pro-ferm
deeper immunity to ageing

PRODUCT DATA SHEET

pro-ferm Day Cream

moisturizing – protective – anti-wrinkle

EFFICIENCY

- Intensive moisturizing – 24 hours long *
- Stimulates cell regeneration and reduces the depth of expression lines and wrinkles *
- Increases the natural protective functions of the skin **

The skin becomes smoother, silkier, reviving its youthful appearance.

* Tests in-vivo
** Tests in-vitro

ACTIVE INGREDIENTS

Glucaferm®
The natural immunostimulant protects and mobilizes the Langerhans cells of the epidermis, which reactivate the skin's defense and repair system. This direct action on the Langerhans cells stimulates an effective skin regeneration that strengthens the skin against the signs of aging. In addition, the skin is effectively protected against internal and external assaults as well as light-induced skin aging.

Hyaferm®
A natural carrier molecule consisting of hyaluronic acid, which transports the active ingredient Glucaferm® deep into the skin to the Langerhans cells. In addition, Hyaferm® is a very effective moisturizer that hydrates the skin and retains its elasticity.

Aloe Vera
Aloe Vera is an herbal ingredient with moisturizing, regenerating and soothing properties.

Vitamin E
A skin vitamin that protects the skin from free radicals and stimulates cell regeneration.

Vitamin A – Palmitate
The physiological storage form of vitamin A in the body. It stimulates the regeneration of prematurely aged skin, stimulates collagen production and increases skin elasticity.

Vitamin C – Palmitate
A natural antioxidant that protects the skin from free radicals.

Peanut Oil
A vegetable lipid high in unsaturated fatty acids and minerals. Makes the skin smooth and silky.

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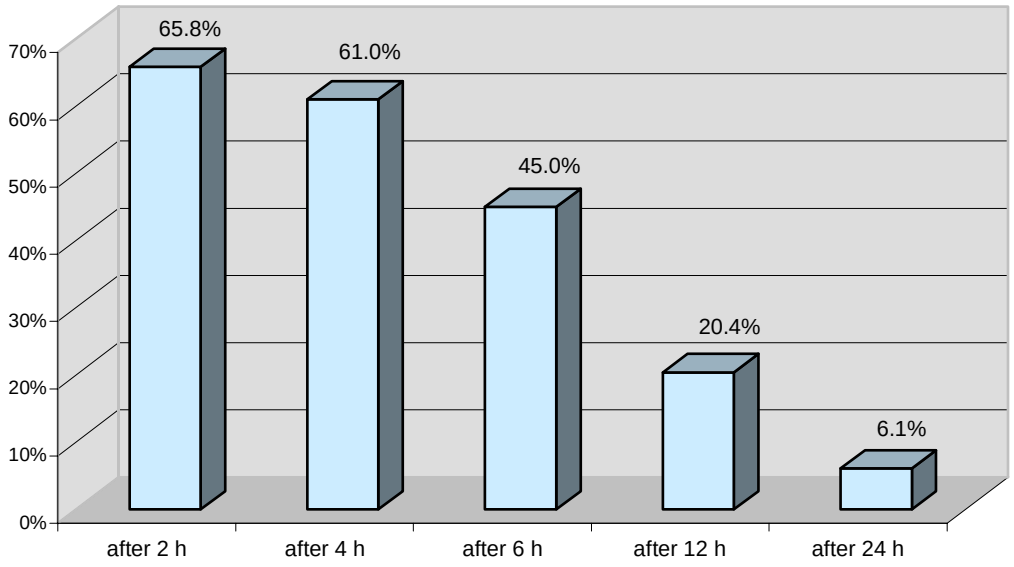
APPLICATION	After cleansing, gently massage a small amount into the face and neck. For best results use every morning.
TOLERABILITY	Dermatologically tested. Also suitable for sensitive skin.
CONTENT	Jar – 50 ml
INGREDIENTS	Aqua [Water], Caprylic/Capric Triglyceride, Glycerin, Dicaprylyl Ether, Petrolatum, Cetearyl Alcohol, Glyceryl Stearate, Cyclopentasiloxane [Cyclomethicone], Dimethicone, PEG-100 Stearate, Tocopheryl Acetate, Sodium Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Cyclohexasiloxane [Cyclomethicone], Betaglucan, Sodium Hyaluronate, Isohexadecane, Aloe Barbadensis Leaf Juice, Polysorbate 80, Retinyl Palmitate, Arachis Hypogaea (Peanut) Oil, PPG-2 Methyl Ether, Ascorbyl Palmitate, 2-Bromo-2-Nitropropane-1,3-Diol, Deceth-8, Phytic Acid, Iodopropynyl Butylcarbamate, Tocopherol, Benzyl Alcohol, Butylphenyl Methylpropional, Hexyl Cinnamal, Citronellol, Linalool, Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde, Alpha-Isomethyl Ionone, Fragrance.

After a single application: **Moisturizes for 24 hours**



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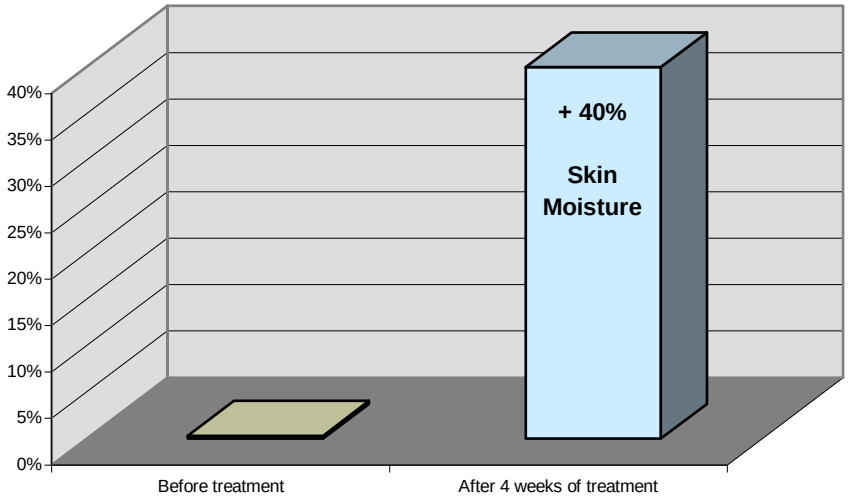
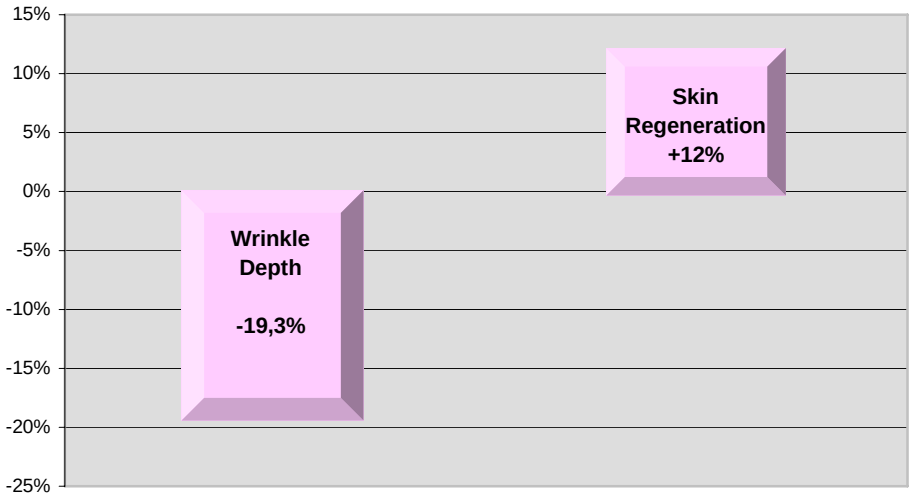
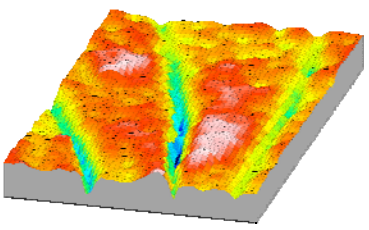
After 4 weeks of treatment: Increase in Skin Moisture



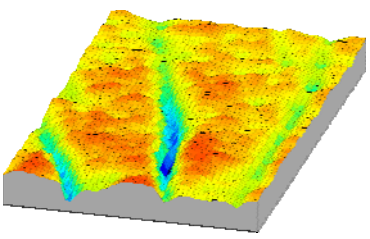
After 4 weeks of treatment: Anti-Aging Effects



Reduction of Wrinkle Depth



Before treatment



After 4 weeks of treatment

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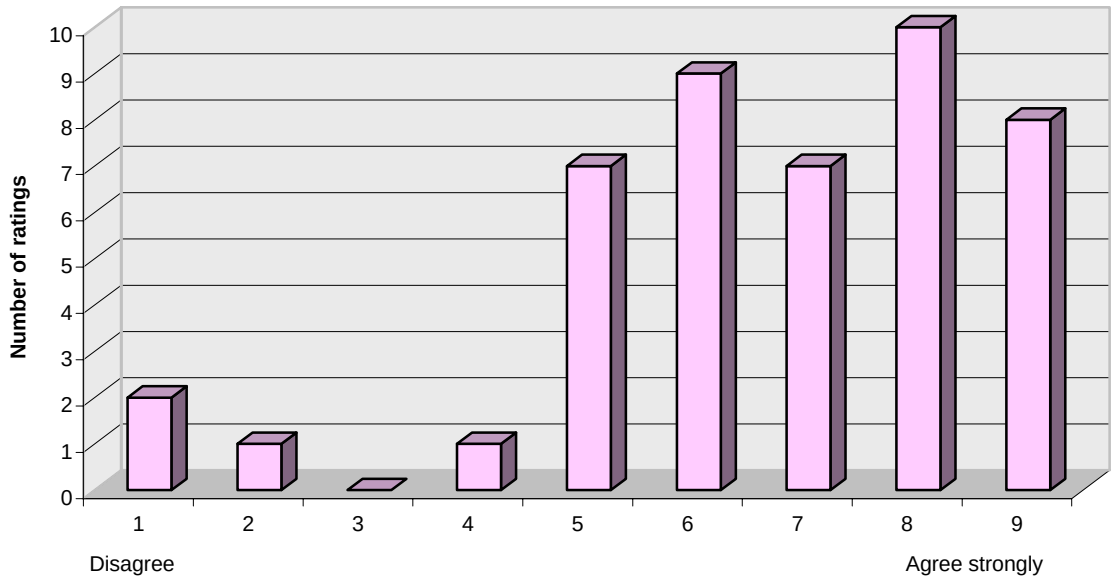
After 8 weeks of treatment: **Anti-Aging Effects**

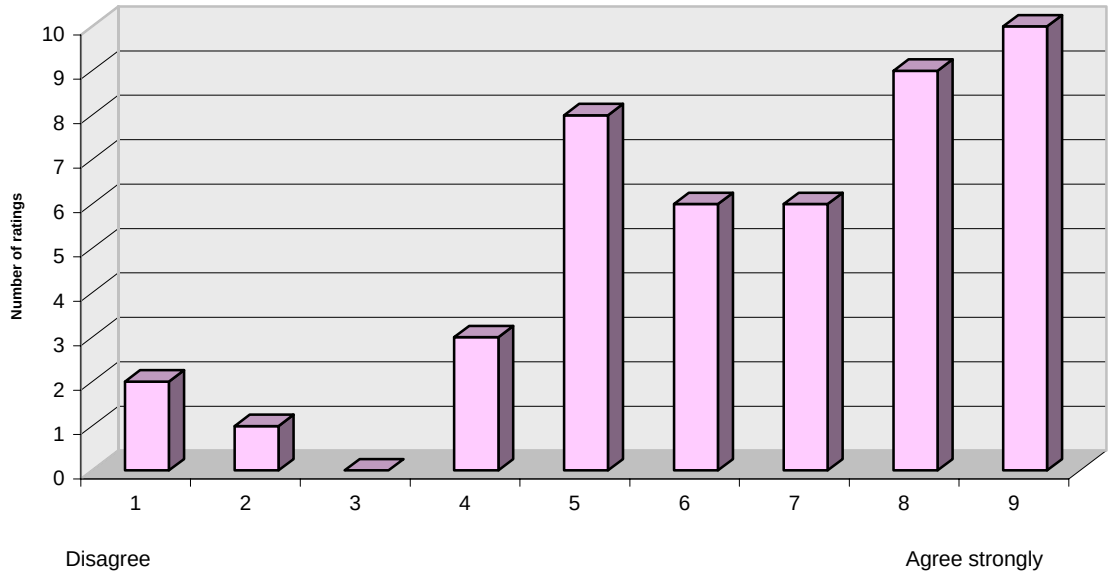
The FIRMNESS of my skin is improved?

Rating by test subjects aged 30 to 60 years after 8 weeks of treatment



The ELASTICITY of my skin is improved?

Rating by test subjects aged 30 to 60 years after 8 weeks of treatment



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PRODUCT DATA SHEET

pro-ferm Night Cream

regenerating – firming – nourishing

EFFICIENCY

- Stimulates cell regeneration *
- Increases the skin's firmness and elasticity *
- Strengthens the skin's immune system **

Supplies the skin with in-depths regeneration. Skin is visibly more toned and firmer, regains its elasticity and resilience.

* Tests in-vivo
** Tests in-vitro

ACTIVE INGREDIENTS

Glucaferm®
The natural immunostimulant protects and mobilizes the Langerhans cells of the epidermis, which reactivate the skin's defense and repair system. This direct action on the Langerhans cells stimulates an effective skin regeneration that strengthens the skin against the signs of aging. In addition, the skin is effectively protected against internal and external assaults as well as light-induced skin aging.

Hyaferm®
A natural carrier molecule consisting of hyaluronic acid, which transports the active ingredient Glucaferm® deep into the skin to the Langerhans cells. In addition, Hyaferm® is a very effective moisturizer that hydrates the skin and retains its elasticity.

Aloe Vera
Aloe Vera is an herbal ingredient with moisturizing, regenerating and soothing properties.

Vitamin E
A skin vitamin that protects the skin from free radicals and stimulates cell regeneration.

Vitamin A – Palmitat
The physiological storage form of vitamin A in the body. It stimulates the regeneration of prematurely aged skin, stimulates collagen production and increases skin elasticity.

Peanut Oil
A vegetable lipid high in unsaturated fatty acids and minerals. Makes the skin smooth and silky.

APPLICATION

After cleansing, gently massage a small amount into the face and neck. For best results use every evening.

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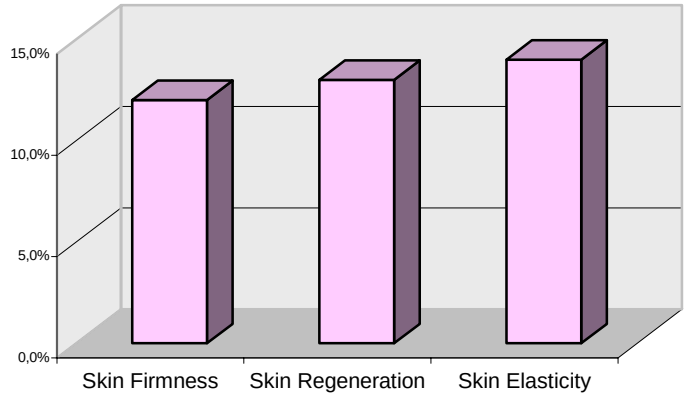
TOLERABILITY	Dermatologically tested. Also suitable for sensitive skin.
CONTENT	Jar – 50 ml
INGREDIENTS	Aqua [Water], Hydrogenated Polydecene, Glyceryl Stearate, PEG-20 Glyceryl Stearate, Glycerin, Hydrogenated Coco-Glycerides, Cetyl Alcohol, Dimethicone, Hydrogenated Castor Oil, Tocopheryl Acetate, Ricinus Communis (Castor) Seed Oil, Sodium Hyaluronate, Betaglucan, Retinyl Palmitate, Arachis Hypogaea (Peanut) Oil, Aloe Barbadensis Leaf Juice, Glyceryl Polyacrylate, PPG-2 Methyl Ether, Tetrasodium Iminodisuccinate, 2-Bromo-2-Nitropropane-1,3-Diol, Deceth-8, Tocopherol, Iodopropynyl Butylcarbamate, Methylparaben, Benzyl Alcohol, Butylphenyl Methylpropional, Hexyl Cinnamal, Citronellol, Linalool, Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde, Alpha-Isomethyl Ionone, Parfum [Fragrance].

After 4 weeks of treatment: **Anti-Aging Effects** (In-vivo tests)



After 8 weeks of treatment: **Anti-Aging Effects** (Ratings by test subjects)

The FIRMNESS of my skin is improved	**82%**

The ELASTICITY of my skin is improved	**80%**

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PRODUCT DATA SHEET

pro-ferm Revitalizing Treatment

revitalizing– intensive regenerating – nurturing

EFFICIENCY

- Promotes intensive regeneration of stressed skin.*
- Supplies the skin with new vitality and suppleness.
- Combines skin repair with protection and intensive care.*

* Tests in-vivo

ACTIVE INGREDIENTS

Glucaferm®
The natural immunostimulant protects and mobilizes the Langerhans cells of the epidermis, which reactivate the skin's defense and repair system. This direct action on the Langerhans cells stimulates an effective skin regeneration that strengthens the skin against the signs of aging. In addition, the skin is effectively protected against internal and external assaults as well as light-induced skin aging.

Hyaferm®
A natural carrier molecule consisting of hyaluronic acid, which transports the active ingredient Glucaferm® deep into the skin to the Langerhans cells. In addition, Hyaferm® is a very effective moisturizer that hydrates the skin and retains its elasticity.

Allantoin
A recognized, multiactive agent, which intensively promotes the regeneration of stressed skin. As a result, Allantoin contributes to the maintenance of a healthy and vital skin. It smoothes the skin and supplies it with new suppleness.

Aloe Vera
Aloe Vera is an herbal ingredient with moisturizing, regenerating and soothing properties.

Vitamin E
A skin vitamin that protects the skin from free radicals and stimulates cell regeneration.

Vitamin A – Palmitat
The physiological storage form of vitamin A in the body. It stimulates the regeneration of prematurely aged skin, stimulates collagen production and increases skin elasticity.

Vitamin C – Palmitat
A natural antioxidant that protects the skin from free radicals.

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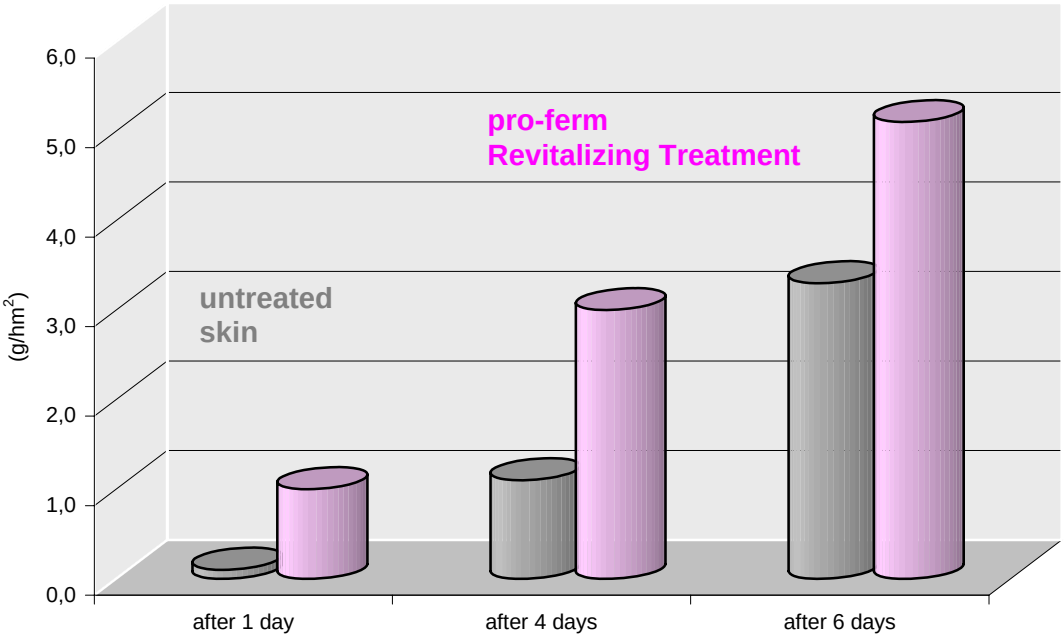
APPLICATION	Use once or twice a week, or whenever skin needs an extra boost. Massage gently into clean skin, avoiding contact with eyes, and leave overnight.
TOLERABILITY	Dermatologically tested. Also suitable for sensitive skin.
CONTENT	Jar – 50 ml
INGREDIENTS	Aqua [Water], Caprylic/Capric Triglyceride, Cetearyl Alcohol, Cetyl Palmitate, Glyceryl Stearate, Oleyl Erucate, Betaglucan, PPG-3 Myristyl Ether, Ceteareth-12, Ceteareth-30, Propylene Glycol, Glycerin, Sodium Hyaluronate, Tocopheryl Acetate, Carbomer, Allantoin, Retinyl Palmitate, Arachis Hypogaea (Peanut) Oil, Aloe Barbadensis Leaf Juice, PPG-2 Methyl Ether, Ascorbyl Palmitate, 2-Bromo-2-Nitropropane-1,3-Diol, Deceth-8, Iodopropynyl Butylcarbamate, Tocopherol, Benzyl Alcohol, Butylphenyl Methylpropional, Hexyl Cinnamal, Citronellol, Linalool, Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde, Alpha-Isomethyl Ionone, Parfum [Fragrance].

Skin Regeneration after Irritation
(Repair of the Skin Barrier Function))



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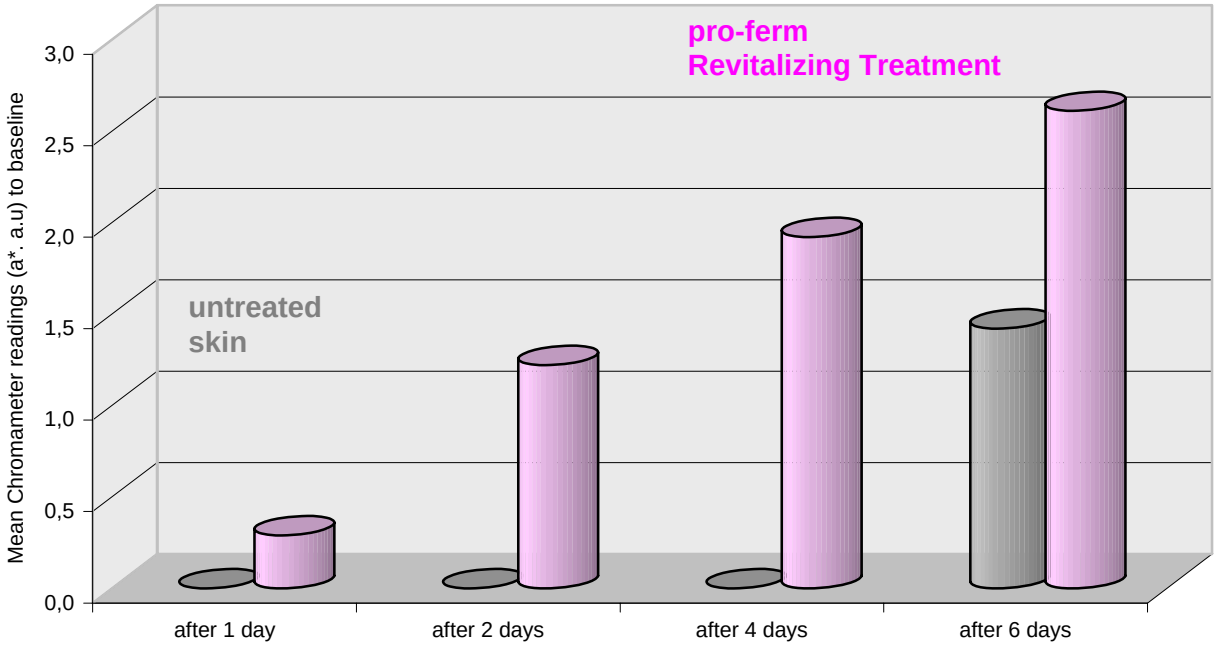
Decrease of Redness after Irritation



After 4 weeks application: **Cell Renewal and Skin Hydration**

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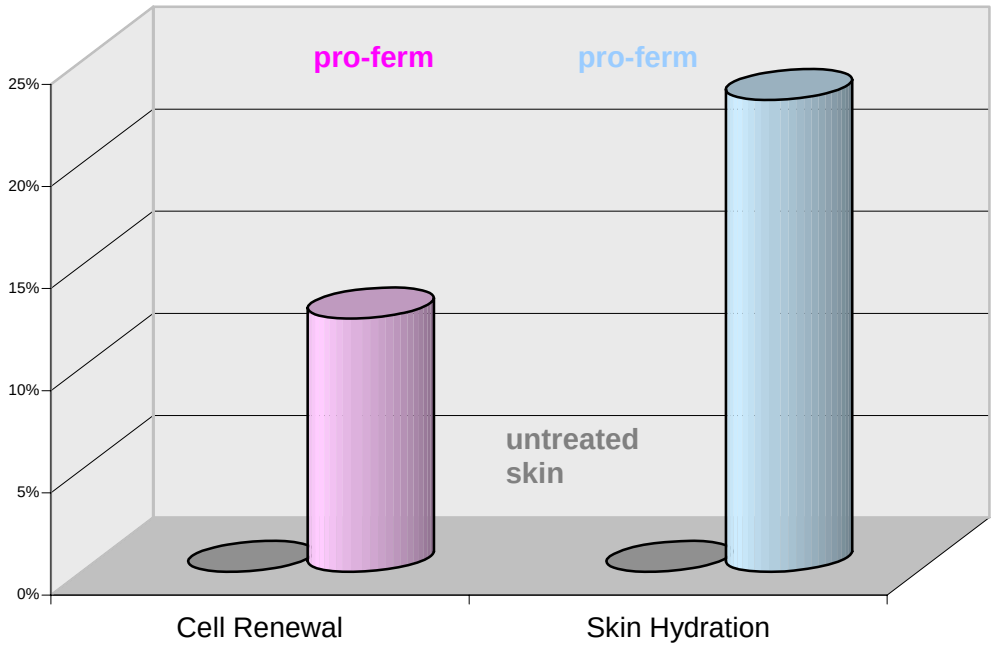

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PRODUCT DATA SHEET

pro-ferm Eye Cream

anti-wrinkle – firming – moisturizing

EFFICIENCY

Intensive firming care for the eye contour area

- Specially developed for the sensitive skin around the eyes
- Reduces wrinkles and crow's feet, intensively moisturizes, firms and smoothes the skin around the eyes*
- Free of fragrance

* Tests in-vivo

ACTIVE INGREDIENTS

Glucaferm®
The natural immunostimulant protects and mobilizes the Langerhans cells of the epidermis, which reactivate the skin's defense and repair system. This direct action on the Langerhans cells stimulates an effective skin regeneration that strengthens the skin against the signs of aging. In addition, the skin is effectively protected against internal and external assaults as well as light-induced skin aging.

Hyaferm®
A natural carrier molecule consisting of hyaluronic acid, which transports the active ingredient Glucaferm® deep into the skin to the Langerhans cells. In addition, Hyaferm® is a very effective moisturizer that hydrates the skin and retains its elasticity.

Vitamin E
A skin vitamin that protects the skin from free radicals and stimulates cell regeneration.

Squalane
Squalane is an oil component extracted from olive oil that is very similar to the human skin surface fat. It prevents loss of moisture and softens and smoothes the skin, improving the structure of the hydrolipid film.

Cera Alba
Purified beeswax, smoothes and nourishes the skin; has moisturizing and lipid regulating effects.

APPLICATION

Apply mornings before make-up and evenings before night cream. Using the fingertips, lightly pat a small amount around the eye contour area.

TOLERABILITY

Dermatologically tested.
Also suitable for sensitive skin.

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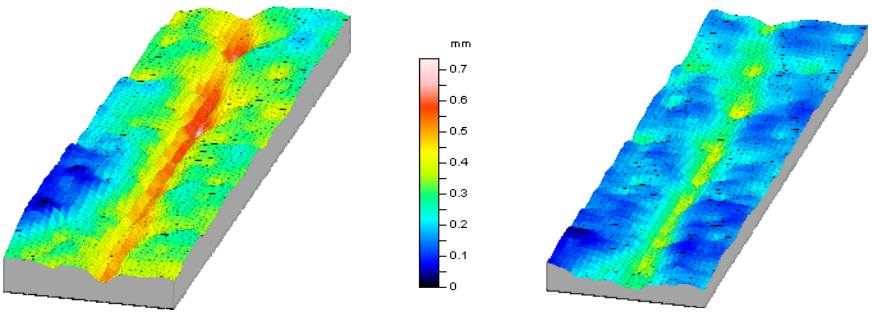
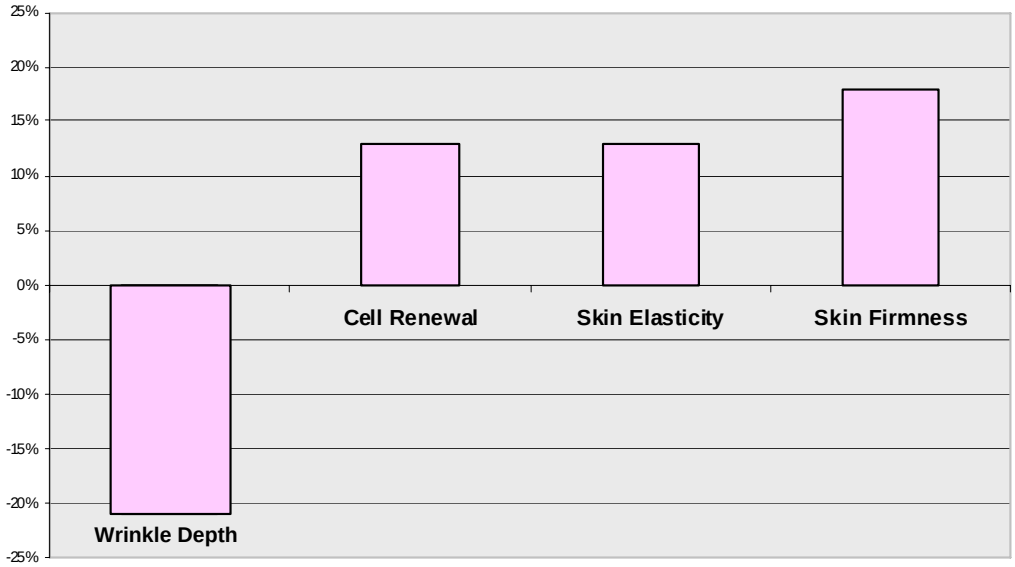
CONTENT	Jar – 15 ml
INGREDIENTS	Aqua [Water], Caprylic/Capric Triglyceride, Cetearyl Alcohol, Cetyl Palmitate, Propylene Glycol, Oleyl Erucate, Squalane, Tocopheryl Acetate, Cera Alba [Beeswax], Cetearyl Glucoside, Saccharide Isomerate, Sodium Hyaluronate, Betaglucan, Methylparaben, Hexamidine Diisethionate, 2-Bromo-2-Nitropropane-1,3-Diol, Citric Acid, Propylparaben.

After 4 weeks of treatment: **Reduction in Wrinkle Depth**



After 4 weeks of treatment:: **Anti-Aging Effects**



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PRODUCT DATA SHEET

pro-ferm Instant Lifting Serum

lifting – contouring – instant perfecting

EFFICIENCY

Facial Instant Effect Serum

- Firms facial contours*
- Reduces existing wrinkles*
- Perfects the optical appearance of the skin with the aid of light-reflecting agents

Integral light-reflecting agents act immediately to bring radiance to the skin, whilst over time the skin's firmness and elasticity are improved, reducing the depth of wrinkles.

* Tests in-vivo

ACTIVE INGREDIENTS

Glucaferm®
The natural immunostimulant protects and mobilizes the Langerhans cells of the epidermis, which reactivate the skin's defense and repair system. This direct action on the Langerhans cells stimulates an effective skin regeneration that strengthens the skin against the signs of aging. In addition, the skin is effectively protected against internal and external assaults as well as light-induced skin aging.

Hyaferm®
A natural carrier molecule consisting of hyaluronic acid, which transports the active ingredient Glucaferm® deep into the skin to the Langerhans cells. In addition, Hyaferm® is a very effective moisturizer that hydrates the skin and retains its elasticity.

Algae Extract
A skin-firming ingredient that causes an immediate firming effect of the skin and long-term strengthening of the connective tissue.

Vitamin E
A skin vitamin that protects the skin from free radicals and stimulates cell regeneration.

Allantoin
A recognized, multiactive agent, which intensively promotes the regeneration of stressed skin. As a result, Allantoin contributes to the maintenance of a healthy and vital skin. It smoothes the skin and supplies it with new suppleness.

Panthenol
Provitamin B, has a skin-regenerative effect, retains moisture and soothes.

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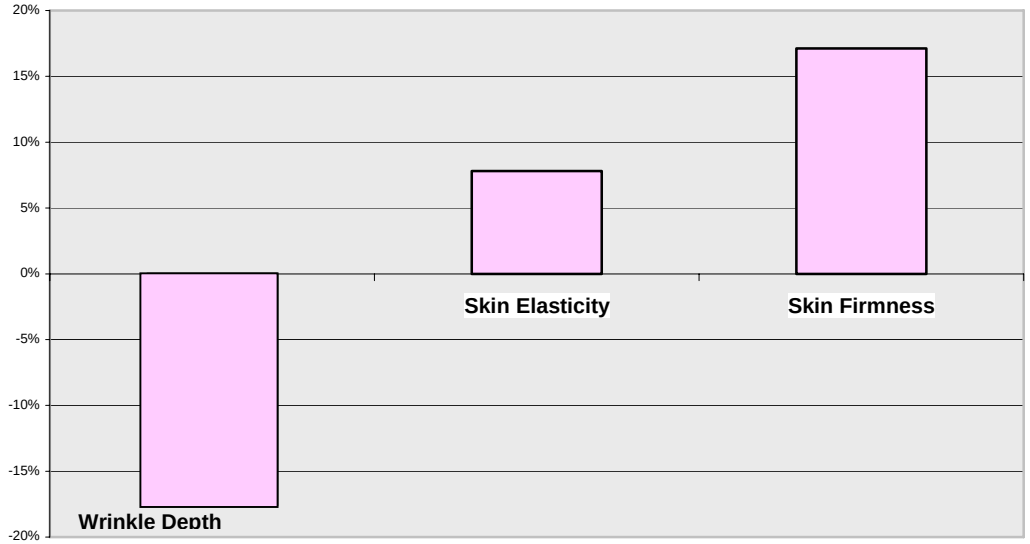
ACTIVE INGREDIENTS	Sweet almond oil A soft, smoothing and nourishing oil, rich in vitamins, minerals and essential unsaturated fatty acids Avocado Oil A high-grade oil that protects the skin against dehydration and refines facial contours. This ingredient contains a high amount of essential fatty acids and vitamins. Alumina Mineral light filter and light reflecting material that improves the optical appearance of the skin.
APPLICATION	Using the fingertips, gently massage a small amount into the face and neck, avoiding contact with eyes. Use daily or when your skin needs a special lift. Very dry skin types can use the serum before apply pro-ferm Day Cream as well.
TOLERABILITY	Dermatologically tested. Also suitable for sensitive skin.
CONTENT	Dispenser – 30 ml
INGREDIENTS	Aqua [Water], Pentylene Glycol, Alumina, Panthenol, Prunus Amygdalus Dulcis (Sweet Almond) Oil, Persea Gratissima (Avocado) Oil, Phenoxyethanol, Pullulan, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, Betaglucan, Sodium Hyaluronate, Sorbitan Laurate, Tocopherol, Helianthus Annuus (Sunflower) Seed Oil, Algae [Algae Extract], Polyglyceryl-10 Laurate, Benzoic Acid, Allantoin, Dehydroacetic Acid, Parfum [Fragrance].

After 4 weeks of treatment: **Anti-Aging Effects**



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PRODUCT DATA SHEET

pro-ferm Skin Balm for Men

soothing – protecting – repairing

EFFICIENCY

- Soothes razor burn and dryness.*
- Encourages healing of minor nicks and cuts.*
- Strengthens the skin's defense mechanisms.**

* Tests in-vivo
** Tests in-vitro

ACTIVE INGREDIENTS

Glucaferm®
The natural immunostimulant protects and mobilizes the Langerhans cells of the epidermis, which reactivate the skin's defense and repair system. This direct action on the Langerhans cells stimulates sustainable skin regeneration and repair of damaged tissue. In addition, the skin is effectively protected against internal and external stress factors due to the reactivation of its own defense system.

Hyaferm®
A natural carrier molecule consisting of hyaluronic acid, which transports the active ingredient Glucaferm® deep into the skin to the Langerhans cells. In addition, Hyaferm® is a very effective moisturizer that hydrates the skin and retains its elasticity.

Vitamin E
A skin vitamin that protects the skin from free radicals and stimulates cell regeneration.

APPLICATION

Use after shaving and whenever skin feels dry or delicate. Massage into clean skin, avoiding contact with eyes.

TOLERABILITY

Dermatologically tested.

CONTENT

Dispenser – 60 ml

INGREDIENTS

Aqua [Water], Alcohol Denat., Propylene Glycol , Caprylic/Capric Triglyceride, Cyclopentasiloxane [Cyclomethicone], Cyclohexasiloxane [Cyclomethicone], Betaglucan, Dimethicone PEG-7 Isostearate, Tocopheryl Acetate, Sodium Hyaluronate, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, Carbomer, Triclosan, PPG-2 Methyl Ether, 2-Bromo-2-Nitropropane-1,3-Diol, Deceth-8, Iodopropynyl Butylcarbamate, Benzyl Alcohol, Parfum [Fragrance].

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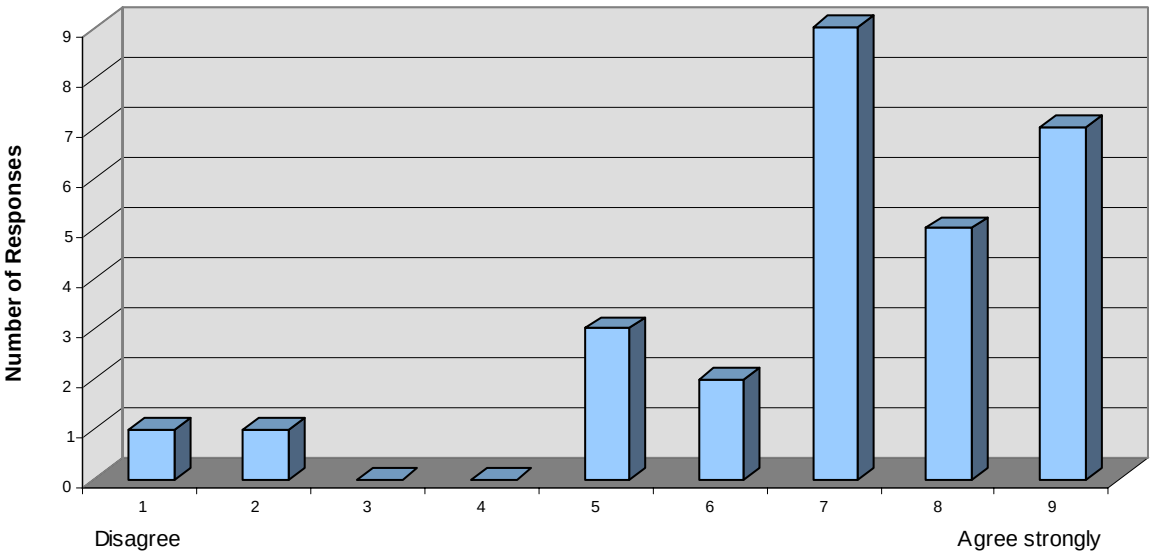
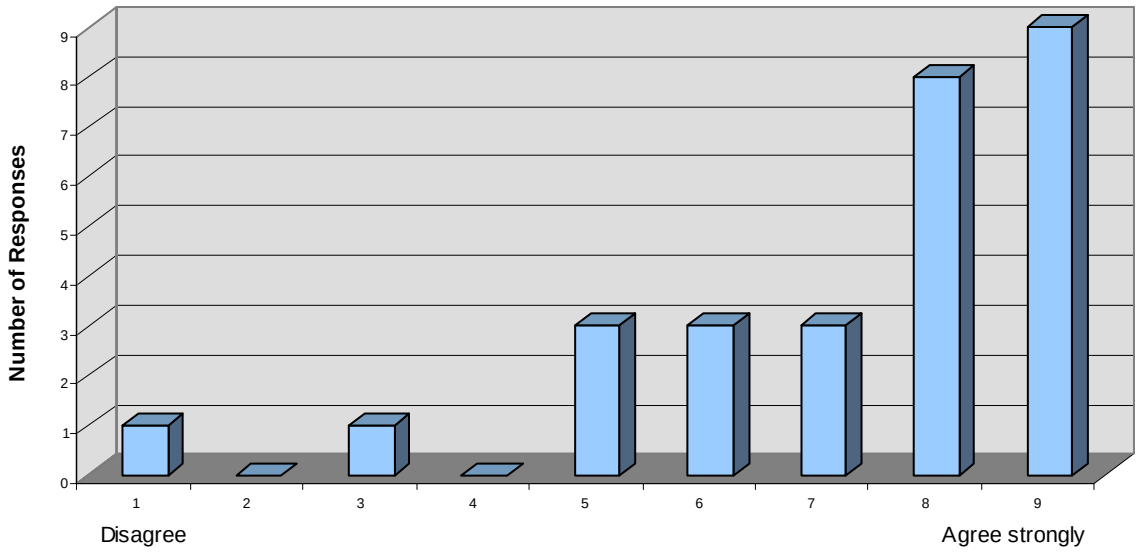
REDNESS and SKIN IRRITATION is reduced?*



RAZOR BURN is reduced?*



*Ratings from test persons aged 35 to 65 years after eight weeks of application.

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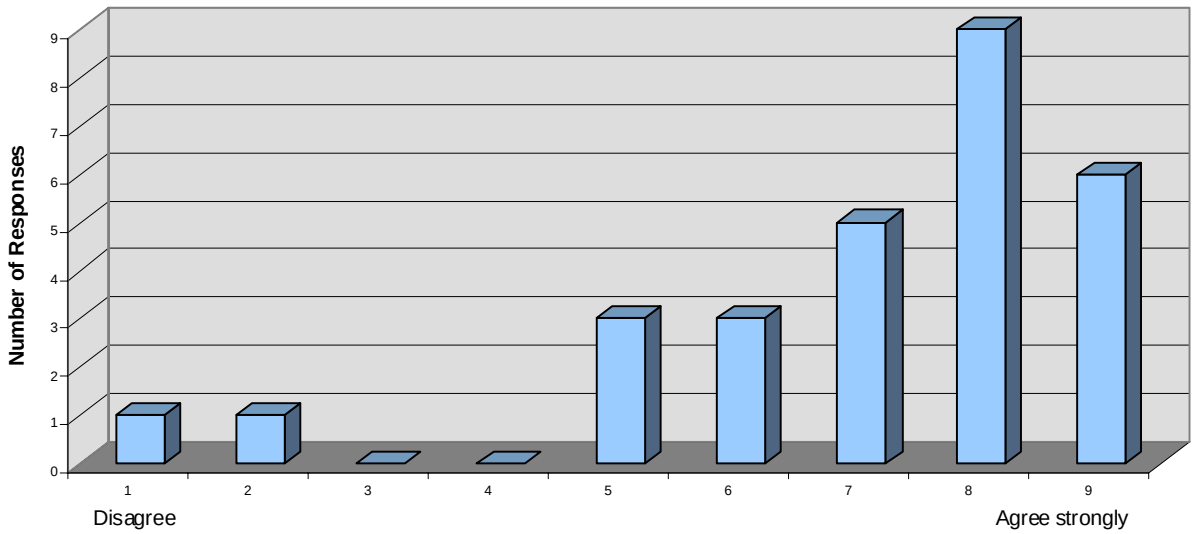
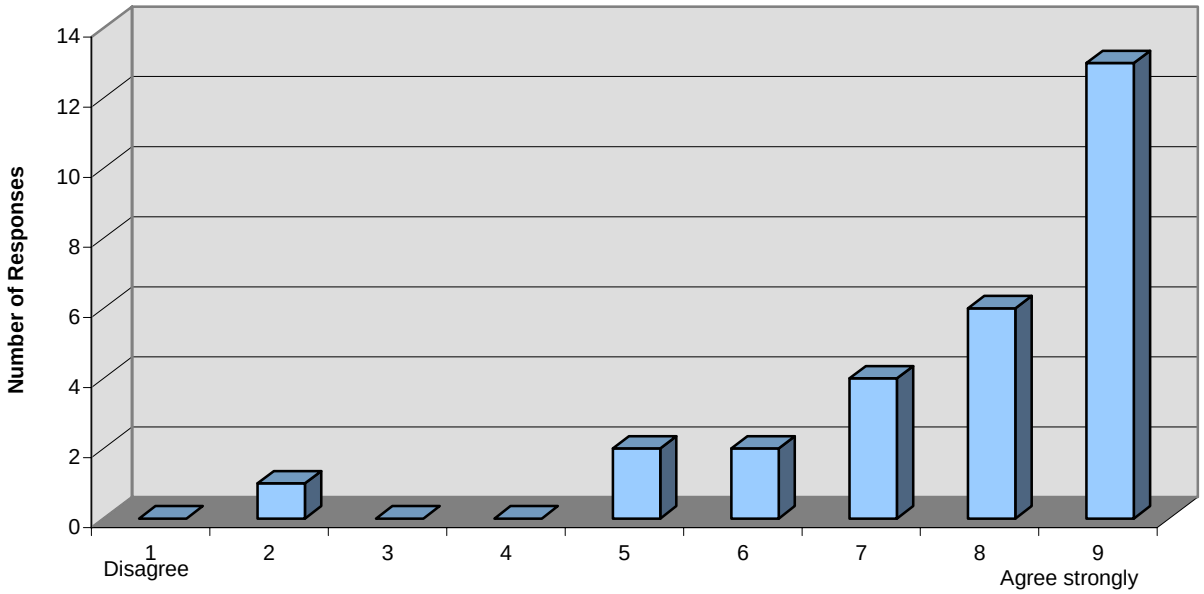
HEALING of CUTS and NICKS is improved?*



My skin is SMOOTHER?*



*Ratings from test persons aged 35 to 65 years after eight weeks of application.

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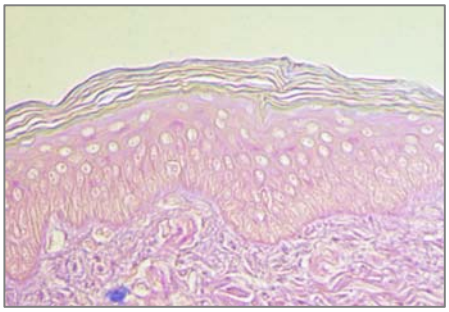
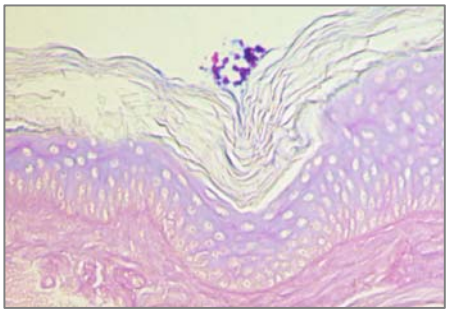
Reactivates the skin's own defense system



untreated skin



after application of
pro-ferm

Skin protection and reinforcement of the skin's defense mechanism through activation of the skin's immune system

The Glucaferm®-Hyaferm®-Composite – active agent complex of pro-ferm – can be recognized by its blue color. Remnants of the formulation are still visible on the skin surface.

The composite penetrates right into the cellular layers of the epidermis, the location of keratinocytes, Langerhans cells and macrophages (the intercellular matrix is stained blue).

This direct action on the most important cells of the skin's defense and repair system stimulates a sustainable skin regeneration and accelerates the natural healing process of the skin.

The retention of moisture in the outmost layer of the epidermis (stratum corneum) is clearly visible. This is due to the water binding property of Hyaferm®. The skin is firmer and moisturized.

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pro-ferm
deeper immunity to ageing

PRODUCT DATA SHEET
(Preliminary)

pro-ferm Self-Tanning Moisturizer

for a natural-looking tan: protective – moisturizing – anti-aging

EFFICIENCY

Anti-Aging Self-Tanning Lotion – for face and body

- Gives the skin a natural and even tan, which can be individually increased in intensity by repeated applications. – The self-tanning effect develops within 2-3 hours.
- Intensive moisturizing.
- Stimulates the skin's own defence and repair mechanisms.
- Provides powerful protection against free radicals and light-induced aging of the skin.
- Helps maintain the elasticity and tone of the skin.

ACTIVE INGREDIENTS

Dihydroxyacetone (DHA) – Self-tanning component 1
DHA is a sugar obtained from glycerine, which also occurs in the human metabolism. DHA binds to proteins and amino acids in the horny layer and thereby forms brown pigments (melanoids), which colour the upper horny layer brown. The active substance DHA does not penetrate into the deeper layers of the skin and is thus a good alternative to sun and solarium. Due to the natural peeling process of the skin, this skin coloration disappears again after a few days.

Erythrulose – Self-tanning component 2
The natural sugar erythrulose occurs in numerous plants and various types of lichens. It produces a brown coloration of the upper horny layer in the same way as the self-tanning substance DHA. In this case, however, the tanning effect first develops after about two days. On the other hand, it also tans much longer.

Glucaferm®
The natural immunostimulant protects and mobilizes the Langerhans cells of the epidermis, which reactivate the skin's defense and repair system. This direct action on the Langerhans cells stimulates an effective skin regeneration that strengthens the skin against the signs of aging. In addition, the skin is effectively protected against internal and external assaults as well as light-induced skin aging.

Hyaferm®
A natural carrier molecule consisting of hyaluronic acid, which transports the active ingredient Glucaferm® deep into the skin to the Langerhans cells. In addition, Hyaferm® is a very effective moisturizer that hydrates the skin and retains its elasticity.

pro-ferm
deeper immunity to ageing

ACTIVE INGREDIENTS	Hydrolyzed Algin Algae extract, membrane polysaccharide of brown algae consisting of Natural Moisturizing Factors (NMF), which are present in the human skin and are responsible for its natural moisture content. This moisture-binding active ingredient keeps the hydrolipid film of the skin intact, and thus protects the skin from moisture loss and the penetration of foreign substances. Chlorella Vulgaris Extract Protein-containing algae extract, rich in amino acids, protects collagen and elastin from enzymatic degeneration and helps maintain the elasticity and tone of the skin. Hemp Oil This skin-protective and calmative care oil excels through its high content of skin vitamin E and essential fatty acids. Anti-oxidative (protects against free radicals), provides moisture and is also anti-inflammatory. Furthermore, hemp oil balances low ceramide levels and thus diminishes the depth of wrinkles. Ceramides are found naturally in the double-lipid layer of the skin. Maris Aqua (Sea Water) Sea water provides the skin with minerals.
APPLICATION	Apply evenly to the cleaned and dry skin and allow absorption to take place. Also, properly distribute and rub in any residues left around the hairline and eyebrows. Wash hands thoroughly with soap and water after use.
NOTE	Not every kind of skin tans with self-tanning agents. They do not work in about 10 % of the population. The intensity of coloration depends upon the thickness of the horny layer. For this reason, apply sparingly to highly keratinised regions, such as the knee, elbows and heels, as they tan more intensively. Regular use ensures that the tanning effect will be revitalized and the color tone intensified.
TOLERABILITY	Dermatological tests will be performed after release of the samples. (There is no reason to doubt that the skin tolerability will be good.)
CONTENT	Bottle – 200 ml

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INGREDIENTS	Aqua [Water], Glycerin, Isohexadecane, Dihydroxyacetone, Caprylic/Capric Triglyceride, Cannabis Sativa Seed Oil, Cetearyl Alcohol, Hydrogenated Polyisobutene, Erythrulose, Butylene Glycol, Dimethicone, Sodium Citrate, Cetyl Alcohol, Glyceryl Stearate, PEG-75 Stearate, Betaglucan, Sodium Hyaluronate, Steareth-20, Ceteth-20, Xanthan Gum, Hydrolyzed Algin, Chlorella Vulgaris Extract, Maris Aqua [Sea Water], Methylparaben, Chlorphenesin, Phenoxyethanol, Limonene, Linalool, Butylphenyl Methylpropional, Hexyl Cinnamal. Benzyl Salicylate, Alpha-Isomethyl Ionone, Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde, Citronellol, Hydroxycitronellal, Benzyl Benzoate, Geraniol, Citral, Parfum [Fragrance].

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PRODUCT DATA SHEET

pro-ferm Body Lotion

Lifting – Smoothing – Moisturizing

EFFICIENCY

Lifting Body Lotion

- Exclusive Anti-Aging Body Lotion
- Intensive lifting properties, moisturizing and soothing (ideal for the sensitive skin)
- Reactivates the skin's own defense and repair mechanisms
- Provides effective protection against free radicals and light-related ageing of the skin.
- Lends the skin a smooth, silky and soft appearance

ACTIVE INGREDIENTS

Glucaferm®
The natural immunostimulant protects and mobilizes the Langerhans cells of the epidermis, which reactivate the skin's defense and repair system. This direct action on the Langerhans cells stimulates an effective skin regeneration that strengthens the skin against the signs of aging. In addition, the skin is effectively protected against internal and external assaults as well as light-induced skin aging.

Hyaferm®
A natural carrier molecule consisting of hyaluronic acid, which transports the active ingredient Glucaferm® deep into the skin to the Langerhans cells. In addition, Hyaferm® is a very effective moisturizer that hydrates the skin and retains its elasticity.

Fomes Officinalis (Mushroom) Extract
A botanic agent with moisturizing and astringent abilities. It augments skin's tone and firmness substantially.

Fig Extract
A lifting and moisturizing ingredient, rich in the anti-aging substances, vitamin A and beta-carotene.

Vitamin E
A skin vitamin that protects the skin from free radicals and the resulting oxidative ski aging process. Furthermore, vitamin E has cell regenerating and connective tissue repairing properties.

Vitamin C
An effective antioxidant that protects the skin from free radicals and lightens pigmented moles.

Bisabol
A skin soothing ingredient derived from the essential oil of chamomile that counteracts redness of the skin. It is especially suited for sensitive skin types.

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ACTIVE INGREDIENTS	Sweet almond oil A soft, smoothing and nourishing oil, rich in vitamins, minerals and essential unsaturated fatty acids
APPLICATION	Apply the body lotion generously to your skin after showering or bathing.
TOLERABILITY	Dermatologically tested. Also suited for sensitive skin.
CONTENTS	Bottle – 200 ml

INGREDIENTS

Aqua [Water], Hydrogenated Polydecene, Dicaprylyl Ether, Glycerin, Cocoglycerides, Fomes Officinalis (Mushroom) Extract, Butylene Glycol, Prunus Amygdalus Dulcis (Sweet Almond) Oil, Cetyl Alcohol, Glyceryl Stearate, Dimethicone, PEG-75 Stearate, Ficus Carica (Fig) Fruit Extract, Betaglucan, Bisabolol, Sodium Hyaluronate, Tocopheryl Acetate, Xanthan Gum, Ceteth-20, Steareth-20, Acrylates/Vinyl Isodecanoate Crosspolymer, Ethylhexylglycerin, Tocopherol, Ascorbyl Palmitate, Alcohol, Olus [Vegetable Oil], Phenoxyethanol, Methylparaben, Ethylparaben, Limonene, Linalool, Butylphenyl Methylpropional, Hexyl Cinnamal, Benzyl Salicylate, Alpha-Isomethyl Ionone, Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde, Citronellol, Hydroxycitronellal, Benzyl Benzoate, Geraniol, Citral, Eugenol, Parfum [Fragrance].

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pro-ferm+

internal skincare

pro-ferm+ is a pharmaco-nutritional support program for the skin. It is designed to be used in conjunction with the pro-ferm cosmetics range but can also be used as a stand-alone supplement. Its comprehensive formulation contains 8 groups of micro- and phyto-nutrients, which work together to support skin regeneration and renewal.

Actives in pro-ferm+

1. The trace elements zinc, copper, manganese and selenium are all co-factors for the antioxidant enzymes that are the body's first line of defence against damaging free radicals. Copper, zinc and manganese are also essential for the formation of the extra-cellular matrix which supports the skin.

2. Mixed carotenoids. These migrate into the skin where they protect it against UV radiation, and impart a delicate golden colouration.

3. B vitamins, essential for energy transformation in all body cells including skin cells.

4. The vitamin E group of compounds contribute valuable additional antioxidant support.

5. GLA, an essential precursor of the complex lipids that keep skin lubricated.

6. 8 essential amino acids, critical for the formation of proteins in the extra-cellular matrix.

7. Spirulina peptides that help regulate circulation in the skin and other tissues.

8. Phycocyanin, an extraordinary compound with anti-inflammatory and anti-cancer properties.

As most of these nutrients are well documented elsewhere, the following article focuses on the less familiar elements in pro-ferm+; mixed carotenoids and phycocyanin. There is also a brief reference to the spirulina peptides, which have not yet been intensively researched.

Mixed Carotenoids

Spirulina contains up to 4 g carotenoids per kilo of dried material, making it one of the richest sources of these valuable compounds. They include:

- Alpha-carotene -- traces
- Beta-carotene -- 1,700 mg/kg
- Xanthophylis -- 1,000 mg/kg
- Cryptoxanthin -- 556 mg/kg
- Echinenone -- 439 mg/kg
- Zeaxanthin -- 316 mg/kg
- Lutein -- 289 mg/kg

These compounds have a range of protective effects in the body including a significant degree of cancer risk reduction. They are well known to induce apoptosis and redifferentiation in a wide variety of cancer cell lines (1-5). In addition to this, they migrate into the skin where they act as UV absorbers and free radical quenchers, protecting against sunburn and sun-induced skin damage (6-9). Finally, the uniquely high levels of both lutein and zeaxanthin make this probably the best single nutritional eye protector yet discovered (10).

Phycocyanin

This bright blue pigment, which is only revealed once the green chlorophyll content of Spirulina has been removed, has recently been shown to have a variety of positive actions in the body. These include strong antioxidant (11), anti-inflammatory (12-15) and anti-cancer effects (12, 16-17).

Whole Spirulina

As spirulina contains carotenoids and phycocyanin which both have chemo-protective properties, one would expect whole spirulina extracts to show similar if not enhanced risk reduction. This is exactly what other researchers have found (18-22). Perhaps the most interesting of these reports was an intervention study in which spirulina extracts were given to patients with oral leukoplakia, a precancerous condition. In this study (23), the extract induced regression of the pre-cancerous lesion in a significant number of cases.

Spirulina's anti-allergy effects have been documented in patients with allergic rhinitis (14); and its anti-viral activity has been shown in vitro, although not in vivo (24-28). Finally, spirulina has also been shown to be an excellent source of iron, especially important in women of child-bearing age (29-30); and to act as a vasco-modulator, beneficially impacting on blood pressure and on peripheral resistance vessels (31).

Overview – the relevance pro-ferm+ to Skin

The various actives in spirulina combine to act on the skin in a number of different but mutually enhancing ways. These include the up-regulation of the skin's antioxidant and UV-absorbing defences, the down-regulation of inflammation, improved circulation and improved anti-cancer defences. The other actives in **pro-ferm+** confer additional antioxidant capacity, and improved cellular energetics. The overall effect is an improvement in skin tone and texture, particularly in those whose life-style combines excessive stress with less than perfect nutrition.

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Laboratory of Viral Pathogenesis, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA; (2) Earthrise Farms, Calipatria, CA; (3) Division of Newborn Medicine, Department of Pediatrics, Tufts University, Boston, MA, USA. 7th IAAA Conference, Knysna, South Africa April 17, 1996.

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SELECTED ABSTRACTS

Reddy MC, Subhashini J, Mahipal SV, Bhat VB, Srinivas Reddy P, Kiranmai G, Madyastha KM, Reddanna P. **C-Phycocyanin, a selective cyclooxygenase-2 inhibitor, induces apoptosis in lipopolysaccharide-stimulated RAW 264.7 macrophages**. Biochem Biophys Res Commun. 2003 May 2;304(2):385-92.

C-Phycocyanin (C-PC) is one of the major biliproteins of Spirulina platensis, a blue green algae, with antioxidant and radical scavenging properties. It is also known to exhibit anti-inflammatory and anti-cancer properties. However, the mechanism of action of C-PC is not clearly understood. Previously, we have shown that C-PC selectively inhibits cyclooxygenase-2 (COX-2), an inducible isoform that is upregulated during inflammation and cancer. In view of the reported induction of apoptosis in cancer cells by cyclooxygenase-2 inhibitors, the present study is undertaken to test the effect of C-PC on LPS stimulated RAW 264.7 mouse macrophage cell line. These studies have shown a dose dependent reduction in the growth and multiplication of macrophage cell line by C-PC. This decrease in cell number appears to be mediated by C-PC induced apoptosis as evidenced by flow cytometric and confocal microscopic studies. Cells treated with 20 micro M C-PC showed typical nuclear condensation and 16.6% of cells in sub-G(o)/G(1) phase. These cells also showed DNA fragmentation in a

dose dependent manner. The studies on poly(ADP ribose) polymerase (PARP) cleavage showed typical fragmentation pattern in C-PC treated cells. This C-PC induced apoptosis in RAW 264.7 cells appears to be mediated by the release of cytochrome c from mitochondria and independent of Bcl-2 expression. These effects of C-PC on RAW 264.7 cells may be due to reduced PGE(2) levels as a result of COX-2 inhibition.

Reddy CM, Bhat VB, Kiranmai G, Reddy MN, Reddanna P, Madyastha KM. **Selective inhibition of cyclooxygenase-2 by C-phycocyanin, a biliprotein from Spirulina platensis.** Biochem Biophys Res Commun. 2000 Nov 2;277(3):599-603.

We report data from two related assay systems (isolated enzyme assays and whole blood assays) that C-phycocyanin a biliprotein from Spirulina platensis is a selective inhibitor of cyclooxygenase-2 (COX-2) with a very low IC(50) COX-2/IC(50) COX-1 ratio (0.04). The extent of inhibition depends on the period of preincubation of phycocyanin with COX-2, but without any effect on the period of preincubation with COX-1. The IC(50) value obtained for the inhibition of COX-2 by phycocyanin is much lower (180 nM) as compared to those of celecoxib (255 nM) and rofecoxib (401 nM), the well-known selective COX-2 inhibitors. In the human whole blood assay, phycocyanin very efficiently inhibited COX-2 with an IC(50) value of 80 nM. Reduced phycocyanin and phycocyanobilin, the chromophore of phycocyanin are poor inhibitors of COX-2 without COX-2 selectivity. This suggests that apoprotein in phycocyanin plays a key role in the selective inhibition of COX-2. The present study points out that the hepatoprotective, anti-inflammatory, and anti-arthritic properties of phycocyanin reported in the literature may be due, in part, to its selective COX-2 inhibitory property, although its ability to efficiently scavenge free radicals and effectively inhibit lipid peroxidation may also be involved. Copyright 2000 Academic Press.

Mao TK, Van de Water J, Gershwin ME**. Effects of a Spirulina-based dietary supplement on cytokine production from allergic rhinitis patients**. J Med Food. 2005 Spring;8(1):27-30.

Spirulina represents a blue-green alga that is widely produced and commercialized as a dietary supplement for modulating immune functions, as well as ameliorating a variety of diseases. We have previously shown that the in vitro culture of Spirulina with human peripheral blood mononuclear cells (PBMCs) modulated the production of cytokines. In the present study, we evaluated the impact of a Spirulina-based dietary supplement (Earthrise Nutritionals, Inc., Irvine, CA) on patients with allergic rhinitis by assessing the production of cytokines [interleukin (IL)-4, interferon (IFN)-gamma, and IL-2] critical in regulating immunoglobulin E-mediated allergy. In a randomized double-blinded crossover study versus placebo, allergic individuals were fed daily with either placebo or Spirulina, at 1,000 mg or 2,000 mg, for 12 weeks. PBMCs isolated before and after the Spirulina feeding were stimulated with phytohemagglutinin (PHA) prior to determining the levels of cytokine from cell culture supernatants. Although Spirulina seemed to be ineffective at modulating the secretion of Th1 cytokines (IFN-gamma and IL-2), we discovered that Spirulina, administered at 2,000 mg/day, significantly reduced IL-4 levels by 32% from PHA-stimulated cells. These results indicate that Spirulina can modulate the Th profile in patients with allergic rhinitis by suppressing the differentiation of Th2 cells mediated, in part, by inhibiting the production of IL-4. To our knowledge, this is the first human feeding study that demonstrates the protective effects of Spirulina towards allergic rhinitis.

Remirez D, Ledon N, Gonzalez R. **Role of histamine in the inhibitory effects of phycocyanin in experimental models of allergic inflammatory response**. Mediators

Inflamm. 2002 Apr;11(2):81-5.

It has recently been reported that phycocyanin, a biliprotein found in the blue-green microalgae Spirulina, exerts anti-inflammatory effects in some animal models of inflammation. Taking into account these findings, we decided to elucidate whether phycocyanin might exert also inhibitory effects in the induced allergic inflammatory response and on histamine release from isolated rat mast cells. In in vivo experiments, phycocyanin (100, 200 and 300mg/kg post-orally (p.o.)) was administered 1 h before the challenge with 1 microg of ovalbumin (OA) in the ear of mice previously sensitized with OA. One hour later, myeloperoxidase activity and ear edema were assessed. Phycocyanin significantly reduced both parameters. In separate experiments, phycocyanin (100 and 200 mg/kg p.o.) also reduced the blue spot area induced by intradermal injections of histamine, and the histamine releaser compound 48/80 in rat skin. In concordance with the former results, phycocyanin also significantly reduced histamine release induced by compound 48/80 from isolated peritoneal rat mast cells. The inhibitory effects of phycocyanin were dose dependent. Taken together, our results suggest that inhibition of allergic inflammatory response by phycocyanin is mediated, at least in part, by inhibition of histamine release from mast cells.

Bhat VB, Madyastha KM. **C-phycocyanin: a potent peroxyl radical scavenger in vivo and in vitro.** Biochem Biophys Res Commun. 2000 Aug 18;275(1):20-5.

C-Phycocyanin (from Spirulina platensis) effectively inhibited CCl(4)-induced lipid peroxidation in rat liver in vivo. Both native and reduced phycocyanin significantly inhibited peroxyl radical-induced lipid peroxidation in rat liver microsomes and the inhibition was concentration dependent with an IC(50) of 11.35 and 12.7 microM, respectively. The radical scavenging property of phycocyanin was established by studying its reactivity with peroxyl and hydroxyl radicals and also by competition kinetics of crocin bleaching. These studies have demonstrated that phycocyanin is a potent peroxyl radical scavenger with an IC(50) of 5.0 microM and the rate constant ratios obtained for phycocyanin and uric acid (a known peroxyl radical scavenger) were 1.54 and 3.5, respectively. These studies clearly suggest that the covalently linked chromophore, phycocyanobilin, is involved in the antioxidant and radical scavenging activity of phycocyanin.

Mathew B, Sankaranarayanan R, Nair PP, Varghese C, Somanathan T, Amma BP, Amma NS, Nair MK. **Evaluation of chemoprevention of oral cancer with Spirulina fusiformis**. Nutr Cancer. 1995;24(2):197-202.

The blue-green microalgae Spirulina, used in daily diets of natives in Africa and America, have been found to be a rich natural source of proteins, carotenoids, and other micronutrients. Experimental studies in animal models have demonstrated an inhibitory effect of Spirulina algae on oral carcinogenesis. Studies among preschool children in India have demonstrated Spirulina fusiformis (SF) to be an effective source of dietary vitamin A. We evaluated the chemopreventive activity of SF (1 g/day for 12 mos) in reversing oral leukoplakia in pan tobacco chewers in Kerala, India. Complete regression of lesions was observed in 20 of 44 (45%) evaluable subjects supplemented with SF, as opposed to 3 of 43 (7%) in the placebo arm ($p < 0.0001$). When stratified by type of leukoplakia, the response was more pronounced in homogeneous lesions: complete regression was seen in 16 of 28 (57%) subjects with homogeneous leukoplakia, 2 of 8 with erythroplakia, 2 of 4 with verrucous leukoplakia, and 0 of 4 with ulcerated and nodular lesions. Within one year of discontinuing supplements, 9 of 20 (45%) complete responders with SF developed recurrent lesions. Supplementation with SF did

not result in increased serum concentration of retinol or beta-carotene, nor was it associated with toxicity. This is the first human study evaluating the chemopreventive potential of SF. More studies in different settings and different populations are needed for further evaluation.

Dasgupta T, Banejee S, Yadav PK, Rao AR. **Chemomodulation of carcinogen metabolising enzymes, antioxidant profiles and skin and forestomach papillomagenesis by Spirulina platensis**. Mol Cell Biochem. 2001 Oct;226(1-2):27-38.

Numerous reports have revealed an inverse association between consumption of some selective natural products and risk of developing cancer. In the present study the effect of 250 and 500 mg/kg body wt. of Spirulina was examined on drug metabolising phase I and phase II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase and lipid peroxidation in the liver of 7-week-old Swiss albino mice. The implications of these biochemical alterations have been further evaluated adopting the protocol of benzo(a)pyrene induced forestomach and 7,12 dimethylbenz(a)anthracene (DMBA) initiated and croton oil promoted skin papillomagenesis. Our primary findings reveal the 'Monofunctional' nature of Spirulina as deduced from its potential to induce only the phase II enzyme activities associated mainly with carcinogen detoxification. The glutathione S-transferase and DT-diaphorase specific activities were induced in hepatic and all the extrahepatic organs examined (lung, kidney and forestomach) by Spirulina pretreatment (significance level being from $p < 0.05$ to $p < 0.005$) except for the low dose treatment in forestomach. With reference to antioxidant enzymes viz., superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and reduced glutathione were increased significantly by both the chosen doses of Spirulina from $p < 0.01$ to $p < 0.005$. Chemopreventive response was quantitated by the average number of papillomas per effective mouse (tumor burden) as well as percentage of tumor bearing animals. There was a significant inhibition of tumor burden as well as tumor incidence in both the tumor model systems studied. In the skin tumor studies tumor burden was reduced from 4.86 to 1.20 and 1.15 by the low and high dose treatment respectively. In stomach tumor studies tumor burden was 2.05 and 1.73 by the low and high doses of Spirulina treatment against 3.73 that of control.

Wu LC, Ho JA, Shieh MC, Lu IW. **Antioxidant and antiproliferative activities of Spirulina and Chlorella water extracts**. J Agric Food Chem. 2005 May 18;53(10):4207-12.

Liver fibrosis is a chronic liver disease that will further develop to cirrhosis if severe damage continues to form. A potential treatment for liver fibrosis is to inhibit activated hepatic stellate cell (HSC) proliferation and, subsequently, to induce HSC apoptosis. It has been reported that antioxidants are able to inhibit the proliferation of HSCs. In this study, the aqueous extract of spirulina was chosen as the source of antioxidant to investigate the inhibitory effect on the proliferation of HSC. The growth inhibitory effects of aqueous spirulina and chlorella extract on human liver cancer cells, HepG2, were also studied and compared in pairs. Results indicated that the total phenol content of spirulina was almost five times greater than that of chlorella (6.86 +/- 0.58 vs 1.44 +/- 0.04 mg tannic acid equivalent/g of algae powder, respectively). The antioxidant activity of spirulina determined by the ABTS*+ method was higher than chlorella (EC50: 72.44 +/- 0.24 micromol of trolox equivalent/g of spirulina extract vs 56.09 +/- 1.99 micromol of trolox equivalent/g of chlorella extract). Results of DPPH* assay also showed a similar trend as the ABTS*+ assay (EC50: 19.39 +/- 0.65 micromol of ascorbic acid equivalent/g of spirulina extract vs 14.04 +/- 1.06 micromol of ascorbic acid equivalent/g of chlorella extract). The aqueous extracts of these two algae both showed antiproliferative effects on HSC and HepG2, but spirulina was a stronger inhibitor than chlorella. Annexin-V staining showed that aqueous extract of spirulina induced apoptosis of

HSC after 12 h of treatment. In addition, the aqueous extract of spirulina triggered a cell cycle arrest of HSC at the G2/M phase.

Subhashini J, Mahipal SV, Reddy MC, Mallikarjuna Reddy M, Rachamallu A, Reddanna P. **Molecular mechanisms in C-Phycocyanin induced apoptosis in human chronic myeloid leukemia cell line-K562**. Biochem Pharmacol. 2004 Aug 1;68(3):453-62.

C-Phycocyanin (C-PC), the major light harvesting biliprotein from Spirulina platensis is of greater importance because of its various biological and pharmacological properties. It is a water soluble, non-toxic fluorescent protein pigment with potent anti-oxidant, anti-inflammatory and anti-cancer properties. In the present study the effect of highly purified C-PC was tested on growth and multiplication of human chronic myeloid leukemia cell line (K562). The results indicate significant decrease (49%) in the proliferation of K562 cells treated with 50 microM C-PC up to 48 h. Further studies involving fluorescence and electron microscope revealed characteristic apoptotic features like cell shrinkage, membrane blebbing and nuclear condensation. Agarose electrophoresis of genomic DNA of cells treated with C-PC showed fragmentation pattern typical for apoptotic cells. Flow cytometric analysis of cells treated with 25 and 50 microM C-PC for 48 h showed 14.11 and 20.93% cells in sub-G0/G1 phase, respectively. C-PC treatment of K562 cells also resulted in release of cytochrome c into the cytosol and poly(ADP) ribose polymerase (PARP) cleavage. These studies also showed down regulation of anti-apoptotic Bcl-2 but without any changes in pro-apoptotic Bax and thereby tilting the Bcl-2/Bax ratio towards apoptosis. These effects of C-PC appear to be mediated through entry of C-PC into the cytosol by an unknown mechanism. The present study thus demonstrates that C-PC induces apoptosis in K562 cells by cytochrome c release from mitochondria into the cytosol, PARP cleavage and down regulation of Bcl-2.

Schwartz J, Shklar G. **Regression of experimental hamster cancer by beta carotene and algae extracts.** J Oral Maxillofac Surg. 1987 Jun;45(6):510-5.

The effect of algae extract on tumor regression was studied. Phycotene (extract of Spirulina and Dunaliella algae) 250 micrograms in 0.1 ml MEM (minimum essential medium) was injected locally into DMBA (7, 12 dimethylbenz(a)anthracene)-induced squamous cell carcinomas of hamster buccal pouch in 20 animals. DMBA-induced carcinomas in 20 hamsters were injected locally with beta carotene 250 micrograms in 0.1 ml MEM; DMBA-induced carcinomas in 20 animals were injected locally with canthaxanthin, 250 micrograms in 0.1 ml MEM, and DMBA-induced carcinomas in 20 animals were injected locally with 13-cis-retinoic acid, 250 micrograms in 0.1 ml MEM. Twenty animals with DMBA-induced carcinomas were sham-injected controls using 0.1 ml MEM. The various agents were injected into the tumor bearing right buccal pouches twice-weekly for four weeks. Total tumor regression was found in 30% of phycotene animals, 20% of beta carotene animals and 15% of canthaxanthin animals after four weeks. Partial tumor regression was found in the remaining 70% of phycotene animals, 80% of beta carotene animals and 85% of canthaxanthin animals. None of the 13-cis-retinoic acid animals had total tumor regression, but 70% showed partial regression. No tumor regression was found in the DMBA control group and the sham-injected group.

Schwartz J, Shklar G, Reid S, Trickler D**. Prevention of experimental oral cancer by extracts of Spirulina-Dunaliella algae.** Nutr Cancer. 1988;11(2):127-34.

An extract of Spirulina-Dunaliella algae was shown to prevent tumor development in hamster buccal pouch when a 0.1% solution of 7,12-dimethylbenz[a]anthracene (DMBA) in mineral oil

was applied topically three times weekly for 28 weeks. The algae extract was delivered by mouth in continued dosages of 140 micrograms in 0.4 ml mineral oil three times per week. After 28 weeks, the animals given vehicle and untreated controls all presented gross tumors of the right buccal pouch. Animals fed canthaxanthin presented a notably and statistically significant reduction in tumor number and size compared with controls. Animals fed beta-carotene demonstrated a smaller but statistically significant reduction in tumor number and size. The algae animals presented a complete absence of gross tumors. However, microscopic sections of the buccal pouch in the algae group showed localized areas of dysplasia and early carcinoma-in-situ undergoing destruction.

Shklar G, Schwartz J. **Tumor necrosis factor in experimental cancer regression with alphatocopherol, beta-carotene, canthaxanthin and algae extract**. Eur J Cancer Clin Oncol. 1988 May;24(5):839-50.

Regression of established hamster buccal pouch carcinoma has recently been demonstrated in association with an induction of tumor necrosis factor alpha in macrophages. Regression of hamster buccal pouch tumors has also been demonstrated following the local injection of alphatocopherol, canthaxanthin and an extract of Spirulina-Dunaliella algae. The current study demonstrates that cancer regression is also accompanied by a significant induction of tumor necrosis factor in macrophages in the tumor area, suggesting a possible mechanism of tumor destruction. One hundred and forty young, male adult hamsters were divided into seven equal groups of 20 animals. Epidermoid carcinomas were induced in right buccal pouches by 14 weeks of painting, three times per week, of a 0.5% solution of 7,12-dimethylbenz(a)anthracene. Groups 1 and 2 were untreated and sham injected controls. Groups 3-7 had injected twice weekly into the right buccal pouches 0.1 ml (1.9 mg/ml of 13-cis-retinoic acid, canthaxanthin, algae extract, beta-carotene and alphatocopherol. After 4 weeks the tumors in groups 3-7 demonstrated varying degrees of regression and the animals were sacrificed and the right buccal pouches excised. Tumor necrosis factor alpha (TNF-alpha) was demonstrated by immunohistochemical techniques. A very significant increase in TNF-alpha positive macrophages was found in the tumor-bearing pouches of animals in groups 5-7. Smaller numbers of TNF-alpha-positive macrophages were found in group 4 pouches and a very slight increase in group 3 pouches.

Pardhasaradhi BV, Ali AM, Kumari AL, Reddanna P, Khar A. **Phycocyanin-mediated apoptosis in AK-5 tumor cells involves down-regulation of Bcl-2 and generation of ROS.** Mol Cancer Ther. 2003 Nov;2(11):1165-70.

C-phycocyanin, which is a major biliprotein of the blue-green algae, has been shown to possess cyclooxygenase-2 inhibitory activity. We have studied the effect of phycocyanin on a rat histiocytic tumor line. AK-5 cells are induced into apoptotic death program when treated with phycocyanin, which involves the activation of caspase-3. Phycocyanin-mediated apoptotic death is induced through the generation of reactive oxygen radicals. Free radical scavengers inhibited phycocyanin-induced apoptotic death in AK-5 cells. Bcl-2, an inhibitor of apoptosis, is shown to regulate ROS generation. Bcl-2 gene-transfected AK-5 cells are resistant to phycocyanin-induced death. Overexpression of Bcl-2 inhibited the production of ROS in phycocyanin-treated AK-5 cells. Thus, our observations demonstrate phycocyanin-induced apoptotic death in AK-5 cells, which is inhibited by Bcl-2 expression through the regulation of free radical generation. Phycocyanin, a natural product, could therefore be a possible chemotherapeutic agent through its apoptotic activity against tumor cells.

Subhashini J, Mahipal SV, Reddy MC, Mallikarjuna Reddy M, Rachamallu A, Reddanna P. **Molecular mechanisms in C-Phycocyanin induced apoptosis in human chronic myeloid leukemia cell line-K562**. Biochem Pharmacol. 2004 Aug 1;68(3):453-62.

C-Phycocyanin (C-PC), the major light harvesting biliprotein from Spirulina platensis is of greater importance because of its various biological and pharmacological properties. It is a water soluble, non-toxic fluorescent protein pigment with potent anti-oxidant, anti-inflammatory and anti-cancer properties. In the present study the effect of highly purified C-PC was tested on growth and multiplication of human chronic myeloid leukemia cell line (K562). The results indicate significant decrease (49%) in the proliferation of K562 cells treated with 50 microM C-PC up to 48 h. Further studies involving fluorescence and electron microscope revealed characteristic apoptotic features like cell shrinkage, membrane blebbing and nuclear condensation. Agarose electrophoresis of genomic DNA of cells treated with C-PC showed fragmentation pattern typical for apoptotic cells. Flow cytometric analysis of cells treated with 25 and 50 microM C-PC for 48 h showed 14.11 and 20.93% cells in sub-G0/G1 phase, respectively. C-PC treatment of K562 cells also resulted in release of cytochrome c into the cytosol and poly(ADP) ribose polymerase (PARP) cleavage. These studies also showed down regulation of anti-apoptotic Bcl-2 but without any changes in pro-apoptotic Bax and thereby tilting the Bcl-2/Bax ratio towards apoptosis. These effects of C-PC appear to be mediated through entry of C-PC into the cytosol by an unknown mechanism. The present study thus demonstrates that C-PC induces apoptosis in K562 cells by cytochrome c release from mitochondria into the cytosol, PARP cleavage and down regulation of Bcl-2.

Shih SR, Tsai KN, Li YS, Chueh CC, Chan EC. **Inhibition of enterovirus 71-induced apoptosis by allophycocyanin isolated from a blue-green alga Spirulina platensis**. J Med Virol. 2003 May;70(1):119-25.

Enterovirus 71 infection causes significant morbidity and mortality in children, yet there is no effective treatment. In this study, a protein-bound pigment, allophycocyanin purified from blue-green algae is first reported to exhibit anti-enterovirus 71 activity. Allophycocyanin neutralized the enterovirus 71-induced cytopathic effect in both human rhabdomyosarcoma cells and African green monkey kidney cells. The 50% inhibitory concentration of allophycocyanin for neutralizing the enterovirus 71-induced cytopathic effect was approximately 0.045 +/- 0.012 microM in green monkey kidney cells. The cytotoxic concentrations of allophycocyanin for rhabdomyosarcoma cells and African green monkey kidney cells were 1.653 +/- 0.003 microM and 1.521 +/- 0.012 microM, respectively. A plaque reduction assay showed that the concentrations of allophycocyanin for reducing plaque formation by 50% were approximately 0.056 +/- 0.007 microM and 0.101 +/- 0.032 microM, when allophycocyanin were added at the state of viral adsorption and post-adsorption, respectively. Antiviral activity was more efficient in cultures treated with allophycocyanin before viral infection compared with that in the cultures treated after infection. Allophycocyanin was also able to delay viral RNA synthesis in the infected cells and to abate the apoptotic process in enterovirus 71-infected rhabdomyosarcoma cells with evidence of characteristic DNA fragmentation, decreasing membrane damage and declining cell sub-G1 phase. It is concluded that allophycocyanin possesses antiviral activity and has a potential for development as an anti-enterovirus 71 agent. Copyright 2003 Wiley-Liss, Inc.

Inhibition of HIV-1 replication by an aqueous extract of Spirulina (arthrospira platensis). by Ayehunie S. (1), Belay A. (2), Hu Y. (1), Baba T. (1,3), Ruprecht R. (1). (1) Laboratory of Viral Pathogenesis, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA,

USA; (2) Earthrise Farms, Calipatria, CA; (3) Division of Newborn Medicine, Department of Pediatrics, Tufts University, Boston, MA, USA. 7th IAAA Conference, Knysna, South Africa April 17, 1996.

Water extract of Spirulina platensis (Arthrospira platensis) inhibits HIV-1 replication in human derived T-cell lines and in human peripheral blood mononuclear cells. A concentration of 5-10 (mcg/ml was found to reduce viral production and/or syncytium formation of about 50%, and a concentration of 100 (mcg/ml showed a 90-100% inhibition without cytotoxicity. The 50% inhibitory concentration (IC50) for cell growth was computed to be between 2-6.5 mg/ml depending on the cell types used; the therapeutic index was >100. The extract also blocked Rauscher murine leukemia virus (RLV)-induced plaques by >95% at concentrations ranging from 75-150 (mcg/ml; the 50% reduction in plaque formation (the 50% effective concentration EC50) was at a concentration of 9-30 (mcg/ml. The extract directly inactivated HIV-1 infectivity when preincubated with virus prior to addition to human T-cell lines at similar inhibitory concentrations.

Hayashi T, Hayashi K, Maeda M, Kojima I.**Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga Spirulina platensis**. J Nat Prod. 1996 Jan;59(1):83-7.

Bioactivity-directed fractionation of a hot H2O extract from a blue-green alga Spirulina platensis led to the isolation of a novel sulfated polysaccharide named calcium spirulan (Ca-SP) as an antiviral principle. This polysaccharide was composed of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, sulfate, and calcium. Ca-SP was found to inhibit the replication of several enveloped viruses, including Herpes simplex virus type 1, human cytomegalovirus, measles virus, mumps virus, influenza A virus, and HIV-1. It was revealed that Ca-SP selectively inhibited the penetration of virus into host cells. Retention of molecular conformation by chelation of calcium ion with sulfate groups was suggested to be indispensable to its antiviral effect.

Hayashi K, Hayashi T, Kojima I. **An extract from spirulina is a selective inhibitor of herpes simplex virus Type 1 Penetration into HeLa Cells.** 1993. Phytotherapy Research, Vol. 7. 76-80. Japan.

The water soluble extract of spirulina achieved a dose-dependent inhibition of the replication of herpes simplex virus type 1 (HSV-1) in HeLa cells within the concentration range of 0.08-50 mg/mL. This extract proved to have no virucidal activity and did not interfere with adsorption to host cells. However, the extract affected viral penetration in a dose-dependent manner. At 1 mg/ml the extract was found to inhibit virus-specific protein synthesis without suppressing host cell protein synthesis if added to the cells 3 hours before hamsters at doses of 100 and 500 mg/kg per day.

Hayashi K, Hayashi T, Kojima I. **A natural sulfated polysaccharide, calcium spirulan, isolated from Spirulina platensis: in vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities.** AIDS Res Hum Retroviruses. 1996 Oct 10;12(15):1463-71.

A sulfated polysaccharide named calcium spirulan (Ca-SP) has been isolated from a sea alga, Spirulina platensis, as an antiviral component. The anti-human immunodeficiency virus type 1 (HIV-1) and anti-herpes simplex virus type 1 (HSV-1) activities of Ca-SP were compared with

those of dextran sulfate (DS) as a representative sulfated polysaccharide. Anti-HIV-1 activities of these agents were measured by three different assays: viability of acutely infected CD4-positive cells, or a cytopathology assay; determination of HIV-1 p24 antigen released into culture supernatants; and inhibition of HIV-induced syncytium formation. Anti-HSV-1 activity was assessed by plaque yield reduction. In addition, their effects on the blood coagulation processes and stability in the blood were evaluated. These data indicate that Ca-SP is a potent antiviral agent against both HIV-1 and HSV-1. Furthermore, Ca-SP is quite promising as an anti-HIV agent because even at low concentrations of Ca-SP an enhancement of virus-induced syncytium formation was not observed, as was observed in DS-treated cultures, Ca-SP had very low anticoagulant activity, and showed a much longer half-life in the blood of mice when compared with that of DS. Thus, Ca-SP can be a candidate agent for an anti-HIV therapeutic drug that might overcome the disadvantages observed in many sulfated polysaccharides. When the role of chelation of calcium ion with sulfate groups was examined by removing calcium or its replacement by sodium, the presence of calcium ion in the molecule was shown to be essential for the dose-dependent inhibition of cytopathic effect and syncytium formation induced by HIV-1.

Puyfoulhoux G, Rouanet JM, Besancon P, Baroux B, Baccou JC, Caporiccio B**. Iron availability from iron-fortified spirulina by an in vitro digestion/Caco-2 cell culture model**. J Agric Food Chem. 2001 Mar;49(3):1625-9.

Iron deficiency, one of the most important nutritional problems in the world, can be caused not only by foods deficient in iron but also by poor availability of dietary iron. Iron food fortification in combination with highly available iron from supplements could effectively reduce this deficiency. The aim of this study was to examine the iron availability from iron-fortified spirulina. We have used an in vitro digestion/Caco-2 cell culture system to measure iron spirulina availability and made a comparison with those of beef, yeast, wheat floor, and iron sulfate plus ascorbic acid as a reference. Iron availability was assessed by ferritin formation in Caco-2 cells exposed to digests containing the same amount of iron. Our results demonstrate a 27% higher ferritin formation from beef and spirulina digests than from digests of yeast and wheat flour. When iron availability was expressed per microgram of iron used in each digest, a 6.5-fold increase appeared using spirulina digest in comparison with meat. In view of this observed high iron availability from spirulina, we conclude that spirulina could represent an adequate source of iron.

Kapoor R, Mehta U. **Iron status and growth of rats fed different dietary iron sources**. Plant Foods Hum Nutr. 1993 Jul;44(1):29-34.

The present study was carried out to investigate the availability of iron from spirulina, whole wheat, whole egg and standard ferrous sulphate in terms of haemoglobin formation, serum and tissue iron levels. Male albino Wistar rats were first depleted of iron by giving low-iron diet (9 ppm) and bleeding 1-2 ml blood at weekly intervals for a period of 21 days. The anaemic rats were repleted with iron sources at a level of 35 ppm for 21 days. Rats receiving whole egg gained significantly ($p < 0.01$) higher weight than the rest of the three groups. The increase in haemoglobin was significantly higher with ferrous sulphate than with whole wheat ($p < 0.05$), spirulina and whole egg ($p < 0.01$). Feeding of ferrous sulphate, whole egg and spirulina produced significantly higher tissue iron levels than feeding of whole wheat. Thus, availability of iron from spirulina and whole egg were found to be comparable to that of the standard.

Suetsuna K, Chen JR**. Identification of antihypertensive peptides from peptic digest of two microalgae, Chlorella vulgaris and Spirulina platensis**. Mar Biotechnol (NY). 2001 Jul;3(4):305-9.

The peptidic fractions that inhibited angiotensin I-converting enzyme (ACE) were separated from the peptic digests of 2 microalgae, Chlorella vulgaris and Spirulina platensis, by ion exchange chromatography and gel filtration. Oral administration of peptidic fractions into spontaneously hypertensive rats at 200 mg/kg of body weight resulted in marked antihypertensive effects. Further separation of the peptidic fractions by ODS high-performance liquid chromatography furnished the following active peptides: Ile-Val-Val-Glu (inhibitory against ACE with an IC(50) of 315.3 microM), Ala-Phe-Leu (63.8 microM), Phe-Ala-Leu (26.3 microM), Ala-Glu-Leu (57.1 microM), and Val-Val-Pro-Pro-Ala (79.5 microM) from C. vulgaris; Ile-Ala-Glu (34.7 microM), Phe-Ala-Leu, Ala-Glu-Leu, Ile-Ala-Pro-Gly (11.4 microM), and Val-Ala-Phe (35.8 microM) from S. platensis.

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