A “Superfruit” extract from the Ayurvedic Tradition: Authenticated and Redefined

INTRODUCTION

An interesting trend in the use of “food as medicine”, is the emergence and classification of some lesser known fruits as “superfruits”, with benefits that go far beyond nourishment alone. Amla fruit enjoys a special place in Ayurveda, as a nurturing food, that is credited with a number of health benefits. In the Ayurvedic tradition, the fruit forms an integral part of medicinal preparations that are used to support wellness and healthy aging.

A method of making a healthful preparation with Amla was described as early as in the 1st century AD in a Sanskrit text. Records of the use of Amla have also been found in Arabic, Iraqi, Tibetan, and Egyptian texts, as well as in the Siddha (Indian), and Unani systems of health care (1,2).

Researchers in the last few decades established the beneficial role of Amla extract as a biological antioxidant. Its high antioxidant power was attributed to the presence of ascorbic acid (Vitamin C). However, some experts have questioned the presence of this heat labile vitamin in such extracts, and perhaps even in the fresh fruit. An effort to resolve this confusion and enable authentication and efficacy validation of this healthful herb led to the identification of a valid biomarker for Amla (3).

Amla fruits are regarded as an “adaptogen”, defined by NIKOLAI LAZEROV in 1947 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”. Herbs that function in this manner are classified as rejuvenators, qi tonics, rasayanas, or restoratives, in traditional systems of medicine. Amla is used as a primary ingredient in the Ayurvedic rasayana tonic “Chyawanprash”, used to support strength and immune functions, and in the composition “Triphala” in conjunction with chebulic and belleric myrobalans, to support digestive health (4).

AUTHENTICATING AMLA EXTRACTS

The fruits of Emblica officinalis are reported to contain low molecular weight hydrolysable tannins – Emblicanin A and Emblicanin B, along with Pedunculagin and Punigluconin (5). Low levels of beta-glucogallin and other mucic acid gallates have also been reported in aqueous extracts of the fruit (6), while ascorbic acid has been long believed to be the major active constituent (7,8).

However, a detailed literature search reveals inconsistent reports with respect to the presence of ascorbic acid in Amla. A proprietary Amla extract, Saberry™ *, is standardized to contain a minimum of 10% beta-glucogallin and 50% gallates (3). Conventionally, Amla extracts were standardized using ascorbic acid as the biomarker. However, recent research...

* A trademark of Sabinsa Corporation, patents pending
revealed that Amla does not contain ascorbic acid in consistent amounts, and sometimes, only in trace quantities, rendering its validity as a biomarker questionable. Beta-glucogallin is a more powerful antioxidant molecule, as compared to Ascorbic acid. The proprietary preparation is a light colored, water soluble powder and is processed from carefully chosen, fresh Indian gooseberries using solvent free technology that preserves the natural goodness of the fruits.

**UNRAVELING THE PHYTOCHEMISTRY OF AMLA**

**Tannin Chemistry**

A new HPLC method enabled the elucidation of seven major peaks in Amla extract (3). The aqueous extract of the fresh fruits of Amla was subjected to preparative reverse phase column chromatography, and the seven fractions obtained were individually lyophilized and spotted on HPTLC (Figure 1). Earlier researchers identified Fraction 3 in Figure 1 as “Emblicanin A”. However, spectral studies including mass spectrometry and nuclear magnetic resonance (NMR), established that Fraction 3 is beta-Glucogallin (Figure 2) and not Emblicanin A.

Further, peak 2 identified as “Emblicanin B” by earlier researchers, has now been correctly identified as mucic acid 1,4-lactone 5-O-gallate (Fraction 2) (5).

**Is Ascorbic Acid a Valid Biomarker?**

Fresh juice of Amla fruit grown in diverse locations was tested for ascorbic acid content. These samples contained either no ascorbic acid at all, or only trace amounts of the compound, up to a maximum of 4.0% w/w. Fraction 1 (in Figure 1), corresponding to ascorbic acid peak was isolated by preparative HPLC, and characterized using mass and NMR spectroscopy. Surprisingly, Fraction 1 on resolution showed four peaks one of which had the same retention time as ascorbic acid. On analysis by LC-MS in negative mode the “ascorbic acid peak” could be separated into four components, constituted by mucic acid gallates (3).

These studies revealed that the consistent presence of ascorbic acid in Amla is questionable, and also facilitated a validated HPLC method to detect any naturally available ascorbic acid in Amla extracts.

**ANTIOXIDANT POTENTIAL OF SABERRY™**

Although ascorbic acid was not detected, Saberry™ showed superior antioxidant activity as compared to conventional amla extract, and pure ascorbic acid at the levels supposedly present in amla extract. Therefore it is logical to conclude that ascorbic acid is not the most optimal biomarker to explain the biological potential of Amla. Beta-Glucogallin is thus a relevant biomarker, and its content truly reflects the antioxidant potential of Amla (3) (Figure 4).

**QUANTIFYING THE ANTIOXIDANT POTENTIAL OF SABERRY™**

The suite of ORAC assays 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 of nutritional products. The ORAC unit is expressed as micromole standard per gram or liter. The acceptable precision of the ORAC assay is 15% relative standard deviation.

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**Figure 1 – HPLC and HPTLC profile of Amla Extract**

**Figure 2 – Beta-Glucogallin (Fraction 3)**

**Figure 3 – Mucic acid 1,4-lactone 5-O-gallate (Fraction 2)**

**Figure 4 – Comparative antioxidant potential**
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. Peroxyl ORAC assay, which was originally conceived by Richard Corder and later augmented and modified, is regarded as the best available test for measuring antioxidant capacity against peroxyl radical. Trolox, a water soluble Vitamin E analog, is used as the calibration standard.

Hydroxyl ORAC measures prevention of hydroxyl radical formation. Peroxynitrite ORAC measures the ability of a substance to prevent peroxynitrite radical formation. Several studies have linked peroxynitrite to the incidence of Alzheimer’s disease, Parkinson’s disease and other degenerative neurological diseases. Superoxide ORAC measures the capability of a substance to quench the superoxide anion. 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 prevent the formation of these radicals as well. The human body combats superoxide by producing the enzyme superoxide dismutase (SOD). Exogenous antioxidants can act against superoxide either by stimulating SOD or by directly quenching superoxide. Normal oxygen is electronically in a triplet state. A very reactive form of oxygen, present in electronic singlet state, is harmful to cells. SOAC or Singlet Oxygen Absorbance Capacity measures the capability of a substance to quench this reactive species.

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) (9,10).

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/100 g ^ (ORAC Total + NORAC), HORAC of 34,500 µmol CAE/100 g ^ and SOAC value of 135,100 µmol VitE/100 g ^ (11).

The enzyme superoxide dismutase (SOD, EC 1.15.1.1), catalyzes the dismutation of superoxide hydrogen and hydrogen peroxide. As such, it is an important antioxidant defense in nearly all cells exposed to oxygen, functioning as a super scavenger of superoxide anions. 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 SOD. Saberry™ has a level of activity of 102 (kunits SOD eq/g). This finding is relevant in validating the efficacy of the extract in supporting healthy aging.

**HEALTHFUL APPLICATIONS**

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

The “antiaging” applications stem from the antioxidant activity of hydrolysable tannins in the extract. Oxidative stress resulting from free radical pathology is implicated in the aging process. Vital components of the cell such as the mitochondria (the energy centers), functional proteins, lipids and DNA are damaged by free radicals. Cross linking and glycation of connective tissue proteins, such as collagen, results in the formation of advanced glycation end products (AGE) which accumulate with age, and induce stiffening of cartilage and extracellular matrix, resulting in cataracts and diabetes mellitus, as well as in the cardiovascular system aging is associated with a decrease in elasticity and an increase in stiffness of the arteries. Glucose tolerance progressively declines with age, and there is a high prevalence of type 2 diabetes, cardiovascular disease and metabolic syndrome in the aging population. Kidney functions, liver functions and sensory perception also deteriorate with age. Malabsorption of vital nutrients in the elderly, results in a compromised immune system and lowered resistance to infection.

In the skin, the appearance of wrinkles is a result of inflammation and the action of enzymes such as matrix metalloproteinases (MMPs) that degrade connective tissues. Both these causative factors are triggered by the ravages of chronological aging, and exposure to environmental factors such as ultraviolet radiation. Amla extracts have been shown to inhibit aldose reductase, an enzyme that has been implicated in the development of cataracts (12). Valid research on the beneficial role of aqueous Amla extract in protecting healthy liver functions, and potentially beneficial in preventing hepatocarcinogenesis (13), counteracting toxicity due to heavy metals (14) or ingested chemicals (15); and gastrointestinal health, including the prevention of gastric ulcers (16,17), is available from peer reviewed publications. The usefulness of Amla extract in maintaining healthy blood lipid levels (18), blood sugar levels (19) and renal functions (20) has also been validated.

An interesting observation on its adaptogenic effects was made by researchers who studied the effects of the tannoid principles of Amla fruit on free radical pathology in the brain in animal models. The results showed that Amla extract normalized stress-induced perturbations in oxidative free radical scavenging enzymes in rat brain frontal cortex and striatum. In the animal models studied, chronic stress, prevalent over a period of 21 days, induced significant increase in rat brain frontal cortical and striatal superoxide dismutase (SOD) activity, concomitant with significant reduction in catalase (CAT) and glutathione peroxidase (GPX) activity. The change in these enzyme activities was accompanied by an increase in lipid peroxidation, in terms of increased levels of thiobarbituric acid-reactive products. Administration of Emblica tannoids (10 and 20 mg, po) in conjunction with the stress procedure, induced a tendency towards normalization of the activities of SOD, CAT and GPX in both the brain areas, along with reduction in lipid peroxidation. The authors postulated that the rasayana reffects of Amla are potentially linked to this mechanism (21). For example, Amla has traditionally been used in hair oils and is believed to decelerate greying. A recent research report links grey hair with reduced catalase activity in the hair follicles, leading to uncontrolled accumulation of hydrogen peroxide. The resultant oxidative stress impairs methionine sulfoxide repair, leading to loss of pigmentation in the hair shaft (22).

Recent research revealed that Amla extract helps to elevate the mitochondrial activity of human skin fibroblasts and

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**Table I** – Photoprotective activity of Saberry™ against UVA and UVB rays

<table>
<thead>
<tr>
<th>Activity</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Melanin Inhibition in B16 F1 Cells IC50</td>
<td>12 µg/ml</td>
</tr>
<tr>
<td>UV B protection in Swiss 3T3 Cells EC50 (effective concentration for 50% UV protection)</td>
<td>41.2 µg/ml</td>
</tr>
<tr>
<td>UV A protection in Swiss 3T3 Cells EC50 (effective concentration for 50% UV protection)</td>
<td>14 µg/ml</td>
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TE/g: Trolox Equivalent / 100 g; VitE/g: alpha-tocopherol lu Equivalent / 100 g; CAE/g: Caffeic Acid Equivalent / 100 g

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promotes production of pro-collagen (23) thus supporting the cosmetic applications of the extract, as an “anti-aging” cosmeceutical or nutricosmetic ingredient. Investigations with the branded Saberry™ established its in vitro efficacy in offering protection against skin damage by ultraviolet (UV) radiation (Table 1). This finding suggests potential applications in sun care and “after sun” care formulations both from within, in the form of nutritional compositions, and from the outside as “cosmeceutical” formulations (24). Multifunctional benefits are anticipated, based on the antioxidant, anti-inflammatory and “protective” effects of the extract. Potential skin tone lightening effects have also been demonstrated, based on the ability of the extract to inhibit the enzyme tyrosinase, a key component of the melanogenesis pathway (24).

Saberry™ was also shown to efficiently inhibit 5-alpha reductase, the enzyme that is implicated in the progression of androgenic alopecia, further supporting the traditional use of Amla in hair care (22).

No major reported toxicities have been associated with Amla fruit, which has a long history of food and topical use. Furthermore, an independent panel of expert toxicologists, recently affirmed Saberry™ as GRAS (Generally Recognized as Safe) for use in food products (25).

CONCLUSIONS

Amla extract finds numerous applications in contemporary preventive health care. Furthermore, a newly identified biomarker, beta-glucogallin, enables its authentication and scientific validation.

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