Saberry™

Multifaceted Antioxidant

SABINSA CORPORATION

OUR INNOVATION IS YOUR ANSWER®

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Saberry™

ORAC Dense Phytonutrient™

Product Write Up

Saberry™ is a proprietary, patent pending, extract of fruits of *Emblica officinalis* Gaertn. (synonymous with *Phyllanthus emblica*) (Euphorbiaceae), more commonly known as, Indian Gooseberry. The fruits of *Emblica officinalis* Gaertn. known in India as *Amla* (Sanskrit name *Amalaki*), are consumed as fruit, or in the form of food products. In the Ayurvedic tradition, Amalaki is regarded as "one of the best rejuvenating herbs."

Saberry™ from Sabinsa Corporation is standardized to contain a minimum of 10% β-Glucogallin and 50% Gallates.[1] Conventionally, Amla extracts used in dietary supplements were standardized using ascorbic acid as the biomarker. However, recent research has revealed the fact that Amla does not contain ascorbic acid in consistent amounts, and sometimes, only in trace quantities, rendering its validity as a biomarker, questionable. Saberry™ is the result of efforts to prepare an authenticated Amla extract, standardized using a valid biomarker, β-glucogallin. In-house studies revealed that β-glucogallin is a more powerful antioxidant molecule, as compared to Ascorbic acid.

Saberry™ is a light colored powder and is processed from carefully chosen, fresh Indian gooseberries by a novel extraction technology, to retain the natural goodness of the fruits.

Amla Fruits

Saberry™

AMLA IN THE AYURVEDIC TRADITION:

Amla fruits are regarded as an adaptogen.[2] In 1947, Nikolai Lazarev defined an adaptogen as an agent that allows the body to counter adverse physical, chemical, or biological stressors by raising nonspecific resistance toward such stress, thus allowing the organism to "adapt" to stressful circumstances. The term "adaptogen" is used by herbalists to refer to a natural product that potentially increases the body's resistance to stress, trauma, anxiety and fatigue. In traditional systems of medicine, such herbs have been called rejuvenating herbs, qi tonics, rasayanas, or restoratives. According to Ayurvedic texts, "rasayana" is generally used to rejuvenate the general health of the body, or aims at enabling the body's maximum potential.

Amla is considered to be a "rasayana" herb in Ayurveda. In India, the fruit is pickled with salt, oil, and spices, and also converted into jams and preserves. It is used as a primary ingredient in the Ayurvedic rasayana tonic "Chyawanprash", and in the herbal composition "Triphala" where it is mixed with chebulic and belleric myrobalans. The Charaka Samhita, the main text of Ayurvedic herbal medicine, describes emblica and chebula myrobalans as possessing the same virtues, though they have slightly different "nature."

THE SCIENCE BEHIND SABERRY™ :

The fruits of *Emblica officinalis* have been reported to contain low molecular weight hydrolysable tannins- Emblicanin A and Emblicanin B, along with Pedunculagin and Punigluconin.[3] Low levels of β-Glucogallin and other Mucic acid gallates have also been reported in aqueous extracts of *Emblica officinalis.*[4] The fruits of Amla have also been considered rich in vitamin C content. In 2006, Scartezzini *et al* proposed a reliable HPLC-DAD (High Performance Liquid Chromatography and Diode Array Detection) for identification and quantification of Ascorbic acid, and further indicated that high antioxidant activity is due to a large percentage of presence of Ascorbic acid.[5] In recent years Raghu *et al* compared Ascorbic acid content of the fruits by conventional colorimetric estimation, specific enzymatic method and derivative of Dehydroascorbic acid and concluded that 100 g of fresh fruit contain 34-38 mg vitamin C.[6] The presence and quantity of Ascorbic acid in Amla has however remained a dubious issue for a long time.[3]

To elucidate the actives behind the beneficial effects of Amla, and their contribution towards its antioxidant activity, scientists at Sabinsa Corporation chose to revisit the chemistry of the Indian Gooseberry.

I: TANNIN CHEMISTRY

The research team at Sabinsa Corporation developed a new HPLC method for the characterization and analysis of the various constituents of Amla Extract. By this method, seven major peaks were obtained for the Amla extract. The aqueous extract of the fresh fruits of Amla was processed using preparative reverse phase column chromatography, and the 7 fractions obtained were individually lyophilized and spotted on HPTLC. (Fig 1)

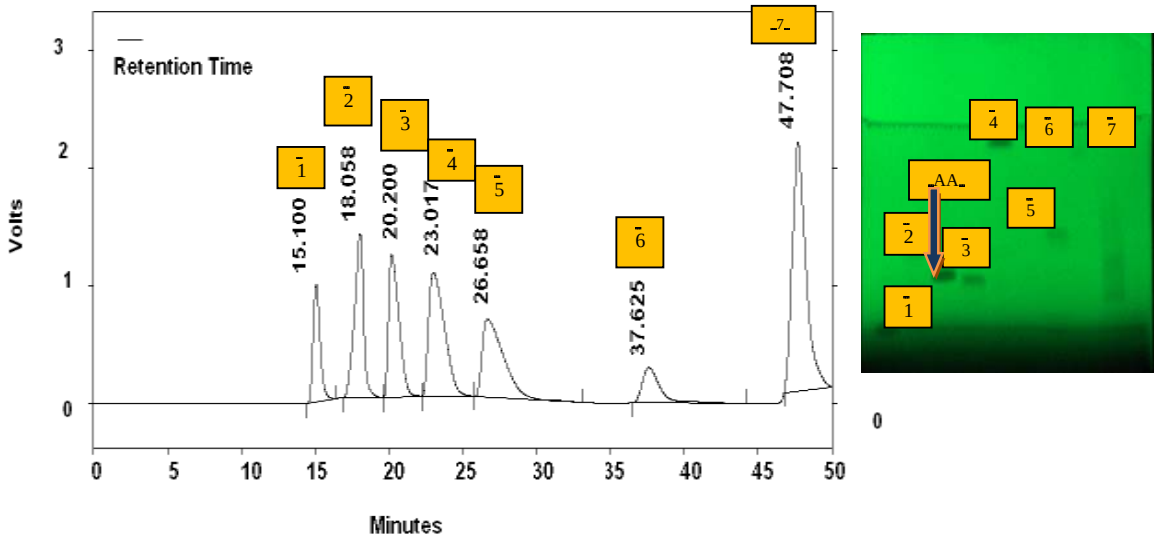
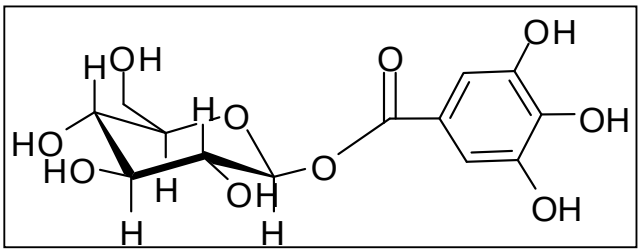


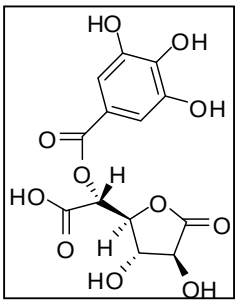
Fig1: HPLC and HPTLC profile of Amla Extract

According to previously published reports[3,7] Fraction 3 was concluded to be Emblicanin A. However, using spectral studies including Mass spectrometry and NMR, it was established that Fraction 3 was β-Glucogallin and not Emblicanin A.



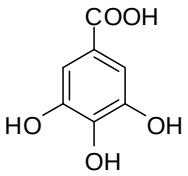
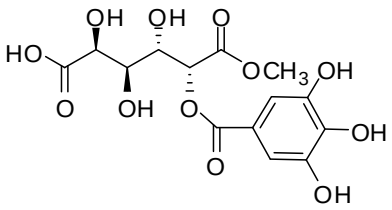
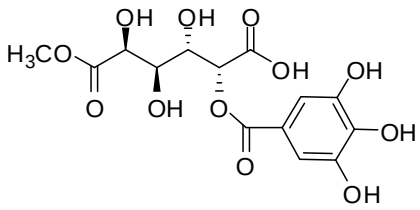
β-Glucogallin (Fraction 3)

Using the same HPTLC profile it was observed that the peak 2 corresponds to Emblicanin B on TLC and was lyophilized to get a hygroscopic white powder.[7] This compound was unstable in water and hence it was immediately analyzed as a solution. It gave a pseudo molecular ion peak (M-H) of *m/z* 343. By a direct comparison of NMR data with previously published literature reports, the compound was identified as Mucic acid 1,4-lactone 5-*O*-gallate[4] and not 2, 3, 4, 6-bis-(S)-hexahydroxydiphenoyl-2-keto-glucono-lactone (Emblicannin B).[3]



Mucic acid 1,4-lactone 5-*O*-gallate (Fraction 2)

Spectral analysis of lyophilized Fractions 4 and 5 revealed them to be Gallic acid and Mucic acid methyl ester 2-*O*-gallates respectively.[4]

		
Gallic acid (Fraction 4)	**Mucic acid 1-methyl ester 2-*O*-gallate (Fraction 5)**	**Mucic acid 6-methyl ester 2-*O*-gallate (Fraction 5)**

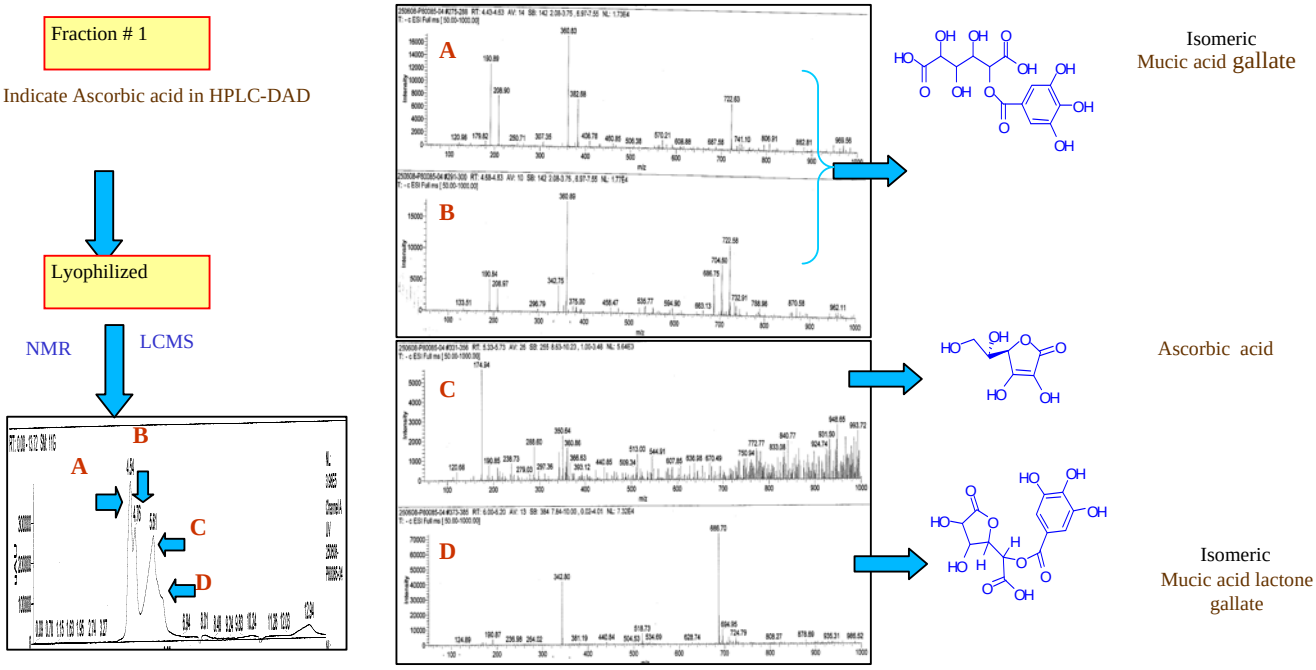
The studies summarized above, led the research team at Sabinsa Corporation to believe that β-Glucogallin and Mucic acid gallates are the predominant active molecules in Amla, and that these molecules are significant contributors to the healthful effects of Amla.

II. INVESTIGATING THE ASCORBIC CONTENT IN AMLA FRUIT EXTRACTS:

Fraction 1, corresponding to Ascorbic acid peak and isolated by preparative HPLC, was evaluated by Mass and NMR spectra. It was found that Fraction 1 contained more than one compound which was different from Ascorbic acid. Fraction 1 was resolved into FOUR peaks -1a, 1b, 1c and 1d by using a modified HPLC method (Table 1).

Analyzing by this method, it was found that Peak 1c and Ascorbic acid had the same RT. However, peak 1c was not a sharp peak and showed a tailing peak 1d. To ascertain whether peak 1c is Ascorbic acid (M.W. 176), extract was analyzed by LC-MS in negative mode. This showed that the so called Ascorbic acid peak consisted of four components, which were found to be mainly Mucic acid gallates as shown below.

Table 1: HPLC profile of Fraction 1 of Amla Extract

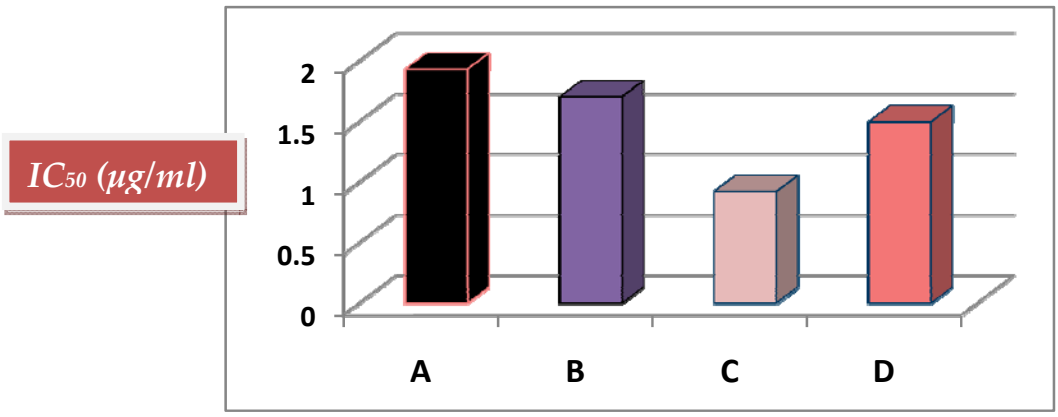


In house studies at Sabinsa Corporation showed that the consistency in the presence of Ascorbic acid in Amla is questionable. A new method of HPLC analysis was developed to detect naturally available Ascorbic acid in the Amla extract.

In order to ensure that there was no chemical degradation of any Ascorbic acid, the fresh juice of Amla fruits were obtained and handled with optimum care. Various batches of fruits were processed under similar conditions and their Ascorbic acid content evaluated. A variety of Amla extract samples were studied and it showed either complete absence of Ascorbic acid or trace amounts to a maximum of 4.0% w/w. Even though Ascorbic acid was not detected, Saberry™ did show potential antioxidant activity. This indicated that the Ascorbic acid is not the most optimal biomarker that reflects the biological potential of Amla. Thus β-Glucogallin is a more optimal and relevant biomarker, and reflects the antioxidant potential of Amla, more accurately than Ascorbic acid.

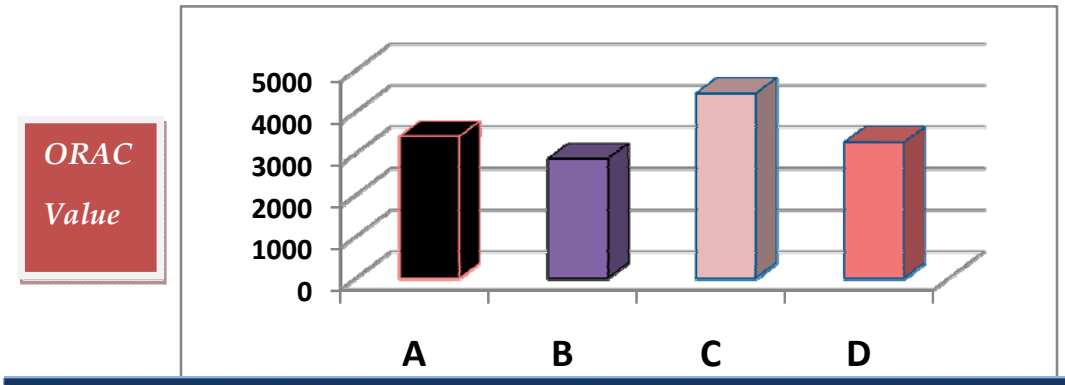
ANTIOXIDANT POTENTIAL OF SABERRY™:

Comparative DPPH Scavenging Activity



Lower the IC_{50} greater is the Antioxidant potential.

Comparative ORAC values



Greater the ORAC value, greater is the Antioxidant potential.

Ascorbic Acid

B. Amla Extract

C. β Glucogallin

D. Mucic Acid Gallate

Based on the enhanced antioxidant potential of β-Glucogallin over Ascorbic acid as evinced in the above graphs, and the inconsistent availability of Ascorbic acid in Amla in general, we concluded that it would be pertinent and more accurate to concentrate on the antioxidant potential of β-Glucogallin.

SABERRY™ - "ORAC DENSE PHYTONUTRIENT"

Based on scientific evidence, daily antioxidant intake should increase to 3,000 to 5,000 ORAC units per day to reach a significant antioxidant capacity in blood plasma and other tissues. According to the U. S. Department of Agriculture (USDA), current intake is about 1,200 units per day.

Brunswick Laboratory in Wareham, Massachusetts, evaluated the power of Saberry™ using state-of-the-art ORAC tests. The suite of ORAC assays - ORAC, HORAC, NORAC, SORAC and SOAC – are validated tests to measure broad-spectrum antioxidant power. They are recognized as the premier antioxidant tests available today by the industry, and are used extensively by leading manufacturers.

The ORAC unit is expressed as micromole standard per gram or liter. The acceptable precision of the ORAC assay is 15% relative standard deviation. The ORAC analysis provides a measure of the scavenging capacity of antioxidants against "peroxyl radical" which is one of the most common reactive oxygen species (ROS) found in the body.

ORAC Values of Saberry™

ORAC hydro (H-ORAC) (µmol TE/100g)	*ORAC lipo (L-ORAC) (µmol TE/100g)*	*ORAC total (H-ORAC + L-ORAC) (µmol TE/100g)*	*HORAC (µmol CAE/100g)*	*NORAC (µmol TE/100g)*	*SORAC (SOD) (kunitsSODeq/100g)*	*SOAC (µmol VitE/100g)*
267,800	400	268,200	34,500	90,400	10,200	135,100

Broad spectrum antioxidant activity is based on the values of $ORAC_{Total}$ [hydrophilic (H-ORAC) and lipophilic (L-ORAC) - Peroxyl Radical Absorbance Capacity)], HORAC (Hydroxyl Radical Absorbance Capacity), NORAC (Peroxynitrite Radical Absorbance Capacity), SOAC (Singlet Oxygen Absorbance Capacity), and SOD (superoxide dismutase equivalent activity, corresponding to Superoxide Radical Absorbance Capacity).

Saberry™ is a leader among water soluble phytonutrients in terms of broad spectrum antioxidant activity, showing a combined ORAC value of 358,600 µmol TE/100g* ($ORAC_{Total}$ + NORAC), HORAC of 34,500 µmol CAE/100g* , SOD capacity of 10,200 kunits SOD eq/100g and SOAC value of 135,100 µmol VitE/100g*. (see page 15 for a detailed explanation of these values)

Arithmetic Addition yields a value of: 538,400/100g

* TE/g : Trolox Equivalent/100g, VitE/g : alpha- tocopherol Equivalent/100g, CAE/g: Caffeic Acid Equivalent/100g

The enzyme superoxide dismutase (SOD, EC 1.15.1.1) catalyzes the dismutation of superoxide oxygen and hydrogen peroxide. As such, it is an important antioxidant defense in nearly all cells exposed to oxygen. It acts as a super scavenger of superoxide anions by ferreting out and destroying them throughout the body. The human body often lacks SOD, transferring the burden of defense on to intake of exogenous dietary antioxidants.

During the testing, Saberry™ was found to contain a class of compounds that act in a manner similar to superoxide dismutase (SOD). Saberry™ has a level of activity of 102 *kunitsSOD eq/g.*

NUTRACEUTICAL APPLICATIONS OF SABERRY™

Saberry™ has significant potential in the following applications:

- Antioxidant/healthy aging support
- As digestive aid
- In supporting cardiovascular health and wellness
- In diabetes management support
- In supporting healthy liver functions/detoxification

1. Antioxidant Potential:

As discussed previously Saberry™ exhibits significant antioxidant activity and is "ORAC Dense". Literature reports validate the antioxidant potential of Amla *in vivo.*

- Lipid peroxidation inhibition:. Amla extract inhibits radiation-induced lipid peroxidation (LPO) in microsomes, and protects SOD in mitochondria.

  For the LPO experiment, Amla extract was added in aqueous solution. The extent of LPO was measured in terms of thiobarbituric acid reactive substances. It was observed that the amla extract acts as a very good antioxidant against *γ*-radiation induced LPO. Similarly, Amla extract was found to inhibit the damage to antioxidant enzyme SOD in rat liver microsomes. The antioxidant activity of the Amla extract was found to be both dose- and concentration-dependent. The authors concluded that Amla extract being water-soluble, may scavenge the free radicals responsible for initiating lipid peroxidation.[9]

- The effects of amla on low-density lipoprotein (LDL) oxidation and cholesterol levels were investigated by Hyun Ju Kim *et al* (2005) in Japan, both *in vitro* and *in vivo* using Cu^{2+}-induced LDL oxidation and cholesterol-fed rats.[8] The ethyl acetate extract of Amla was found to significantly inhibit thiobarbituric acid reactive substance (TBARS) level, an index of oxidation, in Cu^{2+}-induced LDL oxidation. In addition, the extract significantly reduced total, free and LDL-cholesterol levels in a dose-dependent manner, in treated animals, with potential applications in supporting cardiovascular health and wellness.

2. Digestive Aid:

Amla fruit has been traditionally used as a digestive aid in India. It also forms an important constituent of Triphala – an Ayurvedic preparation recognized for its role in supporting digestive functions. Amla is thought to improve the stimulation of gastric juices and also support detoxification. Amla is also reported to have anti-bacterial and astringent properties that potentially support the management of infections and help in the healing of ulcers.[10]

Rafatullah S. (2002) demonstrated the gastro-protective effects of Amla *in vivo* in rats. The ethanolic extract of Amla was reported to offer protection against ethanol induced depletion of stomach mucus, and reduction in non-protein sulphydryl concentration. These results indicate that Amla extract offers antisecretory, antiulcer, and cytoprotective properties.[11]

3. Antidiabetic Property Of Amla

P. Suryanarayan *et al* (2004) investigated the beneficial role of Amla extract in diabetes induced complications.[12] Aldose reductase (AR) has been a drug target because of its involvement in the development of secondary complications of diabetes, including cataract. Amla extract inhibited rat lens AR and recombinant human AR with IC_{50} values 0.72 and 0.88 mg/ml respectively. However they found that ascorbic acid did not inhibit AR even at 5mM concentration (i.e., approx. 0.8 mg/ml. Further, they demonstrated that the hydrolysable tannins of Amla were responsible for AR inhibition, as enriched tannins of the extract exhibited remarkable inhibition against both rat lens and human AR with IC_{50} of 6 and 10µg/ml respectively. Amla tannins (water extract of Amla) are reported to be 100 times more effective than quercetin in inhibiting Aldose reductase. Furthermore, the isolated tannins not only prevented AR activation in rat lens organ culture, but also reduced sugar-induced osmotic changes in rat lens. The authors report that the results indicate that tannoids of *E. officinalis (Amla)* are potent inhibitors of AR and suggest that exploring the therapeutic value of natural ingredients that people can incorporate into everyday life may be an effective approach in the management of diabetic complications.[12] Rao T.P. *et al* (2005) reported the efficacy of amla extract in relieving oxidative stress, and improving glucose metabolism in diabetic rats.[13] Yokozawa et al. (2007)[29] demonstrated that administration of Amla extract helps in the management of age related renal dysfunction in aging animal models, through inhibiting oxidative stress and inflammatory pathways. Renal dysfunction is a common secondary complication in diabetes.

4. Hepatoprotectant Potential Of Amla

The research conducted at the Amala Cancer Research Centre in Kerala, India, has found that an extract of *Phyllanthus emblica* significantly inhibited hepato-carcinogenesis induced by N-nitrosodiethylamine (NDEA) in experimental animals.[14]

Amla is used in Indian system of medicine for the treatment of liver ailments. *Emblica officinalis* has the potential to suppress carcinogen-induced response in rat liver.[15]

Fibrosis of the liver is a state of complicated end stage alteration of structure and function due to different etiologies. There is no established therapy, and the treatment options require long term administration of putative anti-fibrotics which must be free of side effects.

Emblica officinalis (fruit) exract reduced the severity of hepatic fibrosis induced by carbon tetrachloride (CCl_4) and thioacetamide (TAA). Improved liver function was observed by measuring the levels of aspartate aminotransaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin in serum.[16]

According to the authors, the antifibrotic activity of Amla extract could be attributed to its mechanistic intervention in several cellular events which in turn provides beneficial effects against this multifactorial process of fibrogenesis. Mechanisms include reduction of oxidative stress, stabilization the cell membrane against fibrotic challenge, and inhibition of Cytochrome P450 2E1 (CYP2E1) mediated bioactivation.

DOSAGE FORM AND SUGGESTED USE LEVEL (Nutraceutical Applications):

Saberry™ can be used in the form of capsules or tablets .

Saberry™, being water soluble, can also be conveniently incorporated into various beverages. Thus Saberry™ finds interesting applications in health and wellness beverages, citrus fruit juices, sports drinks etc.

Depending on the intended use, typically we suggest that Saberry™ be used at levels ranging from 200mg to 1000mg, per day, in two divided doses.

COSMECEUTICAL APPLICATIONS OF SABERRY™:

Saberry™ can find useful applications in

- Anti aging Nutricosmetic potential
- Sun Care and After Sun Care formulations
- Hair Care Applications

1. Anti Aging Nutricosmetic Potential:

Several theories have been proposed to account for age-related changes in cell functioning and physiological capability.

The “Free Radical Theory of Aging” was propounded several decades ago, by Prof. Harman D. (1956). Recent scientific evidence validates the supportive role of dietary interventions in healthy aging and longevity.

Free radicals are molecules with one or more unpaired electrons; they seek stability by taking electrons from other molecules (a process called oxidation). As a consequence, free radicals damage the molecules from which they take electrons, leading to cell damage, impaired functioning, and even cell death. The prime molecules in the body that are damaged by free radicals are DNA, lipids, and proteins.

A. Amla Extract offers broad-spectrum protection against heavy metals

Amla extract helps protect the skin from the damaging effects of free radicals and heavy metal-induced oxidative stress.

Several animal studies have shown that amla can help prevent a toxic build-up of heavy metals caused by frequent exposure to metals like aluminium, lead and nickel. When vitamin C alone was used, equivalent to that found in amla fruit only partial protection from heavy metals was provided.

A standardized extract of *Phyllanthus emblica* (Emblica) was found to have a long/lasting and broad/ spectrum antioxidant activity. The product has no pro-oxidation activity induced by iron and/or copper because of its iron and copper chelating ability. These play a significant role in the use of Amla extract in anti-aging formulations.

Amla has even been proven to almost completely prevent DNA and cell damage from arsenic poisoning.[17]

In laboratory tests done on animals it was also shown to prevent cellular damage resulting from lead[18, 19, 20], aluminum[19], nickel[21], cadmium[22], and chromium[23] toxicity.

B. Amla Extract and MMPs

With increasing age, collagen synthesis becomes lower and MMP-1 levels become higher in naturally aged human skin, and these alterations cause changes such as skin wrinkling and loss of elasticity[24] (Varani *et al* 2000). Therefore, control of collagen metabolism may be useful for a variety of therapeutic and cosmetic applications.

Takashi Fujii *et al* (2008) demonstrated that amla extract increased procollagen type I C-peptide (PIP) and Tissue inhibitor of metalloproteinase-1 (TIMP-1) production, and decreased Matrixmetalloproteinase-1 (MMP-1) production, concomitant with elevated mitochondrial activity in the fibroblast, in a concentration dependent manner.[25]

The above study has shown that Amla extract helps elevate the *mitochondrial activity* of human skin fibroblasts and promotes production of procollagen and has a number of potential cosmetic applications, particularly as an Antiaging ingredient.

2. Sun Care And After Sun Care Formulations:

Saberry™ was also found to possess significant potential as a UVA and UVB protectant.

Photoprotective Activity Against UVA And UVB Rays Saberry™	
Melanin Inhibition in B16 F1 Cells IC50 (µg/ml)	**12 µg/ml**
UV B protection in Swiss 3T3 Cells EC50 (µg/ml) **(effective concentration for 50% UV protection)**	**41.2 µg/ml**
UV A protection in Swiss 3T3 Cells EC50 (µg/ml) **(effective concentration for 50% UV protection)**	**14 µg/ml**

With the added advantage of its potential antioxidant activity, Saberry™ can help in the management of Sunlight induced photodamage.

3. Hair Care Applications

Amla has a long tradition in being used for improving the health of hair and scalp. It is widely used in hair care preparations as a natural hair conditioner.

The most commonly implicated androgen in hair loss is dihydrotestosterone (DHT), which is a very potent form of testosterone. It is created in the body by the conversion of testosterone by the enzyme 5-alpha reductase. Well, it stands to reason that if we could decrease the amount of this enzyme, 5-alpha reductase, in the blood or in the hair follicles, then less testosterone would be converted to DHT, and therefore DHT levels would be lower. Inhibitors of 5-alpha reductase thus help in hair fall management.

Saberry™ was found to exhibit 5-alpha reductase inhibitory activity (about 80% inhibition at a concentration of 250 µg/ml), validating its potential applications in hair care products.

DOSAGE FORM AND SUGGESTED USE LEVEL (Nutricosmetic Applications):

Saberry™ can be used in the form of capsules or tablets Nutricosmetic applications.
For topical application it can be conveniently used in the form of creams, sprays, serums, gels, lotions etc.

We suggest that Saberry™ be used at levels of 0.2% to 1.0% w/w in topical formulations.

SAFETY AND TOXICITY:

No major reported toxicities have been associated with the fruit, which has a long history of food use. In toxicity studies in rats, no toxicity was observed in single - or chronic-dose administration. Additionally, no detrimental effect was noted on liver or renal function.[26] No chromosomal aberrations were found following 7- and 14-day treatment regimens in rats with crude fruit extract.[27] In another experiment, no toxicity or mutagenicity were observed in rats even at the highest doses administered.[28]

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A NOTE ON ORAC

Peroxyl ORAC *– originally conceived by Richard Cutler, advanced and modified by Drs. Cao, Prior and Brunswick Laboratories - is regarded as the best available test for measuring antioxidant capacity against peroxyl.*
ORAC $_{hydro}$ reflects water soluble anti-oxidant capacity.
ORAC $_{lipo}$ is the lipid soluble anti-oxidant capacity.
ORAC $_{total}$ is the sum of ORAC $_{hydro}$ ORAC $_{lipo.}$
Trolox, a water soluble Vitamin E analog, is used as the calibration standard ORAC result is expressed as micromole Trolox equivalent (TE)/gram.

Hydroxyl ORAC *– Technically, this is an oxygen radical prevention capacity assay – it quantifies the ability of a substance to prevent the formation of hydroxyl rather than absorb it after its formation.*

Peroxynitrite ORAC *– Several studies have linked peroxynitrite to Alzheimer's disease, Parkinson's disease, and other degenerative neurological diseases.*

Superoxide ORAC *– Superoxide anion is uniquely harmful among radicals because it is the precursor to other radicals such as hydrogen peroxide and hydroxyl. By quenching superoxide, antioxidants also prevent the formation of these other radicals.*
The human body compensates for superoxide by producing the enzyme superoxide dismutase (SOD). Exogenous antioxidants can act against superoxide either by stimulating SOD or by directly quenching superoxide.

SOAC *– Singlet Oxygen Absorbance Capacity. Normal oxygen is electronically in a triplet state. A very reactive form of oxygen, present in electronic singlet state, is harmful to cells.*

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