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Original article

Formulation and Evaluation of a Topical Herbal Gel Containing *Rosmarinus Officinalis* for Anti-inflammatory Activity

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Abstract
From antiquated uses to contemporary pharmaceutical technologies, topical medication administration has undergone substantial change. It has long been known that products can have systemic or local effects when applied topically. The development of devices that can carry solutes across this barrier in a targeted manner and with measured accuracy at specific sites within the skin and beyond has been made easier by our growing understanding of the architecture and physiology of the skin. In contrast to diclofenac sodium, which showed an inhibition of 86.09% at 800 µg/mL, Rosmarinus officinalis ethanolic extract showed a concentration-dependent inhibition of protein denaturation, reaching a maximum inhibition of 71.08% at a dosage of 500 µg/mL. The topical gel formulations were prepared using a variety of gelling agents at different concentrations. Out of the six formulations that were made, F4 and F6 showed outstanding physicochemical characteristics, including ideal pH, good spreadability, appropriate viscosity, and no signs of skin irritation. The findings imply that F4 and F6 are the ideal polymeric bases for topical distribution and that rosemary extract may have a notable anti-inflammatory impact.
Keywords. Topical Gel, Rosmarinus Officinalis, Anti-Inflammatory, Inhibition of Albumin Denaturation, Skin Irritation.

Introduction

One of the most popular ways to deliver medications through the skin is through topical drug delivery devices. One of the easiest and most convenient places to administer medication is the skin. Chemicals are supposed to be able to enter and pass through the stratum corneum, which has long been considered the main barrier [1, 2]. Different pharmacological dose forms, including semisolids, liquids, sprays, and solid powders, are used in topical drug delivery systems. The most commonly used semisolid formulations for topical medication administration are gels, creams, and ointments [3]. Rosemary (Rosmarinus officinalis) belongs to the family Lamiaceae and was traditionally classified under the genus Rosmarinus L. In traditional medicine, rosemary has been used orally to treat dysmenorrhea, renal colic, and muscle spasms [4,5]. Rosemary has anti-inflammatory, anticancer, antithrombotic, antinociceptive, antidepressant, and antiulcerogenic qualities in addition to its antioxidant activities [6,7]. *R. officinalis* is used for several medicinal diseases, including respiratory, dermatological, neurological, circulatory, menstrual, hepatic, gastrointestinal, reproductive disorders, and genitourinary [4]. The ursolic, oleanolic, micromeric, and carnosic acids found in the rosemary extract are thought to be responsible for its anti-inflammatory qualities. It has been discovered that the bioactive compound carnosic acid inhibits nitric oxide, a pro-inflammatory mediator that initiates or exacerbates the inflammatory process [9].

There are several benefits when topical gels or formulations are contrasted with other traditional dosing forms. Topical gels are less hazardous and more effective than other dosage forms. Since topical gels are applied directly to the skin or the affected area, they are the ideal option for treating local infections and skin issues. Topical gels work directly at the place of action. Topical gels increase the drug's bioavailability by reducing gastrointestinal distress and pharmaceutical metabolism. Topical gels stop drug-drug and food-drug interactions. Due to their composition of two phases, gels exhibit a greater penetration power [10]. Topical gels are semi-solid, homogeneous solutions used to treat and cure skin diseases. The medicine or active ingredient was released quickly because gels are naturally hydrophilic. A gel is made up of two components: a liquid medium in sufficient amounts to form a rigid structure capable of immobilizing the liquid continuous phase and a three-dimensional, cross-linked substance. The structural network of the gel is made up of both organic macromolecules and inorganic particles.

Polymers that serve as gelling agents are those whose structural network or texture provides gels for the application of topical medications. From the extensive assortment of polymers with established mucoadhesive qualities, two were chosen for creating a mucoadhesive topical gel using hydroxypropyl methyl cellulose (HPMC) and Carbopol. Carbopol® provides a number of benefits when it comes to creating controlled-release delivery systems, including sufficient mucoadhesive qualities and gel-forming capabilities. Its ionic nature and high pH sensitivity may be the cause of this. Carbopol® showed a firmly packed matrix at pH 1.2, but at pH 6.8, all of its carboxyl groups disintegrate to form an expanding gel [11]. The substituent type in cellulose derivatives is widely acknowledged to result in differing properties among them. Cellulosic derivative-based polymers, like hydroxypropyl methylcellulose (HPMC), are known to have mild

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mucoadhesive qualities because they lack carboxyl groups that donate protons, which restricts the creation of hydrogen bonds [12]. The goal of this study is to create and evaluate a topical herbal gel that contains Rosmarinus officinalis extract for its anti-inflammatory properties. It will utilize Carbopol 934 and HPMC polymers, and will also evaluate the gel's physicochemical characteristics, conduct a skin irritation study, and perform a rheological analysis.

Figure 1. Rosmarinus officinalis plant

Materials And Methods

Materials

Fresh leaves of Rosemary (Rosmarinus officinalis L.) were gathered from Al-Jabal Al-Akhdar in Cyrenaica, Libya. Carbomer (Carbopol® 934P) Hydroxypropyl methylcellulose (HPMC K 4m). Propylene glycol (PG), Ethanol (96%). All utilized chemicals were of analytical grade.

Methods

Collection and Authentication

R. officinalis leaves were gathered from agricultural farms located in Al-Jabal Al-Akhdar, Libya. The gathered materials were cleaned, shade-dried at ambient temperature, and ground using an electric blender (Figure 2).

A B

Figure 2. A. leaves of the rosemary plant, B. Powder of the rosemary plant

Extraction method

One hundred grams of dried leaf powder were put into a sealed container, and solvent (ethanol with a concentration of 95%) was added at a ratio of 1:5. It was allowed to sit for three days before being shaken on a G.F.L. shaker. The liquid component is decanted, and then it is filtered. After that, the extract was concentrated using a rotary evaporator for evaporation. In the end, the extract was dried. The final dry extract was stored at 4 °C in a dark bottle in the refrigerator until use [13].

Characterization of rosemary extract

Preliminary Phytochemical Investigation (Qualitative Analysis):
The ethanolic and aqueous extracts were screened for phytochemicals using standard methods to determine the presence of various phytochemical constituents [14].

Saponin test

Crude extracts of approximately 10 ml were shaken and heated until boiling. The development of a stable, persistent froth and a creamy mass of small bubbles signaled the presence of saponins.

Tannins test

Crude extracts (approximately 10 ml) were filtered after being boiled in a water bath. The filtrate was mixed with three drops of a 1% ferric chloride solution. The presence of tannins was indicated by the formation of a dark green solution.

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Flavonoids test
To 10 ml of crude extract, diluted HCl and NaOH were introduced. A yellow solution turned colorless, indicating the presence of flavonoids.

Terpenoids test
Approximately 10 mL of crude extract was combined with 2 mL of chloroform ($CHCl_3$) after being filtered. Three milliliters of concentrated sulfuric acid (H_2SO_4) were then carefully added to create a coating. Terpenoids were detected by the interface, developing a reddish-brown hue.

Alkaloid test
For two minutes, 2% H_2SO_4 was added to approximately 10 milliliters of crude extract. After filtering the mixture, a few drops of Dragendroff's reagent were added. Alkaloids were detected by the orange-red precipitate.

Inhibition of albumin denaturation
The albumin denaturation inhibition method was used to investigate the anti-inflammatory properties of rosemary (Rosmarinus officinalis). A small amount of 1 N HCL was used to alter the pH of the reaction mixture, which included the test extract and a 1% aqueous solution of albumin fraction. After 20 minutes of incubation at 37 ◦ C, the sample extract was heated to 51 ◦ C for an additional 20 minutes. Turbidity was determined at 660 nm after the samples had cooled. Three repeats of the experiment were conducted. The following method was used to calculate the % inhibition of protein denaturation:[15]
Percentage inhibition= (abs Control-Abs Sample) × 100/ Abs control

Preparation of topical gel
A topical gel was formulated by mixing precisely measured quantities of hydroxylpropyl methylcellulose (HPMC) and Carbopol 934P into distilled water that was being stirred vigorously until the two substances were fully dispersed. Polymer molecules were fully hydrated, and then a precisely measured amount of rosemary extract (1% w/w) was added to the polymer dispersion while stirring continuously to guarantee a uniform distribution. To create a uniform gel, Carbopol was neutralized with 1N NaOH while stirring continuously. The gel was kept in a container at 4 °C until it was used [16]. Every formulation was prepared three times.

Table 1. Composition of rosemary extract gel formulations

Formula code	Rosemary extract (%w/w)	CP (%w/w)	HPMC (%w/w)	PG (%v/v)	Distilled water (mL)	NaOH (mL)
F1	1%	1%	-	0.25%	100	q.s.
F2	1%	2%	-	0.25%	100	q.s
F3	1%	-	1%	0.25%	100	q.s
F4	1%	-	2%	0.25%	100	q.s
F5	1%	0.5%	0.5%	0.25%	100	q.s
F6	1%	1%	1%	0.25%	100	q.s

Characterization of topical gels
Physical appearance
The color, homogeneity, consistency, and phase separation of the rosemary-containing gel formulations were visually assessed [17].

Measurement of pH
The pH of the generated gel compositions was measured using a digital pH meter. One gram of gel was dissolved in ten millilitres of distilled water. Each formulation's pH was measured three times, and the average values were calculated [9].

Spreadability test
For the purpose of assessing the gel's spreadability, about one gram of it was positioned at the middle of a glass plate measuring 17 cm x 17 cm. Another glass plate of the same dimensions was placed over this one. A mass of 1000 g was gently placed on the upper surface of the upper plate in order to distribute the gel across the two plates. The weight was removed after a minute, and the spread area's diameter (in centimetres) was calculated [18].

Skin irritation test
This test was conducted on all the prepared formulations to demonstrate the gel's compatibility with the skin. It was tested on human volunteers to determine whether there is any irritancy issue that could render the gel unsuitable for use. Five human volunteers were selected to verify the skin irritancy test. Topically,

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one gram of the gel under investigation was applied to the hand, covering an area of almost 2 square inches. The five volunteers who took part in this test consented to their participation by signing an informed consent document. For approximately 24 hours, observations for redness, lesions, irritation, edema, and any indications of skin irritancy were carried out at regular intervals and documented [19].

Assessment of the viscosity

The gels' viscosity was measured using a Brookfield Digital viscometer (model DV2T, Brookfield Engineering Laboratories, INC., USA) featuring a cone and plate measuring system with spindle 52. The sample was positioned in the space between the plate and the cone, and this gap was progressively filled. The sample underwent dynamic shear rates of 10, 50, 100, and 200 rpm, and its viscosity was measured. All measurements were conducted under isothermal conditions at room temperature [20].

Analysis of statistics

Graph Pad Prism, Trial version 8.0.1, was used for statistical analysis. The information is displayed as mean ± standard deviation (SD). When determining the student's "t" test, the variation between each group's mean values and standard deviation was used. Statistical significance was defined as a P-value probability of less than 0.05.

Results

Screening for phytochemicals

Extracts were found to contain flavonoids, alkaloids, terpenoids, and tannin. No saponins were found in the ethanolic extracts of Rosmarinus officinalis (Table 2).

Table 2. Results of phytochemical screening

Chemical Component	Result
Tannins	+
Saponins	-
Flavonoids	+
Terpenoids	+
Alkaloids	+

Notes: + present; - Absent

In-vitro anti-inflammatory study

The present findings showed that an extract of Rosmarinus officinalis prevented the denaturation of the protein albumin in a concentration-dependent way at concentrations ranging from 100 to 500 µg/mL. As a reference drug, diclofenac sodium was utilized within a concentration range of 75 to 800 µg/mL, as shown in Table 3.

Table 3. Effect of albumin denaturation

Reference drug (µg/mL)	Absorbance (nm)	Inhibition %	leaves extract conc(µg/mL)	Absorbance (nm)	Inhibition %
Control	0.3905±0.02	-	control	0.3905±0.04	-
75	0.2342±0.01	*40*	*100*	0.2731±0.02	30.06
150	*0.1945*±0.02	50.19	*200*	0.2243±0.03	42.56
300	0.*1356*±0.01	*65.27*	*300*	0.1610±0.01	58.77
600	0.*1138*±0.01	*70.85*	*400*	0.1287±0.02	67.04
800	0.*0543*±0.02	*86.09*	*500*	0.1129±0.01	71.08

Formulations of topical gels

While formulating, two gelling agents were utilized at varying concentrations and proportions to create six distinct gel formulations. In this instance, two types of gelling agents were used: Carbopol (CP) and Hydroxypropyl methylcellulose (HPMC). The following two gelling agents were used: a. Carbopol 934 (at 1% and 2% concentrations), b. HPMC K4 m (1% and 2% concentrations), a mixture of 1% and 2% concentrations of Carbopol 934 and HPMC K4 m.

Characterizations of topical gels

Physical Appearance

As per the assessment of blank and loaded formulation clarity, consistency, and homogeneity of formulations F4 and F6, depicted in (Figure 3 and Table 4).

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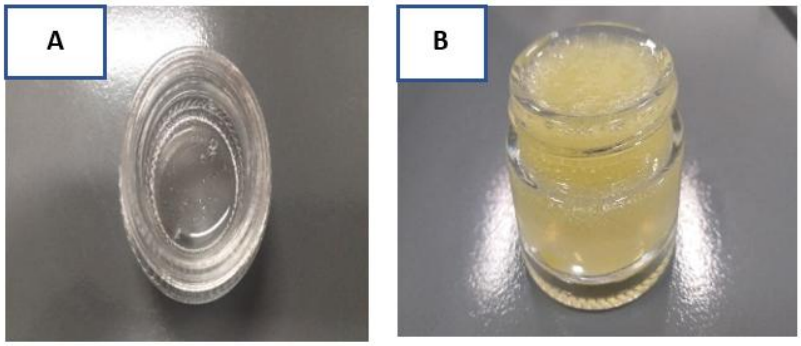

Figure 3. A. Blank of topical gel. B. Rosemary extract-loaded gel

Table 4. Evaluation of blank gel containing CP and HPMC

Parameter	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Transparency	Transparent	Opaque	Transparent	Transparent	Transparent	Transparent
Gel appearance	Thin	Thick	Thin	Good	Thin	Good
Gel consistency	NO	Smooth	Smooth	Smooth	Smooth	Smooth
Color	NO	NO	NO	NO	NO	NO
Odor	NO	NO	NO	NO	NO	NO
Phase separation	NO	NO	NO	NO	NO	NO
Type of smear Removal	NO	NO	NO	NO	NO	NO

Measurement of pH

The acceptable pH values ranged from 5 to 5.7 for the best formulations, F4 and F6. It can be found in (Table 5).

Spreadability

The spreadability of the F4and F6 was 7.50±0.01 and 7.51±0.05, as shown in (Table 5).

Table 5. pH and spreadability of selected topical gel compared with diclofenac gel

Formula	**pH value**	**Spreadability (cm)**
F4	5.15±0.1	7.50±0.01
F6	5.04±0.05	7.51±0.05
Diclofenac gel	5.33±0.2	7.6±0.01

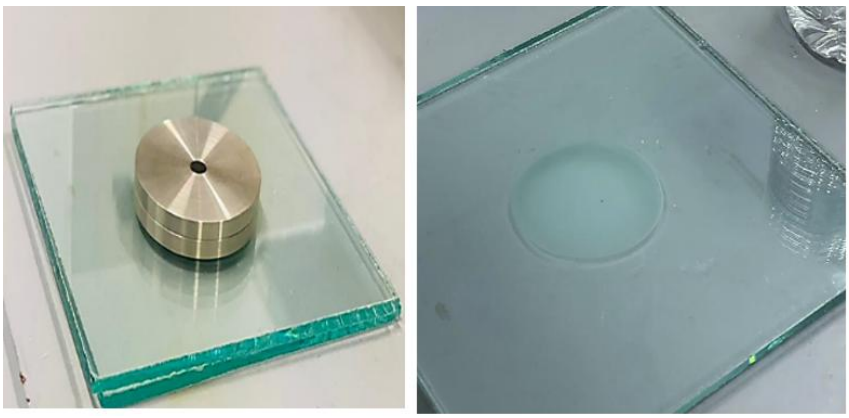

Figure 4. Spreadability of selected topical gel formulations

Skin irritation study

(Figure 5) illustrates that certain gel formulations (F4 and F6) were determined to be irritation-free.

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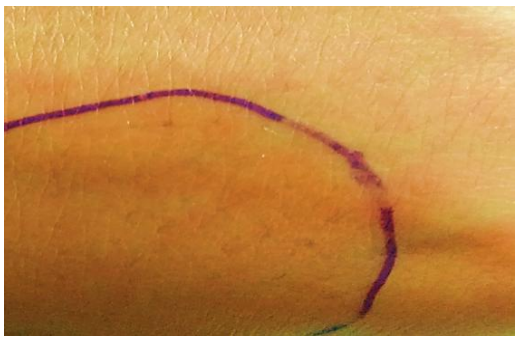

Figure 5. Application of rosemary gel on the skin

Rheological properties

Both F4 and F6 showed shear-thinning behavior (pseudoplastic flow), according to the viscosity investigation (Figure 6).

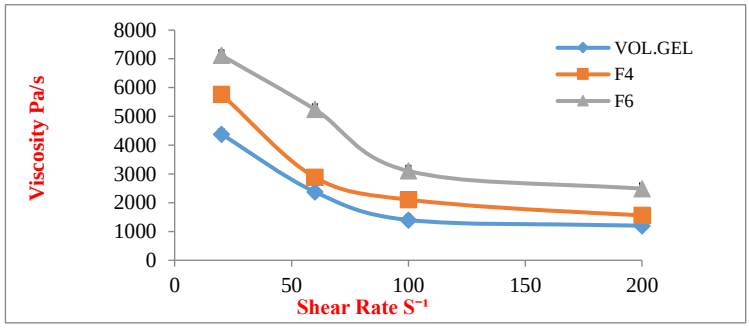

Figure 6. Apparent viscosity of HPMC 2% (F4), CP: HPPMC 2% (F6) topical gel, and Voltaren gel. Viscosity was measured at shear rates between 10 and 200 s²

Discussion

Phytochemical screening

The rosemary plant was extracted in this experiment using the cold immersion method in an organic solvent. On the other hand, the extraction procedures are as follows: Using a rotavapor system, rosemary leaves are dried, crushed, and extracted using organic solvents (95% ethanol). Ethanol is selected as the extraction solvent because its yield is the highest and its residual solvent is non-toxic [6]. Tannin, terpenoids, alkaloids, and flavonoids were found in the extracts; saponins were not found in the ethanolic extracts of Rosmarinus officinalis. Flavonoids, an organic compound soluble in water, play a variety of roles in plants, protecting them from the harmful effects of UV rays and parasites. Plant extracts and their phytoconstituents have been shown to have anti-inflammatory, antibacterial, and antioxidant qualities; flavonoids and tannins are important classes of chemicals that function as key antioxidants [21].

In-vitro Anti-inflammatory study

In this study, the anti-inflammatory activities of the herbal extract of Rosmarinus officinalis were evaluated in vitro using the protein (albumin) denaturation bioassay. Proteins lose their secondary and tertiary structures when exposed to an external stress or chemical, such as heat, a strong acid or base, a concentrated inorganic salt, or an organic solvent. Protein denaturation is the term for this process. Most biological proteins lose their biological function when they are denatured. One well-established cause of inflammation is albumin denaturation. As part of the investigation into the workings of the anti-inflammatory action, the extract's ability to prevent protein denaturation was investigated. The current study showed that Rosmarinus officinalis extracts inhibited protein (albumin) denaturation in a concentration-dependent manner over the 100–500µg/mL concentration range. Diclofenac sodium was employed as a reference medication and showed concentration-dependent reduction of protein denaturation at concentrations between 75 and 800 µg/mL. (Table 3) displays the anti-inflammatory properties of rosemary extract.

Experimental design of topical gels

Six distinct gel formulations were created during the formulation process using two gelling agents at various ratios and concentrations. In this case, Carbopol (CP) and Hydroxypropyl methylcellulose (HPMC) were used as these two kinds of gelling compounds were used. The following two gelling agents were employed: a. Carbopol 934 (1% and 2% concentrations), b. HPMC K4 m (1% and 2% concentrations), a combination of 1% and 2% concentrations of Carbopol 934 and HPMC K 4 m. Every formula was created in accordance with the design of the experiment. Polyethylene glycol PG is used as a plasticizer at a fixed concentration.

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To prevent brittleness and stickiness, PG was added to every formulation at a constant proportion of 2%w/w. PG was used as a plasticizer, according to earlier reports. According to reports, the intermolecular cohesive forces between polymer chains are decreased when a plasticizer is inserted between them and interacts with the functional groups of the polymers [22].

Characterizations of topical gels:

Physical Appearance

The best formulations gel (F4 and F6) demonstrated the most acceptable physical qualities based on the examination of blank formulation clarity, consistency, and homogeneity (Table 4). The extract-loaded gel was light green in color, but the blank gel was transparent (Figure 3).

Measurement of pH

The optimal formulations F4 and F6 have pH values between 5 and 5.7, which is regarded as satisfactory, to reduce the possibility of skin irritation because the pH of mature skin is 5.5 [22]. The optimal gel formulation's pH was measured and compared to that of diclofenac gel. It was located in (Table 5). This demonstrated that the skin was not irritated.

Spreadability

The mechanical properties of formulations meant for topical administration have a significant impact on product performance. The mixture should be easy to apply to the skin. The force needed to distort the sample during compression [23] is known as spreadability. A good gel exhibits great spreadability and requires less time to spread; comparable and satisfactory findings (Table 5) were obtained for both the diclofenac gel and the designed gel, suggesting ease of spreadability by modest shear (Figure 4). Regarding pH value and spreadability, the formulations did not differ statistically significantly ($P<0.05$).

Skin irritation study

Volunteers accept the gel formulation's lack of skin irritation. The in-vitro skin irritation test method was used to conduct the skin irritation test. There are several disadvantages to using animals in experimental pharmacological research, such as ethical conundrums and a lack of rationale when suitable alternatives are available. Therefore, in-vitro skin irritation methods were employed in this work to investigate the skin irritation of the optimized gel. It was discovered that certain gel formulations (F4 and F6) were irritation-free. These findings attest to the gels' good tolerance and suitability for topical use.

Rheological properties

The topical gel must have a sufficient viscosity in order to extend the residence period. Selected gel formulae (F4 and F6) have had their viscosities measured and compared to diclofenac gel. Measurements of viscosity were made using increasing shear rate values. Both F4 and F6 showed shear-thinning behavior (pseudoplastic flow) in the continuous shear rheology research, which is favored to promote the active ingredient's diffusion. The viscosities of both formulations increased more than dicolfenac gel at lower shear rates, but they dropped as the shear rate increased (see Figure 6, which shows viscosity vs shear rate). When stress was applied, they demonstrated the same level of application ease as dicolfenac gel. The previously established spreadability finding is validated by viscosity data. Both dicolfenac gel and herbal gel (F4 and F6) have rheograms that demonstrate shear-thinning behavior that is ideal for topical application [24].

Conclusion

The current study concludes that choosing the right polymers and medication is essential to creating a topical drug delivery system. According to physical compatibility investigations, certain polymers, like Carbopol 934 and HPMC K 4 m, were found to be compatible with the medication Rosmarinus officinalis. The gel's physical characteristics, pH, viscosity, and spreadability were found to be impacted by the different concentrations of the two polymers. Gel formulations made with Carbopol 934 and HPMC demonstrated anti-inflammatory efficacy, acceptable homogeneity, and no skin irritation. The formula of choice, however, turned out to be the HPMC2% and carbopol 934: HPMC2% based gel, which demonstrated the highest percentage of homogeneity, gel appearance, good spreadability, and rheological qualities. When compared to a commercial medication (diclofenac gel), the ethanolic extract of Rosmarinus officinalis demonstrated a notable anti-inflammatory effect.

Conflict of interest. Nil

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