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Design, formulation, and evaluation of a herbal gel contains melissa, sumac, licorice, rosemary, and geranium for treatment of recurrent labial herpes infections

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ABSTRACT

Background: The herpes simplex virus is a human pathogen which can cause skin or mucous membrane infections. Melissa, sumac, licorice, rosemary, and geranium have antimicrobial, antiviral, anti-inflammatory, and local analgesic effect. Shortening recovery period of recurrent herpes labialis and control of viral protein formation are the other effects of these herbs. The aim of this study is design, formulation, and evaluation of the gel containing extracts of these five herbs.

Materials and Methods: In this experimental study after photochemical and macroscopic evaluation of these medicinal herbs, the semisolid concentrated extracts were incorporated in gel bases. Mucoadhesive gels were prepared using carbopol 940, sodium carboxymethylcellulose (Na CMC) and hydroxypropyl methylcellulose K4M as bioadhesive polymers. Physicochemical tests, viscosity, mucoadhesive strength measurement, and *in vitro* drug release study were carried out on formulations F_{10} (carbopol 940, 0.5% and Na CMC, 3%) and F_{11} (carbopol 940, 1% and Na CMC, 3%).

Results: Polyphenol content of extracts mixture was measured 210.8 ± 13.68 mg GAE/g. pH of formulations was 6.0 ± 0.2. 14 gel formulations were prepared. Physical appearance, homogeneity, and consistency of F_{10} and F_{11} were good. Mucoadhesion and viscosity of F_{11} was more than F_{10}. Study of release profiles in F_{10} and F_{11} formulations showed drug release from F_{11} was slower.

Conclusion: The best formulation for treatment and shortening recovery period of recurrent labial herpes infections should exhibit high value of mucoadhesion, show controlled release of drug. F_{11} with the highest viscosity and mucoadhesion and the lowest release rate was considered as the best formulation.

Key Words: Herpes labialis, licorice, melissa, rosemary, sumac

INTRODUCTION

Herpes simplex virus (HSV) is a double-stranded DNA virus which causes skin infections and has subtypes of HSV-I and HSV-II. Type I is associated with upper back infections but type II is associated with lower back infections in both sexes. HSV infections have two stages; primary infections in which the virus hides in a ganglion and secondary infections with disease recurrence. Local damages such as ultraviolet, abrasion, and fraction or systemic

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changes such as fever, fatigue, and menstruation lead to viral activation and return to skin surface through neurons and particular skin lesions such as macule, papule, pustule, and wound develop but ameliorate over 10–14 days.[1,2]

As a result of extensive studies over the past 2 decades, a large number of antiviral agents against HSV (II) were detected such as acyclovir, pancyclovir, valacyclovir, docosanol, and Famciclovir.[3] These drugs are virustatic and cytotoxic and used for recurrent herpes simplex labialis. Central nervous system complications, gingival hyperplasia, rash, acne, hives, kidney failure, changes in the menstrual cycle, phlebitis in the injection area, and joint pain are the side effects of these drugs which are usually seen in oral and parenteral dosage forms.[4,5]

In a study, acyclovir topical cream causes serious functional and superficial deformities in rat embryo in the first trimester of pregnancy.[6] The main problem with these drugs is drug resistance and is why topical acyclovir is outdated today for treatment of herpes simplex labialis.[1,2] Acyclovir accelerates healing of herpetic lesions but cannot prevent virus latent phase in sensory ganglia and frequency and severity of relapses.[7] Medications such as penciclovir and docosanol costs more than others.[4,8]

Above facts have led to the researches for discovery of new drugs, especially herbal-traditional medicines that are potentially less toxic and have less side effects and lower cost. Different herbs from worldwide have been identified an antiviral agents. We are going to mention some of these herbs for preparation of these formulations below:

Melissa officinalis L. (*Lamiaceae*) is used for the treatment of recurrent herpes labialis for years. Active compounds of this herb are polyphenols (Rosmarinic acid, protocatechuic acid, caffeic acid, chlorogenic acid, and hydroxycinnamic acid derivatives), flavonoids (luteolin glycoside, quercetin, apigenin, and kaempferol), essential oils 0.1%–0.2% containing aldehyde monoterpenes (citronellal 30%–40%), citral (20%–30%), and sesquiterpenes.[9,10]

Studies indicate that antiviral activity of this herb is due to occupation of viral receptors by polyphenol compounds. Accordingly, virus cannot adhere to cell membrane. This effect can shorten recovery period of recurrent herpes labialis.[3,11] However, citral and citronellal stop the production of viral proteins.[4] Essential oil of melissa has antimicrobial, antiviral, anti-inflammatory, decongestant, and spasmolytic effects.[9,10]

Glycyrrihiza glabra L. root has a triterpene named Glycyrrhizin which is 50–100 times sweeter than sugar[10] and this sweetener gives a pleasant taste to the product. Hydrolysis of glycyrrhizin produces glycyrrhizic acid which prevents the conversion of cortisol to cortisone in peripheral tissues and induces its anti-inflammatory effect.[9,10]

On the other hand, it contains large amounts of glabridin and hispaglabridins A, B, and isoflavones that act as estrogen-receptors agonists which induce local analgesic and anti-inflammatory effect.[12,13] Glycyrrhizin inhibits reproduction and growth of DNA and RNA viruses including HSV. Glycyrrhizin and glabridin generate nonreactive oxygen species and inhibit the activity of phospholipase A2 enzyme at the site of inflammation which leads to acceleration of healing of inflammation area.[13]

Rhus coriaria L. (*Anacardiacea*) or Sumac contains tannins (gallotannin, Gallic acid and…), flavonoids, bioflavonoids, resins, and essential oils.[14] The herb decreases gingival inflammation and viral proliferation and has positive effect on wound healing. There is amplification in antimicrobial effect due to abundant tannins and its astringent effect. Moreover, the astringency avoids secretions and helps for consistency and appearance.[3,15,16]

Geranium or *Pelargonium roseum* R. Br (*Geraniaceae*) has analgesic and anti-inflammatory effects due to compounds such as geraniol, citral, and citronellal.[17] In a study, the anti-infection of polyohenol extract and inhibition of virus proliferation and growth have been surveyed in the medium.[17,18]

Rosemary or *Rosmarinus officinalis* L. (*Lamiaceae*) has antiviral, antibacterial, anti-inflammatory, and antioxidant effects because of compounds such as rosmarinic acid. The herb's compounds heal the wound by increasing local blood flow. Local pain is one of the complications that patients always complain about recurrent herpes labialis that rosemary can be applied for this purpose. Rosemary is very aromatic and gives a graceful smell and taste to the product.[10-12]

Since herbal remedies are more accepted in world for their fewer side effects and lower costs and also there is no certain cure for recurrent herpes labialis, design of a proper formulation prepared from concentrated

extract of medicinal herbs can be beneficial to decrease herpes simplex symptoms and disease.

MATERIALS AND METHODS

This experimental study was supported by Isfahan University of Medical Sciences as a thesis research project numbered 393142.

Collection and identification of medicinal plants

Dry leaf of *Melissa officinalis* L., dry root of *Glycyrrihiza glabra* L., and dry fruit of *Rhus coriaria* L. were bought from medicinal plants market. Rosemary's shoots were collected and dried from medical herbal Garden of Isfahan University of medical sciences. Herbal species were identified and authenticated at pharmacognosy department of Isfahan school of pharmacy. Geranium essence was provided from Barij Essence Pharmaceutical Company (Isfahan, Iran).

Chemicals

Folin–ciocalteau's reagent, sodium bicarbonate, gallic acid, carbopol 940, sodium carboxymethylcellulose (Na-CMC), hydroxypropyl methylcellulose (HPMC K4M), and PEG 400 were purchased from Merck Company (Germany). Ethanol 96% from Ararat Company was used in this research. Potassium sorbate was prepared from Sigma-Aldrich Chemie Gmbh Company (USA).

Extraction

Rosemary, licorice, and sumac were extracted by percolation method. A total of 1500 g of each plant materials were wetted in 4 L of ethanol 70% for 2 h. Then, herbal components were percolated by 8 L of ethanol 70%. After 48 h, the extracts were collected and then concentrated by rotary evaporator (Heidolph VV 2000).[19,20]

Melissa leaves were extracted by infusion method. A total of 1500 g of ground powder of Melissa was wetted for 24 h and then was infused in 90°C purified water for 15 min and then in 70°C purified water for 30 min. Finally, aqueous extract was filtered by filter paper No. 2 and after collection, it was concentrated by rotary evaporator (Heidolph VV 2000).[12,19,20]

Determination of extract pH

pH of extracts were measured by calibrated digital pH meter (Metrohm 632, Swiss). Measurements were exactly after extraction then 1 week, 2 weeks, 1 month, 3 months, and 6 months after extraction. The measurements were repeated on every four samples three times and the results were reported.[20]

Polyphenol content quantification

Folin–ciocalteau colorimetric method is used for quantification of total phenolics. Polyphenols concentration was calculated by standard curve and the linear equation which was obtained lastly. Phenolic content was determined as gallic acid equivalent expressed by mg gallic acid per g of extract.[19,20]

For drawing calibration curve, 20 µl of each 50, 150, 250, and 500 mg/l concentrations of gallic acid stock solution and blank were diluted with 1.58 ml of purified water, and then, 100 µl of Folin–ciocalteau's reagent was added and mixed well. After 8 min, 300 µl of 20% sodium carbonate was added to solutions and put at room temperature for 2 h in dark place. This method was also repeated on the concentrated extract with concentration of 5 g/l. Finally, the absorbance of samples was measured at 765 nm by UV-VIS spectrophotometer (UV mini 1240, Shimadzu), and the standard curve was drawn. The results were reported in triplicate experiments.[19,20]

Preparation of gel formulation

We used concentrated extract of melissa, rosemary, sumac, licorice, and geranium essence for preparation of gel formulations. Carbopol 940, Na CMC, and HPMC K4M were applied as gelling agent in formulations. Among 14 formulations, 8 formulations had better properties compared to others, so they were selected for further tests.

Carbopol 940 gel

Potassium sorbate was dissolved in purified water 50°C. 0.5, 1, 1.5, and 2 g of carbopol 940 were dispersed in purified water 40°C by a mixer at 1200 rpm for 30 min.[19,20] Herbal extracts and essential oil were dispersed separately in PEG 400 and added to gel base and mixed well. The pH was then adjusted to pH, 6 using triethanolamine and stirred slowly until a clear and transparent gel was obtained.

Sodium carboxymethylcellulose gel

Potassium sorbate was dissolved in purified water 50°C. 1, 2, 3, 4, and 5 g of Na-CMC were dispersed in purified water 50°C by a mixer at 1200 rpm for 30 min. Herbal extracts and essential oil were dispersed separately in PEG 400 and added to gel base and mixed well.

Hydroxypropyl methylcellulose gel

Potassium sorbate was dissolved in purified water 50°C. 3, 4, and 5 g of HPMC K4M were dispersed in

the amounts of purified 60°C water by magnetic mixer at 1200 rpm for 30 min until prepared homogenous dispersion. Then, remaining amount of water was poured coldly and mixed well and kept at refrigerator for 24 h until homogenous gel was obtained (hot/cold technique). Herbal extracts and essential oil were dispersed separately in PEG 400 and added to gel base and mixed well.

Carbopol and sodium carboxymethylcellulose gel

First, potassium sorbate was dissolved in purified water 50°C. Then, specified amounts of carbopol 940 and Na-CMC were dispersed at purified water 40°C and mixed well. Herbal extracts and essential oil were dispersed separately in PEG 400 and added to gel base and mixed well.

Evaluation of physicochemical characteristics

Macroscopic study

Formulations were checked within 48 h of preparation and macroscopic balance **(**the absence of palpable and follicular particles, color, and transparency**)**.[20]

Microscopic study

Formulations were checked in terms of uniformity, gel texture, and air bubble by optical microscope with a magnification of 10 and 40 within 48 h.[19,20]

Centrifuge test

Formulations stability was investigated against gravity by centrifugal device (centrifuge 5430). Each formulation was centrifuged separately inside a tube with 10 cm in length and 1 cm in diameter for 5, 15, 30, and 60 min at 2000 rpm. Finally, each formulation was checked in terms of sedimentation.[19,20]

pH determination test

First, pH meter was calibrated with standard buffers (pH 4 and 7). pH of products was measured 48 h, 1 week, 2 weeks, 1 month, 3 months, and 6 months after preparation. The test was repeated three times.[20,21]

Determination of formulations viscosity

Brookfield DV-III viscometer was used for the determination of viscosity. At first, viscometer was calibrated by Brookfield Viscal Kit. Gel samples were placed at room temperature for 30 min. Then, they were poured in apparatus container. Number 74 spindle was attached then viscosity was determined at 25°C and 100–250 rpm. The results were reported in average after triplicate experiments.[19,20]

Thermal stress test

The test was done as primary stability studies. Packaged products were under thermal stress in aluminum-coated tubes. The samples were placed at oven at 30°C ± 2°C and relative humidity of 60% ± 5% for 6 months. Gel formulations were evaluated at the times of 24 h, 1 week, 1 month, 3 months, and 6 months.[19,20]

Thermal changes test

In this test, products were placed at refrigerator (2°C–8°C), room temperature (25°C), and oven (45°C–50°C) then the apparent quality of products was evaluated after 24 h, 1 week, 1 month, 3 months, and 6 months.[19,20]

Freeze and thaw test

Prepared tubes of each formulation were placed at 25°C for 48 h and at −8°C for 48 h for 6 consecutive periods. At the end of the period, the apparent quality of products was evaluated.[19,20]

Cooling and heating test

In this test, prepared tubes of each formulation were placed in 6 consecutive periods which include 48 h at 25°C and 48 h at 4°C. At the end of the period, the apparent quality of products was evaluated.[19,20]

Mucoadhesion test at ex vivo conditions

SANTAM (STM-1, Iran) apparatus which has 2 metal jaws was used. Two patches of cow's mucous were fixed on the jaws. Certain amount of gels (200 mg) was dispersed on this piece of mucosa after moisturizing the mucous membranes with purified water. After 2 min contact with mucosa, the upper jaw moved until detachment of gel and mucous membrane. The rate of detachment was 10 mm/min and the surface of fixed mucosa was 235 mm. The detachment/mucoadhesion force was measured in terms of MPa. Results of each sample were repeated three times.[19,20]

Quantification of total polyphenols in formulations

Forty-eight hours after preparation of formulations, 1 g of gel was dispersed at phosphate buffer, pH 6.8, and diluted to 10 ml in volumetric flask. Total polyphenols content was measured by Folin–ciocalteu's method using gallic acid standard curve.[20,22]

Determination of *In-vitro* drug release

In-vitro drug release was determined using Franz diffusion cell and synthetic membrane. 1 g of test

sample was dispersed uniformly on membrane surface; finally, it was fixed on cell. cell receiver phase contained phosphate buffer, pH 6.8. The temperature of 37°C was controlled by pumped water bath circulating between 2 shells encompassed the chamber.

Franz diffusion cell was placed at receiver phase space by a magnetic stirrer to obtain sink conditions. This set was also put on a magnetic mixer then the cell mouth was covered by parafilm to avoid evaporation from donor phase. A volume of 1 ml samples were taken at specified time intervals. After each sampling, the aliquots were replaced by fresh phosphate buffer, pH 6.8 subsequently to gain the same volume of receiver phase during the experiment. The test was repeated three times for each sample, and the absorbancies were measured by standard curve of apparent concentration after performing Folin–ciocalteau's method.[20,23] Apparent concentration is converted to actual concentration by equation below:

$C_n = C + (C_{n-1})\ V/V_t$

1. C_n: Actual concentration in sample *n*
2. C: Apparent concentration in sample *n*
3. C_{n-1}: Actual concentration in sample $n - 1$
4. V_t: Volume of receive phase
5. V: Sample volume.

Drug release kinetic studies of gel formulations

Data of drug release from F_5 and F_6 were determined in zero-order model, first-order model, and Higuchi model to investigate release mechanism.

In zero-order model (equation 1), total released polyphenols does not depend on primary polyphenol compounds and cumulative percentage graph of released polyphenols against time is linear. In first-order model (equation 2), log of remained drug against time is linear. In Higuchi model (equation 3), diagram of cumulative percentage graph of released polyphenols against square root of time is linear.

$Q_t = K_0\ t$ (equation 1).

$\text{In}\ Q_t = \text{In}\ Q_0 - K_1\ t$ (equation 2).

$Q_t = K_h\ t^{1/2}$ (equation 3).

In zero order and Higuchi equations, Q_t is the amount of released drug at specified time t, in first-order equation, Q_t is the remained drug at time t, and Q_0 is initial amount of drug in gel. K_0, K_1, and K_h are the constants of the equations.[24]

We used Korsmeyer- Peppas equation to determine the mechanism of drug diffusion. Equation formula is presented as following:

$$Log\left(M_t / M_\infty\right) = \text{Log}(K) + n\,\text{Log}(t)$$

M_∞ = The amount of drug released after infinite time.

M_t = Cumulative amount of drug released at any specified time (t).

K = Release rate constant.

n = Indicates the type of diffusion.

When n value is 0.5 or less, the Fickian diffusion phenomenon dominates, and *n* value between 0.5 and 1 is non-Fickian diffusion (anomalous transport). The mechanism of drug release follows case-II transport when the n value is 1 and for the values of n higher than 1, the release is characterized by super case-II transport. Non-Fickian drug release means that the drug is released from the gel through diffusion mechanism and also another process called chain relaxation.[25]

Each of the above tests was repeated three times for each formulation, then the best formulation was determined according to the analysis of statistical results.

Evaluation of gels test

Flavoring agents were tested using panel test by Latin-square method on selected formulation (F6). According to this method, 30 healthy volunteers were asked to apply the last formulations with no flavorant and final formulations which had lemon powder, orange, cherry, and peppermint on their lips. Then, they were asked to score their points of view as assigned numbers of 1–5 (excellent = 5, good = 4, fair = 3, poor = 2, very poor = 1).[26]

RESULTS

Determination of herbal extracts

Weight and percentage of concentrated herbal extract, percentage of dry matter in concentrated herbal extracts, and percentage of dry matter in total extract by freeze-drying method were measured after extraction, concentration, and removal of hydro-alcoholic extract [Table 1].

pH was measured at different times, and it was 6.1 ± 0.2. Polyphenol content was expressed by gallic acid standard curve ($Y = 0.1308x - 0.041$, $R^2 = 0.993$)

as 168.9 ± 13.53, 139.8 ± 16.42, 98.2 ± 13.82, and 102.38 ± 8.44 mg GAE per 1 g of dry extract for Sumac, licorice, rosemary and melissa extracts, respectively. Polyphenol content of the mixture of herbal extracts was obtained 210.8 ± 13.68 mg GAE per 1 g of dry extract.

About 2.5% of each concentrated extract of all herbs and 0.002% of geranium essence were added into gel base of carbopol 940, Na CMC, and HPMC by PEG 400 as a co-solvent [Table 2].

The results of the study of various gels

Consolidation of F_1, F_2, F_3, F_5, and F_6 was low and did not have the stability enough to remain on the mucous membrane. Uniformity and physical features of F_2, F_3, F_4, F_7, F_8, and F_9 were low. F_{12}, F_{13}, and F_{14} compatibility were not good due to the interactions of pH. Eventually, these formulations were excluded, and the tests were carried out on formulations F_{10} and F_{11}.

Physicochemical characteristics and stability of F_{10} and F_{11} were all appropriate at centrifuge test, thermal stress test, thermal changes test, and freeze and thaw test.

Table 3 shows the results of determination of pH, drug content, mucoadhesive strength measurement, and determination of viscosity carried out on F_{10} and F_{11}.

Drug content of F_{10} and F_{11} was obtained 71.31 ± 0.1 and 71.86 ± 0.3 mg GAE per 1 g of dry extract, respectively.

The results of mucoadhesive strength measurement by Santam apparatus have been shown in Table 3. Mucoadhesive strength of F_{11} was more than F_{10}. The viscosity of F_{10} and F_{11} was determined by a Brookfield DV-III Rheometer. The viscosity of F_{11} was 3 ± 0.2 N/mm^2. *In vitro* drug release was evaluated on F_{10} and F_{11} by Franz diffusion cell. The results have been shown in Figure 1 as cumulative percentage of drug release against time. This

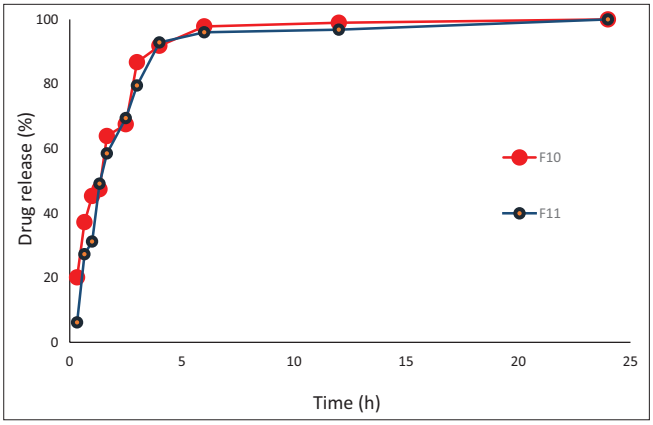

Figure 1: Percentage of cumulative drug release of formulations F10 and F11.

Table 1: Results of herbal extracts analysis

Herbal extract	Concentrated extract (g)	Concentrated extract (%)	Dry concentrated extract (%)	Dry extract by freeze drying method (%)	pH
Sumac extract	522.92±0.1	34.86	63.1	61.23	5.9±0.2
Licorice extract	481.95±0.1	32.13	45.5	43.56	6.0±0.2
Rosemary extract	395.9±0.2	28.29	53.8	55.8	5.9±0.2
Melissa extract	275.7±0.1	18.38	69.5	63.32	6.1±0.1

Table 2: Composition of gel formulations with different polymers (Carbopol 940, Na-CMC and HPMC K4M)

Ingredients (g)	Formulations													
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	F_{14}
Carbopol 940	0.5	1	1.5	2	-	-	-	-	-	0.5	1	-	-	-
Na-CMC	-	-	-	-	1	2	3	4	5	1	3	-	-	-
HPMC	-	-	-	-	-	-	-		-	-	-	3	4	5
Sumac extract	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Licorice extract	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Rosemary extract	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Melissa extract	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Geranium ess. oil	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
PEG 400	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Potassium sorbate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
TEA	qa	qs	qs	-	-	-	-	-	-	qs	qs	-	-	-
Purified water qs to	100	100	100	100	100	100	100	100	100	100	100	100	100	100

determination was based on gallic acid standard curve in phosphate buffer (Y = 0.1293x + 0.0121, R^2 = 0.999). Time needed for release of 50% of all drug content from F_{11} was almost 80 min, and for F_{10} was approximately 70 min, and after 24 h, 100% of drug content was released from both formulations [Figure 1]. For *in vitro* release kinetic study, the dissolution profile of F_{10} and F_{11} was fitted to zero-order, first-order, and Higuchi equations to determine the kinetic modeling of drug release. Release data of F_{10} and F_{11} showed R^2 value of 0.9682 and 0.9704 for first-order, respectively. For explanation of their kinetic, first-order kinetic model was suitable [Table 4]. To describe the mechanism of drug release from the gels, *in vitro* release data were fitted into Korsmeyer-Peppas equation. Drug diffusion for all formulations was non-Fickian type.

Table 3: Results of determination of pH, drug content, mucoadhesive strength and viscosity (at 100 rpm, 25°C) in formulations F_{10} and F_{11} (Mean±SD)

Physicochemical characteristics	F_{10}	F_{11}
pH 48 h after preparation	6.0±0.2	6.1±0.2
Drug content (mg GAE/g)	71.31±0.1	71.86±0.3
Mucoadhesive strength (N/mm^2)	5.2±0.1	8.2±0.1
Viscosity (Cps in 100 rpm)	1800±41	4440±37

Results of taste evaluation using panel test have been shown in Figure 2.

DISCUSSION

In recurrent herpes labialis, activated virus comes to the skin surface because of cutaneous trauma and systemic changes through peripheral neurons and creates skin lesions which recover in 7–10 days.[1,2] Antiviral compounds have many problems in the systemic use,[4,5] they increase drug resistance and have not much impact on incubation and recovery period. The aim of using herbs in this study is the treatment of recurrent herpes labialis with minimal complications and highest efficiency and lowest cost then reducing recovery period.

The polyphenol compounds of these herbs can inhibit viral replication or protein formation and reduce recovery period.[10,11] In addition, they have local anti-inflammatory and local analgesic, antiseptic, and anti-oxidant effects.[12,13,17] The double-ring terpenoids present in the plant accelerate wound healing by increasing local blood flow; on the other hand, the herb is highly aromatic which gives pleasant smell and taste to the product.[10,12]

Table 4: Drug release and drug release kinetics of gel formulations (F_{10} and F_{11})

Formulations	Kinetic of drug release								
	Cumulative drug release (%)	Zero- order model		First- order model		Higuchi model		Peppas parameters		
		K_0	R^2	K_1	R^2	K_h	R^2	*n*	K	R^2
F_{10}	100±2.1 (24 h)	0.3591	0.9074	0.0042	0.9682	6.471	0.9237	0.827	1.29	0.975
F_{11}	100±1.2 (24 h)	0.3939	0.9415	0.0046	0.9704	6.909	0.9082	0.875	0.863	0.966

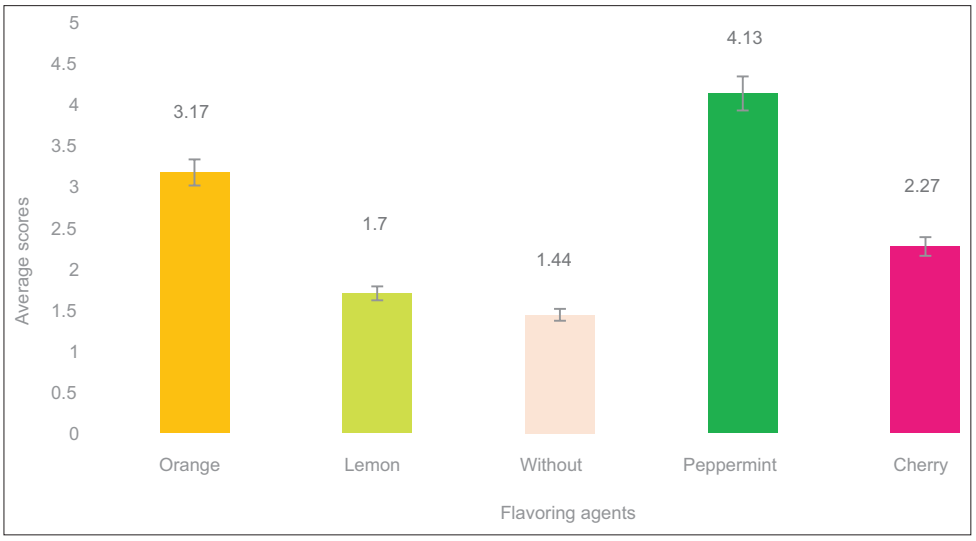

Figure 2: The results of taste evaluation.

Polyphenol content in hydroalcoholic extract of sumac, licorice, rosemary, and melissa was measured 168.9 ± 13.53, 139.8 ± 16.42, 98.2 ± 13.82, and 102.38 ± 8.44 mg GAE per 1 g of dry extract and 210.8 ± 13.68 mg GAE per 1 g of dry mixture of herbal extract according to the equation of standard curve (Y = 0.1308x– 0.041, R^2 = 0.993).

According to other studies conducted by Balouri *et al*., polyphenol content has been reported 181.41 ± 9.89 and 116.51 ± 9.19 mg GAE per 1 g of dry methanolic extract of rosemary and Melissa, respectively. In this study, polyphenol content of rosemary and melissa was reported 193.60 ± 8.48 and 195.11 ± 11.31 mg GAE per 1 g of dry extract, respectively, which was extracted by sonication method.[27] In studies by Cakmak *et al*., polyphenol content of shoots and root of *Glycyrrhiza echinata* L. was reported 146.30 ± 4.58 and 114.13 ± 3.22 mg GAE per 1 g of dry extract, respectively.[28]

According to the study by Al-Muwaly *et al*.,[29] phenolic content of aqueous, hydro-alcoholic and methanolic Sumac extracts was measured 136.67 ± 12.58, 222.56 ± 23.79, and 570.21 ± 82.20 mg GAE per 1 g, respectively. In another study by Kossah *et al*.,[30] polyphenol content of two species of Sumac, *Rhus coriaria* L (Syrian Sumac), and *Rhus typhina* L (Chinese Sumac) was measured 159.32 ± 12.31 and 150.68 ± 11.98 mg GAE per 1 g of dry extract, respectively.[30] Reasons of differences in the amount of these compounds are source of collection, weather, several plant species, extraction method, solvent type, temperature, and optimum duration of time. At high temperatures and longer times, polyphenol content decreases due to oxidation, polymerization, and transformation of polyphenol substances.[30] Polyphenol content of the mixture of herbal extracts did not follow algebraic sum because of interactions between extracts and increased probability of oxidation and polymerization.

Gels are better options for their high aqueous content, lower dermal irritations, less mechanical abrasion and more accepted appearance for using on and around lips compared to other topical drugs.[31] Therefore, in this study, it was decided to use hydrophilic polymers for preparation of hydrophilic gels.

In this study, an equal mixture of concentrated hydro-alcoholic extracts of Melissa, Sumac, Rosemary and licorice was added to hydrophilic gel in the amount of 2.5% and Geranium essence in the amount of 0.002%. A total of 14 formulations were prepared by gelling polymers such as carbopol 940, Na CMC, and HPMC K4M. F_{10} and F_{11} were selected because of acceptable macroscopic and microscopic characteristics, and other tests were performed on them.

pH of product should be close to the pH of local area to decrease local irritation. Lips and skin surface have pH 6.2. pH of selected formulations were in the range of pH of local area.[32]

Centrifuge test, thermal changes, cooling and heating test and freeze and thaw test concluded favorable results. During the tests, products were at stable conditions.

Viscosity of F_{11} was more. The increase in carbopol 940 amount caused increase of viscosity. Viscosity affects the release of drug from the gel, while viscosity increases the rate of drug release decreases.

Mucoadhesion results from Santam apparatus showed that mucoadhesion of F_{11} was measured 8.2 ± 0.1 N/mm^2 but mucoadhesion of F_{10} was obtained 5.2 ± 0.1 N/mm^2. Higher amount of carbopol 940 is responsible for higher mucoadhesion. Carbopol is a gelling agent with high-molecular weight and more hydrogen bonds. After swelling at water, it gets a thousand times lager then creates an adhesive surface. Carbopol 940 is a mucoadhesive gel which increases contact between the mucous membrane and the drug and boosts residents and effectiveness.[33]

In vitro drug release was studied on each of the formulations F_{10} and F_{11} using Franz diffusion cell. Drug release from F_{10} was slower than F_{11}. Time needed for 50% release of drug was 70 and 80 min for F_{10} and F_{11}, respectively. Drug release from F_{11} was more than F_{10} due to higher amounts of carbomer and higher viscosity. With increase in polymer amount, the gel becomes thicker, and water penetration is limited and results in reduction in drug release. Finally, persistence of gel on local area slows down drug release rate.[32] As noted, carbopol has the main role in the release of drug from gel due to high molecular weight and degree of crosslinking. Increase of cabopol raises mucoadhesion, viscosity, and decreases release rate.[20,33]

The kinetic parameters calculations of drug release from F_{10} and F_{11} have been shown in Table 4. The regression coefficient (R^2) of first-order kinetic for F_{10} and F_{11} was calculated 0.969 and 0.971, respectively,

which was higher than regression coefficients (R^2) of other models. As a result, the drug release kinetics follows the first-order model. This represents that the rate of drug release depends on time, and release rate is not constant. In fact, most of the drug is quickly released in the beginning, and then, the release rate is reduced until it is constant.[20,23] In this formulation "*n*" was >0.5, which indicates the mechanism of drug release follows non-Fickian or anomalous model. Non-Fickian release of drug means that diffusion method and relaxation of polymer chains are the mechanisms of release.[25]

Most of topical drugs for treating recurrent herpes labialis such as topical ointment of acyclovir are applied on lesions five times a day. Since more than 90% of drug is released after about 4 h from gel base, it's better to use the product five times a day on lesions according to Figure 1.

According to the results, F_{11} with the highest viscosity and mucoadhesion and the slowest rate of release is more appropriate than F_{10} for the treatment of recurrent herpes labialis. F_{11} with peppermint as flavoring agent received the highest score in panel test with Latin-square method. The next level, lemon flavoring agent, scored the most points.

We suggested effective mucoadhesive herbal gel to the pharmaceutical market considering helpful and efficient results for preventing, treating, and speed-up healing of recurrent herpes labialis.

CONCLUSION

Acyclovir and other-related compounds of this group while widespread use in the treatment of lesions, have no effects and also no anti-inflammatory, analgesic, and antihistaminic effects. However, in this study, rosemary and licorice are responsible for anti-inflammatory, local analgesic, repairing, and wound healing effects.

According to the results of evaluation tests such as, mucoadhesion, viscosity, and drug release profile, F_{11} containing Na CMC 3% and carbopol 940, 1% was selected for its acceptable mucoadhesion and viscosity. Study of drug release profile in F_{11} indicates herbal extracts can be released through a 24 h period.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, and financial or nonfinancial in this article.

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