

INNOVATIVE BIOCOMPOSITE ANTIFUNGAL HYDROGELS BASED ON HYDROXYETHYLCELLULOSE, KERATIN AND OREGANO ESSENTIAL OIL

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Abstract

*The increasing resistance to conventional antifungal therapies necessitates novel topical formulations. This study developed biocomposite hydrogels based on hydroxyethylcellulose (HEC), keratin, and oregano essential oil (OEO) from *Origanum syriacum* var. *bevanii*. Eight hydrogels (H1-H8) with varying HEC (3-3.5%), keratin (0-1.6%), and OEO (0-0.06%) were prepared. GC-MS identified thymol (24.44%), γ -terpinene (20.54%), *p*-cymene (15.45%), and carvacrol (12.65%). Rheological data were quantified using the Ostwald-de Waele model. The consistency index was strongly influenced by composition and temperature. A medium concentration of keratin and OEO represents the optimal formulation ratio, offering rheological behavior suitable for the intended therapeutic purpose. Antifungal activity evaluated against *Aspergillus brasiliensis*, *Fusarium oxysporum*, and *Phanerochaete chrysosporium* showed low OEO concentrations (0.003-0.006%) were ineffective. Hydrogels with 0.06% OEO (H7, H8) achieved complete suppression of *A. brasiliensis* and *P. chrysosporium* (score 0); H7 also fully inhibited *F. oxysporum*. A clear OEO concentration threshold above 0.006% is required for efficacy, with keratin acting as a functional encapsulant. These biocomposite hydrogels offer promising rheological and antifungal properties for further development.*

Key words: antifungal, biocomposite, hydrogels, keratin, oregano oil.

INTRODUCTION

Species of the genus *Trichophyton* are dermatophytes responsible for the development of pathogenic processes in keratinized tissues (skin, nail and hair). These fungi thrive by metabolizing keratin, using specialized enzymes (Moskaluk et al., 2022). Understanding dermatophyte pathogenesis in relation to keratin is crucial for the development of effective treatments and preventive strategies (Bitencourt et al., 2016). Although the availability of conventional therapy is generous (iodine, salicylic acid, chlorhexidine, griseofulvin, amphotericin B, thiabendazole, trichlorfon, enilconazole, terbinafine), there are

recommendations regarding the use of keratin-based products and other innovative treatments to enhance antifungal efficacy and overcome the problems of antifungal resistance to conventional therapy (Bangia et al., 2019; Jegathees et al., 2025; Shao et al., 2025).

The integration of nanotechnology into antifungal formulations, such as silver nanoparticles and eugenol-loaded nanocapsules, has shown potential in increasing the efficacy of treatment against resistant strains of *Trichophyton* (Marquez et al., 2025; Malakootikhah et al., 2025). Extracts from medicinal plants and their incorporation into nanomaterials have been explored for their antifungal properties, offering an alternative to

conventional synthetic drugs (Bezerra et al., 2024). Our recent clinical studies on patented antifungal gel based on collagen, clay and thymbra essential oils represent a first-line therapeutic alternative in the treatment of *Trichophyton* infection in calves, based on its superior clinical efficacy, lack of relapses and incredibly good safety (unpublished results). Starting from that promising results, new innovative formulation was developed based on cellulose, keratin, and essential oil. Oregano oil, derived from the plant *Origanum vulgare*, is known for its antifungal properties and has been explored as a natural treatment for various fungal infections, including those caused by *Trichophyton* species. Interest in oregano oil also stems from its potential to overcome resistance problems associated with conventional antifungal drugs (Wu et al., 2023; Waller et al., 2023; Dimitrijevic et al., 2025). Its efficacy is attributed to compounds such as carvacrol and thymol, which disrupt fungal cell membranes and inhibit essential enzymes, leading to cell death (Scandorieiro et al., 2024). Another novel approach to combat *Trichophytosis* is keratin-based treatment; the use of keratin-based biomaterials has shown promise in wound healing and drug delivery, offering potential benefits for the treatment of dermatophytosis. These materials can be engineered to release antifungal agents in a controlled manner, more effectively targeting infected areas (Costa et al., 2022; Sharma, 2022). *A. brasiliensis*, *F. oxysporum*, and *P. chrysosporium* were used as filamentous fungal models in a preliminary evaluation of the antifungal activity of the developed hydrogels, without being considered taxonomically, biologically, or clinically equivalent to species of the genus *Trichophyton*.

Biocomposite hydrogels offer a promising platform for the controlled release of antifungal agents, combining the mechanical properties of polymers with the biological activity of natural extracts (Hamed et al., 2024; Mederle et al., 2024).

The aim of this study is to develop and evaluate the antifungal activity of innovative hydrogels based on hydroxyethylcellulose (HEC), keratin and oregano essential oil. This is a preliminary research to develop an possible antifungal treatment.

MATERIALS AND METHODS

Obtaining of the origanum essential oil (OEO)

The botanical material used in this study was obtained from the Medicinal and Aromatic Plants collection garden of Hatay Mustafa Kemal University. After harvest, the leaves of *Origanum syriacum* L. var. *bevanii* (Holmes) Ietsw. were subjected to hydrodistillation using a Neo-Clevenger-type apparatus for 2 hours. Then oregano essential oil was obtained and characterized.

Preparation of hydrogels

The initial 6 hydrogels, coded H1 ÷ H6, based on hydroxyethyl cellulose (HEC), keratin and oregano essential oil were prepared according with composition presented in Table 1.

Table 1. Compositions of biocomposite hydrogels

Biocomposite codes	HEC, %	Keratin, %	Oregano oil, %
H1	3.5	0	0
H2	3.5	0.8	0.003
H3	3.5	1.6	0.006
H4	3	0	0
H5	3	0.8	0.003
H6	3	1.6	0.006

The HEC powder was gradually added to the amount of ultrapure water required for preparation, stirring continuously, until a viscous, slightly whitish hydrogel was formed, which became transparent within one hour, with HEC concentrations of 3 and 3.5%, respectively. Keratin (Merck, Darmstadt, Germany), in concentrations of 0.8 and 1.6% (w/v), oregano oil, in concentrations of 0.003% and 0.006% (v/v) were homogenized into the HEC hydrogels. Due to low efficiency of antifungal activity, two hydrogels were prepared consisting of 3.5% HEC, 1.6% keratin and 0.06% Oregano oil coded H7 and 3% HEC, 1.6% keratin and 0.06% Oregano oil coded H8. They were evaluated only by antifungal activity.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Oregano Oil

The volatile composition of the essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS) on a Thermo Scientific Focus system. Chromatographic separation was

conducted on a TR-5MS capillary column (60 m × 0.25 mm i.e., 0.25 µm film thickness) with helium as the carrier gas at a constant flow rate of 1.0 mL/min. The mass spectrometer was operated in electron impact (EI) mode at 70 eV, and data were acquired in full scan mode over the mass range of m/z 50-650. The following temperature settings were applied: injector port at 220°C, ion source at 220°C, and MS transfer line at 250°C. A 1 µL sample was injected in split mode (250:1 split ratio). The oven temperature was programmed to rise from 50°C to 220°C at a rate of 3°C/min. Compound identification was performed by comparing the obtained mass spectra with the Wiley mass spectral library. All data acquisition and processing were conducted using Xcalibur 2.1 software.

Rheological evaluation of hydrogels

The designed hydrogels, namely H1-H6, were maintained after preparation at a temperature of 23°C for 24 hours, and then their rheological properties were evaluated.

For the rheological analysis, a rotational viscosimeter RM100 PLUS (Lamy Rheology Instruments) was used, and the measurements were performed with a coaxial cylindrical system type MS-DIN11.

The rheological experiments were performed at two set temperatures. Firstly 23°C, corresponding to the conditioning temperature for semisolid formulations, and secondly, 37°C (physiological skin temperature). The temperature was kept constant throughout the measurements by using a Julabo Corio CD circulating water-bath. Due to the strong influence of temperature variations on the rheological characteristics, constant thermal conditions were maintained during the experiment. Also, to ensure thermal and mechanical equilibrium, each hydrogel was left unstirred for 10 minutes in the temperature controlled vessel before starting the analysis.

The analysis parameters were set using the RheoTex software. The hydrogels flow properties were evaluated by applying shear rates ranging from 0.4 to 78 s⁻¹, correlated with rotational speeds in the 0.3-60 rpm interval. This interval was selected because it best resembles conditions related to technological or biopharmaceutical operations, specifically

handling, application or administering (spreading on the skin), as well as the release of active ingredients (Ghica et al., 2016). For each applied shear rate, the viscosimeter allowed the recording of the corresponding shear stress value and dynamic viscosity.

Antifungal activity testing

Three reference fungal strains were used as models: *Aspergillus brasiliensis* (ATCC 16404), obtained from Sanimed, Romania; *Fusarium oxysporum* (ATCC 7808) and *Phanerochaete chrysosporium* (ATCC 24725), both obtained from DSMZ, Germany. All strains were maintained as pure cultures on Sabouraud Dextrose Agar (Scharlau, cat. no. 01-165-500), sterilized by autoclaving at 121°C for 15 minutes and poured into 90 mm aseptic Petri dishes under laminar airflow.

Biocomposite hydrogels were equilibrated at ambient temperature (approximately 23°C) for 4 hours prior to testing. Sterile filter paper discs (IDL GmbH, 125 mm diameter) were cut to 3 cm diameter and completely immersed in each gel formulation to achieve uniform impregnation. Antifungal activity was assessed by a disc diffusion method adapted for viscous gel matrices. For *A. brasiliensis*, 0.7 mL of inoculum prepared in sterile distilled water was pipetted and spread with a Drigalski spatula. For *F. oxysporum* and *P. chrysosporium*, plates were inoculated at four peripheral points using a sterile loop from stock cultures. Each gel-strain combination was tested duplicate, with two non-impregnated blank discs per plate as controls. Impregnated discs were applied aseptically and gently pressed onto the inoculated surface.

Plates were incubated at 28°C for 14 days. Antifungal activity was assessed by scoring fungal growth on the disc surface using a semi-quantitative scale: score 0 - no growth; score 1 - trace growth (<10%); score 2 - mild growth (10-30%); score 3 - moderate growth (30-60%); score 4 - heavy growth (60-100%).

RESULTS AND DISCUSSIONS

The essential oil composition of *Origanum syriacum* L. var. *bevanii* (Holmes) Ietsw was analyzed by GC/MS, and 30 compounds representing 96.25% of the total oil were identified as Figure 1 and Table 2 shows.

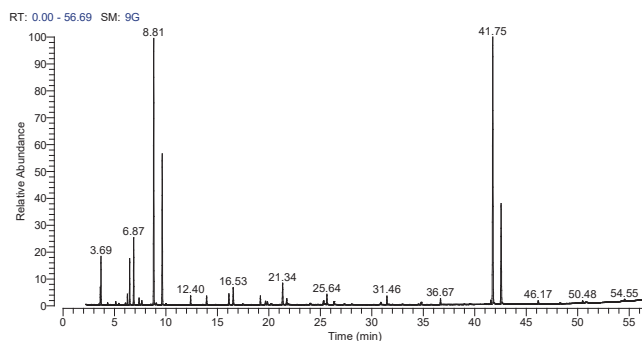


Figure 1. GC/MS chromatogram of the essential oil obtained from *Origanum syriacum* L. var. *bevanii* (Holmes) Ietsw

Table 2. Chemical composition of essential oil from *Origanum syriacum* L. var. *bevanii* (Holmes) Ietsw. identified by GC/MS

RT	Compound Name	Area, %
3.62	α -pinene	0.84
3.69	α -phellandrene	2.76
4.33	camphene	0.32
5.12	β -pinene	0.43
5.42	Sabinene	0.16
6.06	3-carene	0.24
6.48	β -myrcene	3.53
6.87	α -terpinene	4.65
7.39	Limonene	0.74
7.65	terpinen-4-ol	0.43
8.81	γ -terpinene	20.54
9.64	p-cymene	15.45
10.00	α -terpinolene	0.16
16.11	3-octanol	1.32
16.53	trans-sabinene hydrate	1.28
19.68	cis-sabinene hydrate	0.82
19.86	linalool	0.43
20.2	linalyl acetate	0.11
21.34	trans-caryophyllene	2.39
21.9	dihydrocarvone	0.21
24.01	humulene	0.28
25.48	germacrene d	0.18
26.33	β -bisabolene	0.50
27.33	δ -cadinene	0.12
30.88	thymyl acetate	0.33
34.81	caryophyllene oxide	0.43
39.5	spathulenol	0.29
40.98	α -cadinol	0.22
41.75	thymol	24.44
42.56	carvacrol	12.65

The major constituents were found to be thymol (24.44%), γ -terpinene (20.54%), p-cymene (15.45%), and carvacrol (12.65%). Other notable compounds included α -terpinene (4.65%), β -myrcene (3.53%), and α -phellandrene (2.76%).

The high percentage of thymol and carvacrol indicates that the oil belongs to the thymol/carvacrol chemotype, which is characteristic of the genus *Origanum*, particularly *O. syriacum*. The presence of high amounts of γ -terpinene and p-cymene is significant, as these are known biosynthetic precursors of thymol and carvacrol.

The relatively low levels of monoterpene alcohols (linalool, sabinene hydrate) and sesquiterpenes (caryophyllene, humulene, germacrene D) suggest that the oil is dominated by aromatic monoterpenoids. This composition is consistent with previously reported data for *Origanum syriacum* and supports its potential use as an antimicrobial and antioxidant agent due to the known bioactivity of thymol and carvacrol.

The rheological evaluation of topical semisolid formulations provides relevant information regarding consistency, which directly influences conditioning, handling, administering, spreadability and residence time at the application site. These are essential parameters in ensuring the improved therapeutical activity of the designed formulations, specifically the anti-fungal effect and regeneration of damaged skin.

The influence of the designed hydrogels composition, as well as of the temperature at which the rheological experiments were performed, is shown through the graphical representation of shear stress as a function of shear rate (Figures 2 and 3).

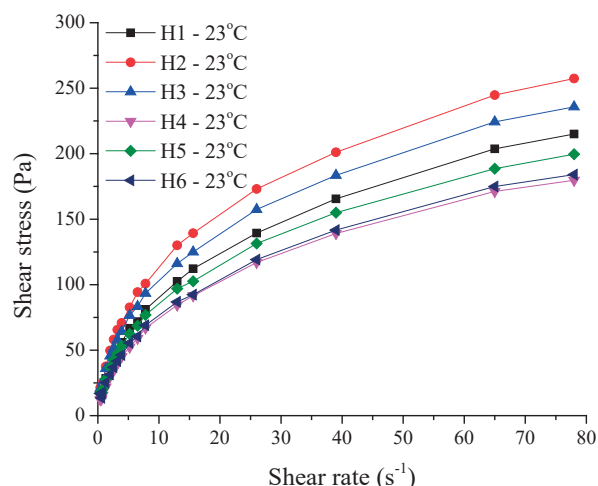


Figure 2. Cumulative rheological profiles of shear stress versus shear rate for hydrogels H1-H6 tested at 23°C

The Ostwald-de Waele model (Eq. 1) was applied to the experimental data:

$$\tau = K \cdot \dot{\gamma}^n \quad (1)$$

where: τ represents the shear stress (Pa); $\dot{\gamma}$ - shear rate (s^{-1}); K - consistency index ($Pa \cdot s^n$); n - flow behavior index (dimensionless) (Ghica et al., 2016).

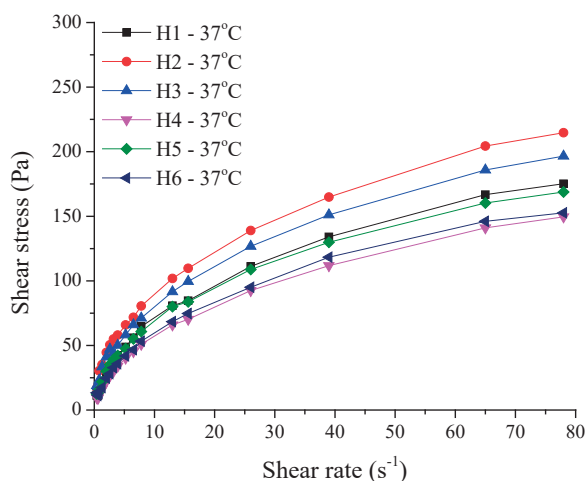


Figure 3. Cumulative rheological profiles of shear stress versus shear rate for hydrogels H1-H6 tested at 37°C

Table 3. Rheological parameters characteristic to Ostwald-de Waele model and the determination coefficient for hydrogels H1-H6 tested at 23 and 37°C

Code	Consistency index, K (Pa·s ⁿ)		Flow index, n		Determination coefficient, R ²	
	23°C	37°C	23°C	37°C	23°C	37°C
H1	30.04	21.92	0.45	0.49	0.9945	0.9942
H2	39.68	32.29	0.43	0.44	0.9928	0.9977
H3	35.96	28.62	0.43	0.45	0.9949	0.9975
H4	23.86	17.33	0.47	0.50	0.9938	0.9961
H5	29.18	22.68	0.44	0.47	0.9954	0.9959
H6	25.17	18.85	0.46	0.49	0.9954	0.9958

From Table 3, the values of the determination coefficient are higher than 0.99 (values between 0.9928 and 0.9977) which indicated that the experimental data obtained during the rheological characterization well fitted the Ostwald-de Waele model.

The values of the rheological parameters characteristic to Ostwald-de Waele model and of the determination coefficient R^2 for the tested formulations at both temperatures, 23 and 37°C, are shown in Table 3.

By analyzing the obtained values of the flow behavior index, n , it is observed that it is smaller than 1 (between 0.43 and 0.50) for all systems tested at both working temperatures, indicating a pseudoplastic non-Newtonian behavior, characterized by shear thinning. This behavior can be attributed to shear-induced orientation and progressive deformation of the gel molecular network along the direction of flow. As the shear rate increases, the effect becomes increasingly pronounced, leading to a reduction in internal friction because of a weakened

intermolecular interaction (Tudoroiu et al., 2023).

Pseudoplastic behavior is a critical quality attribute that is particularly relevant in the design of topical formulations, both in terms of packaging and application onto the skin surface, while also contributing to the formation of a uniform film. At high shear rates corresponding to the extrusion of the gel from the container, the formulation flows easily, facilitating administration. At low shear rates corresponding to spreading on the skin surface, the gel becomes more viscous, progressively recovering its original rheological properties (Ghica et al., 2016).

It can be observed that the incorporation of keratin and, to a lesser extent, oregano essential oil into the formulations does not significantly affect the degree of pseudoplasticity in either the 3 or 3.5% hydrogels, as reflected by the comparable values of the flow behavior index, n . Also, an increase in temperature does not produce significant changes in pseudoplastic character.

The consistency index values are influenced by both the working temperatures and the composition of the hydrogels. Specifically, increasing the temperature leads to a decrease in the consistency index, by factors ranging from 1.23 (H2) to 1.37 (H1 and H4).

For the 3.5% hydroxyethylcellulose formulations, the smallest decrease in consistency index is noticed for hydrogel H2 (containing medium levels of keratin and oregano oil), while the largest decrease was recorded for the hydrogel H1, formulated only with HEC. A similar trend is observed for the 3% HEC formulations.

Concerning the hydrogels composition, it was observed that while maintaining the concentration of keratin and oregano oil constant and varying the concentration of the hydroxyethylcellulose biopolymer from 3 to 3.5%, the consistency index increases by approximately 1.26 times (H1 versus H4) to 1.43 times (H3 versus H6) at 23°C, and 1.27 times (H1 versus H4) to 1.52 times (H3 versus H6) at 37°C.

Regarding the hydrogel composition, increasing the concentration of the biopolymer hydroxyethylcellulose from 3 to 3.5%, while maintaining the same concentrations of keratin

and essential oil, resulted in higher values of the consistency index. Specifically, the consistency index increased by approximately 1.26 (H1 versus H4) to 1.43 times (H3 versus H6) at 23°C, and by approximately 1.27 (H1 versus H4) to 1.52 times (H3 versus H6) at 37°C.

For the same hydroxyethylcellulose concentrations (3 or 3.5%), the addition of a medium quantity of keratin (0.8%) and oregano oil (0.003%) led to the maximum values of the consistency index, K, at both experimental temperatures (hydrogels H5 and, respectively H2). The increase is more pronounced in comparison with the formulations containing only HEC, as follows: at 23°C, the consistency index is 1.22 times higher than H4 and 1.32 times higher than H1, while at 37°C, 1.31 times higher than H4 and, respectively, 1.47 times higher than H1. Compared with the hydrogels containing a double amount of keratin and oregano oil (H3 and H6 respectively) the increase of the consistency index is less evident, with ratio values ranging between 1.10 and 1.16 at 23°C, and between 1.13 and 1.20 at 37°C.

Hydrogels containing double amounts of keratin and essential oil (H3 and H6) exhibit higher values of the consistency index compared to hydrogels based on HEC (H1 and H4, respectively), at both working temperatures. Thus, at 23°C, the consistency index of H3 is 1.20 times higher than that of H1, while that of H6 is 1.05 times higher than that of H4. At 37°C, the ratios are 1.31 relative to H1 and 1.09 relative to H4, respectively. The results highlight that the addition of keratin in a higher amount (1.6%) contributes to the strengthening of the gel network; however, the effect is less pronounced compared to the medium concentration of 0.8%. This behavior could be attributed to the disulfide bonds (–S–S–) present in cysteine from keratin and the –OH groups of hydroxyethylcellulose.

Antifungal activity of biocomposite hydrogels

The antifungal evaluation of the initial series (H1–H6) revealed limited efficacy at the OEO concentrations employed. Blank control discs confirmed full fungal colonization (score 4, 100% coverage) for all three strains, validating inoculum viability. Among impregnated formulations, only H5 (3% HEC, 0.8% keratin, 0.003% OEO) demonstrated partial inhibition

(score 3, 30-60% growth reduction) against *Fusarium oxysporum*. All other formulations, including H6 at the highest OEO concentration (0.006%), produced no measurable inhibition (score 4) against any of the tested strains (Table 4).

Table 4. Antifungal efficacy scores of formulations H1-H6 and optimized formulations H7-H8

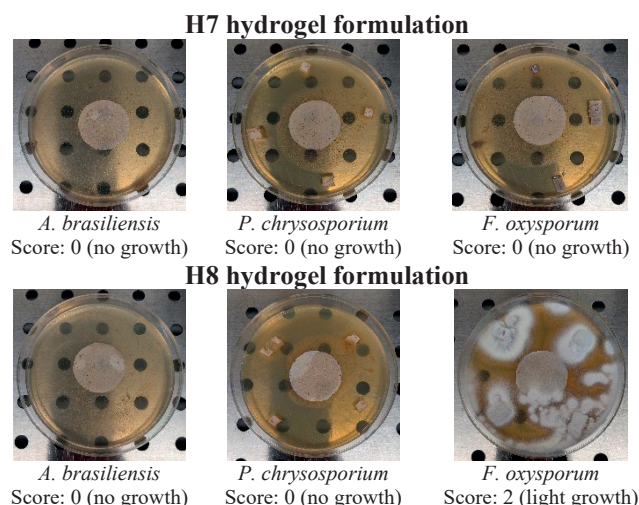
Formulation	<i>A. brasiliensis</i>	<i>F. oxysporum</i>	<i>P. chrysosporium</i>
H1	4	4	4
H2	4	4	4
H3	4	4	4
H4	4	4	4
H5	4	3	4
H6	4	4	4
H7 (+)	0	0	0
H8 (+)	0	2	0

The limited activity of the initial series is consistent with published minimum inhibitory concentration data for OEO, which generally indicate that effective concentrations against *Aspergillus* and *Fusarium* species exceed 0.01% (v/v) (Mezzomo et al., 2025). The partial inhibition observed for H5 against *F. oxysporum* suggests a synergistic contribution of the higher keratin concentration (1.6%), which may facilitate the controlled interfacial release of carvacrol and thymol through disulfide and non-covalent interactions within the polymer matrix (Saul et al., 2011).

The initial formulations (H1-H6), containing 0.003-0.006% OEO, showed limited antifungal efficacy, with only H5 achieving partial inhibition against *Fusarium oxysporum* (score 3), suggesting a synergistic effect between the higher keratin concentration and OEO at sub-threshold levels.

The optimized formulations H7 and H8, containing an approximately ten-fold higher OEO concentration, achieved complete fungal suppression (score 0) against *A. brasiliensis* and *P. chrysosporium*. Against *F. oxysporum*, H7 also achieved complete inhibition, whereas H8 showed mild residual growth (score 2). The incomplete inhibition of *F. oxysporum* by H8 reflects the intrinsic multidrug resistance of this species, attributed to constitutive efflux pump activity and a reinforced cell wall that restricts penetration of exogenous compounds (Al-Hatmi et al., 2017) (Table 5).

Table 5. Fungal growth on H7 and H8 formulations



The sterility of both H7 and H8 was confirmed on non-inoculated control plates, which showed no microbial growth, ruling out contamination as a confounding factor (Table 5). The results confirm a clear OEO concentration threshold for effective antifungal activity in HEC/keratin gel matrices and support the role of keratin as a functional encapsulant for the controlled delivery of phenolic terpenes. The formulations H7 and H8 will be further tested on *Trichophytosis*.

CONCLUSIONS

The innovative biocomposite hydrogels based on hydroxy-ethylcellulose, keratin and oregano essential oil were developed and tests by rheology and microbiology.

The main components of oregano oils were thymol (24.44%), γ -terpinene (20.54%), p-cymene (15.45%), and carvacrol (12.65%).

It can be appreciated that a medium concentration of keratin and oregano essential oil represents the optimal formulation ratio, capable of offering the hydrogels a rheological behavior suitable for the intended therapeutic purpose.

The antifungal evaluation of biocomposite hydrogels based on hydroxyethylcellulose, keratin and oregano essential oil demonstrated a clear concentration-dependent response.

The concentrations of oregano essential oil used in this study, 0.003% and 0.006%, respectively, seem insufficient to induce significant antifungal activity against all tested strains. The higher concentration of oregano oils (10 times)

proved to improve antifungal activity of the hydrogels.

The different resistance of fungal strains confirms the need to adapt antifungal formulations according to the biological particularities of the target fungal agent.

The applicability of studied hydrogels in current practice must also be evaluated from the perspective of treatment costs, the labor required for uniform application over the entire affected body surface, as well as the quantity of product required per animal, aspects that may influence the feasibility of its use in commercial farms.

ACKNOWLEDGEMENTS

This research was supported by the Romanian Government via the Ministry of Research and Education, through CCCDI-UEFISCDI, project number PN-IV-P8-8.3-PM-RO-TR-2024-0059.

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