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FORMULATION AND BIOACTIVITY OF LICHEN USNEA SP. EXTRACT AND TiO_2 NANOPARTICLES MIXTURE IN A HYDROGEL MATRIX

Maulidiyah Maulidiyah[1,✉] , Muhammad Nurdin[1,2], Wa Ode Nasyrah Rafiiqa[1], Akhmad Darmawan[3], Tien Tien [4], Muh Edihar[5] and La Ode Agus Salim [5]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Halu Oleo, Kendari 93232 - Southeast Sulawesi, Indonesia.

[2]Nickel Research Institute, Universitas Muhammadiyah Kendari, Jl. K.H. Ahmad Dahlan No. 10 Kendari, Southeast Sulawesi, Indonesia

[3] Research Center for Drug Ingredients and Traditional Medicine-BRIN

[4]Department of Biochemisttry, Faculty of Medicine, Universitas Halu Oleo, Universitas Halu Oleo, Kendari, Indonesia

[5]Department of Chemistry, Faculty of Science, Technology and Health, Institut Sains Teknologi dan Kesehatan (ISTEK) 'Aisyiyah Kendari, Kendari, 93116-Southeast Sulawesi, Indonesia.

✉Corresponding author: maulid06@yahoo.com

ABSTRACT

Hydrogel-based wound dressing is a widely developed treatment method due to its superior properties, including high fluid absorption and moisture retention, which help reduce the risk of infection. This study aims to develop a hydrogel formulation that combines Lichen *Usnea* sp. extract as an anti-inflammatory agent and TiO2 nanoparticles as an antibacterial agent, optimizing the synergy between organic and inorganic materials to accelerate the wound healing process. The hydrogel was synthesized using Lichen *Usnea* sp. and TiO2 nanoparticles, along with 5% PVA, 20% PEG 400, 10% HPMC, and 4% DMSO as the base materials. The extract and nanoparticles were formulated in varying concentrations of 1%, 3%, and 5% (v/v). Histopathological analysis and post hoc LSD tests demonstrated significant activity in all variations compared to the placebo bandage (negative control). The highest collagen density was observed at the 5% (v/v) concentration, showing effectiveness comparable to the betadine bandage (positive control). These findings highlight the potential of hydrogel-based wound dressing with Lichen *Usnea* sp. extract and TiO2 nanoparticles as a promising wound healing agent, leveraging the synergy of organic and inorganic materials. The results of this study also contribute to SDG-3 (Good Health and Well-Being).

Keywords: Diabetes Mellitus; Hydrogel Plaster; Diabetic Ulcer, Lichen Usnea sp., TiO_2 Nanoparticles

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INTRODUCTION

Chronic wounds, especially diabetic ulcers, remain a significant global health burden, affecting over 6% of the diabetic population. Existing dressings often fail to accelerate healing effectively or prevent infection, necessitating novel bioactive formulations. Hydrogel Plaster is considered more effective for wound healing than conventional dressings due to its three-dimensional hydrophilic polymer network, which can absorb large volumes of fluid.[1,2,3] Hydrogel Plaster is made using Polyvinyl Alcohol (PVA) as the backing, Polyethylene Glycol (PEG 400) as a plasticizer, and Hydroxypropyl Methyl Cellulose (HPMC) as the gel base.[4,5] These bandages are designed to retain large amounts of aqueous solutions and provide an optimal moist environment for wound healing.[6] Hydrogels can be synthesized with various network structures and enhanced with additional therapeutic agents to accelerate healing. Several studies have shown that Hydrogel Plaster modified with antimicrobial agents and nanoparticles, such as silver nanoparticles (Ag-NPs), can significantly improve wound healing by preventing infections and supporting tissue regeneration.[7–11] Furthermore, new technologies, such as ultrasound to enhance hydrogel adhesion to the skin, have shown promising results in strengthening the bond between the bandage and the skin, ensuring more effective and long-lasting application.[4] This research aims to

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maximize the performance of Hydrogel Plaster by formulating it with antimicrobial agents. Specifically, Lichen *Usnea* sp. extracts (anti-inflammatory) and titanium dioxide (TiO_2) nanoparticles (antibacterial). Lichen is a plant widely used for medicinal purposes. The usnic acid, which gives this plant its yellow pigment, has various benefits, including antioxidant, antifungal, anticancer, and antidiabetic properties.[12–15] Based on literature studies[16,17], Lichen *Usnea sp.* exhibits anti-inflammatory solid activity, particularly in inhibiting TNF-α (Tumor Necrosis Factor-alpha). Studies show that *Usnea* sp. extract has an IC50 value between 1.40 and 5.70 μM for inhibiting TNF-α, indicating its potential as an effective anti-inflammatory agent.[18] The presence of nanoparticles in Hydrogel Plaster formulated with Lichen *Usnea* sp. extract is expected to enhance the wound healing performance for diabetes mellitus.[11] TiO_2 nanoparticles are a material with unique properties, offering many benefits such as antibacterial and antifungal activities due to their ability to facilitate the formation of reactive oxygen species (ROS), which can damage the cell membranes of bacteria, causing wound infections.[19,20] Based on this description, we aim to develop a Hydrogel Plaster Made from Lichen *Usnea* sp. and TiO2 for Healing Ulcers in Diabetes Mellitus.

EXPERIMENTAL

Preparation of Lichen Usnea sp.

The preparation of Lichen *Usnea* sp. samples begins with cleaning the samples from adhering dirt using running water. After the lichen samples are thoroughly washed, they are dried, then cut into small pieces and ground into a fine powder.

Extraction and Phytochemical Testing

Extraction of Lichen *Usnea* sp. is carried out using the maceration method with acetone solvent for 3×24 hours. Every 24 hours, the extract is filtered, then remacerated using fresh acetone solvent. The acetone extract obtained is evaporated using a rotary evaporator. Phytochemical screening is then conducted to test for the presence of terpenoids, tannins, and flavonoids.[14,21,22]

Synthesis of TiO_2 Nanoparticles

TiO2 nanoparticle synthesis is performed by mixing 15 mL ethanol, 2 mL distilled water, and 1 mL acetic acid (0.5 M) into a reflux flask containing 4 mL titanium tetra-isopropoxide (TTIP), 0.5 mL acetylacetone, and 15 mL ethanol. The mixture is refluxed for 3 hours at 50 °C. The TiO_2 sol is stirred using a magnetic stirrer for 1 hour at 50 °C.[23]

Formulation of Lichen Usnea sp. Extract and TiO_2 Nanoparticles Mixture

The formulation of the mixture of Lichen *Usnea* sp. extract and TiO_2 involves mixing TiO_2 nanoparticles solid into 10 mL Lichen *Usnea* sp. extract with varying ratios of 1%, 3%, and 5% (v/v). Characterization is then conducted using FTIR and PSA.

Preparation of Hydrogel Plaster

The preparation of hydrogel plaster begins by dissolving polyvinyl alcohol (PVA) in distilled water, then drying it at 50 °C for 8 hours. Lichen *Usnea* sp. extract and TiO_2 nanoparticles (1%) are dissolved in 96% ethanol, then mixed into hydroxypropyl methyl cellulose (HPMC) dissolved in distilled water, with the addition of Polyethylene Glycol (PEG 400) plasticizer (40% of the polymer composition). The mixture is stirred for 1 hour, then poured onto a backing membrane and dried at room temperature for 24 hours. The formed layer is peeled off, where aluminum foil is placed at the bottom, stored in a desiccator, and the layer is attached with adhesive to the dressing layer. The same treatment is also done for the 3% and 5% extract mixtures.[24]

Testing of Hydrogel Plaster on Experimental Animals

To test the performance of the hydrogel plaster, 30 male mice (Mus musculus) weighing 14-28 grams each, and meeting ethical clearance requirements, are used (Fig.-1). The application of the hydrogel plaster is carried out with adaptation for one week on the experimental mice. After the adaptation period, initial blood sugar levels are examined by taking a drop of blood from the tail of the mice and testing it using a glucometer. All samples with normal blood sugar levels are injected with IP aloxan at a dose of 0.0015 g per mouse to induce diabetes and adapted for five days. After five days, blood sugar levels are

examined again in the experimental animals. When the mice have developed diabetes, the next step is to create an incision wound on the back of the mice, about 1.5 cm long and 0.2 cm deep into the subcutaneous tissue. The mice are then treated with each treatment, aimed at five mice: K1: hydrogel plaster formula 1%; K2: hydrogel plaster formula 3%; K3: hydrogel plaster formula 5%; K+: betadine drug administration; K-: placebo hydrogel plaster (without extract formulation), and KN: mice left healthy (not injected with aloxan and not receiving additional treatment). The treatment involves covering the incision wound with the hydrogel plaster for five consecutive days. On the sixth day, excision is performed on the mice's skin, then the wound tissue is taken and made into slides with Hematoxylin and Eosin (HE) staining for histopathological examination by observing collagen density. Microscopic examination is performed from five fields of view to assess collagen density based on scoring 0: no collagen growth, 1: low collagen growth (50%). The data obtained from scoring collagen density in mice (Mus musculus) are analyzed using normality and homogeneity tests, and if there are significant differences with a significance level of $p > 0.05$, the analysis is continued using one-way ANOVA and post hoc LSD tests. Data analysis is performed using the SPSS application.

Fig.-1: Acclimatization, Intervention Administration, And Experimental Animal Preparation: (a) Mice Adaptation Process; (b) Blood Sugar Level Examination; (c) Incision Wound Creation; (d) Covering the Incision Wound Using Hydrogel Plaster

RESULTS AND DISCUSSION

Extraction and Phytochemical Testing

The maceration extraction method applied to 50 grams of coarsely ground Lichen *Usnea* sp. yielded 2.507 grams of acetone extract. Based on Table 1, the phytochemical test results show that the *Lichen Usnea sp*. extract is positive for terpenoids, tannins, and flavonoids. This finding is consistent with the literature[22,25], which states that *Lichen Usnea sp.* contains alkaloids, terpenoids, tannins, and flavonoids.

Table-1: Phytochemical Test Results of Acetone Extract of *Lichen Usnea* sp.

Sample	Compound Content				
	Alkaloid (Bouchardat reagent)	Terpenoid	saponin	Tanin	Flavanoid
Acetone extract of Lichen *Usnea* sp.	-	+	-	+	+

Characterization of Fourier Transform Infrared (FTIR)

FTIR characterization is used to identify the presence of functional groups based on the wavenumbers produced by each functional group. Each functional group has different absorption peaks. The success of the extraction of Lichen *Usnea* sp. can be determined based on the analysis of functional groups using FTIR, as shown in Fig.-2.

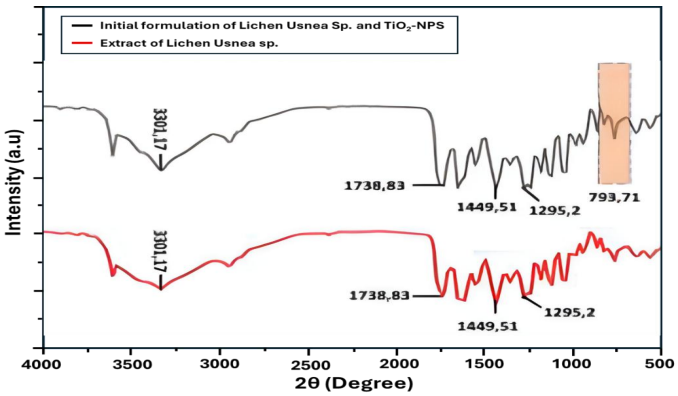

Fig.-2: FTIR Characterization Results of Acetone Extract of Lichen *Usnea* Sp. and the Formula of Extract and TiO_2 NPs 3%

Based on Fig.-1, absorption peaks are obtained, indicating the presence of various types of functional groups in both the acetone extract of Lichen *Usnea* sp. and the formula of the extract and TiO_2-NPs 3%. All spectra show several absorption features ranging from 500 cm^{-1} to 4000 cm^{-1}. The main absorption features appear at 3650-4590 cm^{-1} (O-H)[26–28], 1760-1690 cm^{-1} (C=O)[29], 1470-1340 cm^{-1} (C-H alkane)[26], 1300-1050 cm^{-1} (C-O)[27], 995-675 cm^{-1} (C-H alkene)[30], and 400-850 cm^{-1} (Ti-O).[31,32] The presence of the Ti-O group in the formula of extract and TiO_2-NPs 3% is indicated by the absorption peak in the range of 400-850 cm^{-1} at 793.71 cm^{-1}. This is consistent with the literature[31], indicating that TiO_2 is contained in the formula of the extract and TiO_2-NPs.

Characterization of Particle Size Analyzer (PSA)

PSA characterization was conducted to determine the particle size of the formula of Lichen *Usnea* sp. extract and TiO_2 NPs 3% (v/v), as well as the particle size of synthesized TiO_2. Based on Fig.-3 (a), the particle size of the 3% (v/v) formula is found to be 246.1 nm in diameter, while the particle size of TiO_2 obtained is 20.17 nm, consistent with the literature [journal]. This indicates the successful synthesis of TiO_2.

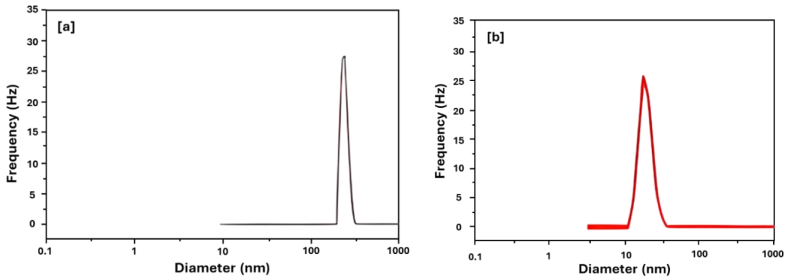

Fig.-3: Particle Size Distribution of the Formula: (a) TiO_2 Particle Size, (b) *Lichen Usnea* sp. Extract and TiO_2 NPs 3%

Table-2 shows various methods that have been developed in synthesizing TiO_2 nanoparticles along with the respective particle sizes obtained. This research also employs the acid-assisted sol-gel method with TiO_2 particle sizes obtained that are not significantly different.

Histopathological Test

Based on the scoring results of collagen density in diabetic mice, the results obtained are as shown in Fig.- 4 and Table-2. Table-3 shows the mean collagen density results in the treatment groups with the mean collagen density values in K1 (1.6±0.548), K2 (2.0±0.707), K3 (2.4±0.548), K+ (2.8±0.447), K- (1.4±0.548), and KN (3.0 ± 0.00). Before conducting the collagen density data analysis, normality and homogeneity tests were performed. The results obtained from the normality test show a significance value of $p > 0.05$, indicating normally distributed data. In the homogeneity test, based on the mean values, a

significance value of $p > 0.05$ was obtained, indicating homogeneous data, so the data analysis continued with the one-way analysis of variance (ANOVA) method.

Table-2: Various Methods of TiO_2 Nanoparticle Synthesis

No.	Synthesis Methods of TiO_2 Nanoparticles	Particle Sizes	References
1	Green nanochemistry	10 – 22 nm	[33]
2	Hydrothermal	15,2 nm	[34]
3	Sonochemical	10 – 25 nm	[35]
4	Acid-assisted sol-gel	5 nm	[36]
5	Acid-assisted sol-gel	20 nm	This Work

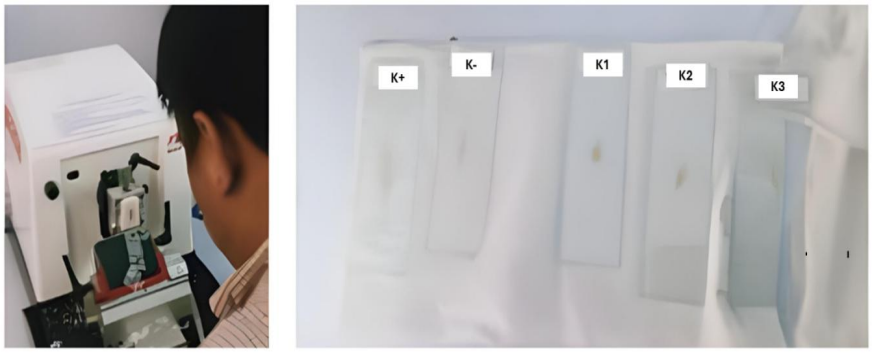

Fig.-4: Histopathological Test of Lichen *Usnea* sp. Extract and TiO_2 NPs 3%

Table-3: Results of Collagen Density Examination Based on Scoring

Treatment	Scoring	Meant ± SD
K1	8	1,6 ± 0,548
K2	10	2,0 ± 0,707
K3	12	2,4 ± 0,447
K+	14	2,8 ± 0,548
K-	2	0,4 ± 0,548
KN	15	3,0 ± 0,00

Explanation: 0 = No growth, 1 = Low growth, 2 = Moderate growth, and 3 = Dense growth.

The results of data analysis using the one-way ANOVA method show a significance value of $p < 0.05$. This indicates that there is a significant difference in the test results data for all treatment groups, so the analysis continued with the post-hoc LSD test to determine whether there were significant differences after treatment in each treatment group. Based on the results of the post-hoc LSD test, the significance value between each treatment group and the negative control group is $p < 0.05$, indicating a significant difference between the negative control group and each treatment group. This indicates that hydrogel patches with *Lichen Usnea sp.* extract and TiO_2 nanoparticles with ratios of 1%, 3%, and 5% (v/v) have activity on collagen growth in the wound healing process. Furthermore, a significance value was obtained between the treatment group (K3) and the positive control group with a p-value > 0.05, indicating a significant effect from group K3. This indicates the effectiveness of hydrogel patches with *Lichen Usnea sp.* extract and TiO_2 nanoparticles with a ratio of 5% (v/v) on collagen growth in the wound healing process. This research also describes the histopathological overview of collagen density in diabetic mouse wound healing.

Figure-3 shows the microscopic appearance of histopathological tissue that has been treated, where in the K+ group (a), there is good collagen growth, unlike the K- group (b), where there is minimal collagen growth, almost none visible. This is because the tissue treated with the placebo patch still undergoes the inflammatory phase, so collagen is not yet prominently visible.[37,38] In the microscopic images of the groups treated with hydrogel patches containing *Usnea* sp. lichen extract and TiO_2 nanoparticles at 1%, 3%, and 5% (c, d, and e), it is evident that the tissue is filled with collagen, albeit with different density levels.

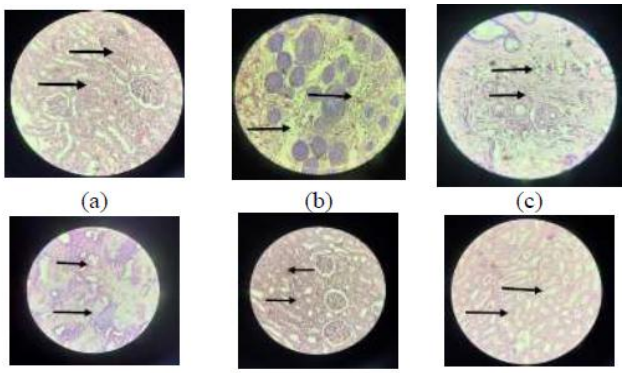

Fig.-4: Histopathological Image (HE, Magnification 400×) of Collagen Density in Diabetic Mice (Mus musculus) using Hydrogel Patches with Lichen *Usnea* sp. Extract and TiO2 Nanoparticles with Treatment K+ using Betadine (a), K- using Placebo (b), K1 using 1% Formula (c), K2 using 3% Formula (d), K3 using 5% Formula (e), and Healthy Mice (f)

The 5% formula shows maximal collagen density compared to the 1% and 3% formulas. Overall, the results indicate that hydrogel patches with 1%, 3%, and 5% extract can increase collagen density compared to the negative control group (K-). Based on the obtained results, this research has the potential to contribute to scientific knowledge in the field of science as well as to address societal issues, particularly regarding the treatment of diabetic wounds. This research also has the opportunity to obtain intellectual property rights (IPR) such as copyrights. Furthermore, the research results can be published in accredited journals, which can serve as a reference source for further research. This study demonstrates clear alignment with Sustainable Development Goal (SDG) 3: Good Health and Well-Being through the development of bioactive hydrogel dressings that enhance diabetic wound healing. This contribution is in line with the broader objectives of the SDGs, particularly SDG 3, by promoting innovative healthcare solutions that address chronic diseases and improve the quality of life in vulnerable populations.

CONCLUSION

Based on the data analysis discussed earlier, it can be concluded that the TiO_2 used as an antibacterial agent in the patch has a particle size of 20.17 nm, indicating successful TiO_2 nanoparticle synthesis. On the other hand, characterization results using FTIR indicate that the Lichen *Usnea* sp. extract contains various functional groups such as O-H, C-H alkane, C-H alkene, C=O, and C-O. Additionally, hydrogel patches with Lichen *Usnea* sp. extract and TiO_2 nanoparticles demonstrate good effectiveness in healing diabetic wounds, especially at a concentration of 3%. These findings support the objectives of SDG 3: Good Health and Well-Being, by contributing to the development of innovative and effective wound care strategies for managing chronic health conditions.

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CONFLICT OF INTERESTS

The authors declare there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related with *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

M. Maulidiyah https://orcid.org/0000-0001-5643-9854
M. Nurdin https://orcid.org/0000-0002-6727-9283
W.O.N. Rafiiqa https://orcid.org/0009-0005-4756-3681

A. Darmawan https://orcid.org/0000-0001-9352-5233
T. Tien https://orcid.org/0000-0003-4677-6106
M. Edihar https://orcid.org/0009-0000-9270-2672
L.O.A. Salim https://orcid.org/0000-0001-6553-2057

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