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# Investigation of hydrogel materials enriched with plant and fungal extracts for potential biomedical applications

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## Abstract

This study presents the characterization of hydrogels enriched with natural extracts of *Withania somnifera* (ashwagandha) and *Lentinula edodes* (shiitake) with respect to their potential biomedical applications. The obtained materials were evaluated in terms of swelling capacity, stability in different incubation media (Ringer's solution, PBS, distilled water), antioxidant activity, as well as morphological and chemical changes after incubation. The results demonstrated that all hydrogels preserved their structural integrity over a 7-day period, while their properties depended on the type and ratio of incorporated extracts. Samples enriched with *Lentinula edodes* showed higher antioxidant activity but underwent more pronounced morphological changes, whereas hydrogels containing *Withania somnifera* were more structurally stable. FT-IR analysis confirmed the preservation of the polymeric backbone after incubation. Overall, the results indicate that the investigated hydrogels can serve as carriers of natural bioactive compounds, and their tunable composition offers promising potential for applications in tissue engineering and regenerative medicine.

**Keywords:** hydrogels, *Withania somnifera*, *Lentinula edodes*, antioxidant activity, biomaterials, incubation, stability

## 1. Introduction

Hydrogels are three-dimensional, crosslinked polymer networks capable of absorbing and retaining large amounts of water or biological fluids without losing their structural integrity. Their unique properties, such as softness, flexibility, high permeability to small molecules, and tunable chemical structure, make them highly suitable for biomedical applications (Ahmed, 2015; Hoffman, 2002; Peppas et al., 2000). They have been widely studied and applied as wound dressings, scaffolds for tissue engineering, controlled drug delivery systems, and biosensors (Caló & Khutoryanskiy, 2015; J. Li & Mooney, 2016; Xue et al., 2025). A key advantage of hydrogels in biomedical use is their stimuli--responsiveness. Hydrogels can respond to changes in pH, temperature, ionic strength, or enzymatic activity, allowing the design of "smart" therapeutic systems that adapt their behavior to in vivo conditions (Qiu & Park, 2001). This property enables controlled and sustained release of bioactive compounds, enhancing therapeutic efficacy while minimizing side effects (Alven & Aderibigbe, 2021; Liu et al., 2017).

In recent years, there has been growing interest in enriching hydrogels with natural bioactive compounds, such as plant and fungal extracts. These compounds, which include polyphenols, alkaloids, and polysaccharides, are known for their strong antioxidant, anti-inflammatory, and immunomodulatory activities (Borges et al., 2013; Choudhary et al., 2017; Gupta & Rana, 2007). Their incorporation into hydrogels serves a dual purpose: protecting tissues against oxidative stress and inflammation, and enhancing the intrinsic bioactivity of the hydrogel material itself (Kozarski et al., 2015).

*Withania somnifera* (ashwagandha), a medicinal plant with a long history of use in Ayurvedic medicine, has been extensively studied for its antioxidant, adaptogenic, and neuroprotective effects (Wasser, 2002). Similarly, *Lentinula edodes* (shiitake) mushrooms are rich in bioactive polysaccharides, particularly lentinan, as well as phenolic compounds, all of which contribute to their antioxidant, immunomodulatory, and anticancer properties (Vetvicka & Vetvickova, 2014; Zhu et al., 2015). The integration of such extracts into hydrogel matrices represents a promising approach for regenerative medicine and chronic disease therapy. Recent studies suggest that bioactive hydrogels combining natural compounds with synthetic or semi-synthetic matrices can enhance wound healing, improve tissue regeneration, and provide safer alternatives to conventional pharmacological treatments (Z. Li & Tan, 2014; Zhao et al., 2025).

In this study, we characterize hydrogels modified with plant- and fungus--derived extracts, focusing on their swelling capacity, mass stability in different incubation media, and preservation of antioxidant activity. Furthermore, surface morphology and chemical stability (FT-IR) analyses were performed to evaluate the structural integrity and functionality of the materials.

## 2. Materials and methods

### 2.1. Materials

Ashwagandha (*Withania somnifera*) extract was purchased from Olimp Labs (Poland) in the form of dietary supplement capsules (200 mg per capsule, including 10 mg of withanolides). Dried shiitake mushrooms (*Lentinula edodes*) were purchased from Green Pagoda (Poland, food-grade quality) and used as a natural source of fungal bioactive compounds.

Poly(vinyl alcohol) (PVA, Mw 13,000–23,000, 87–89% hydrolyzed), poly(ethylene glycol) diacrylate (PEGDA, average Mn 700, stabilized with 100 ppm MEHQ and 300 ppm BHT), and 2-hydroxy-2-methylpropiophenone


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(≥96.5% purity, HPLC, Mw = 164.20 g/mol) were supplied by Sigma-Aldrich (USA). All reagents were used as received without further purification.

## 2.2. Methods

### 2.2.1. Synthesis of hydrogel materials

Hydrogel systems were prepared using extracts obtained from *Withania somnifera* root (WS, ashwagandha) and *Lentinula edodes* fruiting bodies (LE, shiitake). The raw materials (Fig. 1) were subjected to hot-water extraction under continuous stirring on a magnetic stirrer with a heating function for 30 minutes, followed by centrifugation to remove insoluble fractions. The resulting clear extracts (WS, LE) were used as the aqueous phase in subsequent synthesis steps. Because the *Withania somnifera* source was a commercial capsule formulation, the presence of excipients such as microcrystalline cellulose, magnesium salts of fatty acids, and silicon dioxide cannot be fully excluded as a factor affecting extract composition; however, centrifugation removed insoluble fractions before UV crosslinking. This issue should be eliminated in future studies by using a purified standardized extract.

Next, poly(vinyl alcohol) (PVA) solutions at 5% (m/v) were prepared in WS and LE extracts. After complete dissolution of the polymer, the systems were designated as PVA–WS and PVA–LE, respectively.

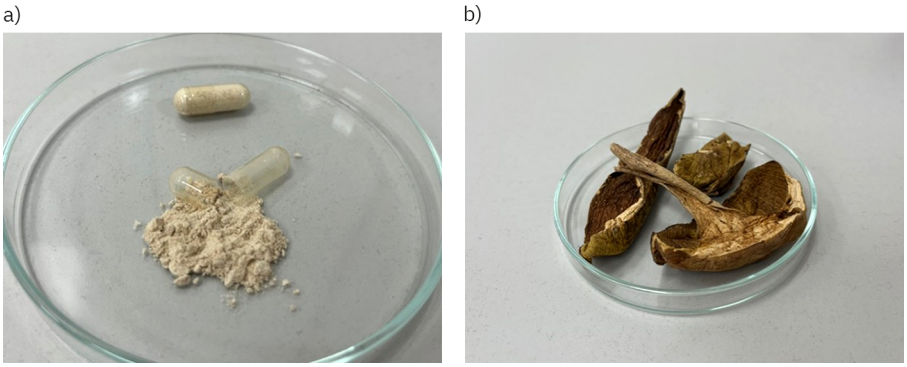


**Fig. 1.** Raw materials used for the synthesis of hydrogel systems: (a) powdered ashwagandha root (*Withania somnifera*), (b) dried shiitake fruiting bodies (*Lentinula edodes*); source: own elaborations

For crosslinking, poly(ethylene glycol) diacrylate (PEGDA 700) was used, while 2-hydroxy-2-methylpropiophenone served as the photoinitiator. Hydrogel compositions were obtained by mixing PVA–WS and PVA–LE solutions in various ratios, as summarized in Table 1, followed by the addition of PEGDA and the photoinitiator. The mixtures were then exposed to UV irradiation, which enabled the formation of stable, crosslinked hydrogel networks. The resulting hydrogel systems (samples 1–7) differed in the relative contribution of plant- and fungus-derived extracts, allowing the evaluation of how the type and ratio of bioactive components influenced the final material properties.

**Table 1.** Composition of hydrogel samples containing *Withania somnifera* (WS) and *Lentinula edodes* (LE) extracts; source: own elaborations

| Sample number | PVA – WS, mL | PVA – LE, mL | Photoinitiator, µL | PEGDA, mL |
|---|---|---|---|---|
| **1** | 10 | – | 100 | 2 |
| **2** | 9 | 1 | | |
| **3** | 6 | 4 | | |
| **4** | 5 | 5 | | |
| **5** | 4 | 6 | | |
| **6** | 1 | 9 | | |
| **7** | – | 10 | | |

#### 2.2.2. Characteristics of hydrogel materials

Swelling behavior is a critical parameter for determining the water absorption capacity and structural stability of hydrogel systems, which directly influences their potential application as wound dressings, drug delivery platforms, or implantable biomaterials. The swelling tests were performed by immersing dried hydrogel samples in three media: Ringer's solution, phosphate-buffered saline (PBS), and distilled water. At predetermined time intervals (5 min, 1 h, 24 h, and 7 days), samples were gently blotted to remove surface liquid and subsequently weighed. The swelling ratio was calculated to assess both the absorption capacity and structural integrity of the hydrogels.

Incubation experiments were conducted to evaluate the stability, degradation profile, and release behavior of the hydrogel systems under simulated physiological conditions. Samples were placed in Ringer's solution, PBS, and distilled water and incubated at 37°C. At defined time intervals (15 min, 1 h, 24 h, and 7 days) the pH of the incubation media was measured using a calibrated pH meter. This allowed for monitoring of potential bioactive compound release and for assessment of long-term material stability under physiological conditions.

The antioxidant properties of the hydrogel samples were determined to evaluate their capacity to neutralize reactive oxygen species. The assay was performed using potassium permanganate ($KMnO_4$) as an oxidizing agent. Hydrogel-containing media were transferred into Petri dishes, stirred magnetically, and supplemented with controlled volumes of $KMnO_4$ solution. After 30 minutes, changes in color intensity and transparency were recorded as qualitative indicators of antioxidant potential, reflecting the release of bioactive compounds from the hydrogel matrices.

Fourier-transform infrared spectroscopy (FTIR) was employed to investigate the chemical structure, functional groups, and potential molecular interactions within the hydrogels. Samples were analyzed in the spectral range of 4000–400 $cm^{-1}$, and the resulting absorption spectra were used to identify characteristic peaks corresponding to functional group vibrations. These data provided insights into the incorporation of plant and fungal extracts, as well as possible structural modifications of the polymeric network.

Microscopic analysis was performed to examine the morphology and distribution of bioactive components within the hydrogel structure. Samples with varying amounts of incorporated extracts were placed on glass slides and observed under a light microscope equipped with 10×, 40×, and 100× objectives. Images were captured at 500× magnification, enabling evaluation of pore structure, distribution of plant-derived additives, and homogeneity of the material. These observations allowed for correlating microstructural features with the functional performance of the hydrogels.

## 3. Results and discussion

### 3.1. Swelling studies

The swelling behavior of the hydrogel materials in different media is presented in Fig. 2.

Figure 2 shows the swelling ratio of the obtained hydrogels in Ringer's solution, PBS, and distilled water. In all cases, swelling exhibited a biphasic profile: (i) an initial rapid uptake of water due to diffusion into free network spaces and (ii) a slower stage corresponding to elastic counterforces of the polymer chains. The highest swelling ratios were observed in distilled water. This is explained by the absence of ions in the medium, resulting in a strong osmotic gradient that drives water influx into hydrophilic groups of the hydrogel. In contrast, in PBS and Ringer's solution, swelling was lower due to ionic charge screening and


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possible secondary ionic crosslinking with divalent cations ($Ca^{2+}$, $Mg^{2+}$) present in Ringer's solution. This effect has been widely reported for polyelectrolyte hydrogels, where ionic strength reduces osmotic pressure and limits expansion of the network. A single deviation from this general trend was observed for Sample 2 incubated in Ringer's solution, for which the swelling ratio at 24 h was lower than the values recorded after 1 h and 7 days. This result should be interpreted with caution, as it most likely reflects experimental variability and/or local heterogeneity of the hydrogel specimen rather than a reproducible change in the polymer network. Possible factors include local differences in crosslinking density, uneven sample thickness, or slight differences in removing surface liquid before weighing. Moreover, the ionic composition of Ringer's solution

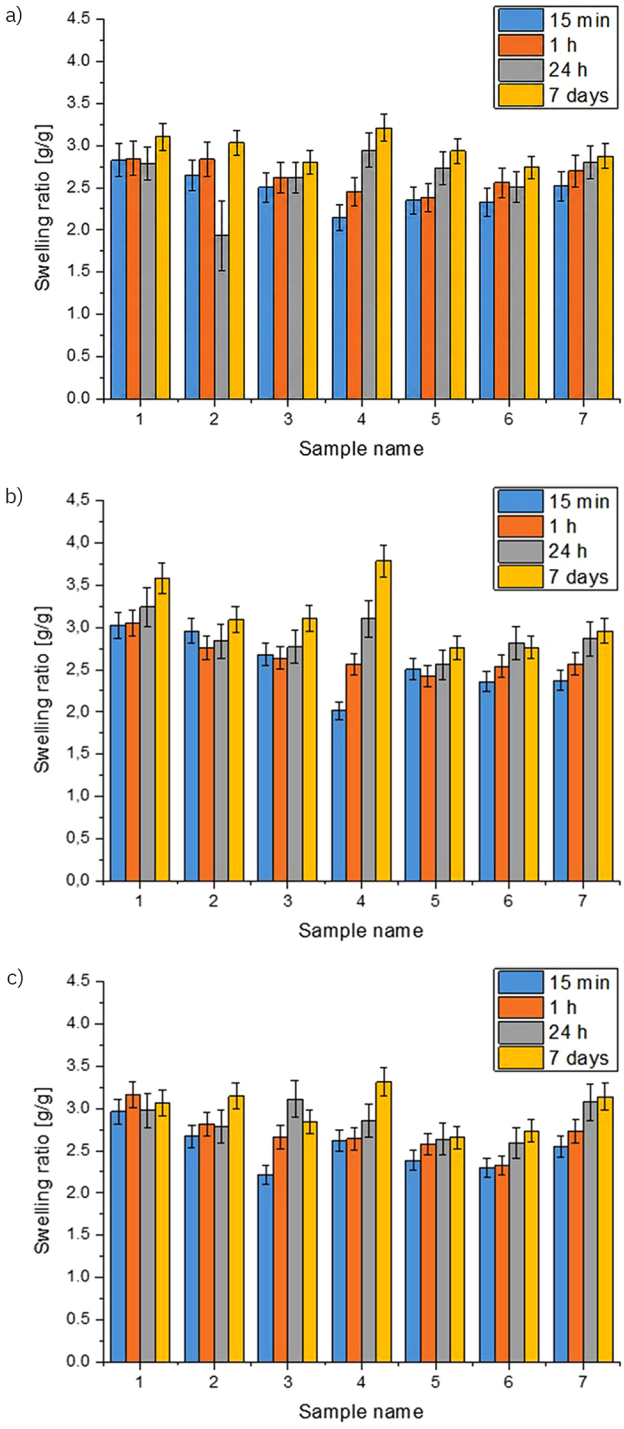


**Fig. 2.** Swelling ratio of the obtained hydrogel materials in different media: (a) Ringer's solution, (b) PBS solution, (c) distilled water; source: own elaborations

may intensify local variations in swelling due to interactions between ions and functional groups of the hydrogel. The observed behavior demonstrates that the hydrogels are medium-sensitive systems, and that swelling properties can be tailored by controlling ionic composition, which is relevant for designing smart drug delivery carriers and wound dressings.

### 3.2. Incubation studies

Figure 3 presents the results of incubation studies, showing the stability and behavior of the hydrogel materials in Ringer's solution, PBS, and distilled water.

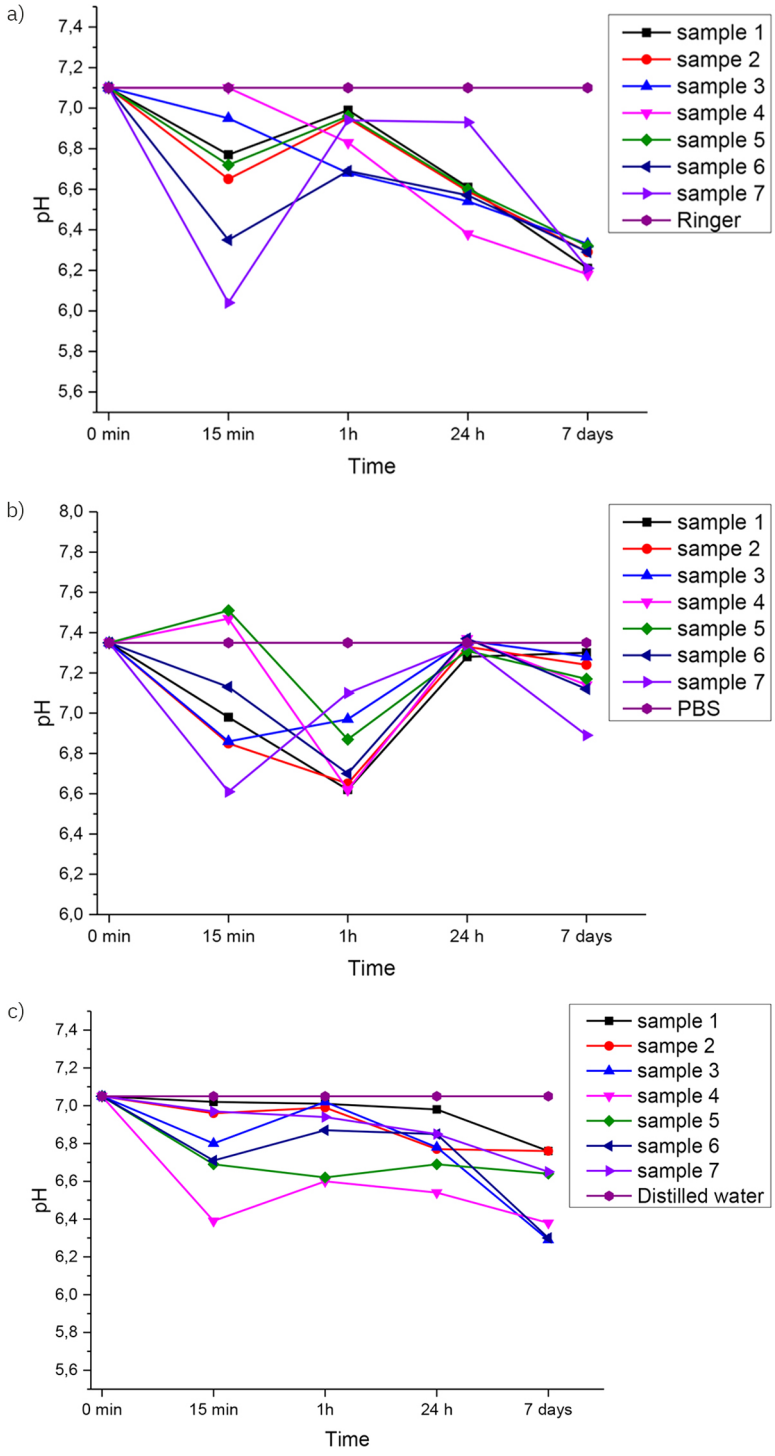


**Fig. 3.** Incubation studies of the obtained hydrogel materials in different media: (a) Ringer's solution, (b) PBS solution, (c) distilled water; source: own elaborations


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The incubation studies allowed for the evaluation of the stability of the obtained hydrogels and their effect on the pH of surrounding media, which indirectly reflects both degradation of the polymeric matrix and the release of bioactive compounds from plant and fungal extracts. In all cases, the initial pH values were within the physiological range (≈7.0–7.4), but subsequent measurements revealed changes depending on both the sample composition and the incubation medium. The non-monotonic pH profile observed at early time points may reflect an initial burst release of acidic low--molecular-weight extract constituents from the hydrogel surface, followed by re-equilibration of the medium and partial compensation by buffering/ion--exchange effects, particularly in PBS and Ringer's solution.

The strongest pH decreases were observed in Ringer's solution, likely due to the presence of divalent ions ($Ca^{2+}$, $Mg^{2+}$), which can interact with released metabolites and enhance acidification. PBS, owing to its buffering capacity, effectively minimized pH fluctuations – after an initial drop to about 6.6–6.8, stabilization near neutral values was observed. In distilled water, the decrease in pH was more pronounced than in PBS but less drastic than in Ringer's solution, confirming the significant role of ionic composition in shaping hydrogel stability.

In terms of sample composition (Table 1), hydrogels with a high proportion of fungal extract (samples 6 and 7) showed the strongest acidification, whereas systems with balanced ratios of both extracts (samples 3–5) exhibited more stable profiles. Samples dominated by plant extract (1–2) showed moderate changes, suggesting less intensive release of acidic metabolites. Overall, the incubation studies confirmed that the obtained hydrogels remain structurally stable for at least 7 days, while the observed pH variations result mainly from the gradual release of bioactive compounds. PBS proved to be the most favorable medium in terms of stabilizing incubation conditions, making it a suitable model environment for further biocompatibility studies. The results also indicate that the ratio of *Withania somnifera* to *Lentinula edodes* extracts can modulate the release profile and behavior of hydrogels under physiologically relevant conditions.

### 3.3. Antioxidant activity

To further evaluate the functional properties of the obtained systems, the antioxidant potential of the incorporated plant and fungal extracts was examined using the $KMnO_4$ reduction assay. This simple visual test provides a qualitative indication of the ability of released bioactive compounds to neutralize oxidizing agents. Table 2 summarizes the results for reference samples of pure extracts, while Table 3 presents the visual assessment of antioxidant activity for hydrogel samples after incubation in different media.

**Table 2.** Visual assessment of antioxidant activity of plant and fungal extracts based on $KMnO_4$ reduction for reference samples; source: own elaborations

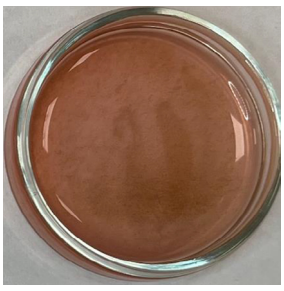
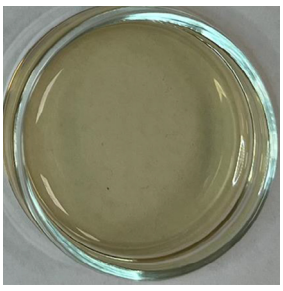
| Reference sample | | |
|---|---|---|
| **Distilled water and $KMnO_4$** | **Ashwagandha root extract and $KMnO_4$** | **Shiitake mushroom extract and $KMnO_4$** |
|  |  |  |

**Table 3.** Visual assessment of antioxidant activity of plant and fungal extracts based on $KMnO_4$ reduction for samples after incubation in different media; source: own elaborations

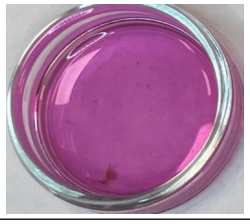
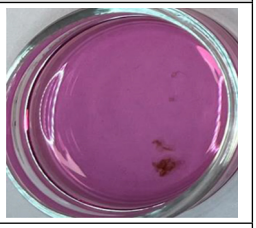
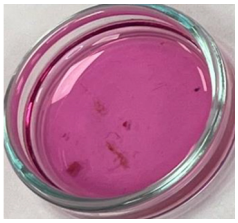
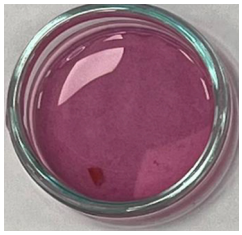
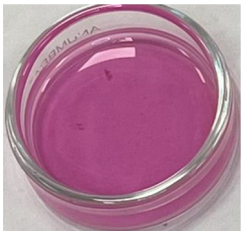
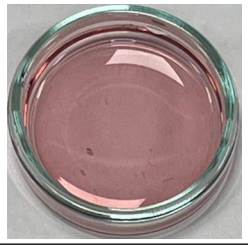
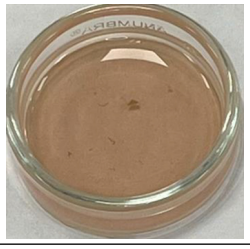
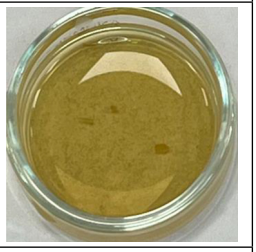
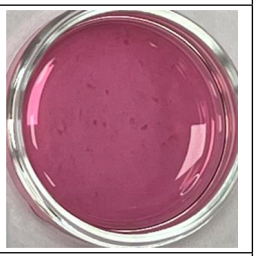
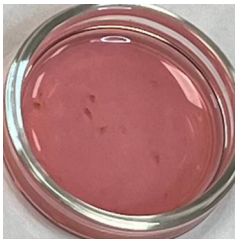
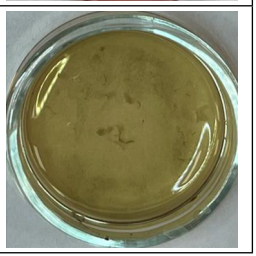
| Sample name | Distilled water | PBS fluid | Ringer fluid |
|---|---|---|---|
| 1 |  |  |  |
| 2 |  |  |  |
| 3 |  |  |  |
| 4 |  |  |  |
| 5 |  |  |  |
| 6 |  |  |  |
| 7 |  |  |  |



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Table 2 presents the antioxidant activity of ashwagandha (*Withania somnifera*) and shiitake (*Lentinula edodes*) extracts assessed by $KMnO_4$ reduction. Both extracts showed strong discoloration of permanganate solution, confirming their ability to donate electrons and scavenge free radicals. These results are consistent with previous studies demonstrating antioxidant properties of withanolides and phenolic compounds from ashwagandha (Gupta & Rana, 2007) as well as β-glucans and phenolics from shiitake (Kozarski et al., 2015; Wasser, 2002).

Table 3 shows the results after hydrogel incubation in different media. Antioxidant activity was preserved in all samples, but activity was slightly reduced in distilled water, likely due to leaching of active compounds. In PBS and Ringer's solution, higher activity was retained, suggesting that salts help stabilize phenolic structures and reduce their susceptibility to oxidation. This protective role of ionic environments for plant-derived antioxidants has also been reported in recent studies (Borges et al., 2013). Thus, the hydrogels act as effective carriers of natural antioxidants, with the ability to maintain their bioactivity during incubation in physiological-like conditions. When comparing the samples, clear differences related to composition can be observed. Hydrogels enriched predominantly with *Lentinula edodes* extract (samples 6 and especially 7) exhibited the strongest discoloration of $KMnO_4$, indicating the highest antioxidant potential. This effect is consistent with the higher content of phenolic compounds and polysaccharides in the fungal extract. In contrast, samples containing mainly *Withania somnifera* (samples 1–2) showed weaker but still evident antioxidant activity, while the intermediate compositions (samples 3–5) displayed moderate activity levels. These findings confirm that the ratio of plant to fungal extract strongly influences the antioxidant performance of the hydrogels, with LE-rich systems providing the most pronounced effect.

### 3.4. Fourier-transform infrared spectroscopy (FTIR)

Spectroscopic analysis was carried out for the hydrogel samples both before and after incubation. The results are presented in Fig. 4.

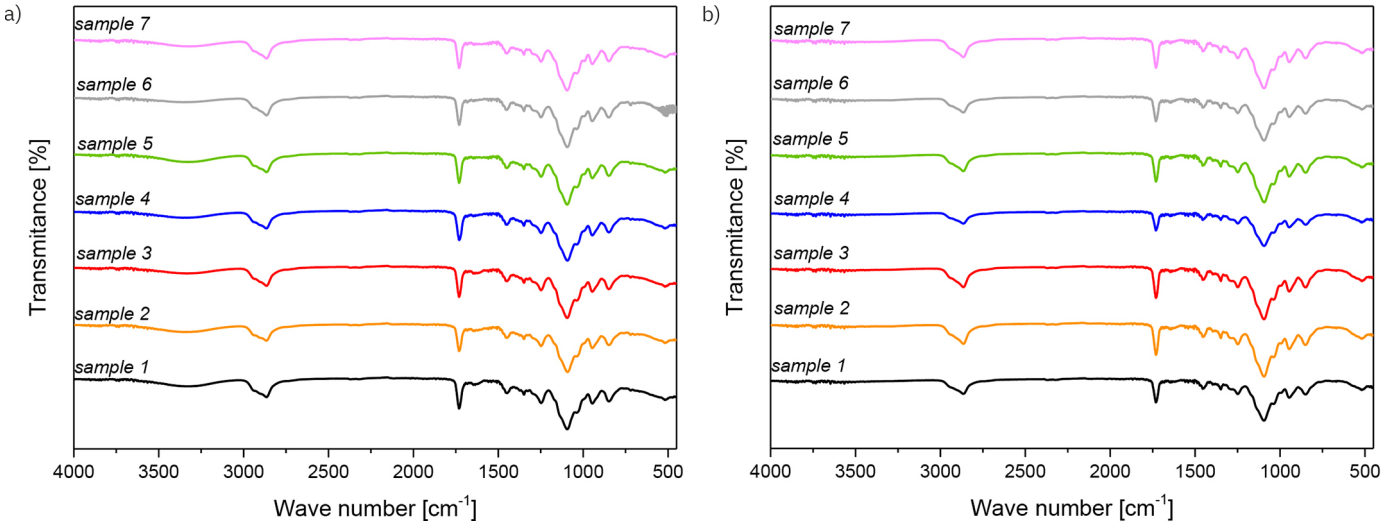



**Fig. 4.** Comparison of FT-IR spectra for samples before and after incubation in PBS liquid; source: own elaborations

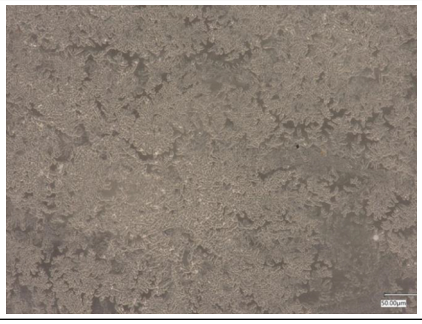
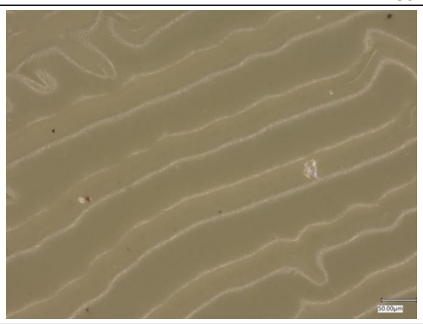
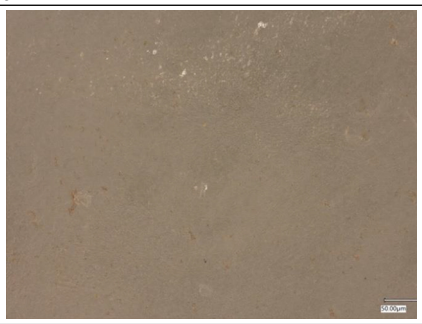
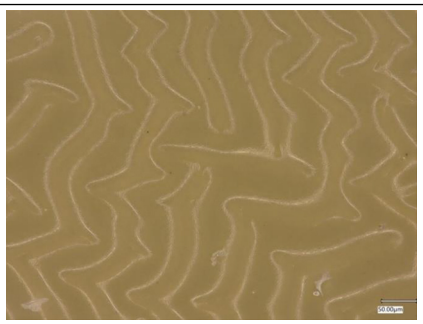
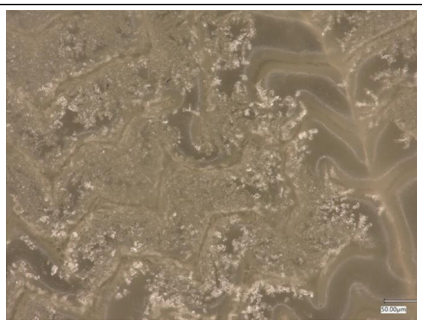
Figure 4 presents the FT-IR spectra of the hydrogels before and after incubation in PBS. The broad band observed in the 3200–3500 $cm^{-1}$ region was assigned mainly to –OH stretching vibrations originating from PVA, PEGDA, and hydroxyl-containing compounds derived from the extract. The band in the 1650–1700 $cm^{-1}$ region was attributed primarily to carbonyl-containing groups, while signals observed in the 1540–1650 $cm^{-1}$ range were not directly

assigned to the synthetic polymer matrix, but rather to extract-derived constituents, possibly including proteinaceous compounds or other nitrogen-containing biomolecules. After incubation, slight decreases in the intensity of hydroxyl-, carbonyl-, and amide-related bands were observed, together with minor shifts in the carbonyl region. These spectral changes may indicate partial release or leaching of low-molecular-weight bioactive extract components into the incubation medium, as well as surface interactions between the hydrogel matrix and PBS ions. Importantly, no disappearance of key absorption bands or formation of new peaks was detected, which confirms that the main chemical backbone of the hydrogel remained intact after incubation. Therefore, the observed variations should be interpreted as minor changes associated with ion-polymer interactions and partial release of extract-derived components rather than degradation of the polymer network (Barth, 2007; El-Azazy, 2018).

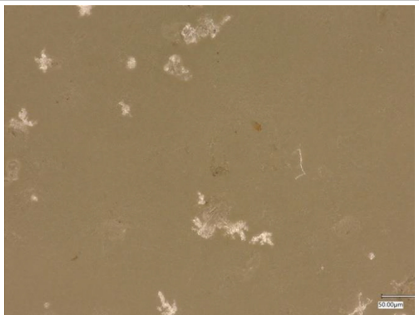
### 3.5. Microscopic observations

In Table 4 are presented the results of microscopic observations of the obtained hydrogels, carried out before and after incubation. The images allow for a comparative assessment of the material structure and provide a general overview of possible changes occurring during the incubation process.

**Table 4.** Comparison of surface morphology of hydrogel samples before and after incubation at a magnification 500×; source: own elaborations

| Before incubation | After incubation |
|---|---|
|  |  |
| Sample 1 | |
|  |  |
| Sample 2 | |
|  |  |
| Sample 3 | |

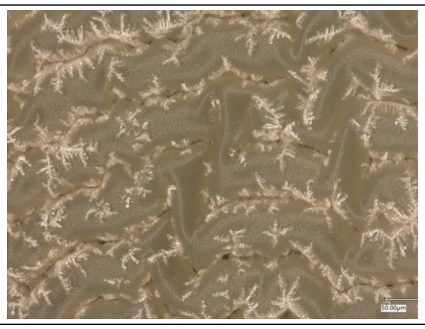

https://doi.org/10.37705/TechTrans/e2026017

| Before incubation | After incubation |
|---|---|
|  |  |

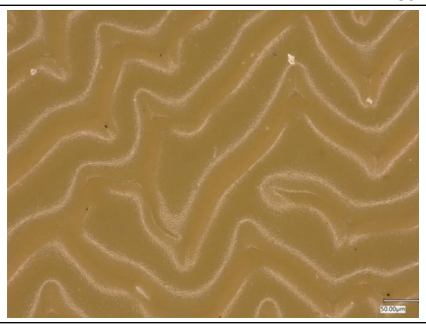
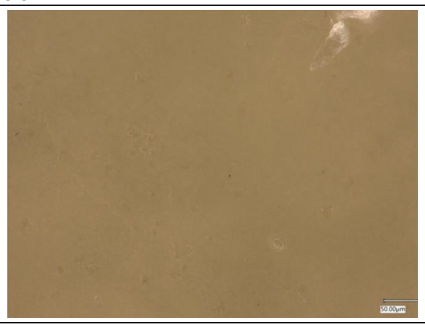
Sample 4

| | |
|---|---|
|  |  |

Sample 5

| | |
|---|---|
|  |  |

Sample 6

| | |
|---|---|
|  |  |

Sample 7

Microscopic analysis at 500× magnification provided insights into the morphology of hydrogel samples before and after incubation (Table 4). The optical microscopy results should be treated as preliminary; future studies will include SEM characterization to provide a more detailed evaluation of pore architecture, surface roughness, and extract-related microstructural heterogeneity.

Before incubation, all samples exhibited a relatively smooth and homogeneous structure with characteristic wavy patterns typical of crosslinked PVA-based hydrogels. This uniform arrangement indicates a stable and compact polymeric network formed during UV-induced crosslinking. After incubation, noticeable differences in morphology were observed, reflecting interactions with the incubation media and the influence of incorporated extracts. In general,

the surfaces became more heterogeneous, with visible roughening, formation of micro-aggregates, and in some cases the appearance of porous or crystalline--like structures. These changes suggest partial leaching of bioactive compounds and reorganization of the hydrogel matrix under aqueous conditions. Samples with a balanced ratio of *Withania somnifera* and *Lentinula edodes* extracts (3–5) showed moderate structural alterations, maintaining visible traces of the original wavy microtexture, which may indicate good stability of the hybrid matrix. By contrast, samples containing predominantly WS extract (1–2) exhibited smoother post-incubation surfaces, likely due to lower content of phenolic and polysaccharide structures that can crystallize or aggregate. On the other hand, LE-rich hydrogels (samples 6–7) revealed the most pronounced morphological changes, including the presence of crystalline deposits and heterogeneous surface roughening, consistent with the stronger release and interaction of fungal-derived compounds with the medium.

## 4. Summary

The conducted investigations demonstrated that hydrogels enriched with plant- and fungus-derived extracts exhibit good structural stability and preserved bioactivity under incubation conditions. Swelling behavior and pH variations confirmed that the systems are sensitive to the ionic composition of the surrounding medium, which may be exploited in the design of smart release systems. Antioxidant assays with $KMnO_4$ revealed that the materials effectively transferred and retained antioxidant properties from the natural extracts, particularly in the case of *Lentinula edodes*-rich samples. Microscopic observations showed that incubation led to different levels of surface modifications–ranging from relatively stable structures in *Withania somnifera*--based samples to more heterogeneous and porous morphologies in fungal--based hydrogels. FT-IR spectra confirmed the preservation of key functional groups and the absence of chemical degradation of the polymeric backbone. Collectively, these findings suggest that the developed hydrogels represent a promising class of biomaterials, whose properties can be tailored by adjusting the ratio of plant and fungal extracts, providing versatile opportunities for applications in regenerative medicine, wound healing, and controlled drug delivery systems.

The presented investigations should be considered as a preliminary but important step toward the development of functional hydrogel biomaterials enriched with plant and fungal extracts. In particular, the antioxidant activity tests provided an initial qualitative confirmation that bioactive compounds released from the hydrogel matrices retained their ability to interact with an oxidizing agent. However, further research is planned to expand these findings through quantitative antioxidant assays, detailed release profile analysis, SEM-based morphological characterization, and biological studies, including cytocompatibility and wound-healing-related in vitro tests. The continuation of this work will enable a more complete evaluation of the relationship between hydrogel composition, extract release, antioxidant performance, and potential biomedical functionality.

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