Dihydroquercetin, Larch Arabinogalactan and Larch Oleoresin in cosmetic and hygiene products.

It is proposed to the use larch wood extracts or natural compounds in particular antioxidant Dihydroquercetin (taxifolin), polysaccharide Arabinogalactan and Arabinogalactan combining with Dihydroquercetin (taxifolin), wood oleoresin comprising oil and resin for applications in cosmetic and hygiene products for exposing numerous benefits within skin care and hygiene care. Conifer wood species, especially those from the family of Pinaceae are considered rich sources of above mentioned natural compounds. The emphasis is put on residues of wood transformation such as bark, butt logs, roots and knotwood as these materials represent particularly rich resources for flavonoids, particularly Dihydroquercetin (taxifolin), polysaccharides, particularly Arabinogalactan and Arabinogalactan combining with Dihydroquercetin (taxifolin) and wood oleoresin, particularly oil and resin.

Applications for larch wood extracts, in particular the polysaccharide Arabinogalactan and flavonoid Dihydroquercetin (taxifolin), are found in the food, pharmaceutical and cosmetic industries. Larch arabinogalactan, a water-soluble polysaccharide deriving mainly from plant genus Larix, is the source of dietary fiber, but has also confirmed effects as prebiotic [1,2]. The flavonoid constituents of larch wood in particular the flavonoid Dihydroquercetin (taxifolin) is known to possess good antioxidant and anti-inflammatory activities [3-5]. The oleoresins of the coniferous trees are well known in the flavor, fragrance, cosmetic and pharmaceutical industries. At the present time, the oleoresins of various species of larch have been studied in detail to consider actual content of biological active natural compounds [6-8]. There is information on the composition of the oleoresin terpenoids as extractive substances of the trunk part of the European larch and the heart and sapwood and the bark of the Larix cajanderi, Larix czekanowskii, Larix dahurica, Larix gmelinii, Larix kamtschatica, Larix russica, Larix sibirica, Larix sukaczewii. The neutral substances composing 50% of the weight of the larch oleoresin were represented by hydrocarbons and oxygen-containing compounds deterpenes (16 and 34%, respectively). Hydrocarbons are presented by monoterpene hydrocarbons, sesquiterpenes and diterpene hydrocarbons and aldehydes. The main components of the neutral substances are bicyclic compounds deterpenes or deterpenoids with the labdane structure: epimanool (~15%), and larixol (~40%) and its monoacetate (larixylacetate ~28%), making up about one-third of the neutral substances. In the acidic fraction of the oleoresin, isopimaric acid (40%) predominates [9]. Diterpenoids are constituents of natural resins, such as colophony resin, which is gained from conifer trees like spruce, firs and pines. Larches belong to the family Pinaceae, as already mentioned.

Cosmetic, including hygiene care is a global billion-dollar industry that markets and sells beauty and healthy products. However, environmental and health worries associated with manufactured goods undermine consumer confidence. Larch wood extracts flavonoid Dihydroquercetin (taxifolin), polysaccharide Arabinogalactan and Arabinogalactan combining with Dihydroquercetin (taxifolin) and wood oleoresin comprising oil and resin are presented potential to solve these concerns.

The skin is one of the most important organs of the body and creates a first line of organism defense against the external environment. Owing to the constantly increasing requirement for cosmetic and hygiene active natural compounds, it is intended in this article to identify active larch wood extracts which (i) have as little irritation potential as possible for the skin, hair, oral cavity (ii) have a high free radical-deactivating and anti-inflammatory effects and (iii) have a potential of bioavailability-enhancing agents (iv) are also suitable for the preparation of cosmetic and/or hygiene formulations or preparations.

Dihydroquercetin (taxifolin) is provided for reducing or inhibiting free radical oxidative damage, harmful microbial and inflammatory effects, thus cosmetic and/or hygiene compositions include an effective amount of Dihydroquercetin (taxifolin) extract.

The combination of a Dihydroquercetin (taxifolin) and Arabinogalactan is provided as one extract to reduce or inhibit free radical oxidative damage, harmful microbial and inflammatory effects resulting in skin, hair and oral care benefits. In one aspect, the polysaccharide Arabinogalactan can be defined as a fiber containing significant amounts of natural antioxidants, mainly Dihydroquercetin (taxifolin) associated naturally to the fiber matrix. The antioxidant capacity must be an intrinsic property, derived from natural constituents of the material not by added antioxidants or by previous chemical or enzymatic treatments. It

has been found that surprisingly and unexpectedly the combination of Arabinogalactan and Dihydroquercetin (taxifolin) synergistically can effectively be used for oral and topical applications and serve as a natural pool of nutrients and growth factors that support skin and oral health.

Larch Arabinogalactan is the suitable polymer, nonionic, water-soluble or water-dispersible polymer to provide support to skin texture and hydration and preserve skin elasticity and considered to be bioavailability-enhancing and surface active agent, which can function as surfactant, emulsifier, foam modulator, and/or active ingredient dispersion agent. Larch Arabinogalactan can be defined as a fiber containing significant amounts of natural antioxidants, mainly Dihydroquercetin (taxifolin) associated naturally to the fiber matrix as already described above and to enjoy a special status in the repository of cosmetic and hygiene ingredients that nurture skin and oral health and wellbeing.

Larch wood oleoresin extract in forms of oil and/or resin is the another class of natural actives that support skin and oral health comprising neutral part of oil and resin especially derived from wood oleoresin for application in cosmetic and hygiene preparations or formulations in order to provide active role in limiting hair loss, stimulating skin pigmentation, and preserving skin or oral health, to provide the body's early defense in response to trauma, inflammation or infection, the acute phase response (APR), which is a complex set of systemic reactions seen shortly after exposure to a triggering event. Larch wood oleoresin can be combined with Dihydroquercetin (taxifolin) naturally as one extract or syntactically.

Molecular biology plays a pivotal role in innovating cosmeceuticals. Ingredient development now begins with the identification of molecular targets. For example, the importance of free radicals in association with skin aging has led in recent years to an intensive search for active substances which eliminate the harmful effects of free radicals and thus protect the tissue from oxidative damage. Skin ageing manifests as age spots, more specifically as melasma, dyschromia, melanomas, and wrinkling, mainly attributed to free radical damage to the tissues that triggers cross linking and glycation of structural proteins, and proinflammatory enzyme systems. The use of flavonoids, in particular, in cosmetics or pharmacy is known per se. Natural antioxidants that quench free radicals are an essential component of anti-ageing formulations. They potentially offer protection against damage to the tissues, and against the detrimental effects of environmental and other agents. Biochemical reactions that accelerate the progression of skin ageing have their roots in inflammatory processes, as inflammation generates micro-scars that develop into blemishes or wrinkles.

Various types of inflammatory mediators may influence melanin synthesis by affecting the proliferation and functioning of melanocytes, pigment-producing skin cells, and normal cutaneous blood circulation. Natural “antiinflammatory” agents are therefore included in anti-ageing formulations in order to soothe, heal and protect skin tone and integrity.

An increasing amount of scientific evidence supports the beneficial “anti-ageing” effects of several phytonutrients at the molecular level. For example, plant flavonoids inhibit the age-related atherosclerotic deposits in animals by influencing vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemotactic protin-1 (MCP-1) gene expression [10]. Results indicate that Dihydroquercetin (taxifolin), like other flavonoids, has an active role in limiting hair loss, stimulating skin pigmentation, and preserving skin health. The potent bio-activities and relatively low toxicity of Dihydroquercetin (taxifolin) makes it a suitable compound for use in cosmetics [11].

Dihydroquercetin (taxifolin) (or 3,5,7,3’, 4’-pentahydroxyflavanone, or (2R, 3R)- 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-chroman-4-one) occurs in various waste residues of conifer species (Larix cajanderi, Larix dahurica, Larix gmelinii, Larix russica, Larix sibirica, Pinus pinaster ssp atlantica) and in Silybum marinum seeds (used for the preparation of the silymarin complex and containing silymarin flavonolignans which are biogenetically formed by oxidative addition of coniferyl alcohol to Dihydroquercetin (taxifolin). It has a chiral bond between cycle B and the two others cycles. Relating to the bioflavonoid group, it possesses a wide spectrum of biological activities [12]. It shows capillary-protecting, anti-inflammatory and gastro-protective action, decreases spasms of sleek muscles of the intestine, increases functions of the liver, possesses antiradiation protective activity. Dihydroquercetin (taxifolin) has also been shown to have potential applications in reducing skin inflammation [10]. These findings suggest that Dihydroquercetin (taxifolin) is effective for the treatment of atopic dermatitis (AD) by preventing the

production of inflammatory cytokines and by reducing skin inflammation [13]. Furthermore, Dihydroquercetin (taxifolin) and its rhamnoside isomers isolated for the first time from kempas could be potent compounds for preventing dental caries [14].

Dihydroquercetin (taxifolin) possess superior antioxidant activity to suppress affects of free radicals [15 - 21]. It has been shown that Dihydroquercetin (taxifolin) [Fig.1,2] and phenolic acids have a high UV absorption activity [22,23]. Dihydroquercetin (taxifolin) acts as antioxidant with mechanisms involving both free radical scavenging and metal chelation [Table 1]. Indeed, excess levels of metal cations of iron, zinc and copper in the human body can promote the generation of free radicals and contribute to the oxidative damage of cell membranes and cellular DNA; by forming complexes with these reactive metal ions, they can reduce their absorption and reactivity. It has to be underlined that though most flavonoids chelate Fe2+, there are large differences in the chelating activity. In particular, the Dihydroquercetin (taxifolin) chelates more efficiently Fe2+ than the corresponding flavonoid quercetine [24]. It have been demonstrated in numerous studies in vitro and ex vivo that Dihydroquercetin (taxifolin) inhibits lipid peroxidation, a process that often leads to atherosclerosis [25-27]. In an animal study, Dihydroquercetin (taxifolin) inhibited the peroxidation of serum and liver lipids following exposure to toxic ionizing radiation [28]. Dihydroquercetin (taxifolin)'s inhibitory effects on lipid peroxidation are enhanced by both vitamin C and vitamin E [29].

Recent research findings lend credence to the fact that metabolism, gene expression, and ageing intersect at the molecular level. Nuclear receptors sense a variety of environmental triggers, including dietary components and steroid hormones, and influence metabolic and the ageing process. An increasing amount of scientific evidence supports the beneficial "anti-ageing" effects of several phytonutrients at the molecular level.

For example, Dihydroquercetin (taxifolin) can modulate the expression of several genes, including those coding for detoxification enzymes, cell cycle regulatory proteins, growth factors, and DNA repair proteins. Dihydroquercetin (taxifolin) significantly activates Antioxidant Response Element. ARE (Antioxidant Response Element) in the promoter region of the human NQO1 gene contains AP-1 or AP-1-like DNA binding sites, and AP-1 proteins have been implicated in the formation or function of this and other ARE complexes. Also, ARE-binding proteins in inducing cerebral MT-1 expression and implicates MT-1 as one of the early detoxifying genes in an endogenous defense response to cerebral ischemia and reperfusion [30,31].

One of the important ways in which Dihydroquercetin (taxifolin) may limit the cytokines plain is by preventing elevation of oxidized glutathione concentration and the oxidized/reduced glutathione ratio induced by inflammatory cytokines [32]. Dihydroquercetin (taxifolin) prevents calcium influx, the last step in the cell death process. By inducing the expression of antioxidant defense enzymes, it has the potential to have long-lasting effects on cellular function. This, in turn, could be highly beneficial to cells exposed to chronic oxidative stress [33]. Dihydroquercetin (taxifolin) processes benefit results in both intracellular and extracellular environments. Studies in erythrocytes, mast cells, leucocytes, macrophages and hepatocytes have shown that Dihydroquercetin (taxifolin) renders cell membranes more resistant to lesions. Dihydroquercetin (taxifolin) protects the inner walls of the blood vessels and capillaries against destructive enzymes, decay and free radical damage [34].

Dihydroquercetin (taxifolin) had been evaluated by different studies as the small-molecule regulator of signalling 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. Dihydroquercetin (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 [35 - 37]. Dihydroquercetin (taxifolin) had a significant inhibitory effect on the production of cytokines, formation of ROS and NO, and change in intracellular Ca2+ levels in dendritic cells of bone marrow and spleen [38]. Dihydroquercetin

(taxifolin) was attributed to its inhibitory effects on tyrosinase enzymatic activity, despite its effects on increasing tyrosinase protein levels [39].

Studies indicate that Dihydroquercetin (taxifolin) is highly safe and efficacious. In fact, research suggests that dihydroquercetin is even safer than its nutritional cousin, quercetin [40,41]. No toxic effects were observed in rats that were treated with high levels of dihydroquercetin for long periods of time [42-49].

Due to their low aqueous solubility, the use of flavonoids such as Dihydroquercetin (taxifolin) in cosmetic and\or hygiene preparations requires adapted and specific formulations. Since these formulations must also satisfy the constraints associated with their final usage, the compromise between acceptability, concentration and stability is often difficult to reach.

Higher arabinogalactan content often goes hand in hand with higher amount of flavonoid substances, in particular with Dihydroquercetin (taxifolin) [50]. More water soluble forms of flavonoids such as the combination of a Dihydroquercetin (taxifolin) and Arabinogalactan, wherein the polysaccharide Arabinogalactan can be defined as a fiber containing significant amounts of natural antioxidants, mainly Dihydroquercetin (taxifolin) associated naturally to the fiber matrix to serve as a natural pool of nutrients and growth factors that support skin and oral health, wherein Arabinogalactan is a suitable polymer, which is also nonionic, water-soluble or water-dispersible polymer.

Water-soluble Arabinogalactan is a typical useful surface active agent is disclosed above in the context of the bioavailability-enhancing agent that includes a solubilizing agent.

Surface active agents generally are an important aspect of the cosmetic and oral compositions, as they can function as surfactants, emulsifiers, foam modulators, and/or active ingredient dispersion agents. Their selection for compatibility with the active ingredient constituents is important. Suitable surface active agents, include those that were discussed in the context of the bioavailability/solubility enhancing agent above, are those which are reasonably stable and foam throughout a wide pH range.

Larch tree extract Arabinogalactan and Arabinogalactan combining with Dihydroquercetin (taxifolin) provides moisture, elasticity, and radiance to skin that boosts collagen production and prevents skin ageing by ‘locking in’ moisture and creating a natural plumping effect. Both Arabinogalactan and Arabinogalactan combining with Dihydroquercetin (taxifolin) provide many desirable skin and oral care effects, and by itself was shown, under clinical conditions, to reduce the appearance of fine lines and wrinkles. It is also proven to provide anti-oxidative properties to help protect skin against UV-induced oxidative damage.

Arabinogalactan has a number of benefits as compared with other polysaccharide polymers. Arabinogalactan is water-soluble, occurs naturally with a narrow molecular weight distribution. While not wishing to be bound by any particular theory, it is believed that because Arabinogalactan is highly branched it is not subject to viscosity problems, as compared to other polymers. Arabinogalactan also stabilizes emulsions. It has been observed in photomicrographs of oil-in-water systems containing Arabinogalactan, the oil-in-water emulsion can be characterized as having smaller and more uniform oil droplets. The ability of Arabinogalactan to produce smaller, more uniform droplets tends to enhance the stability of Arabinogalactan-containing systems over time and is generally known to enhance performance properties. These emulsions have application in cosmetic, personal care, food and industrial applications.

Studies have shown Arabinogalactan to have numerous benefits within skincare, however the object of present invention to use preferably Arabinogalactan combining with Dihydroquercetin (taxifolin) for the purposes already mentioned above and to use for the purposes including:

Skin Cell Renewal Efficacy. Skin keratinocytes in the lower level of the epidermis undergo mitosis. These newly formed cells gradually push the existing cells upwards. The older keratinocytes are eventually pushed to the surface where they are sloughed off. Through surface exfoliation, these dead skin cells are removed to reveal younger skin underneath. A higher rate of skin exfoliation may indicate faster cell turnover rates, and a faster cell turnover rate can lead to a reduction in fine lines and wrinkles. Conducted clinical studies into Arabinogalactan properties show Arabinogalactan as both a primary exfoliant as well as its role as an exfoliant enhancer, and in both cases Arabinogalactan displayed significant properties as

an exfoliant and, when used in combination with lactose, properties as an exfoliant enhancer (by acting as a film-former, and increasing the functionality of the lactic acide by holding it to the skin).

Reduction of Fine Lines and Wrinkles. Faster skin cell turnover, as evidenced by enhanced exfoliation, may help explain Arabinogalactan's effect on fine lines and wrinkles, by bringing younger skin to the surface more rapidly. International Resources Inc conducted a study using 15 panellists to evaluate the effect of Arabinogalactan on fine lines and wrinkles in the crow's feet area of the face. In an 8 week, full face, randomized, double-blind, positive-controlled study, Arabinogalactan was proven to reduce fine lines and wrinkles by 19%. Product performance was assessed using both trained evaluators and instrumentally (using silicon replicas with subsequent image analysis) [51].

Reduce transepidermal water loss (TEWL). TEWL is the measurement of the water loss from a body that passes through the skin epidermis through diffusion, which then evaporates into the atmosphere. This measure is used to define skin barrier characteristics. Reduction of TEWL (i.e. lower TEWL readings) indicates that the skin barrier is more effective in retaining moisture in the skin, allowing it to feel more moisturised. A test conducted by International Research Services Inc in Port Chester, NY, on 21 subjects which measured TEWL after one application of a product containing 2% Arabinogalactan as against a placebo showed a statistically significant reduction in TEWL levels after only 2 and 4 hours, indicating that Arabinogalactan successfully helped to maintain skin barrier function.

Provides film forming and skin tightening - yielding an instant skin radiance, as well as having been shown to increase cell metabolic activity by increasing ATP production.

Increases dispersion of UV filters. Arabinogalactan has been shown to improve dispersion of inorganic sunscreen particles (such as titanium dioxide) leading to a more uniform and effective transference onto the skin surface. This leads to less clumping of the sunscreen particles and therefore more efficient packing of the sun protection per UV level.

Larch Arabinogalactan is defined as the class of long, densely branched low and high-molecular polysaccharides with molecular weight range 3,000- 120,000. Arabinogalactan consist of a main chain of b-D-(1fi3)-galactopyranose units (b-D-(1fi3)-Galp) where most of the main-chain units carry a side chain on C-6 [fi3,6)-Galp-(1fi]. Almost half of these side chains are b-D-(1fi6)-Galp dimers, and about a quarter are single Galp units. The rest contain three or more units. Arabinose is present both in the pyranose (Arap) and furanose (Araf) forms, attached to the side chains as arabinobiosyl groups [b-L-Arap-(1fi3)-LAraf-(1fi] or as terminal a-L-Araf e.g. a single L-arabinofuranose unit or 3-O-(β-L-arabinopyranosyl)-α-L-arabinofuranosyl units [88-91].

The conifers are an important source of diterpenoids making one third of neutral wood oleoresin. The main components of the neutral substances of larch wood oleoresin are bicyclic compounds deterpenes or deterpenoids with the labdane structure: epimanool (~15%), and larixol (~40%) and its monoacetate (larixylacetate ~28%). A variety of biological activities have been associated with labdane diterpenes including antibacterial, antifungal, antiprotozoal, enzyme induction, anti-inflammatory modulation of immune cell functions, as well as cytotoxic and cytostatic effects against human leukemic cell lines [52]. In addition to the (antimicrobial, enzyme and endocrine related) properties mentioned above, it is interesting that many labdane type diterpenes also exhibit significant properties against cancer cells.

After the group separation of the larch wood oleoresin, 12.5% of monoterpene hydrocarbons, 0.75% of sesquiterpenes, 18% of diterpene hydrocarbons and aldehydes, 13.5% of diterpene alcohols, and 32.5% of resin acids were obtained. The qualitative and quantitative analysis of the monoterpenes established that the monoterpene fraction contained: a-pinene (20.5%), camphene (0.3%), 8-pinene (23.2%), 3-carene (49.8%), myrcene (0.3%), limonene (1.1%), and 8-phellandrene (2.3%). In the sesquiterpenoid fraction we identified 16 compounds: cyclosativene, longicyclene, alfa-longipinene, sibirene, longifolene, y-elemene, a,y and e-murolenes, beta-selinene, d-, y-, and e-cadinenes, a-humulene, calamenene, and the methyl ether of thymol, the main components being delta- and gamma-cadinenes and longifolene. From the fraction of diterpene hydrocarbons and aldehydes presented by dehydroabietane, a mixture of dehydroabietinal and abietinal and palustral. By chromatography of the diterpene alcohols were determined epimanool, larixyl acetate, and larixol. The analytical GLC of the mixture of resin acid methyl

esters showed the presence in them of the esters of the acids palustric and (or) levopimaric (7.2%), isopimaric (86.2%), dehydroabietic (2.0%), abietic (4.6%), and neoabietic (traces).

It had been known and recorded in the early history of medical practice that natural products such as oleoresins appeared to have some beneficial effects when applied to a variety of human ailments. Ancient remedies in the form of liniments, salves, poultices and tonics often had contained an ingredient such as turpentine, balsam tar, pine tar, rosin, gum resins, and the like. Because such ingredients tended to irritate the skin, the ingredient was employed in small quantities and in a highly diluted state.

It was discovered that concentrated solutions of deterpenoids and rosin acids when applied to traumas of the skin and underlying tissue, rather than irritate and exacerbate the wound area, the solution promoted rapid healing without development of scar tissue. The study of the dermatological application of concentrated oleoresin solutions was extended to include treatment of a broad variety of traumatic and degenerative skin disorders. There was consistent evidence that oleoresin groups of compounds (i.e., abietic acid derivatives), can act as an unusually effective therapeutic agent in the treatment of skin injuries. Burns, ulcers, infections, abrasions and wounds were treated with concentrated solutions of oleoresin. The wide potential of resin acids as bioactive agents gave rise to a growing effort in the search for new applications of the natural forms and their derivatives. In some of these compounds, the antimicrobial activity is associated to the presence in the molecules of functional groups such as the hydroxyl, aldehyde, and ketone or to their cis or trans configurations. The resin acid family covers a spectrum of antimicrobial activities against several microorganisms, from bacteria to fungi [54]. Concentrations of 25-35 weight percent of resin acids in olive oil were applied as a treatment of lymphangitis-cellulitis, small and large abscesses, carbuncles, adenitis of the inguinal and auxillary lymph glands, phlebitis, and a variety of ulcers including varicose, traumatic, indolent, arteriosclerotic, decubitus and diabetic ulcers. A 15 weight percent resin acids in olive oil terminated pain and infection in the otitis media without mastoid involvement.

Another class of natural actives that support skin hydration are the natural long chain alcohols, such as epimanool, larixyl acetate, and larixol, derived from neutral part of larch oleoresin.

The body's early defense in response to trauma, inflammation or infection, the acute phase response (APR), is a complex set of systemic reactions seen shortly after exposure to a triggering event. The APR is induced by protein hormones called cytokines acting as messengers between the local site of injury and the hepatocytes synthesing the acute phase proteins such as serum amyloid A. Most cytokines have multiple sources, targets and multiple functions. The proinflammatory cytokines can be divided into two major groups with respect to acute phase proteins inductions, the Interleukin-l (IL-l) type (including Tumor Necrosis Factor-a, TNF-a) and the IL-6 type cytokines (including IL-6 cytokine). These cytokines are secreted primarily by monocytes activated by bacterial toxins or in response to local tissue injury. Larixyl acetate proved to be highly active against Leukotriene Biosynthesis (LT) biosynthesis. The abietane-type diterpene dehydroabietinol showed high LT formation inhibitory activity. Isopimaric acid proved to be a potent inhibitor of 5-LOX mediated LT biosynthesis. The LT biosynthesis inhibitory potential of palustric acid was less pronounced, however, this compound also possessed moderate COX-2 inhibitory activity. Some of these diterpenes are known to possess antimicrobial, anti-ulcer and cardiovascular activities.

The preferred larch wood oleoresin in form of oil or resin is combined with Dihydroquercetin (taxifolin) naturaly as one extract according technological process known per se. However, larch wood oleoresin in form of oil or resin can be synergistically enriched with Dihydroquercetin (taxifolin.

Table 1. The results *in vitro* obtained are presented in the following order: the antioxidant capacities as determined by the FRAP, TEAC, and deoxyribose assays. All the samples investigated were found to exhibit antioxidative properties.

The FRAP assay takes advantage of electron-transfer reactions. Herein, a ferric salt, $Fe(III)(TPTZ)_2Cl_3$ (TPTZ =2,4,6-tripyridyl-s-triazine), is used as an oxidant. The reaction detects species with redox potentials <0.7 V [the redox potential of $Fe(III)(TPTZ)_2$], so FRAP is a reasonable screen for the ability to maintain redox status in cells or tissues. Reducing power appears to be related to the degree of

hydroxylation and extent of conjugation in flavonoids. However, FRAP actually measures only the reducing capability based on ferric iron, which is not relevant to antioxidant activity mechanistically and physiologically.

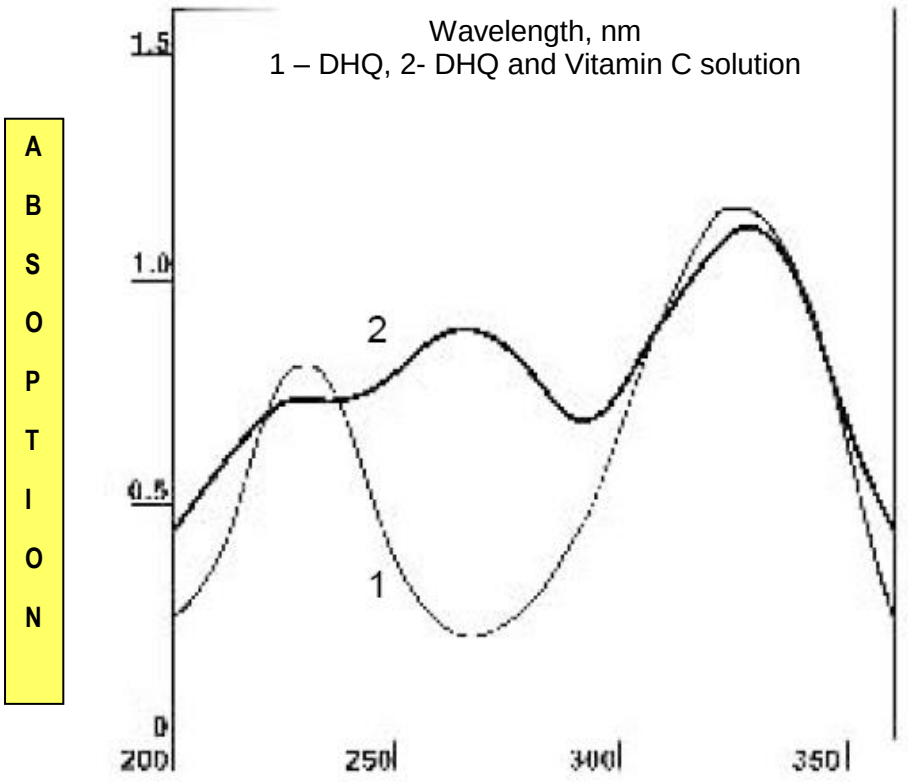
The TEAC assay is based on the formation of ferrylmyoglobin radical (from reaction of metmyoglobin with H_2O_2), which may then react with ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6)-sulfonic acid] to produce the $ABTS^{*+}$ radical. $ABTS^{*+}$ is intensively colored, and AC is measured as the ability of the test species to decrease the color by reacting directly with the $ABTS^{*+}$ radical. Results of test species are expressed relative to Trolox.

Deoxyribose assays: Hydroxyl radicals, generated by reaction of an iron-EDTA complex with H_2O_2 in the presence of ascorbic acid, attack deoxyribose to form products that, upon heating with thiobarbituric acid at low pH, yield a pink chromogen. Added hydroxyl radical "scavengers" compete with deoxyribose for the hydroxyl radicals produced and diminish chromogen formation. A rate constant for reaction of the scavenger with hydroxyl radical can be deduced from the inhibition of color formation. For a wide range of compounds, rate constants obtained in this way are similar to those determined by pulse radiolysis. It is suggested that the deoxyribose assay is a simple and cheap alternative to pulse radiolysis for determination of rate constants for reaction of most biological molecules with hydroxyl radicals.

Sample	FRAP [a]	TEAC [b]	Deoxyribose [c]
Dihydroquercetin	180.73 ± 11.05	462.99 ± 28.56	52.29 ± 3.03
Larch Arabinogalactan consisting with Dihydroquercetin	41.91 ± 0.82	51.62 ± 5.59	35.46 ± 2.08

Values expressed as mean value ± standard deviation (n=3)
[a] mmol Fe (II)/L FW
[b] mmol Trolox/L FW
[c] IC50 value

Figure 1. UV absorption capacity of DHQ and Vitamin C]



A mixture of Dihydroquercetin (DHQ) and Vitamin C (1:1) has advantages over DHQ alone. Vitamin C has λmax 265 nm, which compensates for λmin of DHQ. It is also important that vitamin C at a certain level stabilizes DHQ, preventing its rapid inactivation by UV light and free radicals. Additionally, these compounds act synergetically against free radicals to prevent the damage of the skin and prolong the shelf life of the cosmetic formulation. DHQ protects vitamin C proven capability to stimulate collagen growth in the dermis.

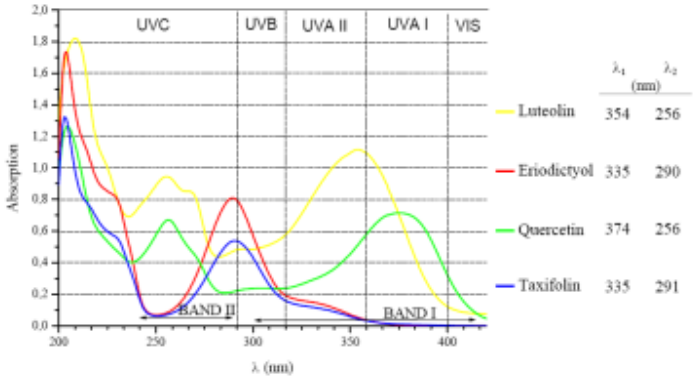
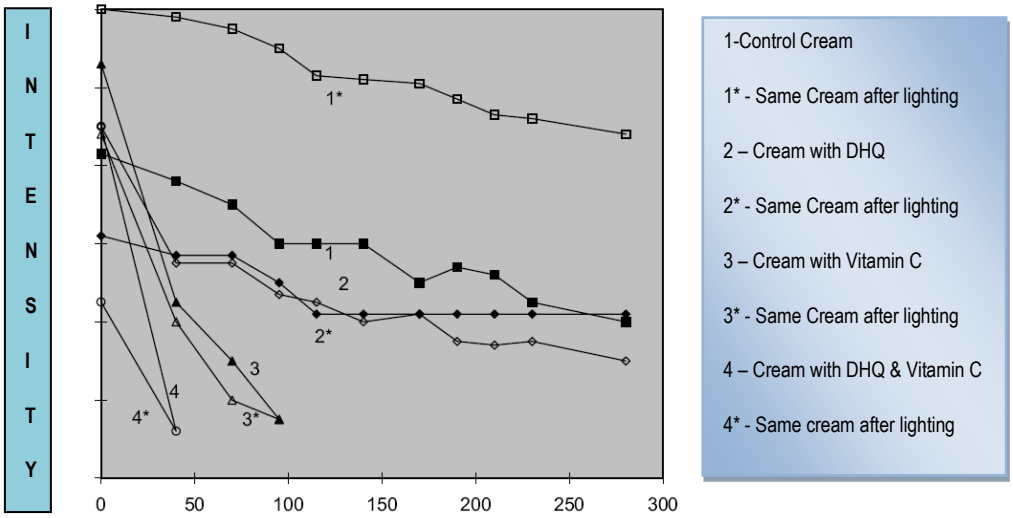
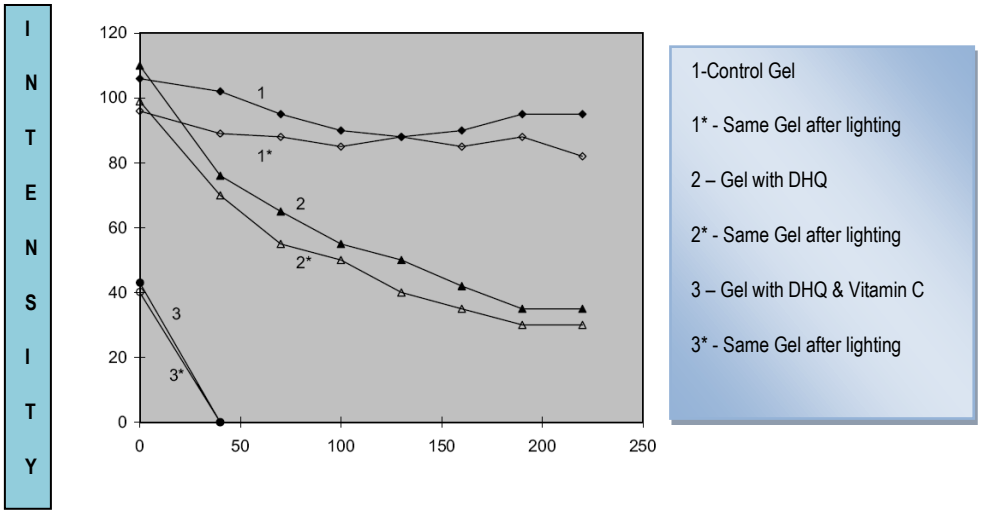


Figure 2. UV absorption capacity of cosmetic crème composition with DHQ and Vitamin C



Kinetic curve of nitroxyl probe restoration in cream samples
DHQ concentration – 0.05%, Vitamin C concentration – 0.05%
***Direct exposure of sun rays on the sample during 2 hours prior induction of probe**



Kinetic curve of nitroxyl probe restoration in gel samples
DHQ concentration – 0.05%, Vitamin C concentration – 0.05%
***Direct exposure of sun rays on the sample during 2 hours prior induction of probe**

DHQ & Vitamin C solution features

- Inhibition of hyalutonidase activity;
- Lysosome stabilization;
- Collagen formation and stabilization;
- Participation in metall ion exchanging;
- Protection from oxidation damage by ROS;
- Decreasing of capillaries toxicosis evidence under the treatment by anticoagulants, salicylic acid and its derivatives;
- Vaso- strengthening effect;
- Antitumoral effect;
- Phytoestragenic activity.

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