GR

ORIGINAL SUBMISSION

000002

Natreon
...in synergy with nature

2-D Janine Place • New Brunswick, NJ 08901
P.O. Box 7398 • North Brunswick, NJ 08902-7398
Tel: 732-296-1080 • Fax: 732-296-1075
Email: info@natreoninc.com • www.natreoninc.com

July 8, 2013

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Subject: GRAS Notification for Amla concentrate (Capros®)

Dear Sir/Madam:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Natreon Inc hereby provides notice of a claim that the food ingredient Amla concentrate (Capros®) described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be Generally Recognized As Safe (GRAS), based on scientific procedures.

As required, please find enclosed three copies of the notification. If you have any questions or require additional information, please feel free to contact me by phone at 732-296-1080 or by email at sanni@natreoninc.com.

Sincerely,

(b) (6)

Sanni Raju, Ph.D., R.Ph.
Chairman and CEO

RECEIVED
JUL 17 2013
Division of Biotechnology and GRAS Notice Review

Natreon
...in synergy with nature

2-D Janine Place • New Brunswick, NJ 08901
P.O. Box 7398 • North Brunswick, NJ 08902-7398
Tel: 732-296-1080 • Fax: 732-296-1075

GRAS NOTIFICATION

I. Claim of GRAS Status

A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

Natreon Inc. (the notifier) has determined that Amla concentrate, a standardized spray-dried powder of the juice of the edible fruits of *Emblica officinalis* Gaertn, is Generally Recognized As Safe, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use as a food ingredient. Therefore, the use of Amla concentrate (Capros®) is exempt from the requirements of premarket approval.

Signed,

(b) (6) [redacted] Date 07/08/2013

Sanni Raju, Ph.D., R.Ph.
Chairman and CEO

B. Name and Address of Notifier:

Sanni Raju, Ph.D., R.Ph.
Chairman and CEO
Natreon, Inc.
2-D Janine Place
New Brunswick, NJ 08901
USA

Tel: (732) 296-1080
Fax: (732) 296-1075
Email: sanni@natreoninc.com

C. Common or Usual Name of the Notified Substance:

The common name of the substance of this notification is Amla concentrate. The concentrate is a spray-dried powder of the juice of the edible fruits of *Emblica officinalis* Gaertn (Amla). The trade name of the substance is Capros®.

D. Conditions of Intended Use in Food

Capros®, a standardized concentrate of Amla (Indian gooseberry) fruit juice, is intended for use as a flavoring agent (flavor enhancer) [21 CFR§170.3(o)(11)] [1] and as an antioxidant for consumer in the following food categories: non-alcoholic beverages and processed fruits and fruit juices at use levels up to 150 mg/serving (reference amounts customarily consumed, 21 CFR 101.12). Amla concentrate is not proposed for uses in foods that are intended for infants and toddlers, such as infant formulas or foods formulated for babies or toddlers, as well as it is not intended for use in meat and poultry products that come under USDA jurisdiction. The intended use of Capros® in the above mentioned food categories, is estimated to result in a maximum daily intake of 868 mg/person (15 mg/kg body weight/day for an individual weighing 60 kg).

E. Basis for GRAS Determination:

In accordance with 21 CFR 170.30, the intended use of Amla concentrate (Capros®) has been determined to be Generally Recognized As Safe (GRAS) based on scientific procedures. The determination is supported by the opinion of the Expert Panel. A comprehensive search of the scientific literature was also utilized for this determination. There exists sufficient qualitative and quantitative scientific evidence, including human and animal data to determine safety-in-use for Amla concentrate (Capros®). The source material of the concentrate has been used as a foodstuff and is listed as a major fruit in the USDA's inventory of perennial edible fruits of the tropics. The safety determination of Capros® is based on the totality of the available evidence. The safety-in-use of Amla concentrate is supported by multiple animal and human studies that have been performed with Amla preparations. The safety of Amla is well established in the literature based on the dietary consumption of foods containing Amla preparations. On the basis of scientific

[1] "Flavor enhancers": Substances added to supplement, enhance, or modify the original taste and/or aroma of a food, without imparting a characteristic taste or aroma of its own.

procedures[2], Natreon, Inc. considers the consumption of Amla concentrate (Capros®), as a food ingredient to be safe at levels up to 868 mg/person/day.

F. Availability of Information:

The data and information that forms the basis for this GRAS determination will be provided to Food and Drug Administration upon request or will be available for FDA review and copying at reasonable times at the above mentioned offices of:

Sanni Raju, Ph.D., R.Ph.
Natreon Inc.
2-D Janine Place
New Brunswick, NJ 08901

Tel: (732) 296-1080
Fax: (732) 296-1075
Email: sanni@natreoninc.com or info@natreoninc.com

II. Detailed Information About the Identity of the Notified Substance:

Capros® is a standardized spray-dried powder of the juice of the edible fruits of *Emblica officinalis*, commonly known in India as Amla.

A. Chemical name:

Not applicable. However, the primary active components of Capros® are tannins such as Emblicanin A, Emblicanin B, Punigluconin, Pedunculagin and other gallotannoids (low molecular weight hydrolysable tannins).

B. Trade Name:

The subject of this notification will be marketed as Capros®

C. Scientific name of Source Material:

Emblica officinalis Gaertn (syn: *Phyllanthus emblica*)

D. Synonyms:

Amla; Amla extract; Indian gooseberry; *Emblica officinalis*

E. Chemical Abstract Service Number:

Amla concentrate has not been assigned a CAS number

F. Molecular Weight

Not defined

G. Physical Characteristics

Capros® is a yellow powder with a characteristic odor and astringent taste.

H. Typical Specifications and Composition

000006

[2] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

Typical food grade specifications of Amla concentrate (Capros®) are presented in Table 1. Analytical data from five manufacturing lots prepared using water are presented in Appendix I. Some typical chromatograms used to identify the product are included in Appendix II. General compositional analysis of Capros® is presented in Table 2. Additional details of compositional analysis are included in Appendix III. The major components of Amla concentrate occur in the fruit, all of which are present naturally in the human diet via consumption of the more common Amla fruit, and other fruits and fruit products. The $ORAC_{FN}$ value of Capros® was found to be 47,110 μmole TE/g.

Table 1. Specifications of Amla concentrate (Capros®)

Parameter	**Characteristics**
Description	Yellow free flowing powder
Identification	To comply by HPLC/HPTLC
Water-soluble extractive value	≥ 80% w/w
Assay	
Low molecular weight hydrolysable tannins	≥ 60% (w/w)
Gallic acid	≤ 4%
Water (Karl-Fischer)	≤ 6% (w/w)
Sulfated ash	≤ 6% (w/w)
Heavy metals	
Lead	≤ 3 ppm
Arsenic	≤ 1 ppm
Cadmium	≤ 1 ppm
Mercury	≤ 0.1 ppm
Microbiological assays	
Aerobic bacteria (no pathogens)	< 5000 cfu/g
Yeast and Mold	< 500 cfu/g
Enterobacteriaceae	< 100 cfu/g
Candida albicans	Absent in 1 g
Escherichia coli	Absent in 1 g
Staphylococcus aureus	Absent in 1 g
Salmonella	Absent in 1 g
Pseudomonas aeruginosa	Absent in 1 g

Table 2. Compositional analysis of Capros®

Assay	**Typical value / 100 g**
Carbohydrate	88.13 g
Fat total	0.29 g
Fat saturated	0.17 g
Fat monounsaturated	0.05 g
Protein (actual)	2.04 g
Amino acids	5.10 g
Total dietary fiber	8.74 g
Water	≤ 6 g
Ash	<6.00 g
Calories	334.71

I. Manufacturing process

Capros®, Amla concentrate is manufactured according to current good manufacturing practices (cGMPs). This process is schematically presented in Figure 1. The starting material for the preparation of Capros® are fruits from the plant *Emblica officinalis* Gaertn. The process that yields Capros® (at 4.2% by weight of the native fruit) involves harvesting the fruit from the *E. officinalis* tree and preparing a finely pulped sample that is subsequently extracted with a very dilute aqueous solution that contains selected salts as identified below. The salts are critical to the process since they impede microbiological growth while also preventing the degradation of the desired antioxidant constituents by hydrolytic enzymes (glycosidases) that are naturally present in the fresh Amla fruit. The Amla concentrate consists primarily of an enriched mixture of natural antioxidants of low molecular weight, hydrolysable tannoids.

More specifically, the dilute aqueous solutions contain salts at levels ranging from 0.1 to 5%, and the specific salts that perform well were identified as sodium chloride, potassium chloride, calcium chloride, and magnesium chloride. Alternatively, the extracting solutions can be buffered with citrate or phosphate. The extraction process occurs with controlled heating to 70±5°C which facilitates the destruction of the indigenous hydrolytic enzymes. Following the extraction itself, the resulting content must be filtered prior to being held under refrigeration (at ~10°C) for three days. The final processing step is the spray drying or vacuum drying to yield the amorphous powder. The resulting material is analyzed to establish its chemical composition. The mixture contains low to medium molecular weight tannoids with Emblicanin-A and Emblicanin-B being the major tannoids. Punigluconin, Pedunculagin, Rutin, and gallo-ellagitannoids are other substances identified as being present in Capros®. The extraction procedure is monitored throughout the process to ensure a consistent and high quality Capros® product. Processing aids, including salts and fumed silicon dioxide (anti-adherent) used in the manufacturing are of food grade/USP quality as specified in the current Food Chemical Codex or USP.

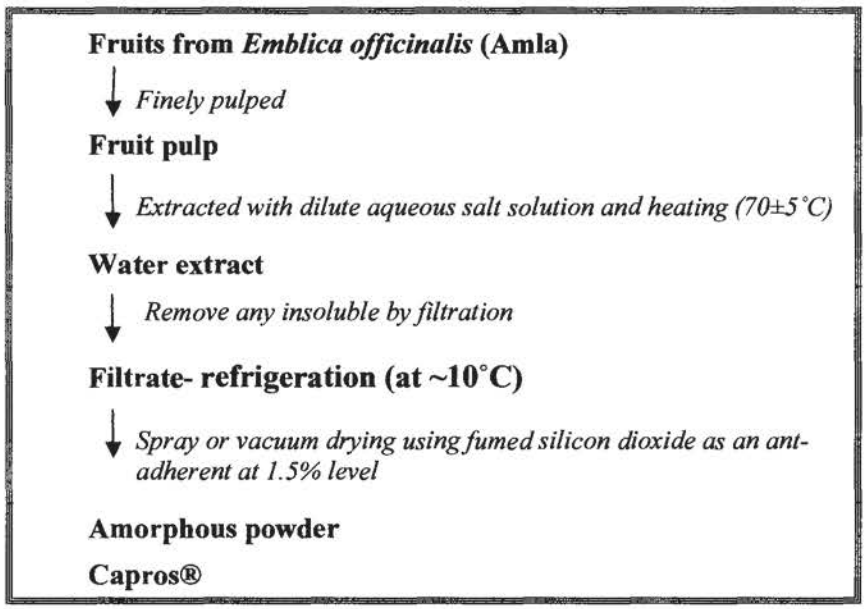


Figure 1. Manufacturing process of Amla Concentrate (Capros®)

000008

J. Intended Technical Effects

Amla concentrate is intended for addition to selected foods as a flavoring agent (flavor enhancer) [21 CFR 170.3(o)(11)][3] and as an antioxidant ingredient in the diet. The use of Amla concentrate is intended for the general population at the levels identified in this document for addition to non-alcoholic beverages and processed fruits and fruit juices at use levels up to 150 mg/serving (reference amounts customarily consumed, 21 CFR 101.12).

III. Summary of the Basis for the Notifier's Determination that Amla Concentrate is GRAS

The safety of Capros® under the intended conditions of use is supported by data demonstrating compositional similarity of the Amla concentrate (Capros®) itself, Amla fruit and its commonly used preparation and by the available preclinical and clinical trials conducted with Amla concentrate and other Amla preparations and/or Amla fruit itself. A comprehensive search of the publicly available scientific databases (PubMed, Toxline, etc.) for safety and toxicity information of Amla, *Emblica officinalis* was conducted through March 2013 and was utilized for this assessment. The intake of Capros® from the proposed uses was determined using the serving size data (21 CFR 101.12) and the mean and 90th percentile of each type of food of interest from the CSFII 1994-96 database. The intended use of Capros® in the above mentioned food categories, is estimated to result in a maximum daily intake of 868 mg/person. Based on a critical evaluation of the pertinent data and information summarized here and employing scientific procedures, it is determined that the addition of Amla concentrate to non-alcoholic beverages and processed fruits and fruit juices at use levels up to 150 mg/serving meeting the specification cited above and manufactured according to current Good Manufacturing Practice, is GRAS under the conditions of intended use as specified herein.

In arriving at this decision that Amla concentrate is GRAS for the proposed use, the Panel relied upon the conclusions that neither Amla nor any of its constituents or degradation products poses any toxicological hazards or safety concerns at the intended use levels, as well as on published toxicology studies and other articles relating to the safety of the product. It is also the opinion of the Expert Panel that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

[3] "Flavor enhancers": Substances added to supplement, enhance, or modify the original taste and/or aroma of a food, without imparting a characteristic taste or aroma of its own.

IV. Basis for a Conclusion that Amla Concentrate is GRAS for its Intended Use.

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF AMLA CONCENTRATE (CAPROS®)

TABLE OF CONTENTS

1. EXECUTIVE SUMMARY 7
1.1. Background 7
1.2. Description, Manufacturing Process and Specifications 8
1.3. Chemistry 8
1.4. Traditional and Current Uses 9
1.5. Technological Effects 11
1.6. Proposed Uses and Food Categories 11
1.6.1. Estimated Daily Intake 11
1.7. Comparison of Capros® Vs Amla Fruit 12
2. SAFETY-RELATED DATA 13
2.1. Toxicology 13
2.1.1. Specific Studies with Capros® (Emblica™) 14
2.1.1.1. Acute Toxicity 14
2.1.1.2. Primary Eye Irritation Study in Rabbits 14
2.1.1.3. Phototoxicity 14
2.1.1.4. Sensitization 14
2.1.1.5. Ames Assay 14
2.1.1.6. Subchronic Toxicity 15
2.1.2. Other Animal Studies 16
2.1.3. Human studies 18
2.2. Biochemical/Pharmacological Action 22
2.2.1. *In vitro* and Animal Studies 22
2.2.2. Human Efficacy Studies 23
3. SUMMARY AND DISCUSSION 25
4. CONCLUSION 27
5. REFERENCES 28
6. APPENDIX I 33
7. APPENDIX II 34
8. APPENDIX III 37
9. APPENDIX IV 38

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS FOR AMLA CONCENTRATE (CAPROS®)

1. EXECUTIVE SUMMARY

A comprehensive search of the scientific literature for safety and toxicity information on Amla (*E. officinalis*) fruit and its preparation was conducted through March 2013 and critically evaluated to determine the Generally Recognized As Safe (GRAS) status of Amla (*Emblica officinalis* Gaertn fruit) concentrate (Capros®) for its intended uses as a flavoring agent (flavor enhancer) [21 CFR§170.3(o)(11)] and as an antioxidant for consumer in non-alcoholic beverages and processed fruits and fruit juices at use levels up to 150 mg/serving is GRAS. The exposure from added Capros® in the proposed food uses in the total U.S. population is estimated as 868 mg/day. As described below, the weight of evidence clearly supports the safety and GRAS status of Amla concentrate (Capros®), when produced in accordance with cGMP to established food-grade specifications, for its intended use in non-alcoholic beverages and processed fruits and fruit juices. No studies were identified showing any adverse effects when this amount of Amla concentrate or other similar preparations is added to the diet.

1.1. Background

Emblica officinalis is a deciduous small to medium size tree native to tropical South-Eastern Asia. The tree is a graceful ornamental, normally reaching a height of 60 ft and in rare instances of 100 ft (Chaudhuri et al., 2002). The fruit of this plant also known as Amla (Indian gooseberry) is nearly spherical, light greenish yellow, quite smooth and hard on appearance, with six vertical stripes or furrows. The fruits are about one inch diameter in size and weights about 5 to 6 g (Patel and Goyal, 2012). The fruit has a sour, bitter and astringent taste. The United States Department of Agriculture's inventory of perennial edible fruits of the tropics has listed Amla as a major fruit (Martin et al., 1987). *Emblica officinalis* is also grown in the U.S. (Morton, 1987).

The *E. officinalis* plant has long been and remains to this day enormously popular in India for its fruits (Amla) that are commonly consumed as food and its extract is used in various prepared foods. In India, two rasayana[4] products, Chyawanprash and Triphala, contain Amla as the predominant ingredient (Dharmananda, 2006b). Not only is Amla broadly consumed in India, but it has been noted to be used in over 20 countries of the world for enhanced well-being (Ghosal et al., 2002). It has been reported that over 50% of the total sales of Ayurvedic[5] products in India is Chyawanprash and that the advantages to be derived from consumption of this product are commonly recognized among the Indian population. Virtually all producers of Ayurvedic-type formulations in India include Chyawanprash among their offerings (Dharmananda, 2006a).

---

[4] Rasayan, a Sanskrit word [with literal meaning: *Path* (ayana) *of the Juice* (rasa), or Elixir vitae], is used to describe chemistry and alchemy.

[5] Ayurveda (*Ayur* = long life; *veda* = knowledge)

1.2. Description, Manufacturing Process and Specifications

General descriptive parameters and properties of Amla concentrate (Capros®) produced by Natreon, Inc. are summarized in Table 1. The product is a spray-dried powder of the juice of the edible fruits with characteristic odor and astringent flavor. Detailed information about the identity of Amla concentrate along with specifications and manufacturing are summarized earlier in Section II (page 3). Spray drying is a process that is commonly used in the food industry. Products such as cheese powder, instant coffee, food colors, food flavors, spices, milk-based products, tomatoes, and many others are produced and marketed as spray-dried food products. This production process is consistent with what is described in FDA's guidance entitled: "Draft Guidance for Industry: Assessing the Effects of significant Manufacturing Process Changes, Including Emerging Technologies, on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances, Including Food Ingredients that are Color Additives" in that aqueous extract and spray-drying are commonly employed processes in the food industry. [See Section IV and Appendix I in this guidance document.][6]

1.3. Chemistry

The extensive consumption of Amla by human beings for centuries, and associated physical well-being, has prompted substantial interest in the chemical characterization of the fruit and its extract (Bhattacharya, et al., 1999; Pathak, 2002). Chemical structures of active constituents of Amla are presented in Figure 2. According to Bhattacharya et al. (1999) research performed during the second and third quarters of the 1900s yielded the conclusion that the biological effects of Amla fruits, including the well-recognized antioxidant clinical effects, were a consequence of the high vitamin C content. It was also thought that the presence of hydrolysable tannins--with gallic and ellagic acids--inhibited the degradation/oxidation of vitamin C (Bhattacharya, et al., 1999). This long-standing belief was determined to be incorrect following a comprehensive chromatographic and spectroscopic analysis with chemical transformations of the chemical constituents (Ghosal et al., 1996). Neither free nor conjugated vitamin C was detected. These investigators reported that the fruit contains low molecular weight hydrolysable tannins- Emblicanin A and B, along with Pedunculagin and Punigluconin. Subsequent investigations established that the significant antioxidant activity of Amla and its extract was due to the presence of a mixture of low molecular weight hydrolysable tannins that are derived from gallic and ellagic acids (Chaudhuri, 2005; Dharmananda, 2006b; Ghosal et al., 2002). However, the presence and quantity of ascorbic acid in Amla remained a dubious issue for a long time (Scartezzini et al., 2006; Raghu et al., 2007).

[6] The complete document is available at:
http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm300661.htm

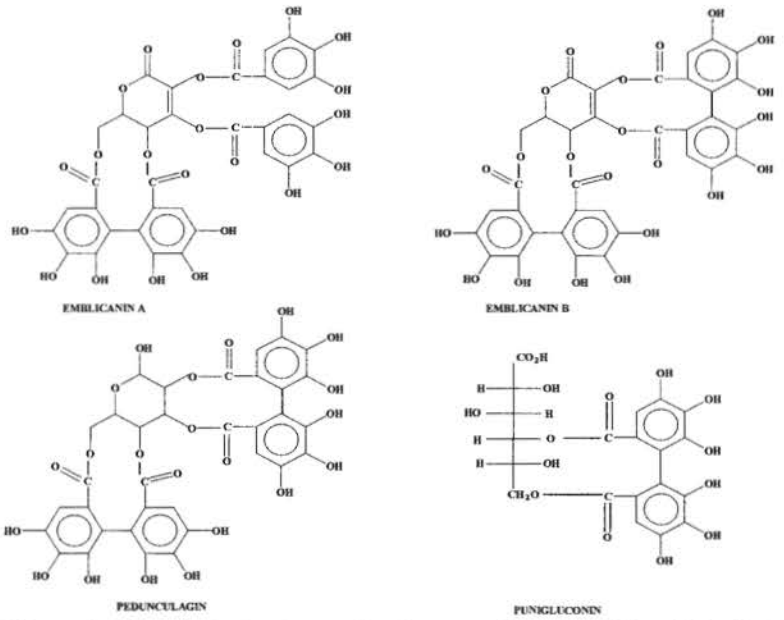


Figure 2. Chemical structure of active constituents of *E. officinalis* fruit

1.4. Traditional and Current Uses

The perceived benefits of ingestion of *E. officinalis* fruit (Amla) has provided sustained motivation over generations to consume this fruit and its preparation. The use of Amla as the predominant ingredient in popular rasayanas is reported to date to ancient times as both a home remedy used extensively throughout India and as prescribed by Ayurvedic practitioners. Rasayanas, especially *Emblica*-containing chyawanprash, have been consumed for thousands of years. Several references to ancient writings are reported by Ojha (2003) where *Emblica* as consumed in chyawanprash was acknowledged for its health benefits. Other standard Indian health references include listings of Amla. The Ayurvedic Pharmacopoeia of India lists Amla when consumed either as fresh fruit pulp or as dried fruit pulp for multipurpose applications (Ayurvedic Pharmacopoeia, 2001).

In a 1919 publication in the journal Lancet, Chick, et al. reported that the consumption of dried fruits of *Phyllanthus emblica* (Amla) by Indian troops was effective in both preventing and curing scurvy (Chick et al., 1919). The authors stated that the native population in India had long consumed the fruit as an antiscorbutic, and that this food was commonly bought and sold at the native markets. There was further reference to an 1837 Indian medical journal whereby an outbreak of scurvy at Nassirabad, Rajputana in 1833-1834 was effectively treated with the dried fruits of Amla. The publication also reported that the minimum daily dose of the dry fruit needed to be effective was 14 grams. With very specific information documenting the consumption of *E. officinalis* (Amla), there remains little doubt of the extensive use of Amla as a food product. Amla has been consumed by large numbers of Indians for centuries, and the consumption of Amla has carried forward to modern times not only in India but in Japan, Europe and the U.S.

In India and other parts of Southeast Asia, the ripe fruits (Amla) are commonly consumed fresh or in the form of its juice. Eating the highly acidic, fresh, raw fruit, followed by water, produces a sweet and refreshing aftertaste (Morton, 1987).

Additionally, food preparations from dried fruits are also very common. The fresh juice of Amla in combination with two other fruit juices [Myrobalans- chebulic (*Terminalia chebula*) and Beleric (*Terminalia belerica*)] in the form of a decoction known as *Triphala* (three fruits) is also commonly consumed as a cooling and *sherbet.* Additionally, the fruit is commonly pickled with salt, oil and spices or preserved in sugar; these preparations are considered a delicacy. In Indian homes it is a common practice to cook the fruits whole with sugar and saffron and give one or two to children every morning (Morton, 1987). Fresh Amla are often baked in tarts, added to other foods as seasoning during cooking, and the juice is used to flavor vinegar. The composition of Amla as a food is presented in Table 3 (Morton, 1987).

Table 3. Food Value Per 100 g of Edible Portion*

Composition	Value
Moisture	77.1 g
Protein	0.07 g
Fat	0.2 g
Carbohydrates	21.8 g
Fiber	1.9 g
Ash	0.5 g
Calcium	12.5 mg
Phosphorus	26.0 mg
Iron	0.48 mg
Carotene	0.01 mg
Thiamine	0.03 mg
Riboflavin	0.05 mg
Niacin	0.18 mg
Tryptophan	3.0 mg
Methionine	2.1 mg
Lysine	17.0 mg
Ascorbic Acid**	625 mg
Fat	0.2 g

*Adapted from Morton (1987) - As reported by the Finlay Institute Laboratory, Havana. **The ascorbic acid ratings vary immensely. Analyses in Puerto Rico, showed 625 mg; fruits from one tree in Avon Park, Florida, showed only 467 mg, while 2 adjacent trees in Homestead, Florida, showed 1,130 and 1,325 mg; and Dr. Margaret Mustard reported an average of 1,561.0 and a high of 1,814 mg in 7 samples analyzed.

Both ripe and half-ripe Amla are candied whole and also made into jams, other preserves, sweetmeats, pickles and relishes. Amla are combined with other fruits in making chutney. In Indonesia, Amla are added to impart acidity to many dishes, often as a substitute for tamarinds. Amla by itself or along with vegetables is used in the preparation of curry that is commonly consumed in India. Furthermore, individuals over the age of 40 in India are said to consume 1 to 3 teaspoons of Chyawanprash (or 10 g to 30 g) per day directly, as part of their diets in the form of a bread spread or mixed in warm milk (Dharmananda, 2006a; Anonymous, 2006). In recent years, an extract from

Amla fruit is becoming popular in Western countries as a dietary supplement (Poltanov et al., 2009). As a dietary supplement in the U.S., Amla and its preparations are marketed under the names Indian Goosberry extract, AmlaMax™, SunAmla™, Anwala Churna, etc. The recommended dose of these products ranges between 3 to 6 g per day. In some reports, the recommended dose has been extrapolated from a common dose of vitamin C of 1 g/day.

1.5. Technological Effects

The characteristic flavor enhancing effects of Amla and its preparations are known to influence the sensory perception of food. The sour taste of Amla fruit and its preparations is well recognized. It is noted that the intended uses of Capros® are for addition as a flavoring agent (flavor enhancer) [21 CFR 170.3(o)(11)] and as an antioxidant to foods.

1.6. Proposed Uses and Food Categories

Natreon, Inc. proposes to use Capros® as a flavoring ingredient in non-alcoholic beverages and processed fruits and fruit juices at use levels up to 150 mg/serving (reference amounts customarily consumed, 21 CFR 101.12). It is recognized that there are Standard of Identity requirements for some of these specified foods and these foods will not be referred by their commonly recognized names. The proposed use levels of Capros® and food categories are presented in Table 4.

1.6.1. Estimated Daily Intake

The estimates of possible daily intake of Capros® from its proposed uses were calculated using the FDA guidelines using serving size data and the mean and 90th percentile of each type of food of interest from the CSFII 1994-96 database (Smiciklas-Wright et al., 2002). Based on USDA CSFII surveys for quantities of foods consumed daily, the mean and 90th percentile consumption of Capros® from the proposed uses in beverages and processed fruit juices was determined (Table 4). The CSFII data provides intake of different juices. However, it is anticipated that individuals drinking one kind of fruit juice are unlikely to drink the other kind of fruit juice at the same time. Hence, for the determination of fruit juice intake, only one fruit category, i.e., total orange juice, is considered as representative for other fruit juices intake. The intended use of Capros® at levels of 150 mg *per* serving will result in mean and 90th percentile intake of 444.87 and 867.49 mg/person/day, respectively. For safety assessment purposes, the maximum intake of 868 mg/person/day of Capros® (14.47 mg/kg bw/day, for an individual weighing 60 kg) is considered as appropriate.

Table 4. Intended Use Levels (150 mg/serving) and Possible Daily Intake of Capros® Based on USDA Data[1]

Food category	Consumption of food product (g/day)		Use levels (mg/kg)	Serving size; RACC (g)	Daily intake by adult (mg/person)	
	Mean	90th %			Mean	90th %
Beverages (non-alcoholic)	507	947	625	240	319.87	591.87
Processed fruits and fruits juices[2]	200	441	625	240	125.00	275.62
				Total (mg/person/day)	444.87	867.49

[1]The daily intake calculations are based on USDA data (CSFII) and mean portion size; [2]Total orange juice intake considered to represent intake of other fruit/vegetable juices; RACC – Reference Amounts Customarily Consumed Per Eating Occasion (21 CFR §101.12)

1.7. Comparison of Capros® Vs Amla Fruit

The available information as discussed earlier suggests that the current and traditional use of Amla fruit has a robust history. However, the proposed use of aqueous hot water extract/concentrate (subject of current GRAS) lacks such a history. As the Natreon production process does not chemically alter any of the components, it is likely that the Natreon product (Capros®) is substantially equivalent to the historically consumed forms of Amla. In order to address this similarity, Natreon has taken an approach to scientifically bridge the chemical identity by showing that the subject of this GRAS notice is similar (substantially equivalent) to Amla fruit.

Natreon performed a study to compare the primary (active) chemical constituents of Capros® with Amla preparations derived from the same lot of fruits using different methods such as spray drying, lyophilization and Amla juice. A comparison of the constituents of Amla fruit and its preparations with Capros® did not reveal any new constituents. This suggests that the Amla extract is substantially equivalent to the Amla fruit and its preparations as consumed. The levels that Natreon proposes to add to food products is expected to be similar to what would be consumed by consuming the fruit in its many different products.

For the comparison, tannins such as Emblicanin A, Emblicanin B, Punigluconin and Pedunculagin, and total gallotannoids (low molecular weight hydrolysable), along with methionine and total amino acids were quantitatively analyzed (Appendix IV). The results of this analysis (Table 5) showed that the levels of Emblicanin A is lower, while that of Emblicanin B is higher in Capros® as compared to the other three products. This is expected because some of Emblicanin A gets converted to Emblicanin B, during production-scale drying, which is also an antioxidant (hence the name, Cascading antioxidant). Similarly, for the same reason, Capros® has higher levels of Punigluconin and lower levels of Pedunculagin as compared to other preparations (Table 5). Despite these changes, the total amount of low molecular weight hydrolysable tannins remains at 94% in Capros® as compared to Amla juice. These results suggest that the active ingredients of Capros® are substantially equivalent to other Amla preparations. A complete study report is included as Appendix IV.

Table 5. Comparison of Active Constituents of Capros® with other Amla Preparations

Preparations	Emblicanin A (µg/20 µg sample)	Emblicanin B (µg/20 µg sample)	Punigluconin (µg/20 µg sample)	Pedunculagin (µg/20 µg sample)	Total Low Mol. Wt. Hydrolysable Tannins, µg/20 µg sample
Capros®	3.496	7.970	2.754	3.682	17.902
Amla/Spray dried	7.950	4.156	1.714	5.276	19.096
Amla/Lyophilized	8.364	3.876	1.442	5.264	18.946
Amla juice	8.384	4.066	1.174	5.456	19.080

2. SAFETY-RELATED DATA

As described above, Amla and its preparations have been commonly used as food for centuries, particularly in India and other Southeast Asian countries. The safe uses of Amla and its products for generations have diminished the perceived need for detailed scientific inquiry to establish the safety of Amla in more recent times. Even so, there has been interest in better understanding what health consequences are available from Amla and how it functions as it does. These interests have prompted several experimental *in vitro,* animal and human studies with Amla or its extract to evaluate efficacy as an antioxidant or for different health conditions. A comprehensive search of the published literature did not reveal any significant adverse effects of Amla or its preparations. As in addition to efficacy, relevant safety end-points were also included in some of the published studies with Amla and its preparations, these studies are evaluated from safety and tolerability points of view. An attempt has been made to interpret the findings from such studies and their relevance to the current GRAS assessment. Relevant biological and toxicological studies on Amla and its preparations are included in the following section in the order of their importance and in support of the conclusions drawn in this determination.

2.1. Toxicology

The safety profile (acute toxicity, eye irritation, phototoxicity, sensitization and mutagenicity) of a standardized preparation of *E. officinalis* fruit called Emblica™ (a trade name product of Merck KGaA, Darmstadt, Germany and EM Industries, Inc., USA) was investigated and published by Chaudhuri (2002). The product used in these studies is identical to Capros®. The available information described in the publication by Chaudhuri (2002) as well as some of the study reports, including subchronic toxicity, provided by Natreon were reviewed and the findings from these studies are presented below. In addition to these investigations, effects of *E. officinalis* fruit (Amla) extract are also reported in other studies. These studies are summarized in the following sections.

2.1.1. Specific Studies with Capros® (Emblica™)

2.1.1.1. Acute Toxicity

In an acute limit-test oral toxicity study, the LD_{50} of Capros® (Emblica™) was reported as >5,000 mg/kg (Chaudhuri, 2002; Product Safety Labs, 2000). The study was conducted in compliance with the good laboratory practice as defined in 21 CFR part 58: U.S. FDA Good Laboratory practice Standards and in accordance with Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food, Guidelines for Acute Oral LD50 Toxicity Studies. In this study, 10 Sprague Dawley rats, 5 male and 5 female, were tested with observations being made over 14 days. All animals survived, gained weight, and appeared to be healthy with no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for the animals when necropsied after the 14-day observation period.

2.1.1.2. Primary Eye Irritation Study in Rabbits

Chaudhuri (2002) also reported a primary eye irritation test conducted with rabbits to determine the potential for Capros® (Emblica™) to produce irritation from a single instillation via the ocular route. Under the conditions of this study, Capros® (Emblica™) is classified as minimally irritating to both the unrinsed and rinsed eyes of rabbits.

2.1.1.3. Phototoxicity

A phototoxicity test was conducted with 20 human subjects to determine the potential for Capros® (Emblica™) to produce phototoxicity from a single dermal application. Under the conditions of this study, the test substance is classified as nonphototoxic when tested on human subjects at 2% dilution in distilled water (Chaudhuri, 2002).

2.1.1.4. Sensitization

In a repeat insult patch test, potential for Capros® (Emblica™) to produce primary irritation and primary sensitization were investigated with 100 human subjects. Under the conditions of the study, the test substance is considered as a nonprimary irritant and a nonprimary sensitizer to the human skin (Chaudhuri, 2002).

2.1.1.5. Ames Assay

A salmonella mutagenicity test was conducted to determine the potential for Capros® (Emblica™) at 50 and 100 μg/plate to produce mutagenicity. Under the conditions of the study, the test substance is considered as a nonmutagenic material (Chaudhuri, 2002). In addition to this, a complete report of a mutagenicity study conducted for Merck KGaA was provided by Natreon. Based on the report, the mutagenic potential of Capros® (Emblica™) were investigated using *Salmonella typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537, and *Escherichia coli* WP2 uvrA. The plate incorporation test with and without addition of liver S9 mix from Aroclor 1256 pretreated rats was used. Capros® (Emblica™) was dissolved in water and tested at concentrations ranging from 5 to 5000 μg/plate. Precipitation of the test material on the agar plates and toxicity to the bacteria was not observed. Each treatment with the substances used as positive controls led to a clear increase in revertant colonies,

thus, showing the expected reversion properties of all strains and good metabolic activity of the S9 mix used. With and without addition of S9 mix as the external metabolizing system, Capros® (Emblica™) was not mutagenic under the experimental conditions described.

2.1.1.6. Subchronic Toxicity

In an unpublished 90-day feeding study, potential adverse effects, if any, of Capros® were investigated in rodent (rat) and non-rodent (rabbit) species (Muruganandam and Das, 2000). In this study, Sprague Dawley rats (10/sex/group) as well as New Zealand white rabbits (3/sex/group) were divided into three groups (control and two treatment groups). Animals of either species in control group received vehicle (water), while treatment groups received Capros® at dose levels of 100 and 500 mg/kg bw/day via oral gavage. During the course of study, the animals were observed for clinical signs. The food and water intake was measured, as was the total body weight and organ weights. Hematology and clinical chemistry parameters were measured at termination. The clinical signs did not show any treatment related clinical signs in rats as well as in rabbits as evaluated by behavior, appearance, diarrhea or any discharge from orifices. Similarly, no treatment-related adverse effects of Capros® were noted on food consumption, water intake or body weight gains in any of the treatment groups in either species. A significant increase in the feed intake was noted in rats receiving Capros® at the dose of 100 mg/kg and a significant increase in the feed and water intake was observed in the rabbits receiving 100 and 500 mg/kg Capros®, respectively. However, these changes were not considered as adverse.

The hematological examination revealed an increase in the mean hemoglobin concentration and red blood cell (RBC) count in both rats and rabbits treated with Capros®. However, the increases in the levels of hemoglobin and RBC were well within the reported physiological range of the rats and rabbits. It should be noted that these experiments were not well designed and did not follow the current guidelines or GLP for such studies. No leukocytosis or leukopoenia was noticed in rats and rabbits of any group in the study. The differential white blood cell count also did not show any abnormality in their population percentage as compared to the control group. The mean value of platelet count in rabbits receiving 100 mg/kg was significantly higher in comparison to vehicle-treated rabbits; however, at the 500 mg/kg dose, no such increase was noted.

Various biochemical markers (glutamic oxaloacetic transaminase-GOT, glutamic pyruvate transaminase-GPT, Glucose, Cholesterol, high density lipoprotein-HDL, Total protein and Albumin) in the experimental groups of rats and rabbits revealed that hepatic function of the experimental groups was not adversely affected by Capros® treatment. In rabbits treated with Capros® (500 mg/kg), a significant decrease in GOT level as compared to control was noted. The total protein and albumin values were not reduced in experimental rats compared to the control group. Blood urea nitrogen (BUN) and plasma creatinine levels were found to be decreased in both species following treatment with Capros®. However, the decreased values were found to be within the physiological range (0.2-0.8 mg/dl) of the animals. Control animals had the creatinine levels at the upper side of the limit. The decreases in clinical chemistry parameters (GOT, BUN, creatinine) are believed to be non-specific and are not indicative of any adverse effect. The significant changes noted were either limited to one sex, lacked dose-response, were within the

normal laboratory ranges, and/or were not supported by any other changes in related clinical parameters. The investigators reported that no histopathological examinations were conducted as there were no toxic effects observed in the present study.

2.1.2. Other Animal Studies

In a chronic toxicity study, Jaijoy et al. (2012) investigated the possible adverse effects of the standardized water extract from Amla fruits. The extract was standardized by high performance liquid chromatography (HPLC) using gallic acid as a reference standard. In the chronic toxicity study, seven week old male and female Sprague-Dawley rats were randomly divided into five groups of 10 rats each. According to the WHO guidelines and the OECD 452, the extract was administered orally at concentrations of 300, 600 and 1,200 mg/kg bw/day to the three subsequent treatment groups for consecutive 270 days, while the control group received distilled water. In order to assess reversibility and/or accumulation effects, the extract at the dose of 1,200 mg/kg was given once daily to the satellite group of rats for 270 days, and kept for another 28 days post treatment. No change in behavior or toxic signs was noted during the experimental period. Body weight and the body weight gain in the treatment groups of female and male rats were significantly lower than those of their control groups. In the 600 and 1,200 mg/kg dose groups, body weight in the male treatment group significantly decreased on the 180th day, while the average body weight of the satellite group stayed normal until the end of the experiment. In addition, body weight and weight gain of all the treatment groups significantly decreased on the 270th day (Jaijoy et al., 2012).

Hematological examinations revealed significant increase in platelet counts in the female rats treated at the dose level of 600 mg/kg bw/day, while no change in the male rats was noted. A significant increase in eosinophils and neutrophils was found at the 300 mg/kg/day dose in the female and male treatment group, respectively. Furthermore, a significant decrease in neutrophils, and an increase in lymphocytes was observed in the female satellite group. However, these values were also within the normal range indicating that the Amla water extract does not affect hematopoiesis and leukopoiesis in rats. The clinical chemistry parameters such as glucose, BUN, creatinine, total protein, albumin, total and direct bilirubins, SGOT, SGPT, and ALP were evaluated. In general, the investigators considered clinical blood chemistry values more or less than one fold from the normal values to represent abnormality of kidney, liver and pancreas function. The results showed significant differences among the experimental groups in albumin, SGOT, and ALP. Nevertheless, these significant values also fell within the normal ranges indicating the healthy status of liver and kidney in the treatment rats. Overall, there were no significant differences in these values between the treatment and control groups (Jaijoy et al., 2012).

Among the organ weights, kidney and heart weights of the female treatment group with 300 and 600 mg/kg bw/day doses respectively were significantly lower than that of their control. The satellite female group showed a significant increase in the heart and liver weights. The organ weights of male groups revealed significant weight decrease in the brain and spleen for the 300 mg/kg bw/day dose, and in brain, heart, livers and spleen for the 600 mg/kg bw/day dose. At the dose of 1,200 mg/kg/day, significant weight decrease was found in the brain. Necropsy and histopathological examinations were performed to further confirm whether or not the internal organs or tissues had been

affected. However, no macroscopic or microscopic changes were detected in the internal organs or tissues in any of the treatment rats. The NOAEL of the extract was considered as 1,200 mg/kg bw/day, the highest dose tested (Jaijoy et al., 2012). The findings from Jaijoy et al. (2012) study are applicable to the present GRAS determination of Capros® as both products are water extracts of Amla.

In two separate studies, Yokozawa et al. (2007a; 2007b) investigated the effects of Amla on oxidative stress, renal function and lipid profile in young (2 months old) and aged (13 month old) male rats. Wistar rats (young and aged male) were administered (gavage) two grades of Amla extract [an enzymatic Amla fruit juice extract, SunAmla (Taiyo Kagaku Co., Ltd., Japan), and a polyphenol-rich fraction of Amla fruits derived from an ethyl acetate (EtOAc) extraction]. The SunAmla preparation is a dry powder. It is prepared as a water extract of Amla fruit that has been hydrolyzed with pectinase followed by centrifugation, collection of the supernatant and spray drying. In this study, the Amla extracts in water were given orally by gavage to rats for 100 days. SunAmla was administered at a dose level of 40 mg/kg bw/day, while EtOAc extract of Amla was given at 10 mg/kg bw/day). The contents of polyphenols were 11.8 and 8.0 mg/kg bw/day, respectively. The oral administration of SunAmla or EtOAc extract of Amla significantly reduced thiobarbituric acid-reactive substance levels of serum, renal homogenate, and mitochondria in aged rats, suggesting that Amla would ameliorate oxidative stress under aging. The investigators concluded that Amla may be beneficial in preventing age-related kidney diseases (Yokozawa et al., 2007a). The lipid levels, such as cholesterol and triacylcglycerides, in serum and liver were markedly elevated in aged control rats, while administration of Amla resulted in significantly decreased levels of these lipids. (Yokozawa et al., 2007b).The results of these studies indicate that administration of Amla extract at a dose of 10 or 40 mg/kg/day for 100 days did not cause adverse effects (Yokozawa et al., 2007a; 2007b).

Mathur et al. (1996) investigated hypolipidaemic effect of the fruit juice of *E. officinalis* in cholesterol-fed rabbits (rendered hyperlipidaemic by an atherogenic diet and cholesterol feeding). In this study, the fresh Amla juice was administered to rabbits at a dose level of 5 ml/kg bw/day for 60 days. Serum cholesterol, triglycerides, phospholipid and LDL levels were lowered by 82%, 66%, 77% and 90%, respectively. Similarly, the tissue lipid levels showed a significant reduction following Amla juice administration; aortic plaques were regressed. Amla juice treated rabbits excreted more cholesterol and phospholipids, suggesting that the mode of absorption was affected. The investigators concluded that Amla juice is an effective hypolipidaemic agent.

Biswas et al. (1999) reported that dietary administration of a crude aqueous extract of Amla reduced significantly the cytotoxic effects of sodium arsenite. In this study, the crude extract (685 mg/kg bw) of Amla juice was given daily by gavage to Swiss albino mice for 7 and 14 days, followed by a single dose of sodium arsenite (2.5 mg/kg bw). The animals were killed after 24 hours and chromosome preparations made following a schedule of colchicine-fixative-air drying-Giemsa. The endpoints screened were chromosomal aberrations and damaged cells. The crude extract reduced arsenic damage bringing the cells almost to the normal level. The results of this study suggest that aqueous extract of Amla does not cause cytotoxic effects or chromosomal aberrations.

2.1.3. Human studies

In a prospective randomized double-blind, placebo and positive controlled clinical trial (Usharani, P. and Sravanti, I.V. Nizam Institute of Medical Sciences, Hyderabad, India, accepted for publication in the Journal of Diabetes, Metabolic Syndrome and Obesity, Dovepress), a total of 80 Type 2 diabetic subjects were divided into four groups (20/group). In this study, Groups I and II received, Capros® at a dose of 500 mg and 1000 mg/day. Group III received Atorvastatin 10 mg at night plus placebo in the morning. The fourth group (Group IV) received placebo. All groups received the respective dosage daily for a period of 12 weeks. The diabetes in all subjects was stabilized (for 8 weeks prior to screening) by treatment with Metformin. In addition to efficacy related parameters, hematological and clinical chemistry parameters were investigated. All the subjects underwent complete physical examination, and safety evaluations at baseline and at the end of the treatment. Samples were collected after an overnight fast of 12 hours after the last dose of medication for determination of hemoglobin, HbA1c, blood urea and serum creatinine, liver function test, and lipid profile (total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C). The changes noted in these parameters at the end of 12 weeks are summarized in Table 6. The safety related parameters did not reveal any significant changes. The results of this study in type-2 diabetic population suggest that daily administration of Capros® at a dose of 1000 mg/day for 12 weeks is safe.

Table 6. Effects of Capros® at dose levels of 500mg/day and 1000 mg/day for 12 weeks on different blood parameters in type 2 diabetcs stabilized on Metformin

Parameters	CAPROS® 500 mg n=20		CAPROS® 1000 mg n=20		Atorvastatin 10 mg n=20		Placebo n=20	
	PreTT	Post TT	PreTT	Post TT	PreTT	Post TT	PreTT	Post TT
Systolic BP(mmHg)	119.40 ±4.55	118.90 ±3.92	118.20 ±4.25	119.40 ±2.90	121.00 ±2.86	118.40 ±2.30	120.1 ±2.20	117.90 ±1.89
Diastolic BP(mmHg)	79.40 ±3.44	78.40 ±3.35	77.90 ±3.58	76.50 ±3.36	80.00 ±3.37	78.10 ±3.14	79.50 ±2.50	78.00 ±1.83
Heart rare (bpm)	70.05 ±3.41	69.95 ±3.12	72.85 ±3.90	73.20 ±4.89	70.10 ±3.37	69.90 ±3.11	69.85 ±1.75	69.65 ±1.09
Hemoglobin (gm/dl)	12.72 ±1.29	12.59 ±1.23	12.75 ±1.43	12.63 ±1.31	12.51 ±1.06	12.43 ±0.99	12.87 ±0.81	12.88 ±0.82
WBC Count (/mm3)	7040 ±624.4	7000 ±618.1	6580 ±101.0	6708 ±693.6	6675 ±516.9	6705 ±505.2	6735 ±567.8	6700 ±501.6
Platelet Count (10^5/ mm3)	2.50 ±0.29	2.47 ±0.34	2.83 ±0.64	2.72 ±0.69	2.42 ±0.55	2.45 ±0.50	2.60 ±0.42	2.62 ±0.46
Blood Urea (mg/dl)	22.95 ±4.83	23.15 ±4.77	25.30 ±7.04	23.83 ±4.82	19.80 ±3.58	20.80 ±4.17	20.40 ±3.70	20.15 ±3.28
Serum Creatinine (mg/dl)	0.98 ±0.07	0.98 ±0.08	1.01 ±0.10	1.01 ±0.08	0.96 ±0.11	0.95 ±0.11	1.00 ±0.08	0.99 ±0.08
AST(SGOT) (U/L)	23.35 ±4.32	23.85 ±3.99	28.65 ±7.55	26.80 ±5.87	21.50 ±2.42	21.50 ±2.95	21.70 ±2.66	22.40 ±2.68
ALT(SGPT)(U/L)	25.10 ±3.24	26.25 ±3.24	26.00 ±7.45	26.90 ±6.31	23.95 ±4.03	24.80 ±4.74	23.10 ±3.49	23.50 ±3.39
Alkaline Phosphatase (U/L)	175.4 ±22.1	176.6 ±25.7	178.7 ±20.1	181.9 ±19.0	173.5 ±18.6	171.1 ±21.9	162.8 ±17.9	163.0 ±17.9
Total Bilirubin (mg/dl)	0.21 ±0.08	0.19 ±0.08	0.26 ±0.05	0.26 ±0.05	0.23 ±0.08	0.26 ±0.06	0.22 ±0.06	0.21 ±0.06

PreTT = Pretreatment; PostTT = Post treatment

In a second prospective randomized double-blind and placebo controlled clinical trial (Usharani, P. and Sravanti, I.V. Nizam Institute of Medical Sciences, Hyderabad, India, (2012), being prepared for publication), a total of 54 type 2 diabetic subjects were screened and 40 selected subjects were divided into two groups (20/group). In this study, Group I received Capros® at a dose of 1000 mg/day and Group II received the placebo capsules. All groups received the respective dosage daily for a period of 12 weeks. The diabetes in all subjects was stabilized (for 8 weeks prior to screening) by treatment with Glimiperide. In addition to efficacy related parameters, hematological and clinical chemistry parameters were investigated. All the subjects underwent complete physical examination, and safety evaluations at baseline and at the end of the treatment. Samples were collected after an overnight fast of 12 hours after the last dose of medication for determination of hemoglobin, HbA1c, blood urea and serum creatinine, liver function test, and lipid profile (total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C). The changes noted in these parameters at the end of 12 weeks are summarized in Table No.7. The safety related parameters did not reveal any significant changes. The results of this study in type-2 diabetic population suggest that daily administration of Capros® at a dose of 1000 mg/day for 12 weeks is safe.

Table No.7: Effect of CAPROS® 1000mg/day and placebo on blood parameters in type 2 diabetics stabilized on Glimiperide (All values expressed as Mean±SD)

Parameters	CAPROS® 1000mg (n=20)		PLACEBO (n=20)	
	Pretreatment	**Post treatment**	**Pretreatment**	**Post treatment**
Systolic BP(mmHg)	117.33±4.22	118.6±3.32	117.9±2.79	118.5±2.91
Diastolic BP(mmHg)	76.8±3.00	75.3±2.85	77.4±2.84	76.7±2.45
Heart rate (bpm)	76.6±2.76	75.8±3.49	74.3±1.87	75.15±2.37
Hemoglobin (gm/dl)	13.15±0.79	13.2±0.71	13.17±0.73	13.22±0.58
WBC Count ($/mm^3$)	6310±913.01	6300±890.89	6405±664.49	6525±542.78
Platelet Count (lakh/ mm^3)	2.6±0.58	2.67±0.56	2.76±0.51	2.78±0.47
Blood Urea (mg/dl)	25.3±4.55	26.0±4.96	27.15±3.95	28.65±3.72
S.Creatinine (mg/dl)	1.01±0.09	1.00±0.09	1.01±0.09	0.94±0.24
AST(SGOT) (U/L)	28.25±4.48	29.25±4.10	26.7±3.44	27.1±2.95
ALT(SGPT)(U/L)	26.60±4.96	27.55±4.43	27.7±3.03	28.1±3.86
Alkaline Phosphatase (U/L)	182.7±18.25	181.7±16.50	178.2±31.48	177.0±28.90
Total Bilirubin(mg/dl)	0.59±0.11	0.54±0.15	0.58±0.16	0.59±0.14

As seen from Table 7, there were no significant changes in vital, hematological, renal and hepatic functions with both treatments. All subjects tolerated therapy well. There were no serious adverse events recorded in the study. Three patients in CAPROS® group complained of dyspepsia and two patients in placebo group complained of mild headache.

In a third prospective randomized double-blind and placebo controlled clinical trial (Usharani, P. and Sravanti, I.V. Nizam Institute of Medical Sciences, Hyderabad, India, (2012) being prepared for publication), a total of 59 borderline metabolic syndrome subjects (21 in Capros® 250mg group, 20 in Capros® 500mg group and 18 in placebo group) completed the study. In this study, Groups I received, Capros® at a dose of 500 mg/day, Group II received Capros® 1000mg/day and Group III received the placebo capsules for a period of 12 weeks. These subjects were not on any other medication. In addition to efficacy related parameters, hematological and clinical chemistry parameters were investigated. All the subjects underwent complete physical examination, and safety evaluations at baseline and at the end of the treatment. Samples were collected after an overnight fast of 12 hours after the last dose of treatment for determination of hemoglobin, HbA1c, blood urea and serum creatinine, liver function test, and lipid profile (total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C). The changes noted in these parameters at the end of 12 weeks are summarized in Table No.8. The safety related parameters did not reveal any significant changes. The results of this study in borderline metabolic syndrome population suggest that daily administration of Capros® at a dose of 1000 mg/day for 12 weeks is safe.

Table No.8: Effect of CAPROS® 500mg/day, CAPROS® 1000mg/day and placebo on blood safety parameters in pre-metabolic syndrome subjects (All values expressed as Mean±SD)

Parameters	CAPROS® 250mg (n=20)		CAPROS® 500mg (n=21)		Placebo (n=18)	
	PreTT	Post TT	PreTT	Post TT	PreTT	Post TT
Heart rate (bpm)	73.7±2.68	73.85±1.98	75.95±2.99	76.52±2.52	75.06±3.15	75.17±2.23
Hemoglobin (gm/dl)	12.79±1.11	12.82±1.29	13.28±1.06	13.24±1.02	13.39±0.87	13.58±0.96
WBC Count (/mm^3)	7090±827.11	7135±797.55	7019±953.74	7085±930.74	7055.5±669.7	7033.3±673.8
Platelet Count (lakh/ mm^3)	2.87±0.55	2.99±0.55	2.87±0.67	2.86±0.68	2.90±0.47	2.98±0.5
Blood Urea (mg/dl)	23.5±4.01	24.4±3.39	24.10±4.32	24.86±3.81	24.83±4.45	26.06±4.52
S.Creatinine (mg/dl)	1.04±0.12	1.06±0.11	1.0±0.12	1.04±0.10	0.96±0.10	1.05±0.09
AST(SGOT) (U/L)	23.5±3.85	24.0±3.99	24.29±5.17	24.48±4.85	24.44±4.12	24.06±3.76
ALT(SGPT)(U/L)	24.45±4.51	25.4±3.07	23.81±4.43	24.14±4.03	25.78±2.84	25.22±4.08
Alkaline Phosphatase (U/L)	186.3±25.50	188.4±24.90	185.24±20.16	187.81±21.18	179.78±24.67	180.11±24.20
Total Bilirubin(mg/dl)	0.55±0.16	0.54±0.15	0.56±0.16	0.53±0.14	0.58±0.16	0.54±0.16

Data presented in Table No.8 shows that there were no significant changes in heart rate, hematological, renal and hepatic functions with all treatments. All subjects tolerated therapy well. There were no serious adverse events recorded in the study. Two subjects in each of CAPROS® 250mg and CAPROS® 500mg groups complained of dyspepsia and three subjects in the placebo group complained of mild diarrhea.

In a fourth randomized, double-blind, placebo-controlled clinical study (Srikanta Pandir, Ram B.Ghosh, Tuhin K.Biswas; J.B.Roy State Ayurvedic Medical College and Hospital, Kolkata, India, 2008; submitted for publication) total of forty two normal long-time smoker volunteers enrolled for the study. Twenty subjects from Capros® 250mg BID treated group and ten subjects from the placebo group completed the 60 day treatment. A total of twelve patients from both groups dropped out from the present study. The main reasons for study withdrawals were protocol violations and lost follow-up link. No treatment emergent adverse events were reported to the doctors by those who had completed the study.

Table 9: Mean (SD) levels of objective variables at baseline and 60 days of treatment of Capros (250 mg BID) in smokers

Parameters	Capros (n=20)			Placebo (n=10)		
	Baseline	60 days	%Δ	Baseline	60 days	%Δ
HAEMOGRAM						
Hemoglobin (mg/dl)	14.06 (0.79)	14.42 (0.82)	2.6*	13.48 (1.00)	13.41 (0.94)	-0.5
PCV (%)	40.95 (2.41)	42.01 (2.52)	2.6*	40.36 (2.88)	39.66 (2.87)	-1.7
MCV (%)	87.76 (6.13)	86.61 (5.86)	-1.3	86.39 (8.28)	86.93 (7.62)	0.6
MCH (%)	30.18 (2.65)	29.75 (2.21)	-1.4	28.92 (3.45)	29.40 (2.63)	1.7
MCHC (%)	34.36 (1.16)	34.35 (0.94)	-0.02	33.40 (0.99)	33.82 (0.64)	1.3
RBC (millions/cmm)	4.68 (0.36)	4.86 (0.35)	3.9*	4.71 (0.60)	4.58 (0.34)	-2.8
ESR (mm in 1 st hr.)	21.41 (5.99)	20.06 (6.59)	-6.3	27.10 (9.59)	26.90 (10.52)	3.7
WBC (thousands/cmm)	8.588 (1.68)	8.288 (1.56)	-3.5	8.17 (2.23)	8.47 (2.45)	-3
Neutrophil (%)	60.06 (9.75)	63.71(6.02)	6.1	66.90 (6.40)	64.80 (4.47)	-1.3
Lymphocyte (%)	34.76 (8.68)	32.06 (5.14)	-7.8	29.50 (5.87)	31.50 (5.30)	-3.1
Eosinophil (%)	3.76 (1.95)	2.94 (1.85)	-21.9	3.20 (1.81)	3.10 (1.73)	6.8
Platelet (x 10^5)	1.294 (0.47)	1.534 (0.40)	18.6	1.627 (0.55)	1.578 (0.39)	-3.1
FBS (mg/dl)	99.0 (6.6)	98.2 (8.4)	-0.8	93.4 (10.9)	94.6 (8.7)	1.3

Another double blind, placebo-controlled, crossover study was carried out in 12 healthy volunteers, by P. Usharani and I.V.Sravanthi at Nizam Institute of Medical Sciences, Hyderabad, India, to evaluate the effect of Capros® 500mg BID dosing for 14 days on cold-pressor induced cardiovascular changes. Pharmacodynamic parameters like heart rate, augmentation index, sub-endocardial viability ratio, radial and aortic blood pressure were recorded at baseline and end of treatment. After washout period of 10-14 days, subjects crossed over to the other treatment and same test procedure was repeated again.

Safety assessments were done at baseline and at end of treatment and Capros® at 1000mg/day caused no adverse effects.

Yet another double blind, placebo-controlled, crossover study was carried out in 10 healthy volunteers, by P. Usharani and I.V.Sravanthi at Nizam Institute of Medical Sciences, Hyderabad, India, to evaluate the effect of Capros® 500mg BID dosing for 14 days on mental stress induced cardiovascular changes. Pharmacodynamic parameters like heart rate, augmentation index, sub-endocardial viability ratio, radial and aortic blood pressure were recorded at baseline and end of treatment. After washout period of 10-14 days, subjects crossed over to the other treatment and same test procedure was repeated again. Safety assessments were done at baseline and at end of treatment and Capros® at 1000mg/day caused no adverse effects.

In a pilot clinical trial, Antony et al. (2008) investigated the effect of Amla extract (Amlamax™) on the lipid parameters and inflammation. The extract was prepared by water extraction of fresh fruits. For this open label study, male and female human subjects with total cholesterol level 190-310 mg/dL and the c-reactive protein (CRP) level in the range of 1.5-5 mg/L were selected. The subjects were divided in two groups- a group of 22 (Group I; age range 26-72 years) and another group of 17 subjects (Group II; age range 32-60 years) and were given Amla extract at a dose level of 500 mg or 1000 mg/day, respectively, for 6 months. All subjects were screened at the beginning of the trial and at the end of the 3rd and 6th months. The parameters investigated included lipid profile (total cholesterol, triglyceride, HDL, LDL and VLDL), hematology (hemoglobin, neutrophil, lymphocytes, eosinophil, total count), fasting blood glucose, CRP and TSH (thyroid stimulating hormone). In addition to these biochemical investigations, physical parameters such as blood pressure and body weight were also recorded. Blood samples collected at the end of 3 and 6 months showed a reduction in total and LDL cholesterol levels and enhancement of HDL cholesterol. In addition, blood CRP levels, a marker for inflammation, were also significantly reduced. The results of this study suggest that administration of Amla extract at dose level of 1000 mg/day did not cause any adverse effects.

2.2. Biochemical/Pharmacological Action

Because Amla and its products have been consumed for many years with no evidence of safety concerns, very few studies have been conducted on individual substances found in Amla. However, due to recent increased interest, several studies have been published regarding the health benefits of Amla and its products. Some of these studies (both animal and human) have end points that point to the safety of Capros®. It should also be noted that the products tested were not all prepared in the same way. As a result some differences in the test result may be attributed to the variations in the product that was tested.

2.2.1. *In vitro* and Animal Studies

Amla or its preparations has been used for its antioxidant activity or for its purported antimutagenic, cytoprotectant, antitumor, antifungal, antimicrobial, hypolipidemic, and antiatherosclerotic effects. Some of these biological effects of the fruit or its preparations reported in the literature are summarized in Table 10.

Table 10. Biochemical and Pharmacological Actions of Amla or its preparations

Reference	Biological Activity
Jose and Kuttan 1995; Ghosal et al.,1996; Kumar and Muller, 1999	Antioxidant activity *in vitro*
Yadav, 1987	Protection against radiation-induced chromosome damage, *in vitro*
Giri and Banerjee, 1986; Dhir et al., 1990, 1991: Roy et. al., 1992: Nandi et al., 1997.	Antagonistic activity against genotoxic chemicals, anticlastogenicity *in vitro*
Roy et al., 1991; Gulati et al., 1995; Jose et al., 1997, 1999	Hepatoprotective activity, prevention of hepatocarcinogenesis, *in vitro* and *in vivo*
Sankarnarayanan and Jolly 1993	Antimicrobial activity, *in vitro*
Suresh and Vasudevan 1994	Enhancer of natural killer cell (NK) activity, *in vitro*
Asmawi et al., 1992; Jantan et al., 1996; Ihantola-Vormisto et al., 1997	Anti-inflammatory activity, *in vivo* and *in vivo*
El-Mekkawy et al., 1995; Kusumoto et al., 1995	Inhibition of human immunodeficiency virus-1 (HIV-1) reverse transcriptase, *in vitro*
Thakur 1985; Mand et al., 1991; Jacob et al., 1988; Mathur et al., 1996	Hypolipidaemic, *in vivo* and *in vitro*
Thorat et al., 1995	Prevention of experimental acute pancreatitis, *in vivo*

2.2.2. Human Efficacy Studies

Similar to the animal and *in vitro* studies, there has been much interest in establishing the efficacy of Amla and its preparations as an antioxidant in human investigations. In these studies, one encounters references to human testing of varying durations and dosages in the form of the dried fruit, the raw fruit or pulp, or as an antioxidant-rich extract. The human studies of Amla extract and its preparations are summarized in Table 11. In some cases, it is unclear as to the actual compositions that were tested. With test materials identified as chyawanprash, the dosage was first converted to Amla content (which comprises 90% by weight of chyawanprash), which is subsequently converted to the antioxidant extract equivalent by using the 4.2% conversion reported by Ghosal (2000) as the actual yield due to the extraction process. The true percentage of the extract found in the native fruit could be slightly higher, so the estimated daily exposures reported for some entries in Table 7 could be slightly understated. The dosages considered were determined based on the probable Amla extract equivalent which serves as a more direct comparator with Capros®. In these investigations, no dose-related effects emerged. The studies, taken in their totality, indicate no safety concerns associated with the use of Amla products.

Table 11. Summary of Selected Human Testing Studies with *Emblica officinalis* and Its Extract

Reference	Number of subjects	Amla exposure (mg/day)	Duration	Findings; Comments
Pharma Intel, 2002	24 and 24	500 and 1000 mg Capros	6 weeks	Exhibited reduced total cholesterol & LDL, but not dose-related; no serious adverse effects, but minor effects--- weakness, irregular bowels, backache--- reported by 3 subjects
Yadav, et al, 2003	25 m	1512[a] mg as Amla extract	2 months	Genoprotective effects observed.
Manjunatha, 2001	10 m	567[b] mg as Amla extract	8 weeks	Effective in lowering blood sugar & improves blood lipid profile but statistically weak; no adverse effects
Paranjpe, et al., 2000	22	7.6[c] mg as Amla extract	28 days	Blend of materials imparted significant benefit in treatment of piles
Chawla, et al, 1982	38	378[d] mg as Amla extract	28 days	Effective in treating dyspepsia; loose stools & vomiting reported by 3 patients
Jacob, et al., 1988	35 m	2100 mg as Amla extract[e]	28 days	Effective in reducing cholesterol in normal & hyperlipidemic men, considering both LDL & VLDL; health benefit reversed after discontinuing administration of test material
Mishra, et al., 1983	14	336 mg as Amla extract[f]	60 days	Effective treatment for diabetes with no adverse effects
Singh & Sharma, 1971	20	378 mg as Amla extract[g]	7 days	Effective in treating gastric disorders; no adverse effects noted
Chen, et al,, 2009	17	450 mg in tablets	4 months	Reduced oxidative stress in uremic patients with no adverse effects noted
Antony, et al., 2008	25 and 26	500 and 1000 mg	6 weeks	Effective in reducing C-reactive protein, total & LDL cholesterols & increasing HDL with no adverse effects reported

[a] 40 g of chyawanprash, which was noted to be 90% amla, were administered; assuming that 4.2% amla is the antioxidant extract as reported by Ghosal (Ghosal, 2000), the calculated amount of EO extract is 1512 mg.

[b] 15 g of chyawanprash were administered; assuming that it is 90% amla as reported by Ojha (Ojha, 2003), of which 4.2% is EO extract as represented above, the calculated exposure to EO extract was 567 mg.

[c] 180 mg of amla powder were administered; this amount is equivalent to 7.6 mg of EO extract by using the above calculation.

[d] 9 g amla were administered, which corresponds to 378 mg EO extract.

[e] Based on administration of 50 g of amla.

[f] Based on administration of 8 g of amla.

[g] Based on administration of 9 g of amla.

3. SUMMARY AND DISCUSSION

Amla concentrate (Capros®), a standardized *Emblica officinalis* Gaertn fruit (Amla) preparation, is intended for use as a flavoring agent (flavor enhancer) and as an antioxidant at use levels up to 150 mg/serving (reference amounts customarily consumed, 21CFR 101.12) in non-alcoholic beverages and processed fruits and fruit juices. Capros® is a spray-dried powder of the juice of the edible fruits (Amla) with characteristic odor and astringent flavor. Compositional analysis of Capros® demonstrated that all constituents such as carbohydrates, fats, proteins and minerals found in the product are common components of the human diet and as such, are regularly consumed. The intended use of Capros® in the specified food categories will result in mean and 90th percentile estimated daily intakes of 445 and 868 mg/person/day, respectively.

The source material of Capros®, Amla (Indian gooseberry) has been used as a foodstuff and is listed as a major fruit in the USDA's inventory of perennial edible fruits of the tropics. There are several traditional as well as current uses of Amla and its preparation, particularly in Southeast Asian countries, that support the safety-in-use. The traditional human uses of Amla fruit described in Ayurveda claims a number of health benefits. Amla fruit (ripe and dried) is commonly consumed in India. The juice prepared from the fruits is a common and preferred beverage in India during the summer. Amla is used in making curries and is also added to several food preparations for its flavoring effects. Amla pickles and preserves are considered to be a delicacy. In addition to its safe uses as a foodstuff, Amla preparations have been investigated for its efficacy and safety in experimental studies.

The safety profile of Capros® was investigated in acute toxicity, eye irritation, phototoxicity, sensitization and mutagenicity studies (Chaudhuri, 2002). The findings from these studies did not reveal adverse effects of Capros®. The findings from these studies suggest that Capros® is practically non-toxic (LD50 > 5 g/kg bw), non-phototoxic, non-irritant, not a sensitizer and not mutagenic. The results of an unpublished subchronic toxicity study in rats and rabbits with Capros® did not reveal any treatment related adverse effects at dose levels of 100 and 500 mg/kg bw/day. Some significant changes noted in this study following Capros® administration were not considered as adverse as the values were either limited to one sex, lacked dose-response, were within the normal laboratory ranges, and/or were not supported by any other changes in related clinical parameters. In a chronic toxicity study with a water extract of Amla fruit, the NOAEL of the extract was determined as 1,200 mg/kg bw/day, the highest dose tested. In six randomized double-blind, placebo controlled trials in type-2 diabetes, pre-metabolic syndrome subjects, long term smokers as well as healthy volunteers, administration of Capros® at dose levels up to 1000 mg/person/day for 12 weeks did not cause any adverse effects. The results of other efficacy studies of Amla preparations in humans did not reveal any tolerability or safety concerns. A search of the literature related to potential allergenic effects of Amla did not reveal any reports related to allergenicity.

There is sufficient qualitative and quantitative scientific information, including current uses as a foodstuff, to determine the safety-in-use of Capros® in the above mentioned applications. The GRAS status of Amla concentrate (Capros®) for its intended use is supported by:

- It is a well-characterized preparation that is manufactured in accordance with cGMP and meets appropriate food-grade specifications. The method of manufacture involves spray drying of Amla fruit juice extract. Spray drying is not uncommon in food production and is consistent with the draft guidelines entitled ; "Draft Guidance for Industry: Assessing the Effects of significant Manufacturing Process Changes, Including Emerging Technologies, on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances, Including Food Ingredients that are Color Additives"
- Its safety for the intended use in non-alcoholic beverages and processed fruits and fruit juices is supported by published and unpublished studies that include: (a) current use of Amla and its preparation as a foodstuff, (b) a 90-day rat and rabbit study, in which no adverse effects were noted at dose level of 500 mg/kg bw/day, (c) a chronic rat study with similar product in which the NOAEL of the extract was determined as 1,200 mg/kg bw/day, (d) human clinical studies in which no adverse effects were noted at levels up to 1000 mg/day for up to six months;
- Additional published animal and human studies with Amla and its preparations, further corroborate the safety;
- The chemical profile is the same for Capros® as for fresh Amla fruit and other Amla products;
- The data supporting the safety of Amla and its preparation are available in the published literature

The safety of Capros® is supported by data demonstrating compositional similarity of the Amla concentrate (Capros®) itself, Amla juice, Amla/Spray dried, Amla/Lyophilized and by the available preclinical and clinical trials conducted with Amla concentrate and other Amla preparations and/or Amla fruit itself. The totality of available evidence clearly supports the safety of Capros® for its intended use in non-alcoholic beverages and processed fruits and fruit juices. On the basis of scientific procedures[7] and exposure from natural sources, the consumption of Capros® as a flavoring agent (flavor enhancer) [21 CFR§170.3(o)(11)] and as an antioxidant resulting in an estimated daily 90th percentile exposure of 868 mg/person is considered safe. The intended uses are compatible with current regulations, *i.e.,* Capros® will be used in non-alcoholic beverages and processed fruits and fruit juices, when not otherwise precluded by a Standard of Identity, at use levels up to 150 mg/serving and it is produced according to current good manufacturing practices (cGMP) to a food-grade specification.

[7] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

4. CONCLUSION

Based on a critical evaluation of the publicly available data summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively concluded that *Emblica officinalis* Gaertn fruit (Amla) concentrate (Capros®), meeting the specifications cited herein, and when used as a flavoring agent (flavor enhancer) [21 CFR§170.3(o)(11)] and as an antioxidant at maximum use levels of up to 150 mg/serving (reference amounts customarily consumed, 21 CFR 101.12) in non-alcoholic beverages and processed fruits and fruit juices, when not otherwise precluded by a Standard of Identity as described in this monograph and resulting in the 90th percentile all-user estimated intake of 868 mg Capros®/person/day is safe and GRAS.

It is also the opinion of the Expert Panel that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, the Expert Panel concludes that *Emblica officinalis* Gaertn fruit (Amla) concentrate (Capros®), when used as described, is GRAS based on scientific procedures.

Signatures

(b) (6)

Robert L. Martin, Ph.D. — July 16, 2013 Date

(b) (6)

Madhusudan G., Soni, Ph.D., FACN, FATS — July 12, 2013 Date

(b) (6)

Sanni Raju, Ph.D., R.Ph. — 07/08/2013 Date

5. REFERENCES

Anonymous, 2006. Chyawanprash India's Oldest Longevity Tonic. Article available at: http://www.kitchendoctor.com/Ayurveda/Chyawanprash.html.

Antony, B., Benny, M., Kaimal, T.N.B. 2008. A pilot clinical study to evaluate the effect of *Emblica officinalis* extract (Amlamax™) on markers of systemic inflammation and dyslipidemia. Indian J. Clin. Biochem. 23, 378-381.

Asmawi, M.Z., Kankaanranta, H., Moilanen, E., Vappatalo, H., 1992. Antiinflammatory activities of *Emblica officinalis* GAERTN leaf extracts. J. Pharm. Pharmacol. 45, 581–584.

Ayurvedic Pharmacopoeia of India, 2001. Vol I, Part 1, The Controller of Publications, Department of Health and Family Welfare, Government of India, Nirman Bhavan, New Delhi. Also see the website: www.indianmedicine.nic.in.

Bhattacharya, A., Chatterjee, B., Ghosal, S., and Bhattacharrya, S. K., 1999. Antioxidant activity of active tannoid principles of *Emblica officinalis* (Amla), Indian Journal of Experimental Biology 37:676-680.

Biswas, S., Talukder, G., Sharma, A., 1999. Protection against cytotoxic effects of arsenic by dietary supplementation with crude extract of *Emblica officinalis* fruit. Phytother. Res. 13, 513-516.

Chawla, Y. K., Dubey, P., Singh, R., Nundy, S., Tandpm, B. N., 1982. Treatment of Dyspepsia with Amalaki (Emblica officinalis Linn.)-an Ayurvedic Drug. Indian J. Med. Res. 76(Suppl):95-98.

Chaudhuri, R.K., 2002. Emblica cascading antioxidant: A novel natural skin care ingredient. Skin Pharmacol. Appl. Skin Physiol. 15, 374–380.

Chaudhuri, R., 2005. Phyllanthus Tannins. In Cosmeceuticals and Active Cosmetics: Drugs Versus Cosmetics, 2nd ed., Editors: Elsner, P. and Maibach, H. I., Taylor and Francis, pp 465-488.

Chen, T.S., Liou, S.Y., Chang , Y.L., 2009. Supplementation of Emblica Officinalis (Amla) Extract Reduces Oxidative Stress in Uremic Patients. American Journal of Chinese Medicine 37(l):19-25.

Chick, H., Hume, E. M., and Skelton, R. F., 1919. The antiscorbutic value of some Indian dried fruits. Lancet 2:322-323.

Chopra, R.N., Nayar, S.L., Chopra, I.C., 1956. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi, India.

Dharmananda, S., 2006b. Emblic Myrobalans: Amla Key Herb of Ayurvedic Medicine. Startgroup Manuscript Publishing. Article available at: http://www.itmonline.org/arts.amla.htm.

Dhir, H., Agarwal, K., Sharma, A., Talukder, G., 1991. Modifying role of *Phyllanthus emblica* and ascorbic acid against nickel clastogenicity in mice. Cancer Lett. 59, 9–18.

Dhir, H., Roy, A.K., Sharma, A., Talukder, G., 1990. Protection afforded by aqueous extracts of *Phyllanthus* species against cytotoxicity induced by lead and aluminium salts. Phytoter. Res. 4, 172–176.

DiNovi, M.J., Kuznesof, P.M., 1995. Estimating Exposure To Direct Food Additives And Chemical Contaminants in the Diet. Available at http://www.cfsan.fda.gov/~dms/opa-cg8.html Website visited: February 26, 2013.

DSHEA, 1994. Dietary Supplements Health and Education Act of 1994. US Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, DC.

El-Mekkawy, S., Meselhym, R., Kusumoto, I.T., Kadota, S., Hattori, M., Namba, T., 1995. Inhibitory effects of Egyptian folk medicines on human immunodefience virus (HIV) reverse transcriptase. Chem. Pharm. Bull. 43, 641–648.

FASEB, 1988. Estimation of Exposure to Substances in the Food Supply, Anderson, S.A., Editor, FDA Contract No. 223-84-2059, Life Sciences Research Office, Bethesda, MD.

FDA. 2006. *Estimating Dietary Intake of Substances in Food.* Guidance document. Available at: http://www.fda.gov/food/guidancecomplianceregulatoryinformation/guidancedocuments/foodingredientsandpackaging/ucm074725.htm Website visited: February 26, 2013.

Ghosal, S., Tripathi, V. K., Chauhan, S., 1996. Active constituent of *Emblica officinalis*: part 1- the chemistry and antioxidant effects of two new hydrolysable tannins, emblicanin A and B. Indian J. Chem. 35B, 941-948.

Giri, A.K., Banerjee, T.S., 1986. Antagonistic activity of herbal drug (*Phyllanthus emblica*) on cytological effects of environmental chemicals on mammalian cells. Cytologia 51, 375–380.

Gulati, R.K., Agarwal, S., Agrawal, S.S., 1995. Hepatoprotective studies on *Phyllanthus emblica* Linn. and quercetin. Ind. J. Exp. Biol. 33, 261–268.

Ihantola-Vormisto, A., Summanen, J., Kankaanranta, H., Vuorela, H., Asmawi, M.Z., Moilanen, E., 1997. Anti-inflammatory activity of extracts from leaves of *Phyllanthus emblica*. Planta Med. 63, 518–524.

Jacob, A., Pandey, M., Kapoor, S., Saroja, R., 1988. Effect of the Indian gooseberry (amla) on serum cholesterol levels in men aged 35-55 years. Eur. J. Clin. Nutr. 42, 939–944.

Jaijoy, K., Soonthornchareonnon, N., Lertprasertsuke, N., Panthong, A., Sireeratawang, S., 2010. Acute and chronic oral toxicity of standardized water extract from the fruit of Phyllanthus emblica Linn. Int. J. Appl. Res. Nat. Prod. 2010; 3(1): 48-58. 2010; 3(1): 48-58.

Jantan, I.B., Kang, Y.H., Suh, D.Y., Han, B.H., 1996. Inhibitory effects of Malaysian medicinal plants on the platelet-activating factor (PAF) receptor binding. Nat. Prod. Sci. 2, 86–89.

Jose, J.K., Joy, K.L., Kuttan, R., 1999. Effect of *Emblica officinalis, Phyllanthus amarus* and *Picrorrhiza kurroa* on *N*-nitrosodiethylamine induced hepatocarcinogenesis. Cancer Lett. 136, 11–16.

Jose, J.K., Kuttan, R., 1995. Antioxidant activity of *Emblica officinalis*. J. Clin. Biochem. Nutr. 19, 63–70.

Jose, J.K., Kuttan, G., George, J., Kuttan, R., 1997. Antimutagenic and anticarcinogenic activity of *Emblica officinalis* GAERTN. J. Clin. Biochem. Nutr. 22, 171–176.

Kirtikar, K.R., Basu, B.D., 1935. *Indian Medicinal Plants*. 2nd ed. Vol. 1-4. 1935. Reprint. Delhi: Periodical Experts. pp. 2220-2221.

Kumar, C. S., Muller, K., 1999. Medicinal plants from Nepal; II. Evaluation as inhibitors of lipid peroxidation in biological membranes. J. Ethnopharmacol. 64, 135–139.

Kusumoto, I.T., Nakabayashi, T., Kida, H., Miyashiro, H., Hattori, M., Namba, T., Shimotohno, K., 1995. Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-1) protease. Phytother. Res. 9, 180–184.

Manjunatha, S., Jaryal, A. K., Bijlani, R. I., Sachdeva, U., and Gupta, S. K., 2001. Ayurvedic Herbal Combination Chyawanprash Lowers Blood Sugar and Cholesterol Greater Than Vitamin C in Small Study. Indian Journal of Physiology and Pharmacology 45:71-79.

Mand, J.K., Sonli, G.L., Gupta, P.P., Singh, R., 1991. Effect of amla (*Emblica officinalis*) on the development of atherosclerosis on hypercholesterolemic rabbits. J. Res. Edu. Ind. Med. 10, 1–7.

Martin, F.W., Campbell, C.W., Ruberte, R.M., 1987. Perennial edible fruits of the tropics: an inventory. United States Department of Agriculture, Agricultural Research Service, Washington, DC, p. 27.

Mathur, R., Sharma, A., Dixit, V.P., Varma, M., 1996. Hypolipidaemic effect of fruit juice of *Emblica officinalis* in cholesterol-fed rabbits. J. Ethnopharmacol. 50, 61-68.

Mishra, S. K., Pandey, S. K., Sharma, S. H., and Singh, G. D., 1 983. Effect of Amalaki-Haridra on Madhumeha. Nagarjun, January 1983, pp 105-107.

Morton, J., 1987. Emblic. p. 213–217. In: Fruits of warm climates. Julia F. Morton, Miami, FL.

MRCA, 1965. Market Research Corporation of America (MRCA), in conjunction with the "Food intake and nutritive value of the diets of men, women and children in United States, Spring 1965". A Preliminary Report by the Consumer and Food Economics Research Division, Agricultural Research Division. United States Department of Agriculture, Washington, DC.

Muruganandam, A. V., Das, S. N., 2000. Sub-chronic Toxicity Studies of Capros®. Indian Herbs Research & Supply Co Ltd. Unpublished study.

Nandi, P., Talukder, G., Sharma, A., 1997. Dietary chemoprevention of clastogenic effects of 3,4-benzo(a)pyrene by *Emblica officinalis* GAERTN fruit extract. Brit. J. Cancer 76, 1279–1283.

Ojha, J. K., 2003. Chyawanprash From Vedic to Genomic Era, Chaukhamba Sanskrit Pratishthan, New Delhi, India.

Paranjpe, P., Patki, P., Joshi, N., 2000. Efficacy of an Indigenous Formulation in Patients with Bleeding Piles: a Preliminary Clinical Study. Fitoterapia 71:41-45.

Patel, S.S., Goyal, R.K., 2012. *Emblica officinalis* Geart: A comprehensive review on phytochemistry, pharmacology and ethnomedicinal uses. Research Journal of Medicinal Plant, 6:6-16.

Pathak, R. K., 2002. Emblica officinalis. Indian Council of Agricultural Research. Article available at: http://ecoport.org/ep?Plant=8537&entityType=PLME**&entityDisplayCategory=full Website visited: February 22, 2013.

Pharma Intel Drug Research & Consultancy Group, 2002. Randomized Controlled Clinical Trial of Capros® Oral Capsule in Hyperlipidemia. Unpublished study.

Poltanov, E.A., Shikov, A.N., Dorman, H.J., Pozharitskaya, O.N., Makarov, V.G., Tikhonov, V.P., Hiltunen, R., 2009. Chemical and antioxidant evaluation of Indian gooseberry (*Emblica officinalis* Gaertn., syn. *Phyllanthus emblica* L.) supplements. Phytother. Res. 23(9):1309-1315.

Raghu, V., Patel, K., Srinivasan, K., 2007. Comparison of ascorbic acid content of *Emblica officinalis* fruits determined by different analytical methods. J. Food

Roy, A.K., Dhir, H., Sharma, A., 1991. Comparative efficacy of *Phyllanthus emblica* fruit extract and ascorbic acid in modifying hepatotoxic and renotoxic effects induced by metals *in vivo*. Int. J. Crude Drug Res. Int. J. Pharmacognosy (Netherlands). 29, 117–126.

Roy, A.K., Dhir, H., Sharma, A., 1992. Modification of metal-induced micronuclei formation in mouse bone marrow erythrocytes by *Phyllanthus* fruit extract and ascorbic acid. Toxicol. Lett. 62, 9–17.

Natreon (2013). Information on General description, specifications, composition and manufacturing of Capros™.

Sankarnarayanan, J. Jolly, C.I., 1993. Phytochemical, antibacterial and pharmacological investigations on *Momordica charantia* Linn., *Emblica officinalis* Gaertn and *Curcuma longa* Linn. Indian J. Sci. 1, 6-13.

Scartezzini, P., Speroni, E., 2000. Review on some plants of Indian traditional medicine with antioxidant activity," J Ethnopharmacology, 71, pp 23-43.

Scartezzini, P., Antognoni, F., Raggi, M. A., Poli, F., Sabbioni, C., 2006. Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of *Emblica officinalis* Gaertn. J. Ethnopharm. 104, 113-118.

Shanmugasundaram, K.R., Seethapathy, P.G., Shanmugasundaram, E.R., 1983. Anna Pavala Sindhooram- an antiatherosclerotic Indian drug. J. Ethnopharmacol. 7, 247-265.

Singh, B. N., Sharma, P. V., 1971. Effect of Amalaki on Amlapitta. J. Res. Ind. Med. 5(2):223-229.

Smiciklas-Wright, H., Mitchell, D.C., Mickle, S.J., Cook, A.J., Goldman, J.D., 2002. Foods Commonly Eaten in the United States: Quantities Consumed Per Eating Occasion and in a Day, 1994-1996. U.S. Department of Agriculture NFS Report No. 96-5, 252 pp. Available online at website: www.barc.usda.gov/bhnrc/foodsurvey/Products9496.html Website visited: March 28, 2013.

Suresh, K., Vasudevan, D.M., 1994. Augmentation of murine natural killer cell and antibody dependent cellular cytotoxicity activities of *Phyllanthus emblica*, a new immunomodulator. J. Ethnopharmacol. 44, 55–60.

Thakur, C.P., 1985. *Emblica officinalis* reduces serum, aortic and hepatic cholesterol in rabbits. Experientia 41, 423–424.

Thorat, S.P., Rege, N.N., Naik, A.S., Thatte, U.M., Joshi, A., Panicker, K.N.S., Bapat, R.D., Dahanukar, S.A., 1995. *Emblica officinalis*: a novel therapy for acute pancreatitis—an experimental study. HPB Surgery 9, 25–30.

Unander, D.W., Webster, G.L., Blumberg, B.S., 1990. Records of usage or assays in Phyllanthus (Euphorbiaceae). I. Subgenera Isocladus, Kirganelia, Cicca and Emblica. J. Ethnopharmacol. 30, 233-264.

Yadav, J. S., Thakur, S., Chadha, P., 2003. Chyawanprash Awaleha: A Genoprotective Agent for Bidi Smokers. Int. J. Hum. Genet. 3(1):33-38.

Yadav, S.K., 1987. Protection against radiation induced chromosome damage by *Emblica officinalis* fruit extract. Caryologia 49, 261–265.

Yokozawa, T., Kim, H.Y., Kim, H.J., Okubo, T., Chu, D.C., Juneja, L.R., 2007b. Amla (*Emblica officinalis* Gaertn.) prevents dyslipidaemia and oxidative stress in the ageing process. Br. J. Nutr. 97, 1187-1195.

Yokozawa, T., Kim, H.Y., Kim, H.J., Tanaka, T., Sugino, H., Okubo, T., Chu, D.C., Juneja, L.R., 2007a. Amla (*Emblica officinalis* Gaertn.) attenuates age-related renal dysfunction by oxidative stress. J. Agric. Food Chem. 55, 7744-7752.

Zhang, Y. J., Tanaka, T., Yang, C. R., Kouno, I., 2001. New phenolic constituents from the fruit juice of *Phyllanthus emblica*. Chem. Pharm. Bull. 49, 537-540.

6. APPENDIX I

Analytical data from five manufacturing lots

Specifications of Capros® from five different manufacturing batches

Parameter	Standard*	Lot #	Lot #	Lot #	Lot #	Lot #
Description	Yellow free flowing powder	(b) (4)				
Identification	To comply with HPLC/HPTLC	Confirms	Confirms	Confirms	Confirms	Confirms
Water-soluble extractive value, % w/w	≥ 80%	95.7%	95.8%	95.7%	96.3%	96.2%
Assay						
Low molecular weight hydrolysable tannins, % w/w	≥ 60%	61.6%	70.9%	61.6%	64.4%	70.7%
Gallic acid	≤ 4%	0.5%	0.27%	1.08%	0.86%	0.51%
Water (Karl-Fischer), % w/w	≤ 6%	3.5%	3.7%	3.35%	3.25%	2.50%
Sulfated ash, % w/w	≤ 6%	3.2%	4.8%	3.95%	5.2%	4.78%
Heavy metals, ppm						
Lead	≤ 3	0.87	<0.5	<0.5	<0.5	0.54
Arsenic	≤ 1	<0.10	<0.10	<0.10	<0.10	<0.10
Cadmium	≤ 1	<0.10	<0.10	<0.10	<0.10	<0.10
Mercury	≤ 1	<0.01	<0.01	<0.01	<0.01	<0.01
Microbiological assays, cfu/g						
Aerobic bacteria (no pathogens)	< 5000	70	60	50	70	150
Yeast and Mold	< 1000	≤ 10	≤ 10	≤ 10	≤ 10	80
Enterobacteria	≤ 100	≤ 10	≤ 10	≤ 10	≤ 10	
Escherichia coli	Absent in 1 g	Absent	Absent	Absent	Absent	Absent
Candida albicans	Absent in 1 g	Absent	Absent	Absent	Absent	Absent
Salmonella species	Absent in 10 g	Absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent in 10 g	Absent	Absent	Absent	Absent	Absent
Staphylococcus aureus	Absent in 10 g	Absent	Absent	Absent	Absent	Absent

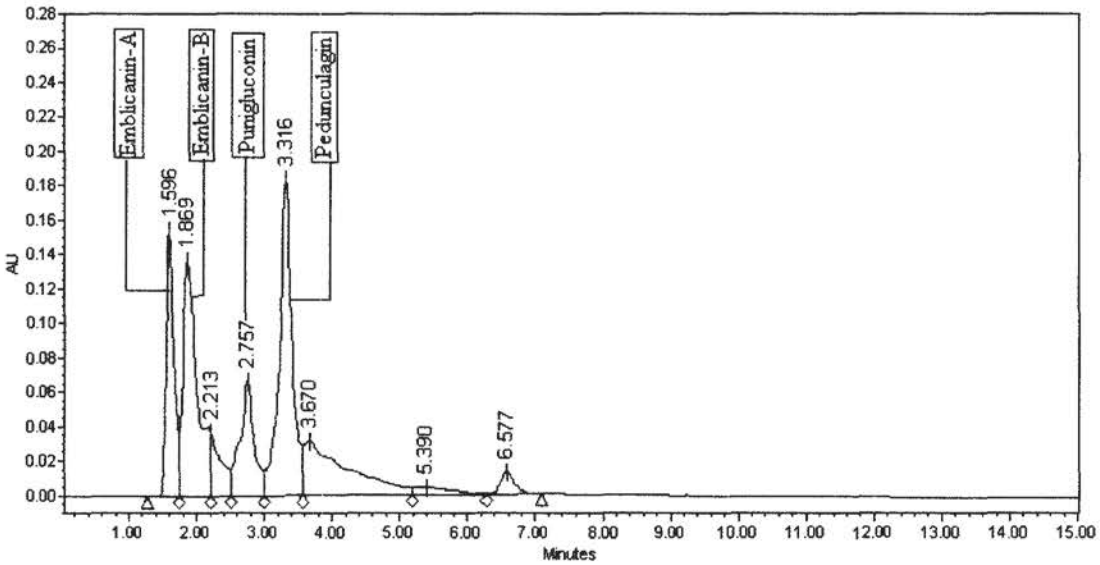
*Standard specifications for marketed product.

7. APPENDIX II

Typical chromatograms of Capros® - 5 Different Lots

Natreon
...in synergy with nature.

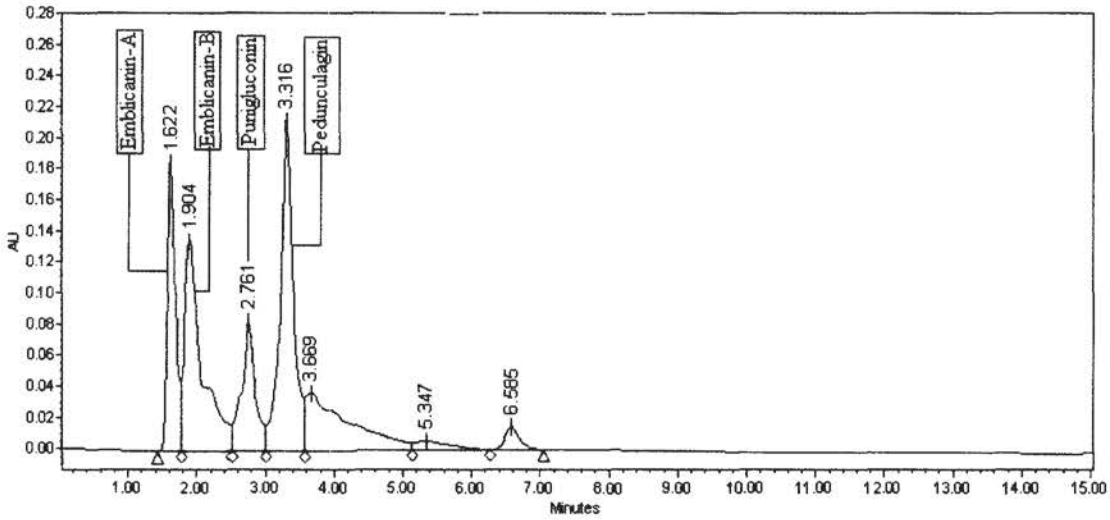
CONFIDENTIAL



HPLC chromatogram of Capros; Batch No: CA-1303143; Mfg.dt: March 2013

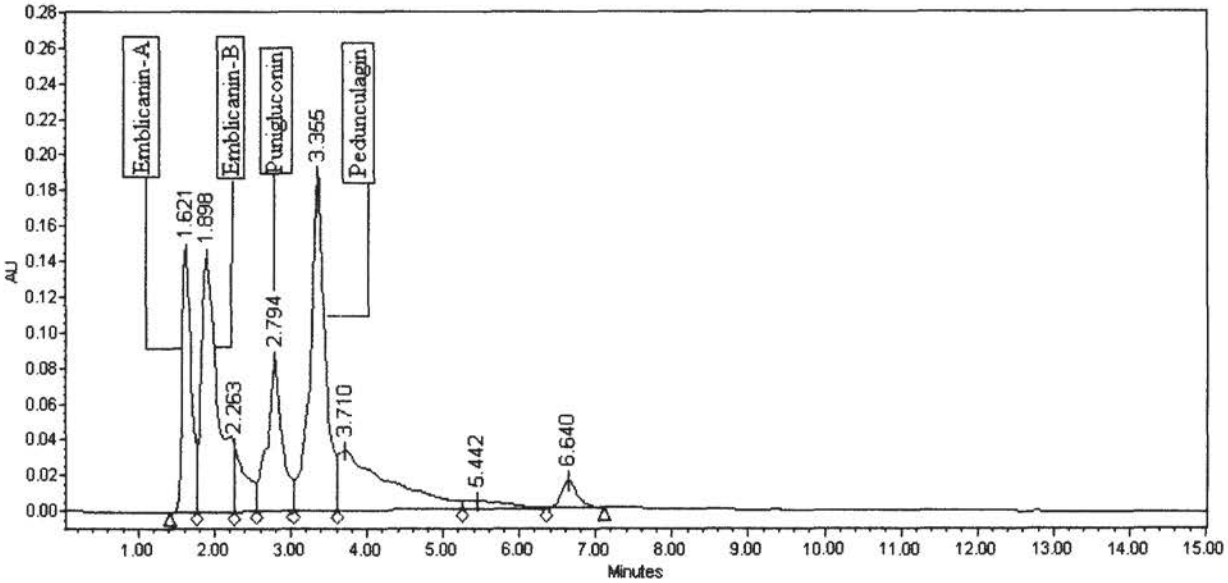
Natreon
...in synergy with nature.

CONFIDENTIAL



HPLC chromatogram of Capros; Batch No: CA-1303144; Mfg.dt: March 2013

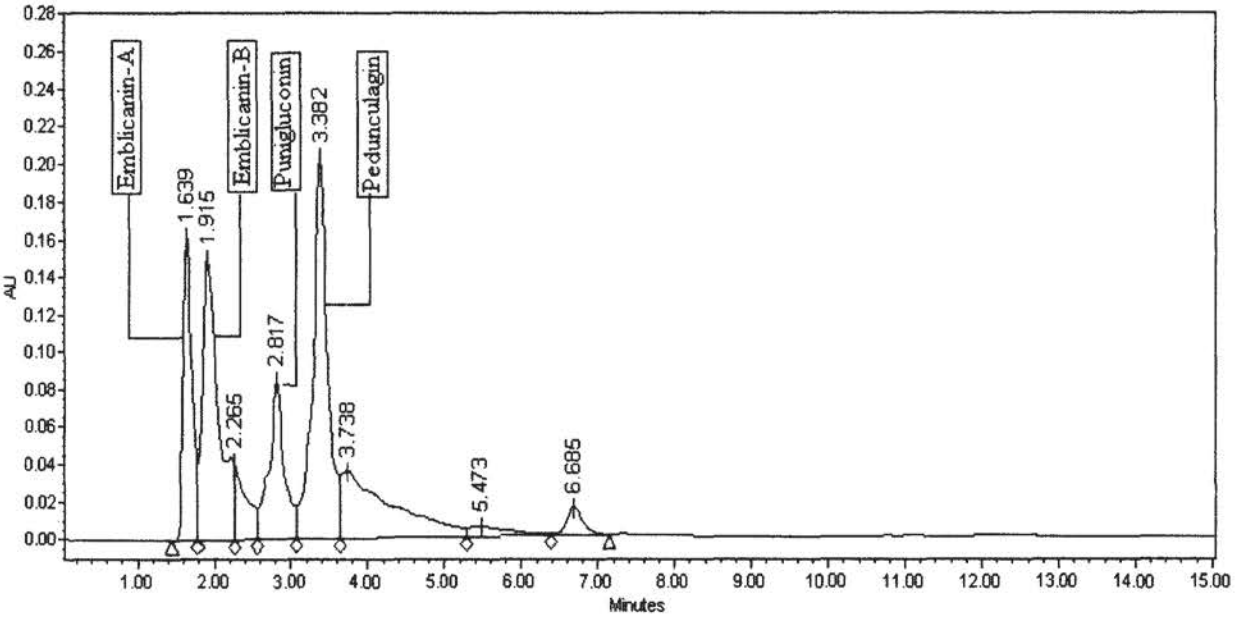
000038



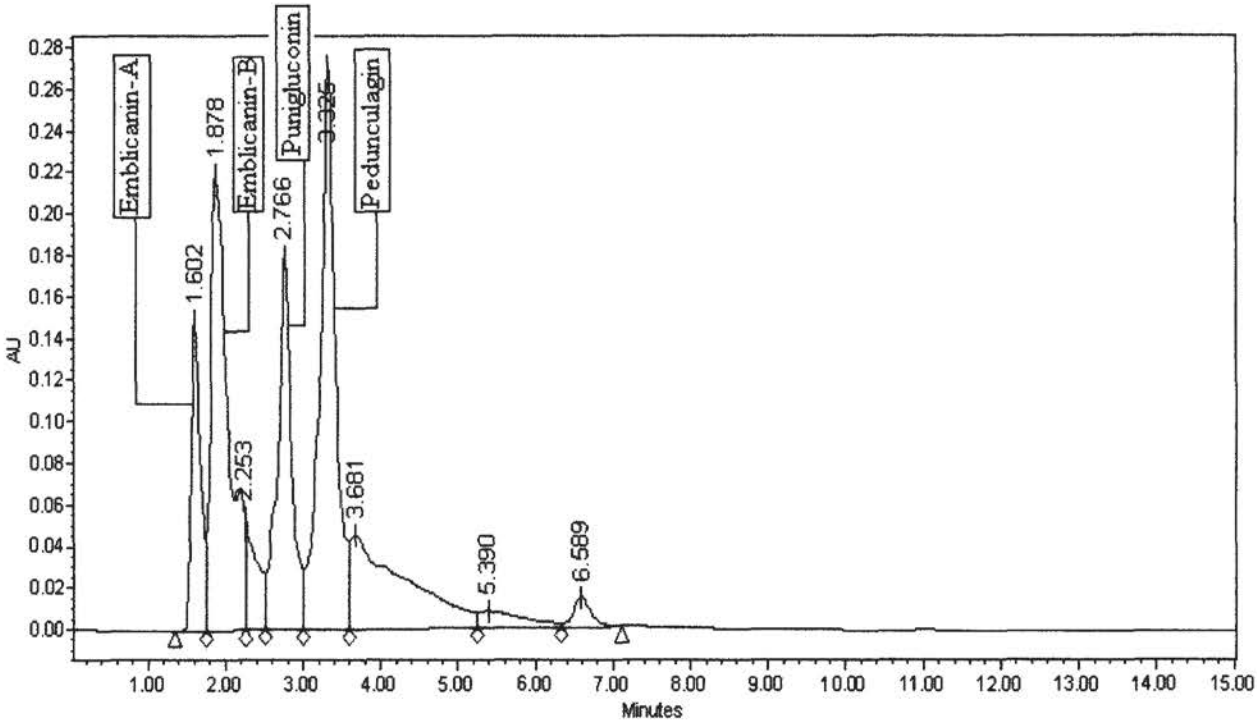
HPLC chromatogram of Capros; Batch No: CA-1303145; Mfg.dt: March 2013

Natreon
...in synergy with nature.

CONFIDENTIAL



HPLC chromatogram of Capros; Batch No: CA-1303146; Mfg.dt: March 2013



HPLC chromatogram of Capros; Batch No: CA-1205136; Mfg.dt: May 2012

8. APPENDIX III

Compositional Analysis- Nutrient Content of Capros®

N

CAPROS® (*Phyllanthus emblica*)

INGREDIENT INFORMATION NUTRIENT CONTENT

PRODUCT NAME: (BOTANICAL)	*Phyllanthus emblica*
BRAND NAME:	CAPROS®
COMPANY REPRESENTATIVE:	Lucien Hernandez

- The following values are based upon amount per 100 grams of ingredient.
- Values in bold italics represent the intended product. All other items are incidental minor components. Minor constituent values are typical, but will vary dependent on the raw material processed.
- Heavy Metal Impurities (arsenic, cadmium, lead, mercury and tin) are included for informational purposes. Calculated as ppm and not based on amount (per 100 gm).

Nutrients	Amount (per 100 gm)	Nutrients	Amount (per 100 gm)
Total Carbohydrates	88.13 g	Sugars	26.50 g
Cholesterol	NA	Water	≤ 6.00 g
Calories	334.71	Aluminum	NA
% of Calories from fat	2.59	Boron	NA
Protein (Actual)	2.04 g	Calcium	80.00 mg
Amino Acids	5.10 g	Chloride	6.30 mg
Fat – Total	0.288 g	Chromium	0.06 mg
Fat – Saturated	0.170 g	Copper	1.50 mg
Fat – Monounsaturated	0.053 g	Fluoride	NA
Total Dietary Fiber	8.74 g	Iodine	NA
Vitamin A	NA	Iron	20.00 mg
Thiamine (B1)	0.05 mg	Magnesium	60.00 mg
Riboflavin (B2)	NA	Manganese	2.10 mg
Niacin (B3)	0.20 mg	Molybdenum	NA
Vitamin B6	NA	Phosphorus	12.00 µg
Vitamin B12	NA	Potassium	12.00 mg
Folic Acid	NA	Selenium	0.50 mg
Pantothenic Acid	NA	Sodium	5.50 mg
Biotin	NA	Zinc	1.65 mg
Vitamin C	0.50 g	Caffeine	NA
Vitamin D	NA	Alcohol	NA
Vitamin E	NA	Ash	≤ 6.00 g
Vitamin K	NA	Silicon	25.00 µg

Heavy Metals	Amount
Arsenic	< 1.00 ppm
Cadmium	< 1.00 ppm
Mercury	< 1.00 ppm
Lead	< 2.00 ppm
Tin	< 1.00 ppm

Natreon, Inc.
2-D Janine Place
New Brunswick, NJ 08901
T: 732-296-1080
F: 732-296-9898
email: info@natreoninc.com
www.natreoninc.com

caprōs

We believe this information to be accurate and reliable; however, no warranty, either express or implied, is made and no freedom from liability, from Patents, Trademarks or other limitations should be inferred.

9. APPENDIX IV

Comparison of Chemical Constituents of Capros® with Amla Preparations

COMPARATIVE TANNIN CONTENT AND ANTIOXIDANT ACTIVITY OF CAPROS®, AMLA JUICE, SPRAY-DRIED AMLA JUICE AND LYOPHILIZED AMLA JUICE

PREPARATION OF JUICE:

a) Fresh fruits of Amla were collected from local market (Ultadanga) on November 15, 2006.
b) Pericarp and seed were separated
c) Wet weight of pericarp was taken
d) Pericarp was grinded with 2% NaCl and 25% water by mixer-grinder
e) The juice was squeezed with a fine meshed net
f) Juice was then used as is or spray-dried or lyophilized into powder.
g) Amla juice was kept in air tight container for further use

ANALYTICAL METHODS:

HOPLC and HPTLC methods were used for chemical analysis and antioxidant activity was measured using DPPH and ABTS radivals.

RESULTS:

Table 1: HPLC RESULTS (Area Under the Curve, Low Molecular Weight Hydrolysable Tannins):

Sample Name	Emblicanin A		Emblicanin B		Peunigluconin		Pedunculagin	
	Area	Area %	Area	Area %	Area	Area %	Area	Area %
Capros®	3794494	17.48	8653456	39.85	2989772	13.77	3997034	18.41
Amla/Spray dried	5368702	39.75	2805999	20.78	1157405	8.57	3564194	26.39
Amla/Lyophilized	7189597	41.82	3332422	19.38	1240421	7.21	4525824	26.32
Amla/MCC/Lyophi	8363919	41.92	4056509	20.33	1171539	5.87	5442513	27.28

HPLC concentration of sample 1 mg / ml

Table 2:HPLC RESULTS (Quantitative, Low Molecular Weight Hydrolysable Tannins)

Sample Name	Emblicani n A (µg/20 µg sample)	Emblicani n B (µg/20 µg sample)	Punigluconi n (µg/20 µg sample)	Pedunculagi n (µg/20 µg sample)	Total Low Mol. Wt. Hydrolysabl e Tannins, µg/20 µg sample
Capros®	3.496	7.970	2.754	3.682	17.902
Amla/Spray dried	7.950	4.156	1.714	5.276	19.096
Amla/Lypholize d	8.364	3.876	1.442	5.264	18.946
Amla juice	8.384	4.066	1.174	5.456	19.080

Table 3: HPTLC RESULTS: [Total Gallotannoids]

Sample Name	Area	
	15 microgram sample (Total area under the chromatogram)	25 microgram sample (Total area under the chromatogram)
Capros®	24159.9	34791.0
Amla/Spray dried	23989.9	31155.9
Amla/Lyophilized	27595.5	35293.8
Amla/MCC/Lyophi	23047.3	33923.9

Sample concentration 5 mg / ml; Sample applied 3mcl and 5mcl

Table 4: HPTLC RESULTS: [Amino Acids]

Sample Name	% of Methionine	% of Total amino acids
Capros®	0.134	5.64
Amla/Spray dried	0.330	7.40
Amla/Lyophilized	0.480	7.64
Amla/MCC/Lyophi	0.440	8.60

Sample concentration 5 mg / ml; Sample applied 5mcl

Table 5: RESULTS OF ANTIOXIDANT ACTIVITY:

Sample Name	DPPH (IC50) µg / ml	ABTS (IC50) µg / ml
Capros®	10.32	2.99
Amla/Spray dried	9.95	2.85
Amla/Lyophilized	9.82	2.70
Amla/MCC/Lyophi	9.90	2.90

COMMENTS:

Although differences were seen in the percentage of individual tannins, the amount of total tannins and the overall antioxidant activity remained almost the same between Capros®, spray-dried Amla juice, lyophilized Amla juice and Amla juice itself and thus Capros® may be considered equivalent to Amla fruit in its tannin content and antioxidant activity.

Signed,

(b) (6) [redacted] Date 07/08/2013

Sanni Raju, Ph.D., R.Ph.
Natreon, Inc.
Chairman and CEO

Natreon
...in synergy with nature

2-D Janine Place • New Brunswick, NJ 08901
P.O. Box 7398 • North Brunswick, NJ 08902-7398
Tel: 732-296-1080 • Fax: 732-296-1075
Email: info@natreoninc.com • www.natreoninc.com

August 27, 2013

Moriama J. Ramos Valle, M.S.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

Subject: GRAS Submission for Amla Concentrate

Dear Ms. Ramos Valle,

I hereby confirm that only pages 19, 20 and 21 of the GRAS submission for Amla Concentrate from
Natreon, Inc. have confidential information on our clinical studies which are pending publication and all
other pages can be considered non-confidential public information.

Please let me know if you have any questions.

Thank you.

Sincerely,

(b) (6)

Sanni Raju, Ph.D., RPh.
CEO & Chairman

RECEIVED
AUG 27 2013
OFFICE OF
FOOD ADDITIVE SAFETY

Natreon
...in synergy with nature

2-D Janine Place • New Brunswick, NJ 08901
P.O. Box 7398 • North Brunswick, NJ 08902-7398
Tel: 732-296-1080 • Fax: 732-296-1075
Email: info@natreoninc.com • www.natreoninc.com

August 30, 2013

Moriama J. Ramos Valle, M.S.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

Subject: GRAS Submission for Amla Concentrate

Dear Ms. Ramos Valle,

I am sorry for the confusion.

I hereby confirm that pages 34, 35 and 36 of the GRAS submission for Amla Concentrate from Natreon, Inc., labeled confidential, can be considered non-confidential public information. In addition, information contained on all other pages of this submission is also non-confidential public information.

This letter supersedes all other communications regarding this matter.

Please let me know if you have any questions.

Thank you.

Sincerely,

(b) (6)

Sanni Raju, Ph.D., R.Ph.
CEO & Chairman

RECEIVED
AUG 30 2013
OFFICE OF
FOOD ADDITIVE SAFETY

SUBMISSION END