Original Article

Formulation and Evaluation of the Novel Herbal Antibacterial Gel to the Treatment of Cutaneous Burn Infections

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Abstract

Due to the rapid development of antibiotic resistance, the strong need for alternative strategies to tackle this problem is inevitable. The objective of this study was to prepare and evaluate the antibacterial effects of a pharmaceutical gel containing herbal extracts including *Lawsonia inermis* (henna) and *Matricaria chamomilla*. Using hydroxypropyl methylcellulose (HPMC), carboxymethylcellulose (CMC), and propylene glycol (PEG), the pharmaceutical gel was formulated and the physical properties of the formulation were specified at 37 ± 2°C. The total phenolic content (TPC) of extracts was determined using the Folin–Ciocalteu method and expressed as mg gallaic acid (GA) per gram extract (Ex). The release of the polyphenol compounds from the optimum formulation was investigated using the Franz cell device. Eventually, the disc diffusion method was used to evaluate the antibacterial activity of optimum formulation against the two pathogenic bacteria strains *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results showed that the optimum formulation was stable at least for 3 months. The TPC of the aqueous extract of henna leaves, the hydroalcoholic extract of chamomile flowers, and the optimum formulation was 57.8, 181.08, and 202.75 mg GA/g Ex, respectively. Nearly 80% of the phenolic compounds in the optimum formulation were released over 4 h. The phenolic compounds have inhibitory effects on the growth of *S. aureus* and *P. aeruginosa.* On the basis of this finding, the formulation had excellent stability, viscosity, homogeneity, extrudability, and antibacterial activity which can be employed as a topical pharmaceutical gel in cutaneous burn infection treatment.

Keywords: *Antibacterial gel, burn infections, Lawsonia inermis, Matricaria chamomilla*

Introduction

Burns are defined as non-mechanical damage to the skin caused by heat, sunlight, chemicals, electricity, and nuclear radiation. The burn is a critical care problem starting from the moment of injury.[1] Healing of burn wound infection is a dynamic process and any postponement in the initiation of the response to infection can result in bacteremia, sepsis, or multiple organ dysfunction syndromes.[2] The responsible microorganisms for burn infections are mostly characterized by Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[3]

Today, increasing health problems caused by drug resistance in pathogenic microorganisms are a big challenge for the pharmaceutical and biomedical areas in a way that the primary sources of hospital infections such as multidrug-resistant *P. aeruginosa* led to considerable mortality, morbidity, and prolonged treatment costs in burn infections.[4]

In contrast, a few available antibiotics in market are effective against these bacteria, e.g. Avycaz and Zerbaxa.[5-7] Therefore, many strategies devised to reduce the aforementioned shortcomings of antibiotics and topical antibacterial therapy seem to enhance therapeutic efficiency.[8]

Since ancient times, there were ideas that natural compounds had healing potential and can be used for medicinal purposes. Medicinal plants have made significant strides during the past decade through the development of herbal pharmaceutical product along with extending the potential number of viable solutions to tackle antibiotic resistance.[9] The antibacterial activity of plant extracts is mainly related to the sulfur-comprising compounds in the aqueous phase or their essential oil fraction.[10,11]

Chamomile (*Matricaria chamomilla* L.) belongs to the family of Compositae and naturalized in west Asia, France, Germany, and Brazil. According to the finding, its flowers have anti-inflammatory and antibacterial properties.[12] Its extract contains several

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bioactive compounds such as terpenoids, monoterpenoids, sesquiterpenoids, and phenolics.[13] This plant has been used in herbal medicine as an anti-inflammatory and antispasmodic substance. It is also employed in the treatment of antibacterial, antibiofilm, and antifungal diseases.[14,15] Studies show that combination of *M. chamomilla* ethanol extract with diclofenac demonstrated significant antinociceptive effect in rats.[16] Furthermore, its ethanol extract was active against *S. aureus* strains,[17] and cutaneous use of chamomile extract could accelerate burn wounds healing.[18]

Lawsonia inermis is a perennial shrub of Lythraceae family (commonly known as henna) which is used for medicinal and cosmetic purposes. Henna is native to India, Arabian Peninsula, and North Africa. Its leaves contain 1.3–22% Lawson or hydroxy naphthoquinone which is the active ingredient and the color compound. The antibacterial and fungicidal effects of henna have long been known and reported in myriad studies.[19] Henna leaves display *in vitro* anti-inflammatory properties,[20,21] and Lawsone showed good wound healing activity.[22] In addition, several studies demonstrated aqueous extracts of henna leaves showing good inhibitory effects against both Gram-positive and Gram-negative bacteria.[23-25]

Based on the aforementioned studies, the potent antibacterial and anti-inflammatory activities of chamomile and henna extract and the combination of them in the formulation can hold therapeutic promise in the management of burn infection conditions. The purpose of this study was to develop a topical pharmaceutical dosage form based on HPMC, CMC, and PEG encapsulated with hydroalcoholic extract of chamomile and aqueous extract of henna for bacterial infection treatment. By adjusting the proportions of polymers, wide varieties of hydrogels with various viscosity, homogeneity, and extrudability properties were obtained and characterized. Based on the best physicochemical properties of formulation, the phenolic compound release behavior and intrinsic antibacterial properties of optimum hydrogel were tested to investigate the successful treatment of burn infection.

Materials and Methods

Materials

Mueller Hinton agar, Folin–Ciocalteu reagent, GA, HPMC, CMC, PG, and sodium carbonate (Na_2CO_3) were purchased from Sigma-Aldrich, USA.

Two pathogenic strains of bacteria, namely, *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853), were supplied from the Microbial Control Laboratory, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Procurement of plant material

The samples of *M. chamomilla* and *L. inermis* were collected from Kermanshah and Kerman Province, Iran, respectively. It was identified by Dr Mirtajeddini, Bahonar University, Kerman, and a voucher specimen for *M. chamomilla* (No. 075 101 003) and *L. inermis* (No. 167 003 001) was deposited at the Herbarium of the School of Pharmacy and Pharmaceutical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Preparation of the plant extract

Primarily, leaves of henna and aerial parts of *M. chamomilla* including flowers, leaves, and the end of the stem were washed to remove dirt via deionized water and then air-dried and ground using a mechanical grinder. The powder was then placed in a dark space at room temperature. Next, 50 g of henna leaves powder was mixed with 500 mL distilled water in Erlenmeyer flask under stirring at room temperature for 24 h. In the case of chamomile extraction, 50:50 ratios of distilled water and ethanol were added about one-tenth of the weight of the henna-powdered plant based on its adhering property for 72 h. The macerated mixture was filtered through Whatman grade No. 42 filter paper to get the appropriate aqueous or hydroalcoholic extract. Finally, the extracts were concentrated, lyophilized, and stored at 4°C for further studies.

Preparation of gel formulation

Preparation of gel formulations was done according to Shukr *et al.* with some modifications. For this purpose, HPMC and CMC were used as gelling agents, PG as humectant, and benzalkonium chloride as preservative. Table 1 shows different formulations containing henna and chamomile extract as an active ingredient with total concentrations of 5% (w/v).[26]

The amount of preservative was considered constant. The gelling agent (HPMC or CMC) was mixed with PG and dissolved in an adequate quantity of phosphate buffer at pH 5.8 (equal to skin's pH) by stirring, after which the preservative

Table 1: Amounts of gel formulation compositions (% w/v) using different polymers

Formulation number	CMC	HPMC	PG	Benzalkonium chloride	Lyophilized henna Ex	Lyophilized chamomile Ex
F1	2.5	—	10	0.1	5	5
F2	3	—	10	0.1	5	5
F3	3.5	—	10	0.1	5	5
F4	4	—	10	0.1	5	5
F5	4.5	—	10	0.1	5	5
F6	3.5	—	7.5	0.1	5	5
F7	4.5	—	7.5	0.1	5	5
F8	—	3.5	7.5	0.1	5	5
F9	—	4.5	7.5	0.1	5	5

was added to the prepared gel. In order to use henna and chamomile extracts in the gel formulation, lyophilized extracts were dissolved in phosphate buffer and added to the gel with a final concentration of 5 w/v% in each formulation.

Physical evaluation of the gel formulations

The optimum formulation was chosen according to a comparative evaluation of the physical properties of the prepared gels. These parameters were tested in all formulations:

- pH was measured using a digital pH meter (827 pH Lab, Metrohm, Switzerland).
- The color was tested via a white surface as the gel background.
- Homogeneity was checked through observing the presence of any particle in the formulations.
- Extrudability was determined by means of the method of Aiyalu *et al.*, where 20 g of gel in a collapsible tube was pressed from the crimped end with prevention of any rollback. The percentage of the extruded gel was then calculated.[27]
- Viscosity was evaluated using the viscometer (DV-111ULTRA, Brookfield, USA).

Stability studies

The stability studies were carried out under different temperature conditions (25 and 40°C) for 3 months. All the evaluation parameters, namely, pH, viscosity, and appearance were, studied at time intervals of 30, 60, and 90 days.

Total phenolic assay

The total phenolic contents (TPCs) of henna and chamomile extracts were determined at a concentration of 5% w/v using the Folin–Ciocalteu method.[7] Briefly, 0.2 mL of henna or chamomile extracts was added to 1 mL of freshly prepared Folin–Ciocalteu reagent, to which 0.8 mL of sodium carbonate (7.5% w/v) was added after 10 min. After 2 h incubation at room temperature, the absorbance of the reaction mixture was recorded by an ultraviolet–visible (UV–Vis) spectrophotometer at 760 nm. GA was used as standard and TPC was explained as mg GA/g Ex equivalents. The calibration equation for GA at different concentrations is as follows (1):

$$y = 0.0037x + 0.0081, \quad (1)$$

$$R^2 = 0.9987,$$

where x is the absorbance of the sample and y is the GA equivalent (mg GA/g Ex).

The release of phenolic compounds

The extract release from optimum formulation was determined using a Franz cell device at 37°C in the phosphate buffer medium at pH=5.8. The amount of phenolic compound was determined using the Folin–Ciocalteu method as already mentioned. The release of the phenolic compound was measured across the dialysis membrane (12 kDa) using Franz diffusion cell, with a diffusional area of 2 cm^2 and a receptor volume of 50 mL. The membrane was soaked in the receptor compartment, and 5 g of the gel was placed on the membrane surface in the donor compartment. The receptor compartment of the cell was filled with 50 mL of phosphate buffer pH 5.8 and 37°C.

An aliquot of 1 mL was collected from the receptor side at intervals of 0, 15, 30, 60, 90, 120, 180, and 240 min and replaced by the same volume of fresh buffer in the receptor to maintain the constant volume. The concentration of phenolic compounds in the samples was determined through the use of a UV–Vis spectrophotometer and GA calibration curve. All experiments were done in triplicate and results expressed as means ± standard deviation (SD).

Determination of the antibacterial activity of gel

The antibacterial activity of optimum formulation, 5% (w/v) henna, and chamomile extracts was tested by the disc diffusion method against the two pathogenic strains of bacteria *S. aureus* and *P. aeruginosa*. In this test, clindamycin disc and preservative (0.1%) were used as the control. The bacterial suspension (1.5×10^8 CFU/mL) was swabbed onto sterile Mueller-Hinton Agar plates using a sterile cotton swab. Disks soaked in 5% henna or chamomile extract, optimal gel formulation, 0.1% preservative, and clindamycin disc were placed in the environment of Muller-Hinton agar. The plates were incubated at 37°C for 24 h, after which time, the inhibition zone of bacteria was measured.

The minimal inhibitory concentrations (MICs) of the henna and chamomile extracts were determined using the standard broth dilution method. Live cells of experimented pathogenic bacterial strains at final concentrations of 1.5×10^7 CFU/mL were inoculated into 48-well plates followed by 100 µL of henna and chamomile extract. After incubation for 24 h at 37°C, their concentrations were recorded. The pure medium and the medium containing bacteria were, respectively, used as the negative and positive controls, respectively. The MIC was calculated based on the lowest concentration of extract or gel formulations inhibiting the bacterial growth.

Results and Discussion

Hydrogel dressing is 3D networks of polymers with cooling and soothing effects in burns treatment for skins which can adsorb water several times the gel weight.[28] For reinforcement of prepared hydrogel, CMC was essential. The extract of henna and chamomile was used as an antibacterial ingredient facial gel.

The stability studies of the different parameters of hydrogels are exhibited in Table 2. Among the prepared formulations, formulation number 6 was selected as the optimum formulation for further calculation of drug release due to the better appearance and physical properties. Physical stability parameters of optimum formulation such as appearance, pH, and viscosity were investigated at 37 ± 2°C within 3 months. According to the results obtained in this study, after 3 months

no significant change in pH, viscosity, and appearance was observed in an optimal gel formulation. The pH of hydrogel was measured to be in a range of 5.6–5.9, which was in compliance with human skin pH (4.0–6.0).

Determination of TPCs

Polyphenol compounds (PCs) are one of the most important factors in the antioxidant capacity of plants. Since the therapeutic properties of plants are related to the amount of PC, determining the amounts of phenolic compounds during storage and processing is important in the production of products with high potential against free radicals.[29] Total phenolic compounds in plants normally have antioxidant activity. Hence, they are an important factor in health promotion because of their antioxidant and antibacterial activity. In fact, their beneficial free radical scavenging came from the hydroxyl group of phenolic compounds, which acts as a hydrogen donor.[30] These compounds were studied in the aqueous extract of henna leaves, hydroalcoholic extract of chamomile flowers, and optimum formulation using the Folin–Ciocalteu method. The results are shown in Table 3. The TPCs in various compounds such as flavonoids, coumarins, and gallic acid derivatives, obtained from henna and chamomile extracts, are documented by many publications, although just a few studies have investigated the amounts of the phenolic compounds in a mixture of these two plants. Our observations confirmed the results obtained by Mongi and co-workers.[31] It could be concluded that the high quantity of phenolic compounds in chamomile flower extract is due to the use of hydroalcoholic solvent as a higher polar solvent than less polar solvents such as alcohol which is more efficient in extracting the phenolic compounds.[32]

In vitro phenolic compounds release

Phenolic compounds were considered as the effective ingredient index in the optimum formulation. When it comes to the measurement of total phenols, the colorimetric Folin–Ciocalteu method seems to be the best due to its simplicity, saving time and cost-effective.[29,33,34] As shown in Table 4 and Figure 1, nearly 80% of the phenolic compounds in the optimal formulation were released over a period of 4 h due to the slow and regular erosion of the polymeric matrix in the gel and the presence of PG which can play a penetration enhancer role and facilitate drug release from the 3D matrix of the gel. In order to investigate the effects of polymer on total phenol content, the base formulation without henna and chamomile extract was checked out similar to its release process. The results showed that the basic formulation did not interfere with the observed total phenol content.

Kinetics and mechanism of drug release

Regarding the *in vitro* release profile, data were subjected to different release kinetic models such as zero-order Eq. (2), first-order Eq. (3), and Higuchi Eq. (4)[35,36]:

$$Q = K_0 t, \tag{2}$$

$$\ln(100 - Q) = \ln(Q_0) - K_1 t, \tag{3}$$

$$Q = K_H t^{\frac{1}{2}}, \tag{4}$$

where Q is the phenolic compound (active ingredients) released at time t, Q_0 is the percent of active ingredients remaining to be released, and k_0, k_1, and k_H are the coefficients of the equations. The experiments to determine the kinetics of release were carried out in triplicate.

According to the zero-order kinetic, the release velocity of active ingredient is constant and independent of its concentration in formulation, whereas first-order kinetics indicate the release

Table 2: Physicochemical properties of prepared gel formulations comprising pH, viscosity, color, homogeneity, and extrudability

	pH	Viscosity (cP)	Color	Homogeneity	Extrudability
F1	5.8	716	Brown	+++	+++
F2	5.8	847	Brown	+++	+++
F3	5.9	1650	Brown	+++	+++
F4	5.8	2400	Brown	++	+
F5	5.8	2740	Brown	+	+
F6	5.8	2700	Brown	+++	+++
F7	5.6	2823	Brown	+	+++
F8	5.8	2940	Brown	+++	+
F9	5.7	3261	Brown	++	+

+: Good, ++: very good, +++: excellent

Table 3: TPC of the aqueous extract of henna leaves, hydroalcoholic extract of chamomile flowers, and optimum formulation F6 (results are presented as mean ± SD (n = 3))

Sample	Henna leaves Ex	Chamomile flowers Ex	Optimum formulation
Total phenol content (mg GA/g Ex)	57.8±1.2	181.08±2.57	202.75±3.78

Results are presented as mean ± SD (n=3)

velocity dependent on the concentration. The Higuchi model describes the release of active ingredient from formulation by surface erosion. Figure 2 shows the analyzed release data using mathematical models for zero-order kinetic, first-order kinetic, and Higuchi kinetic. The best fit model was determined by the highest R^2 value, as shown in Table 5. The highest regression value was calculated for first-order model, indicating the drug release with diffusion controlled.[37]

Table 4: Correlation coefficient of different kinetic models for phenolic compounds release (n = 3)

Zero order	Higuchi	First order
Y=0.3407x+4.0311	Y=5.5799x–9.9828	Y=0.0029x+2.0176
R^2=0.9782	R^2=0.9587	R^2=0.9973

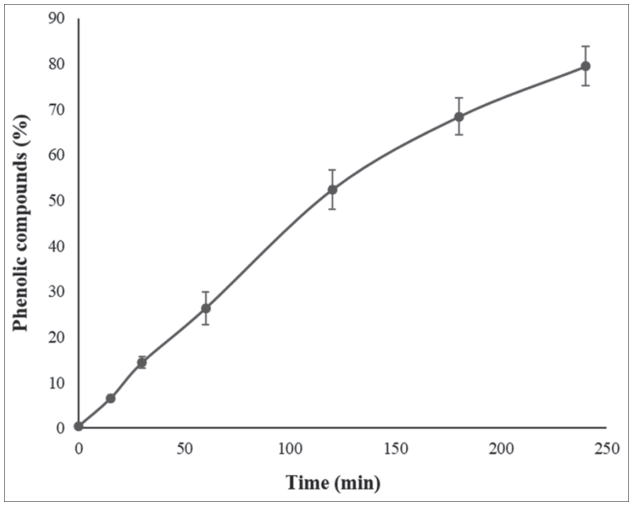

Figure 1: The percentage of cumulative release of phenolic compounds from the optimum formulation using Franz diffusion cell through a cellulose acetate membrane for 4 h at 37°C (results are presented as mean ± SD (n = 3))

Antibacterial activity

Maintaining antibacterial properties after formulating extracts plays a crucial role in burn infection treatment. Thus, the antibacterial properties of the prepared henna aqueous extract, chamomile hydroalcoholic extract, and optimum formulation against *P. aeruginosa* and *S. aureus* were evaluated in this part. Table 5 summarizes the results of inhibition zones of phenolic compounds in extracts and optimum formulation against two pathogenic bacteria under disk diffusion. Preservative (0.1%) and clindamycin disk were used as controls. Because clindamycin is ineffective against Gram-negative bacteria and only kills Gram-positive bacteria, it was used as both negative and positive controls in the test. The antibacterial activity of extracts is also presented in Figure 3. The MIC values of *S. aureus* and *P. aeruginosa* were 50 µg/mL (chamomile extract), 5 µg/mL (henna extract), and 1 µg/mL (optimum formulation). According to the obtained results, the phenolic compounds had higher effects on Gram-negative bacteria *P. aeruginosa* in comparison to Gram-positive bacteria *S. aureus*. Interestingly, fewer antibiotics are effective against *P. aeruginosa*.[38] This may be attributed to the thicker cell wall of Gram-positive bacterium causing more resistance to be attacked by polyphenol active groups than *P. aeruginosa*.[39] The antibacterial activities of the henna and chamomile leaf

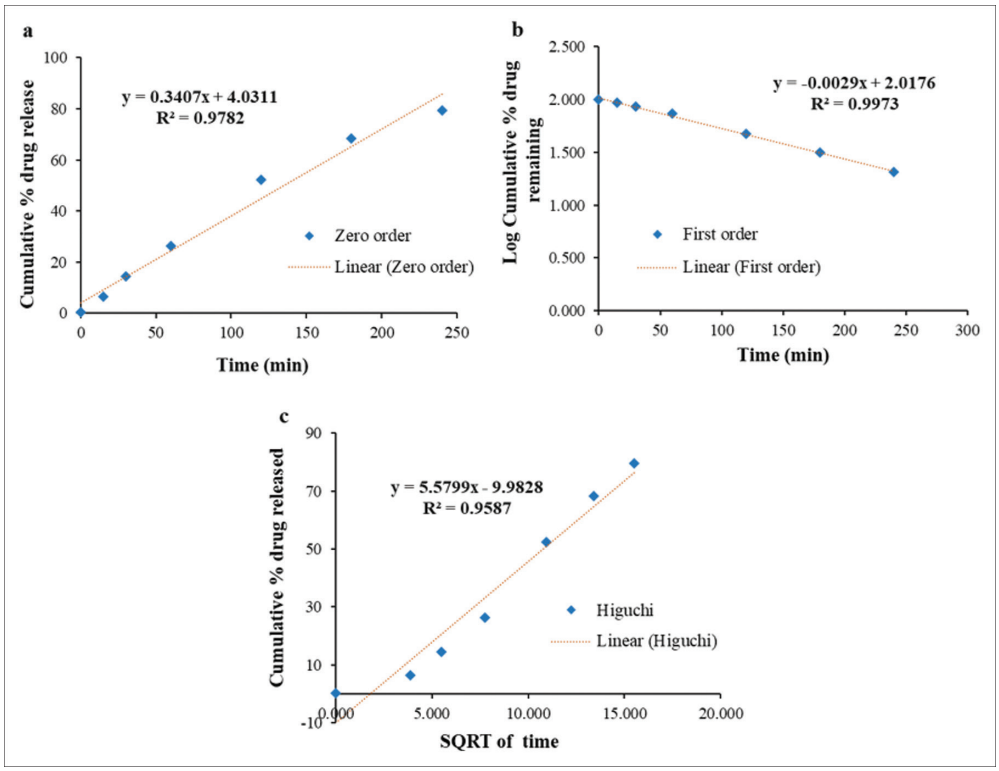

Figure 2: Release kinetic model for optimized formulation: (A) zero-order kinetic, (B) first-order kinetic, and (C) Higuchi kinetic

Table 5: Inhibition zone diameter of henna Ex, chamomile Ex, and optimum formulation against *S. aureus* and *P. aeruginosa* (results are presented as mean ± SD (*n* = 3))

Test samples	Inhibition zone diameter (mm), mean ± SD	
	S. aureus	*P. aeruginosa*
Henna Ex (5%)	11.6±0.057	16.3±0.11
Chamomile Ex (5%)	14.3±0.057	22.6±0.057
Optimum formulation	25.1±0.15	36±0.36
Preservative 0.1%	17.3±0.15	19.6±0.057
Clindamycin disk	35.3±0.057	0

Results are presented as mean ± SD (*n*=3)

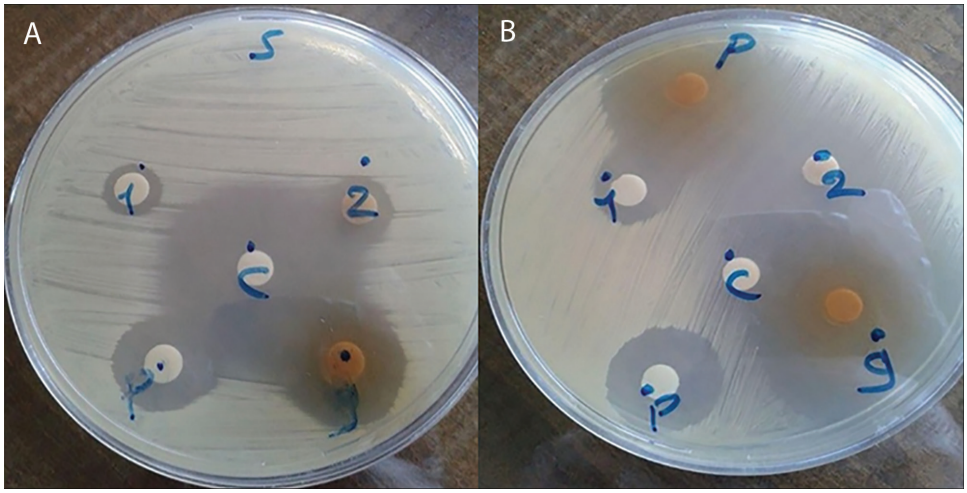

Figure 3: Antibacterial effects of phenolic compounds against (A) *S. aureus* and (B) *P. aeruginosa*. 1: henna Ex disk, 2: chamomile Ex disk, g: optimum formulation disk, P: preservative, C: clindamycin disk

extracts have been reported against both Gram-negative and Gram-positive bacteria.[12,40] It is worth mentioning that various conditions comprise extract concentration, type of the solvent, and environmental conditions of plant growth that could affect the antibacterial activities of extracts.[41] Antibacterial activity of formulation containing chamomile and henna extracts is to be caused by the synergistic activity of secondary metabolites such as phenols, flavonoids, terpenoids, quinones, and fatty acids, which is in good agreement with calculated total phenolic compounds. The probable antibacterial mechanism of chamomile is based on the abundant amount of active ingredients such as α-bisabolol and chamazulene, which gave dual bactericidal and bacteriostatic antibacterial action. Besides, it is assumed that sesquiterpenoid compounds of chamomile with the ability to interrupt cell wall permeability barrier and inhibition of cell membrane enzymes could induce antibacterial effects.[42] Similar to chamomile extract, henna is a rich source of polyphenol compounds such as lawsone, gallic acid, tannic acid, mucilage, and mannitol; the free hydroxyl group of these compounds with the capability to combine with the bacterial cell wall carbohydrates and proteins could attach to the sites of the enzyme, rendering them inactive.[43-45] The antibacterial test demonstrated the validity of this topical gel formulation and confirmed the findings in the past studies, which showed that these extracts were effective in the treatment of burn wound infections.

Conclusion

In this study, we prepared and evaluated aqueous-based gel topical dosage formulation for burn infection treatment. It was found that the phenolic compounds as effective ingredients of chamomile and henna extracts have a good potency against burning infection-inducing bacteria. Therefore, gel formulation is developed from the blend of gelling agent polymers (PG and CMC) and these extracts. Formulation exhibited good stability, viscosity, homogeneity, greater extrudability, and acceptable kinetics in the release of the phenolic compound. It is concluded that the topical gel possesses excellent antibacterial activity which is an essential attribute required for wound treating. Finally, the enhanced antibacterial activity of the formulation than primary extracts and its burst release for 4 h can be effective at the initial stage of the infection when Gram-positive bacteria are predominant at the wound site.

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Conflicts of interest

The authors declare no conflict of interest, financial, or otherwise.

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