

Original Research Article

Assessment of the activity of *Origanum syriacum* extract as an antibacterial agent in Carbopol-based gel

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Abstract

Purpose: To determine the most potent extract of *Origanum syriacum* and subsequently incorporate it in the development of a gel suitable for use as an antibacterial gel.

Methods: Aqueous and ethanol extracts were prepared by maceration of the dried plant powder. Phytochemical analysis and microbial assessment of the extracts were performed using standard methods, and the most potent (ethanol) extract was prepared into gels containing varying amounts of Carbopol, code-named F1 to F10. The gel's physical properties (homogeneity, pH, and viscosity) as well as its effect against *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 35984, and *Staphylococcus aureus* were characterized.

Results: Both extracts possessed similar phytonutrients. Tannins, carbohydrates, glycosides, phenols, resins, flavonoids, terpenoids, sterol and essential oils were present while saponins, anthraquinones and anthocyanins were absent in both extracts. At 100 and 50 %, the ethanol extract was more effective as it showed a higher diameter of inhibition against *S. epidermidis* (18 and 15 mm), *E. coli* (15 and 13 mm) and *S. aureus* (17 and 15 mm) compared to 10 µg gentamycin whose inhibition diameter was 18, 15 and 17 mm against *S. epidermidis*, *E. coli* and *S. aureus*, respectively. Furthermore, the pH of the developed formulations ranged from 6.71 to 7.1. Formula F7 was the optimized formula and resulted in the disappearance of viable bacterial colonies.

Conclusion: The ethanol extract of *O. syriacum* exhibits higher antimicrobial efficacy and produces a gel with suitable characteristics, including appearance, viscosity, and pH, which proves microbiologically effective in reducing bacterial infections. Therefore, *O. syriacum* may be incorporated into suitable pharmaceuticals to enhance its applicability.

Keywords: Marjoram, Antibacterial, Extraction, Gel formation, Assessment

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INTRODUCTION

Plant extracts are the principal source of antioxidants due to the existence of active components such as phenolic acids and flavonoids [1]. These phytochemicals scavenge free radicals and preserve cells from

deterioration by decreasing oxidative reactions. Plant extracts are widely employed as vital sources of compounds that possess various biological activities, including antioxidants, anti-inflammatory, antitumor, antiviral and antimicrobial activities [2]. The World Health Organization (WHO) has paid great attention to

the utilization of medicinal herbs for the purpose of caring for the general health of humans [3]. *Origanum syriacum* (Marjoram) belongs to the family Lamiaceae. It is distinguished by the existence of secretory trichomes retaining essential oils comprised of carvacrol and thymol. It is employed in traditional medicine as an antispasmodic and in the therapy of colds, coughs and toothaches [4].

The human skin is exposed to internal and external chemical and physical factors throughout life. These factors sometimes lead to cracks, wounds and destruction of the surface and internal layers of the skin, causing acute or chronic skin diseases [5]. Sometimes, microbes such as bacteria, fungi and viruses spread inside the body, causing localized diseases. Bacteria represent the majority of microbes that are endemic to the human skin for a transient or continuous period [6].

Previously, some studies have focused on the efficacy of different extracts of Marjoram against some pathogenic microorganisms [7]. The current study aimed to assess the activity of leaf extracts of *O. syriacum* before and after being incorporated into a gel to achieve the beneficial application of the extract in pharmaceuticals.

EXPERIMENTAL

Preparation of aqueous extract

Origanum syriacum powder was purchased from the local market in Baghdad, Iraq, in February 2025 and identified by the Botanical Experts at Al-Zahraa University for Women. The powder (50 g) was combined with 200 mL of water in a 1 L conical flask. The mixture was stirred continuously while incubating at 37 °C for 6 h. Thereafter, the solution was first filtered through several layers of a fine cloth (medical gauze) to get rid of coarse impurities, and further filtered using a Buechner funnel (pickling pressure) from the air. The filtrate was dried using a spray dryer and kept in the refrigerator at 2 °C. Extract (8 g) was stored in an opaque glass container, tightly closed until use.

Preparation of *O. syriacum* ethanolic extract

Origanum syriacum powder (50 g) was weighed into a beaker containing 200 mL of 70 % ethanol and placed in a vibrating incubator at 37 °C for 6 h. The solution was filtered using a two-step method involving several layers of fine cloth (medical gauze) to remove coarse impurities at first and subsequently through a filter paper. The filtrate was dried, employed a spray dryer and

stored in the refrigerator. The resulting extract (7 g) was stored in an opaque, tightly closed glass container until use.

Phytochemical analysis

Test for tannins, resins and saponins

The presence of tannins was determined using the method of Majhi and coworkers. Briefly, 400 mg of the extract was ultrasonicated with 5 mL of water for ¼ h at 70 °C. After cooling and filtering, drops of 0.1 % FeCl₃ were added. The presence of tannins was characterized by a blue-black or brownish-green coloring [8].

To test for resins, 400 mg of the extract was combined with 5 mL of water, sonicated for ¼ h, and then filtered. The filtrate's turbidity revealed resins [8].

In order to test for saponins, 400 mg of the extract was combined with 5 mL of water. The mixture was then ultrasonicated for ¼ h at 80 °C, filtered, cooled, and shaken until a stable, persistent froth formed, signifying the existence of saponins [9].

Test for phenols, flavonoids and quinones

To ascertain the presence of phenolic compounds, 400 mg of the extract was combined with 5 mL of 95 % ethanol, ultrasonicated for ¼ h, and thereafter filtered. Then, 2 mL of water and drops of 5 % FeCl₃ were combined with the filtrate. green color signified the existence of phenols [8].

The presence of flavonoids in the extracts was confirmed by combining 200 mg of the extract with 1 mL of a 2 % NaOH solution, followed by the addition of a few drops of diluted acid. This resulted in a yellow-colored fluid that was concentrated and the existence of flavonoids was then demonstrated by the addition of dilute acid that turned the solution colorless [10].

To determine the presence of quinones in the extracts, 5 mL of 95 % ethanol was combined with 400 mg of the extract and ultrasonicated for ¼ h. Following filtration, 1 mL of concentrated H₂SO₄ was added to 1 mL of filtrate. Presence of quinones was confirmed by the appearance of a red color [11].

Assay for steroids, glycosides and terpenoids

In order to check for steroids, 400 mg of the extract and 5 mL of 95 % ethanol were mixed

and ultrasonicated for ¼ h, then filtered and dried using evaporation. One milliliter each of glacial acetic acid and chloroform was added to dissolve a few milligrams of the dried extract, then combined with 1 mL of H₂SO₄ and added to the test tube's side. Presence of steroids was revealed by green color [10].

To check for glycosides, 5 mL of 95 % ethanol was combined with 400 mg of the extract and ultrasonicated for ¼ h at 30 °C, then filtered and dried by evaporation. One milliliter of glacial acetic acid, a few drops of 2 % FeCl₃, and 1 mL of H₂SO₄ were added to dissolve a few milligrams of the extract. Glycosides were recognized by the formation of a brown ring [10].

In the test for terpenoids, 400 mg of extract was combined with 5 mL of chloroform, then ultrasonicated for ¼ h. After filtering the mixture, 2 mL of H₂SO₄ was added to the test tube via the side. Terpenoids were detected by a reddish-brown [12].

Test for anthraquinones, anthocyanins and essential oils

Four milliliters (4 mL) of benzene were mixed with 400 mg of the plant extract to test for anthraquinones. After filtration, 10 % NH₃ solution was combined with the mixture. The appearance of a reddish-violet tint after shaking suggests the existence of anthraquinones [13]. To check for anthocyanins, 400 mg of the extract was ultrasonicated for ¼ h after 5 mL of 95 % ethanol was added. After heating 1 mL of the extract with 1 mL of sodium hydroxide (NaOH) for 5 minutes, the extract turned bluish-green, indicating the presence of anthocyanin [14]. Finally, to ascertain essential oils in the extracts, a solution containing 400 mg of the extract combined with 5 mL of ethanol (95 %v/v) was ultrasonicated for ¼ h. Subsequently, the filtrate was mixed with 0.1 mL of 1 M NaOH and a few drops of 1 M HCl. Appearance of a white precipitate confirmed the existence of essential oils [14].

Microbiological testing of the extract

The preserved bacterial strains *Escherichia coli* (ATCC 25922), *Staphylococcus epidermidis* (ATCC 35984) and *Staphylococcus aureus* (NCTC 8325) were activated using blood agar media. Mueller–Hinton agar was employed as media. The media quantity was sterilized using an autoclave at 121° C for ¼ h, then cooled, and then divided into petri dishes. Sterile filter paper discs 4 mm in diameter were

impregnated with 50 µl of each extract, except in negative control, which was impregnated with 50 µl ethanol and positive control with gentamycin disc and placed on inoculated agar surface and incubated in the basin at 37° C for a day [15].

Formulation of gel

The extract, which proved to be more effective from the results of microbiological tests, was incorporated into the gel. For the preparation of 100 g of gel, 0.1 g of triethanolamine was added to 60 mL of water containing preservatives (to bring the pH to between 6.8 and 7.5), and properly mixed. Preservatives used were paraben derivatives, namely methylparaben Sodium (sodium Nipagin M; Clariant®, Switzerland) and propyl paraben Sodium (Nipazol M sodium; Clariant®, Switzerland). To form a gel, different percentages of Carbopol 940 (Sigma Aldrich, USA) were added to the mixture and stirred continuously with a glass rod until the formation of a gel. Five grams (5 g) of *O. syriacum* extract was added while stirring, followed by gradual addition of polyethylene glycol with stirring till complete mixing was attained. The volume was made up to 100 g with water. Formulas are shown in Table 1.

Table 1: Formula of gel (per 100 g) containing 5 g of *O. syriacum* ethanol leaf extract (100 %) and 0.1 g triethanolamine, 0.2 g Nipagen sodium, and 0.1 g Nipazol sodium

Formula	Carbopol 940 (g)	Polyethylene glycol (g)
F 1	0.3	20
F2	0.6	20
F3	0.7	20
F4	0.8	20
F5	1.0	20
F6	1.1	20
F7	1.3	20
F8	1.4	20
F9	1.6	20
F10	1.7	20

Characterization of gel

Homogeneity

Gel formulations were tested for homogeneity and appearance using an established protocol [16].

pH analysis

The pH of the gel formulation was assessed employing a digital pH meter (Sartorius,

Germany). To determine the precise pH reading of the gel formulation, 5 g of the gel was dissolved in 100 mL of H₂O and the pH was read off the meter [16].

Viscosity

The gel's viscosity was determined with a Viscometer (Brookfield viscometer, USA), spindle 4, speed 20 rpm [16].

Microbiological assessment of the gel

Investigations were done to assess the effect of *O. syriacum* extracts (aqueous and ethanol) on *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 35984, and *Staphylococcus aureus* NCTC 8325, employing the disc diffusion method [15]. The reading time for the results was determined after 24 hours by measuring the inhibition zone diameters. Five participants were given similar instructions to follow the same process. A stable surface was chosen for the placement of the nutrient agar plate. For two seconds, participants were required to position all four fingertips, apart from the thumb, at an almost horizontal angle on the agar plate. The thumb was then placed on the same side of the plate, taking up the space in the centre. Then, the second hand's fingertips were similarly arranged on the other side of the plate. The steps above were taken both before and after applying the gel. The established procedure was approved by the Ethics Committee according to the Council for International Organizations of Medical Sciences (CIOMS) international ethical guidelines for health-related studies involving humans and approval was obtained from the Ethical Committee (approval no. PH0250511).

Statistics analysis

The significance was assessed by t-tests. A *p*-value < 0.05 was assumed as statistically significant.

RESULTS

Phytochemical analysis of the extracts

Table 2 revealed the findings of the detection of active components in the aqueous and ethanol extracts. The result shows the presence of tannins, carbohydrates, glycosides, phenols, resins, flavonoids, terpenoids, sterol and essential oils, while saponins, anthraquinones, and anthocyanins are absent in both extracts. Both extracts possess similar phytonutrients.

Table 2: Chemical qualitative tests of *O. syriacum* extracts

Test for	Aqueous extract	Ethanol extract
Tannins	+	+
Carbohydrate	+	+
Glycosides	+	+
Phenols	+	+
Resins	+	+
Flavonoids	+	+
Saponins	-	-
Terpenoids	+	+
Sterols and steroids	+	+
Anthraquinones	-	-
Anthocyanins	-	-
Essential oils	+	+

(-): absent; (+): present.

Antimicrobial screening of *O. syriacum* extracts

The results of the antimicrobial tests (Table 3) revealed that the ethanol extract of *O. syriacum* is more effective as it showed a diameter of inhibition of 18 and 15 mm at a concentration of 100 % and 50 %, respectively against *S. epidermidis*, while the aqueous extract, at the same concentrations, gave an inhibition diameter of 12 and 11 mm, towards the same bacteria when compared with the standard antibiotic (Gentamycin), which gave an inhibition diameter of 17 mm. A similar trend was observed for *E. coli*, resulting in an inhibition diameter of 15 mm and 13 mm at 100 % and 50 % concentration of ethanol extract, respectively, while a lower diameter of inhibition (12 mm) was only observed at 100 % concentration of aqueous extract against *E. coli*. In addition, inhibition diameters of 17 mm and 15 mm at 100 % and 50 % concentration of ethanol extract, respectively, were observed against *Staphylococcus aureus*. On the other hand, the aqueous extract gave an inhibition diameter of 20 and 17 mm compared to the standard antibiotic, which gave an inhibition diameter of 17 mm, indicating that the aqueous extract is more effective against *S. aureus* compared to the ethanol extract.

Gel formulation analysis

The results from Table 3 revealed that the ethanol extract has a higher efficacy; therefore, it was used to formulate the antibacterial gel. Table 4 presents the gel formulation results of the ethanol extract of *O. syriacum*. The pH of the developed formulations ranged from 6.71 to 7.1. Aside from that, however, the viscosities of formulations F1 to F6 ranged from 8000 to 23,000 cps, which was lower than what was required.

Table 3: Microbiological activity of *O. syriacum* extracts against selected bacteria

Bacteria	Inhibition Zone Diameter (mm)±SD*					Gentamycin (10 mcg)
	Aqueous extract		Ethanol extract		Negative control	
	50%	100%	50%	100%		
<i>E. coli</i> ATCC 25922	-	12±0.06	13±0.05	15±0.05	2±0.03	17±0.03
<i>S. epidermidis</i> ATCC 35984	11±0.10	12±0.04	16±0.10	18±0.07	2±0.05	17±0.02
<i>S. aureus</i> NCTC 8325	17±0.05	20±0.07	15±0.03	17±0.06	1±0.05	17±0.10

*n: 3, SD: standard deviation

In contrast, the viscosities of the formulations F8 to F10 were quite high. These formulations were used to make the sample. As shown in Table 4, the formulations F1 – F5 that were prepared with Carbopol 940, ranging from 0.3 to 1.2 grams, did not result in a suitable viscous gel formation that was suitable. On the other hand, the formulation F7 had positive features, such as a viscosity of 29,000 cps. Furthermore, the gels that were generated after that were made up of a significantly higher quantity of Carbopol and had a viscosity that was far higher than the requirements that were stated during the production process. Formula F7, composed of 5 g of extract, 1.3 g of Carbopol, polyethylene glycol (20 g), Nipagin M Sodium (0.2 g) and Nipasol M Sodium (0.1 g), was shown to be the optimized formula and further subjected to microbiological study, where no bacteria appeared after using the formulated gel.

Table 4: Physical characteristics of formulations

Formulation	pH (6-8)	Viscosity (centipoise) 25000-35000 cps
F1	6.85	8,000
F2	6.71	9,100
F3	6.78	10,000
F4	6.84	12,500
F5	6.72	18,000
F6	6.65	23,000
F7	6.86	29,000
F8	6.73	40,000
F9	6.91	44,000
F10	7.1	51,000

DISCUSSION

Origanum syriacum has flavonoids that are water-soluble ingredients that have a therapeutic impact that is attributed to medicinal plants. The glycosides kill germs, the resins have antiseptic action, and the tannins are useful in treating burns, wounds, and mucous membrane healing [17].

The biological activity examination against gram-positive *Staphylococcus epidermidis* revealed that the ethanol extract of *O. syriacum* at 100 % had a higher efficacy compared to the 50 % and higher than Gentamycin, as well as higher than the aqueous extract of marjoram, which affected

Dermatitis. This study is consistent with the findings of previous studies. Also, the efficiency of the ethanol extract in inhibiting bacterial growth is due to the presence of ethanol in the extract, which precipitates many biologically active compounds, including alkaloids, flavonoids, phenols, and tannins [17].

According to the current studies, *Origanum syriacum* comprises a variety of phytochemical components, which is in accord with previous investigations demonstrating that *O. syriacum* ethanol extract retains terpenoids, flavonoids, carotenoids, and phenols such as thymol and carvacrol. Consequently, it is beneficial to note that the ethanol extract may have more efficacy than a single bioactive constituent and enhance the bioavailability [18].

The antimicrobial usefulness of these oils is attributed to their hydrophobic nature that eases the penetration into the cell membrane of bacteria; it leads to a modification in the characteristics of the cell membrane, an increase of proton passive flux across, electron flow in the cell, active transport, and coagulation in the cell contents [17]. The delocalized electron system permits the hydroxyl group included in oils to release protons, causing antimicrobial activity. Furthermore, several investigations indicate the synergistic antibacterial impact of essential oils on bacteria [19]. Many studies on *O. syriacum* have been performed on essential oils, and few investigations have employed ethanol extract [18].

Ethanol extract was chosen for inclusion in gel due to its high antibacterial activity, which is consistent with the results of previous studies that used ethanol extracts of some plants in semi-solid preparations without altering their antimicrobial capacity [20,21]. Furthermore, the pH of the new formulation is within the range suitable for use on the skin, as reported by previous studies on herbal antibacterial gels [22].

The current study was the first trial to incorporate Syrian oregano into a gel as an antibacterial agent, and it demonstrated significant antibacterial activity. However, Badikova *et al* prepared an anti-cavity dental gel employing

Origanum vulgare. The antimicrobial activity of the synthesized gel was assessed using the disc diffusion method, and some formulations revealed significant antimicrobial activity [23].

CONCLUSION

This study shows that the ethanol extract of *O. syriacum* possesses a higher antimicrobial efficacy against selected bacterial species compared to the aqueous extract. Formula F7 produces a gel with appropriate characteristics regarding appearance, viscosity, and pH and proves microbiologically effective in reducing bacterial infection. Therefore, *O. syriacum* may be incorporated into suitable pharmaceuticals to enhance its applicability.

DECLARATIONS

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Conflict of interests

The authors declare no conflict of interest.

Contributions of authors

All authors reviewed and approved the final version of the manuscript.

Availability of data and materials

Data is available upon request.

Use of Artificial Intelligence/large language models

The authors confirm that no AI-generated content was employed.

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