Abstract:

Title: OPTIMAL BIOLOGICAL MARKER FOR THE BIOLOGICAL POTENCY OF EMBLICA OFFICINALIS GAERTN. (AMLA) FRUIT-METHODS AND PRODUCTS THEREOF

STR#1

(57) Abstract: Disclosed is the most optimal biomarker for the BIOLOGICAL POTENCY of Emblica Officinalis Gaertn. (Amla) fruit and products standardized for 5% and above w/w of the said biomarker. Further, the products described herein contain from about 0.0001 0% to about 4% of free ascorbic acid depending on the raw material used. The optimal biomarker for amla described herein above is represented by STR#1.
OPTIMAL BIOLOGICAL MARKER FOR THE BIOLOGICAL POTENCY OF Emblica Officinalis Gaertn. (AMLA) FRUIT-METHODS AND PRODUCTS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS
This application claims the benefit of US Patent application No. 12/353,381 filed on January 14, 2009, at the United States Patents and Trade Mark Office, the contents of which is incorporated herein in their entirety by reference.

[PARA 001] BACKGROUND OF THE INVENTION

[PARA 002] FIELD OF THE INVENTION

[PARA 003] The invention in general relates to Emblica Officinalis Gaertn. (Amla) fruit. More specifically, the present invention relates to novel optimal biomarkers for the biological potency of amla fruits and products thereof.

[PARA 004] DESCRIPTION OF PRIOR ART

[PARA 005] The fruits of E. officinalis are reported to contain hydrolysable tannins, emblicanin A and emblicanin B, along with pedunculagin and punigluconin [Ghosal, S.; Tripathi, V. K.; Chauhan, S. Active constituent of Emblica officinalis: part I-the chemistry and antioxidant effects of two new hydrolysable tannins, emblicanin A and B. Indian J. Chem. 1996, 35B, 941-948]. Important patent references in this regard include:

A. Process for obtaining a stabilized antioxidant formulation from Emblica officinalis fruit comprising extracting the finely pulped fruit with a dilute aqueous or alcoholic water salt solution at a temperature of about 70°C to 5°C to form an extract containing solution, filtering, and drying to provide the desired antioxidant blend as a powder has been discussed in WO/2000/048551, US6124268, US6235721 to Ghosal, Shibnath.

B. The main constituents from the pericarp of Emblica officinalis discussed here include

1. Emblicanin-A: 2, 3-di-O-galloyl-4, 6-(S)-hexahydroxydiphenoyl-2-keto-glucono-lactone;
2. Emblicanin-B: 2, 3, 4, 6-bis-(S)-hexahydroxydiphenoyl-2-keto-glucono-lactone;
3. Punigluconin: 2, 3-di-O-galloyl4, 6-(S)-hexahydroxydiphenoyl gluonic acid;
4. Pedunculagin: 2, 3, 4, 6-bis-(S)-hexahydroxydiphenoyl-D-glucose;
5. Rutin: 3', 4', 5, 7-tetrahydroxyflavono 3-O-rhamnoglucoside;
5. Gallo-ellagitannoids;
6. Gallic acid and
7. Ellagic acid.

C. Sun protective and skin lightening dosage forms of Emblica officinalis extract comprising Emblicanin A, Emblicanin B, Pendunculagin and Punigluconin, preferably in an amount of >40% by weight of the extract have been discussed in US20050089590, EPI 560561, US20030198612 and US20040086560.


[PARA 007] Similar views on ascorbic acid contents in Emblica officinalis to bring about the biological effects of amla were discussed in "A much higher concentration of Ascorbic acid is required to effect the same inhibition as Amla"-S. M. Khopde et al., Current Science, Vol. 81, No. 2, 25 July 2001.

[PARA 008] However, in 2006, Scartezzini et al. proposed a reliable HPLC-DAD for the identification and quantification of ascorbic acid and further indicated that high antioxidant activity is due to a large percentage of the presence of ascorbic acid

[PARA 009] Recently, Raghu et al. compared ascorbic acid content of the fruits by conventional colorimetric estimation and specific enzymatic method and as the o-phenylene diamine derivative of dehydroascorbic acid and found contents of 34-38 mg of vitamin C equivalent to 100 g of fresh weight (Raghu, V.; Platel, K.; Srinivasan, K. Comparison of ascorbic acid content of Emblica officinalis fruits determined by different analytical methods. J. Food Compos. Anal. 2007, 20, 529-533.)

[PARA 001 0] Zhang et al have reported the principal phenolic constituents of Emblica officinalis fruit juice as mucic acid 2-O-gallate, mucic acid 1,4-lactone 2-O-gallate and mucic acid 1,4-lactone 5-O-gallates. These along with 1-O-galloyl-β-D-glucose (β-glucogallin) have been suggested as possible antioxidants in the juice together with vitamin C.

[PARA 001 1] The inventors of the present invention revisited the amla chemistry to identify and confirm the essential biomarkers of the fruit that confer its vital properties.

[PARA 001 2] Accordingly, it is the principle object of the present invention to investigate thoroughly the chemistry of amla and unravel unknown data if any and also confirm the essential biomarkers of the fruit that confer its vital properties.

[PARA 001 3] It is also another object of the present invention to develop a product standardized in terms of the most optimal biomarker molecule as identified by the chemical studies on amla fruit.
The present invention fulfills the aforesaid objectives and provides further related advantages.

SUMMARY OF THE INVENTION

The present invention relates to the most optimal biomarker for the biological potency of Emblica Officinalis Gaertn. (Amla) fruit and products standardized for 5% and above w/w of the said biomarker represented by STR#1. Further, the products described herein above contain from about 0.0001 0 % to about 4% of free ascorbic acid depending on the raw material used. In specific embodiment of the present invention, the product standardized for 5% and above w/w of the said biomarker represented by STR#1 represents an aqueous extract from Emblica Officinalis Gaertn. fruit (Amla fruit). More specifically, the aqueous extract also comprises from about 50% to greater than 50% total gallates including mucic acid 1, 4-lactone 5-O-gallate, mucic acid 2-O-gallate, mucic acid 6-Methyl ester 2-O-gallate, mucic acid 1-Methyl ester 2-O-gallate and ellagic acid.

STR#1

The advantages of the present invention include

1. The invention that β-glucogallin is the most optimal biomarker for the efficiency of Emblica Officinalis Gaertn. (Amla) fruit along with co-eluting mucic acid gallates, rather than previously reported hydrolysable tannins such as Emblicanin A and Emblicanin B, which have been shown absent in amla fruit by the current inventors.
2. The demonstration of poor, variable presence of ascorbic acid adding new dimensions to the myth that amla is rich in ascorbic acid and ascorbic acid is the most optimal biomarker for amla efficacy.

3. A product comprising the compound represented by STR#1, said compound used as the most optimal biomarker for the efficiency of Emblica Officinalis Gaertn. (Amla) fruit, wherein said product is standardized to contain 5% and above w/w of the biomarker represented by STR#1 and from about 0.0001% to about 4% free ascorbic acid depending on the raw material used.

![STR#1]

[PARA 001 8] Other features and advantages of the present invention will become apparent from the following more detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way of example, the principle of the invention.

[PARA 001 9] BRIEF DESCRIPTION OF THE DRAWINGS

Fig 1 shows the preparative HPLC chromatogram (at 280 nm) of freeze-dried aqueous extract of E. officinalis fruits.
Fig 2 shows the TLC of isolated peaks with ascorbic acid (spots from left to right: 1, fraction 1; 2, fraction 2; AA, ascorbic acid; 3, fraction 3; 4, fraction 4, 5, FRACTION 5, 6, FRACTION 6, 7, FRACTION 7)

Fig 3 shows the HPLC chromatogram of (A) ascorbic acid (RT 4.57 min) and (B) PEAK 3 (STR#1) (RT 12.6 min).

Fig 4 shows the HPTLC profile of E. officinalis fruit extract.

Fig 5 shows the compounds from E. officinalis fruit extract: 1. β-glucogallin; 2. mucic acid 1,4-lactone 5-O-gallate; and 3. mucic acid 2-O-gallate.

Fig 6 shows the HPLC chromatogram of (A) Ascorbic Acid With Retention Time 4.57 Min ; (B) Fraction 1; (C) Peak 1 Spiked With Ascorbic Acid.

Fig 7 shows the LC-ESIMS of Peak 1.

Fig 8 shows the comparative anti-oxidant potential in terms of ORAC values, where greater the ORAC value greater is the anti-oxidant activity.

Fig 9 shows the comparative anti-oxidant potential in terms of DPPH scavenging activity, where lower the IC50 values, greater is the Antioxidant activity.

Fig 10 shows the comparative UVB protection values [EC50µg/ml] (represented on Y axis) of A=Ascorbic acid; B= β-glucogallin and C=Mucic acid gallates (represented on X Axis)

Fig 11 shows the HPLC chromatogram of β-glucogallin and ascorbic acid in amla juice.
In the most preferred embodiment, the present invention relates to the most optimal biomarker for the biological potency of *Emblica Officinalis* Gaertn. fruit (Amla fruit) and products standardized for 5% and above w/w of the said biomarker represented by STR#1. Further, the products described herein above contain from about 0.0001% to about 4% of free ascorbic acid depending on the raw material used.

In specific embodiment of the present invention, the product standardized for 5% and above w/w of the said biomarker represented by STR#1 represents an aqueous extract from *Emblica Officinalis* Gaertn. fruit (Amla fruit). More specifically, the aqueous extract also comprises from about 50% to greater than 50% total gallates including mucic acid 1,4-lactone 5-O-gallate represented by STR#2, mucic acid 2-O-gallate represented by STR#3, mucic acid 6-Methyl ester 2-O-gallate represented by STR#4, mucic acid 1-Methyl ester 2-O-gallate represented by STR#5 and ellagic acid represented by STR#6.
The aforesaid most preferred embodiments are explained in detail by specific examples described herein below.

EXAMPLE I

CHEMICAL ANALYSIS OF Emblica Officinalis FRUITS
The present inventors separated the extract of *Emblica officinalis* into seven major fractions using Preparative Shimadzu HPLC system equipped with Class Vp software with binary gradient (LC8A) pump, UV-VIS dual wavelength detector (SPD-I OAVp), system controller (SCL-I OAVp) and Rheodyne injector 7725i with 5mL sample loop. Separation was done on Phenomenex Jupiter C18 column 300A(250x50 mm, 15µm) with mobile phase A-water (0.1 % formic acid) and B- methanol. A gradient was used as 0-35 min 5% B, 35-40 min 100% B, 40-50min. 100% B, 50-51 min. 5% B and 51-60min.5% B. Flow rate was 35ml/min and monitoring at 240 and 280nm.

**FICl** shows the preparative HPLC profile of *Emblica officinalis* as discussed herein above. **FIG.2** shows HPTLC profile of the seven peaks along with standard ascorbic acid. **FIG. 3** shows the different retention times for ascorbic acid and peak 3. The HPTLC profile as seen in **FIG. 4** elucidated by the present inventors perfectly matches with reported HPTLC profile for Emblica officinalis [J. Sep. Sc. 2000, 30, 1250-1254]. Peak 3 obtained by the present inventors indeed corresponded to Emblicanin A by TLC profiling (**FIG.2**). Further HPLC profiling (**FIG. 3**) of Peak 3 reported by the present inventors and ascorbic acid, clarify that peak 3 is not ascorbic acid owing to the variant retention time values of ascorbic acid being 4.6 and Peak 3 being 12.6. Identification of peak 3 isolated by the current inventors using $^1$H, $^{13}$C NMR & Mass yielded a structure (STR#1) different from the one reported for Emblicanin A in prior art (STR#001). STR#1 corresponded to 1-0-galloyl- β-D-glucose (β-glucogallin).

![Structure](STR#1)
Under stringent conditions of temperature and light, the present inventors isolated, freeze dried and subjected Peak 2 of FICl to NMR studies. $^1$H, $^{13}$C NMR & Mass yielded a structure (STR#2) for Peak 2. However peak 2 corroborated with emblicanin B in HPTLC studies. This led to conclusion that Emblicanin B, represented by STR#002 is actually Peak 2 with correct structure (STR#2) indicating it to be Mucic acid 1,4-lactone 5-O-gallate.
Ascorbic acid has been estimated previously by various methods. Retention time of Ascorbic acid (FIG. 6A) and peak 1 (FIG. 6B) were nearly same. Standard ascorbic acid when spiked with peak 1 (FIG. 6C) was found to elute nearly at the same retention time. To evaluate the presence of ascorbic acid, peak 1 isolated by preparative HPLC from Emblica officinalis extract was freeze dried, and was successfully separated into mixture of four constituents on reverse phase HPLC and LCMS analysis was done using the same method. However on LCMS studies ascorbic acid presence was revealed FIG. 7. The $^{13}$C NMR of peak 1 established it to contain (STR#3), mucic acid 2-O-gallate.

**EXAMPLE 2: BIOLOGICAL POTENCY OF Emblica Officinalis FRUITS IN TERMS OF ANTI-OXIDANT ACTIVITY** (Figs 8 and 9).

**I. Oxygen Radical Absorbance Capacity (ORAC)** - ORAC value is a measure of inhibition of peroxyl radicals. ORAC value is represented as Trolox equivalents where the product is compared with Trolox, a standard inhibitor of peroxyl radicals. Higher the ORAC value, higher is the peroxyl radical inhibition. ORAC value is represented as Trolox equivalents per gm or liter by calculating the area under the curve formed by the inhibition of quenching of fluroscein dye induced by the peroxyl radical generator, azobis.
II. DPPH scavenging - Generation of free radicals in skin due to various stress conditions such as UV exposure, pollution, ageing etc, result in induction of melanin synthesis. The quenching of the stable free radical Diphenylpicryl hydrazyl (DPPH) in a methanolic reaction mixture by the antioxidant is an indication of the antioxidant potential.

[PARA 0032] In terms of both DPPH scavenging assay and ORAC values (Figs. 8 and 9), it is evident that β-glucogallin is a much better anti-oxidant than ascorbic acid or mucic acid gallates. With proof of (i) the absence of hydrolysable tannins such as Emblicanin A and Emblicanin B (ii) higher relative content of β-glucogallin over ascorbic acid in amla juice (Fig 11, Table A); (iii) from about 0.0001 0% to a maximum of about 4% of free ascorbic acid in amla; (iv) the enhanced anti-oxidant potential of β-glucogallin over ascorbic acid and mucic acid gallate (Mucic acid 1, 4-lactone 5-O-gallate); and (v) the relative unstable nature of mucic acid gallates, it is clear that the biological activity of amla is concentrated in β-glucogallin which represents the most optimal biological marker.

Table A

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Collection Amla Samples</th>
<th>β-Glucogallin mg/ml</th>
<th>Ascorbic acid mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Uttar Pradesh (Krishna + Narendra)</td>
<td>6.20</td>
<td>2.19</td>
</tr>
<tr>
<td>02</td>
<td>Maharasstra (Krishna)</td>
<td>6.52</td>
<td>2.85</td>
</tr>
<tr>
<td>03</td>
<td>Tamilnadu (BSR-1)</td>
<td>10.62</td>
<td>3.04</td>
</tr>
<tr>
<td>04</td>
<td>Rajasthan (Chakia)</td>
<td>9.52</td>
<td>2.85</td>
</tr>
<tr>
<td>05</td>
<td>Karnataka (Kanchan)</td>
<td>6.35</td>
<td>2.74</td>
</tr>
</tbody>
</table>
[PARA 0033] EXAMPLE III: BIOLOGICAL POTENCY OF Emblica Officinalis FRUITS IN TERMS OF UVB PROTECTANT ACTIVITY (Fig 10)

[PARA 0034] Varying concentrations of the test sample in the cell culture medium were added to the 24 hr monolayers of cells in a 96 well flat bottomed clear plates and then exposed to UV irradiation dose of 0.05 J cm-2. Each concentration was applied with 'n' value of 12. The plate layout was made in such a way that six of the replicates were exposed to UV and six unexposed. The unexposed region of the plate was covered by aluminum foil. After exposure, the cells were incubated in a CO2 incubator for 48 hrs and developed by the NRU staining techniques to analyze the cell viability. The percentage of UV protection was calculated with respect to the cytotoxicity in treated cells as compared to that of the untreated cells.

[PARA 0035] UV source: UV lamp of 14.7W, 0.3A, 55V with an intensity of 33.3 µW cm-2 was obtained from Sankyo Denki Co., Ltd, Japan.

[PARA 0036] EXAMPLE III: PRODUCT (SABERRY™) REPRESENTED BY THE PRESENT INVENTION (able B)

[PARA 0037] Table B

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>RESULTS OF ANALYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>DESCRIPTION</td>
<td>Beige colored odorless hygroscopic powder</td>
</tr>
<tr>
<td>IDENTIFICATION</td>
<td>To comply by TLC</td>
</tr>
<tr>
<td>SOLUBILITY</td>
<td></td>
</tr>
<tr>
<td>Water solubles (%w/w) (1% w/v solution in water)</td>
<td>Not less than 90.0% w/w</td>
</tr>
<tr>
<td>Residue on Ignition</td>
<td>Not more than 15% w/w</td>
</tr>
<tr>
<td>Moisture content in KF (%w/w)</td>
<td>Not more than 5% w/w</td>
</tr>
</tbody>
</table>

13
<table>
<thead>
<tr>
<th>Product</th>
<th>ORAC hydro (µm TE/g)</th>
<th>ORAC lipo (µm TE/g)</th>
<th>ORAC total (µm TE/g)</th>
<th>NORAC (µm CAE/g)</th>
<th>SOD kunitSODEq/g</th>
<th>SOAC (µm VITE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SABERRY™</td>
<td>2678</td>
<td>4</td>
<td>2682</td>
<td>345</td>
<td>904</td>
<td>102</td>
</tr>
</tbody>
</table>

1. ORAC ANALYSIS-Oxygen Radical Absorbance Capacity
2. ORAC hydro- Water soluble antioxidant capacity.
3. ORAC lipo—Lipid soluble antioxidant capacity.
4. NORAC-Peroxynt πte absorbance capacity
5. HORAC-Hydroxy l radical absorbance capacity
6. SOD-Superoxide dismutase activity
7. SOAC-Singlet oxygen absorbance capacity
In-house clinical data on the antioxidant capacity of SABERRY™.

Study Design: Two healthy subjects and four unhealthy subjects (age: 40 to 50) were recruited to participate in this study. All healthy participants were in good health as determined by the following eligibility criteria: 1) no history of cardiovascular, hepatic, gastrointestinal, or renal disease; 2) no alcoholism; 3) no antibiotic or supplemental vitamin and/or mineral use for at least 4 wk before the start of the study and 4) no smoking. The unhealthy individuals were obese and frequent smokers.

A 10 mL blood sample (zero baseline samples) was obtained from fasting subjects at the start of the study, following which they were given 200 mg per day of SABERRY™ for a period of one month. Blood samples (10 mL) were collected again after one month. ORAC assay and ROS scavenging assay were conducted for all of the serum samples.

Oxygen Radical Absorbance Capacity (ORAC)

Principle: The Oxygen Radical Absorbance Capacity (ORAC) assay depends on the free radical damage to a fluorescent probe through the change in its fluorescence intensity. The change of fluorescence intensity is an index of the degree of free radical damage. In the presence of antioxidant, the inhibition of free radical damage, which is reflected in the protection against the change of probe fluorescence, is a measure of its antioxidant capacity against the free radical.

Procedure:

25μl of each of the serum samples was pipetted into each well followed by 150μl of 10X10^2M (final cone) AAPH reagent (2,2'-Azobis (2-amidinopropane).
dihydrochloride) made in 75mM potassium phosphate buffer (pH 7.4). Then 150µl of disodium fluorescein dye (final cone.) 4.8X10^-7M was added and mixed before the initial reading (fo) was taken. Fluorescence reading were taken (Fluostar Optima Microplate Reader) at 485/520nm after every 1 minute for 35 minutes (fi, ..., f3s). 25µl of phosphate buffer (75mM) was pipetted instead of antioxidant in the blank. Trolox standard from 12.5 - 200 µM was also kept. The number of wells being used in the experiment should not exceed 20 to reduce the error due to time lag. Difference between duplicates also occurs due to the time lag and to avoid this, the experiment is to be repeated thrice with %CV not more than 15.

**[PARA 0046] Calculation:** The final ORAC values were calculated by using a quadratic regression equation \( K = a + bX + cX^2 \) between the trolox concentration \( Y \) (µM) and the net area under the Fluorescence decay curve \( X \) and were expressed as micromoles of trolox equivalents per liter or per gram of sample (µmol TE/g or µmol TE/L).

The Area under curve \( \text{AUC} = (1 + fi/fo + f2/fo + .... + f3s/fo) \) ——— eq 1

Where \( fo \) is the initial fluorescence reading at 0 min and \( fi \) is the fluorescence reading after 1 min.

The data were analyzed by applying eq 1. The net AUC was obtained by subtracting the AUC of the blank from that of the sample. The value calculated using the net AUC of the sample and the quadratic regression equation was divided by the weight of the sample in g/L. The final value obtained is the ORAC value of the sample expressed as µmol trolox equivalents (TE)/g.

**[PARA 0047] Result CTable D]:**
### Table D

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Health condition</th>
<th>ORAC value (μmol trolox equivalents (TE)/g) Before treatment</th>
<th>ORAC value (μmol trolox equivalents (TE)/g) After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1</td>
<td>Healthy</td>
<td>3100 ± 100</td>
<td>3210 ± 100</td>
</tr>
<tr>
<td>Subject 2</td>
<td>Healthy</td>
<td>3520 ± 110</td>
<td>3690 ± 108</td>
</tr>
<tr>
<td>Subject 3</td>
<td>Unhealthy</td>
<td>1312 ± 140</td>
<td>1600 ± 120</td>
</tr>
<tr>
<td>Subject 4</td>
<td>Unhealthy</td>
<td>1223 ± 120</td>
<td>1552 ± 102</td>
</tr>
<tr>
<td>Subject 5</td>
<td>Unhealthy</td>
<td>1556 ± 121</td>
<td>1842 ± 112</td>
</tr>
<tr>
<td>Subject 6</td>
<td>Unhealthy</td>
<td>1426 ± 142</td>
<td>1782 ± 122</td>
</tr>
</tbody>
</table>

Note: Higher ORAC value indicates better efficacy

**[PARA 0048] Conclusion:**

**[PARA 0049] The serum ORAC values for healthy individuals did not vary significantly before and after treatment with SABERRY™. However, for unhealthy individuals, the serum ORAC values increased significantly in the range of 10 - 25%. In conclusion, SABERRY™ is good for the maintenance of the body’s antioxidant potential. The reduced levels of antioxidant potential in unhealthy conditions were raised significantly and were not increased significantly in healthy conditions.**

**[PARA 0050] Reactive Oxyben Species (ROS) scavenging assay in Swiss 3T3 fibroblast cell line**

**[PARA 0051] Principle:** A cell permeable, non-fluorescent dye, DCFH-DA (dichloro fluorescein diacetate) after intracellular getting de-esterified is converted to dichlorofluorescein upon oxidation by the reactive oxygen species. The scavenging activity of sample is indicated by the decrease in fluorescence (wavelength 485/520nm).

**[PARA 0052] Procedure:** Serum samples (0.05ml) in 96 well black flat-bottomed tissue culture plates were incubated with 100μl of stock solution of DCFH-DA
(2mg/ml in ethanol, diluted to 100 times in phosphate buffered saline) for 1hr at 37°C and the fluorescence reading was taken at wavelength 485/520 nm (Fluostar optima microplate reader).

[PARA 0053] **Calculation:** The optical density (OD) measured is directly proportional to the ROS generated.

[PARA 0054] **Result (Table E):**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Health condition</th>
<th>OD due to ROS generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td>Subject 1</td>
<td>Healthy</td>
<td>0.41</td>
</tr>
<tr>
<td>Subject 2</td>
<td>Healthy</td>
<td>0.39</td>
</tr>
<tr>
<td>Subject 3</td>
<td>Unhealthy</td>
<td>0.81</td>
</tr>
<tr>
<td>Subject 4</td>
<td>Unhealthy</td>
<td>0.72</td>
</tr>
<tr>
<td>Subject 5</td>
<td>Unhealthy</td>
<td>0.96</td>
</tr>
<tr>
<td>Subject 6</td>
<td>Unhealthy</td>
<td>0.84</td>
</tr>
</tbody>
</table>

[PARA 0055] **Conclusion:**

[PARA 0056] The serum ROS levels for healthy individuals did not vary significantly before and after treatment with SABERRY™. However, for unhealthy individuals, the serum ROS levels decreased significantly in the range of 20 - 25%. In conclusion, SABERRY™ is good for reducing the excess ROS levels. The increased levels of ROS in unhealthy conditions were decreased significantly and were not affected in healthy conditions.
[PARA 0057] As an additional embodiment, the present invention also relates to the use of most optimal biomarker represented by STR#1 of Emblica Officinalis Gaertn. fruit (Amla fruit) and products standardized for 5% and above w/w of the said biomarker as peroxynitrite scavengers. Further, the products described herein above contain from about 0.00010 % to about 4% of free ascorbic acid depending on the raw material used. In specific embodiments of the present invention, the product standardized for 5% and above w/w of the said biomarker represented by STR#1 represents an aqueous extract from Emblica Officinalis Gaertn. fruit (Amla fruit). More specifically, the aqueous extract also comprises from about 50% to greater than 50% total gallates including mucic acid 1, 4-lactone 5-O-gallate represented by STR#2, mucic acid 2-O-gallate represented by STR#3, mucic acid 6-Methyl ester 2-O-gallate represented by STR#4, mucic acid 1-Methyl ester 2-O-gallate represented by STR#5 and ellagic acid represented by STR#6. In further specific embodiments, the present invention relates use of the aforesaid product for managing peroxynitrite-mediated damage occurring in Alzheimer’s disease.

[STR#1]

[STR#2]
While the invention has been described with reference to a preferred embodiment, it is to be clearly understood by those skilled in the art that the invention is not limited thereto. Rather, the scope of the invention is to be interpreted only in conjunction with the appended claims.
We claim,

1. A most optimal biomarker for the biological potency of Emblica Officinalis Gaertn. fruit (Amla fruit), said marker represented by STR#1.

![](image1.png)

[STR#1]

2. A product standardized for the compound represented by STR#1, said product comprising 5% and above w/w of the biomarker represented by STR#1.

![](image2.png)

[STR#1]

3. The product according to claim 2, wherein the said product is an aqueous extract of *Emblica Officinalis* Gaertn. fruit (Amla fruit).

4. The product according to claim 2, wherein the said product further comprises from about 0.00010 % to about 4% of free ascorbic acid depending on the raw material used.

5. The product according to claim 3, wherein said product comprises from 50% to greater than 50% total gallates also including mucic acid 1,4-lactone 5-O-gallate represented by STR#2, mucic acid 2-O-gallate represented by STR#3, mucic acid 6-Methyl ester 2-O-gallate represented by STR#4, mucic acid 1-Methyl ester 2-O-gallate represented by STR#5 and ellagic acid represented by STR#6.
6. The product according to claim 2, wherein said product is useful for scavenging peroxynitrite radicals.

7. The product according to claim 2, wherein said product is useful for managing peroxynitrite-mediated damage occurring in Alzheimer's disease.
FIG. 2

(Spotting from left to right: 1: Fraction 1, 2: Fraction 2, A: Ascorbic acid, 3: β-glucogallin, 4: Gallic acid, 5: Fraction 5, 6: Fraction 6, 7: Fraction 7)
FIG. 3 B.

(HPLC fingerprint of Peak-3)
FIG. 4

(HPTLC fingerprint of Amla Extract; 1: β-glucogallin, 2: mucic acid lactone gallate, 3: ellagic acid, 4: gallic acid)
FIG. 5

STR#1

STR#2

STR#3
FIG. 6C

(HPTLC fingerprint of Peak-1 spiked with Ascorbic acid)
FIG. 8

Comparative ORAC values [μmol TE/gm] (represented on Y axis) of A=Ascorbic acid; B=β-glucogallin and C=Mucic acid gallates (Mucic acid 1, 4-lactone 5-O-gallate) [represented on X Axis].
FIG. 9

Comparative DPPH Scavenging values [IC₅₀ µg/ml] (represented on Y axis) of A=Ascorbic acid; B=β-glucogallin and C=Mucic acid gallates (represented on X Axis).
FIG. 10
Comparative UVB protection values [EC₅₀ μg/ml] (represented on Y axis) of A=Ascorbic acid; B=β-glucogallin and C=Mucic acid gallates (represented on X Axis)

<table>
<thead>
<tr>
<th></th>
<th>UVB Protection EC50 values (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>![Graph Bar]</td>
</tr>
<tr>
<td>β-glucogallin</td>
<td>![Graph Bar]</td>
</tr>
<tr>
<td>Mucic acid gallate</td>
<td>![Graph Bar]</td>
</tr>
</tbody>
</table>

(Mucic acid 1, 4-lactone 5-O-gallate)
FIG. 11

- Ascorbic Acid (4.954 mAU, 5.66 min)
- Betaxanthin (4.42 mAU, 12.91 min)
- Gallic Acid (10.897 mAU, 12.34 min)
- Betacyanin (7.41 mAU, 1.12 mAU, 17.91 min)

mAU vs. min