

# Sytenol® A

The First Natural Alternative to Retinol for Anti-aging



SYTHE<>N  
...making innovation work

## Sytenol® A

As we age skin loses its elasticity and becomes thin, weak and prone to wrinkles and hyperpigmentation. How can this be prevented? The main goal of anti-aging products is to decrease and eventually eliminate the appearance of fine lines and wrinkles and provide even-toning effects. The way to achieve this goal is to keep wrinkles from forming in the first place. This means preventing skin from further damage, restoring mainly the extra cellular matrix (ECM), dermal-epidermal junction (DEJ) areas and preserving skin hydration.

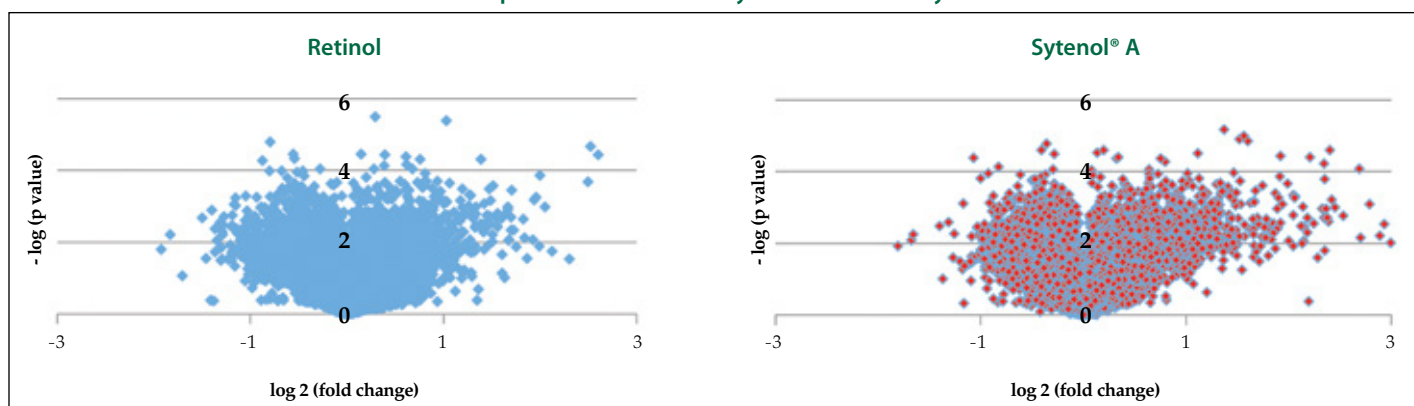
### Product Information

Trade Name	Sytenol® A
INCI Name	Bakuchiol
Chemical Name	Phenol, 4-[(1E, 3S)-3-ethenyl-3, 7-dimethyl-1, 6-octadienyl]
CAS #	10309-37-2
Origin	100% Natural; Extracted and purified from edible seeds
Appearance	Yellow to yellowish brown liquid
Purity	98% min.(typically >99%)
Solubility/Miscibility	Miscible with a wide-range of hydrophobic emollients
Stability	Photochemically & hydrolytically stable
Suggested use level	0.5 to 1%
Storage	Store in original sealed container at +10 to +30 °C; Avoid exposure to light & heat
Regulatory	Approved for use in all major countries –US, Europe, Australia, Canada, Japan, Korea and many others
Patents	US 8,529,967; US 8,859,021; Multiple pending US & European patents

## Sytenol® A - A Functional Analog of Retinol

From the perspective of topically applied compositions, a small molecule without having the negatives of Retinol that safely mimics the properties of Retinol (Fisher *et al.*, *FASEB J*,1002-1013, 1996) is a greatly sought after ingredient. Volcanic plots illustrate the molecular signatures of Retinol and Sytenol® A of a DNA microarray experiment using reconstructed full thickness epidermis. This shows a very similar overall shape, indicating similar overall modulation of gene expressions in the skin substitute model. Multiple comparative studies revealed Sytenol® A to be the true alternative to Retinol (Chaudhuri, *In Cosmeceuticals and Active Cosmetics, 3r Edition, Eds., Maibach et al., Chapter 1, 1-18, 2015*) for anti-aging applications and does not have the inherent safety & stability issues of Retinol. A recent randomized, double-blind clinical study between Sytenol® A and Retinol carried out by UC-Davis Dermatologists demonstrated that both compounds significantly decreased wrinkle surface area and hyperpigmentation, with no statistical difference between the compounds. The Retinol users reported more facial skin scaling, burning and stinging. Authors concluded that Sytenol® A is a more tolerable alternative to Retinol (Dhaliwal *et al*, *British J Dermatology*, 2018 Jun 27. doi: 10.1111/bjd.16918).

Volcanic plots of DNA microarray data: Retinol vs. Sytenol®A



# Clinically Proven to Reduce Multiple Signs of Aging

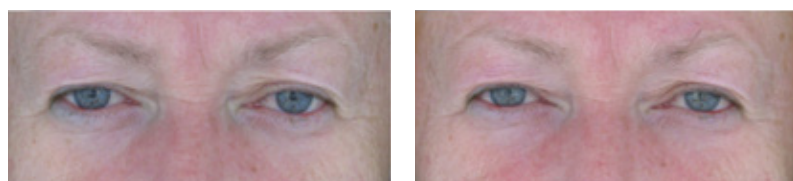
## Protocol:

- **Human volunteers** – 17; 16 Completed; Age – 41 to 60 yrs; Caucasian (14), Hispanics (2)
- **Study duration** – 12 weeks
- **Test sites** – Full face
- **Test substance** – Lotion with 0.5% Sytenol® A; Contains No sunscreen and No moisturizer
- **Application frequency** – About 2 g twice a day
- **Methodology** – Expert grading/Self-assessment by panelists (Grading 0 to 4): (1) Roughness & Dryness; (2) Fine lines & wrinkles; (3) Skin tone; (4) Skin elasticity & firmness; (5) Radiance; (6) Brightening; (7) Overall eye-area appearance; Silicone Replica Analysis: Wrinkle depth & Skin roughness; Photography: Before & after the treatments; Readings were taken at baseline, 4, 8 & 12 weeks.
- **Statistical Analysis** – Statistical significance defined as  $p \leq 0.05$

## Results

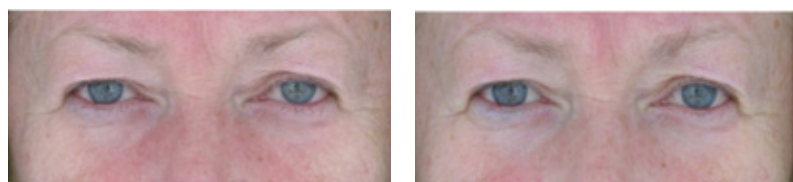
The results clearly showed that, after twelve weeks treatment, significant improvement in lines and wrinkles, pigmentation, elasticity, firmness and overall photo-damage was observed, with no irritating effect on skin. Based on these results and the comparative studies of Retinol and Sytenol® A done by Sytheon, we conclude that Sytenol® A is the first true Retinol-like anti-aging product (Chaudhuri & Bojanowski, Intern J Cosmet Sci, 36(3):221-230, 2014).

### Subject M535: Reduction in wrinkles & skin redness



Before treatment

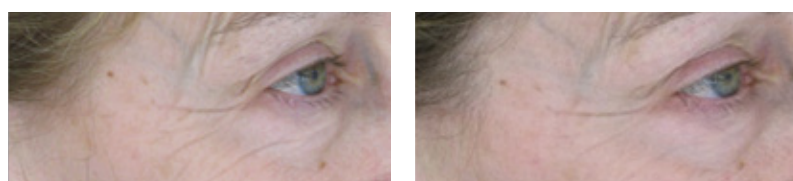
After 4-week treatment



After 8-week treatment

After 12-week treatment

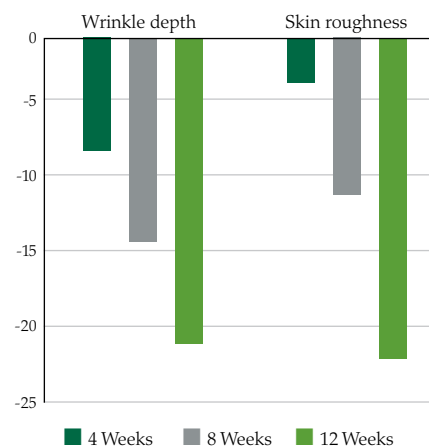
### Subject M572: Reduction in fine lines & wrinkles



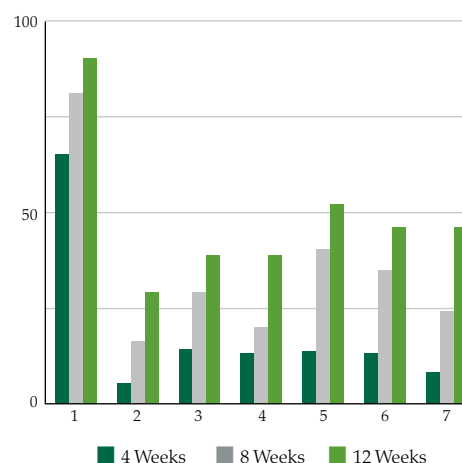
Before treatment

After 12-week treatment

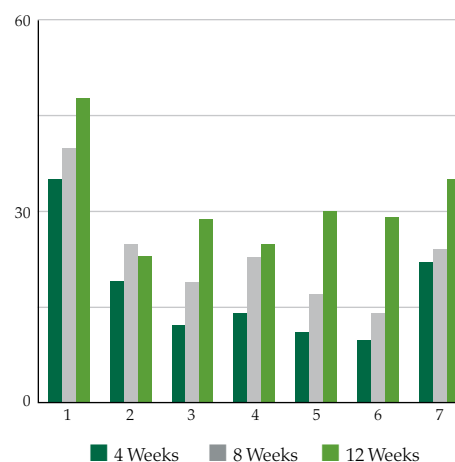
## % Reduction in Wrinkle depth & Skin roughness



## % Score improvement – Expert



## % Score improvement – Panelists



# Science Behind the Product – Restorative Anti-aging

## Comparative Collagen Stimulatory Effects

In aged skin, collapsed fibroblasts produce low levels of collagen and high levels of collagen-degrading enzymes. This imbalance advances the aging process in a self-perpetuating, never-ending deleterious cycle. Treatments such as topical retinol or retinoic acid have been clinically proven to stimulate production of new, undamaged collagen (Fisher *et al.*, *Arch Dermatol*, 144(5):666-672, 2008). The attachment of fibroblasts to this new collagen allows elasticity, which in turn balances collagen production and degradation, thereby slowing, if not reversing, the aging process. We have measured collagen stimulation by ELISA and histochemistry methods. The ELISA assessment employed cell-culture conditioned media from neonatal (type I & IV collagens) or mature (type III collagen) fibroblasts (Chaudhuri & Bojanowski, *Intern J Cosmet Sci*, 36(3):221-230, 2014). Results of this findings, as summarized in the Table (% stimulation vs control) below, demonstrated a significant improvement in collagen stimulation by Sytenol® A as compared to Retinol, hence greater restorative effect is expected.

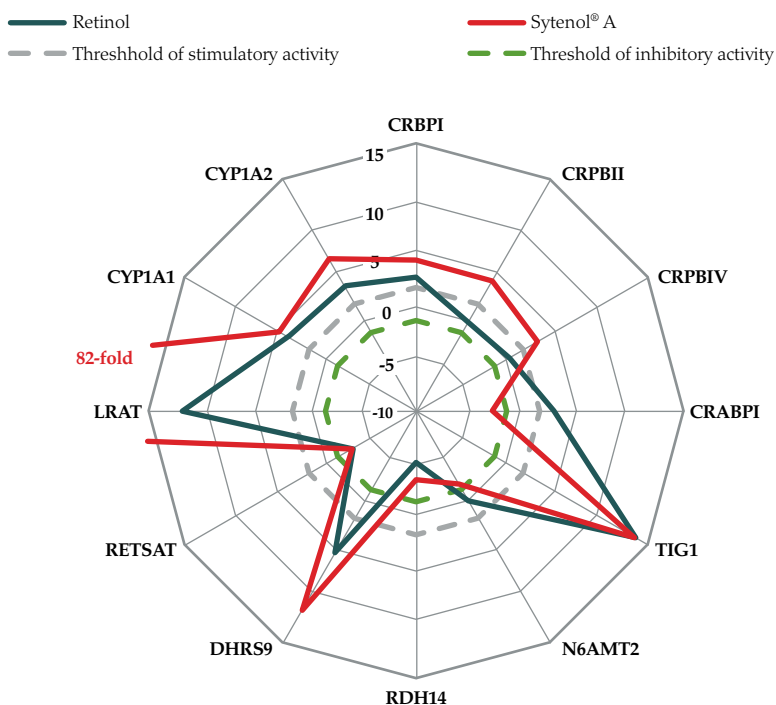
Test material (10 µg/ml)	Collagen I	Collagen III	Collagen IV
Sytenol® A	147	150	119
Retinol	119	148	100

## Modulation of Key Anti-aging Genes

The effects of Sytenol® A and retinol on specific genetic pathways (Full thickness Epiderm tissue from Mattek, Fold change  $\pm > 2$ ;  $p \leq 0.05$ ) relevant to retinol functionality were compared. First, a similar modulation of many (however not all) genes for Retinoid binding and metabolizing genes were observed. A brief description of these genes as well as the impact of Retinol and Sytenol® A on each is presented below. Similarly, many genes involved in the generation and maintenance of the extracellular matrix (ECM) and the dermo-epidermal junction (DEJ) areas were modulated by both Retinol and Sytenol® A (Chaudhuri & Bojanowski, *Intern J Cosmet Sci*, 36(3):221-230, 2014). Skin aging entails drastic changes in the ECM and DEJ. These biological alterations are reflected in the clinical signs of aged skin.

## Retinoid Binding & Metabolizing Genes:

Modulation of retinoid binding & metabolizing genes is essential for Sytenol® A to function like Retinol



**Cellular retinol binding protein (CRBP):** Indispensable for Retinol & Retinoic acid biosynthesis and storage of Retinol.

**Cellular retinoic acid binding protein (CRABP):** Down-regulation by Sytenol® A eliminates Retinoic acid-induced toxicity.

**N-6 adenine-specific DNA methyltransferase 2 (N6AMT2):** Retinoic acid resistance might be combatted by the use of epigenetic modifying agents such as DNA methyl transferase inhibitors. Down-regulation of N6AMT2 by Sytenol® A may reduce Retinoic acid-induced toxicity.

**Tazarotene-inducible gene 1 (TIG1):** TIG1 is down-regulated in acne, rosacea and psoriasis. Up-regulation by Sytenol® A expects to provide skin benefits.

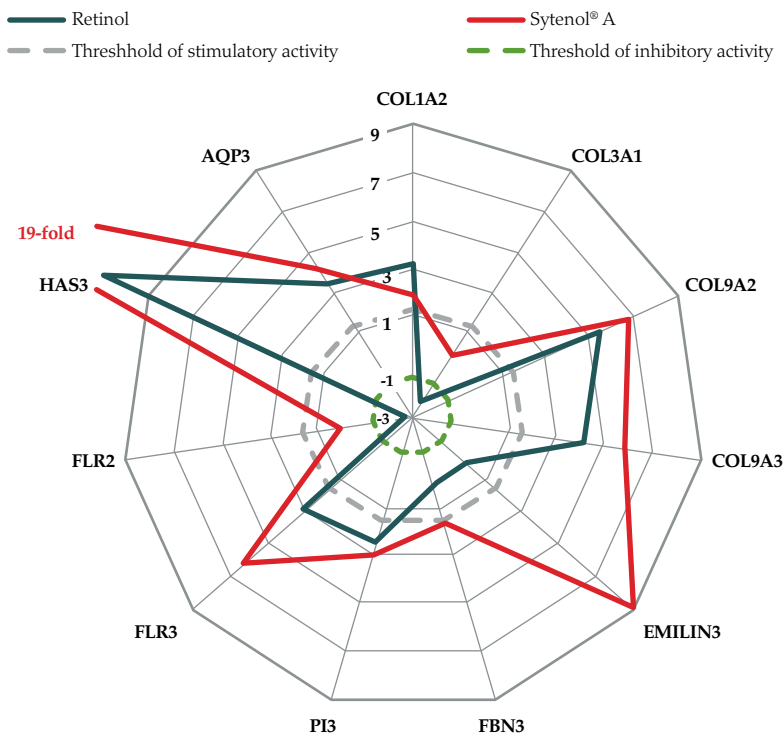
**Retinol dehydrogenase/reductase (RDH14; DHRS9; RETSAT):** Converts Retinol to Retinal and then to retinoic acid. Retinol saturase (RETSAT) is involved in normalization of adipocyte differentiation.

**Lecithin-retinol acyltransferase (LRAT):** Retinol esterification with long-chain fatty acid by LRAT is the key step in Retinol absorption & storage.

**Cytochrome P450 (CYP1A1; CYP1A2):** P450s 1A1 and 1A2 responsible for catalyzing retinol to retinal, the rate-limiting step for the biosynthesis of Retinoic acid.

## Extracellular Matrix Genes:

An intact and hydrated extracellular matrix genes is essential in maintaining youthful skin.



**Collagens:** Responsible for skin strength and elasticity; Degradation leads to wrinkles.

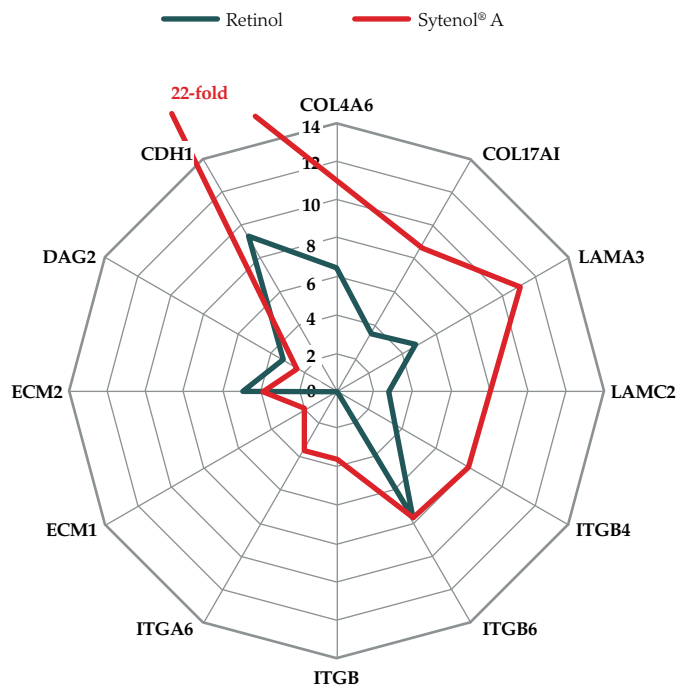
**Elastic Fibers:** (1) Elastin microfibril (EMILIN3) is a component of elastic fibers and localized mainly at the interface and is involved in the process of elastogenesis. (2) Fibrillins (FBN3): constitute the major backbone of multifunctional microfibrils in elastic and nonelastic extracellular matrices. Sytenol® A can maintain the desired level of Elastin required for maintaining the connective tissue homeostasis due to the up-regulation of elastase inhibitor (PI3).

**Fibronectin (FLR3; FLR2):** Maintains the shape of cells and is a prerequisite for matrix stability.

**Hyaluronan & Aquaporin:** Hyaluronans are responsible for the maintenance of a highly hydrated extracellular matrix in tissues & also involved in cell adhesion and supports cell migration. It is synthesized by one of three distinct hyaluronan synthases, such as HAS-3. Aquaporin 3 (AQP3) is the water/glycerol transporting channel protein expressed in the epidermis & helps maintain right levels of skin hydration, elasticity, and barrier recovery. AQP3 protein stimulation confirmed by using EpidermFT full thickness skin substitute

## Dermo-epidermal Junction Genes:

An intact basement membrane at the dermo-epidermal junction genes is essential in maintaining youthful skin



**Type IV Collagen:** Type IV Collagen forms supra molecular networks, which influence cell adhesion, migration, and differentiation.

**Type XVII Collagen:** Type XVII is one of the key anchoring fibrils that fortifies the attachment of the epidermis to the dermis.

**Laminin (LAMA3 and LAMC2):** Integral part of the structural scaffolding; Major non-collagenous proteins in basal lamina. Involved in cell differentiation, migration, adhesion as well as phenotype and survival.

**Integrin (ITGB4, ITGB6, ITGB8, ITGA6):** Defines cellular shape, mobility, and regulate the cell cycle; Key functions - attachment of the cell to the ECM & signal transduction from ECM to the cell.

**ECM Protein (ECM):** Involved in basement membrane and collagen fibril macro-assembly and growth factor binding.

**Dystroglycan (DAG2):** Involved in both assembly and maintenance of basement membrane structure; Loss causes disruption of epidermal differentiation.

**E-Cadherin (CDH1):** Involved in tight junction formation & maintaining stem cell pluripotency; Sun over exposure causes loss of CDH1

## Science Behind the Product - Preventative Anti-aging

Targeting skin concerns early on can effectively prevent damage to the skin and improve skin quality. Slowing down the aging process can be achieved by i) antioxidant protection to limit direct oxidative damage to the cells, proteins, and DNA, ii) controlling inflammation to minimize inflamma-aging & iii) reducing matrix metalloprotease activity to protect ECM and DEJ proteins.

### Defense Against Oxidative Stress

The mechanisms and the sequence of events by which free radicals interfere with cellular functions are not fully understood; but one of the most important events seems to be lipid peroxidation, which results in cellular membrane damage. This damage causes a shift in the net charge of the cell, changing the osmotic pressure, leading to swelling and eventually cell death (Nijvel et al., *Am J Clin Nutr*, 74(4):418-425, 2001). Sytenol® A is a broad-spectrum antioxidant and has an excellent lipid peroxidation inhibitory activity (Chaudhuri & Bou, *C&T*, 130:63-75, 2015) & capable of stimulating antioxidant defense system (Chaudhuri, *EuroCosmetics*, 11-12:20-24, 2015). This dual antioxidant pathways of Sytenol® A helps maintain lipid homeostasis and hence protect skin from oxidative damage. Review "Consequences of Lipid Peroxidation" in the Sytenol® A anti-acne brochure.

### Scavenging of Radicals & Non-radical

Unit <sup>1</sup>	Peroxyl	Hydroxyl	Superoxide	Peroxynitrite	Singlet Oxygen	Lipid Peroxidation <sup>2</sup>
Sytenol® A	15,165	569	204	130	1,325	0.5
Natural Tocopherol	813	Not detected	Not detected	1	1,110	30

<sup>1</sup> μmole Trolox equivalent/g

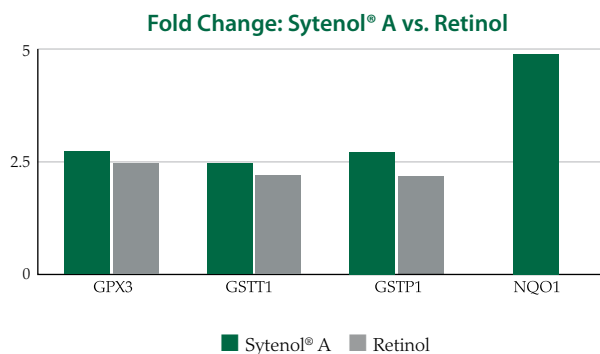
<sup>2</sup> Squalene was used as a substrate for lipid peroxidation inhibitory activity; data is expressed in IC<sub>50</sub> in μg/ml; 60-fold more effective than Tocopherol

### Up-regulation of Antioxidant Defense Genes

#### Protocol:

DNA microarray test using full thickness Epiderm tissue (Mattek)

- Retinol and Sytenol® A were dissolved in DMSO, further diluted in water and applied to full thickness Epiderm tissues
- Incubation for two days
- Selected gene expressions having  $\geq \pm 2$ -fold change
- Statistical significance:  $p = \leq 0.05$



### Role Played by the Antioxidant Defense Genes

- Glutathione peroxidase (GPX3): Reduces lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water
- Glutathione S-transferases (GSST1 and GSTP1): Regulates the intracellular concentrations of the lipid peroxidation products
- NAD(P)H dehydrogenase (quinone)] gene (NQO1): Catalyzes metabolic detoxification of quinones and protects cells against quinone-induced oxidative stress, cytotoxicity, and mutagenicity (Long et al., 60(21):5913-5915, 2000).

### Protection of Mitochondria & Increase in ATP Synthesis

Many studies have established that oxidative stress and mitochondrial dysfunction are two central factors contributing to the aging process. Mitochondrial biogenesis involves multiple processes that need to be tightly coordinated. PGC-1 $\alpha$  is the master regulator of mitochondrial biogenesis & shown to be stimulated by Bakuchiol (Seo et al., *Evid Based Complement Altern Med*, article ID 678028, 2013). Bakuchiol was shown to be very effective in protecting mitochondrial functions against oxidative stress (Haraguchi et al., *Planta Med*, 16:539-544, 2002), preventing mitochondrial lipid peroxidation & inhibiting oxygen consumption originating in lipid peroxidation in a time-dependent manner. Bakuchiol was shown to protect mitochondrial respiratory enzyme activities against both NADPH-dependent and dihydroxyfummarate-induced peroxidation injury.

Bakuchiol is reported to increase ATP synthesis in the hepatocytes of old mice whose ATP synthesis had been reduced by H<sub>2</sub>O<sub>2</sub> treatment and guarded against mitochondrial genome damage (Tsujimoto et al, *Apoptosis*, 12(5):835-840, 2007; Seo etl al., *Evid Based Complement Altern Med*, article ID 678028, 2013).

## Defense Against Inflammation

Skin aging and inflammation are critically linked. The inflammation-induced aging process involves a highly complex chain of events, by which acute inflammation gradually gives way to chronic or silent inflammation. It is this underlying inflammation that ultimately exhausts the body's defense system resulting in collagen and elastin degradation and the breakdown of the skin's barrier function (Woods *et al.*, *Aging Dis*, 3(1):130-140, 2012). The inflammatory cascade of reactions that erode the skin's structure – ultimately deep wrinkles, hyperpigmentation and non-elastic tissues. Sytenol® A controls inflammation by inhibiting/down-regulating pro-inflammatory genes and enzymes (Chaudhuri, *In Cosmeceuticals and Active Cosmetics*, 3rd edition, Eds. Maibach *et al.*, Chapter 1, 1-8, 2015).

## Down-regulation of Pro-inflammatory Genes

### Protocol:

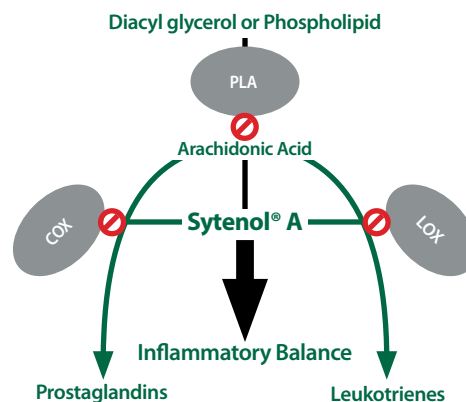
- DNA microarray test using full thickness Epiderm tissue (Mattek)
- Retinol and Sytenol® A were dissolved in DMSO, further diluted in water and applied to full thickness Epiderm tissues
- Incubation for 48 hours
- Selected gene expressions having  $>\pm 2$ -fold change
- Statistical significance:  $p = \leq 0.05$

Gene	Gene Description	Retinol	Sytenol® A
COX1/PTGS1	Cyclooxygenase-1 (Prostaglandin G/H synthase precursor)	-3.4	-3.6
PLAA	Phospholipase A-2-activating protein	No effect	-7.7
PLA2G4A	Cytosolic phospholipase A2	-3.1	-2.6
PTGER2	Prostaglandin E2 receptor EP2 subtype (PGE2)	-2.2	-2.4
PTGER4	Prostaglandin E2 receptor EP4 subtype (PGE2)	-3.0	-6.1
*HPGD/15PGDH	Prostaglandin dehydrogenase 1	+4.1	+21.8

\* HPGD is a catabolic enzyme controlling the biological activities of prostaglandins by converting them into inactive keto-metabolites

## Inhibition of Pro-inflammatory Enzymes

Sytenol® A has inhibitory activity against pro-inflammatory enzymes - phospholipase A2 (PLA2), COX & LOX and dose-dependently reduce formation of LTB4 and TXB2 (Ferrandiz *et al.*, *J Pharm Pharmacol*, 48(9):975-980, 1996). Sytenol® A is effective in inhibiting COX-1  $IC_{50}$  14.7  $\mu$ g/ml and COX-2 ( $IC_{50}$  514  $\mu$ g/ml) activities. Interestingly, at higher dose (50  $\mu$ g/ml), Retinol boosts COX-2 activity while Sytenol® A reduced the activity by 40% (Chaudhuri & Marchio, *Cosmet & Toilet*, 126(7):502-510, 2011). Literature also shows that Sytenol® A has lipoxygenase (LOX) inhibitory activity (Ferrandiz *et al.*, *J Pharm Pharmacol*, 48(9):975-980, 1996).



## Defense Against Matrix Metalloproteases (MMPs)

MMPs play a major role in protein and collagen degradation which affects the structural integrity of the dermis. In normal skin, its production is in balance with their natural inhibitors (TIMPs); however, ultraviolet light is reported to enhance the synthesis of MMP in human skin in vivo leading to collagen destruction leading to an imbalance between the MMPs, and their natural inhibitors (TIMPs) which results in the accelerated destruction of connective tissues and photoaging (Fisher *et al.*, *New Engl J Med*, 337:1219-1228, 1997). Therefore reduction of MMPs would be expected to retard the clinical manifestations of skin aging.

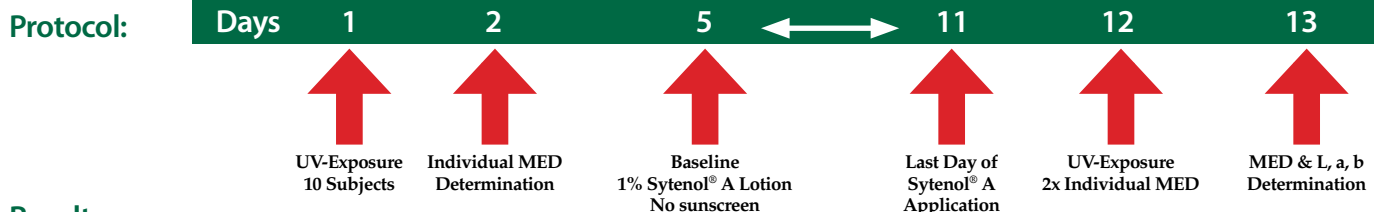
Given the many similar targets of retinol and Sytenol® A, we compared their performances of Sytenol® A and retinol on two key matrix metalloproteases, MMP-1 and MMP-12. As presented in the following Table, Sytenol® A has a significant inhibitory effect on MMP-1 and a markedly stronger inhibitory effect on MMP-12, far exceeding the effect of Retinol (Chaudhuri, *In Cosmeceuticals and Active Cosmetics*, Eds. Maibach *et al.*, Chapter 1, 1-18, 2015). Thus, it is expected that Sytenol® A will provide an even stronger protection of extracellular matrix proteins in vivo against MMPs.

## Inhibition of Matrix Metalloprotease Activity

Matrix metalloprotease	Methods Used	Sytenol® A	Retinol
MMP-1 (collagenase)	Enzcheck collagenase assay kit (Molecular Probe)	50% inhibition at 1 mg/ml	Not determined
MMP-12 (Elastase)	Calbiochem human neutrophil elastase kit	70% inhibition at 1 $\mu$ g/ml	8% inhibition at 1 $\mu$ g/ml

# Clinically Proven to Protect Skin from UV-induced Damage

Erythema, the most familiar manifestation of UV radiation exposure, occurs in a biphasic manner. UVA mediates the early part of this reaction, known as immediate pigment darkening (IPD) and lasts for about half-hour. Delayed erythema, a function primarily of UV-B dosages, begins 2-8 hours after exposure and reaches a maximum in 24-36 hours, with erythema, pruritus, and pain in the sun-exposed areas. A dose of UVB radiation sufficient to induce erythema in human skin results in the formation of about 20 photoproducts per 106 nucleotides.



## Results

As presented in the following Table, the results clearly showed a marked reduction in the manifestation of erythema, as evidenced by practically no difference in the delta (or change) in the L-, a- and ITA values in those areas that were treated with the Sytenol® A containing lotion as compared to the untreated areas.

### Reduction in erythema using 1% Sytenol® A lotion (No Sunscreen)

	Pre-irradiation	Post-irradiation	ΔL or ΔITA or Δa- value
L-value with Sytenol® A	65.69	66.25	-0.56
L-value without Sytenol® A	66.45	60.71	-5.74 (p = <0.001)
ITA with Sytenol® A	43.97	46.83	+2.86
ITA without Sytenol® A	46.05	36.76	-9.29 (p = <0.001)
a-value with Sytenol® A	8.53	8.38	-0.15
a-value without Sytenol® A	8.17	16.32	+8.15 (p = <0.001)

## Formulation Guidelines

Sytenol® A is a lipophilic compound miscible in a wide variety of emollients, such as, caprylic/capric triglycerides, ethyl linoleate, C12-15 alkyl benzoates, squalane, jojoba oils, olive oils, etc. Sytenol® A can be easily formulated into creams, lotions, oils, serums, hydro-alcoholic sprays, etc.

- Sytenol® A should be used at a level of 0.5 to 1.0% (w/w) of finished formulation
- Add Sytenol® A to the formulation after making emulsion with a processing temperature of about 50 °C. Alternately, Sytenol® A can be included in the oil phase
- Addition of a small amount of a chelating agent (0.05%) is helpful in overcoming coloration issue due to the presence of iron or copper
- The finished product must be acidic, preferably having pH below 6 and must be protected from prolonged exposure to heat and light for maintaining product integrity over time.

## Key Publications

1. S Dhaliwal et al., Prospective, randomized, double-blind assessment of topical bakuchiol and retinol for facial photoaging, *British J Dermatology*, 2018 Jun 27. doi: 10.1111/bjd.16918
2. RK Chaudhuri, Bakuchiol; A retinol-like functional compound modulating multiple retinol and non-retinol targets, In *Cosmeceuticals and Active Cosmetics*, 3rd Edition, Eds., RK Sivamani, J Jagdeo, P Elsner & HI Maibach, Francis & Taylor, Boca Raton, Chapter 1, 1-18, 2015
3. RK Chaudhuri & B Ou, Bakuchiol to stabilize retinol and polyunsaturated lipids, *Cosm & Toil*, 130:64-75, 2015
4. RK Chaudhuri & K Bojanowski, Bakuchiol: A retinol-like functional compound revealed by gene expression profiling and clinically proven to have anti-aging effects, *International J Cosmetic Science*, 36(3):221-230, 2014

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