Clinical Data
RetiGen™ Proprietary retinoid blend

Synergie Skin has formulated a proprietary trademarked blend of highly active and stabilised vitamin A with potent antioxidant Coenzyme Q10 and sonically processed Green Tea concentrate. These three powerhouse ingredients work in synergy to:

- regulate cellular processes and cellular communication
- optimise collagen and elastin production
- reduce free radical damage generated by solar radiation
- reduce inflammation
- inhibit tyrosinase for improved skin tone

RetiGen will benefit all skin types and concerns by delivering a cutting edge cosmeceutical blend to target cells for optimal skin health to give a youthful and luminous appearance.

Hydroxypinacolone Retinoate: Next generation Vitamin A

Hydroxypinacolone Retinoate is a cosmetic grade ester of all-trans retinoic acid. This skincare active ingredient belongs to a class of chemical compounds termed retinoids, which are derivatives of Vitamin A capable of binding to retinoid receptors in cells. The binding of retinoid receptors can epigenetically enhance gene expression, which effectively turns key cellular functions on and off. When skin cell retinoid receptors are bound with retinoids, a cascade of mechanisms that benefit skin structures and functions are 'switched on'. This can result in enhanced cell proliferation, biosynthesis of extracellular proteins and glycans, and improved cellular turnover. Stimulating these age defying processes in the skin is critical for fighting and reversing signs of ageing and overall skin balance.

Biological Pathways to youthful skin with Hydroxypinacolone Retinoate

Stimulating cell proliferation and cell turnover are important for normalising cell renewal and repair processes. Furthermore, as we age our skin becomes thinner and less elastic, leading to sagging, loss of thickness, and wrinkles. Hydroxypinacolone Retinoate helps regulate cell processes to renew plumpness, elasticity and hydration to provide a radiant appearance. Moreover, Hydroxypinacolone Retinoate stimulates skin cell proliferation, restoring thickness to skin that has become thinner over time. These processes help reduce lines and wrinkles to promote a youthful appearance, whilst safeguarding skin from further wrinkle development. The effectiveness of Hydroxypinacolone Retinoate at reducing lines and wrinkles can be seen in Figure 1. Hydroxypinacolone Retinoate is also highly recommended for addressing acne due to its ability to regulate oil production. This cosmetic ingredient regulates cell turnover and numerous skin functions, which results in improved skin health, youthfulness and clarity.

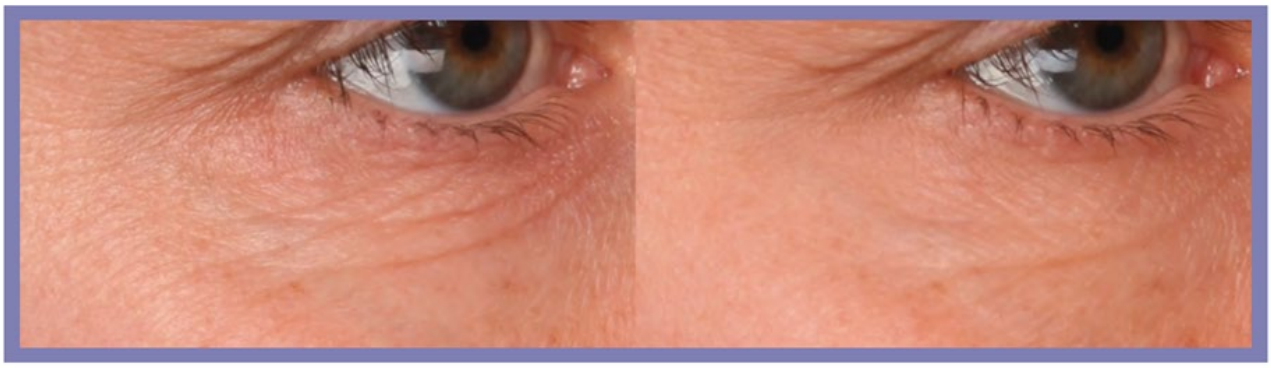


Figure 1 - Dramatic reduction of lines and wrinkles were observed after 14 days Hydroxypinacolone Retinoate application.

Challenges with older generation Vitamin A chemistries

Although the benefits of retinoids have been known for decades, skin irritation, photochemical instability, and toxicity concerns have hindered their use. Retinoic acid is a prescription-only topically applied ingredient recognised for its antiageing benefits, however, it can be irritating to skin. In recent years, milder over-the-counter derivatives have become popular alternatives to Retinoic acid. These derivatives are metabolised to the active form by skin cells. Retinol (Vitamin A) is the most popular topical retinoid used to date, but unless stabilised in liposomes, its skin irritancy and instability to sunlight has limited its scope and appeal. Retinol esters are often used to lower the irritation potential and increase stability, but the trade-off is decreased retinoid activity and reduced potency.

Hydroxypinacolone Retinoate advances opportunities in vitamin A skincare

Hydroxypinacolone Retinoate is a next-generation antiageing product, delivering the performance of retinoic acid, retinol and retinoid derivatives with significantly lower irritation potential. The mechanism of action of Hydroxypinacolone Retinoate is advanced compared to standard retinol derivatives. To interact with retinoid receptors, retinol must first be metabolised to more active forms, such as retinaldehyde and retinoic acid using several enzymatic steps. Hydroxypinacolone Retinoate is unique in that it possesses innate retinoic acid activity, binding directly with retinoid receptors without the need for metabolic breakdown to more biologically active forms.

Hydroxypinacolone Retinoate is dermatologically tested to offer less irritation potential than retinoic acid and most retinol derivatives, providing a gentle, safe and effective antiageing retinoid.

Clinical Results		
Assay	Subject	Result
Cumulative irritation patch	Human clinical panel	No irritation
Local irritation and sensitisation potential assay	Human clinical panel	No adverse experiences
Skin roughness	Human clinical panel	50% improvement
Skin surface scaling	Human clinical panel	40% improvement
Skin irritation potential vs standard retinol	Human skin cells	Lower irritation potential
Toleration under environmental stresses vs unstabilised retinol	Human skin cells	Better toleration
Retinoid gene expression modulation	Human skin cells	Typical retinoid expression
In vitro percutaneous penetration	Human skin	Better toleration
In vivo percutaneous absorption	Human skin	Passed – proven safe

These results add credence to the beneficial safety and irritation profile of Hydroxypinacolone Retinoate as a topical cosmetic. The low irritation profile of Hydroxypinacolone Retinoate versus raw retinol was demonstrated on a 24-hour occlusive patch test and can be seen in Figure 2.

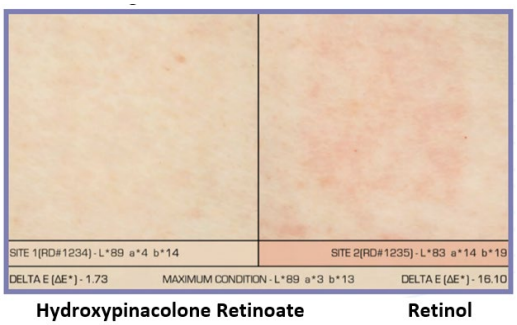
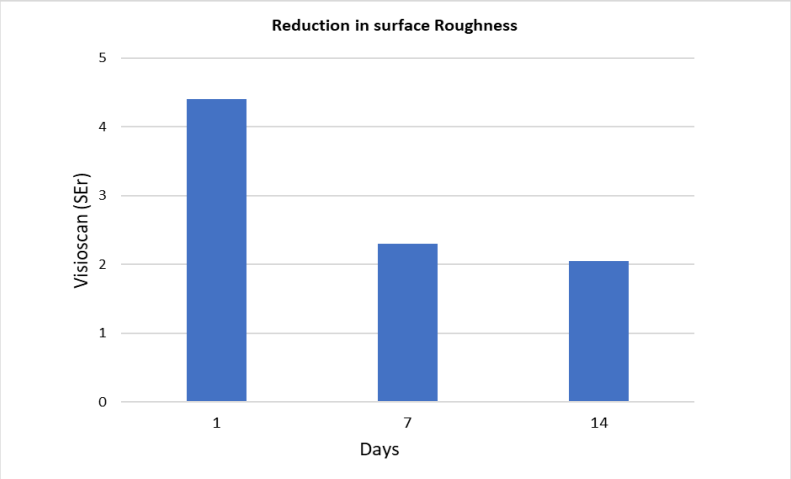


Figure 2 – After application on a 24-hour occlusive patch test, Hydroxypinacolone Retinoate demonstrated a significantly lower irritation profile versus retinol. Test samples were 0.5% retinoid in dimethyl isosorbide.

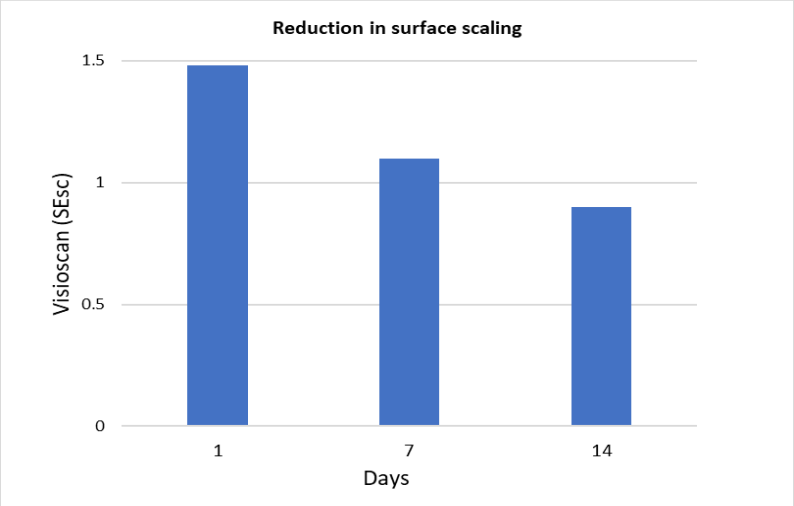
Reduction in surface roughness

- A pilot-scale study to evaluate efficacy of Hydroxypinacolone Retinoate to reduce appearance of fine lines, facial wrinkles, age spots, surface roughness and scaling was performed
- Formulation # G101-235.02 (Skin Lightening Cream) containing 1% Hydroxypinacolone Retinoate
- Measurements performed by a Visioscan instrument
- Results at day 7 and day 14 are statistically significant (p-value < 0.05)
- 50% improvement in skin texture

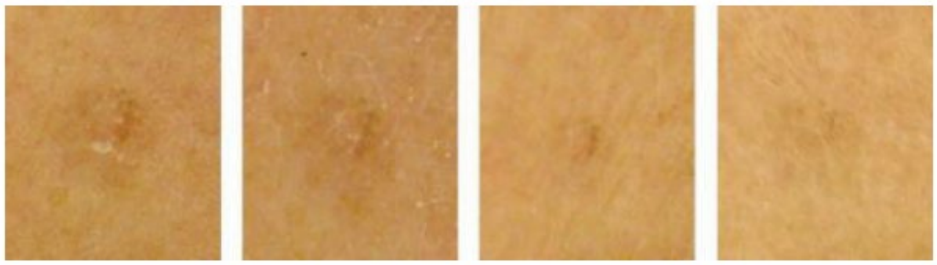


Reduction in skin scaling

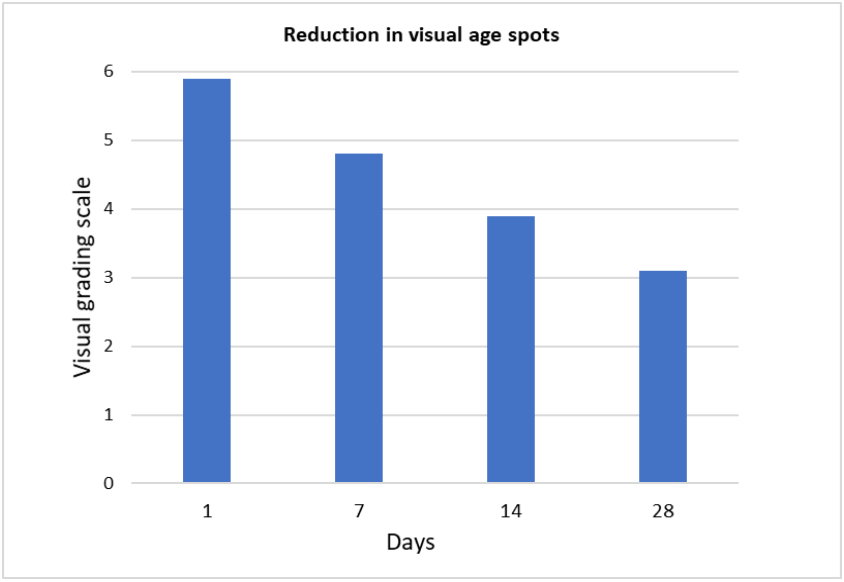
- Visioscan evaluation of the skin surface exhibited decreases in surface scaling
- Indicating reduced dryness and improved overall appearance
- Results at day 7 and day 14 are statistically significant (p-value <0.05)
- 40% improvement in overall appearance



Reduction in age spots



- Contributes to skin lightening and brightening
- Visual grading performed by a trained clinical evaluator using a 0-10 scale (best-to-worst)
- Results at day 7, day 14 and day 28 were statistically significant (p-value < 0.05)
- 50% improvement after 4 weeks



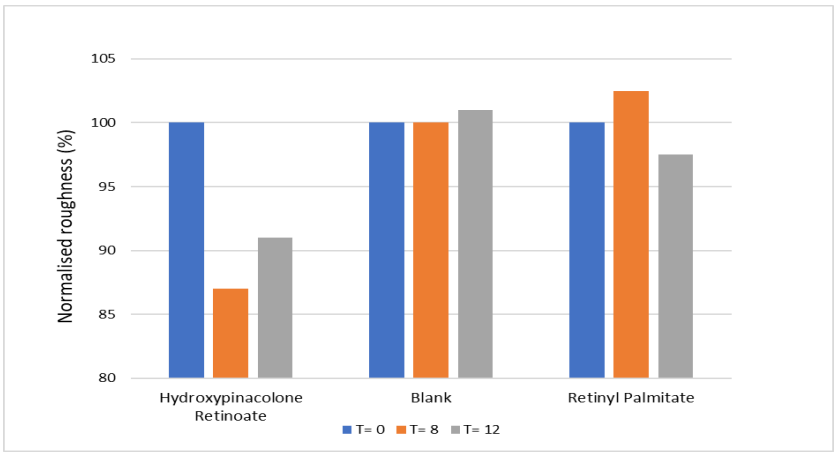
Results: Statistical improvement in texture and UV spots

- Significant differences and improvement in texture (smoothness) and UV spots
    - Texture is primarily an analysis of skin smoothness. Texture measures skin colour and smoothness by identifying gradation in colour from the surrounding skin tone, as well as peaks (yellow) and valleys (blue) on the skin surface that indicate variations in the surface texture
    - UV spots (age spots) occur when melanin coagulates below the skin surface as a result of sun damage. UV spots are generally invisible under normal lighting conditions and in their appearance are enhanced by the absorption of UV light by the melanin
- Overall improvement in facial and hand photographs for subjects applying Hydroxypinacolone Retinoate

Improved texture using Hydroxypinacolone Retinoate over time

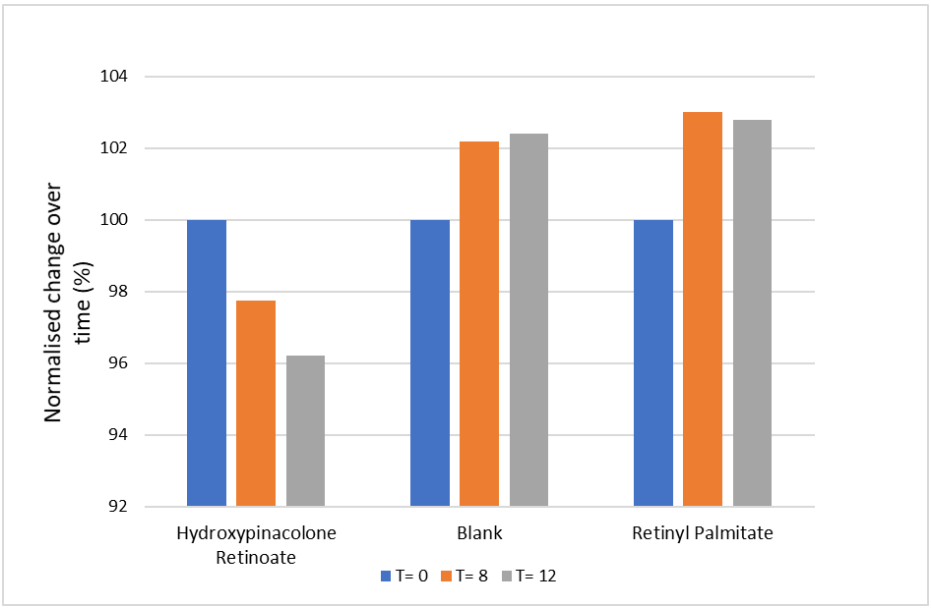
Canfield Texture

- Texture relates to skin smoothness
- Texture measures skin colour and smoothness by identifying gradations in colour from the surrounding skin tone, as well as peaks and valleys on the skin surface that indicate variation in the surface texture
- Significant decrease at T=12 for Hydroxypinacolone Retinoate (p-value <0.05)
- No significant change for blank or Retinyl Palmitate

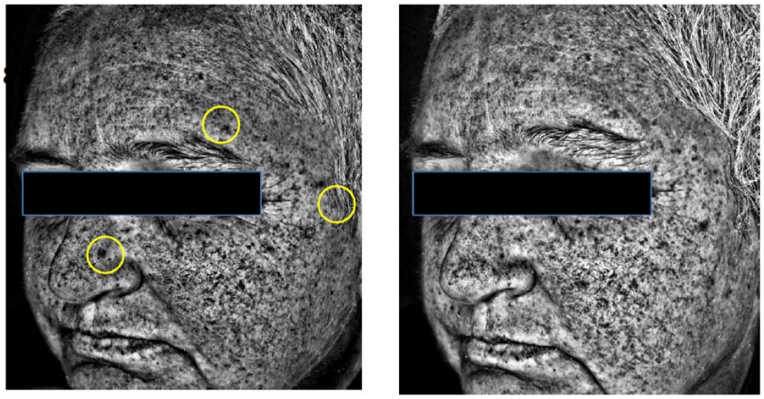


Canfield UV Spots

- UV Spots (age spots) occur when melanin coagulates below the skin surface as a result of sun damage. UV spots are generally invisible under normal lighting conditions. The selective absorption of the UV light by the epidermal melanin enhances its display and detection by VISIA
- Significant decrease at T=12 for Hydroxypinacolone Retinoate (p-value <0.05) and significantly different from blank and Retinyl Palmitate
- No significant change for blank or Retinyl Palmitate

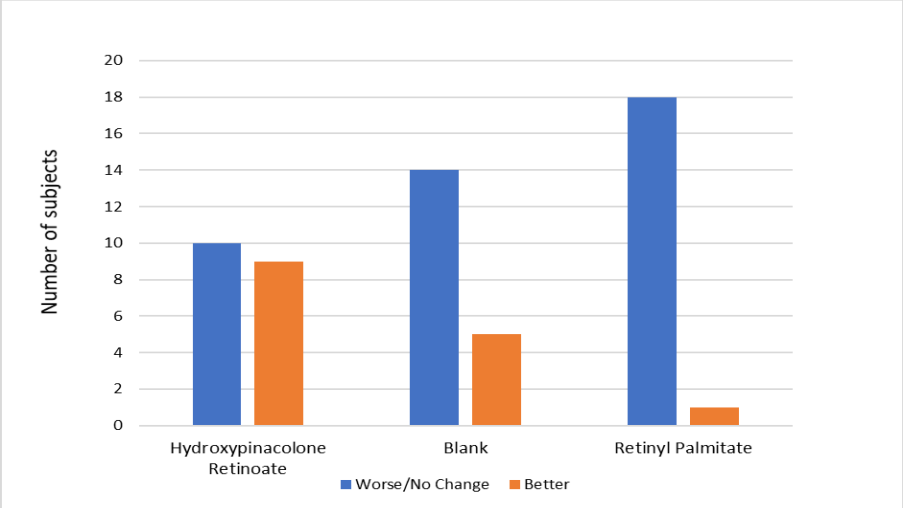


Reduction of UV spots using Hydroxypinacolone Retinoate over time



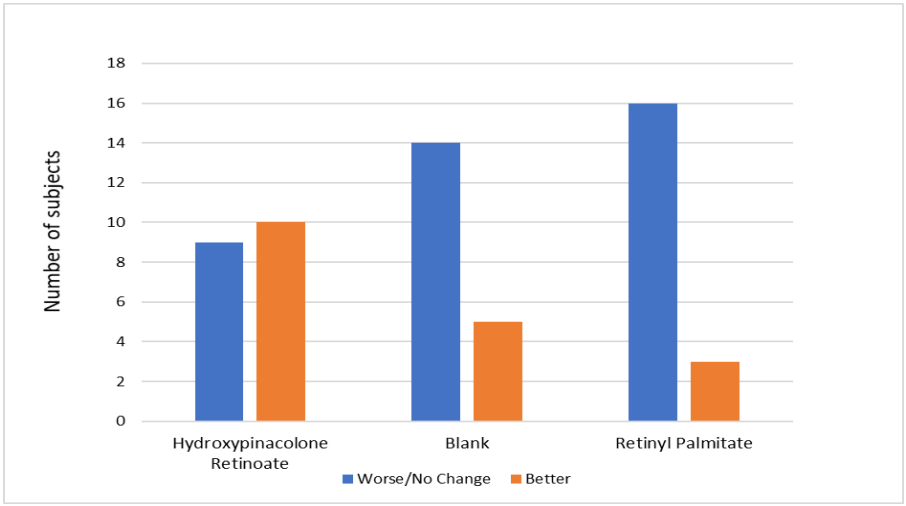
Facial photographic assessment

- Blinded grading of the *face* indicated the Hydroxypinacolone Retinoate serum resulted in more volunteers having noticeable improvement that the other two treatments
- This supports the positive benefits of Hydroxypinacolone Retinoate
- Results on this data was found to be significant (p-value < 0.05)



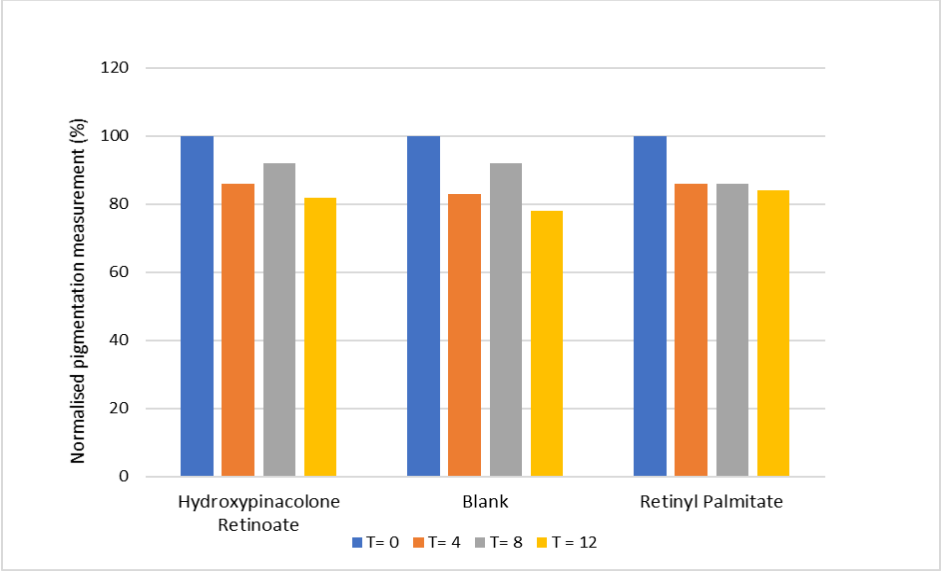
Hand photographic assessment

- Blinded grading of the hand indicated the Hydroxypinacolone Retinoate serum resulted in more volunteers having noticeable improvement that the other two treatments.
- This supports the positive benefits of Hydroxypinacolone Retinoate.
- Results on this data was found to be significant (p-value < 0.05).



Hand pigmentation measurements

- Measurements of skin colour on 5 sites on the back of each hand using CIE colour space values (CIELAB) at T = 0; 4; 8; 12 weeks
- "No change" using Chromameter for the subjects
- Blank had a significant change (p-value < 0.05)
- Hydroxypinacolone Retinoate and Retinyl Palmitate had no significant change
- No irritation is observed and both retinoids prevented darkening of the skin



Performance

Hydroxypinacolone Retinoate combats the appearance of fine lines and wrinkles for a more youthful appearance and improved texture. It also reduces uneven pigmentation improving brightness and luminosity and regulates cell renewal to increase skin firmness and elasticity. Reduction in pore size and excess oil is also observed leading to overall skin clarity. Hydroxypinacolone Retinoate delivers higher performance compared to most retinol derivatives, but with a significantly lower irritation potential.

Coenzyme Q10

Coenzyme Q10 is an important mitochondrial component and naturally occurring lipid-soluble antioxidant present in all human cell membranes. It plays a crucial role in the generation of cellular energy, enhances the immune system, and acts as a free radical scavenger.

Ageing, poor eating habits, stress, and infection – they all affect the organism's ability to provide adequate amounts of CoQ10. After the age of about 35, the organism begins to lose the ability to synthesise CoQ10 from food and deficiency develops. Many researchers suggest that using CoQ10 supplements both in supplements and skincare can aid in the health and vitality of cells.

Antioxidant activity

The protective antioxidant effect is extended to lipids, proteins and DNA in all cells. CoQ10 in its reduced form as Ubiquinol is a potent lipophilic antioxidant that has a great importance as a free radical scavenger. CoQ10 protects the stability of the cell membranes, protects DNA from free radical induced oxidative damage, and is capable of recycling and regenerating other antioxidants, such as tocopherol and ascorbate (Crane 2001). Other important functions of CoQ10 for cell signalling and epigenetic gene expression have also been described (Bhagavan & Chopra 2006).

Another direct demonstration of the elimination of free radicals is shown by topical coenzyme Q 10 treatment of skin in elderly subjects. Damaged cell luminescence is eliminated via free radical scavenging when a skin cream containing coenzyme Q is applied (Hoppe *et al.* 1999).

Topical supplement

CoQ10 plays a key role in the skin protective network. In the skin. Ageing, poor eating habits, stress, and infection all affect the ability to provide adequate amounts of CoQ10 in the skin. After the age of 35, humans lose the ability to synthesise CoQ10 from food and its deficiency develops. CoQ10 increases in the skin from childhood to maturity and then decreases with age, environmental stress and under irradiation with UVA rays. It has been concluded that oral supplementation with CoQ10 may be very helpful to skin health.

CoQ10 has become a key active ingredient in many topical products. There are numerous topical formulations containing CoQ10, claiming to possess of antioxidant effects, skin repair and regeneration abilities as well as anti-wrinkling and antiageing capability (Hojerova *et al.* 2006). Vinson and Anamandla (2006) demonstrated *in vivo* antioxidative effects in the skin of young and middle-aged subjects of two forms of CoQ10 after a single dose and after a long-term supplementation.

Clinical study: Topical treatment with CoQ10-containing formulas improves skin's Q10 level and provides antioxidative effects

Results demonstrate that stressed skin benefits from the topical Q10 treatment by reduction of free radicals and an increase in antioxidant capacity.

Introduction

Skin as the outermost human organ is in direct contact with the environment and is, therefore, exposed to external stress factors. To combat resulting damages, cutaneous cells are constantly involved in tissue regeneration and repair, processes that require a high amount of energy and a well-regulated cellular metabolism. With increasing age, however, energy production as well as mitochondrial activity decline. As a consequence, cell and tissue functions are impaired and visible structural alterations occur including the appearance of lines and loss of elasticity.

Reactive oxygen species (ROS) and free radicals represent predominant causes of damages to cellular components. In aging cells, ROS are frequently generated due to changes in cell respiration. Especially in skin cells, formation of ROS is also promoted by exposition to external insults such as ultraviolet (UV) light, IR light, HEV blue light and pollution.

ROS damage not only affects cell membranes and DNA but also structural and catalytic proteins which play a crucial role in cellular energetic pathways. As a result, the energy metabolism is further impaired by actions of free radicals, which are not only the cause of the aging process, but also its result.

During the process of energy production and in extracellular enzymatic processes, ubiquinone is converted into its reduced form (ubiquinol) which serves specific functions as a lipid-soluble antioxidant. Ubiquinol acts as a radical scavenger and protects mitochondria, lipid membranes, lipoproteins, and also DNA from oxidative damage.

Coenzyme Q10 (Q10), also known as ubiquinone, is an important coenzyme that is present in all human cells. It was originally shown to be a necessary component of the mitochondrial respiratory chain, working as an electron carrier and is crucial for energy production in the human body. It has also been well established that Q10 has many other important functions,

A recent publication showed that loss of Q10 levels in a mouse model leads to gradual loss of mitochondrial function, the development of aging-like disease traits and reduced lifespan. This condition is reversible when ubiquinone levels are restored. This illustrates the importance of Q10 and its epigenetic impact for optimal function of the entire organism.

In skin, endogenous Q10 levels decline with increasing age 14. Additionally, UV-irradiation, which leads to oxidative damage, significantly reduces skin's Q10 levels 20. In this context, the objective of this study was to investigate whether human skin may benefit from a topical Q10 treatment with regards to the two aforementioned important points of action: increase in cellular energy metabolism as well as antioxidant effects.

In Vivo trials with Q10-Containing Formulas

A controlled, randomised study was carried out enrolling 73 healthy, non-smoking, female volunteers (20–66 years).

For the study, two formulas, a cream and a serum, containing Q10 in different concentrations were used (cream 348 µM ubiquinone [formula 1]; serum 870 µM ubiquinone [formula 2]).

During a 5-day preconditioning period and throughout the study, volunteers were required to desist from using skincare products and to avoid excessive contact with surfactants and sun exposure on both forearms. Visits to saunas, solariums, swimming-pools as well as very demanding exercise were prohibited for 1 day prior to measurements. Measurements were performed by trained and experienced personnel after acclimatization for at least 30 minutes.
One inner forearm of each volunteer was used for product treatment (two test areas) and the other forearm was left untreated and utilized as control (one test area). The positioning of treatment locations was left–right randomized and a stencil was used to mark the test areas. Volunteers applied the test formulas twice daily (morning and evening; 2 mg/cm2) for 2 weeks according to written instructions.

Collection of Skin Samples

After 2 weeks of application, the following skin samples were obtained on the morning after the last treatment: (I) uppermost layers of stratum corneum (skin surface), (II) suction blister fluid, and (III) suction blister epidermis (Fig.1).

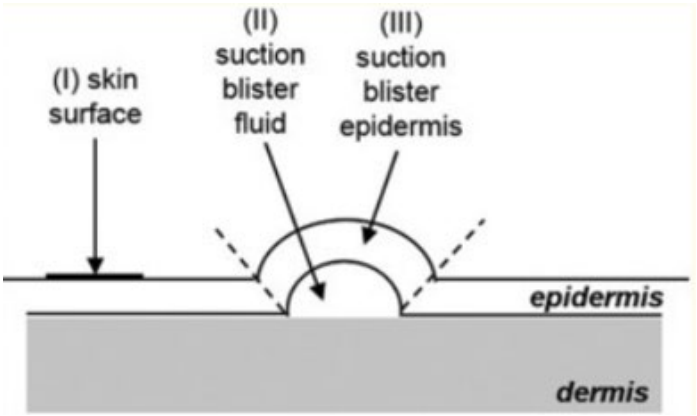


Figure 1 – Schematic illustration of collection of skin samples (not shown to scale). Samples from the skin surface (I) were obtained using adhesive sampling discs (D-Squames). After raising suction blisters, the blister fluid (II) was collected using a sterile syringe. In the last step, suction blister epidermis (III) was harvested using sterile forceps and scissors (III).

Preparation of Extracts from Suction Blister Epidermis and sampling discs

Suction blister epidermis and sampling discs were analysed.

Analysis of Suction Blister Fluids

Freshly isolated suction blister fluid samples were analysed using the FORM Analyzer (Micro-Medical Instrumente GmbH). The concentration of hydroperoxides was determined in untreated skin utilizing the Free Oxygen Radicals Test (FORT, Calligari).

Baseline levels in suction blister fluids were ≤160 FORT units. According to reference values of blood samples given by the manufacturer and obtained from a recent publication 24, increased oxidative stress levels are considered as such starting at a value of 55% over baseline. Thus, the limit value for oxidative stress in our samples starts at 250 FORT units and was displayed by 16 out of 73 volunteers which are depicted in Fig. 2

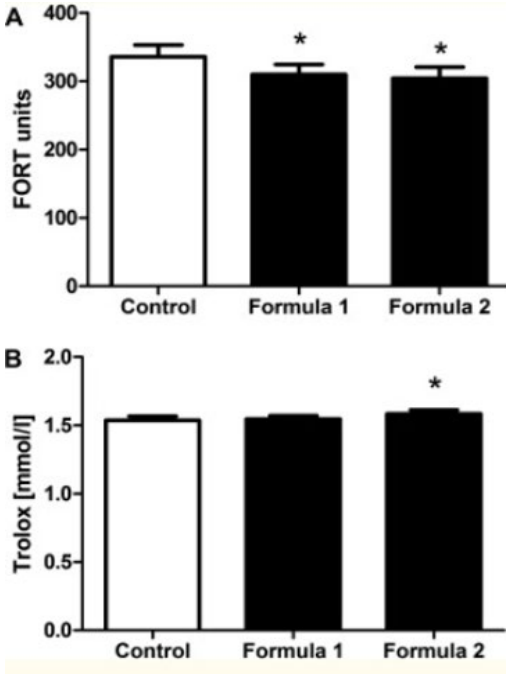


Figure 2 – Antioxidant properties of stressed skin are improved after treatment with Q10-containing formulas. Volunteers displaying elevated oxidative stress (≥250 FORT units) in untreated skin, were analysed following a 14-day treatment with formula 1 and formula 2. The level of free oxygen radicals (A) and the free oxygen radical defence (B) were determined in suction blister fluid obtained from treated compared with untreated control samples. Results are depicted as mean ± SEM (*n = 16)*. Significant differences are marked with an asterisk [*P<0.05 with respect to the untreated control (repeated measures analysis of variance, Dunnett's *post hoc* test)].

1. Quinone Levels are reduced with Age in Human Epidermis

To investigate total quinone (ubiquinone plus ubiquinol) levels and connected biological effects in human skin in more detail, suction blister epidermis material was used for the determination of quinone concentrations. A comparison of samples obtained from young and aged volunteers showed that the quinone content in aged epidermis (8.04 ± 0.26 ng/µg cholesterol) was significantly lower than in young epidermis (9.45 ± 0.37 ng/µg cholesterol) indicating a loss of quinones with age (Fig 3). With this information it was investigated whether the epidermal quinone content can be improved by topical application.

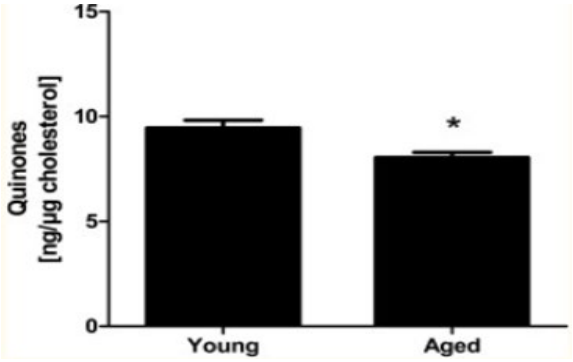


Figure 3 – Age-dependent decline of quinone levels within human epidermis. Quinone concentrations of young (20-25 years; *n=28)* and aged (60-66 years; *n= 28)* volunteers measured in suction blister epidermis obtained from untreated forearm skin. Data are depicted as mean ± SEM. Significant differences are marked with an asterisk [**P<0.05* for comparison between young and aged subjects (Student's *t*-test)].

2. Topical Treatment with Q10 Formulas Increases Epidermal Quinone Content on Multiple Levels

After 14 days of treatment with Q10-containing formulas, quinone levels were assessed in D-Squame® disc samples collected from the skin surface. To take the size of the treatment area into account, values were related to the area of the sample (mm2). The untreated control sample was compared with two individual samples which were obtained from test areas treated with ubiquinone-containing formula 1 or formula 2 (containing more than twice as much ubiquinone than formula 1). The untreated control sample displayed a level of 0.024 ± 0.003 ng quinones/mm2. Compared with the untreated control, treatment with formula 1 resulted in a significant increase to 0.133 ± 0.02 ng quinones/mm2, whereas application of formula 2 led to an even more pronounced and significant augmentation to 0.717 ± 0.083 ng quinones/mm2 (Fig. (Fig.4)

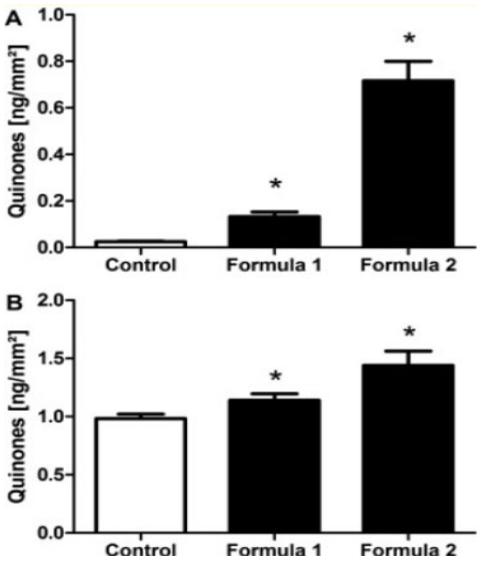


Figure 4 – Increase in quinone levels after treatment with Q10-containing formulas. Following a 14-day treatment with Q10-containing formulas 1 and 2, quinone levels were assessed on the skin surface using samples obtained from D-Sqaumes (A) and within the epidermis using suction blister material (B). Results are shown as mean ± SEM (20-66 years; *n=73).* Significant differences are marked with an asterisk [**P<0.05* with respect to the untreated control (repeated measures analysis of variance, Dunnett's *post hoc* test)].

It was further investigated whether topical treatment can increase quinone levels also in deeper layers of the epidermis. For this purpose, quinone content was analysed in suction blister epidermis obtained from the treated and untreated areas described above. Quinone values determined in the respective D-Squame® disc samples were subtracted to exclude quinone residues present on the skin surface. After treatment with formula 1, quinone levels were significantly increased (1.14 ± 0.06 ng quinones/mm2) compared with the untreated control (0.98 ± 0.04 ng quinones/mm2). Application of formula 2 elevated the quinone content even more (1.44 ± 0.12 ng quinones/mm2) and also showed a significant augmentation compared with the untreated control (Fig. (Fig.33B).

According to this data, topical application of the two test formulas increased quinone levels not only on the skin surface but also within the deeper levels of the epidermis.

Study conclusion:

Skin is constantly exposed not only to intrinsic but also to environmental stressors producing augmented internal ROS concentrations which cause damage throughout the tissue. Therefore, the "Free Radical Theory of Aging" 5 postulating that aging is the result of cellular damage inflicted over time by free radicals is not only one of the most prominent ideas concentrating on the complex phenomenon of aging, but also highly relevant for skin.

Coenzyme Q10 constitutes the only endogenously synthesized lipid-soluble antioxidant. At the same time, it plays a crucial role in cellular energy production. Intracellular synthesis is the major source of human CoQ10 but it can also be delivered through the diet or dietary supplements which can increase human total Q10 levels in plasma.

However, in skin, Q10 is not only found in living cells but also in the skin surface lipids (SSL), which are part of the stratum corneum, forming the outermost barrier of the skin. SSL are composed of a mixture of sebum secreted from sebaceous glands and lipids originating mainly from corneocytes. Due to their location on the skin surface SSL are constantly exposed to UV irradiation, air pollution, chemical oxidants, and microorganisms. SSL Q10 levels have been shown to decline with age and decrease after UV exposure in vitro.

Quinone values on the skin surface were significantly increased after treatment with Q10-containing formulas demonstrating that the powerful antioxidant Q10 can be delivered directly to the uppermost layer of the skin. This data is significant since oral supplementation with Q10 did not result in the enhancement of Q10 content in skin surface lipids. With a topical Q10 treatment, however, short-term environmental stress-induced as well as age-related Q10 deficits, may be counteracted directly at the skin surface. Young individuals displaying normal Q10 values may benefit since spontaneously occurring external oxidative stress can be neutralized quickly. In the aged population already decreased Q10 levels may be replenished. Thus, using Q10-containing topical formulas on a regular basis to protect the outermost skin layer and is recommended for skin at any age.

There is a significant age-dependent decline in quinone levels in suction blister epidermis samples which is well in line with other findings documenting decreased Q10 levels not only in several human organs but also in the epidermis of volunteers aged 30–80 years. With advancing age, the decline of both quinones represents the major issue in skin. Besides the chronological aging process, occasional external stress events inside the epidermis may have an impact on the levels of both quinones as demonstrated by Podda *et al*. in human skin equivalents after UV-irradiation. In skin, both causes of Q10 decline (age-dependent and UV-induced) may be of significant physiological importance given that even small changes in Q10 concentration could result in substantial alterations in skin cell function.

According to the data, topical application of CoQ10 serves to replenish epidermal ubiquinone levels, which may be decreased with aging and environmental stress.

It was also found that, in cases of elevated levels of free radicals in the skin tissue, topical treatment with Q10-containing formulas may significantly reduce the oxidative stress level in cells.

In this context, it is interesting to note that dietary supplementation with ubiquinol is reported to exert beneficial effects in age-related diseases, such as cardiovascular disease, diabetes, age-related hearing loss, and Parkinson's disease. Moreover, decelerated age-related accumulation of oxidative damage was shown in ubiquinol-supplemented mice with accelerated senescence.

In summary, the data presented here show that topically applied Q10 can penetrate the skin, exert antioxidant effects and can support the maintenance of cellular energy levels. These effects are not only beneficial for the aged population suffering from a Q10 deficit but also to replenish the Q10 level in skin which is lost over time through normal environmental stress.

Regular treatment with Q10-containing formulas enables the skin to cope more effectively with short-term insults inflicted by UV and IR irradiation and pollution stress. The broad benefits aid in cellular protection and long-term anti-aging effects for their skin.

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Anja Knott,[1] Volker Achterberg,[1] Christoph Smuda,[1] Heiko Mielke,[1] Gabi Sperling,[1] Katja Dunckelmann,[1] Alexandra Vogelsang,[1] Andrea Krüger,[1] Helge Schwengler,[1] Mojgan Behtash,[1] Sonja Kristof,[1] Heike Diekmann,[1] Tanya Eisenberg,[1] Andreas Berroth,[1] Janosch Hildebrand,[1] Ralf Siegner,[1] Marc Winnefeld,[1] Frank Teuber,[1] Sven Fey,[1] Janne Möbius,[1] Dana Retzer,[1] Thorsten Burkhardt,[1] Juliane Lüttke,[1] and Thomas Blatt[1]

Camellia Sinensis (Green Tea) Leaf Extract

Major sources of dermal damage:

1. Exposure to Oxidative Stress
   - Pollution, sunlight and other insults generate Reactive Oxygen Species (ROS) which disrupt biological processes essential for cellular survival

2. Increased levels of Proteolytic Enzymes
   - Higher levels of MMPs (enzyme which metabolise ECM proteins such as collagen and elastin) are detected during ageing

3. UV-Induced Pigmentation Disorders
   - Age spots in mature skin are caused by cumulative UV-mediated damage

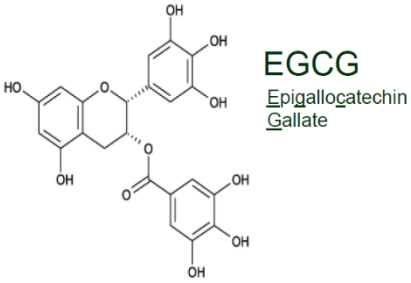
Several Activities are necessary in order to provide good antiageing efficacy:

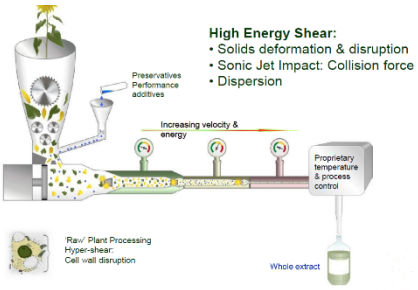
- Camellia Sinensis (Green Tea) Leaf Extract processed via sonic extraction, provides multifunctional antiageing benefits such as:
  1. Strong protection against oxidative stress
  2. Broad-spectrum protection against proteolytic damage
  3. Inhibits tyrosinase activity and melanin production
  4. Modulates inflammatory PLA_2 activity
- Camellia Sinensis (Green Tea) Leaf Extract also has proven stability and long-lasting efficacy

The technology

- Leaves of Green Tea are submitted to the sonic extraction process as part of manufacturing. No chemical additives (glycols, alcohols, etc.) are used during the industrial extraction process.
- Green tea extracts are among the most widely used ancient medicinal agents. They possess antioxidant, anti-inflammatory, wound healing and anticarcinogenic properties.
- Among the different polyphenolic catechins in Green Tea, Epigallocatechin gallate (EGCG) is the most abundant and effective.

- Green tea polyphenols are easily oxidised and lose their activity if not used immediately. The primary goal of topical formulations containing green tea extracts should be to maintain the stability of these beneficial components. This has been overcome using the sonic extraction method.





Efficacy

1. Inhibition of oxidative damage:

- Pollution, sunlight and other insults generate Reactive Oxygen Species (ROS) that disrupt biological processes essential for cellular survival. Two assays were used to evaluate the antioxidant activity of Camellia Sinensis (Green Tea) Leaf Extract

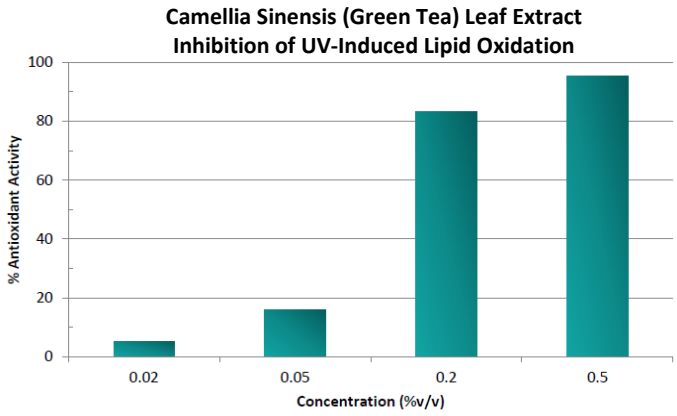
a) UV-Induced Lipid Peroxidation Inhibition

- Camellia Sinensis (Green Tea) Leaf Extract was tested for its ability to inhibit oxidation of phospholipid liposomes exposed to UV light. Rates of oxidation were determined by measuring generation of malonaldehyde, a toxic by-product of oxidized lipids

b) Scavenging/quenching of Singlet Oxygen (SO):

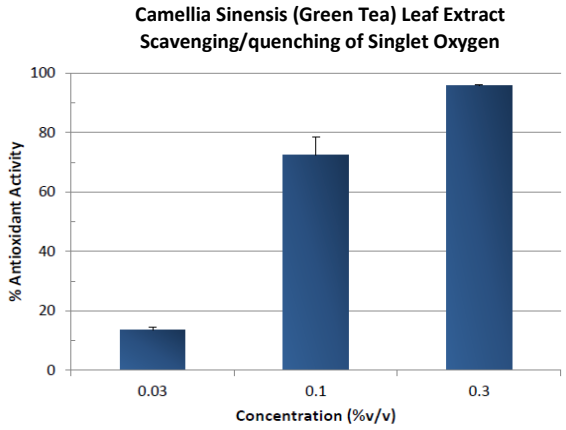
- SO is generated from other reactive oxygen species at sites of inflammation, from peroxyl radicals such as those generated during UV-induced lipid oxidation and simple exposure of the skin to visible light by means of photosensitisation reactions
- Camellia Sinensis (Green Tea) Leaf Extract was tested for its ability to prevent oxidation cause by SO. Reaction mixtures containing a photosensitising dye were exposed to visible light to generate SO. Oxidation of iodide by SO was measured spectrophotometrically

Efficacy 1. Inhibition of oxidative damage: UV-induced lipid peroxidation



- Camellia Sinensis (Green Tea) Leaf Extract is significantly effective at low concentrations achieving almost complete inhibition of lipid oxidation at only 0.5%

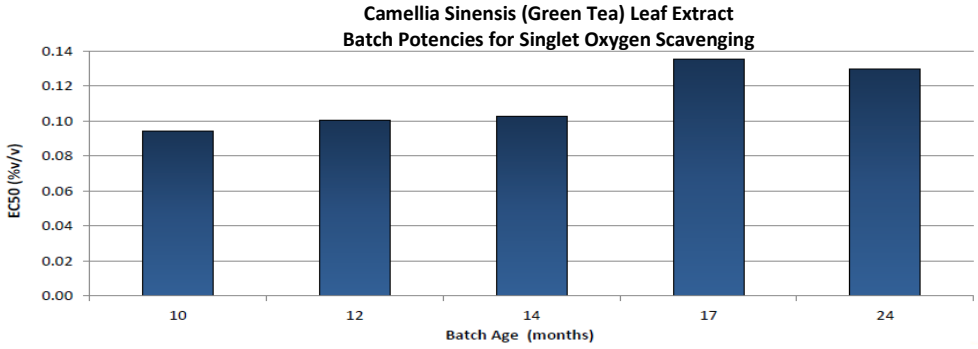
Efficacy 1. Inhibition of oxidative damage: Scavenging/quenching of singlet Oxygen



- Singlet Oxygen is one of the most aggressive Reactive Oxygen Species (ROS) and is especially damaging to mitochondria, the cell's energy source
- Singlet Oxygen damages all classes of biological macromolecules, thereby compromising cell membrane structure, DNA integrity and protein functions

Camellia Sinensis (Green Tea) Leaf Extract proves to be an effective Singlet Oxygen Scavenger!

- Batches of Camellia Sinensis (Green Tea) Leaf Extract of varying ages were tested for efficacy. The calculated EC50 values show only a minimal drop in potency over two years



- Unlike most commercial green tea extracts, Camellia Sinensis (Green Tea) Leaf Extract provides long lasting antioxidant activity

2. Protection of the Extracellular Matrix

The Extracellular Matrix (ECM) provides structural support to dermal cells (fibroblasts). ECM is mainly composed of proteins (e.g., collagen and elastin) and glycosaminoglycans. Camellia Sinensis (Green Tea) Leaf Extract was tested for its ability to inhibit proteases that damage ECM.

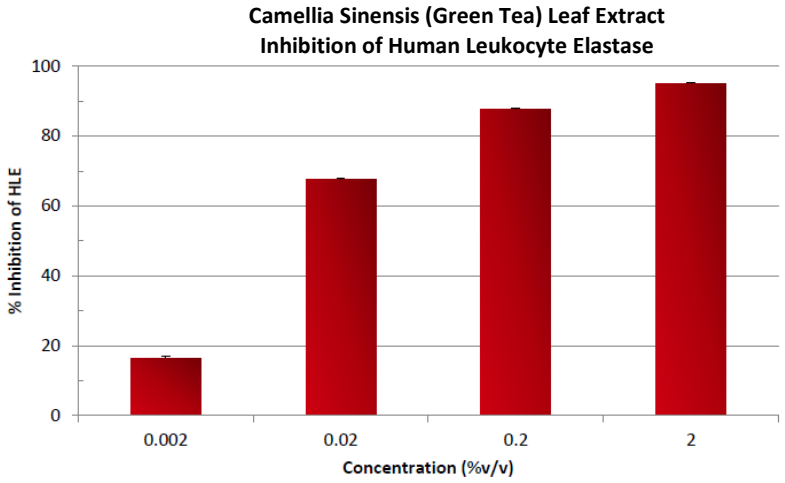
a) Inhibition of Human Leukocyte Elastase:

- This enzyme is a broad-spectrum serine protease released by infiltrating neutrophils during inflammation. It will attack and degrade most components of the ECM
- Excessive Elastase activity is a key indicator of inflammation and is triggered by release of pro-inflammatory mediators (TNF-α, interleukins) from keratinocytes and other skin cells

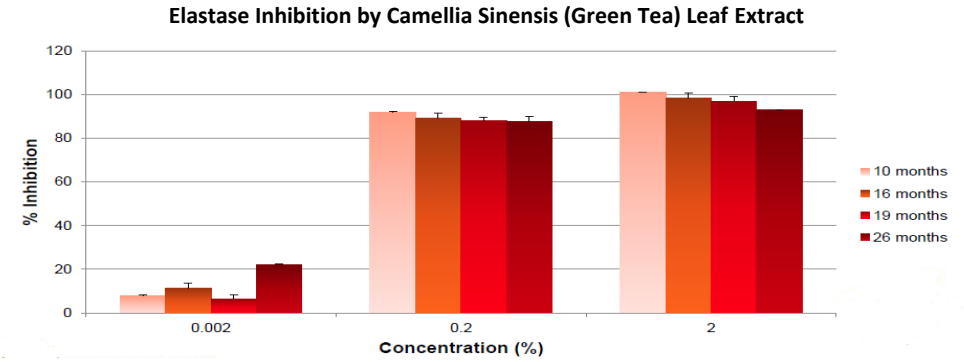
b) Inhibition of Matrix Metalloproteinases (MMP-1 and MMP-8)

- MMPs are released into the ECM by infiltrating white blood cells and also by resident skin cells in response to an inflammatory stimulus. MMP-1 and MMP-8 are especially damaging to type I, II and III collagens

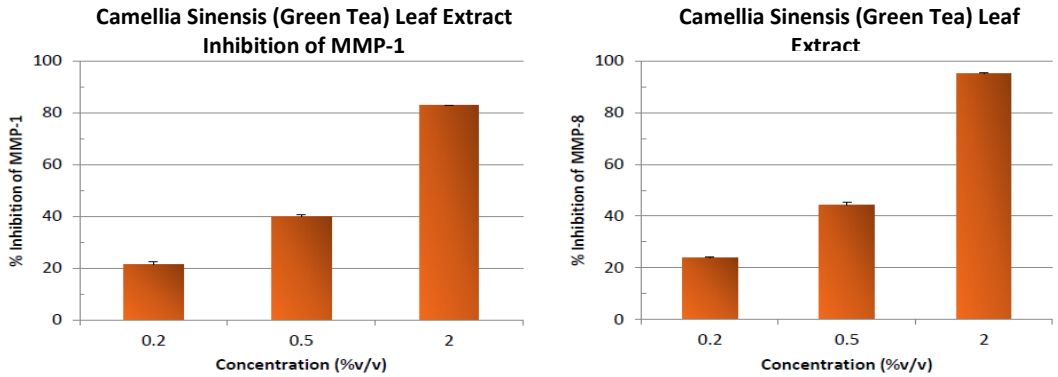
Efficacy 2. Protection of the Extracellular Matrix: Inhibition of Human Leukocyte Elastase:



- With an EC90 of approximately 0.2%, Camellia Sinensis (Green Tea) Leaf Extract is a strong inhibitor of proteolytic damage
- Batches of Camellia Sinensis (Green Tea) Leaf Extract of varying ages were tested for potency vs. human leukocyte elastase. The results were very consistent with the potency for fresh batches of Camellia Sinensis (Green Tea) Leaf Extract



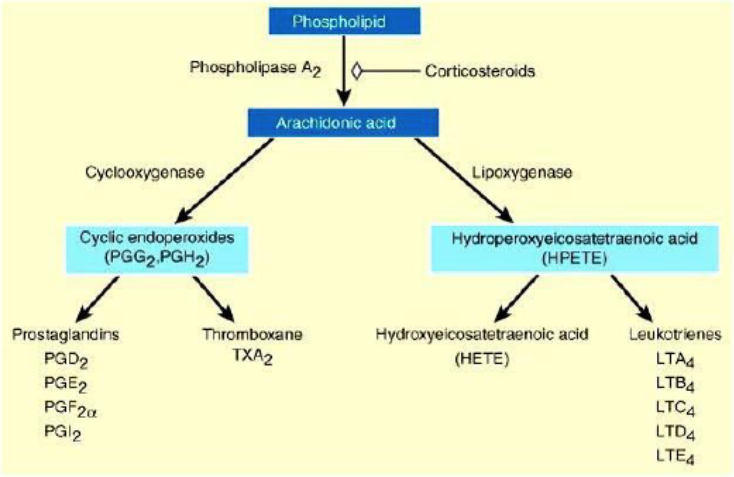
Efficacy 2. Protection of the Extracellular Matrix: Inhibition of MMPs



- Camellia Sinensis (Green Tea) Leaf Extract showed dose-dependent inhibition of MMP-1 & MMP-8

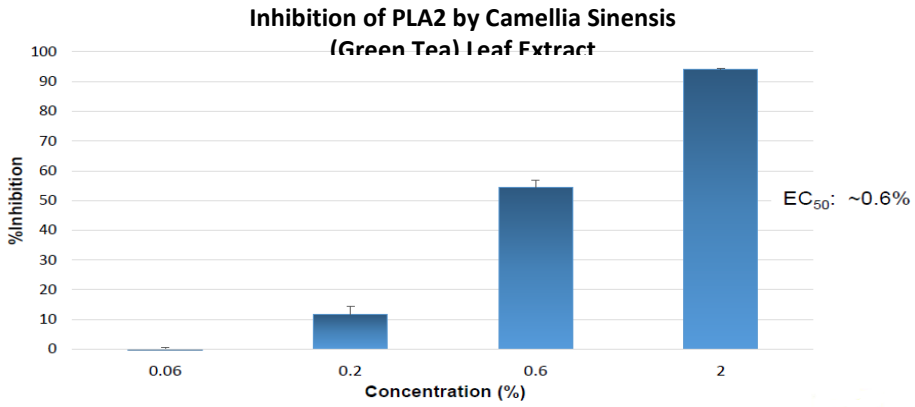
3. Inhibition of Phospholipase A2 (PLA_2)

- PLA_2 enzymes are commonly found in mammalian tissues. Increased presence and activity of PLA_2 can lead to inflammation, itching and pain.



Schematic diagram of arachidonic acid metabolism. LT = leukotriene; PG = prostaglandin; TXA_2 = thromboxane A_2.

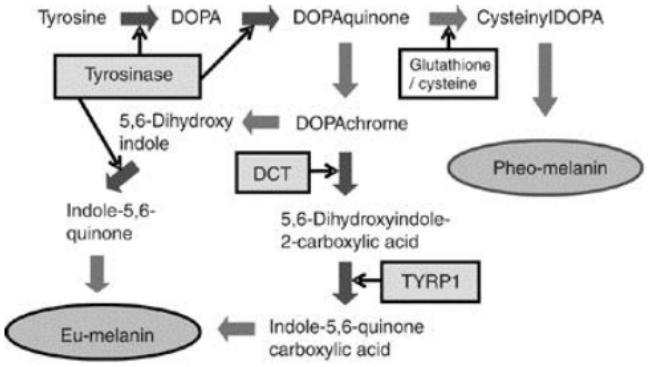
- Inhibitors of PLA_2 are known to have anti-inflammatory efficacy. Camellia Sinensis (Green Tea) Leaf Extract effect on PLA_2 activity was assessed using the EnzChek PLA_2 Assay Kit.



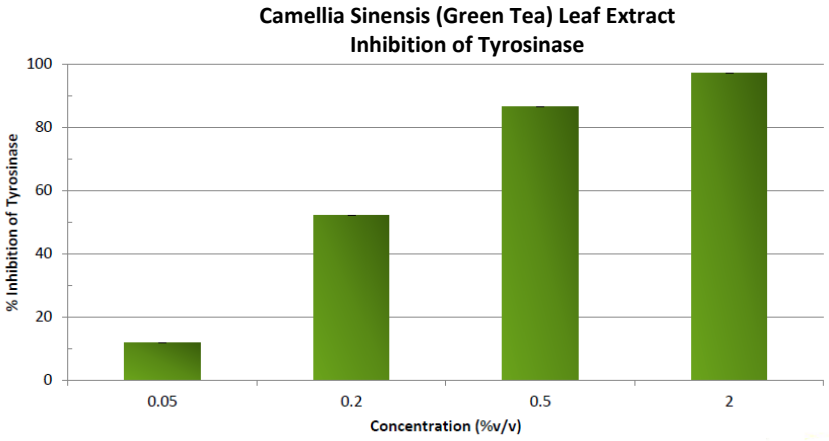
- Camellia Sinensis (Green Tea) Leaf Extract demonstrated dose-dependent inhibition of PLA_2

Inhibition of Tyrosinase

- Melanin production is dependent on initial conversion of tyrosine to DOPAquinone. This reaction is catalysed by the tyrosinase enzyme and is rate limiting for melanin production



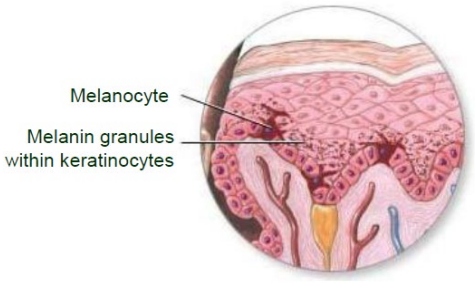
- Camellia Sinensis (Green Tea) Leaf Extract effect on tyrosinase activity was determined by measuring generation of DOPAchrome (OD_{495}) which is formed spontaneously following oxidation of tyrosine by tyrosinase



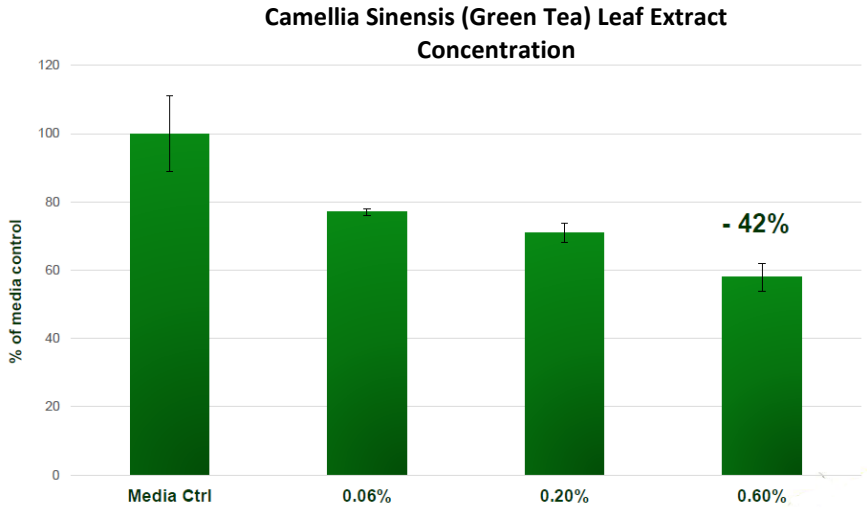
- Tyrosinase activity is efficiently inhibited by Camellia Sinensis (Green Tea) Leaf Extract

Inhibition of Melanin Production

- Melanocytes produce melanin and transfer it to neighbouring keratinocytes in the basal layer of the skin's epidermis



- B16-F10 melanocytes were cultured and treated with different concentrations of Camellia Sinensis (Green Tea) Leaf Extract, with non-treated cells used as control. Following 48h incubation, melanin levels were quantitated



Conclusions

- Camellia Sinensis (Green Tea) Leaf Extract has multiple beneficial effects:
    - Efficient ROS scavenger
    - Strong inhibitor of Elastase and MMPs
    - Modulates inflammatory PLA_2 activity
    - Inhibits tyrosinase activity and melanin production.
- Unlike most commercial green tea extracts, Camellia Sinensis (Green Tea) Leaf Extract produced by sonic extraction, is a stable source of long-lasting antioxidant and anti-inflammatory activities.