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Advancing Wound Care: A Hydrocarbon Ointment Formulated with *Oregano vulgaris* for Antibacterial Action Against *Staphylococcus aureus*

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ABSTRACT

The challenge of wound healing is significantly compounded by the risk of infections, notably those caused by the pathogen, *Staphylococcus aureus*. Oregano (*Oregano vulgaris*) is a plant with strong antibacterial effects mostly due to its thymol and carvacrol components. There are attempts to employ this plant in novel ways. The present study was conducted to formulate and evaluate a hydrocarbon-based herbal ointment derived from oregano, as well as evaluate its antibacterial activity against *S.* aureus. Dried *Oregano vulgaris* leaves were extracted with 96% ethanol by maceration and oregano oil is obtained using the distillation method. The extract was further subjected to phytochemical screening. The ointment was formulated at 3, 6, and 9% concentrations. The formulated ointment was evaluated for physical properties and organoleptic characteristics, such as pH, homogeneity, spreadability, viscosity, and adhesion. The antibacterial activity against *S. aureus* at various concentrations (ranging from $1.5x10^5$ to $1.5x10^8$ CFU/ml) was also evaluated. Remarkably, the results revealed a positive correlation between the concentration of the herbal ointment and its antibacterial activity against *S. aureus*. The 3% *Oregano vulgaris* herbal ointment demonstrated ideal physical properties and antibacterial activity. The findings of the study suggest the effectiveness of oregano as a novel, natural therapeutic agent for treating skin wound infections caused by *S. aureus*. Future studies must seek clinical validation and formulation optimization to harness its full therapeutic benefit

Keywords: Antibacterial, characteristic, ointment, *Oregano vulgaris*, *Staphylococcus aureus*.

Introduction

A wound is a condition in which the continuity of one or more tissues in the body is impaired or the anatomical unit of the tissues is damaged by trauma.[1] The healing process is often accompanied by mild illnesses, such as pyoderma minor, and severe diseases, like necrotic infections due to bacteria, such as *Staphylococcus aureus*.[2] Gram-positive *Staphylococcus aureus* bacteria have a spherical, clustered, grape-like shape. *Staphylococcus aureus* is a pathogenic bacterium with a golden colony color. Skin, nose folds, armpits, and the perineum are all common places to find asymptomatic S. *aureus* colonies. Because *S. aureus* can grow on a variety of media and is tolerant of high salinity, it can be selected using selective media, such as mannitol salt agar (MSA).[3]
Oregano (*Oregano vulgaris*) is an herb of the *Lamiaceae* family that has many benefits, including antioxidant and antibacterial potentials. Its antioxidant and antibacterial activities are affected by thymol and carvacrol.[4] The antibacterial compound of *Oregano vulgaris* acts by inhibiting the growth of the bacterium *S. aureus.*[5] An ointment is a semisolid form for external use on the skin. Some ointments provide the therapeutic effect of the constituent compounds. Ointments without medicinal content are commonly used as protectors, emollients, or lubricants.

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The three primary functions of ointment as a topical medication are as follows: drug carrier, emollient, and skin layer protector.[6]
The present study aimed to formulate hydrocarbon-based *Oregano vulgaris* herbal ointments and *Oregano* essential oil and assess their antibacterial activity against *S. aureus*.

Materials and Methods

Materials

Preparation of the extract and essential oil

Dried *Oregano vulgaris* leaves (500 grams) were extracted using 96% ethanol in a ratio of dried leaves and solvent (1:2). The *Oregano vulgaris* leaf extract was evaporated using a rotary evaporator at a temperature of 30°C.[7] Essential oil was extracted by isolating oregano leaves using steam and water distillation apparatus for 3 hours. Subsequently, the obtained essential oil was stored at 4°C until it was utilized for analysis.

Formulation of ointments

Herbal ointments of *Oregano vulgaris* and essential oil were formulated with different formulations (Table 1). Vaseline was briefly melted using a water bath at 80°C and stirred until homogeneous. The melted ointment base was gradually added to *Oregano vulgare* extract or essential oil according to the formulation by Demilew *et al.*[8] Finally, the matrix was cooled to 20-25ºC.

Physical screening of herbal ointment

The characteristics of each ointment formula were determined by the organoleptic test, homogeneity, pH, viscosity, adhesion power, and spreading power. The organoleptic test was based on the shape, colour, and smell of the herbal ointment by visual observation. The homogeneity of herbal ointment was examined by observation of the ointment smears on the objective glasses. Homogeneity was evidenced

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by the absence of any non-uniform particles in the ointment. Viscosity and pH were measured using a device. Spreading tower and adhesion tests were performed by placing a sample on the object glasses and observing the sample structure on them. The spreading tower was measured transversely, longitudinally, and crosswise. Sample adhesion was determined based on the stickiness of the ointment.[9]

Antibacterial activity testing
The screening of the antimicrobial activity of oregano herbal ointment was evaluated with a minimum inhibitory concentration (MIC) test. *Staphylococcus aureus* inoculum was cultured in the MHA. The test was carried out for three treatments: Vaseline album (negative control), oregano extract, and oregano essential oil. Each test was conducted in triplicates. Each agar plate was perforated and then inoculated with *Staphylococcus aureus* in varying concentrations of $1.5x10^5$, $1.5x10^6$, $1.5x10^7$, and $1.5x10^8$ CFU/mL. Vaseline album, oregano extract, and essential oil (60 µL each) were added to the agar plate wells to determine the inhibition zone. The cultures were then incubated at 37°C for 18-24 hours.

Table 1: Herbal ointment preparation formula *Oregano vulgaris*.

Formulation	Ingredients		
	Vaseline album **(gr)**	**Extract (gr)**	**Essential oil (ml)**
T0	50	0	0
OV1	48,5	1,5	0
OV2	47	3	0
OV3	45,5	4,5	0
EO1	48,5	0	1,5
EO2	47	0	3
EO3	45,5	0	4.5

T0: Ointment based on hydrocarbon without extract content; OV1: Ointment extract of *Oregano vulgaris* (3%) on a hydrocarbon basis; OV2: Ointment extract of *Oregano vulgaris* (6%) on a hydrocarbon basis; OV3: Ointment extract of *Oregano vulgaris* (9%) on a hydrocarbon basis; EO1: Ointment essential oil of *Oregano vulgaris* (3%) on a hydrocarbon basis; EO2: Ointment essential oil of *Oregano vulgaris* (6%) on a hydrocarbon basis; EO3: Ointment essential oil of *Oregano vulgaris* (9%) on a hydrocarbon basis

Results and Discussion

Oregano vulgaris extract contains active compounds such as alkaloids, flavonoids, tannins, and saponins that have antibacterial properties. The results of this research are consistent with those conducted by Wulandari *et al*.,[10] which showed that saponins, alkaloids, and tannins in avocado leaves act as Gram-positive and Gram-negative antibacterials. The alkaloid inhibits the growth of recombinant *S. aureus* by disrupting the cell membrane of Gram-positive pathogenic bacteria.[11] In addition, *O. vulgaris* contains additional antimicrobial and antioxidant substances, such as polyphenol (which includes apigenin, asam rosmarinic, herbacetin, hispidulin, luteolin, naringin, and diosmetin).[12] The results demonstrated that the colour of the *O. vulgaris* extract ointment varied according to concentration, at 3, 6, and 9% (Figure 1A). The colour of the ointment color became more intense after more *O. vulgaris* extract was added to the mixture. Numerous factors can contribute to the variation in ointment colours. For example, as the extract concentration increases, the pigment density of *O. vulgaris* (such as melanin) increases as well, resulting in a darker or more intense colour.[13] Components in the extract may interact differently with the ointment base at various concentrations. These interactions can affect colour, especially if the extract compounds undergo oxidation or other chemical changes.[14] The solubility and dispersion of the extract in the ointment base may differ at various concentrations. Higher concentrations cause less even dispersion, changing the appearance of the colour.[15] Different extract concentrations may slightly change the pH of the ointment, resulting in a colour change, especially if pH-sensitive compounds are present in the extract.[16] Some compounds in the extract may be more stable at specific concentrations and may degrade or change at higher or lower concentrations, which may affect the colour of the ointment. As a comparison, ointment prepared from essential oil showed three identical colors, though the concentration for the formulation was different (Figure 1B). The smell of the herbs in the ointment was also different in each formulation.
As shown in Table 2, ointment formulated from essential oil has a weaker herbal odour compared to ointment formulated from *O. vulgaris* extract. All of the ointment formulations used in this study had a pH of between 4.5 and 6.5 (Table 3). This pH is normal for the skin.[17] The 9% *O. vulgaris* extract formulation produced the most acidic pH ointment (pH 4.54). Because they are not overly acidic, the ointment compositions in this study did not irritate or dry up the skin. Changes in pH play a role in the skin pathogenesis of diseases such as irritant contact dermatitis, atopic dermatitis, ichthyosis, acne vulgaris, and *Candida albicans* infections.[18]
According to SNI 16-4399-1996, prepared ointment formulations had an optimal viscosity, with typical viscosity values ranging between 2,000 and 50,000 (Table 3). This study found that the viscosity of the ointment formulated from *O. vulgaris* extract increased as the concentration increased. This is likely caused by high molecular-weight compounds in the extract, such as proteins, polysaccharides, or other biopolymers. These compounds can interact with each other and the ointment base, forming a more structured and less fluid network as the concentration increases. The results of the present study showed that the 96% ethanol extract had a higher viscosity than the essential oil of *Oregano vulgare*, as illustrated in Table 3. Extracts may contain components that bind water, contributing to increased viscosity. Conversely, the viscosity of ointment formulated from essential oil decreased as the concentration increased. Essential oils are generally complex mixtures of low molecular weight compounds,[19] most of which are hydrophobic,[20] and have a lower interaction capacity with the ointment base,[21] compared to the compounds in *O. vulgaris* extracts.

Figure 1: Organoleptic characteristics of herbal ointments from *Oregano vulgaris* hydrocarbon base. A: Extract ointment; B: Ointment essential oil.

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Table 2: Organoleptic characteristics of herbal ointments from *Oregano vulgaris* hydrocarbon base

Formulation	Parameter				
	Form	**Consistency**	**Color**	**Smell**	**Texture**
T0	Semisolid	Soft	White	Not smelly	Smooth
OV1	Semisolid	Soft	Light green	Weak herbs	Smooth
OV2	Semisolid	Soft	Green	Medium herbs	Smooth
OV3	Semisolid	Soft	Dark green	Strong herbs	Smooth
EO1	Semisolid	Soft	Yellowish white	Weak herbs	Smooth
EO2	Semisolid	Soft	Yellowish white	Weak herbs	Smooth
EO3	Semisolid	Soft	Yellowish white	Weak herbs	Smooth

T0: Ointment based on hydrocarbon without extract content; OV1: Ointment extract of *Oregano vulgaris* (3%) on a hydrocarbon basis; OV2: Ointment extract of *Oregano vulgaris* (6%) on a hydrocarbon basis; OV3: Ointment extract of *Oregano vulgaris* (9%) on a hydrocarbon basis; EO1: Ointment essential oil of *Oregano vulgaris* (3%) on a hydrocarbon basis; EO2: Ointment essential oil of *Oregano vulgaris* (6%) on a hydrocarbon basis; EO3: Ointment essential oil of *Oregano vulgaris* (9%) on a hydrocarbon basis.

Table 3: Physical and chemical Characteristics of herbal ointment prepared from *Oregano vulgaris* hydrocarbon base

Formulation	pH	Viscosity (cPas)	Spreadibility (mm)	Adhesion	Homogeneity
T0	6.11	1700	36	4’ 39”	Soft, no coarse particles
OV1	5.31	3100	41	2’ 04”	Inhomogeneous, no coarse particles
OV2	4.70	3300	44	2’ 56”	Inhomogeneous, no coarse particles
OV3	4.54	3500	47	3’ 17”	Inhomogeneous, no coarse particles
EO1	6.00	2700	42	2’ 46”	Homogeneous, no coarse particles
EO2	6.64	2600	44	2’40”	Homogeneous, no coarse particles
EO3	7.09	2500	46	2’30”	Homogeneous, no coarse particles

T0: Ointment based on hydrocarbon without extract content; OV1: Ointment extract of *Oregano vulgaris* (3%) on a hydrocarbon basis; OV2: Ointment extract of *Oregano vulgaris* (6%) on a hydrocarbon basis; OV3: Ointment extract of *Oregano vulgaris* (9%) on a hydrocarbon basis; EO1: Ointment essential oil of *Oregano vulgaris* (3%) on a hydrocarbon basis; EO2: Ointment essential oil of *Oregano vulgaris* (6%) on a hydrocarbon basis; EO3: Ointment essential oil of *Oregano vulgaris* (9%) on a hydrocarbon basis.

Table 4: Antibacterial activity of various concentrations of ointments from *Oregano vulgare* against *Staphylococcus aureus*

Treatment group	Inhibition zone (mm)			
	$1.5x10^8$ (CFU/ml)	**$1.5x10^7$ (CFU/ml)**	**$1.5x10^6$ (CFU/ml)**	**$1.5x10^5$ (CFU/ml)**
T0	0	0	0	0
OV1	0	0	0	6
OV2	0	0	8	11
OV3	0	3	11	13
EO1	3	4	5	18
EO2	5	9	7	23
EO3	7	29	33	40

T0: Vaseline album; OV1: Extract of *Oregano vulgaris* (3%); OV2: Extract of *Oregano vulgaris* (6%); OV3: Extract of *Oregano vulgaris* (9%); EO1: Essential oil of *Oregano vulgaris* (3%); EO2: Essential oil of *Oregano vulgaris* (6%); EO3: Essential oil of *Oregano vulgaris* (9%).

Therefore, adding more significant amounts of essential oil reduces the intermolecular interactions in the ointment base, decreasing viscosity. The lower the viscosity of a substance, the easier it flows. Ointments with a lower viscosity may be easier to apply to the skin, improving the spread and absorption of the active ingredients.[22] On the other hand, a decrease in viscosity can affect the stability and efficacy of the ointment formulation, including its ability to release the active ingredient.[23] Therefore, adding essential oil to the ointment formulation must be done by considering the balance between the antimicrobial and therapeutic properties of the oil.

As highlighted in Table 3, the ointment meets the standard of > 4 seconds in the adhesion test. The adhesiveness of the ointment from *O. vulgaris* extract differed from that of essential oil. The higher the concentration of the ointment, the more complex it is to penetrate and absorb through the skin. Penetration of active compounds into the skin is influenced by factors such as the rate of transfer of solutes, the material, the size of the treatment surface area, and skin permeability. Skin permeability can be affected by many factors, such as drying, moisturizing, or blocking effects in the formulation that might alter product release at the treatment site.[24] The results showed that ointments of *O. vulgaris* extract were inhomogeneous, while ointments of essential oil were homogeneous (Table 3). The current study evaluated the ointment based on adhesion, homogeneity, pH, viscosity, spreadability, and organoleptic properties. Based on the physical examination, the ointment formulation of *O. vulgaris* extract, at all concentrations, performed primarily better than the ointment formulation of essential oil.

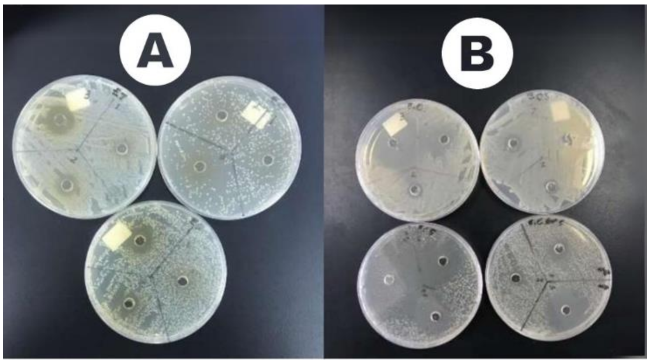

Figure 2: Antibacterial activity of *Oregano vulgaris* against *Staphylococcus aureus* ATCC 25923. A: Extract; B: Essential oil.

The results (Figure 2) obtained from the antimicrobial testing revealed that all ointment formulations showed inhibitory activity against *S. aureus*. The results showed that the control had no inhibitory zone, which indicated that the ointment had no antimicrobial activity against *S. aureus* (Table 4). However, both *O. vulgaris* extract and essential oil inhibited *S. aureus*. The outcome of the current investigation showed that, compared to essential oil, *O. vulgaris* extract can inhibit *S. aureus* growth at a lower concentration in a smaller zone. At concentrations of $5x10^5$ and $1.5x10^6$ CFU/mL, the 9% *O. vulgaris* extract demonstrated a reduction in the size of the inhibitory zone against *S. aureus*, with diameters of 8 and 11 mm, respectively. However, the 3% essential oil demonstrated a $1.5x10^8$ CFU/mL inhibitory zone against *S. aureus*. The 9% essential oil showed diameters of inhibition zones of 7, 29, 33, and 40 mm at concentrations of $1.5x10^8$, $1.5x10^7$, $1.5x10^6$, and $1.5x10^5$ CFU/ml, respectively.

Oregano vulgare extract contains active compounds such as alkaloids, flavonoids, tannins, saponins, and polyphenols that are effective as antibacterials, offering a new alternative in antibiotic development. The variation in extract concentration affects the ointment's colour, viscosity, and homogeneity, providing important insights for better pharmaceutical formulations. The *O. vulgaris* extract demonstrated higher effectiveness in antibacterial activity and better physical characteristics of the ointment than essential oils. Ointments based on *O. vulgaris* extract are compatible with skin pH, showing potential for clinical use without irritation and paving the way for the development of safe topical therapies.

Conclusion

The findings of the present study revealed that the essential oil formulation containing 3% *Oregano vulgare* extract resulted in the most effective ointment. Compared to the herbal ointment formulated with *Oregano vulgare* extract, the essential oil obtained from the extract showed greater antibacterial activity against *Staphylococcus aureus* ATCC 25923. These findings highlight the potential of *Oregano vulgare* essential oil as a potent ingredient in developing antibacterial ointments.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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