; since they have antiseptic properties and can be used for the treatment of fever, wounds, diarrhea, bronchitis, sinusitis, tetanus. In addition, the main chemical compounds present in these plants are recognized for their antimicrobial action [2]. In this work, the interest was focused on two species of Verbenaceae: *Lippia turbinata* Griseb. y *Lippia alba* (Mill.) N.E. Br. ex Britton & P. Wilson. Both species have recognized uses as antimicrobials. Thus, *Lippia turbinata* was incorporated as an official drug in the fifth and sixth Argentine National Pharmacopoeia [3]. Moreover, in a recent work, we have shown the antibacterial effect and chemical composition of *L. alba* and *L. turbinata* extracts [4]. In general, the species of the genus *Lippia* are classified as plants with compounds "generally considered as safe" (GRAS) by the Food and Drug Administration (FDA) [5].

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In the last decade, plant extracts and plant-derived compounds have been investigated to treat multi-drug-resistant (MDR) pathogens. A great number of studies were focused on natural antimicrobials for topical application to control various skin disorders linked with bacterial infections [6-9].

Hydrogels are increasingly popular topical dosage forms due to their simple application and resistance to physiological stress caused by skin flexion, blinking, and mucociliary movement, adopting the shape of the applied area. They are colloidal aqueous dispersions of hydrophilic polymers (gelling agents) that form a three-dimensional network of high water content. These semi-solid formulations are widely studied due to their properties for drug delivery and their use in biomedical devices [10]. In particular, poly(acrylic acid) (Carbopol®) polymers have been extensively studied for the development of hydrophilic matrices and solid dispersions [11, 12].

Given the above, this work aimed to develop antibacterial hydrogels for dermic use that include extracts of *L. turbinata* and *L. alba*; and to evaluate how the presence of the extract affects the gel structure and its physical properties.

Materials and Methods

Material vegetal and plants extraction

Lippia alba and *L. turbinata* were collected from department 25 de Mayo, province of Chaco, Argentina (*L. alba* 60° 4′45” W 26° 54′6” S, *L. turbinata* 60° 6′26” W 26° 53′30” S). Both species were identified by Professor Alberto Bela from the Laboratory of Pharmacobotany (National University of Chaco Austral), and deposited in the Herbarium of the Instituto de Botánica del Nordeste, IBONE, Corrientes, Argentina with the following barcode: CTES0059998 for *Lippia alba* and CTES0059999 for *Lippia turbinata*.

The extracts from dried leaves and flowers were obtained by percolation method employing ethanol 70% (v/v) as extracting solvent. The HPLC characterization of both extracts had already been done [4] and the main compounds found were luteolin and its derivatives.

Preparation of gel formulations

The composition of gel formulations developed is shown in **Table 1**. The formulations included the combination of hydroalcoholic extracts of *L. alba* and *L. turbinata* (1:2 volume ratio). Before incorporating the combination of hydroalcoholic extracts in the formulations, the mixture was dried and re-dissolved in propylene glycol and ethanol 70% (v/v). Formulations without extracts were also prepared, where the amount of extracts was replaced by distilled water, and they were control formulations. No preservatives were used in the formulations developed.

The Carbopol®, sodium carboxymethylcellulose (CMC), and methylcellulose (MC) polymers were wetted in water for 12 hours. Then, the extracts solution was added. It was mixed with a propeller stirrer at 1000 rpm (Decalab Fbr®). Finally, triethanolamine (TEA) was added to formulations with Carbopol®, and stirring was continued for 30 minutes. Sepigel® did not need to be pre-moistened.

Table 1. Percentage composition (% w/w) of studied formulations.

Components	Formulations										
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Carbopol® 934	0.5	1	-	-	-	-	-	-	-	-	-
Carbopol® 940	-	-	0.5	1	-	-	-	-	-	-	-
MC	-	-	-	-	3	4	-	-	-	-	-
CMC	-	-	-	-	-	-	3	4	-	-	-
Sepigel®	-	-	-	-	-	-	-	-	4	6	8
Blend extracts	3	3	3	3	3	3	3	3	3	3	3
Propylene glycol	5	5	5	5	5	5	5	5	5	5	5
Ethanol 70% (v/v)	5	5	5	5	5	5	5	5	5	5	5
TEA*	s.q.	s.q.	s.q.	s.q.	-	-	-	-	-	-	-
Water	s.q.	s.q.	s.q.	s.q.	s.q.	s.q.	s.q.	s.q.	s.q.	s.q.	s.q.
Carbopol® 934	0.5	1	-	-	-	-	-	-	-	-	-
Carbopol® 940	-	-	0.5	1	-	-	-	-	-	-	-

Reference: TEA* was added until pH = 7 or until the formulation became viscous; s.q.: sufficient quantity.

Formulation characterization and stability tests

Batchs constituted by six packages of 30 grams of each gel formulation were conformed. To find out the physical stability of formulations, each batch was divided into two groups with three packages in each group. One group was stored at 40 °C and 75% relative humidity (RH) for six months. We denominated this conditions like "Accelerated Stability (AS)". The other group was kept under ambient conditions (25 ± 2 °C and 56% RH). We denominated this conditions like "Room Conditions (RC)". Both groups were checked visually and characterized by the next physico-chemical and microbiological tests.

Organoleptic characteristics

The description was according to the appearance, color, and smell.

pH

The measurements were made by immersing the electrode in the preparation. A portable HANNA® HI 9811-5 pH meter and conductivity meter was used.

Homogeneity

The determination was performed by observation of a thin layer of product spread on a glass slide, under a stereoscopic binocular magnifying glass (Arcano® ST 30 2L) and optical microscope (LeicaTM DM LB2 equipped with LeicaTM ICC50HD digital camera).

Spreadability

This parameter was evaluated as described by Gandomkar *et al*. [13], with some modifications. The gel sample (25 mg) was placed between two slides and at each minute, weights of 0.5, 1, 2, and 5 grams were added. The diameter of the expanded zone of the gel was measured.

Texture profile

Texture profile analysis (ATP) was performed using a Brookfield CT3 texturometer, using the TA11/1000 probe. The hardness and adhesiveness of the preparations were evaluated. The hardness is defined in this case as the force in grams necessary to penetrate 10 mm into the gel. Adhesiveness is defined as the work required to remove the probe from the surface gel. It is expressed in milli Joules (mJ). The parameters of the test were the following: load cell 4500 g, activation load 1 g, test speed 1 mm/s, return speed 1 mm/s, cycles: 1. The cylinder (sample container) was 30 mm high and 38 mm in diameter.

Antibacterial activity

The antibacterial activity was evaluated by agar diffusion method [14]. Agar Mueller-Hinton media and Petri dishes of 90 mm diameter were used. The plates were incubated at 37 °C for 24 hours. Then, the diameter of the inhibition halo was measured. The strain tested were *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 12213.

Microbiological quality

Hygienic quality control was carried out according to the conditions of Argentinian Pharmacopeia Seventh Edition [15] for products that are not necessarily sterile. For this, 1 g of each gel was dispersed in 10 mL of phosphate buffer pH 7.2, and then the indicated volume of sample was taken for each test. The control was made about viable aerobes, *S. aureus*, *Pseudomonas aeruginosa*, enterobacteria, fungi, and yeast.

Ex vivo skin permeation and retention study

Ex vivo polyphenols permeation test was carried out in Franz diffusion cells. Pig ear skin (total exposed area 2.66 cm^2) was mounted between the donor and receptor compartments from cells. The gel (25 mg) was placed onto the skin, in the donor chamber. The receptor chamber of 27 mL was filled with a buffer phosphate solution pH 7.4. The system was maintained at 32 °C and stirred continuously with the help of a magnetic stirrer. Aliquots of 1 mL were collected from the receptor compartment, at a pre-specified time over six hours and replaced by a new phosphate buffer solution. Samples were stored at -18 °C before analysis. A cell was destined as blank, using saline instead of herbaceous gel. Samples from this cell were used as targets of subsequent studies.

The amount of permeated polyphenols was determined with the Folin-Ciocalteu reagent [4]. The permeated quantities were calculated by interpolation in a calibration curve elaborated with gallic acid (ranging between 2.5 – 0.15 mg Gallic Acid/mL). The cumulative permeated quatities versus time was graphed. The flux of permeation was expressed like the micrograms of gallic acid equivalents per area unit per hour (µg GAE/cm^2.h). The permeated amount at six hours was quantified as micrograms gallic acid equivalents (µg GAE). Also, the permeated percentage were calculated. The permeability coeficient (Kp) is expresed like centimeter per hour (cm/h).

At the end of the permeation assay, the skin was separated from Franz diffusion cells and the formulation that remained on the skin was removed. Then, the skin was cut into small pieces and submerged in 5 mL ethanol 80% (v/v) for 24 hours, to ensure effective extraction of the phenolic content retained in the skin. Then, 100 µL were taken and the amount of polyphenols retained on the skin was determined by the same methodology employed to quantify the amount of polyphenols permeated. The amount retained on the skin was expressed as micrograms of gallic acid equivalent (µg GAE), and the percentage retained into skin were calculated.

Statistical analysis

All determinations were made in triplicate for each sample analyzed and mean values and standard deviations were subsequently reported. Data analysis was done using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test in SPSS 21.0 software. Differences between any two means could be accomplished using Duncan's multiple range test (DMRT). The significance level was set at a P-value lower than 0.05.

Results and Discussion

Organoleptic characters

The formulations showed an brownish olive green color with different intensities according excipient employed. This can be observed in **Figure 1**, where is shown phographies of gels with

Carbopol® 940 1% (F4), MC 3% (F5) and 4% (F6), and Sepigel® 4% (F9), 6% (F10), and 8% (F11). The color of the formulations of Sepigel® is a lighter olive green than the others. When the concentration of Sepigel® increases, the tone becomes even clearer. Generally, when a plant hydroalcoholic extracts is formulated like a gel or cream, the formulation trend to presents yellow, brown or brownish color, regardless of the plant species [16-20].

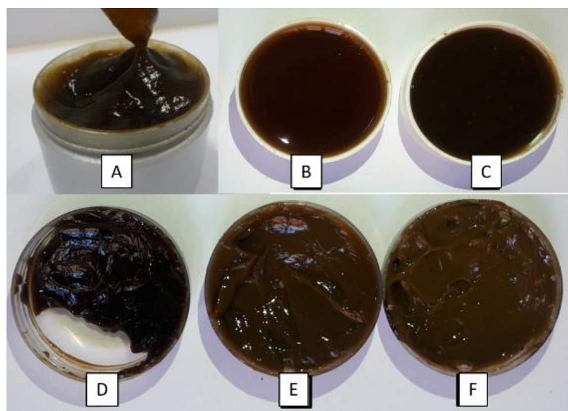

Figure 1. Photography of gel F4 (A), F5 (B), F6 (C), F9 (D), F10 (E), and F11 (F).

All the gels acquired the smell of the extracts, intense, typical of the species, with a slight sensation of mentholated aroma. They presented a homogeneous appearance. All base gels are odorless.

Homogeneity

The gel with Carbopol®, MC, and CMC with incorporated extracts, turned out to be heterogeneous when observed by binocular magnifying glass and microscope. No particles were observed on Sepigel® gels.

When the base gels were observed, either with a binocular magnifying glass or optical microscope, no two phases were observed, this would indicate that the observed particles originated from the presence of the extract. These results are in agreement with results reported by others [21-23], who had seen a heterogeneous gel when they observed microscopically.

pH

In general, Carbopol® gels (F1 to F4) showed pH values higher than the control formulation. In MC and CMC gels (F5 to F8), the inclusion of the combination of extracts did not modify the pH values compared with its control gel. In Sepigel® gels (F9 to F11), with the addition of the extracts, a decrease in the pH values (pH 5.5 at 6.5) with respect to the control formulations (pH = 6.9) was produced.

In papers where Carbopol® 934 and CMC are used for the preparation of gels with plant extracts, pH values close to neutrality were also obtained (pH 6.4-6.9) and when the extract presented acid pH, Carbopol® gels were gelled at a pH value below 6 [21-25].

Spreadability

The formulations prepared with Carbopol® 0.5% (F1 and F3) showed the highest spreadability, followed by the formulation with Sepigel® 4% (F9). In general, the gels with Carbopol® and Sepigel® were more spreadable than their control. The gels with MC and CMC (F5-F8) present less spreadability than their respective control. This may be due to the presence of tannins and phenols in the extracts. According to the Handbook of Pharmaceutical Excipients [26], CMC solutions exhibit stability at pH 7-9; and the MC solutions coagulate (an effect known as salting-out) with phenols and tannins. However, these can be avoided by adding ethanol at 95% (v/v) [27].

Regarding gels with Sepigel®, F9 showed an increase in spreadability compared with the base gel, while F10 and F11 did not suffer important modifications.

Texture profile

The gels were characterized with respect to their hardness and adhesiveness **(Table 2)**. Both properties varied widely according to the polymer type and concentration used. The softest gels were those of Carbopol® and the hardest were those of MC and CMC at 4%.

In all the gels with Carbopol® or Sepigel®, the inclusion of the extract produced a decrease in hardness and adhesiveness. In cellulose derivative's gels, both hardness and adhesiveness, increased except the adhesiveness for F5.

The polymer present in the commercial blend Sepigel®, likewise Carbopol® polymers, is affected by the presence of electrolytes or extreme pH, and this can change its texture. The most drastic change in texture occurs in Carbopol® formulations at 0.5% (F1 and F3), which decreases between 81-84% of the hardness with respect to the base, while Carbopol® gels at 1% (F2 and F4) decreased 62-72% the hardness value. Therefore, when the concentration of Carbopol® is higher, the system is less affected by the addition of the extracts. This is also seen in gels with Sepigel®, where the decrease in hardness was 60.54% in the F9 formulation, 22% in F10, and 12.8% in F11, compared to their control formulations.

The statistical test (T-test) showed that the inclusion of the extract produced a significant modification ($p<0.05$) of the hardness in the formulations, with the exception of F5.

Regarding adhesiveness, Carbopol® 0.5% gels were the most affected because the adhesiveness decreased between 96 and 97% compared to the control gel, while in gels at 1% the adhesiveness decreased between 60 and 80% regarding its control gels. The gels with Sepigel® presented a variation ratio similar to the hardness.

In the formulations containing MC and CMC (F5-F8), the inclusion of the extracts increases these properties. In gels with MC, the hardness increased between 1.5 and 4.4 times compared to their control gels. The adhesiveness practically was not affected in the gel with 3% MC, but increased 4.9 times in the gel with 4% MC (F6). Regarding gels with CMC, both properties increased slightly, both in those at 3% CMC (F5: 1.1 times the

hardness and adhesiveness) and 4% CMC (F6: 1.38 times the hardness and 1.66 times the adhesiveness).

When MC was used, the variation of adhesiveness was only significant in F6. The formulations with CMC had been the least affected in terms of pH with the inclusion of the extract and also generated no significant changes in adhesiveness.

According to the hardness results, the harder formulations (F5-F8) have less extensibility and the less hard ones have greater extensibility.

Table 2. pH values and texture profile from studied formulations.

	Parameters evaluated					
	pH		Hardness (g)		Adhesiveness (mJ)	
	Control gel	Herbal gel	Control gel	Herbal gel	Control gel	Herbal gel
F1	5.9 ± 0	7.5 ± 0.1	69.00 ± 6.77	13.50 ± 1.32	4.76 ± 0.56	0.14 ± 0.09
F2	5.2 ± 0	5.9 ± 0.1	102.00 ± 11.93	28.67 ± 5.39	4.22 ± 0.43	0.84 ± 0.49
F3	5.5 ± 0	7.6 ± 0.1	84.50 ± 10.99	13.83 ± 1.61	3.47 ± 0.45	0.15 ± 0.05
F4	4.5 ± 0	4.8 ± 0.2	112.50 ± 8.39	39.67 ± 1.76	4.00 ± 0.38	1.61 ± 0.15
F5	6.9 ± 0	5.8 ± 0.2	59.00 ± 3.55*	77.50 ± 15.50*	3.87 ± 0.77♠	3.82 ± 0.44♠
F6	6.9 ± 0	6.8 ± 0.2	47.00 ± 2.10	207.00 ± 45.51	2.25 ± 0.53	11.09 ± 3.27
F7	6.9 ± 0	6.9 ± 0.1	77.00 ± 7.54	85.33 ± 35.30	3.95 ± 0.38♣	4.40 ± 1.48♣
F8	6.9 ± 0	6.9 ± 0.2	141.00 ± 10.31	194.67 ± 11.72	7.46 ± 0.64♦	12.39 ± 3.16♦
F9	6.9 ± 0.1	6.5 ± 0.2	93.33 ± 0.29	36.83 ± 2.02	5.93 ± 0.54	2.03 ± 0.43
F10	6.9 ± 0.2	5.5 ± 0.2	119.50 ± 3.77	93.33 ± 4.25	7.18 ± 0.10	5.37 ± 0.44
F11	6.9 ± 0.1	5.5 ± 0.1	146.67 ± 3.88	128.00 ± 2.60	9.23 ± 0.46	7.52 ± 0.30

The symbols *, ♠, ♣, and ♦ indicate that there are no significative differences.

Antibacterial activity

All the gels presented antibacterial activity; the inhibition halo generated against strains of *Staphylococcus* ranged from 8.5 ± 0.0 to 15.3 ± 0.6 mm **(Table 3)**. The more fluid formulations with less consistency showed greater halos of inhibition. These results could be related to a limitation of the technique agar diffusion, since more fluid formulations better diffuse the active ingredients into the culture medium, giving better inhibition halos.

Table 3. Inhibition halo (mm) from formulations.

Formulation	Strains		
	S. aureus ATCC 25923	*S. aureus* ATCC 29213	*S. epidermidis* ATCC 12228
Ciprofloxacin	38.0 ± 0.0	29.0 ± 1.0	29.7 ± 0.6
F1	11.2 ± 0.1	8.25 ± 0.0	9.0 ± 0.0
F2	9.3 ± 0.6	8.7 ± 0.6	10.5 ± 0.5
F3	12.5 ± 0.0	8.5 ± 0.0	8.5 ± 0.0
F4	9.7 ± 0.6	8.7 ± 0.6	9.6 ± 0.6
F5	15.3 ± 0.6	10.6 ± 0.6	12.3 ± 2.0
F6	10.2 ± 0.0	9.0 ± 0.0	11.0 ± 0.0
F7	11.0 ± 0.0	9.0 ± 0.0	12.0 ± 0.0
F8	10.0 ± 0.0	9.0 ± 0.0	13.0 ± 0.0
F9	10.6 ± 0.57	11.6 ± 0.6	10.0 ± 0.5
F10	10.3 ± 0.57	11.3 ± 0.6	9.3 ± 0.6
F11	9.6 ± 0.57	11.0 ± 0.5	9.3 ± 0.6

There is no unanimous criterion to evaluate the antimicrobial potency of natural products and cut values from which the antibacterial activity of natural products can be classified as acceptable. Martins *et al*. [28, 29] propose the following classification criteria for antibacterial activity: weak when the halo is between 3 and 7 mm, moderate when the halo is between 8 and 10 mm, and strong when the halo is equal to or greater than 11 mm. In turn, Koohsari *et al*. [30] consider that halos equal to or greater than 12 mm can be considered as a good inhibitory effect of the extracts. According to these criteria, it can be considered that the gels exhibit moderate to strong inhibition. It should be noted that these halos are generated from 25 mg of gel, which contains 75 μg of phenolic compounds from the mixture of extracts. These values are encouraging because studies about natural products have shown that hydroalcoholic extracts of other *Lippia* species generated inhibition against *Staphylococcus* species from an amount equal to or greater than 2.5 mg [4, 30]. In general, hydroalcoholic extracts of other plant genera have weak or no antibacterial activity [28, 29] or require higher concentrations to produce good inhibition.

On the other hand, it is interesting that the formulations have good activity against these bacteria because *S. aureus* is responsible for some dermatological pathologies [8]. In this way, there may be an alternative available for the prevention and treatment of some dermal infections, to avoid resorting to a synthetic antibiotic as a first option. This is important because the indiscriminate use of antibiotics was one of the factors that led to the current bacterial resistance.

Microbiological quality

The number of registered bacterial colonies, as well as the total count of fungi and yeasts, are below the maximum allowable by Pharmacopoeia Argentina Seventh Edition [15] thus ensuring

quality parameters regarding asepsis in the preparation of the product.

Ex vivo skin permeation assay

The consistency and texture of F1, F2, F6, and F8 formulations were not suitable for skin application. F1 and F2 formulations were discarded because they were very fluid, whereas F6 and F8 were very hard. Formulation F9 was selected for this assay both because of its appropriate rheological characteristics and the fact that it includes fewer polymers.

Table 4 shows the parameters calculated from the permeation curve **(Figure 2)** of the cumulative amount permeated of polyphenols by area, as a function of time (in hours).

The permeation fluxes were between 8.95 ± 1.18 (F7) and 24.40 ± 1.41 μg of GAE/cm^2.hour (F2). The ANOVA of the fluxes values and the Tuckey post hoc test (α = 0.05) showed significant differences between them. At the end of the permeation study, F2 had a higher percentage of permeation (29 ± 3%) and F7 had a lower permeation (18 ± 1%).

The pH value of the vehicle, the solubility of the active principle in the vehicle, and the texture of the gel matrix are three important factors to consider in the evaluation of the penetration of the polyphenols through the membrane skin. The results obtained showed that the formulations that had the lowest hardness had permeated at a higher percentage and had a higher permeation flow. Conversely, those formulations that showed higher values of hardness, were those that presented lower percentage permeation and higher percentage of polyphenols retained in the skin. The statistical analysis determined that there are significant differences in the permeation coefficients, therefore, the matrix that contains the extract affects its skin permeation.

Table 4. Parameters evaluated from the permeation kinetics of polyphenols.

Gel	Flux (μg GAE/cm^2.h)	Permeated amount at 6h (μg GAE)	Permeated %	Retained on the skin (μg GAE)	Retained %	Kp (cm/h)
F2	17.53 ± 2.76^a	373.98 ± 10.82	29 ± 3	135.86 ± 14.84	10.5	0.0135^a
F4	24.40 ± 1.41^b	361.38 ± 53.54	23 ± 5	128.33 ± 4.19	7.9	0.0150^{a,b}
F5	13.54 ± 1.66^{a,c}	225.06 ± 54.94	18 ± 4	175.86 ± 21.66	14.18	0.0110^{b,c}
F7	8.95 ± 1.18^c	214.34 ± 19.17	18 ± 1	179.21 ± 12.54	14.85	0.0075^c
F9	18.61 ± 2.26^a	288.00 ± 51.76	30 ± 6	134.34 ± 10.82	14.09	0.0195^c

Different letters indicate significant difference among formulation evaluated.

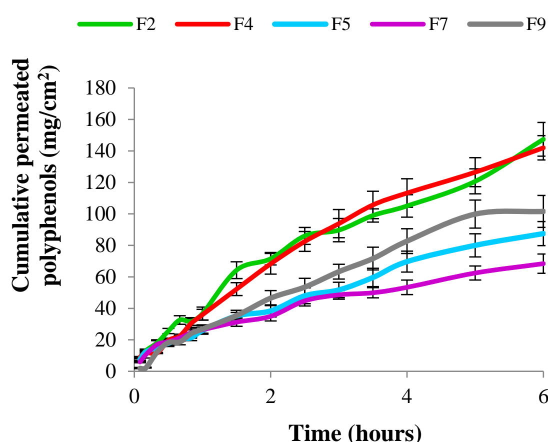

Figure 2. Polyphenol permeation curves in the formulations as a function of time.

According to Torky *et al*. [31] and Iqbal *et al*. [32] due to polyphenols have shown poor permeation, the formulations are adequate for topic application.

Stability study

The initial color of formulations (olive green) was maintained for a week, but then, they turned brown. This change may be due to the oxidation of chlorophyll since the same change could be observed in the extracts stored in dark green glass bottles at 8 °C. In both storage conditions (accelerated stability and room conditions), the gels showed no changes in the overall appearance or odor, and no signs of physical instability were observed.

Studies involving gels with plant extracts made with Carbopol® and cellulose derivatives have shown that they are generally stable under different storage conditions and that no changes in their appearance are observed [21, 22]. However, Aslani *et al*. [33] have reported changes in the physicochemical characteristics of Carbopol® 0.5 and 1% gels with 12% extract.

In both study conditions, the pH presented a significant change at three months, then varied very little. The variations recorded in both storage conditions are similar. Under room conditions, gels with Carbopol® reduced between 1.3 and 1.6 pH units. The gels with CMC decreased by 2.2 pH units for six months, while their control gels remained constant and, the gels with MC decreased by 2.5 units and their control 1.7 pH units. It seems that the most important changes in the formulations, if they happen, would occur within three months after elaboration, given that most of the studies report a stability study for three months, and some few extend the study up to six months.

In gels with plant extracts, the pH value of the different formulations decreased over time. Similar results were observed by Aslani *et al*. [33] in Carbopol® 934 and CMC gels with 12% extract, where the pH decreased slightly during six months, and the pure extract varied in the same measure. There has also been a decrease in pH in Carbopol® 934 gels with 2% extract [34]. However, in the study carried out by Giri and Bhalke [35], herbal

gels of Carbopol® 934 do not showed changes over tree months at different conditions of temperature and humidity. Carbopol® gels decreased extensibility in both conditions, but no significant differences were observed in all cases. Their control gels show the same behavior. The gels with MC and CMC gels tend to decrease the extensibility in both stability conditions, and this is because the preparations become firmer, but this does not happen with their control gels. Sepigel® gels exhibit the same behavior. The tendency observed with all the polymers studied here is that the viscosity increases and the extensibility decreases after a time of elaborated gels, independently of the storage conditions. This is probably related to the loss of moisture that the gels suffer.

Over time, the antimicrobial activity of the gels decreased **(Table 5)**. In most cases, this decrease was minimal during the six months analyzed and the formulations maintained the antibacterial activity against the strains tested.

Table 5. Halos of inhibition (mm) of the formulations determined at different times.

Formulation	Analysis time	Strains		
		S. aureus ATCC 25923	*S. aureus* ATCC 29213	*S. epidermidis* ATCC 12228
F2	Time 0	9.3 ± 0.6	8.7 ± 0.6	10.5 ± 0.5
	Time 6 RC*	6.0 ± 0	8.5 ± 0.5	10.2 ± 1.0
	Time 6 AS**	6.3 ± 0.6	8.1 ± 0.3	9.0 ± 1.0
F4	Time 0	9.7 ± 0.6	8.7 ± 0.6	9.7 ± 0.6
	Time 6 RC*	10.3 ± 0.6	8.3 ± 0.6	9.2 ± 0.3
	Time 6 AS**	6.3 ± 0.6	8.5 ± 0.5	7.3 ± 0.57
F5	Time 0	15.3 ± 0.6	10.7 ± 0.6	12.3 ± 2.1
	Time 6 RC*	12.7 ± 2.1	8.0 ± 1.0	12.8 ± 0.8
	Time 6 AS**	7.3 ± 0.6	7.6 ± 0.6	11.3 ± 0.6
F7	Time 0	17.0 ± 1.0	13.0 ± 1.0	11.6 ± 0.6
	Time 6 RC*	11.0 ± 1.0	11.6 ± 0.6	12.8 ± 0.8
	Time 6 AS**	8.3 ± 0.6	11.0 ± 1,0	11.3 ± 0.6
F9	Time 0	10.3 ± 0.6	11.3 ± 0.6	9.3 ± 0.6
	Time 6 RC*	9.5 ± 0.5	10.3 ± 0.4	9.5 ± 0.5
	Time 6 AS**	10.0 ± 0.5	11.5 ± 0.5	9.0 ± 0.5

*RC: Room Conditions. **AS: Accelerated Stability

The formulations evaluated have presented a microorganism count that was within the limit established by the pharmacopoeia for products that are not necessarily sterile. They did not present contamination by *Escherichia coli* or *Pseudomonas aeruginosa*.

This shows the aptitude of the natural products as preservatives of the formulation for six months. This fact is of interest because the trend today is to use compounds obtained from natural sources, characterized as safe for people and effective against microorganisms, to reduce or eliminate the use of traditional chemical preservatives [36, 37]. This is partly motivated by the growing skepticism of consumers regarding the safety of chemical preservatives combined with the fact that the lasting health of the skin is often associated with the use of natural ingredients.

The use of natural products as preservatives is an alternative to reduce the use of synthetic compounds, with a corresponding decrease in environmental impact in addition to the benefits sought in human health. Plants are a potential source of active molecules; many secondary metabolites are good antimicrobial agents [36, 38-40]. So, the current trend, both from industries and consumers is to resort to natural products as active ingredients and excipients of cosmetic and pharmaceutical formulations.

Conclusion

The inclusion of the combined extracts significantly affects the gel structure. The evaluated gels can transport the combination of extracts with the tested excipients, but it is necessary to incorporate a surfactant or co-solvent to achieve their inclusion. The use of emulsified gelling agents facilitates the vehiculization process. One of these agents, the Sepigel® at 4% gel was most suitable because the system was homogeneous, it presented good spreadability, and retained the antibacterial activity. This formulation did not show microbial contamination for six months. The combination of extracts potentiated the antimicrobial activity of this natural product and it was preserved over time. The formulation is a natural alternative to other current antimicrobials.

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