Natural and synthetic substances are known that impart useful properties to cosmetic products, for example, antioxidant, anti-inflammatory, etc. "Etalon Cosmetics" (Russian Federation) develops actives that make skin healthy, resilient and simply beautiful, addressing the two cornerstones of skin health bringing: — Balance and Protection. We understand our business as creating a bridge between the invisible world and the visible one, between the in-vitro and in-vivo, from the cells to the skin to make visible and perceptible beauty and wellness.

But what is beauty and well-being really all about? Healthy skin is the basis of both. Keeping skin healthy is a complex topic which requires more than just a marketing story namely scientific know-how and expertise. To make products smart, relevant and sustainable, highly effective and goal-oriented ingredients substantiated by science and studies are needed.

"Etalon Cosmetics" (Russian Federation) as a company grounded in science has dedicated years of research to learning about the science behind **TAXIFOLIN** (*syn*. DIHYDROQUERCETIN) to fully understand the extent of its benefits.

**TAXIFOLIN** is a safe, natural molecule with strong antioxidant and anti-inflammatory activity, continued displaying a variety of health-promoting properties and modulating diverse biological functions. **TAXIFOLN** has opportunities to reach consumers in search of general health and wellness protection, as well as those who opt for targeted solutions, looking for the ultimate wellness solutions.

The main raw material used to obtain Taxifolin is the butt of larch. Generally, TAXIFOLIN-RICH EXTRACT is recovered from saw logs of larch tree by chipping or grinding the wood after debarking and extracting in thermo-vacuum system of using energy to heat solvent the purified water – ethanol solution (<420C) in contact with wood particles in order to extract Taxifolin from the wood particles.



The molecular structure of TAXIFOLIN is due to the chirality of the molecule and the spatial arrangement of its functional groups with optically active functional components, which are very sensitive to the process of their interconversion, called racemization, which leads to the disappearance of optical activity, loss of natural, i.e. native biological activity of the Taxifolin molecule. Therefore, the methods for isolating TAXIFOLIN and obtaining its native form require extremely "careful" conditions of the technological process, excluding racemization and polymerization of molecule, at the same time excluding the presence of any impurities in the form of fractions of larch oil, resin and their compounds, saponins associated with the Taxifolin molecule.

With more than 60 years of scientific research behind it, showing its benefits, this ingredient has the science to back its benefits. TAXIFOLIN can be used in oral as well as topical applications for improved skin health and appearance. TAXIFOLIN contributes an unmatched variety of physiological functions for improvement of both health and aesthetic appearance of human skin. In brief, TAXIFOLIN supports increased presence of collagen and elastin, improves skin micro-circulation, elevates skin hydration and elasticity by upping dermal hyaluronic acid generation and, furthermore, balances pigmentation for brighter skin complexion and quenches inflammatory processes.

The mechanism of action of biologically active substances usually consists in their combination with specific receptors. Receptors can be viewed as sections of cell membranes containing complex organic molecules that are sensitive to certain substances. Each receptor has a characteristic spatial structure of the site interacting with a biologically active substance, and their structures must correspond to each other according to the key-lock principle. Most biologically active molecules, for example TAXIFOLIN molecule, have a close relationship between the spatial structure and biological activity, that is, the stereospecificity of action.

In order for a substance to be optically active, only one condition is required - the molecule must have neither a center nor a plane of symmetry. In the simplest case, this is determined by the presence in the molecule of the so-called asymmetric (chiral) atom. TAXIFOLIN molecule corresponds to these rules.

The recognition regions of inducible transcription factors are called response elements and, as a rule, the same response elements are contained in the structure of the flanking regions of genes encoding functionally similar proteins. Due to this distribution of response elements and the activation of inducible transcription factors under the conditions of one or another action in the cell, a coordinated rearrangement of the expression of a whole group of genes occurs, which leads to an increase in the intracellular amount of proteins that counteract the action of the environment.

**Studies: Reference [1]**

The effect of plant flavonoids on intercellular adhesion molecule-1 (ICAM-1) expression in human keratinocyte was investigated. ICAM-1 is known to mediate skin inflammation. Among the flavonoids tested, **TAXIFOLIN** was the most potent in inhibiting interferon gamma (IFN gamma)-induced ICAM-1 protein as well as mRNA expression in human keratinocytes. Much smaller dosages of TAXIFOLIN were required in primary keratinocytes compared to HaCaT (immortalized cell) to achieve similar levels of inhibition in the inducible ICAM-1 expression. Regulation of inducible ICAM-1 expression by TAXIFOLIN was at transcriptional level by inhibiting the activation of signal transducers and activators of transcription (STAT)1 and protein tyrosine phosphorylation of Janus kinase (JAK)1 suggesting that the JAK-STAT pathway may be the molecular site of action of TAXIFOLIN. Finally, TAXIFOLIN pre-treatment also potently inhibited IFN gamma-induced ICAM-1 expression in a reconstructed human skin equivalent suggesting therapeutic potential of TAXIFOLIN in skin pathological conditions related to increased cell adhesion and inflammation.

The inflammatory or immune response requires the cell-cell interaction between leukocytes and targets cells. The formation of constitutive and inducible adhesion molecule complexes is necessary for the interaction of these cells. Differential expression of intercellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin gene superfamily, in the epidermis plays a critical role in the regulation of cutaneous inflammation, immunologic reactions and tissue repair. ICAM-1 mediates the firm binding of a variety of leukocytes to the target cells via its interaction with lymphocyte function associated antigen 1 (LAF-1) or Mac-1 (CD11b/CD18) expressed on the circulating white blood cells.



Adhesion of T-cells to keratinocytes is a key feature in skin inflammation processes. The present study provides the first evidence that among various flavonoids tested, TAXIFOLIN is a potent inhibitor of IFNQ-induced adherence of Jurkat T-cells to keratinocytes. Such effect of TAXIFOLIN on inducible cell-cell adhesion was mediated via its inhibitory effect on ICAM-1 expression. The maximum effect of TAXIFOLIN on inducible ICAM-1 was observed following 12 h of pre-treatment suggesting that native TAXIFOLIN (not metabolized) is not active in inhibiting inducible ICAM-1 expression. A 12 h period required to achieve the maximal inhibitory effect on ICAM-1 may be due to the rate of uptake of TAXIFOLIN in cells or cellular activation/transformation of TAXIFOLIN being required to achieve the observed effect. The study also provides the first evidence that topical application of a low concentration (**20 umol**) of a naturally occurring flavonoid TAXIFOLIN markedly inhibits IFNQ-induced ICAM-1 expression in a reconstructed human skin model. The observation that much lower dosages of TAXIFOLIN are required in HEK (primary cells) to achieve the comparable effects on inducible ICAM-1 expression observed in HaCaT suggests that data obtained using immortalized cells should be interpreted with caution in terms of the effective concentration.

The marked inhibition of IFNQ-induced ICAM-1 expression by TAXIFOLIN was observed in a 3D human skin model. In conclusion this study provides therapeutic potential of this flavonoid in skin pathological conditions related to increased cell adhesion and inflammation.

**Studies: Reference [2]**

The TAXIFOLIN molecule can modulate the expression of several genes, including those that code for detoxification enzymes, intracellular circulating proteins, cell growth factors, and DNA-repairing proteins. TAXIFOLIN (syn. Dihydroquercetin) significantly activates the induction of transcription factors, which are called antioxidant response elements (ARE). A total of 65 genes, including several detoxifying enzymes (NQO1, GSTM1) and an antioxidant enzyme (thioredoxin reductase1), were activated by TAXIFOLIN at 60 mg.

Phase II detoxification enzymes are known to be responsible for detoxification and elimination of activated carcinogens suggesting that induction of these enzymes is an important biomarker for chemoprevention. We tested a quinone reductase (QR) activity by TAXIFOLIN in HCT 116 colon cancer cells. TAXIFOLIN induced significant QR activity and showed high chemoprevention index (CI) 5.75. To identify the target genes regulated by TAXIFOLIN, DNA microarray was performed with a 3K human cancer chip containing 3096 human genes associated with carcinogenesis. Significant analysis of microarray (SAM) revealed 428 differentially expressed (DE) genes as statistically significant. Among them, 65 genes were up-regulated including important chemo-preventive enzymes such as NQO1, GSTM1 and TXNRD1, and 363 genes were down-regulated in the presence of 60 umol TAXIFOLIN. Since the enzymes up-regulated contain antioxidant response element (ARE) in common, we defined that TAXIFOLIN modulates chemo-preventive genes through activation of the ARE. Transient transfection experiments using ARE-QR-CAT, and XRE-QR-CAT demonstrated that TAXIFOLIN activated ARE only but not xenobiotic response element (XRE) indicating that TAXIFOLIN is a mono-functional inducer. Taken together, TAXIFOLIN acts as a potential chemo-preventive agent by regulating genes via an ARE-dependent mechanism.

**Studies: Reference [3]**

Flavonoids are a group of polyphenolic compounds widely distributed in plants. Their potent bio-activities and relatively low toxicity have rendered them useful ingredients in functional cosmetics. The purpose of the present study was to examine their potential effects on cellular melanogenesis. When tested in murine melanoma B16F10 cells activated by -melanocyte stimulating hormone ( -MSH), TAXIFOLIN inhibited the cellular melanogenesis as effectively as arbutin, one of the most widely used hypo-pigmenting agents in cosmetics. As opposed to its anti-melanogenic effects, TAXIFOLIN rather increased the tyrosinase protein levels in the absence and presence of -MSH. However, flavonoid effectively inhibited tyrosinase-catalysed oxidation of l-dihydroxyphenylalanine in cell-free extracts and in living cells. Furthermore, TAXIFOLIN attenuated cell pigmentation induced by expression of exogenous human tyrosinase. Therefore, the anti-melanogenic effects of TAXIFOLIN is attributed to its inhibitory effects on tyrosinase enzymatic activity, despite its effects on increasing tyrosinase protein levels.

**Studies: Reference [4]**

TAXIFOLIN was shown to inhibit microsomal TG synthesis by 37% and its subsequent transfer into the lumen (-26%). The reduction in synthesis was due to a decrease in diacylglycerol acyltransferase (DGAT) activity (-35%). The effect on DGAT activity was found to be non-competitive and non-transcriptional in nature. Both DGAT-1 and DGAT-2 mRNA expression remained essentially unchanged suggesting the point of regulation may be at the post-transcriptional level. Evidence is accumulating that microsomal triglyceride transfer protein (MTP) is also involved in determining the amount of lumenal TG available for lipoprotein assembly and secretion. TAXIFOLIN was shown to inhibit this enzyme by 41%. Whether the reduction in TG accumulation in the microsomal lumen is predominantly due to DGAT and/or MTP activity remains to be addressed. In summary, TAXIFOLIN reduced apoB secretion by limiting TG availability via DGAT and MTP activity.

**Studies: Reference [5]**

TAXIFOLIN has been reported to down-regulate the expression of intercellular adhesion molecule-1 (ICAM-1), a receptor-mediating firm adhesion with beta2 integrin (e.g., Mac-1) expressed on leukocytes. To evaluate whether TAXIFOLIN could modulate Mac-1-dependent firm adhesion by neutrophils, and the possible mechanism(s) underlying its anti-inflammatory action, its effects on N-formyl-methionyl-leucyl-phenylalanine (fMLP) or phorbol-12-myristate-13-acetate (PMA)-activated peripheral human neutrophils were studied. Pretreatment with TAXIFOLIN (1-100 microM) concentration-dependently diminished fMLP- or (PMA)-induced Mac-1-dependent firm adhesion and up expression of surface Mac-1. Mobilisation of intracellular calcium and production of reactive oxygen species (ROS) signal the upexpression of Mac-1 and firm adhesion by neutrophils. TAXIFOLIN impeded the calcium influx induced by fMLP (a receptor-mediated activator) or AlF(4)(-) (a G protein-mediated activator).

TAXIFOLIN also effectively inhibited the fMLP- or PMA-induced ROS production with 50% inhibitory concentration (IC(50)) less than 10microM, possibly through impairing the activation of NADPH oxidase, a major ROS-generating enzyme in neutrophils, by restricting the activation of p38 mitogen-activated protein kinase (p38 MAPK) and protein kinase C (PKC). In conclusion, we propose that impairment of ROS production by NADPH oxidase through interfering with p38 MAPK- and/or PKC-dependent signals, and antagonism of G protein-mediated calcium influx may account for the inhibition of Mac-1-dependent neutrophil firm adhesion that confers TAXIFOLIN the anti-inflammatory activity.

**Studies: Reference [6]**

TAXIFOLIN (*syn*. Dihydroquercetin – DHQ) had been evaluated by different studies as the small-molecule regulator of signaling cascades as promising anti-inflammatory agent with biological targets such as COX-2, and related pro-inflammatory mediators (cytokines and chemokines, interleukins [ILs], tumour necrosis factor [TNF]-α, migration inhibition factor [MIF], interferon [IFN]-γ and matrix metalloproteinases [MMPs]) implicated in uncontrolled, destructive inflammatory reaction. TAXIFOLIN was effective with relevant biological targets that include nuclear transcription factor (NF-κB), p38 mitogen-activated protein kinases (MAPK) and Janus protein tyrosine kinases and signal transducers and activators of transcription (JAK/STAT) signalling pathways has received growing attention.

In the clinical observation during the experimental period, TAXIFOLIN (TAX) treatment significantly reduced the severity of AD-like lesions induced in NC/Nga mice. Eosinophil and IgE levels decreased after treatment of the animals with TAX. TAX may thus be associated with improvement of eosinophil-related allergic diseases. The expression of cytokines (IL-4, 5 and 13) was significantly inhibited in the TAX-treated group, suggesting that TAX might play an immunoregulatory role associated with AD. In RT-PCR, iNOS and COX-2 expression levels were reduced in the TAX-treated group. In western blotting, the expression levels of iNOS and COX-2 were also reduced in the TAX-treated group. These findings suggest that TAX is effective for the treatment of AD by preventing the production of inflammatory cytokines and by reducing skin inflammation.

**Studies: Reference [7]**

The aim of the present study was to assess the clinical efficacy of proscillaridin-A (C30H42O8), TAXIFOLIN (C15H12O7) and scilliroside ( C32H44O12 ) on various pains of spontaneous volunteer patients. In this study, 250 patients were monitored in coats. The average age of these patients were between 40 and 74 years old. Of these 100 were male and 150 female patients. Also, 60 % of proscillaridin-A (C30H42O8), TAXIFOLIN (C15H12O7) and scilliroside ( C32H44O12 ) solution in pure glycerin was applied as external on the pain area. ASO, CRP and RF higher values of patients were significantly decreased ( p < 0.05 and p < 0.01). Knee, joint, calf, hip, shoulder, upper back, low back (lumbago), tailbone and fibromyalgia paints of patients were significantly reduced (p < 0.05 and p < 0.01). TAXIFOLIN can reduce the musculoskeletal pains. Effects of 60 % of TAXIFOLIN (C15H12O7) on some painful areas and biochemical parameters.

**Studies: Reference [8]**

TAXIFOLIN reportedly exerts multiple biologic effects, but the molecular mechanisms and direct target(s) of TAXIFOLIN in skin cancer chemoprevention are still unknown. In silico computer screening and kinase profiling results suggest that the EGF receptor (EGFR), phosphoinositide 3-kinase (PI3K), and Src are potential targets for TAXIFOLIN. Pull-down assay results showed that EGFR, PI3K, and Src directly interacted with TAXIFOLIN in vitro, whereas TAXIFOLIN bound to EGFR and PI3K, but not to Src in cells. ATP competition and in vitro kinase assay data revealed that TAXIFOLIN interacted with EGFR and PI3K at the ATP-binding pocket and inhibited their kinase activities. Western blot analysis showed that TAXIFOLIN suppressed UVB-induced phosphorylation of EGFR and Akt, and subsequently suppressed their signaling pathways in JB6 Pþ mouse skin epidermal cells. Expression levels and promoter activity of COX-2 and prostaglandin E2 (PGE2) generation induced by UVB were also attenuated by TAXIFOLIN. The effect of TAXIFOLIN on UVB induced signaling pathways and PGE2 generation was reduced in EGFR knockout murine embryonic fibroblasts (MEF) compared with EGFR wild-type MEFs. TAXIFOLIN also inhibited EGF-induced cell transformation. Importantly, topical treatment of TAXIFOLIN to the dorsal skin significantly suppressed tumor incidence, volume, and multiplicity in a solar UV (SUV)-induced skin carcinogenesis mouse model. Further analysis showed that the TAXIFOLIN-treated group had a substantial reduction in SUV induced phosphorylation of EGFR and Akt in mouse skin. These results suggest that TAXIFOLIN exerts chemopreventive activity against UV-induced skin carcinogenesis by targeting EGFR and PI3K.

**Studies: Reference [9]**

The Effect of TAXIFOLIN and MitoPBN on Blue Light-induced DNA Damage: To clarify the role of specific ROS in blue light induced mtDNA damage, we utilized the superoxide scavenging properties of TAXIFOLIN and the carbon-centered radical scavenging properties of the mitochondria-targeted MitoPBN in our model system. In irradiated TAXIFOLIN-fed cells, complete mtDNA protection was observed at 1, 3, and 6 h (p<0.05) compared with irradiated cells in the absence of TAXIFOLIN, which showed extensive damage at 3 and 6 h. By contrast, MitoPBN-fed cells showed no mtDNA protection throughout the 6-h blue light exposure. TAXIFOLIN- and MitoPBN-fed cells maintained in the dark did not show significant mtDNA damage at any of the time points.

**Studies: Reference [10]**

The inhibitory effect of TAXIFOLIN and hydrocortisone on serum aminotransferases may be responsible for inhibition of mucopolysaccharide synthesis which is mainly concerned with the proliferative phase of inflammation. An inhibition of aminotransferases and stimulation of ATP phosphohydrolase have also been observed with salicylates, phenylbutazone, corticoids, indomethacin, glycyrrhetic acid and other anti-inflammatory drugs. TAXIFOLIN was one-eighth as active as hydrocortisone on carrageenin-induced oedema; however, its "therapeutic index" was almost equal to that of hydrocortisone. TAXIFOLIN prevented the increase in serum aminotransferase activity during inflammation.

**Studies: Reference [11]**

The effect of flavonoids on beta-hexosaminidase transport and endocytosis has been studied in cultured human skin fibroblasts. In mucolipidosis II fibroblast cultures, characterized by their preferential secretion of most newly synthesized hydrolases, quercetin and phloretin (200 microM) inhibited beta-hexosaminidase synthesis as well as total culture-associated enzyme activity. **Taxifolin** induced a 2.4-fold increase in the total enzyme activity without altering the intra- and extracellular distribution of the enzyme. TAXIFOLIN inhibited receptor-mediated endocytosis of beta-hexosaminidase by fibroblasts up to 50% of control uptake.

**Studies: Reference [12]**

Dihydroflavonol TAXIFOLIN and its glycoside, astilbin, from Engelhardtia chrysolepis were evaluated as antioxidants and radical scavengers. These dihydroflavonols inhibited superoxide anion production in the xanthine/xanthine oxidase system. Microsomal lipid peroxidation induced by NADPH-cytochrome P-450 reductase was also inhibited by these flavonoids.

Mitochondrial lipid peroxidation was inhibited only by the aglycon (not glycoside). TAXIFOLIN protected peroxy radical-damaged mitochondria with no effect on enzyme activity. Furthermore, TAXIFOLIN and astilbin protected red cells against oxidative hemolysis. These dihydroflavonols were found to be effective for protecting subcellular systems and red blood cells against oxidative stress in vitro.

**Studies: Reference [13]**

TAXIFOLIN is a natural flavonoid and possesses many pharmacological activities including antioxidant and anti-inflammatory. Because flavonoids have been confirmed to fight osteoporosis and promote bone health, the aim of this study was to investigate the effects of TAXIFOLIN on the formation and function of osteoclast. In this study, we examined the effects of TAXIFOLIN on osteoclast using both in vitro and in vivo studies. TAXIFOLIN suppressed the activation of nuclear factor-κB, C-Fos and mitogen-activated protein kinase, and also decreased osteoclast-specific genes expression, including Trap, Mmp-9, Cathepsin K, C-Fos, Nfatc1, and Rank. TAXIFOLIN also prevented reactive oxygen species (ROS) production following RANKL stimulation. In addition, TAXIFOLIN alleviated ovariectomized-induced bone loss by repressing osteoclast activity and decreasing serum levels of tumor necrosis factor-α, interleukin-1β, interleukin-6 and receptor activator of nuclear factor-κB ligand (RANKL) in vivo. Our results indicated that TAXIFOLIN inhibits osteoclastogenesis via regulation of modulation of several RANKL signaling pathways. Therefore, TAXIFOLIN may be considered as a potential alternative therapeutic agent for treating osteoclast-related diseases.

**Studies: Reference [14]**

Nuclear factor erythroid-2 related factor 2 (Nrf2) is a vital transcription factor that regulates the anti-oxidative defense system. Previous reports suggested that the expression of the Nrf2 gene can be regulated by epigenetic modifications. The potential epigenetic effect of TAXIFOLIN (TAX), a potent cancer chemopreventive agent, in skin cancer chemoprotection is unknown. In this study, we investigated how Nrf2 is epigenetically regulated by TAX in JB6 P+ cells. TAX was found to inhibit the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced colony formation of JB6 P+ cells. TAX induced antioxidant response element (ARE)-luciferase activity in HepG2-C8 cells and up-regulated mRNA and protein levels of Nrf2 and its downstream genes heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO1), in JB6 P+ cells. Furthermore, bisulfite genomic sequencing revealed that TAX treatment reduces the methylation level of the first 15 CpGs sites in the Nrf2 promoter. Western blotting showed that TAX inhibits the expression levels of DNA methyltransferase (DNMT) and histone deacetylase (HDAC) proteins. In summary, our results revealed that TAX can induce expression of Nrf2 and its downstream target genes in JB6 P+ cells by CpG demethylation. These finding suggest that TAX may exhibit a skin cancer preventive effect by activating Nrf2 via an epigenetic pathway.

**Studies: Reference [15]**

Tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL) induces apoptosis in many transformed cells but not in normal cells and, hence, has emerged as a novel anticancer agent. Previously, we showed that although most adult T-cell leukemia/lymphoma (ATLL) cells express the TRAIL death receptor DR4 (TRAIL-R1) or DR5 (TRAIL-R2), they are resistant to TRAIL. Thus, in this study, we tried to find natural products that can overcome TRAIL resistance. Among more than 150 materials screened, a dihydroflavonol that was extracted from Blumea balsamifera (BB-1 e.g. methylated form of TAXIFOLIN (syn. dihydroquercetin) exhibited the most striking synergism with TRAIL. Treatment of the TRAIL-resistant ATLL cell line KOB, with a combination of BB-1 and TRAIL, resulted in apparent apoptosis that was not observed on treatment with either agent alone. Furthermore, pretreatment with BB-1 followed by TRAIL further augmented the synergism.

BB-1 increased the level of TRAIL-R2 promoter activity and surface protein expression in a p53-independent manner. TRAIL-R2 siRNA inhibited the synergism, indicating that sensitization was caused by the increase of TRAIL-R2 expression. More interestingly, similar effects were observed in other leukemia cell lines by exactly the same mechanisms. These results suggest that combined treatment with BB-1 and TRAIL may be a new strategy for cancer therapy.

BB-1, the methyl dihydroquercetin (methyl TAXIFOLIN)



The constitutive activation of NFkB is thought to be an important factor in antiapoptotic effects on cancer cells, including ATLL, and NFkB-targeted therapy has received a great deal of attention. Furthermore, a recent report (Aggarwal BB. Nuclear factor-kB: the enemy within. Cancer Cell. 2004;6:203-208.) suggests that the specific down-regulation of NFkB significantly sensitizes cells to TRAIL. To determine whether BB-1 down-regulates NFkB activities, we performed an assay of transcription factors. These results suggest that BB-1 influences c-Rel and p52 but not p50 or p65.

Tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL) induces apoptosis in many transformed cells but not in normal cells and, hence, has emerged as a novel anticancer agent. Although upregulation of TRAIL-R2 by diverse agents, including proteasome inhibitors, bile acids, sulforaphane and methyl dihydroquercetin has been proposed to be important in the sensitization of different cell types to TRAIL induced apoptosis, a mechanistic link has not been conclusively demonstrated. In the former study, knockdown of TRAIL-R2 resulted in a decrease of cell surface TRAIL-R2 expression below base line accompanied by a decreased ability of methyl dihydroquercetin to sensitize to TRAIL

**CLAIMS: TAXIFOLIN – “Etalon Cosmetics”**

The condition of the skin plays an important role in the overall process of well-being. Imbalances in the skin (whatever they are) negatively influence our whole appearance and charisma. TAXIFOLIN is a highly effective multifunctional active ingredient:

Our Taxifolin is a multifunctional agent, suitable for a wide variety of skincare concepts.

• Antioxidant potency

• Increased skin elasticity

• Anti-inflammatory activity

• Improved skin microcirculation

• Anti-photoaging and sun-protection

• Lowered skin pigmentation

It effectively soothes and calms skin, accelerating the reduction of skin redness and irritation after external stresses. Additionally, Taxifolin vitalizes and energizes skin cells and induces the synthesis of collagen, yielding skin smoothing and firming properties, thus making it a potent anti-aging product. Taxifolin is effective in skin lightening, reducing the production of melanin.

Skins’ purpose is to protect us, but all the influences it is exposed to make it hard for skin to perform its job. This is something people see and feel. Skin needs support in many different ways. First and foremost, it needs help in upholding its protective capacity and balance.

Taxifolin works synergistically together to soothe, calm and support skin in maintaining its quality as a physical barrier to our body. Taxifolin is used to give effective support where the skin is challenged by environmental stress, e.g. shaving, depilation or daily hygiene. It helps the skin recover.

Because the skin suffers from various types of damage - such as broken skin or inflammation - that can profoundly change its appearance (e.g. acne, irritation, shaving cuts, ingrown hairs, dryness, etc.), it is necessary to strengthen its internal repair and protection mechanisms. For stronger, more uniform skin -its natural balance.

TAXIFOLIN (“Etalon Cosmetics”): Helps to reconstruct skin tissue; Encourages better metabolism; Detoxifying; Reduces inflammatory processes; Helps to reduce redness; Reduces the formation of free radicals, strengthens internal antioxidant protection. To be used in products such as creams, oils, masks, serums, essences etc.

Our body through our eyes and brain uses natural blue light from the sun to regulate itself and in particular to control the sleep-wake cycles, called circadian rhythms. This regulation is possible thanks to a category of photoreceptors, retinal proteins called opsins, which play a key role in phototransduction. Phototransduction is an essential mechanism by which photons in the light are absorbed and converted into a cellular response. Similar to retinal opsins, recently discovered skin opsins act as photoreceptors for the phototransduction of signals in the skin and contribute to the cutaneous physiology and regulation of circadian rhythms as well as pigmentation. While natural blue light provides benefits for the regulation of certain vital physiological mechanisms, overexposure to artificial blue light has a negative impact on photoreceptors, damaging them. This is how blue light accelerates skin aging. Wrinkles but also especially hyperpigmentation are the most visible consequences.

The oxidation generated by blue light also results in inflammatory and degradation phenomena, in particular due to the release of metalloproteinases. MMP-1 also called collagenase is thus responsible for the breakdown of collagen, an essential component of the ExtraCellular Matrix. The degradation of collagen by the action of MMP-1 released following overexposure to blue light, partly explains the appearance of wrinkles, a sign of premature aging.

Taxifolin provides protection against the release of MMP-1. By protecting collagen against InfraRed, in addition to blue light, Taxifolin offers extensive photoprotection against premature aging induced by sun exposure.

Blue light is not the only cause of skin damage. UVA / UVB are also well known for their deleterious effects. Studied in recent years, InfraRed emitted by the sun (radiation beyond 600 nm) have also proven to be dangerous for the skin because of deep penetration into it. They generate many free radicals that can alter DNA and other cellular components. This can result in accelerated photoaging and impaired cell function.

Taxifolin absorbs blue light, significantly protects the opsins from stress, helps maintain the smooth operation of physiological and vital mechanisms such as pigmentation and circadian rhythms. Taxifolin confirms its antioxidant capacity by significantly inhibiting blue-light-induced MMP-1 release. It thus protects the collagen from breakdown and helps preserve the structure of the ExtraCellular Matrix, participating in the fight against premature aging.

Whatever our skin type, we are susceptible to everyday skin problems such as seasonal dryness, blackheads and inflammation for seborrheic skin, redness for sensitive skin, small cuts during shaving for men, scars etc. When these imperfections are not due to problems of a medical nature, they are due to multiple imbalances that cause the skin’s surface structure to deteriorate: it becomes swollen, red or irregular in certain places, and basically loses its uniformity. These imperfections may come from a lack of oxygenation, dehydration, overproduction of sebum due to bacterial infection, or some form of external threat.

Tiredness and age cause several basic cell mechanisms to slow down, such as cell respiration or micro-circulation, and therefore healing, and can also cause an intensification of inflammatory phenomena. Although these imbalances cause changes that are visible at the skin’s surface, their repair can involve numerous elements and mechanisms located in the first two layers of the skin: the epidermis and the dermis.

Taxifolin first helps to complete the healing of skin damage, from the reduction of inflammation to tissue reconstruction: damage causes inflammation and redness that Taxifolin will endeavor to regulate, while also helping cells to multiply and to synthesize more tissue thanks to a supply of oxygen and nutrients, and a better elimination of toxins. The cells are then able to defend themselves more efficiently.

Taxifolin enables the skin to repair damage by itself more quickly. Thanks to its broad spectrum of antioxidant, anti-inflammatory, energizing and protective properties, Taxifolin rebalances the skin’s complexion.

It acts at various levels in the healing process, which takes place in three stages: reduction in general inflammation, increase in cellular activity and reconstruction of cellular tissue.

To ensure better oxygenation of the cells manufacturing cell tissue, which must also defend themselves against damage due to general oxidation, it directly reduces the production of free radicals and increases the synthesis of enzymes that block their formation. Taxifolin enables the skin to defend itself more effectively against environmental threats.

The inflammation that is triggered by the appearance of a lesion (or an overproduction of sebum) is necessary to combat bacteria in the area. During this phase, leukocytes (white blood cells) infiltrate the wound, remove “waste” (clots, damaged cells and microbes), and release growth factors and cytokines produced by cells in the epidermis. Scientists have selected several of these: the interleukins IL-6, IL1-alpha, VEGF (Vascular endothelial growth factor) and TGF-beta (Transforming Growth Factor-beta). Il-6, IL1-alpha and TNF-alpha are pro-inflammatory cytokines. VEGF and TGF-beta stimulate the proliferation and migration of endothelial cells. TGF-beta also regulates pro-inflammatory cytokines. While this inflammation is necessary, its intensity can eventually be harmful and result in chain reactions that damage the skin. It is therefore necessary to significantly reduce this inflammation, due to the cytokines, and bring it down to a lower level, that does not pose a long-term risk. **Taxifolin (“Etalon Cosmetics”) helps to reduce the long-term inflammation**.

Less than a week after the skin has been broken, it begins to fill the break by manufacturing skin tissue from fibroblasts in the dermis. These cells produce two components essential for the skin’s support: collagen, the fibrous structural protein component, and proteoglycans, macro-molecules composed of a protein and a glycosaminoglycan, which attract water. Fibroblasts are nourished by amino acids released during the breakdown of the blood clot by macrophages, and use the fibrin network as a "matrix" to deposit the collagen; this is the granulation layer. Later the collagen fibres mature. The wound retracts and the granulation tissue, lacking water and blood vessels, forms scar tissue. At the end of the process, epidermal cells capable of division multiply and begin to cover the granulation tissue, starting from the edges of the wound, eventually closing the wound when this initial layer of cells has completely formed. The presence of regulated growth factors (see first part, inflammation) enables keratinocytes to multiply and enhances regeneration of the skin at the epidermal scale. **TAXIFOLIN** was evaluated with the effect on the synthesis of these two essential components: collagen and proteoglycans. Due to the increase in the production of collagen and proteoglycans (may be first fibroplasts), **Taxifolin (“Etalon Cosmetics”) helps to accelerate the reconstruction of skin tissue**.

The skin around the eyes is prone to start sagging and becomes wrinkled during aging. Many people suffer from dark circles, which have a big impact on the healthy look of a person. On top of that, the skin around the eyes is extremely thin and vulnerable. Effective eye care products need to combine actives addressing all these phenomena.

Peptide Complex – Taxifolin / Whey Protein – Polyphenol, Dosage: 0.5%, pH range: 6.0–7.0 /: reduces the visible signs of aging by reactivating skin cells and inducing the production of extracellular matrix molecules in the dermis, such as collagen Type I, hyaluronic acid and fibronectin.

In vivo studies have shown that formulations with peptide complex make the skin firmer and improve its elasticity. Peptide complex quickly and effectively reduces wrinkle depth and improves the structure of the skin.

To ensure better defense of the epidermal cells, thereby enhancing their operation and preventing new inflammation and lesions, **Taxifolin (“Etalon Cosmetics”) works to strengthen the natural defenses of the cells** affected by both internal attacks (physiological lipid peroxidation, in particular from oxygenation) and external attacks (light radiation, bacteria and other microbes entering through wounds). And also to restore a good skin barrier: a well-balanced physical line of defense.

The production of free radicals is twofold: endogenous (metabolism, stress, intense inflammatory reactions, etc.) and exogenous (exposure to light or chemicals, pollution, etc.). However, the production of free radicals attacks our skin in different ways: oxidizing cell membranes (lipid peroxidation), and proteins generally, ultimately damaging the cell DNA. Because the healing process and inflammatory responses lead to increased production of free radicals that destroy cells in the long term, **TAXIFOLIN** was verified for activity in terms of the overall production free radicals, and also its action on the several main antioxidant enzymes Due to its direct and indirect antioxidant action on free radicals, **TAXIFOLIN** strengthens the skin’s natural antioxidant defenses.

|  |  |
| --- | --- |
| **TAXIFOLIN-RICH EXTRACT 92% purity assay (manufacture "Etalon Cosmetics", Russian Federation)** **ORAC Using Multiple Radicals (ORAC-FN): New Horizons in Total Antioxidant Capacity Measurement.** | |
| **ORACFN Test by Brunswick Laboratories, INC (USA)** | |
| **Antioxidant Power Result** ( **μmole TE/gram )** | |
| Against Peroxyl Radicals | 23 075 |
| Against Hydroxyl Radicals | **32 873** |
| Against Peroxynitrite | 975 |
| Against Super Oxide Anion | 7 228 |
| Against Singlet Oxygen | 2 795 |
| **Total ORACFN** | **66 946** |

*TAXIFOLIN has the highest ORAC value among botanical antioxidants. TE - Trolox® (a water-soluble analogue of vitamin E) as a standard by which all other antioxidant compounds are compared.*

Oxidative stress is an imbalance between the levels of free radicals produced by the body and the amount of antioxidants available to neutralize these free radicals. Under situations of oxidative stress, free radicals damage the cell’s membranes, DNA, proteins, etc. Collectively, these increase the body’s inflammatory response.

**Cellular Antioxidant Assay CAA TAXIFOLIN-RICH EXTRACT** / Test by Brunswick Laboratories, INC (USA) /

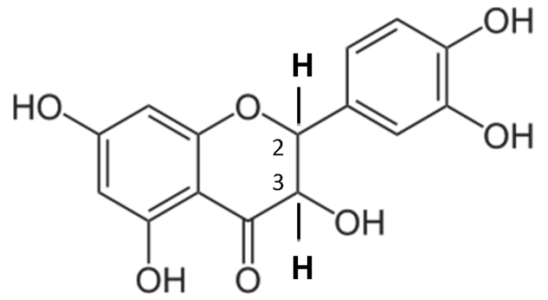
|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Results:**  **Description** | |  | | | **Test** | | | **Result** | |
| **EC50 CAA** | | | | | | | | | |
| TAXIFOLIN rich extract “Etalon Cosmetics” |  | | CAA | 61.17 | | μg/mL | 437.77 | | μmole QE/gram |
|  |  | |  |  | |  |  | |  |

*Cellular Antioxidant Assay measures intracellular antioxidant levels and inhibition of oxidation.*

*It is a preclinical measure of bioavailability that describes the amount of a substance to be absorbed by cells as well as its antioxidant effectiveness within the cell. Quercetin is used as the standard, and the results are expressed as μmole quercetin equivalency per gram (or milliliter) of a tested material.*

Stressors (external and internal; physical, mental and emotional) can cause reactive oxygen species to flourish and the body’s antioxidant cycle cannot keep up the same pace to wipe them out. The damage done by oxidative stress to the body’s cells, proteins and DNA manifests itself in a variety of ways, contributing to accelerated aging and risk factors for disease. The object is to stop free radicals from proliferating and disable their damaging activity. Decades of studies on antioxidants has led to development of the theory that aging itself and its root causes lay in the oxidative stress that develops over time in the body. Today we understand that many chronic health conditions have their origin from oxidative stress, and so to manage these conditions it’s important to manage oxidative stress.

In addition, within the large family of flavonoids, Dihydroflavonols and namely Taxifolin present a unique structural feature known as chirality, which distinguishes them from all other classes of flavonoids. Almost all dihydroflavonols such as Taxifolin have two chiral carbon atoms in position 2 and 3. The term optical activity, which is absolutely referred to Taxifolin native molecule form, is derived from the interaction of chiral materials with polarized light.



Four main functions of TAXIFOLIN are to neutralize free radicals, repair oxidized membranes, decrease reactive oxygen species production, and—via lipid metabolism, short-chain free fatty acids and cholesteryl esters—neutralize reactive oxygen species. The polyphenolic rings of TAXIFOLIN molecule can react and detoxify free radicals, it is also very stable. A robust body of science both at the clinical and mechanistic level show that antioxidant nutrient such as TAXIFOLIN is capable of neutralizing free radicals and limiting cell damage. This dietary polyphenolic compound has been shown to be effective in managing healthy levels of inflammation primarily because of its antioxidant activities.

**Cellular Anti-Inflammatory Assay (NFkB) TAXIFOLIN-RICH EXTRACT** / Test by Brunswick Laboratories, INC (USA) /

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Results: Description** |  | **Analysis** | **Inducer/**  **Stressor** | **Maximum inhibition** **NFkB**  (%) | **Effective Concentration**  (μg/mL) |
| TAXIFOLIN rich extract “Etalon Cosmetics” |  | Cellular Anti-inflammatory Assay (NFkB) | TNF-alfa | **53.79** | 423.98 |

*Cellular Anti-Inflammatory Assay (NFkB) determines the anti-inflammatory potential of a given material in human cells. NFkB (Nuclear Factor kappa B), a protein complex that is involved in cellular responses to stimuli such as stress and free radicals, is used as inflammation biomarker. Such important protein is NF-κB which is implicated as a key transcription factor in the development of tumors, tumor metastasis, angiogenesis (an essential component for tumor growth), and chronic inflammation.*

*In this particular NFkB assay, Tumor necrosis factor alpha (TNF-alfa), a pleiotropic inflammatory cytokine, is introduced to the human cells to trigger cellular inflammation. If an anti-inflammatory material presents in the cellular environment, the material inhibits NFkB activation and the degree of inhibition can be monitored via NFkB expression. NFkB expression level of the human cells, treated with and without test materials, under the stressed condition are therefore monitored and compared. Maximum percentage of NFkB expression inhibition induced by tested materials is reported. The concentration used that induced the maximum inhibition of NFkB expression is also noted.*

Science indicates many health concerns boil down to basic bodily functions like inflammation, which can result in oxidative stress. TAXIFOLIN is a unique catalyzer that is shown to stimulate production of antioxidant enzymes inside cells, therefore protecting cells from free radical damage; and has been shown to help support basic body functions to reduce inflammation and oxidative stress while improving blood vessel health. TAXIFOLIN is effectively used by our body to perpetuate the glutathione recycling mechanism, the body’s way of continuing the antioxidant process. Its antioxidant benefits are fully preserved for bioavailability, and its ability to facilitate the glutathione pathway of antioxidant regeneration is strengthened. TAXIFOLIN is able to stimulate the activities of our antioxidant enzymes such as catalase and glutathione peroxidase. TAXIFOLIN plays a big role in maintaining the homeostasis of the oxidative balance. The truth is that both oxidation and silent inflammation can ravage our health and lead to serious maladies. Fortunately, there’s a great product that is scientifically validated to control both—natural TAXIFOLIN.

Therefore, nutritional antioxidants can decrease lipid and protein oxidation, potentially encouraging quicker recovery and protecting against deterioration to chronic inflammation and diseases.

TAXIFOLIN has a regulating and modulating effect on key functional systems of cells, organs and tissues of the body, including: antioxidant system of cells and tissues; enzymatic systems, including representatives of almost all classes and groups of enzymes (oxido-reductase, hydrolase, lyase, transferase, kinase); receptor apparatus of cells and intracellular information systems; systems of ion transport and ionic homeostasis of the cell.

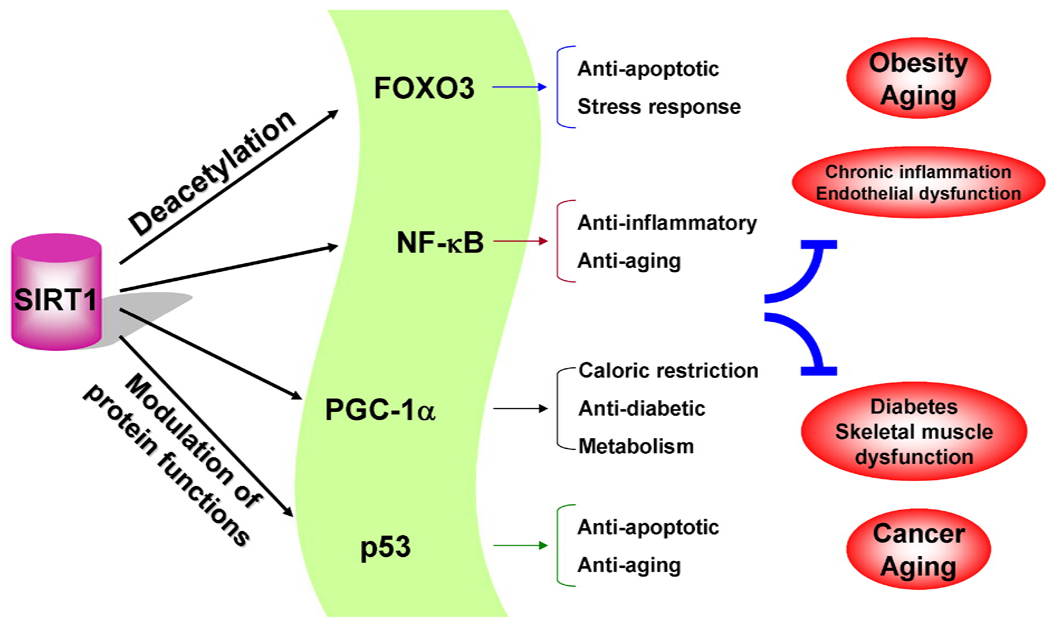
As the average human lifespan increases, discoveries about cellular aging may provide keys to achieve beneficial effects across multiple health outcomes. Many hallmarks of aging occur within the cells, such as the accumulation of DNA mutations, the shortening of telomeres, the accumulation of protein aggregates, as well as changes to the epigenetic landscape of people’s genomes. However, it seems the accumulation is not a solely random process, and opportunities are available to influence its progress. This evidence comes from research in model organisms—from invertebrates such as nematode worms or fruit flies, all the way through to mammals, including rodents and primates—which has shown that mutations in single genes, or interventions that target these genes and their pathways, can extend lifespan significantly, and delay or prevent the onset of age-related conditions.

**Cellular Anti-aging Assay (SIRT1) TAXIFOLIN-RICH EXTRACT** / Test by Brunswick Laboratories, INC (USA) /

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Results: Description** |  | **Analysis** | **Marker** | **Maximum SIRT1 Expression Change (%)** | **Effective Concentration Taxifolin** | **Units** |
| TAXIFOLIN rich extract “Etalon Cosmetics” |  | Cellular Anti-aging Assay | SIRT1 | **30.4** | 14.8 | μg/mL |

*Cellular Anti-aging Assay (SIRT1) measures the anti-aging ability of a material using SIRT1 production in human cells as a biomarker for anti-aging. SIRT1 is a protein that is believed to play important roles in longevity and reduction of age-related diseases.*

*Previous studies have shown that when mammals age, SIRT1 expression decreases, where induction and activation of SIRT1 has been associated with extended lifespan. These studies have triggered the search for SIRT1 activators that may be used as functional agents to promote health and longevity.*



The prevalence of genes involved in pathways related to nutrients and energy demonstrates it is not just important which nutrients people put into their bodies, but also how these nutrients are sensed and metabolized in the cells. This suggests promise exists in taking the target genes and treatments identified and applying them to the development of interventions that support healthier human aging.

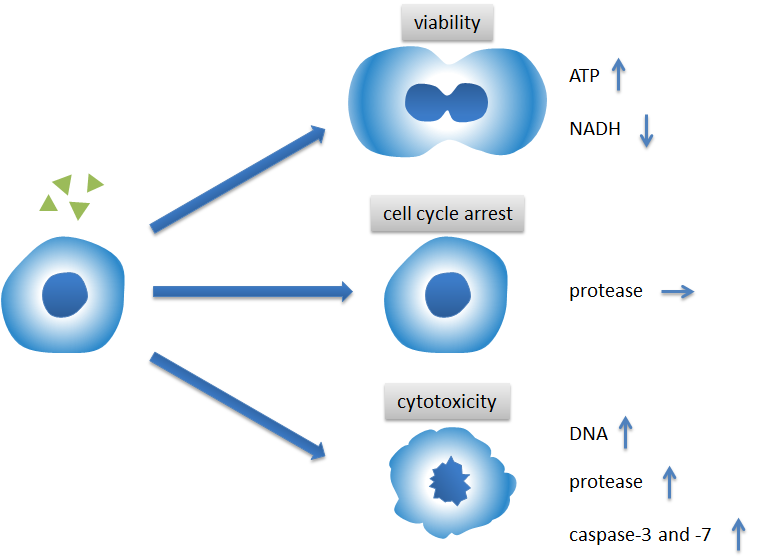
In the redox regulation of cell transcription, TAXIFOLIN supports the protective effect of enzymes of the enzymatic link of the antioxidant system of the cell, in particular, enzymes of the first line of antioxidant defense (peroxidases, catalases, etc.). The ingredient has an indirect antioxidant effect, capable of activating (or stimulating) phase 2 detoxification enzymes in the liver, which act as a defense mechanism, triggering a wide range of antioxidant processes, preventing cell damage. The indirect antioxidant effect of TAXIFOLIN persists even after its elimination from the body, in contrast to the direct antioxidant effect of the ingredient.

**Cells viability ATP method TAXIFOLIN-RICH EXTRACT** / Test by Brunswick Laboratories, INC (USA) /

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Results: Description** |  | **Test** | **Result** | **Units** |
|  |  |  |  |  |
| TAXIFOLIN rich extract E.C. |  | Viability | 990.31 | μg/mL |

*Cells viability ATP method. The adenosine triphosphate (ATP) luminescence assay is a highly effective method for the quantitative evaluation of proliferation and cytotoxicity of cultured human cells. ATP is a marker for cell viability because it is present in all metabolically active cells and the concentration declines rapidly when the cells undergo necrosis or apoptosis.*

*The viability result is expressed as the sample concentration which the number of viable cells in culture based on quantitation of the ATP present is the maximum.*



*The amount of ATP in cells correlates with cell viability. Within minutes after a loss of membrane integrity, cells lose the ability to synthesize ATP, and endogenous ATPases destroy any remaining ATP; thus the levels of ATP fall precipitously.*

|  |  |
| --- | --- |
| **Summary table Efficacy cellular system of**  **TAXIFOLIN-RICH EXTRACT 92% purity assay**  **(manufacture "Etalon Cosmetics", Russian Federation)** | |
| **Total ORACFN** | 66 946 μmole TE/gram |
| **CAA** (Cellular Antioxidant Assay) | 437.77 μmole QE/gram |
| **EC50** (less means more active) | 61.17 µg/mL |
| **Cellular Anti-Inflammatory Assay (NFkB)** determines the anti-inflammatory potential of a given material in human cells. NFkB (Nuclear Factor kappa B), a protein complex that is involved in cellular responses to stimuli such as stress and free radicals, is used as inflammation biomarker. | Maximum inhibition - 53.79%  Effective Concentration –  423.98 µg/mL |
| **Cellular Anti-aging Assay** (SIRT1) determines the impact of a test material on expression/production level of SIRT1 in human cells. SIRT1 serves as an anti-aging biomarker. | Maximum SIRT1 Expression Change (%) 30.4  Effective Concentration  14.8 µg/mL |
| **Cells viability ATP method.** The adenosine triphosphate (ATP) luminescence assay is a highly effective method for the quantitative evaluation of proliferation and cytotoxicity of cultured human cells. | Sample concentration corresponds to ATP present is the maximum.  990.31 μg/mL |

Manufacture "Etalon Cosmetics", (Russian Federation) pays considerable attention to the qualifications of its raw material suppliers, as well as strict adherence to the process parameters in the production of TAXIFOLIN.

INCI/CTFA Name: TAXIFOLIN **INCI Name: DIHYDROQUERCETIN**

IUPAC Name: (2R,3R)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydrochromen-4-one

European Community (EC) Number: 207-543-4 CAS number: 480-18-2

Bio-compatible (COSMOS Reference)

Raw material approved by ECOCERT GREENLIFE according to the COSMOS standard

Recommended dose: 0.5% – up to 5%

pH stability: 5.0 à 9.0 Soluble at 2% in water, dispersible in glycerin, 100% alcohol solution

Thermostability: recommended temperature below 45°C (may be incorporated at a maximum temperature of 60°C but must not be heated for more than 6h)

**EXAMPLES of FORMULATIONS with TAXIFOLIN [I]**

**Cream** - Soft form without impurities and inclusions

**TAXIFOLIN** (Dihydroquercetin), mg/tube, not less 40 mg per 10 gr tube // 200 mg per 50 gr tube

CAS No.480-18-2

**Phospholipid content**, mg/tube, not less (Lipoid E80, Lipoid GmbH Germany) Egg phospholipids with 80 % phosphatidylcholine

CAS No. 93685-90-6 300 mg per 10 gr tube // 1500 mg per 50 gr tube

**Glycine**, mg / tube (Wirud GL 10001, Wirud GmbH Germany)

CAS No. 56-40-6 500 mg per 10 gr tube // 2500 mg per 50 gr tube

**Mass fraction of water**, % 92

Hydrogen index, pH 5,9 Thermo stability Stable

**Gel** - yellowish shade allowed, Tube, contains 10 grams net of the gel

**TAXIFOLIN** (Dihydroquercetin), %, not less 0.04 (40 mg per tube)

CAS No. 480-18-2

**Phospholipid content**, %, not less 3 ( 300 mg / tube)

(Lipoid E80, Lipoid GmbH Germany) CAS No. 93685-90-6

**Glycine**, 500 mg / tube

(Wirud GL 10001, Wirud GmbH Germany) CAS No. 56-40-6

**Ethanol content** (medical grade), %, not more 0,5 (0.4-0.5)

**Water**

Hydrogen index, pH 5,5 – 6,5

Thermo stability Stable

**EXAMPLES of FORMULATIONS with TAXIFOLIN [II]**

Components of the Cream Formulations

Percentage of components in formulation (w/w)

|  |  |
| --- | --- |
| Part A | |
| Dimethicone/PEG-10/15 crosspolymer | 15.00 |
| Cyclopentasiloxane | 5.00 |
| Part B | |
| Distilled water | 63.92 |
| Glycerin | 10.00 |
| Glycosyl trehalose/Hydrogenated starch hydrolysate | 1.00 |
| Butylene glycol | 5.00 |
| Betaine | 0.01 |
| Disodium EDTA | 0.05 |
| Part C | |
| Taxifolin | 0.05 |

*The lotions were prepared by heating part A to 60°C until dissolved. Part B was added to part A while homogenizing at a rate of 3000 rpm for 3 min. Subsequently, part C was added and emulsified under the same condition.*

*Where after parts D, E and F were added together at stirring speed of 3000 rpm for 3 min and then reduced to 1500 rpm for 3 min. The lotions were then cooled with ice water and stirred at 500 rpm until room temperature was reached, and finally degassed*.

Components of the Lotion Formulations

| **Components** | **Percentage of components in each formulation (w/w)** |
| --- | --- |
| Part A | |
| Distilled water | 81.28 |
| Methylparaben | 0.20 |
| Butylene glycol | 1.00 |
| Glycerin | 5.00 |
| Arginine | 0.20 |
| Part B | |
| Cetyl alcohol | 1.00 |
| Butylparaben | 0.10 |
| Tocopheryl acetate | 0.30 |
| Phenoxyethanol | 0.50 |
| Part C | |
| Dimethicone | 2.00 |
| Cyclotetrasiloxane/Cyclohexasiloxane | 1.00 |
| PEG/PPG-18/18 Dimethicone | 0.10 |
| Cyclopentasiloxane | 1.00 |
| Part D | |
| Sodium hyaluronate | 1.00 |
| Carbomer | 5.00 |
| Part E | |
| 3-*O*-Ethyl ascorbic acid | 0.30 |
| Part F | |
| Taxifolin | 0.1 |

*The compositions of part A were mixed together and heated to 80°C until dissolved. Then part B was added to part A while homogenization of different parts was achieved at stirring speed of 3000 rpm. After 3 min, part C was added with continuous stirring and emulsified under the same condition. Then part D was added ahead of part E. After all the components have been added, stirring was continued at a rate of 3000 rpm for 3 min and then reduced to 1500 rpm for 3 min. When the temperature dropped to room temperature, the essences were continuously stirred at 500 rpm for 3 min, and finally degassed.*

Formulations containing taxifolin were stored in a thermostat (Grant Instruments Ltd., Cambridge, U.K.) at 25 and 40°C with 75% relative humidity (RH) for up to 12 weeks, respectively. Formulations were evaluated for various parameters at the time intervals of 0, 1, 2, 4, 8 and 12 weeks.

The viscosity of various formulations was determined at 0, 1, 2, 4, 8 and 12 weeks with a Brookfield viscometer (LVDV-II viscometer, Brookfield Engineering Laboratories, Inc., U.S.A.) fitted with No. 4 spindle. The product temperature to be tested was controlled by a Brookfield circulating water bath with a temperature controller (Massachusetts, U.S.A.). The viscosity of the formulations was determined at a stable temperature of 25°C. An amount of 100 g of the product was placed into a glass container for viscosity reading. In each reading, the viscosity readings were taken every 1 min during 10 min.

The pH of a formulation may influence the stability of active components in the formulation. In this study, the pH of each sample kept in different storage conditions was determined by a portable pH meter (LAQUAact, D-71, Horiba, Ltd., Japan). The samples of cream and lotion formulations were diluted to 1 : 15 (w/w) in distilled water. In the case of the essences, measurements were made at their original concentrations. The pH of each formulation was determined at room temperature, right after the preparation and during the 12 weeks of the stability study. To ensure accuracy, three measurements were taken on each batch.

For the content analysis of taxifolin in all formulated products, degassed acetonitrile-1% acetic acid in water (30 : 70, v/v) was the mobile phase at a flow rate of 1.0 mL/min. The injection volume was 10 µL and analyse was performed at 280 nm. One gram of formulation sample was transferred to a 20 mL volumetric flask and diluted with methanol. Then, the samples were dissolved ultrasonically and filled to volume in the volumetric flask. The solution was filtered with a 0.20 µm polytetrafluoroethylene (PTFE) membrane syringe filter and used for HPLC analysis.

EXAMPLES of FORMULATIONS with TAXIFOLIN [III]

w/w %

soy phospholipids containing phosphatidylcholine (lecithin) from 50 to 100% - 6.0-16.0

water-soluble plant extracts 0.5-12.0

carbohydrates 2.0-5.0

distilled glycerin 2.0-5.0

TAXIFOLIN (dihydroquercetin) 0.5-2.5

vitamin PP (Nicotinamide) 0.1-1.0

water-soluble antioxidants-antihypoxants 0.05-0.5

oil-soluble antioxidants-antihypoxants 0.05-0.5

magnesium ascorbyl phosphate 0.05-0.5

preservative 0.1-1.0

ethyl alcohol 6.0-14.0

deionized water up to 100

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