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Original Article

An Evaluation of Anti-Microbial Properties of Gelatin and Collagen Films Enriched by Aloe Vera and Henna to Build an Organic Band Aid

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Abstract

Background and Aim: Today, the idea of using biodegradable polymers which can naturally replace skin shortly before skin repair while also inhibiting the growth of bacteria causing wound infection is of great interest. The purpose of this study was to investigate the possibility of making degradable films using natural compounds, including chicken gelatin and collagen, henna plant extract, and aloe vera gel.

Materials and Methods: Aloe vera gel and ethanolic extract of the henna plant were prepared and their antioxidants were studied by DPPH (Diphenylpicrylhydrazyl) free radical-scavenging assay. Then, the antimicrobial properties of henna extract and aloe vera gel were evaluated against *Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* using the disk diffusion method along with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests. To prepare the films, the gelatin and collagen extracted from chicken, along with the ethanolic extract of henna and aloe vera gel, was used. Subsequently, the physicochemical and mechanical properties (elasticity, dissolution potency, and moisture level and microscopy investigation) of the films as well as the antimicrobial properties of films against these microbes were investigated.

Results: The results revealed that the anti-microbial effects of henna extract and aloe vera gel alone and in combination with gelatin/collagen matrix varied and had different effects on the tested microbes. Moreover, aloe vera showed stronger antioxidant properties than henna.

Conclusion: When synthesizing gelatin/collagen-based biopolymers, plant compounds and extracts can be used to enhance the antioxidant and antibacterial properties and apply organic band aids to help heal wounds and prevent wound infections.

Keywords: Gelatin/collagen films, Plant compounds, Antioxidant, Organic band

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Introduction

Skin is an immediate normal obstacle that protects inner organs from the outer environment threats such as attacks by microorganisms, contamination, infection, etc. (1). Wounds are caused by tears or defects in the skin induced by physicochemical or thermal injury or an underlying pathological condition (2). Wound healing is a dynamic and complex process that involves numerous interactions between various cells requiring a suitable environment to advance the healing process (3). In this regard, wound colonization is mostly polymicrobial (4-8), involving multiple microorganisms that are potentially pathogenic, leading to the risk of infection (5). Meanwhile, wound care management is very difficult (9). Thus, there is the demand of some means to prevent the growth of microorganisms on wounds (10) to prevent infection. Wound infections are principally engendered by microorganisms such as *Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* (9, 11). Traditionally, plants have proven to be natural remedies for several ailments. In a research conducted by Gandhi and Amnerkar in 2016, plant enzymes revealed a synergistic burn wound healing effect with chitosan by faster wound contraction, shortening of epithelialization time, and increased hydroxyproline content (12). Designing an excellent antibacterial cover is very significant to improve the development of wound dressings (9). Researchers have made various types of wet dressings (9). Due to their non-toxic, biocompatible, moisture-restraining, readily-available and biodegradable properties, currently the most abundant wound dressings are made of chitosan (13), gelatin (14, 15) hyaluronic, collagen, alginates, and cellulose (9, 16, 17)

Gelatin, derived from collagen, has received much consideration in tissue engineering and drug transference usage in recent years (18, 19). Moreover, gelatin hydrogels (20) and gelatin films (21) have been successfully examined to be used as bioactive materials. Henna has also been the subject of many scientific surveys. The extracts of its leaves have exhibited different biological activities, including antidiabetic (22, 23), antifungal (24), antiviral (25), antioxidant (26, 27), and anti-inflammatory (28) properties. Jridi *et al*. (20) suggested that hydrogels or films based on cuttlefish skin gelatin (CSG) combined with AHE, as well as their in vitro antioxidant effects, significantly promote the healing process. It was shown that cuttlefish skin gelatin-gel and film without hydrous extract of henna (L. inermis) exhibit low levels of antioxidant activities possibly associated with the presence of some antioxidant peptides perhaps obtained in the gelatin extraction process (20, 21). Aloe vera and henna are both potent plants with antimicrobial properties. The clinical properties of aloe vera and henna and their application contribute to wound healing in a normal and cost-efficient way. It has been indicated that the total gel extract of aloe vera boosts wound, burn, and bite healing. Furthermore, it has anti-inflammatory, antifungal, hypoglycemic, and gastroprotective properties. In particular, aloe vera's anti-inflammatory and wound-healing properties have been thoroughly investigated (29). Tumalapali et al. (30). indicated that composite dressings of OP-Gel-Aloe can be used as beneficial substances that can contribute to lesion healing. They provided a novel class of wound dressing based on the properties of an ideal wound-dressing device. Tumalapali et al. (30) showed that aloe vera loaded OP-Gel dressings could exhibit noticeable antimicrobial activity as evidenced by the colony count method.

In the present study, the ethanolic extract of henna and aloe vera gel were incorporated in chicken-foot gelatin/collagen films to investigate their effects on microorganisms such as *Candida albicans, Escherichia coli, Enterococcus faecalis* and, *Pseudomonas aeruginosa* in-vitro. To conduct this research, collagen and gelatin were extracted from chicken feet. The antibacterial effects of henna extract and aloe vera gel on the mentioned microbes were investigated. Moreover, anti-oxidant effects of henna extract and aloe vera gel were measured using the DPPH method. After the preparation of the films, the physical features of the films were checked. Furthermore, their effects on the microorganisms were investigated using the zone of inhibition method to investigate the antibacterial effect of the films.

Materials and Methods

Collagen Preparation from Chicken Feet

After washing with cool water and removing contaminants from chicken feet, they were minced into small pieces (average size 0.5 × 0.5 cm^2) by a meat processor. Non-collagenous protein, and fatty as well as mineral materials were isolated from the chicken feet in accordance with the method mentioned by Kittiphattanabawon et al. (31) with some modifications. All the processes occurred at 4°C under persistent stirring. To eliminate the non-collagenous protein materials, the sliced chicken feet were soaked at 0.1 N NaOH solid-to-liquid ratio (1:6, w/v) in a basic sample. The sample was changed every 2 hours. Then, it was washed until becoming clear. Deproteinized samples were defatted using 10% alcohol butyl proportional 1:6 (w/v). The mixture was under stirring for 24 hours and the solution was altered every 6 hours. Finally, the defatted sample was washed with distilled water (D.W) for three times. The non-mineral combination was isolated by soaking defatted sample in 0.1 N HCl proportional 1:6 (w/v). The solution underwent stirring by a mixer for 24 hours. The sample was filtered using double-layer cheese and washed with distilled water. It was treated using one stage acetic acid method. All these phases were performed at 4 ˚C under continuous stirring. The method developed by Kittiphattanabawon *et al*. (31) was used for collagen extraction using acetic acid with slight modifications. The ground samples were soaked at 0.5 M acetic acid solid-to-liquid ratio (1:6, w/v). The samples were filtered using a flaxen double-layer filter.

The extracted collagen was precipitated according to the method developed by Kittiphattanabawon *et al*. NaCl was used for precipitation at the final concentration of 2.6 M in the presence of Tris Hydroxymethyl aminomethane (PH: 7). The sediment was assembled using a centrifuge at 20000×g for 60 minutes at 4 ˚C and dissolved in 0.5 M acetic acid. The sample was dissolved in 10 volumes of 0.1 M acetic acid and D.W. It was then isolated from the liquid bed using a Buchner cone and vacuum pump via a polyvinylidene difluoride (PVDF) nitrocellulose filter. PVDF is an ideal film for western blot functional programs as well as analyzing protein and amino acids subsequence in protein quantity. This film has been used in this research for the first time for the isolation of collagen from the liquid phase. Buchner cone and vacuum pump were employed to facilitate the isolation at this stage.

Gelatin Preparation from Chicken Feet

Specifically, 1 kg of young chicken feet provided by Kaban chicken factory was purchased. The chicken feet were washed thoroughly to remove any contamination. They were poached with 1 L of water for 2 hours. Then, the solution was filtered and maintained in a refrigerator. After three hours, two phases formed. The above phase which was fatty was thrown out while the lower phase was used for film provision.

Preparation of Henna Extract

A total of 50 g of the henna (*Lawsonia Inermis*) leaves powder was suspended in 250 ml of 70% ethanol. The mixture was shaken and covered and then placed at 37 ˚C for 24h. Using filter paper, the ethanolic extract was filtered and maintained in a refrigerator until usage.

Aloe Vera Gel Preparation

A leaf of aloe vera (belonging to the *Liliaceae* family) was cleaned thoroughly with alcohol pads. The plant was cut by a scalpel laterally to extract the gel. After smashing jelly sectors, their entire content was stirred through a laboratory vortex. Then, it was filtered and stirred with vortex again.

Preparation of Industrial Gelatin Solution

4.34 g of gelatin powder (equivalent amount of a teaspoon) that was purchased from a confectionary accessories market was scattered over 150 ml of cool water and was dissolved in a boiling water bath.

Antimicrobial Studies

Antimicrobial studies of both the henna extract and aloe vera gel were separately carried out using the zone of inhibition, MIC and MBC. Moreover, the antimicrobial studies of the films were performed using the zone of inhibition. The microbial acting was investigated versus *Candida albicans* ATCC 10231, a gram-positive bacterial strain *Enterococcus faecalis* ATCC 29212 and two Gram-negative bacterial strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Antibacterial activities of henna extract and aloe vera gel were investigated separately on the mentioned microorganisms in two methods. Standard dilution method was used to study the antimicrobial effect of henna and aloe vera by evaluating visible growth of

microorganisms in Muller Hinton Broth. Serial dilutions of henna extract and aloe vera gel in concentrations ranging from 0.156 mg/ml to 5 mg/ml with adjusted bacterial concentration (10^8 CFU/ml) were used to determine MIC in broth. The control contained only inoculated broth. The tubes were incubated at 37 °C for 24h. The MIC was the lowest concentration of henna and aloe vera where no visible growth was observed in the tubes. They were inoculated of all the remaining transparent tubes on Muller Hinton Agar (MHA). Subsequently, they were incubated at 37 °C for 24h. The MBC was the lowest concentration of henna and aloe vera, and 99.9% of the bacterial population was killed. It was the concentration which did not lead to the growth of any organism on Muller Hinton agar plates (32). In another method, a 0.5 McFarland's standard was prepared from each microorganism. They were inoculated on 6cm Muller Hinton agar plates. 6 mm blank paper discs were put on a clean surface using a sterile forceps. 30µl of each extract and gel were poured on the discs and they were placed on inoculated plates. A sterile distilled water impregnated blank paper disc was used as the negative control, while nalidixic acid (30µg) and ciprofloxacin (5µg) were incorporated in paper discs that were used as the positive control. The plates were incubated at 37°C for 24h. Then, the zone of inhibition was measured to quantify the antibacterial efficacy of aloe vera and henna (32).

In the same way, after preparing the films and selecting the best ones, 6 mm discs prepared from the films (by punching) were placed on the inoculated Muller Hinton agar. The plates were incubated at 37°C for 24h. Then, the zone of inhibition was measured to quantify the antibacterial efficacy of the films.

Measuring the Antioxidant Properties of the Plant Extracts

The free radical scavenging activity of plant extracts was measured using the DPPH free radical-scavenging assay. In the DPPH protocol, methanol is used as a solvent. The solutions were prepared in 10 tubes in concentrations from 1mg/ml to 512 mg/ml of henna extract and aloe vera gel in methanol. Then, 2 ml DPPH was added to all the tubes. The tubes were left in the dark at the room temperature for 30 minutes. Subsequently, the absorbance was recorded at 517 nm. The methanol was used as blank. The experiment was repeated for three times. The obtained OD numbers were converted into inhibition percentage using the following formula and were reported as IC50. The unit is in mg/ml (33).

$$RSC\% = 100 \times [\frac{A\ blank - A\ sample}{A\ blank}]$$

Film Preparation

Different amounts of collagen compounds, gelatin (chicken and industrial), glycerol, henna extract and aloe vera gel along with distilled water were used to make a film with proper strength and physical as well as mechanical properties. Glycerol acted as a softener in the composition of these films and ensured the flexibility of the film (30). After combining the ingredients in 25 ml Falcons, they were vigorously stirred for 15 minutes and then were poured into 6 cm sterile glassy Petri dishes. It took a week to dry the films inside the incubator at 37°C (Table 1).

Analyses of the Physical Properties of the Prepared Film

Rectangles (34 mm × 10 mm) of the dried films were cut and removed from the plate. Then, they underwent analyses of thickness and elasticity by a digital ruler.

Moisture Level

The films were cut off into small segments (34 mm ×10 mm) and put on small aluminum sheets and weighed. Then, they were kept at 60˚C. After the complete drying of films, they were weighed again to measure the lost weight.

Table 1: Components and Quantities Used to Make the Film. The reported numbers were in ml. To volume with water (10), Chicken gelatin (3), Industrial gelatin (1), Glycerol (1.5) and Collagen (0.013).

Specimen	Henna	Aloe vera
1	0.038	0.050
2	0.038	0.025
3	0.012	0.075
4	0.012	0.050
5	0.012	0.025
6	0.025	0.075
7	0.025	0.050
8	0.025	0.025
9	0.025	-
10	0.012	-
11	0.038	-

Dissolution Level

The films were cut off into small segments (1cm ×1cm) and swallowed in 1.5 ml of D.W and finally kept at 36 ˚C.

Microscopy Survey

Small pieces of the films were put on a slide which was observed under the microscope. Homogeneity and dissolution of compounds in the films can represent the optimal quality of the film and material suitability directly affecting film elasticity and strength.

Investigation of Antimicrobial Properties of the Prepared Films

Films with the best stability and elasticity were used to investigate the antimicrobial properties. To this end, small disks of the prepared films were prepared by puncture and put on the surface of inoculated media of Muller Hinton agar plates. Then, the plates were incubated for 24h at 37˚C. The formation of inhibition zones showed the presence of antimicrobial activities of the films against the cultured bacterial strains.

Data of diffusion, MIC and MBC in every plant were separately analyzed using CRD statistical design with three repetitions. After being significant of those traits between the bacteria, the mean compare was carried out by LSD (0.05) using SAS Software. For antioxidant properties, the data were analyzed in nonlinear regression method, and then asymptotic exponential model was fitted. General form of the model is:

$$Y=a\ [1-\exp^{-k(x-x_0)}]$$

Where a: maximum value of Y in top of curve (here: antioxidant properties of extraction), x_0: the mount of x (concentration) when Y=0 and k: rate of approaching of Y to a.

Results and Discussion

The results of the single microbial test of henna and aloe vera have been outlined in **Tables 2 to 5**. This study indicated that the extract of henna and aloe vera gel has strong antimicrobial properties and they have the ability to supply antimicrobial properties of the films and could be used in film preparation. Therefore, different concentrations of the extract and gel were used in the films in this research. The results of analysis of variance showed that the plant product on bacteria was significantly affected (p>0.01, Table 2).

Table 2: Analysis of Variance of Square Mean of Disk Diffusion Test (percentage inhibition) in Different Bacteria.

SOV	df	Henna	df	Aleo vera
Bacteria	2	35.80**	1	79.42 **
Error	6	6.77	4	0.118

Table 3: Mean compares of Disk Diffusion Test (percentage inhibition) on the Disk of Aloe Vera and Henna on Different Bacteria.

Bacteria/Plant product	Aleo vera	Henna
Candida albicans	12.96 a	16.29 a
Pseudomonas aeruginosa	11.82 a	9.01 b
Enterococcus faecalis	8.28 b	00.0 c
Escherichia coli	Not seen	Not seen
LSD (0.05)	2.78	0.77

The results of means comparisons of disk diffusion agar test have been presented in Table 3. In this study, plant products had variable effects on different strains of the tested microbes. Both of the products exhibited larger zones of inhibition for *Candida*, while none of them revealed zones of inhibition for *Escherichia coli.* Thus, mean compares indicated that inhibition of aloe Vera prouduct on *Candida albicans* and Pseudomonas aeruginosa was statistically similar and for Enterococcus faecalis was lower (8.28). However, the results for henna were different. Inhibition of henna products on *Candida albicans* was more than others, and for *Enterococcus faecalis* was zero and had no effect (Table 3).

The results of variance analysis indicated that Aleo vera and henna extraction had significant effects on MBC (minimal bactericidal concentration minimal bactericidal concentration) and MIC (minima inhibitor concentration) on bacteria (Table 4). The results of mean comparisons showed that aleo vera extraction had the highest minimal inhibitor concentration (MIC) on Enterococcus *faecalis* and the lowest effect was

Table 4: Analysis of Variance of Square Mean of Various Effects of Plant products on MIC and MBC Tests of Different Bacteria.

SOV	dF	Mss			
		Aleo vera		Henna	
		MIC	MIC	MBC	MIC
Bacteria	3	38.11	9.53	47.02	11.72
Error	8	0.211	0.045	0.166	0.045

Table 5: Mean Compares of MIC and MBC of Aloe Vera and Henna on Different Bacteria.

Bacteria/Plant product	Aleo vera		Henna	
	MIC	MBC	MIC	MBC
E. faecalis	2.413 a	4.83 a	00.0 c	0.0 c
P. aeruginosa	1.24 b	2.41 b	2.413 a	4.83 a
C. albicans	0.624 c	1.24 c	0.624 b	1.246 b
E. coli	00.0 d	0.0 d	00.0 c	00.0 c
LSD (0.05)	0.148	0.346	0.153	0.289

observed in *Escherichia coli.* Therefore, the highest minimal bactericidal concentration (MBC) was observed in *Enterococcus faecalis*, and similar to the effect of its MIC, the lowest effect of MBC was observed in *Escherichia coli.* However, the effect of the extraction of henna on MIC and MBC was conversely of that of aloe vera extraction. The greatest effect of henna extraction of MIC and MBC was on *Pseudomonas aeruginosa*, and the lowest (MIC and MBC) one was observed on *Enterococcus faecalis* and *Escherichia coli* for two traits (Table 5).

The results of the regression analysis of the antioxidant properties of henna extract and aloe vera gel have been reported in Table 6. Both of the plants exhibited beneficial antioxidant properties. These outcomes demonstrated that the antioxidant activity of aloe vera was greater than that of henna. The asymptotic exponential model predicted values of (the maximum value of antioxidant properties) 89.67 and 76.12 for aloe vera and henna, respectively. Thus, the observed and predicted data of anti-oxidant properties vs concentration of extraction of aloe vera and henna have been indicated in Figure 1. High R^2 and good overlapping and predicted data line on 1:1 line indicated that the model could be used for analyzing the data of bacteria in this paper (Fig. 2).

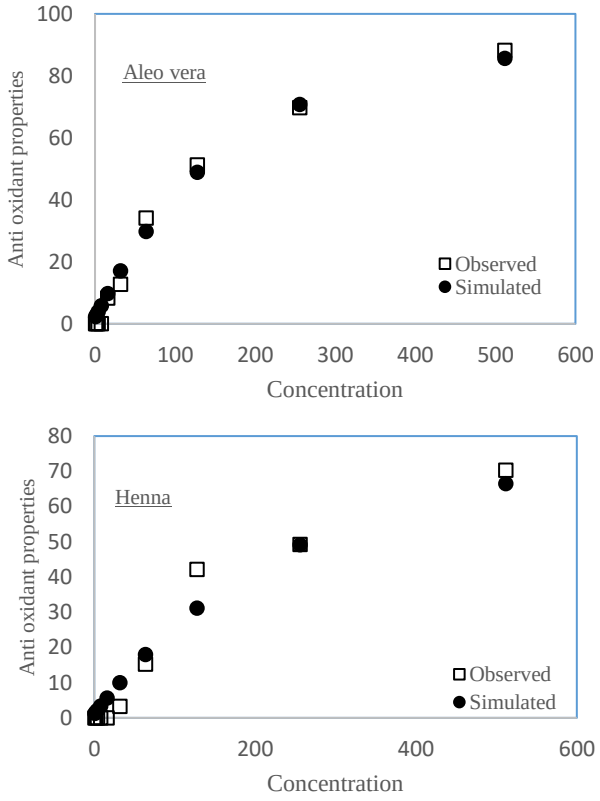

Figure 1. The Observed and Predicted Data of Anti-Oxidant Properties Vs Concentration of the Extraction of Aloe Vera and Henna.

As the thickness and elasticity of the prepared film had direct effects on its settlement on the skin surface, this property was tested. Initially, the thickness of the film cutting pieces was measured using a digital ruler. Then, the films for the tensile strength and elongation were measured as shown in Table 7. This study showed that this increase in length was reversible and could return to original dimensions.

Pieces of the films (34mm ×10mm) were placed on small aluminum foils which were already weighed. Then, they were exposed to 60 ˚C. After 14 days, they were dried and weighed again. The results have been presented in Table 8.

Table 7: Physical Properties of the Films. The reported numbers are in mm.

Sample	1	2	3	4	5	6	7	8	9	10	11
Thickness	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77
Initial length	34	34	34	34	34	34	34	34	34	34	34
Final length	87.9	80.6	78.3	81.7	84.3	80.6	83.5	78.9	80.2	76	86.7

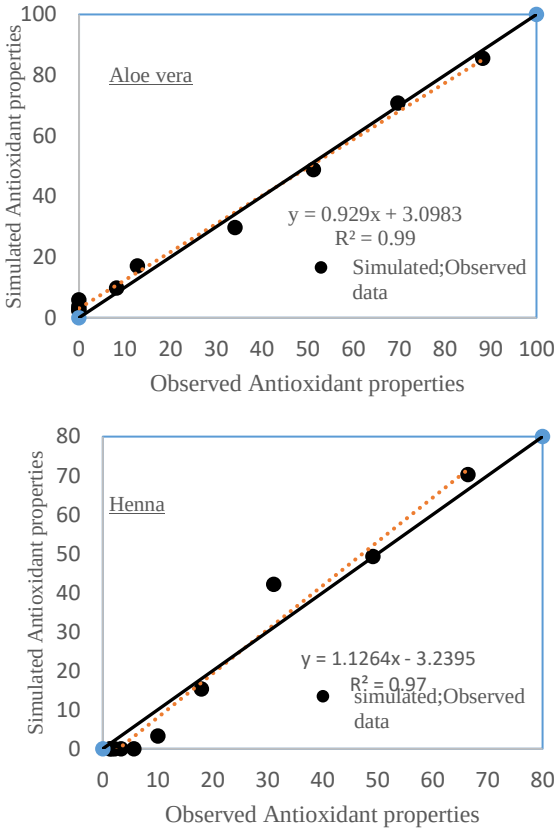

Figure 2. Observed VS. Predicted Data of Antioxidant Properties for Aloe Vera and Henna.

In order to examine the dissolution rate, pieces of 1cm×1cm dimensions were cut from the films and were suspended in micro tubes containing 1.5 ml D. W at 36 ˚C which were checked every half an hour. After 1 hour, the film softened which was at the bottom of the tubes with the micro tube being completely dissolved by rolling. It was found that the compounds used in the film under the microscope did not have uniform distribution (Fig. 3, No: 1, 2, 4, 5 and 9) and were variable. In some films, uniformity was more than others (Fig. 3, No 3, 6, 7, 8, 10 and 11).

Creation of a zone of inhibition around the disks signals the antimicrobial effect on the cultured species. In this research, this test did not affect

Table 9: Anti-Microbial Properties of the Films.The reported numbers are in ml. For C. albicans and E. Faecalis was not seen.

Sample	*P. aeruginosa*	*E. coli*
1	11.23	11.35
2	11	9.25
3	not seen	10
4	not seen	not seen
5	not seen	10.23
6	not seen	not seen
7	not seen	not seen
8	10.94	10
9	9.04	10.43
10	not seen	not seen
11	not seen	10.16

Enterococcus faecalis and *Candida albicans*, while the effects on *Escherichia coli* and Pseudomonas

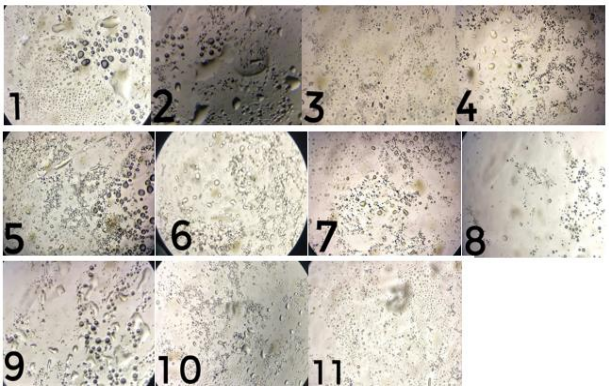

Figure 3. Microscopic Examination of the Film.

aeruginosa were different at distinct concentrations of the extract and gel (Table 9).

Since wound healing depends on reactive oxygen species (ROS), the antioxidant activity of the extract of henna and aloe vera gel was investigated using DPPH radical scavenging (34). Alpha-tocopherol from vitamin E is a potent antioxidant found in aloe vera. Moreover, anthraquinones found in aloe vera offer a radical scavenging activity and are perhaps responsible

Table 8: The Moisture Content.

Sample	1	2	3	4	5	6	7	8	9	10	11
Initial weight	0.1768	0.1273	0.1832	0.1819	0.1825	0.1519	0.3278	0.2297	0.3255	0.1582	0.3455
Secondary weight	0.1375	0.948	0.1492	0.1484	0.1522	0.1023	0.2718	0.1907	0.2339	0.1209	0.2576
Weight difference	0.393	0.325	0.340	0.335	0.303	0.496	0.560	0.390	0.916	0.373	0.869

for the pro-antioxidant activity of aloe vera (29). Mikaehil *et al*. (35) reported that henna leaves are rich in apigenin, apiin, cosmosiin, p-coumaric acid, 2-methoxy-3-methyl-1, 4-naphthoquinone, luteolin, and lawsone which are regarded as great antioxidant composites. In a pilot study, cuttlefish skin gelatin-gel and the films without hydrous extract of henna (L. inermis) exhibited low antioxidant activities possibly associated with the presence of some antioxidant peptides obtained in the gelatin extraction process (20, 21). The results acquired in this study suggested that hydrogels or films based on CSG combined with AHE, as well as them in vitro antioxidant effects, could significantly contribute to the healing process. The observed activities were possibly due to the presence of phenolic compounds in henna leaves. They functioned as good donors of the reactive proton with free radicals to change them into more stable crops and terminate the radical chain reaction (36). Moreover, the antioxidant activities were dose dependent, which increased with the rise of AHE concentration in the gelatin matrix (20). In the present study, both of the plants indicated remarkable antioxidant properties. The antioxidant activity of aloe vera gel started at lower concentrations than henna extract. Furthermore, the inhibition percentage of aloe vera was slightly higher than that of henna.

There are many low molecular weight combinations in aloe vera, with lupeol, cinnamonic acid, salicylic acid and uric acid (30). They have a significant role in the destruction of bacterial cells by interfering in the enzymatic processes (37). Similarly, acemannan, the polysaccharide which is considered as the major agent for the anti-inflammatory property of aloe vera, has an antimicrobial effect (38). It was shown in a research conducted by Tumalapali *et al*. that composite dressings of OP-Gel-Aloe could be noticed as suitable substances with the potential for impressive and manageable lesion healing. They provided a novel class of wound dressing based on the properties of an ideal wound-dressing device. Tumalapali et al. (30). Showed that although zones of inhibition were not observed, aloe vera loaded OP-Gel dressings indicated a significant antimicrobial activity as evidenced by the colony count method. Moreover, curcumin exhibited a stronger antimicrobial activity than aloe vera at a similar drug content. This impact was observed at all concentrations. Nevertheless, the OP-Gel-Aloe Curcumin dressings did not seem to have fared so well. They stated that there might be certain chemical interactions between aloe vera and curcumin that result in a decrease in their biological activity (30). In the present study, the antimicrobial properties were investigated through two methods of disk diffusion testing and MIC as well as MBC determination with both confirming each other. Thus, in the case of the Gram-negative bacteria *Escherichia coli*, none of the henna extract and aloe vera gel created a growth inhibition zone. Moreover, none of the concentrations of the extract and gel affected *Escherichia coli* by MBC and MIC. Furthermore, both of them created a significant growth inhibitory zone on the plate containing *candida albicans* and at low concentrations showed inhibitory and fatal effects. In the case of *Pseudomonas aeruginosa*, both of the plants, especially aloe vera, exhibited a significant anti-microbial effect. Henna extract did not affect the Gram-positive bacteria *Enterococcus faecalis* though aloe vera gel created a growth inhibition zone. In this study, none of the films affected *Enterococcus faecalis* and *Candida albicans*, and they failed to create a growth inhibitory zone. On the other hand, most of the films affected *Escherichia coli* and a few of them affected *Pseudomonas aeruginosa*.

The major characteristic of burns is the formation of a scar, which is made up of burned and traumatized tissues. As a result, prompt removal of scars is essential for the healing of burns (39). Several plants and plant products have been reported to treat skin disorders, including burn wounds (40 - 43). Plants and their extracts possess great potentials for the management and repair of burn wounds. Plant enzymes induce wound healing and renewal of lost tissues through various mechanisms (12). In a research conducted by Gandhi and Amnerkar in 2016, plant enzymes revealed a synergistic burn wound healing effect with chitosan by faster wound contraction, shortening of epithelialization time, and increased hydroxyproline content. Hence, the result supports the traditional use of plant enzymes in skin disorders, including burn wound healing (42). In the present study, the antibacterial properties of henna extract and aloe vera gel on *Pseudomonas aeruginosa*, which is one of the most important factors in burn wound infections in hospitals,

were desirable. Both of these products produced a growth inhibition zone in the disk diffusion test. On the other hand, the application of different values of these extracts and the gel in the films in the next stage of the study did not inhibit the anti-bacterial properties of the products on *Pseudomonas aeruginosa*. Hence, in general, it is probable that aloe vera and henna in combination with gelatin/collagen films have some chemical interactions that lead to a reduction in their antibacterial activity.

Conclusion

Overall, the results acquired in this survey revealed that gelatin-based films with aloe vera gel and henna extracts could significantly improve the treatment process due to antioxidant effects along with their antimicrobial effects in the laboratory. Moreover, the presence of moisture in the films and the ability to be flexible are likely to be effective in placing the dressing on the wound and maintaining the moisture balance of the wound, which can contribute to the healing of wounds. The results of this study indicated that henna extract and aloe vera gel used in the collagen-gelatin matrix can be considered antimicrobial and antioxidant agents for the production of wound-healing agents. However, this study did not evaluate the clinical activity of these extracts. Many factors are involved in clinical evaluations, and comparative as well as controlled clinical studies should be conducted to determine whether plant products are really effective. Along with the advancement of science and technology, it is better to replace synthetic drugs with natural herbal medicines.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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