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Research Article

In Vitro Antibacterial Activity of Aqueous and Acetone Extracts Obtained from Epiphytic Lichens

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ABSTRACT

This study evaluated the antibacterial activity of acetone and aqueous extracts obtained from three macrolichen species *Ramalina farinacea*, *Pseudevernia furfuracea*, and *Platismatia glauca* using the minimum inhibitory concentration (MIC) method against selected Gram-positive and Gram-negative bacteria. Test organisms included *Staphylococcus epidermidis*, *Escherichia coli*, and *Listeria innocua*. Antibacterial activity was detected only in acetone extracts, whereas aqueous extracts showed no inhibition. *R. farinacea* exhibited the strongest activity, with MIC values of 1 mg/mL against all tested bacteria. *P. furfuracea* demonstrated moderate activity (MIC: 2–8 mg/mL), while *P. glauca* showed limited inhibition restricted to *S. epidermidis* (MIC: 1 mg/mL). These findings indicate that both solvent type and lichen species critically influence antibacterial efficacy. The study provides preliminary in vitro evidence supporting the antimicrobial potential of macrolichen-derived extracts and highlights the need for further replicated MIC analyses, expanded bacterial panels, and chemical characterization of active metabolites.

Keywords*: Antibacterial, minimum inhibitory concentration (MIC), Ramalina farinacea (L.) Ach, tree lichen*

Epifitik Likenlerden Elde Edilen Su ve Aseton Ekstraktlarının In Vitro Koşullarda Antibakteriyel Aktivitesi

ÖZET

Bu çalışmada üç makroliken türünden *Ramalina farinacea*, *Pseudevernia furfuracea* ve *Platismatia glauca* elde edilen aseton ve su ekstraktlarının antibakteriyel aktivitesi, minimum inhibitörik konsantrasyon (MIC) yöntemi kullanılarak değerlendirildi. Test mikroorganizmaları arasında *Staphylococcus epidermidis*, *Escherichia coli* ve *Listeria innocua* yer aldı. Antibakteriyel etkinlik yalnızca aseton ekstraktlarında saptanmış, su ekstraktlarının hiçbirinde inhibitör etki gözlenmedi. *R. farinacea* tüm bakterilere karşı 1 mg/mL MIC değeri ile en güçlü aktiviteyi gösterdi. *P. furfuracea* orta düzeyde etki (MIC: 2–8 mg/mL) görüldü. *P. glauca* yalnızca *S. epidermidis* üzerinde sınırlı inhibisyon (MIC: 1 mg/mL) oluşturdu. Bulgular, çözücü türü ve liken türünün antibakteriyel etkinlik üzerinde belirleyici olduğunu gösterdi. Sonuç olarak çalışma, makroliken kökenli ekstraktların in vitro antibakteriyel potansiyeline ilişkin ön veriler sunmakta ve ileri çalışmalar için tekrarlı MIC analizleri, daha geniş bakteri panelleri ve aktif bileşiklerin kimyasal karakterizasyonunun gerekliliğini ortaya koymaktadır.

Anahtar kelimeler**:** *Ağaç likeni, antibakteriyel, minimum inhibitör konsantrasyon (MIC), Ramalina farinacea (L.) Ach*

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Introduction

Lichens are highly organized symbiotic systems, often resulting from the interaction between a fungus (mycobiont) and a photosynthetic partner, a green alga or cyanobacterium. This association enables lichens to withstand harsh ecological conditions and biosynthesize a wide range of structurally diverse metabolites. These natural compounds, including depsides, depsidones, and usnic acid derivatives, are of great interest because they exhibit diverse biological activities, including antioxidant, antimicrobial, and enzyme-inhibiting properties (Zhao et al., 2021).

Pseudevernia furfuracea (L.) Zopf, *Platismatia glauca* (L.) W.L. Culb, and *Ramalina farinacea* (L.) are foliose and shrubby lichens, widely distributed in different regions and climates around the world, and are notable for their chemical composition and pharmacological properties. *Pseudevernia furfuracea* produces atranorin, chloroatranorin, and physodic acid, which have been associated with antimicrobial and cytoprotective effects. *Pseudevernia furfuracea* is rich in caperatic acid and atranorin derivatives, while *R. farinacea* contains high amounts of evernic and usnic acids. These secondary metabolites contribute to the antioxidant and antibacterial potential of these lichens (Moreira et al., 2015; Studzińska-Sroka et al., 2022; Essadki et al., 2024). This metabolite diversity forms the biochemical basis of the ecological resilience and biological activity of lichens.

Increasing pathogen resistance has led to renewed interest in natural products as alternative antimicrobial sources. Recent studies have shown that extracts of *P. furfuracea, P. glauca*, and *R. farinacea* can inhibit the growth of both Gram-positive and Gram-negative bacteria such as *Listeria innocua*, *Staphylococcus epidermidis*, and *Escherichia coli* (Mitrović et al., 2014; Essadki et al., 2024). *L. innocua* is used as a model organism for *Listeria monocytogenes* in laboratory tests, while *S. epidermidis* is a commensal bacterium that can form persistent biofilms on medical devices. *E. coli* is a versatile model widely used in antimicrobial studies. The inhibition observed in lichen-derived bacteria may result from multifactorial mechanisms such as membrane disruption, interference with protein synthesis, or induction of oxidative stress (Essadki et al., 2024).

The aim of this study was to evaluate and compare the antimicrobial potential of acetone and aqueous extracts obtained from P*. furfuracea, P. glauca*, and *R. farinacea* against selected Gram-positive (*L. innocua* and *S. epidermidis*) and Gram-negative (*E. coli*) bacterial strains. The primary aim of this study was to determine the antibacterial activity of selected lichen extracts through a preliminary screening approach. The bacterial panel used in this study was intentionally selected to represent both Gram-positive and Gram-negative microorganisms with different ecological and clinical backgrounds. *Listeria innocua* was included as a model organism for foodborne *Listeria* spp., *Staphylococcus epidermidis* as a clinically relevant opportunistic pathogen associated with biofilm formation, and *Escherichia coli* as a representative Gram-negative bacterium commonly used in antimicrobial screening studies. This selection allowed a comparative evaluation of lichen extracts across different bacterial cell wall structures and application contexts. By examining the inhibitory activities of these macrolichen species, we aimed to identify extracts containing antimicrobial potent metabolites. The findings are expected to contribute to the understanding of the pharmacological value of lichen-derived compounds and to contribute baseline data for future studies investigating natural antimicrobial candidates.

Materials and Methods

Lichen Material Collection Studies

Fresh lichen specimens were collected from the cities of Kocaeli, Bursa and Balıkesir in Türkiye. The samples were carefully placed in paper bags to allow proper aeration and prevent moisture accumulation during transport. For each collection, relevant ecological and geographical data including locality, altitude and substrate type were recorded on the sample envelopes. Detailed information regarding the GPS coordinates, elevation, and sampling dates of the collected specimens is presented in Table 1.

Following collection, all specimens were transferred to the Herbarium of the Faculty of Arts and Sciences at Marmara University for taxonomic identification and reference preservation, under the accession codes *Ramalina farinacea* (L.) Ach (MUFE), *Pseudevernia furfuracea* (L.) Zopf (MUFE) and *Platismatia glauca* (L.) W.L. Culb (MUFE). Before biochemical and microbiological analyses, samples designated for culture were meticulously cleaned to remove any surface debris and subsequently

Table 1. Information on the stations where lichen samples were collected

Lichen	Locality	GPS recording(X/Y)	Altitude	Substrate
Ramalina farinacea (L.) Ach.	Kocaeli-Kartepe	253955/4494483	806	Oak
Pseudevernia furfuracea (L.) Zopf	Bursa-Uludağ	67123/4439382	1266	Larch
Platismatia glauca (L.) W.L. Culb.	Balıkesir-Kazdağları	479437/4391193	1331	Larch

*: L: Linnaeus, W.L. Culb: William Louis Culberson, Ach: Acharius

Figure 1. Extracts of three lichen species prepared using acetone (a) and distilled water (b)

stored at −20 °C until use to maintain sample integrity and prevent degradation of bioactive components.

Studies on the determination of Lichen Species

Taxonomic identification of the collected lichen specimens was performed following conventional systematic approaches. Morphological characteristics and chemical spot tests (K, C, I, P, and N reactions) were evaluated under a stereo microscope (Olympus CX23, Japan) to determine diagnostic features. Identification was carried out using standard lichenological literature, including comprehensive lichen floras and dichotomous keys, with Smith et al. (2009) serving as the primary reference source. Prior to identification, each lichen sample was carefully separated from its substrate and any extraneous materials such as other lichens, mosses, plant residues, or soil particles. The cleaned specimens were then individually labeled and preserved as voucher samples for further examination and documentation.

Preparation of Lichen Extracts

Lichen samples were collected during the summer season and thoroughly cleaned with distilled water to eliminate surface contaminants and debris. The cleaned thalli were air-dried at room temperature for approximately 48 hours and subsequently ground into a fine powder using a laboratory mill. The powdered materials were stored in sterile glass containers at 4 °C until extraction. For solvent extraction, 10 g of the powdered sample was mixed with 100 ml of either acetone or distilled water and kept at ambient temperature for 72 hours with occasional agitation. The crude extracts were obtained by decantation and successive filtration through cheesecloth followed by Whatman No. 1 filter paper to remove particulate matter. Further purification was achieved using membrane filtration with a pore size of 0.45 µm. The acetone extracts were concentrated under reduced pressure using a rotary evaporator at low temperature, while aqueous extracts were freeze-dried under a vacuum of approximately 5 µm Hg. During lyophilization, the samples were first frozen at −80 °C and then subjected to sublimation under ultra-low pressure, allowing direct transition of ice to vapor without melting. The resulting dry extracts were reconstituted in their respective solvents to final concentrations of 0.1 mg/ml and 0.2 mg/ml for antimicrobial assays. All extracts were preserved at −20 °C until further analysis (Srivastava et al., 2013; Bhatta et al., 2020; Furmanek et al., 2024). Photographic images of lichen extracts in Falcon tubes are presented in Figure 1.

Microorganisms

Total three bacteria, two Gram positive *L. innocua, S. epidermidis* and one Gram-negative *E. coli* were used to assess the antimicrobial properties of the test samples. These bacteria were grown on nutrient agar plates at 37 °C, respectively. For use in experiments, the organisms were subcultured in nutrient broth.

Determination of Antimicrobial Activity

The antibacterial properties of the samples were evaluated against *S. epidermidis, E. coli* and *L. innocua* using the MIC method, with bacterial strains obtained from the Culture Collection of the Department of Veterinary, Kepsut Vocational School, Balıkesir University. Both bacteria were incubated in Nutrient Broth (Merck, Germany) at 37 °C for 24 hours before MIC test. The strains were also confirmed for purity (Quinn et al., 2002). Minimum inhibitory concentrations (MIC) tests for herbal extracts were done and evaluated by according to EUCAST guidelines. According to EUCAST, the MIC was determined as the lowest concentration of antimicrobial agent that completely inhibited the growth of bacteria detected with the naked eye on microplates. Bacterial growth appears as turbidity or cell accumulation at the bottom of the well and was also evaluated (Eucast, 2003; Eucast, 2017; Eucast, 2019; Eucast, 2024). MIC determinations were performed as single measurements, as the primary aim of the study was preliminary screening to determine the antibacterial activity of the lichen extracts rather than to conduct replicated quantitative efficacy analyses. *E. coli* ATCC 25922 was included as a reference control strain to validate the performance and reliability of the MIC assay throughout the experimental procedures. Sterility control (broth only), growth control (bacteria without extract), and solvent control were included in each

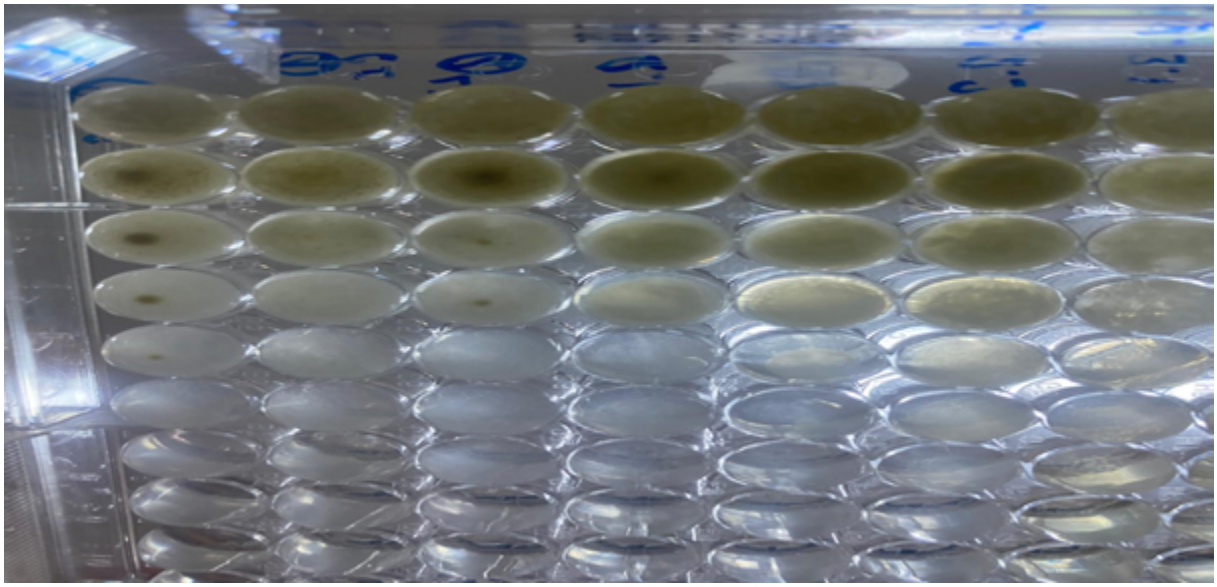

Figure 2. Minimum inhibitory concentrations (MIC) determined for acetone and water-based

experiment. The results of the minimum inhibitory concentration (MIC) tests are presented in Figure 2.

Statistical Analysis

Since MIC determinations were performed as single measurements without biological or technical replication, inferential statistical tests were not applied. Instead, a descriptive comparative analytical approach was used to evaluate differences in antibacterial activity among lichen species, extraction methods, and bacterial strains based on MIC distributions. Antibacterial potency was interpreted by comparing MIC values, activity spectrum, and solvent-dependent extraction effects.

Results

Ramalina farinacea exhibited the highest antibacterial activity, followed by Pseudevernia furfuracea, whereas Platismatia glauca showed limited inhibition restricted to *S. epidermidis.*

Table 2. Minimum inhibitory concentration (MIC) results of lichen extracts against tested bacterial strains.

	Bacterial Types			
	Acetone Extraction (100 mg.ml^{-1})			
Lichen Types	*Staphylococcus epidermidis*	*Escherichia coli*	*Listeria innocua*	*E.coli ATCC 25922* (as control)
Pseudevernia furfuracea (L.) Zopf	2	4	8	2
Platismatia glauca (L.) W.L. Culb	1	-	-	-
Ramalina farinacea (L.) Ach	1	1	1	1
	Water Extraction (100 mg.ml^{-1})			
	Staphylococcus epidermidis	*Escherichia coli*	*Listeria innocua*	*E.coli ATCC 25922* (as control)
Pseudevernia furfuracea (L.) Zopf	-	-	-	-
Platismatia glauca (L.) W.L. Culb.	-	-	-	-
Ramalina farinacea (L.) Ach	-	-	-	-

P. glauca demonstrated antibacterial activity exclusively against *S. epidermidis*, while the third tested species, *R. farinacea*, exhibited inhibitory effects against all bacterial strains evaluated, with comparable levels of activity across species. MIC results of lichen extracts against the tested bacterial strains are presented in Table 2.

When the acetone extracts were compared, *R. farinacea* showed the most pronounced antibacterial efficacy, effectively inhibiting all three bacterial species at lower MIC values. Although *P. glauca* also demonstrated activity against the same bacterial strains, its inhibitory effect against *L. innocua* was notably weaker, as reflected by higher MIC values.

In contrast, water extracts of all three lichen species displayed no inhibitory antibacterial activity. Growth was observed in all wells during the MIC assay, indicating the absence of inhibitory effects. These findings suggest that acetone serves as a more efficient solvent for extracting antibacterial compounds from lichen thalli compared to water, highlighting the importance of solvent polarity in determining the biological activity of lichen-derived extracts.

E. coli ATCC 25922, used as the control strain, exhibited MIC values of 1 mg/mL for *Ramalina farinacea* and 2 mg/mL for *Pseudevernia furfuracea*, while no inhibition was observed for Platismatia glauca, showing a susceptibility pattern comparable to that of the tested *E. coli* isolate.

Antibacterial activity was observed exclusively in acetone extracts, whereas aqueous extracts showed no inhibitory effect against any of the tested bacterial strains. This finding indicates a strong solvent-dependent difference in extraction efficiency and antimicrobial potential.

Among the acetone extracts, *Ramalina farinacea* exhibited the lowest MIC values (1 mg/mL) against all tested bacteria, including *S. epidermidis*, *E. coli*, and *L. innocua*, indicating the highest antibacterial potency and the broadest activity spectrum. *Pseudevernia furfuracea* demonstrated moderate inhibitory activity with MIC values of 2 mg/mL against S. epidermidis, 4 mg/mL against *E. coli*, and 8 mg/mL against *L. innocua*, suggesting reduced efficacy particularly against Gram-negative bacteria. *Platismatia glauca* showed limited antibacterial activity and inhibited only *S. epidermidis* (MIC: 1 mg/mL), with no detectable effect against the other tested strains.

When bacterial susceptibility was compared, *S. epidermidis* was the most sensitive microorganism, being inhibited by all acetone extracts, whereas *E. coli* showed moderate susceptibility and *L. innocua* exhibited comparatively higher MIC values, indicating reduced sensitivity to lichen-derived compounds.

Discussion

Lichen species are known for their ability to synthesize various secondary metabolites such as depsides, depsidones, and usnic acid derivatives; these compounds have remarkable antimicrobial potential (Stocker-Wörgötter, 2010; Furmanek et al., 2022). In this study, it was determined that acetone extracts obtained from the species *Pseudevernia furfuracea* (L.) Zopf, *Platismatia glauca* (L.) W.L. Culb, and *Ramalina farinacea* (L.) Ach exhibited measurable antibacterial activity, whereas no inhibitory activity was detected in water extracts of the same species. This difference proves that solvent polarity has a significant effect on the extraction efficiency of biologically active compounds.

Among the lichen species examined, *Ramalina farinacea* (L.) Ach exhibited the broadest and most consistent antibacterial spectrum, effectively inhibiting the growth of *S. epidermidis*, *E. coli*, and *L. innocua* strains at relatively low MIC values. This broad-spectrum activity is may be associated with the presence of lipophilic secondary metabolites reported in previous studies, which are more effectively extracted by organic solvents similar to acetone. Previous studies have also linked the potent antimicrobial activities of Ramalina lichen species to compounds such as usnic, evernic, and sekikaic acids (Kosanić and Ranković, 2011; Shrestha and St. Clair, 2013).

Pseudevernia furfuracea (L.) Zopf exhibited selective antibacterial activity and was more effective against *S. epidermidis* than against *E. coli*. This difference can be explained by the structural differences between Gram-positive and Gram-negative bacteria. The outer membrane of Gram-negative bacteria acts as a barrier restricting the diffusion of large or hydrophobic molecules, which reduces susceptibility to lichen-derived compounds (Boustie and Grube, 2005). Similarly, *Platismatia glauca* (L.) W.L. Culb showed inhibition only against *S. epidermidis*, suggesting that fewer or weaker antibacterial compounds may be present in the metabolic profile of this species.

The lack of inhibitory activity in all aqueous extracts supports the hypothesis that phenolic and depsidone-type molecules responsible for antibacterial activity have low solubility in polar solvents. Organic solvents such as acetone facilitate the extraction of non-polar compounds, allowing for more accurate detection of in vitro antimicrobial activity (Molnár and Farkas, 2010). Therefore, solvent selection is a critical parameter in assessing the biological activity of lichens.

Overall, the findings indicate that the antibacterial activity of lichen extracts depends on both the species-specific metabolite composition and the physicochemical properties of the solvent. These results are consistent with previous studies highlighting lichens as promising sources of natural antimicrobial compounds with potential pharmaceutical applications (Furmanek et al., 2022; Elkhateeb et al., 2023). The consistent inhibition of *S. epidermidis* by acetone extracts further supports the potential of lichen-derived compounds for the development of natural antimicrobial agents targeting Gram-positive pathogens.

The comparable MIC responses observed for *E. coli* ATCC 25922 and the tested *E. coli* isolate support the metho-

dological reliability of the assay and confirm that the antibacterial activity of lichen extracts reflects true biological effects rather than experimental artifacts.

The absence of antibacterial activity in aqueous extracts and the consistent inhibition observed in acetone extracts emphasize the importance of solvent polarity in the extraction of bioactive lichen metabolites. Acetone is known to solubilize a broader range of secondary metabolites, including depsides, depsidones, and usnic acid derivatives, which may explain the stronger antimicrobial effects observed.

The comparative MIC distribution suggests that *Ramalina farinacea* possesses a higher concentration or diversity of antimicrobial compounds, resulting in broader-spectrum activity. In contrast, the moderate activity of *Pseudevernia furfuracea* and the limited inhibition observed in Platismatia glauca indicate species-specific differences in metabolite composition and antibacterial potency.

Differences in bacterial susceptibility may be associated with structural characteristics of bacterial cell walls. The higher sensitivity of *S. epidermidis* compared with *Escherichia coli* and *L. innocua* may reflect differences in membrane permeability and intrinsic resistance mechanisms.

A limitation of this study is that MIC determinations were performed without experimental replication, and therefore statistical hypothesis testing could not be conducted. This is because the primary aim of the present work was to conduct a preliminary screening study to determine the antibacterial activity of selected lichen extracts. Accordingly, single-measurement MIC assessments were considered sufficient for initial activity detection. In addition, antibacterial activity was evaluated against a limited number of bacterial strains. Future studies including replicated experiments, expanded bacterial panels, and chemical profiling of active compounds will be necessary to confirm and extend these findings.

Conclusion

This study demonstrated that the antibacterial activity of lichen extracts was closely related to both the species characteristics and the extraction solvent used. Among the lichen species examined, *Ramalina farinacea* (L.) Ach showed the most extensive and consistent antibacterial capacity, effectively inhibiting the growth of all bacterial strains tested at relatively low MIC values. *Pseudevernia furfuracea* (L.) Zopf and *Platismatia glauca* (L.) W.L. Culb exhibited selective inhibitory activity, particularly against *Staphylococcus epidermidis*, a clinically important Gram-positive bacterium. The absence of antibacterial activity in aqueous extracts highlights the critical role of solvent polarity in the extraction process. This suggests that active compounds, particularly phenolic and depsidone-type molecules, are more effectively isolated with organic solvents such as acetone. Non-polar lichen metabolites are thought to be largely responsible for antimicrobial effects. The lichen species *Ramalina farinacea* (L.) Ach, in particular, may represent a promising source of biologically active molecules. The present findings demonstrate that acetone extracts of selected macrolichen species possess measurable antibacterial activity, whereas aqueous extracts show no detectable inhibitory effects. Among the tested species, *Ramalina farinacea* exhibited the strongest and broadest antibacterial activity, followed by *Pseudevernia furfuracea* and *Platismatia glauca*. These results highlight the importance of extraction method and species-specific metabolite composition in determining antimicrobial efficacy.

Although the study provides preliminary evidence supporting the antibacterial potential of lichen-derived extracts, further research involving replicated MIC analyses, expanded pathogen panels, and chemical characterization of bioactive compounds is required before pharmaceutical or biotechnological applications can be considered. These findings represent preliminary screening data and should not be interpreted as direct evidence of therapeutic applicability.

These findings indicate the potential of lichen-derived extracts as sources of antimicrobial compounds; however, further studies including chemical characterization, expanded bacterial panels, and in vivo validation are required. Future studies focusing on the purification, chemical characterization, and elucidation of the mechanisms of action of these secondary metabolites will strengthen the scientific basis for the development of lichen-derived natural antimicrobial drugs.

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Author contribution

TT performed data curation, analysis, methodology, project administration, and manuscript writing and revision. OB conducted the antibacterial assays using the MIC method. HKS collected and prepared the lichen samples and contributed to manuscript editing. FK carried out the laboratory extraction of lichen species.

Conflict of interest

The author declared that there is no conflict of interest.

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