Lichen Metabolites

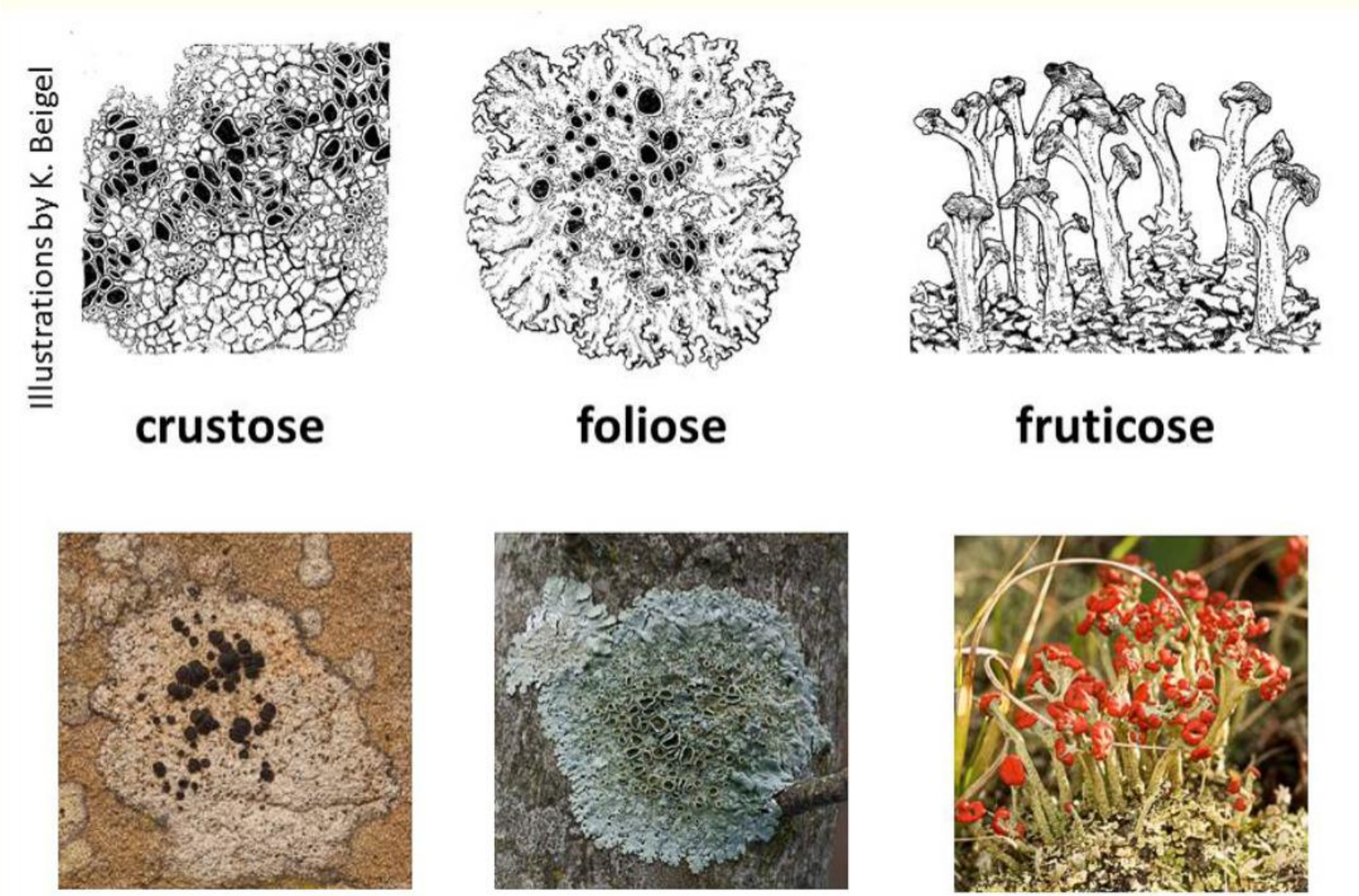
Nature’s Potent Multi-Functional Actives for Cosmetics

BioLichen

WE ARE LICHEN METABOLITES

www.biolichen.com

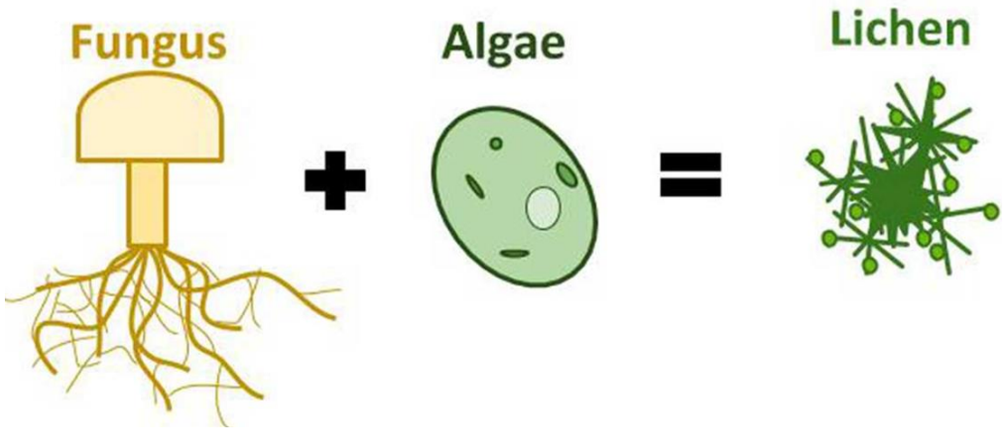
Lichen Types



Lichens are symbiotic organisms

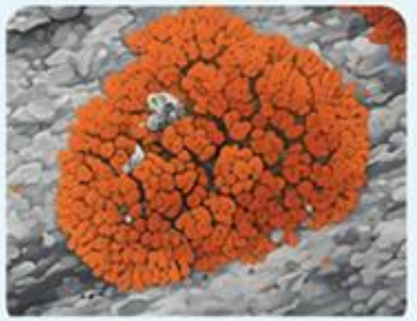
Symbiosis is a close, long-term, and persistent interaction between two or more different biological species.

These relationships are vital for survival.



External morphology:

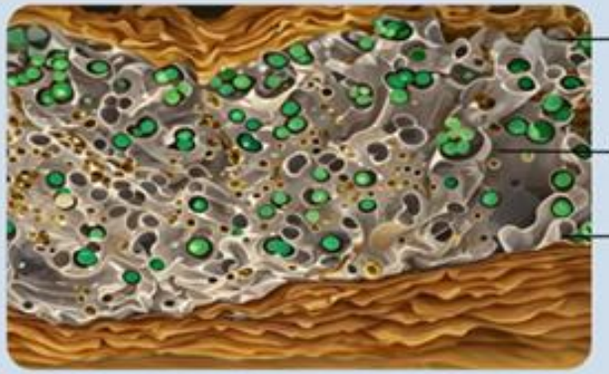
Foliose lichens



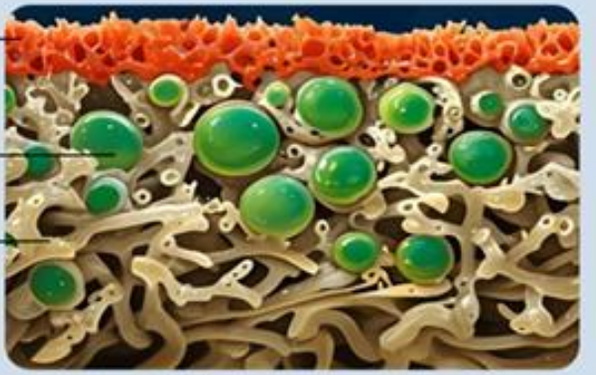
Crustose lichens

Fruticose lichens

Histological structure:



Homoiomerous

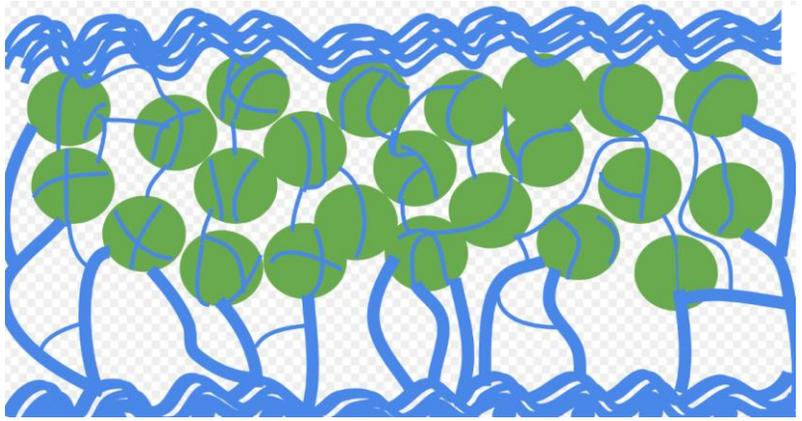


Heteromerous

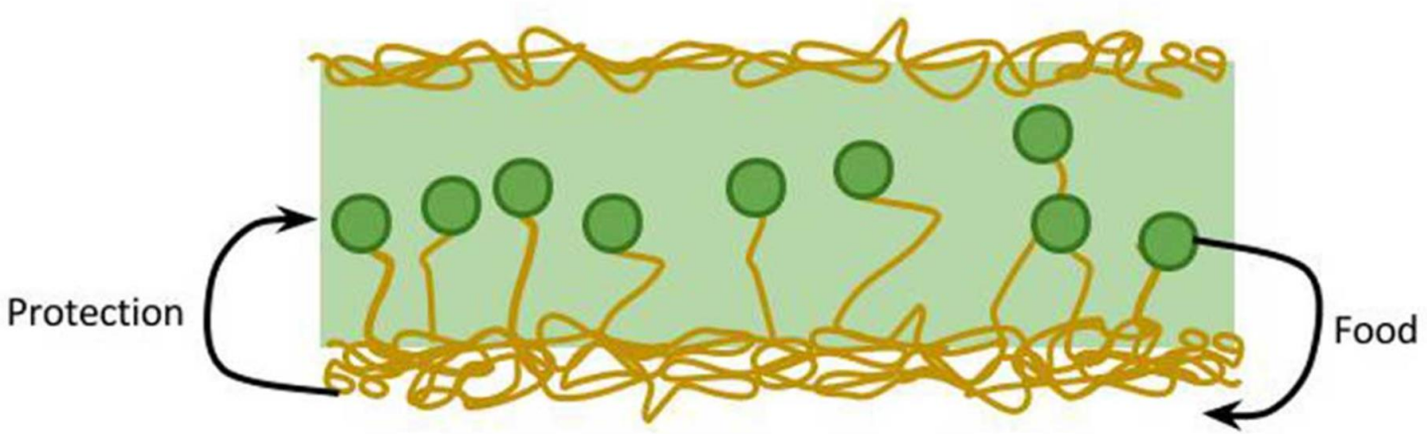
Lichens survive in diverse and nutrient-poor environments.

Lichens typically grow in ecological niches with limited resources; Rocky surfaces, tree bark, and exposed to harsh UV exposure.

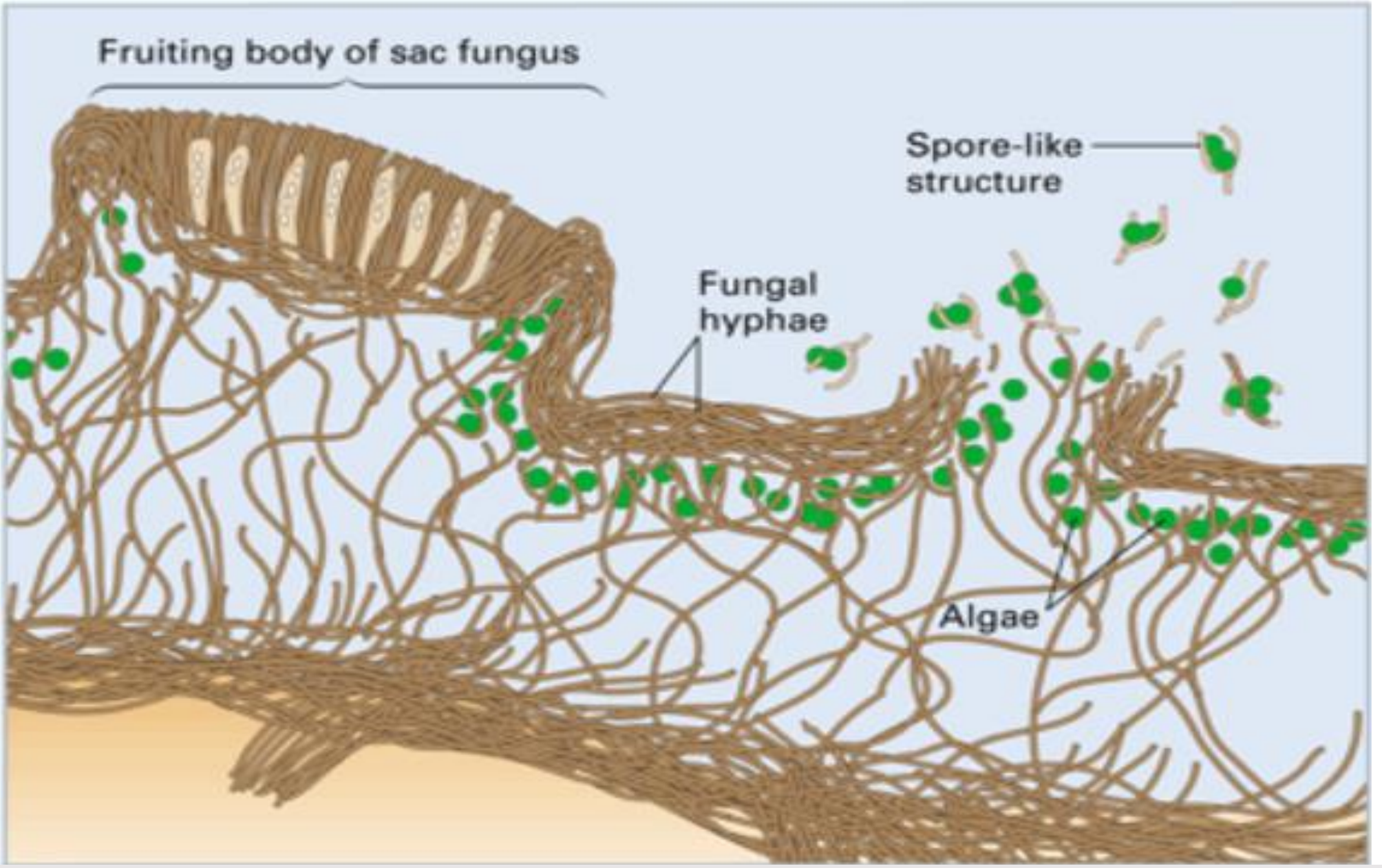
Photosynthesizing algae and fungi work in symphony to survive.



Photosynthetic sugars feed Fungal metabolism to produce Lichen Metabolites



The biological exchange in chemistry is closely related to defense mechanisms, niche competition, self-protective abilities, and adaptive evolution.


Fruiting body of sac fungus
Spore-like structure
Fungal hyphae
Algae

Antimicrobial compounds in lichens:

- Prevent microbial colonization and competition.
- Inhibit the growth of bacteria, fungi, and other microorganisms.
- Reduce competition from other microorganisms, enhancing survival capabilities, facilitating habitation, and reproduction.
- Some antimicrobial compounds also serve to protect their photosynthetic partners from UV radiation.

Lichens used in Traditional Medicine

- Lichens are used in traditional medicine by cultures across the world, particularly in temperate and arctic regions. Knowledge of these medicinal uses is available to us because of the contributions of traditional knowledge holders in these cultures.

- In ancient times, wounded soldiers and patients were known to press lichen into their wounds to help the healing process. Many European cultures have used lichen for curative decoctions.

- Anti-microbial properties of Lichen Metabolites explain the effectiveness in preventing pathogenesis and infection.

LICHEN METABOLITES

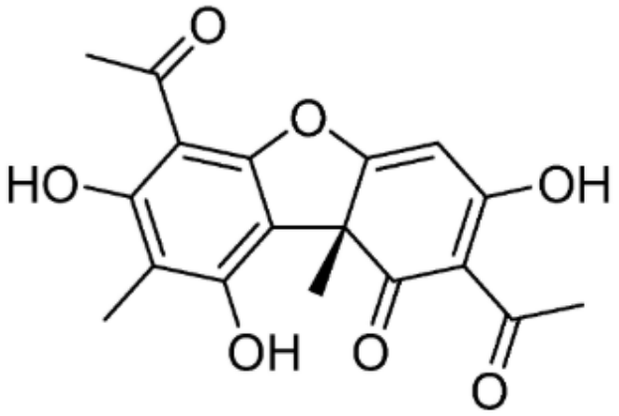
- Unique Molecules only found in Lichen.
- Potent bioactivity.
- Molecules stimulated by its harsh environmental conditions.
- Specific UV screening properties.
- Antimicrobial properties.

The chemical diversity and substantial biological activity of lichens present great potential for the development of novel antimicrobial applications.

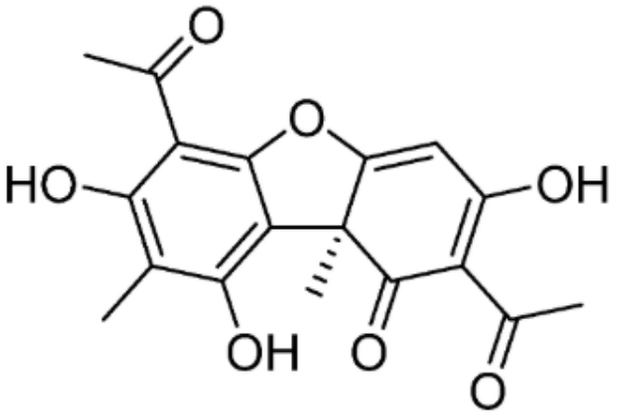
Lichens produce a wide array of structurally unique secondary metabolites, demonstrating high efficacy against bacterial, fungal, and viral pathogens.

This chemical diversity may provide new avenues for drug development and the discovery of natural antimicrobial agents.

Lichen Metabolites in Cladonia Sp.



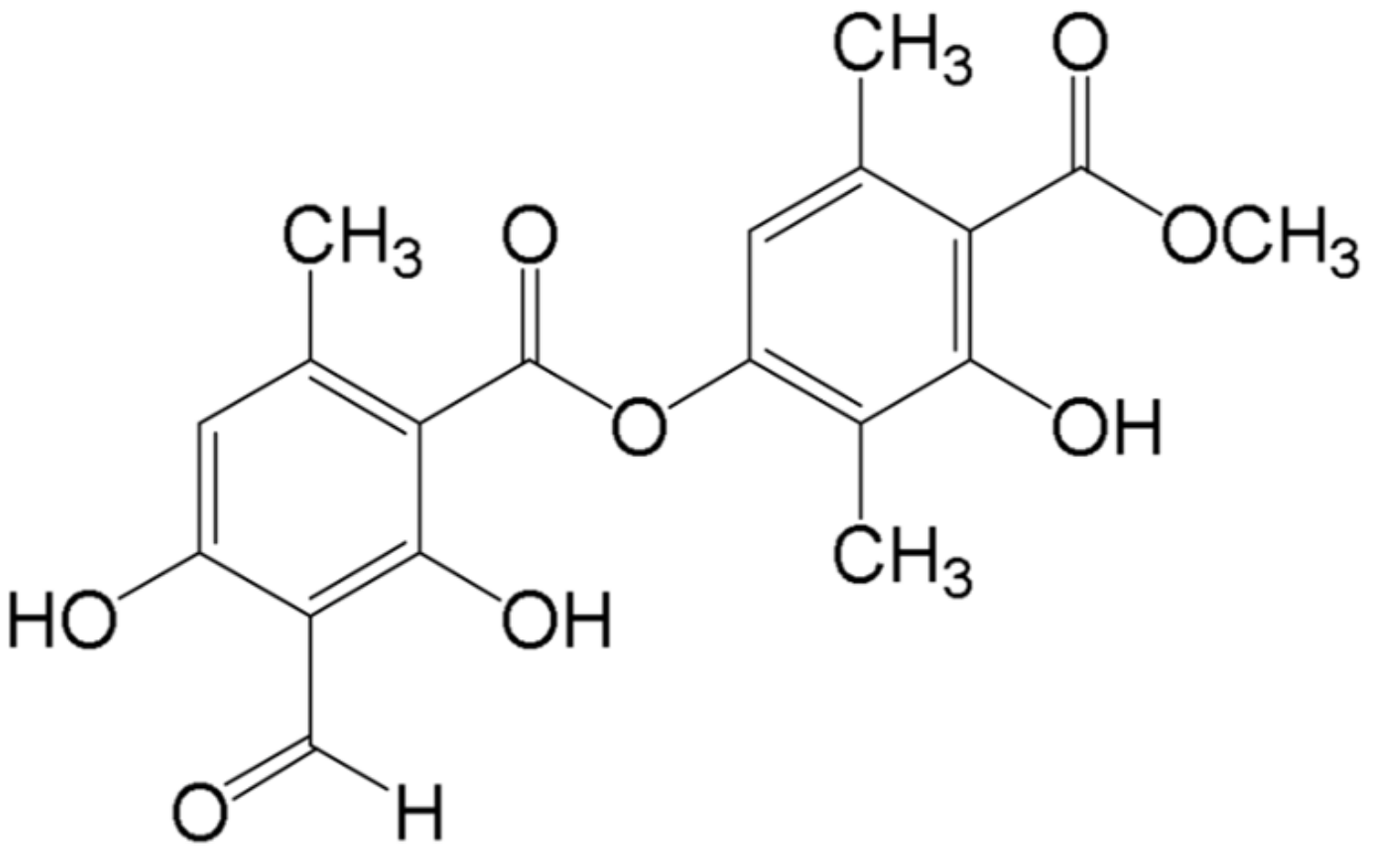
(+)-*(R)*-Usnic Acid



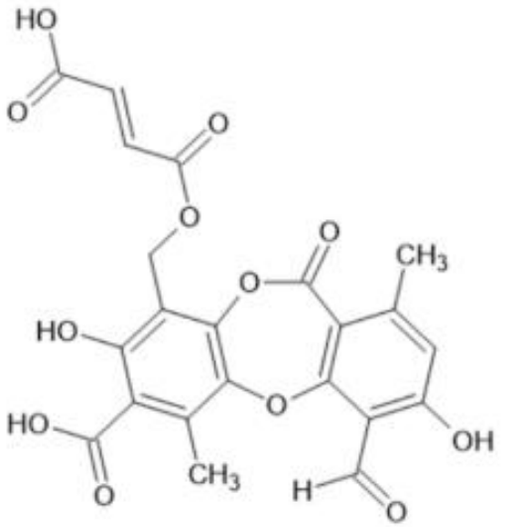
(-)-*(S)*-Usnic Acid

Lichen Metabolites in Cladonia Sp.

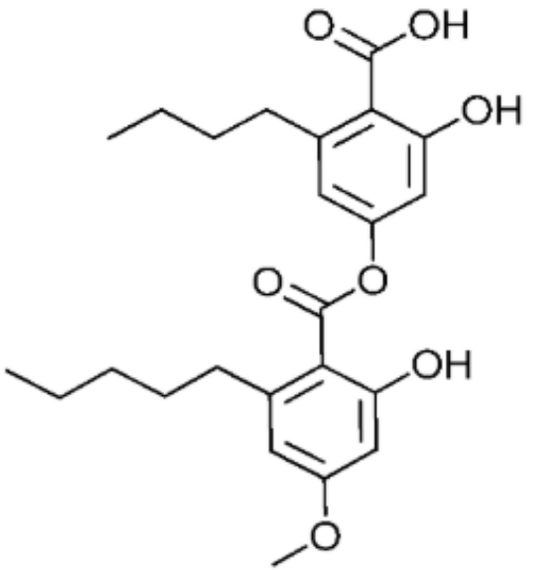
Figure 1. Chemical structure of Atranorin (molecular weight [Mw], 374.34).



Lichen Metabolites in Cladonia Sp.



Fumarprotocetraric acid



Perlatolic acid

Lichen Metabolite Biological Activity:

- Anti-microbial
- Anti-oxidant
- Wound- healing
- Photoprotective
- Reduces ROS stress
- Cytoprotective
- Anti-inflammatory
- Anti-Age (mTOR signaling & anti-glycating)*
- Anti- Cancer - many
- Neurotrophic
- Anti-neurodegenerative
- Anxiolytic/Antidepressant
- Anti-viral
- Anti-parasitic
- Anti-infective
- Anti-biofilm

Recent Publications in Scientific Journals - Atranorin

Atranorin from lichens act as potential inhibitor for cervical tumor proteins TGFbeta and kinase enzyme CDK4/CyclinD3: An anti-cancer intervention therapy. (2025)
Atranorin is a novel potential candidate drug for treating myelodysplastic syndrome. (2024)
Atranorin inhibits NLRP3 inflammasome activation by targeting ASC and protects NLRP3 inflammasome-driven diseases. (2023)
Atranorin driven by nano materials SPION lead to ferroptosis of gastric cancer stem cells by weakening the mRNA 5-hydroxymethylcytidine modification of the Xc-/GPX4 axis and its expression. (2022)
Atranorin Impedes Glioma Invasiveness and Progression by Inhibiting the Epithelial-Mesenchymal Transition and Stemness in Monotherapy and in Combination Therapy With Temozolomide. (2026)
Atranorin, an antimicrobial metabolite from lichen Parmotrema rampoddense exhibited in vitro anti-breast cancer activity through interaction with Akt activity. (2021)
Atranorin, a Secondary Metabolite of Lichens, Exhibited Anxiolytic/Antidepressant Activity in Wistar Rats. (2022)
In Vitro and in Silico Studies of Lichen Compounds **Atranorin** and Salazinic Acid as Potential Antioxidant, Antibacterial, and Anticancer Agents. (2023)
Effects of **atranorin** on the proliferation, apoptosis and metastasis of two different gastric cancer cell lines and exploring underlying mechanisms. (2026)
Murine breast carcinoma 4T1 cells are more sensitive to atranorin than normal epithelial NMuMG cells in vitro: Anticancer and hepatoprotective effects of **atranorin** in vivo. (2016)
Redox properties and cytoprotective actions of **atranorin**, a lichen secondary metabolite. (2011)
Evaluation of wound healing activity of **atranorin**, a lichen secondary metabolite, on rodents. (2013)

Recent Publications in Scientific Journals – Atranorin Cont'd

The Comprehensive Roles of **ATRANORIN**, A Secondary Metabolite from the Antarctic Lichen Stereocaulon caespitosum, in HCC Tumorigenesis. (2019)
Usnic acid and **atranorin** exert selective cytostatic and anti-invasive effects on human prostate and melanoma cancer cells. (2017)
Targeting Ferroptosis with Small Molecule **Atranorin** (ATR) as a Novel Therapeutic Strategy and Providing New Insight into the Treatment of Breast Cancer. (2024)
Bioassay-guided evaluation of wound healing effect of **atranorin** in rodents. (2011)
Antiglycating and Antioxidant Properties of **Atranorin** Isolated from Lichen Cladonia rangiferina. (2025)*
The lichen secondary metabolite **atranorin** suppresses lung cancer cell motility and tumorigenesis. (2017)
Biochemical Properties of **Atranorin**-Induced Behavioral and Systematic Changes of Laboratory Rats. (2022)
Anti-inflammatory and toxicity studies of **atranorin** extracted from Cladina kalbii Ahti in rodents. (2011)
Depsides: lichen metabolites active against hepatitis C virus. (2015)
Antimicrobial and antibiofilm activity of secondary metabolites of lichens against methicillin-resistant Staphylococcus aureus strains from cystic fibrosis patients. (2013)
Bioactive Compounds and Their Influence on Postnatal Neurogenesis. (2023)
Cladonia lichens and their major metabolites as possible natural antioxidant, antimicrobial and anticancer agents. (2014)

Recent Publications in Scientific Journals – Usnic acid

Usnic acid alleviates pulmonary fibrosis in vitro and in vivo by inhibiting the ZNF70-mediated Wnt/β-catenin signaling pathway. (2025)
Usnic Acid induces dual-pathway apoptosis in SKOV-3 ovarian cancer cells via PARP1 inhibition and MAPK pathway activation. (2025)
Usnic acid suppresses inflammation and endoplasmic reticulum stress in a methotrexate-induced pulmonary toxicity model via modulating Nrf2 pathway. (2025)
Usnic acid alleviates inflammatory responses and induces apoptotic signaling through inhibiting NF-κB expressions in human oral carcinoma cells. (2024)
Usnic acid induced changes in biomolecules and their association with apoptosis in squamous carcinoma (A-431) cells: A flow cytometry, FTIR and DLS spectroscopic study. (2022)
Usnic Acid-Loaded Polymeric Micelles: An Optimal Migrastatic-Acting Formulation in Human SH-SY5Y Neuroblastoma Cells. (2022)
Usnic acid impacts energy production and iron metabolism in Mycobacterium tuberculosis H37Rv. (2025)
Usnic acid brief exposure suppresses cariogenic properties and complexity of Streptococcus mutans biofilms. (2025)

Recent Publications in Scientific Journals – Usnic acid Cont'd

Anti-Inflammatory Effects of **Usnic Acid** in Breast Cancer. (2026)
Antibacterial, antibiofilm, and antioxidant impacts of **usnic acid** against vancomycin-resistant enterococcus. (2024)
Nanocomposite gel containing **usnic acid**: Characterization and evaluation of the antibacterial efficacy on Staphylococcus epidermidis. (2025)
Electrospun Nanofibrous Mesh Based on PVA, Chitosan, and **Usnic Acid** for Applications in Wound Healing. (2023)
Usnic Acid extends healthspan and improves the neurodegeneration diseases via mTOR/PHA-4 signaling pathway in Caenorhabditis elegans (roundworm). (2022)*
Supertool for Superbugs—Smart "Nano-ointment of Graphene **Usnic Acid** Nanoparticles" Against Antimicrobial Resistance (AMRs). (2023)
Enhanced wound-healing capability with inherent antimicrobial activities of **usnic acid** incorporated poly(ε-caprolactone)/decellularized extracellular matrix nanofibrous scaffold. (2022)
Multifaceted Properties of **Usnic Acid** in Disrupting Cancer Hallmarks. (2024)
Critical Assessment of the Anti-Inflammatory Potential of **Usnic Acid** and Its Derivatives-A Review. (2023)
Effects of the Dibenzofuran, **Usnic Acid**, on Inhibition of Ocular Biofilm Formation Due to Coagulase-Negative Staphylococci. (2023)
Unassuming Lichens: Nature's Hidden Antimicrobial Warriors. (2025)

Publications in Scientific Journals – Fumarprotocetraric acid

Fumarprotocetraric Acid Inhibits Tau Covalently, Avoiding Cytotoxicity of Aggregates in Cells. (2021).

Fumarprotocetraric acid and geraniin were identified as novel inhibitors of human respiratory syncytial virus infection in vitro. (2024)

Expectorant and antioxidant activities of purified **fumarprotocetraric acid** from Cladonia verticillaris lichen in mice. (2014)

In vitro neuroprotective potential of lichen metabolite **fumarprotocetraric acid** via intracellular redox modulation. (2017)

Links for Effects of **Fumarprotocetraric Acid**, a Depsidone from the Lichen Cladonia verticillaris, on Tyrosinase Activity. (2017)

Antimicrobial and antibiofilm activity of secondary metabolites of lichens against methicillin-resistant Staphylococcus aureus strains from cystic fibrosis patients. (2013)

Table 1.1 Literature sources mentioning data for some lichen substances with different pharmaceutical activity

Lichen compounds	Studied activities	References
Lecanoric acid	Antitumor, antioxidant, antibacterial, antifungal, antidiabetic, anticancer, anti-inflammatory	Gomes et al. (2003), Lopes et al. (2008), Ranković et al. (2008), Honda et al. (2010), Bogo et al. (2010), Choudhary et al. (2011), Ristić et al. (2016b), Studzinska-Sroka and Dubino (2018)
Atranorin	Antimicrobial, antioxidant, anti-inflammatory, anticancer, antineurodegenerative, antidiabetic	Kumar and Muller (1999), Yilmaz et al. (2004), Turk et al. (2006), Ranković et al. (2008, 2014), Melo et al. (2011), Reddy et al. (2016), Thadhani and Karunaratne (2017), Studzinska-Sroka and Dubino (2018)
Zeorin	Antioxidant, antibacterial, antifungal, antidiabetic	Behera et al. (2008), Kosanić et al. (2010), Karunaratne et al. (2014)
Gyrophoric acid	Antimicrobial, anticancer, antioxidant, antidiabetic	Candan et al. (2006), Ranković et al. (2008), Burlando et al. (2009), Choudhary et al. (2011), Kosanić et al. (2014a, b)
Protocetraric acid	Antibacterial, antifungal, antioxidant, anticancer	Tay et al. (2004), Ranković et al. (2008), Honda et al. (2010), Manojlović et al. (2012)
Fumarprotocetraric acid	Antibacterial, antifungal, antioxidant, anticancer, neuroprotective	Yilmaz et al. (2004), Ranković et al. (2008), Kosanić et al. (2014a, b), Fernández-Moriano et al. (2017)
Stictic acid	Antioxidant, antimicrobial, anticancer	Lohézic-Le Dévéhat et al. (2007), Ranković et al. (2008)
Salazinic acid	Antitumor, antibacterial, antifungal, antioxidant, antidiabetic, antiviral	Candan et al. (2007), Burlando et al. (2009), Manojlović et al. (2012), Verma et al. (2012), Odabasoglu et al. (2006)
Usnic acid	Antiviral, antitumor, antioxidant, antibacterial, antifungal, antipyretic, analgetic, anti-inflammatory, hepatotoxic, antiviral, antidiabetic	Lauterwein et al. (1995), Tay et al. (2004), Ranković et al. (2008, 2014), Paudel et al. (2010), Perry et al. (1999), Odabasoglu et al. (2006), Bazin et al. (2008), Burlando et al. (2009), Ramos and Almeida da Silva (2010), Verma et al. (2012), Thadhani and Karunaratne (2017), Vijayakumar et al. (2000), Okuyama et al. (1995)

Usnic Acid mechanisms of antimicrobial action

- Inhibition of RNA, DNA, and Protein synthesis.
- Disruption of the stability of cell membranes.
- Reprogramming Homeostasis – Redox, lipid synthesis, nucleic acid repair – altering metabolic state.
- Inhibition/ disruption of bacterial biofilm.
- Inhibition of the Efflux pump protein (Membrane drug resistant protease activity).

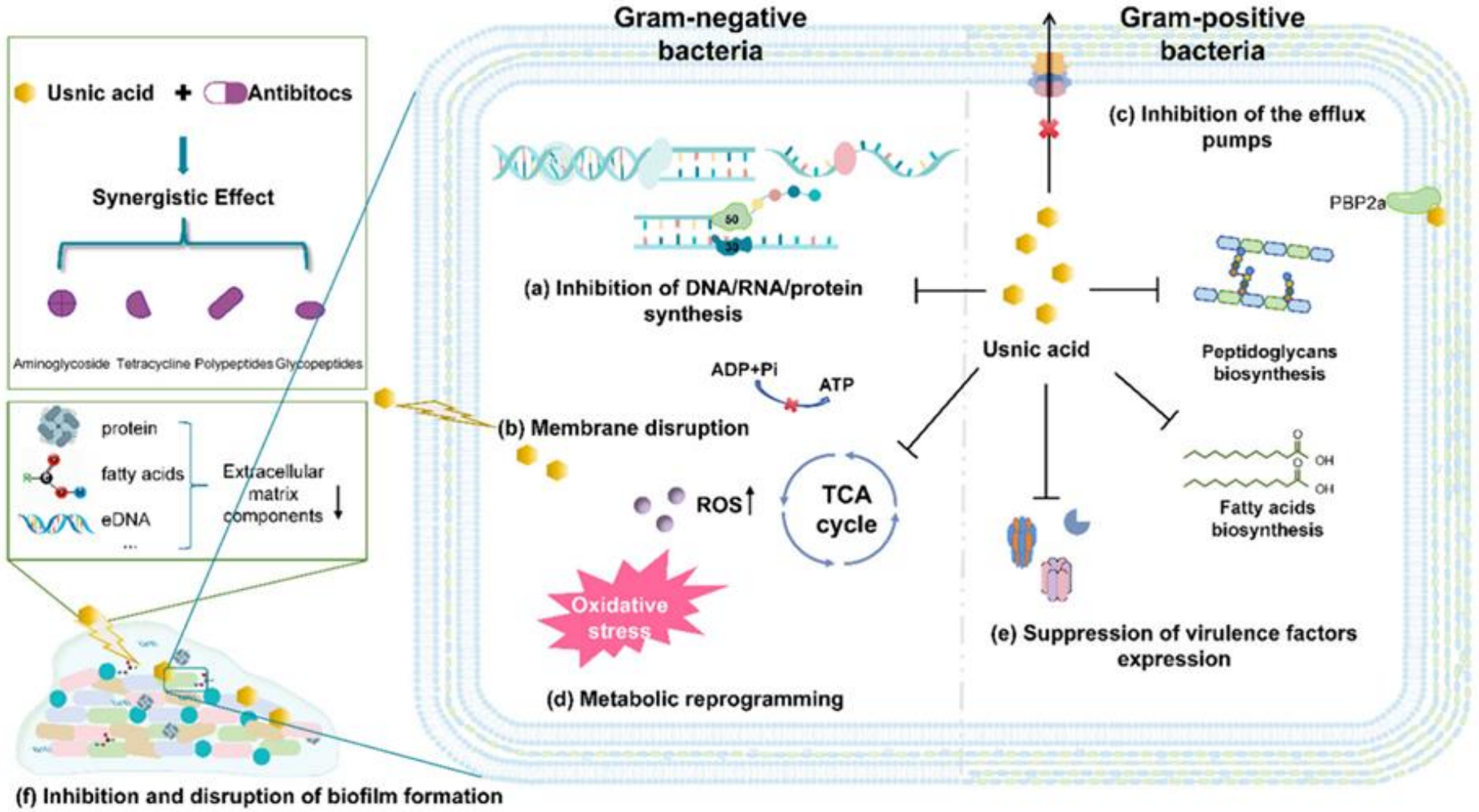


Figure 2. Schematic diagram of the diverse antibacterial mechanisms of usnic acid: (a) inhibition of nucleic acid and protein synthesis; (b) enhancement of cell membrane permeability and (c) suppression of efflux pump activity, thereby promoting drug accumulation; (d) disruption of metabolic pathways leading to disturbance of fatty acid metabolism, peptidoglycan biosynthesis, and oxidative stress; (e) suppression of virulence factor expression; (f) inhibition and disruption of biofilm formation.

COSMOSPRESERVE™

INCI: Triethyl Citrate, Cladonia (Rangiferina/Stellaris) Extract.

Characteristics of CosmosPreserve	
Appearance	Transparent yellow liquid
INCI Name	Triethyl Citrate, Cladonia (Rangiferina/Stellaris) Extract
Recommended Dosage	0.75-4%
pH Range	3-7.0
Analytical Assay (HPLC)	Usnic Acid isomers > 4.0 mg/g, Atranorin > 0.2 mg/g, Fumarprotocetraric acid 0.2 mg/g.
Application Guide	- In emulsion: Preferably applied during the cooldown phase <45°C. - In cold emulsion: Preferably applied in the lipid phase. - In surfactant system: Applied in the final step.

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or   :                          Detector Type: 335
ation:                          Bus Address  : 44
ment : Varian Star #1           Sample Rate  : 10.00 Hz
1    : 1 = 280 nm               Run Time     : 23.947 min

Workstation Multi Instrument Version 6.30 ** 00814-76C8-AEB-2224 **

Speed =    0.87 cm/min   Attenuation = 33          Zero Offset = 3%
Time  =    0.000   min     End Time    = 23.947  min   Min / Tick  = 1.00
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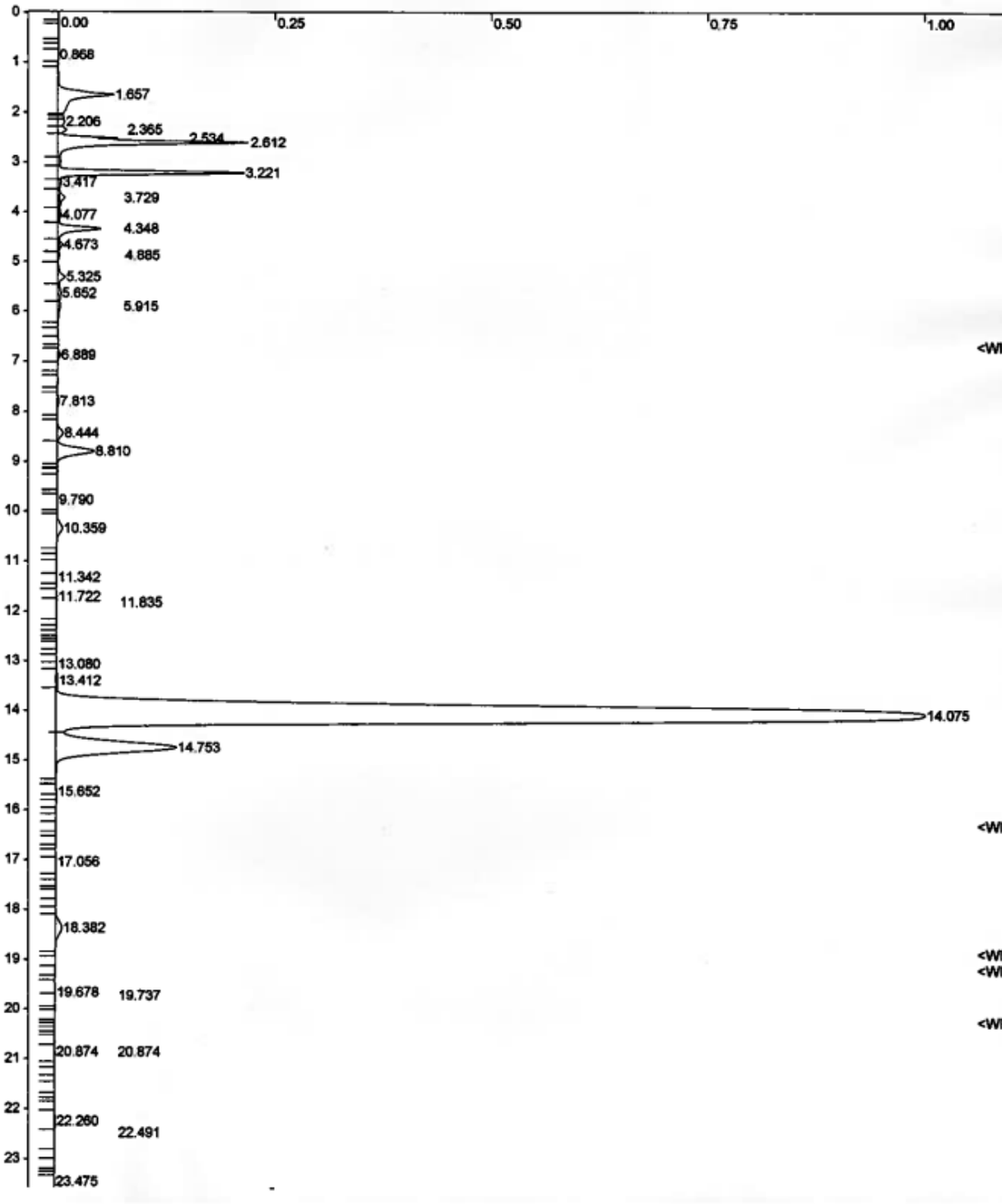
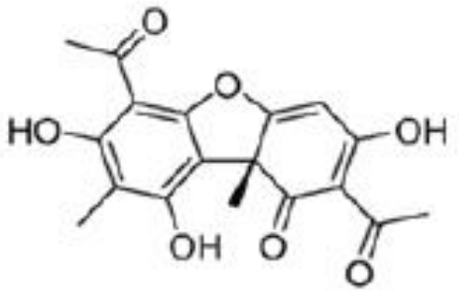
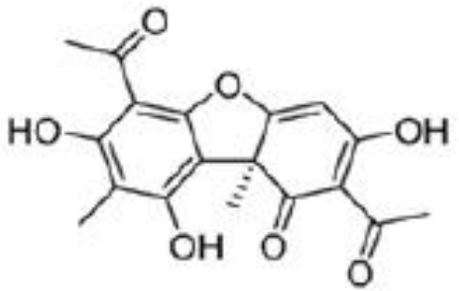
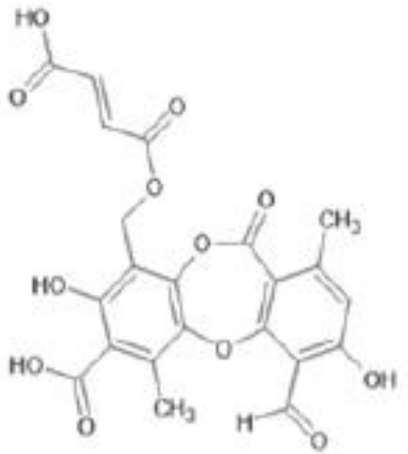


Lichen Metabolite structures in Cladonia spp.

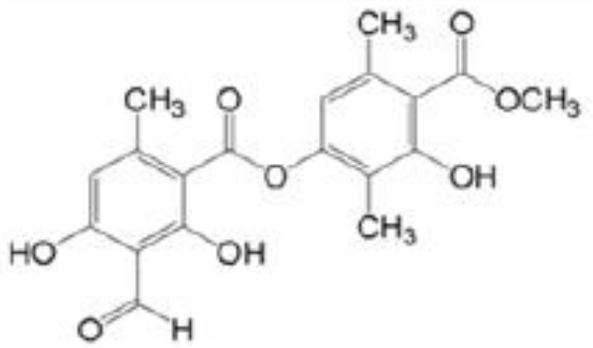




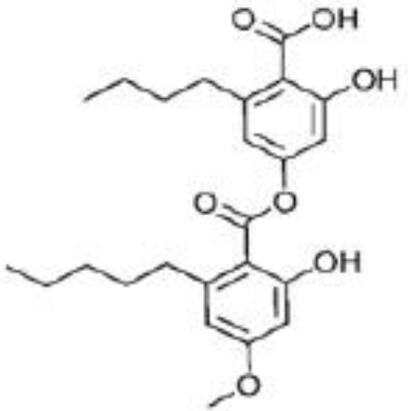
Usnic acid Isomers



Fumarprotocetraric acid



Atranorin



Perlatolic acid

Preservative Effectiveness Testing Results

Formula at pH < 6.5

- Effective at 0.75% - 1.5%
- Broad-spectrum effect
- Passes all AET protocols
- Formulation dependent

Formula pH >6.5

- Effective at 1.5% & higher
- Broad-spectrum effect
- Passes all AET protocols
- Formulation dependent

Typical standard formulations tested for AET

Water, Stearic Acid, Palmitic Acid, Coco-Caprylate, Glycerine, Cetyl Alcohol, Glyceryl Stearate, Linoleic Acid, Linolenic Acid, Sodium Hydroxide, Citric Acid, Tocopherol.

+ CosmosPreserve

Water, MCT oil, Sunflowerseed oil, Glyceryl Citrate, Cetyl Alcohol, Xanthan gum, Tocopherol.

+ CosmosPreserve

Preservative Effectiveness Testing
pH 5.5 & dosage 1% CosmosPreserve

Strain	E. coli	P. aeruginosa	S. aureus	C. albicans	A. brasiliensis
Inoculum (10^5)	4.5 x10^5	4.5 x10^5	4.5 x10^5	3.0 x10^5	3.0 x10^5
CFU Day 7	50	<10	<10	<10	10
Reduction (log)	3.95	>4.65	>4.65	>4.48	4.48
CFU Day 14	<10	<10	<10	<10	<10
Reduction (log)	>4.65	>4.65	>4.65	>4.48	>4.48
CFU Day 28	<10	<10	<10	<10	<10
Reduction (log)	>4.65	>4.65	>4.65	>4.48	>4.48

Preservative Effectiveness Testing
pH 6.5 & dosage 1.5% CosmosPreserve

Strain	E. coli	P. aeruginosa	S. aureus	C. albicans	A. brasiliensis
Inoculum (10^5)	4.5 x10^5	4.5 x10^5	4.5 x10^5	3.0 x10^5	3.0 x10^5
CFU Day 7	400	80	<10	30	20
Reduction (log)	3.05	3.75	>4.65	4.00	4.18
CFU Day 14	<10	<10	<10	<10	<10
Reduction (log)	>4.65	>4.65	>4.65	>4.48	>4.48
CFU Day 28	<10	<10	<10	<10	<10
Reduction (log)	>4.65	>4.65	>4.65	>4.48	>4.48

COSMOSPRESERVE™ COMPONENTS WORK SYNERGISTICALLY

- The composition of TEC and dissolved metabolites collectively affects the antimicrobial load.
- Usnic acid & Atranorin, and other trace lichen metabolites work together.
- Efficacy is dose-dependent and will depend on the final pH.
- Typical dose 1-2% pH 5 to 6.5

Anti-Inflammatory effects of Usnic acid & Atranorin

Literature Review

Atranorin inhibits NLRP3 inflammasome activation by targeting ASC and protects NLRP3 inflammasome-driven diseases

Hao-yu Wang[1,2], Xi Lin[3], Guan-gen Huang[2,3], Rong Zhou[2], Shu-yue Lei[1,2], Jing Ren[2,3], Kai-rong Zhang[2,4], Chun-lan Feng[2], Yan-wei Wu[2,5] and Wei Tang[1,2,3]

Aberrant NLRP3 activation has been implicated in the pathogenesis of numerous inflammation-associated diseases. However, no small molecular inhibitor that directly targets NLRP3 inflammasome has been approved so far. In this study, we show that Atranorin ($C_{19}H_{18}O_8$), the secondary metabolites of lichen family, effectively prevents NLRP3 inflammasome activation in macrophages and dendritic cells. Mechanistically, Atranorin inhibits NLRP3 activation induced cytokine secretion and cell pyroptosis through binding to ASC protein directly and therefore restraining ASC oligomerization. The pharmacological effect of Atranorin is evaluated in NLRP3 inflammasome-driven disease models. Atranorin lowers serum IL-1β and IL-18 levels in LPS induced mice acute inflammation model. Also, Atranorin protects against MSU crystal induced mice gouty arthritis model and lowers ankle IL-1β level. Moreover, Atranorin ameliorates intestinal inflammation and epithelial barrier dysfunction in DSS induced mice ulcerative colitis and inhibits NLRP3 inflammasome activation in colon. Altogether, our study identifies Atranorin as a novel NLRP3 inhibitor that targets ASC protein and highlights the potential therapeutic effects of Atranorin in NLRP3 inflammasome-driven diseases including acute inflammation, gouty arthritis and ulcerative colitis.

Keywords: Atranorin; NLRP3; inflammasome; ASC; acute inflammation; gouty arthritis; colitis

Evaluation of wound healing activity of atranorin, a lichen secondary metabolite, on rodents

Rosana S. S. Barreto,[1,a] Ricardo L. C. Albuquerque-Júnior,[3,a] Rose Nely Pereira-Filho,[3] Jullyana S. S. Quintans,[1] André S. Barreto,[1] Josimari M. DeSantana,[2] Valter J. Santana-Filho,[2] Marcio R. V. Santos,[1] Leonardo R. Bonjardim,[1] Adriano A. S. Araújo,[1] Lucindo J. Quintans-Júnior[,1]*

[1]*Departamento de Fisiologia, Universidade Federal de Sergipe, Brazil,*
[2]*Departamento de Fisioterapia, Universidade Federal de Sergipe, Brazil,*
[3]*Instituto de Tecnologia e Pesquisa, Universidade Tiradentes, Brazil.*

Abstract: The aim of this study was to investigate the wound healing activity of atranorin cream (Patent requested) on excision wounds. Seventy-two male rats were anesthetized and an excisional wound was performed. Then the rats were randomly assigned into three groups: untreated control group; atranorin 1 (group treated with 1% AT ointment); and atranorin 5 (group treated with 5% AT ointment). Six animals of each group were euthanized 3, 7, 14 or 21 days after surgical procedures and the wounded areas were analyzed and removed. Serial histological sections were obtained and stained by histochemical techniques (Hematoxilin-Eosin-HEand Sirius red) and immunohistochemical techniques. Topical application of atranorin reduced wound areas, induced earlier granulation tissue formation, increased cell proliferation, improved collagenization and modulated the myofibroblasts differentiation when compared to control animals. It is suggested that atranorin modulates the wound healing process. These data suggest that this formulation based on atranorin extracted from *Cladina kalbii* AHTI may be a new biotechnological product for wound healing clinical applications.

In Vitro and *in Silico* Studies of Lichen Compounds Atranorin and Salazinic Acid as Potential Antioxidant, Antibacterial and Anticancer Agents

Subhash B. Gaikwad,[a, b] Sachin V. Mapari,[a, b] Ruchira R. Sutar,[a, b] Muntjeeb Syed,[b] Roshni Khare,[b] and Bhaskar C. Behera*[a]

Lichens are symbiotic organisms made up of alga/cyanobacterium and fungus. We investigated antioxidant, antibacterial and anticancer properties of two lichen compounds, atranorin and salazinic acid, and five lichen species: *Heterodermia boryi, Heterodermia diademata, Heterodermia hypocaesia, Parmotrema reticulatum*, and *Stereocaulon foliolosum*. Free radical scavenging, Ferric reducing potential, Nitric oxide scavenging, and Trolox equivalent capacity were used to measure antioxidant activity. Strong radical scavenging action was demonstrated by atranorin and salazinic acid, with IC_{50} values of 39.31 µM and 12.14 µM, respectively. The Minimum Inhibitory Concentration (MIC) assay based on resazurin, was used to measure antibacterial activity. *Parmotrema reticulatum* demonstrated significant antibacterial activity against *Raoultella planticola* with MIC of 7.8 µg/mL. Cytotoxicity assay on breast cancer cell line was used to assess anticancer activity. To further understand the binding locations on the target proteins Er (Estrogen Receptor alpha), EGFR (Epidermal Growth Factor Receptor), mTOR (Mammalian Target of Rapamycin), and PgR (Progesterone Receptor), molecular docking experiments were conducted. Docking study showed that the binding energies of atranorin and salazinic acid with mTOR were −5.31 kcal/mol and −3.43 kcal/mol, respectively. The results suggest that atranorin has the potential to be a multitargeted molecule with natural antioxidant, antibacterial, and anticancer properties.

Redox properties and cytoprotective actions of atranorin, a lichen secondary metabolite

Marcelia Garcez Dória Melo [a], João Paulo Almeida dos Santos [a], Mairim Russo Serafini [a], Fernanda Freitas Caregnato [b], Matheus Augusto de Bittencourt Pasquali [b], Thallita Kelly Rabelo [a], Ricardo Fagundes da Rocha [b], Lucindo Quintans Jr. [a], Adriano Antunes de Souza Araújo [a], Francilene Amaral da Silva [a], José Cláudio Fonseca Moreira [b], Daniel Pens Gelain [b,*]

[a] *Departamento de Fisiologia, Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brazil*
[b] *Centro de Estudos em Estresse Oxidativo, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*

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ABSTRACT

Atranorin (ATR) is a lichenic secondary metabolite with potential uses in pharmacology. Antinociceptive and antiinflammatory actions have been reported, and the use of atranorin-enriched lichen extracts in folk medicine is widespread. Nonetheless, very few data on ATR biological actions are available. Here, we evaluated free radical scavenging activities and antioxidant potential of ATR using various *in vitro* assays for scavenging activity against hydroxyl radicals, hydrogen peroxide, superoxide radicals, and nitric oxide. The total reactive antioxidant potential (TRAP) and total antioxidant reactivity (TAR) indexes and *in vitro* lipoperoxidation were also evaluated. Besides, we determined the cytoprotective effect of ATR on H_2O_2-challenged SH-SY5Y cells by the MTT assay. ATR exerts differential effects towards reactive species production, enhancing hydrogen peroxide and nitric oxide production and acting as a superoxide scavenger; no activity toward hydroxyl radical production/scavenging was observed. Besides, TRAP/TAR analysis indicated that atranorin acts as a general antioxidant, although it demonstrated to enhance peroxyl radical-induced lipoperoxidation *in vitro*. ATR was not cytotoxic, and also protected SH-SY5Y cells against H_2O_2-induced cell viability impairment. Our results suggest that ATR has a relevant redox-active action, acting as a pro-oxidant or antioxidant agent depending on the radical. Also, it will exert cytoprotective effects on cells under oxidative stress induced by H_2O_2.

2.1. Anti-Inflammatory Effects

Inflammation is the body's response to foreign antigens or tissue damage that may result in loss of tissue structure and function.

Different acute doses of usnic acid can reduce swelling in rat foot induced by carrageenan in acute and chronic inflammation rat models and the alleviating effects are dose dependent. However, this phenomenon was significantly reduced only at 100 mg/kg usnic acid [18]. Similar to nonsteroidal anti-inflammatory drugs, usnic acid has an anti-inflammatory mechanism that may inhibit prostaglandin synthesis. Further studies showed that different concentrations of usnic acid and self-microemulsion of usnic acid can reduce tumor necrosis factor-alpha (TNF-α) induced by heat-killed *Escherichia coli* (EC) at varying degrees with a dose–effect relationship [58].

Usnic acid reduces TNF-α level in a dose-dependent manner and inhibits NO production in lipopolysaccharide (LPS)-activated RAW264.7 macrophages [59]. It can inhibit the expression of TNF-α and inducible nitric oxide synthase (iNOS), possibly by inhibiting the nuclear translocation of NF-κB p65 and the degradation of I-κBα and ultimately exerts anti-inflammatory effects. A study [60] explored the anti-inflammatory effects and corresponding mechanisms of usnic acid on RAW264.7 cells stimulated by LPS and found that usnic acid plays an anti-inflammatory role by inhibiting the activation of NF-κB, increasing the production of IL-10 and HO-1, and downregulating the expression of iNOS, IL-6, IL-1β, TNF-α, and COX-2 genes.

Moreover, usnic acid has a neuroprotective effect on Parkinson's disease (PD), affecting motor dysfunction in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD model [61]. The simultaneous administration of MPTP and usnic acid can attenuate MPTP-induced motor dysfunction in a time-dependent manner. Although usnic acid cannot prevent 1-methyl-4-phenyl-pyridine ion (MPP^+)-induced primary neuronal death by blocking the activation of NF-κB to reduce the activation of astrocytes, usnic acid can inhibit the inflammatory signaling pathway induced by MPP^+ and protect dopaminergic neurons.

Table 1. Anti-inflammatory activities and mechanism of usnic acid.

Model	Mechanism or Effect	Concentration	Year	References
Acute and chronic inflammation rat models	Reduce the swelling of rat foot induced by carrageenan in dose dependent. Only effect at 100 mg/kg.	25 mg/kg, 50 mg/kg, 100 mg/kg, p.o.	2000	[18]
Lipopolysaccharide (LPS) activated RAW264.7 macrophages	Inhibit the express of TNF-α and iNOS, possibly through suppression of nuclear translocation of NF-κB p65 and I-κBα degradation; Inhibit LPS-induced TNF-α accumulation and NO production in a dose-dependent manner.	0.5–400 μM; IC_{50}: 4.7 μM (TNF-α), 12.8 μM (NO)	2008	[59]
Lipopolysaccharide (LPS) activated RAW264.7 macrophages	Down-regulating iNOS, COX-2, IL-1β, IL-6 and TNF-α, COX-2 gene expression through the suppression of NF-κB activation and increasing anti-inflammatory cytokine IL-10 and anti-inflammatory mediator HO-1 production.	10 μg/mL, 50 μg/mL and 100 μg/mL	2011	[60]
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced Parkinson's disease model in Male C57BL/6 mice	Inhibit MPP^+-induced glial activation in primary astrocytes by blocking NF-κB activation.	5 or 25 mg/kg dissolved in phosphate buffered saline (PBS) containing 5% Tween-80 (i.p.)	2020	[61]
Tau protein derived hexapeptide AcPHF6 model	Inhibit the aggregation of full-length 2N4R tau protein by a heparin-induced mechanism.	10 μM	2020	[62] *
Human neuroblastoma SK-N-SH cell line (SH-SY5Y)	Exert no significant hepatotoxicity, showed low hepatotoxicity at 40 μM.	10–40 μM	2020	[62] *
Human hepatocyte cells (LO2) cells	Exert no significant hepatotoxicity, showed low hepatotoxicity at 40 μM.	10–40 μM	2020	[62] *
Murine microglial BV2 cells	Reduce NO release in Lipopolysaccharide-stimulated mouse microglia BV2 cells.	10–40 μM	2020	[62] *
Adult male SD rats	Enhance the cognitive ability of okadiac acid induced AD model rats.	5 mg/kg, 10 mg/kg	2020	[62] *

2.5. *Wound Healing*

Wound healing is a complex process divided into three phases: inflammation, proliferation, and remodeling. These involve a variety of coordinated cellular activities, such as migration to wound areas, proliferation, and extracellular matrix deposition and remodeling.

Different concentrations of sodium usnic acid were added to L929 fibroblasts in vitro for the study of its effect on fibroblast proliferation [99]; the results showed that sodium usnic acid can promote wound healing, but not by stimulating the proliferation of fibroblasts. Sodium usnic acid can decrease inflammatory cells and promote the proliferation of fibroblasts and granulation tissues and vascular regeneration in wounds [25]. Moreover, it can promote the early generation of epithelial cells, formation of a well-organized collagen band, and epidermal keratinization and significantly increase VEGF level.

Pagano et al. [100] investigated different adhesive polymer films that improve the bioavailability of usnic acid in injury body; their study was based on the following: excellent inhibition ability of usnic acid against the common pathogens of burn wounds, such as Gram-positive bacteria and anaerobes [16,58,63–67], the anti-inflammatory effect of usnic acid [60], and the ability of usnic acid to stimulate keratinocyte monolayers to promote wound closure.

The effects of the five main polyketones in lichens, including (+)-usnic acid, on cell proliferation or wound healing have been investigated [24]. In this study, neutral red uptake and crystal violet cytotoxicity assays using MM98 malignant mesothelioma cells, A431 vulvar carcinoma cells, and HaCaT keratinocytes showed that usnic acid is highly toxic. However, usnic acid significantly stimulated wound closure in keratinocyte monolayers at subtoxic doses. This result suggests that usnic acid can be used to prevent hyperproliferative syndrome and promote tissue regeneration. Hypertrophic scar is a common fibroblast proliferation disease, characterized by the overexpression of collagen and excessive deposition of extracellular matrix in healing wounds caused by deep burns, inflammatory reactions, and trauma [101]. Inhibiting angiogenesis is an effective strategy for anti-hypertrophic scar treatment [102–104]. Moreover, usnic acid can significantly inhibit hypertrophic scars and scar angiogenesis and considerably reduce the height, color, and scar elevation index of a scar and collagen tissue accumulation markedly improves [105,106]. In vitro experiments have shown that usnic acid can inhibit the proliferation, migration, and formation of human umbilical vascular endothelial cells and inhibit the proliferation of scar fibroblasts. Bruno et al. [23] synthesized several enamine derivatives obtained from usnic acid; some derivatives with low cytotoxicity and high wound healing properties were used to promote wound healing or facilitate anti-aging skin preparation.

Owing to the increasing requirements of injured skin repair after trauma or surgery, many existing methods can no longer meet the needs of people and novel repair methods are constantly emerging in the market. Usnic acid can promote tissue regeneration and prevent excessive proliferation of wounds and can exert its effects at multiple stages of wound healing. How to make usnic acid safe and effective in promoting wound healing deserves further study.

(+)-Usnic Acid as a Promising Candidate for a Safe and Stable Topical Photoprotective Agent

Agnieszka Galanty [1,*], Justyna Popiół [2], Magdalena Paczkowska-Walendowska [3], Elżbieta Studzińska-Sroka [3], Paweł Paśko [4], Judyta Cielecka-Piontek [3], Elżbieta Pękala [2] and Irma Podolak [1]

1 Department of Pharmacognosy, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland; irma.podolak@uj.edu.pl
2 Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland; justyna.popiol@uj.edu.pl (J.P.); elzbieta.pekala@uj.edu.pl (E.P.)
3 Department of Pharmacognosy, Poznań University of Medical Sciences, Święcickiego 4, 60-781 Poznan, Poland; mpaczkowska@ump.edu.pl (M.P.-W.); ela_studzinska@op.pl (E.S.-S.); jpiontek@ump.edu.pl (J.C.-P.)
4 Department of Food Chemistry and Nutrition, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland; p.pasko@uj.edu.pl
* Correspondence: agnieszka.galanty@uj.edu.pl

Abstract: The study aimed to examine whether usnic acid—a lichen compound with UV-absorbing properties—can be considered as a prospective photoprotective agent in cosmetic products. Moreover, a comparison of two usnic acid enantiomers was performed to preselect the more effective compound. To meet this aim, an in vitro model was created, comprising the determination of skin-penetrating properties via skin-PAMPA assay, safety assessment to normal human skin cells (keratinocytes, melanocytes, fibroblasts), and examination of photostability and photoprotective properties. Both enantiomers revealed comparable good skin-penetrating properties. Left-handed usnic acid was slightly more toxic to keratinocytes (IC_{50} 80.82 and 40.12 µg/mL, after 48 and 72 h, respectively) than its right-handed counterpart. The latter enantiomer, in a cosmetic formulation, was characterized by good photoprotective properties and photostability, comparable to the UV filter octocrylene. Perhaps most interestingly, (+)-usnic acid combined with octocrylene in one formulation revealed enhanced photoprotection and photostability. Thus, the strategy can be considered for the potential use of (+)-usnic acid as a UV filter in cosmetic products. Moreover, the proposed model may be useful for the evaluation of candidates for UV filters.

Keywords: usnic acid; photoprotection; normal skin cells; octocrylene

Citation: Galanty, A.; Popiół, J.; Paczkowska-Walendowska, M.; Studzińska-Sroka, E.; Paśko, P.; Cielecka-Piontek, J.; Pękala, E.; Podolak, I. (+)-Usnic Acid as a Promising Candidate for a Safe and Stable Topical Photoprotective Agent. *Molecules* **2021**, *26*, 5224. https://doi.org/10.3390/molecules26175224

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Received: 30 July 2021

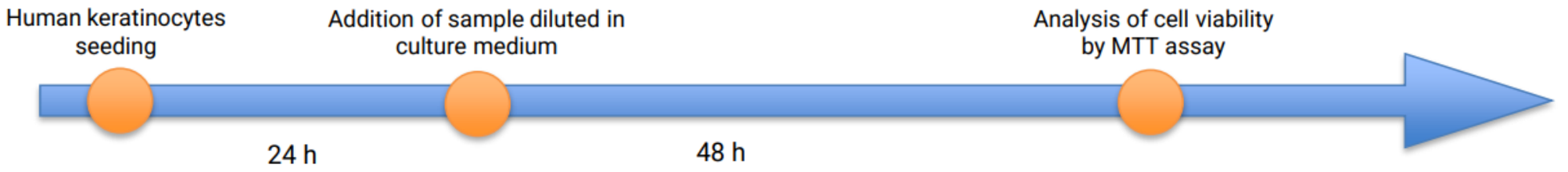
Two new studies:

Analysis of **antioxidant effects** through the protection of ROS after UVA induction.

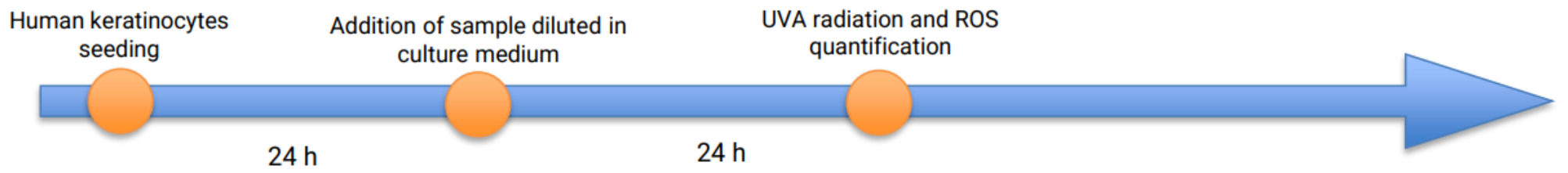
Analysis of the regeneration potential using the **Wound-Healing (Scratch) Assay.**

Analysis of antioxidant effects through the protection of ROS after UVA induction

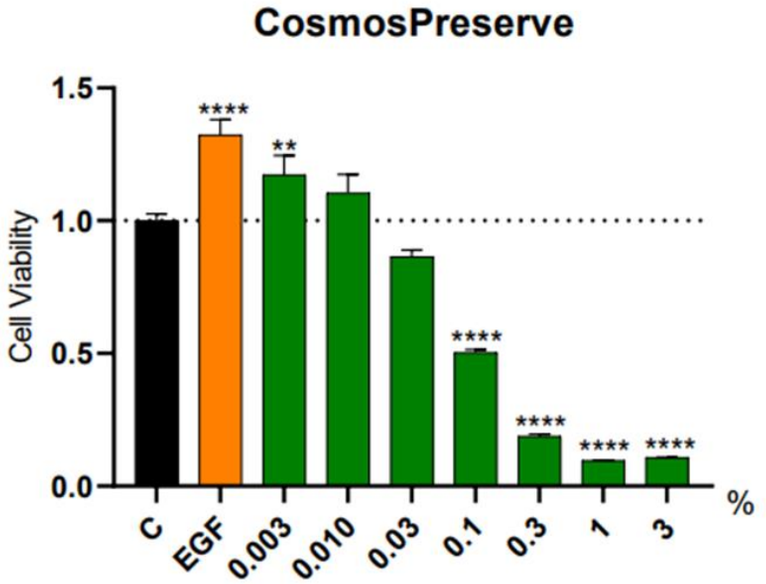
Cell viability



ROS quantification

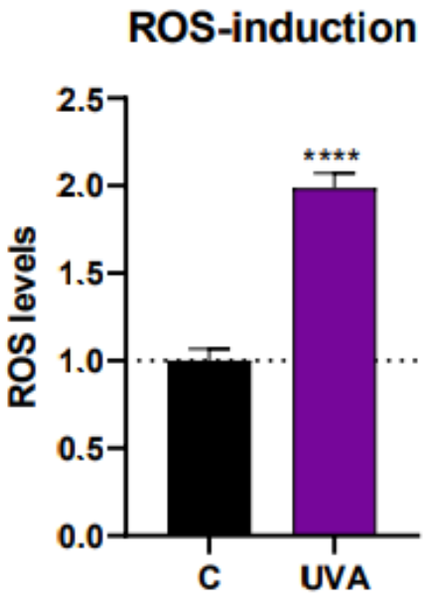


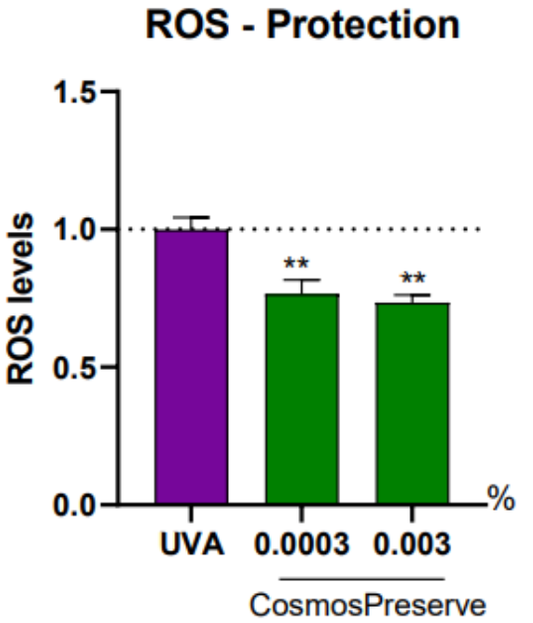
Analysis of antioxidant effects through the protection of ROS after UVA induction



- Epidermal Growth Factor (**EGF**, Possitive Control) induced cell proliferation by increasing cell viability by **32.4 ± 5.0 %** in human keratinocytes.
- Treatment with **CosmosPreserve** at **0.003 %** induced cell proliferation by increasing cell viability by **17.4 ± 5.0 %** in human keratinocytes.
- Therefore, the selected working concentrations were **0.0003 %** and **0.003 %**.

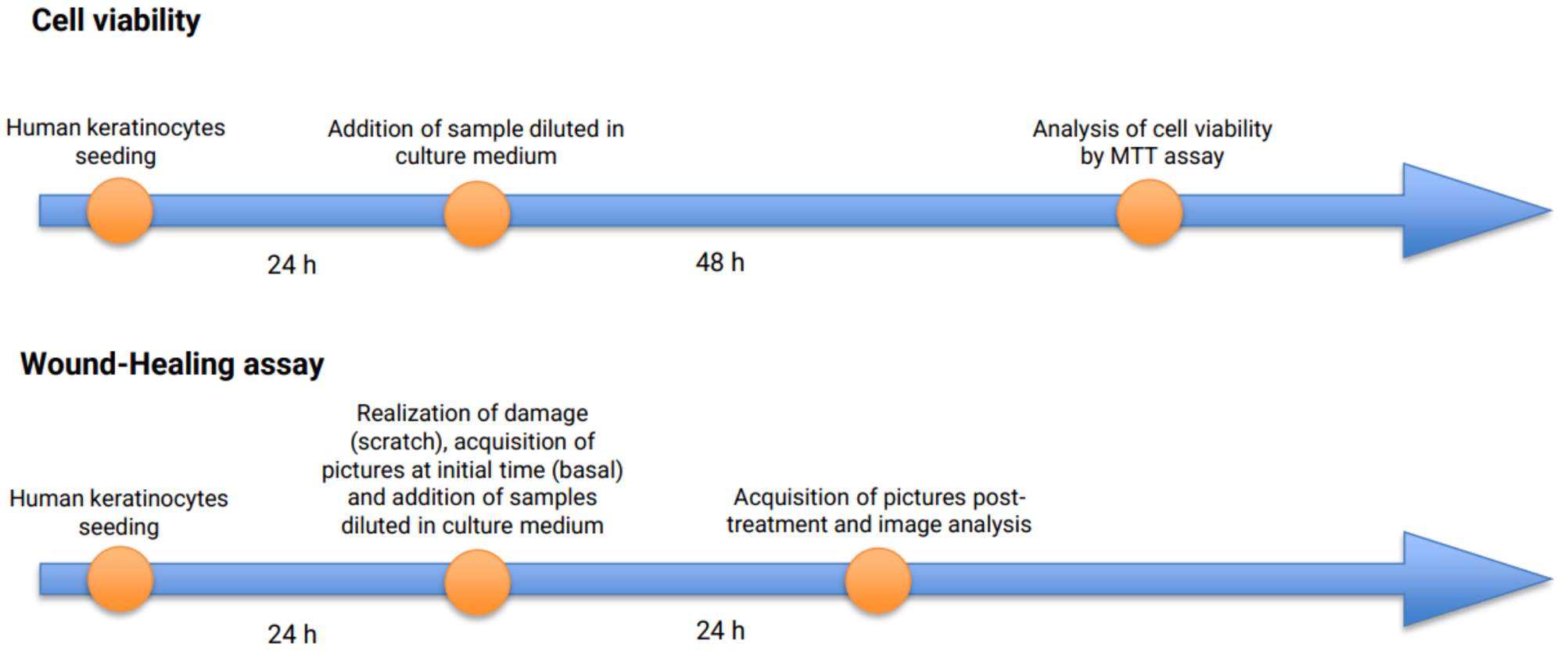
Treatment with CosmosPreserve shows antioxidant potential by decreasing UVA-induced ROS levels in human keratinocytes.



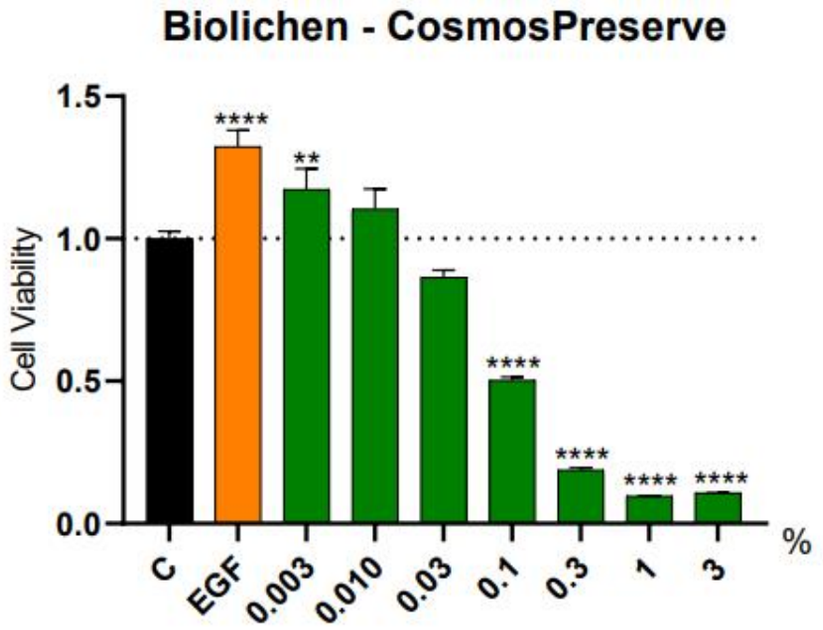


- **UVA** radiation increased ROS levels by **98.6 ± 10.9 %** in human keratinocytes.
- Treatment with **CosmosPreserve** at **0.0003 %** and **0.003 %** reduced UVA-increased ROS levels by **23.4 ± 5.9 %** and **26.6 ± 5.9 %,** respectively, in human keratinocytes.

Analysis of the regeneration potential using the Wound-Healing (Scratch) Assay



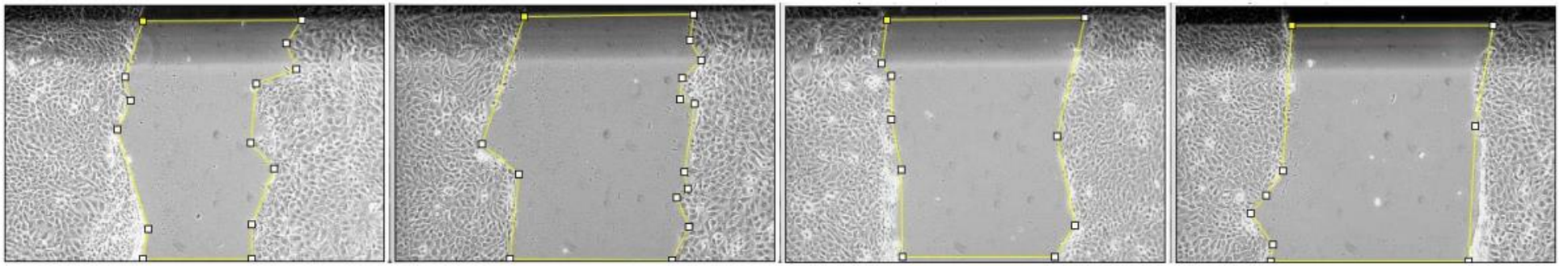
Analysis of the regeneration potential using the Wound-Healing (Scratch) Assay



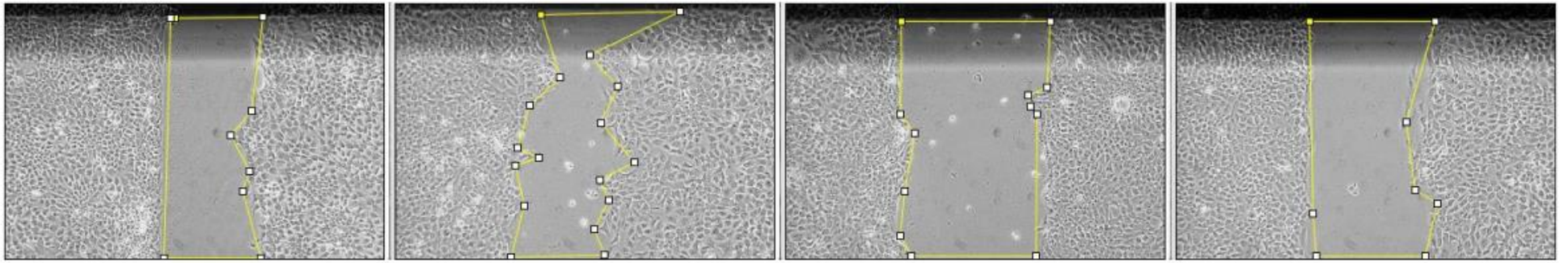
- Epidermal Growth Factor (**EGF**, Possitive Control) induced cell proliferation by increasing cell viability by **32.4 ± 5.0 %** in human keratinocytes.
- Treatment with **Biolichen - CosmosPreserve** at **0.003 %** induced cell proliferation by increasing cell viability by **17.4 ± 5.0 %** in human keratinocytes.

Analysis of the regeneration potential using the Wound-Healing (Scratch) Assay

Control Basal

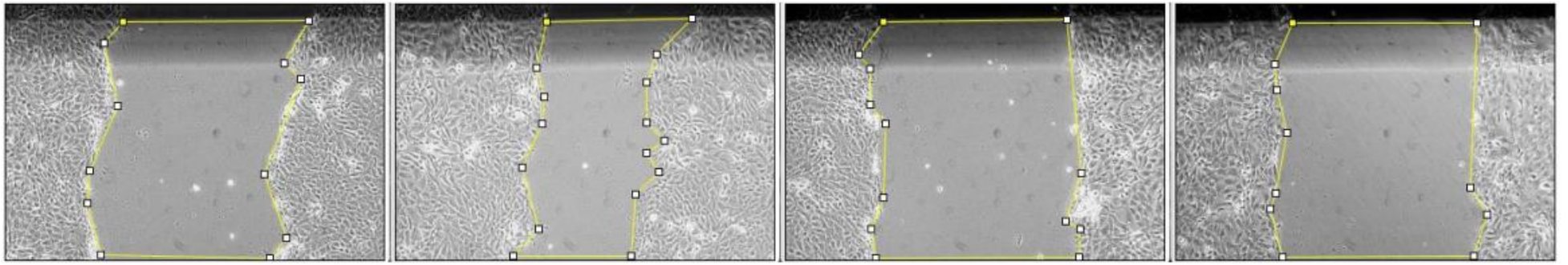


Control 24 H

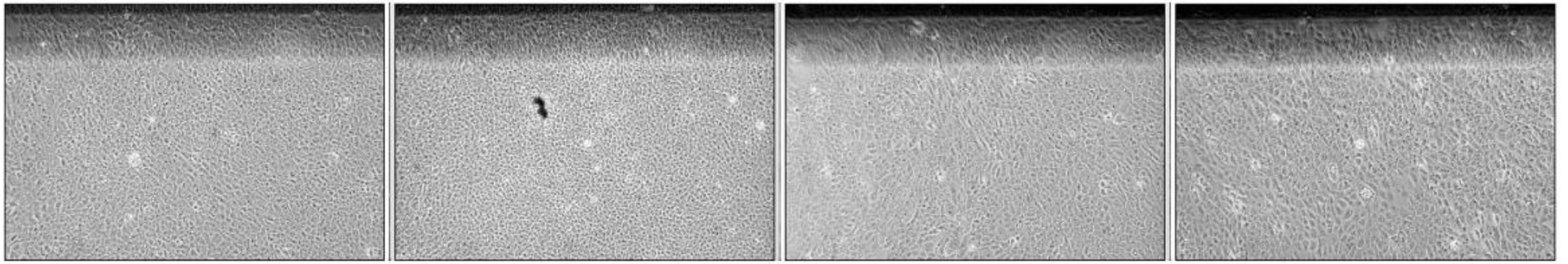


Analysis of the regeneration potential using the Wound-Healing (Scratch) Assay

EGF 20ng/ml
basal

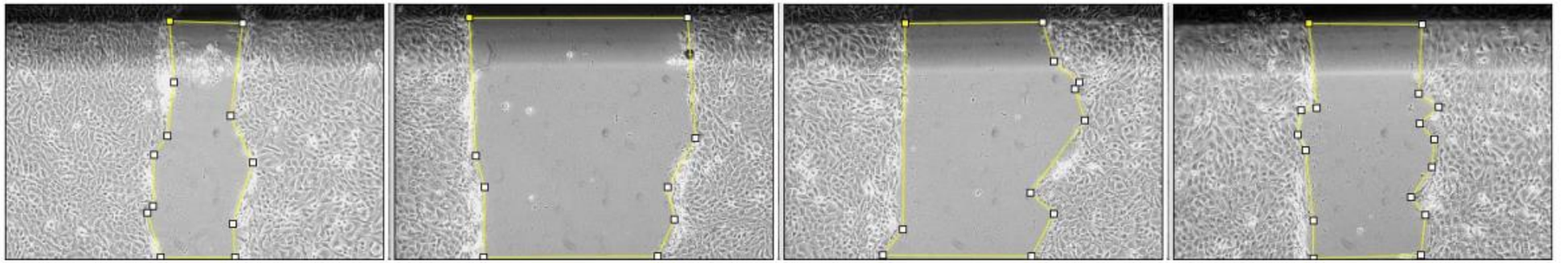


EGF 20ng/ml
24H

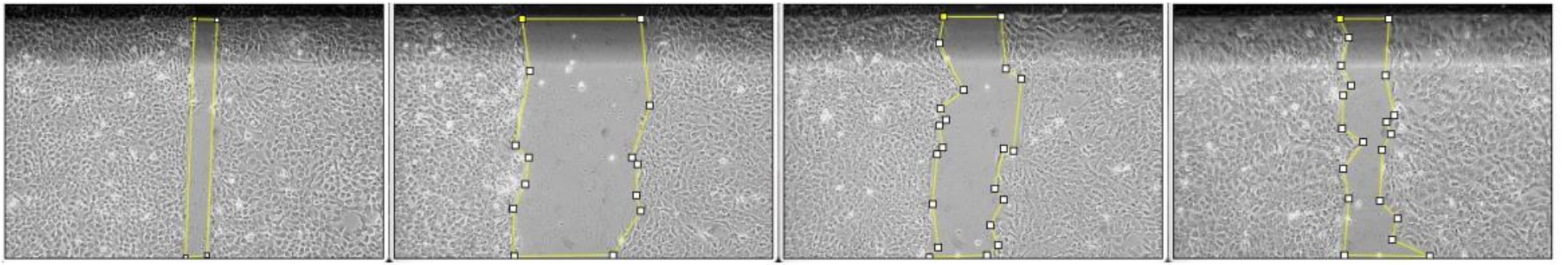


Analysis of the regeneration potential using the Wound-Healing (Scratch) Assay

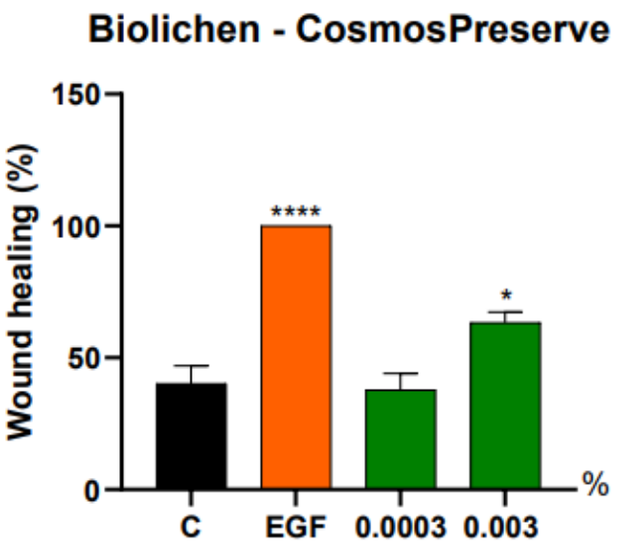
Biolichen – CosmosPreserve 0.003% basal

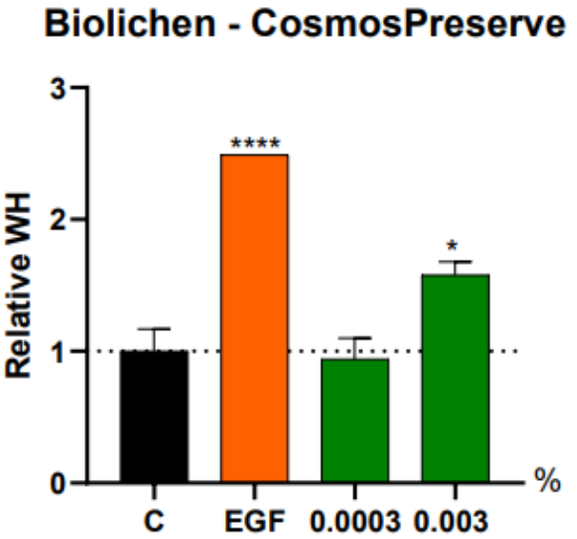


Biolichen – CosmosPreserve 0.003% 24 H



Analysis of the regeneration potential using the Wound-Healing (Scratch) Assay





- Positive control (**EGF**) resulted in significant Wound Healing after 24h, with a value of **149.1 ± 17.9%.**
- Treatment with **Biolichen - CosmosPreserve** exhibited regenerative effects in human Keratinocytes after 24 h when used at **0.003%** (**57.8 ± 19.3%**).

Results for two new studies - CosmosPreserve

ROS / Anti-oxidant study

- Treatment with CosmosPreserve shows antioxidant potential by decreasing UVA-induced ROS levels in human keratinocytes.
- Reduced ROS by 26.6 %

Wound Healing Study

- Treatment with Biolichen - CosmosPreserve shows regenerative potential in human Keratinocytes after 24 h.
- Regenerative effects of 57.8 % after 24 hours.

ASSOCIATION WITH INDIGENOUS COMMUNITIES

- Lichen is a natural resource under the forest canopy in Northern Canada.
- Indigenous caretakers of these territories, people, and communities who live in their traditional hunting and fishing territories.
- Harvest lichen for their economic benefit.
- We conducted a series of training sessions to establish harvesting protocols and to inspire sustainable practices.
- Harvesters are trained to use manual tools to trim the top 3 cm of this lichen mat. Abundant – one will never need to harvest in one place a second time.
- Europelab and our native family partners have developed a program to re-inoculate burnt forested areas ravaged by fire by spreading dried Lichen powder in measurable designated areas. This seeding program will generate important data for the habitat.

Program to re-inoculate Lichen after forest fires - Study

Strong Position

- Largest inventory globally.
- Unique chemistry.
- Validated published science to support claims.
- Efficacy studies in cosmetics.
- Large production capacity.
- Quality control.
- Sustainable model.
- Natural alternative.

FORMULATION VERSATILITY

Compatible with:

Creams & Lotions

Emulsions & Surfactant Systems

Masks & Treatments

Sun Care

Hair Care

Color Cosmetics

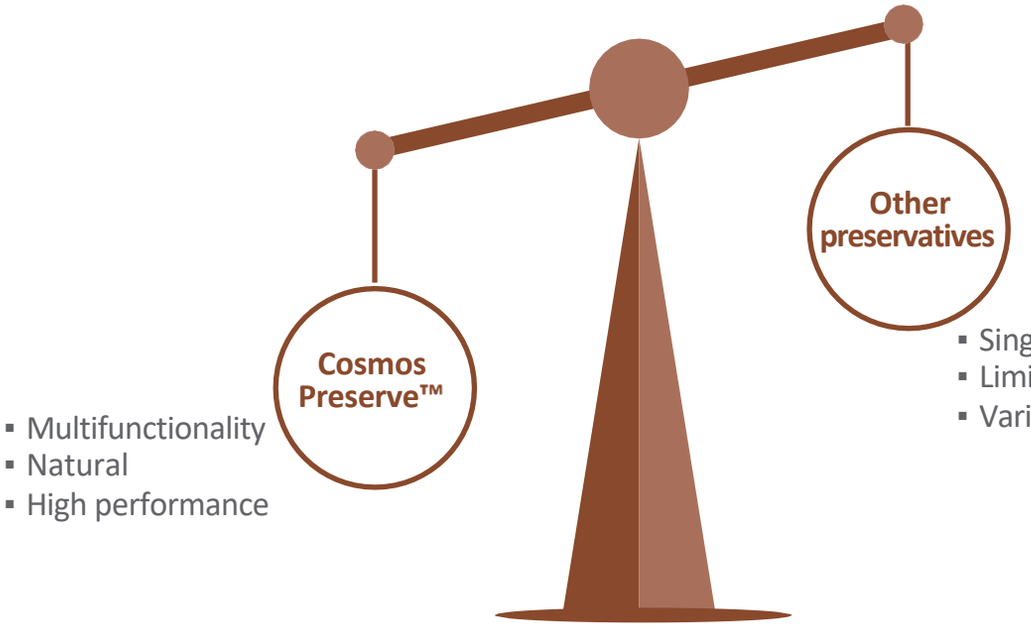
Recommended usage:

- **1–2%** for preservation.
- Up to **4%** for multifunctional skin benefits.

Stable in **pH 4–7**, lipid/surfactant integration.

THE ADVANTAGE: BEYOND PRESERVATION

- Traditional preservatives = single function, safety concerns
- CosmosPreserve™ = natural, effective, multifunctional, performance-driven
- Sets a new standard for “beauty that does more”



ETHICAL & SUSTAINABLE SOURCING

- Collaboration with Indigenous communities
- Sustainable harvesting of Canadian lichens
- Respect for biodiversity & traditional knowledge
- Social & ethical alignment with today’s conscious consumers

“With BioLichen CosmosPreserve™, brands can create innovative, market-leading products that align with the 'skincare that does more' trend.

REGULATORY & COMPLIANCE

- **Patented:** innovative preservation system protected by patent, reinforcing its unique and cutting-edge formulation.
- **COSMOS-certified by ECOCERT®:** BioLichen CosmosPreserve™ meets the strictest standards for natural and organic products.
- **FDA compliant (United States):** meets all regulatory requirements for use in cosmetic formulations in the U.S. market.
- **Health Canada compliant:** approved under Health Canada regulations for ingredients used in cosmetics and personal care.
- **EU compliant:** approved under European regulations for ingredients used in cosmetics and personal care.
- **Technical documentation:** available.

WHY THIS MATTERS FOR BRANDS

- **Formulators face a growing challenge:**

  - ✓ Find alternatives to synthetic preservatives
  - ✓ Meet COSMOS and global compliance
  - ✓ Deliver stable, high-performance formulas
  - ✓ Use clean, natural ingredients
  - ✓ Evidence for Claims

COSMOSPRESERVE™

BioLichen

WE ARE LICHEN METABOLITES

COSMOSPRESERVE™

BioLichen CosmosPreserve™: The Multi-functional Ingredient

BioLichen CosmosPreserve™ is a natural, multi functional active ingredient derived from Cladonia rangiferina and Cladonia stellaris lichens, extracted with Triethyl Citrate.

- INCI Name: Triethyl Citrate, Cladonia (Rangiferina/Stellaris) Extract.

Powerful Preservation & Skin Benefits

BioLichen CosmosPreserve™ uniquely combines broad spectrum antimicrobial efficacy with significant skin health benefits. It's not just a preservative; it’s a skin active ingredient that contributes anti-oxidant, anti-inflammatory, wound healing and cyto-protective values.

COSMOS Organic Certified

BioLichen CosmosPreserve™ is COSMOS Organic certified, meeting stringent standards for natural and organic cosmetics. This certification builds consumer trust and enhances brand credibility.

“Discover how BioLichen CosmosPreserve™ dual action power delivers both effective preservation and visible skin improvements.

REQUEST YOUR SAMPLE TODAY!

Verified by ECOCERT

COSMOS APPROVED

Q&A