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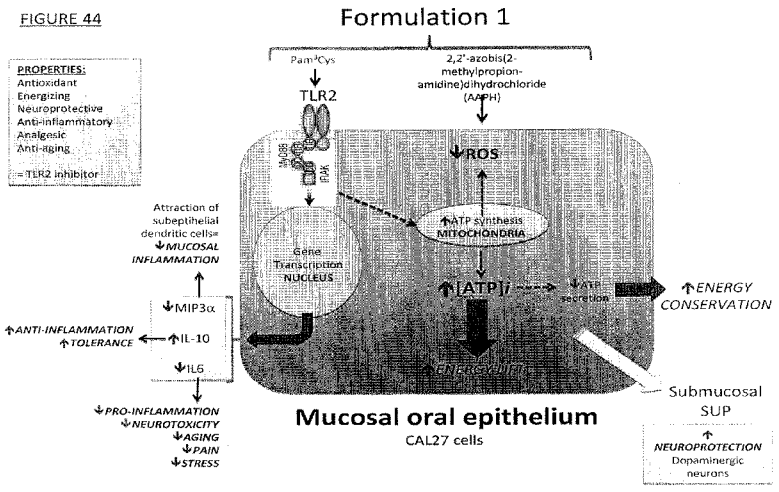
(54) **Title**: FUNCTIONAL FOODS AND BEVERAGES WITH SYNERGISTIC PROPERTIES TO PROMOTE HOMEOSTASIS

FIGURE 44



(57) **Abstract**: The present invention provides compositions having synergistic antioxidant properties in the modulation of Toll-like receptor signaling for the promotion of homeostasis, immunity, energy conservation, protection of neural cells and anti-inflammatory responses, said compositions comprising plant and/or fruit extracts and a natural sweetener with high oxidation potential as defined by ORAC value.

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FUNCTIONAL FOODS AND BEVERAGES WITH SYNERGISTIC PROPERTIES TO PROMOTE HOMEOSTASIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from US provisional applications numbers 61/438,747 filed February 2, 2011 and 61/472,298 filed April 6, 2011, the contents of which is hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to the fields of functional foods, functional beverages and sweeteners useful for the maintenance of homeostasis and energy levels in mammals. More specifically, the present invention relates to functional foods and beverages that can contribute to the maintenance of health and wellness through mechanisms that involve interactions between epithelial cells of the mucosa with both the immune system and the dopaminergic nervous system of the submucosa as well as ATP and ROS generated from cellular responses. Such foods and beverages contribute to reducing the risks of chronic diseases such as cancers, cardiovascular diseases, neurodegenerative diseases, chronic inflammatory diseases, autoimmune diseases and diabetes.

BACKGROUND OF THE INVENTION

Adaptation

[0003] Evolution is driven by a process called adaptation whereby an organism becomes better able to live in its habitat. Adaptedness is the state of being adapted, the degree to which an organism is able to live and reproduce in a given set of habitats. It is acknowledged that best adapted organisms will have the best chance of survival. Adaptedness is best reached through the body's ability to maintain a state of equilibrium, known as homeostasis, which involves the preservation of physiological functions such as body's temperature, respiratory rate, and blood chemistry, within tightly controlled limits.

Homeostasis

[0004] Homeostasis is defined as a balanced physiological state essential for adaptation. Homeostasis is a state in which everything within the cell is in equilibrium and functioning properly. Homeostasis conservation in human body depends on the delicate balance

between cell's capacity to maintain metabolism and demand. In order to prevent diseases and other dysfunctions, tissues in our body accomplishes many metabolic reactions to maintain a constancy of environment for the vitality and well-being. At the cell level, to maintain equilibrium or homeostasis, the cell membranes must be in continuous interaction with both the internal (intracellular) environment and the external (extracellular) environment. When the homeostasis of any component is disturbed, the interaction permits automatic readjustment by giving rise to stimuli that result in restoration of metabolic process. The state of homeostasis requires a good transport of important nutrient for the cell to continue to function properly.

Mechanisms of homeostasis

[0005] In general: Homeostasis processes are involved in the regulation of gut / mucosal epithelial functions and integrity, glucose level / insulin resistance, energy levels, endocrine / stress hormones levels, oxidative stress (respiration), immune / inflammatory state, neurological functions and mitochondria functions. Molecular mechanisms regulating homeostasis involve, among others, TLR activation, calcium influx, ATP production, inflammatory cytokines production, neurotransmitter release, hormonal production, and reactive oxygen species (ROS) production.

[0006] Mitochondria-driven homeostasis: Intake of oxygen and nutrients regulate the physiological responses of adaptation. These responses are carried out mainly by mitochondria of eukaryotic cells, also know as the cell's power producers. Through glycolysis, a process that converts glucose into pyruvate, the energy fuel adenosine triphosphate (ATP) is released. Pyruvate is then used by the mitochondria to generate more ATP, representing 90% of all ATP generated, and reactive oxygen species (ROS) through the reduction of the oxygen from the electron transport chain. ROS include the superoxide, oxygen singlet, hydrogen peroxide and hydroxyl. Intracellular ROS, the majority of which is derived from mitochondria (Finkel and Holbrook, 2000), can act as signaling molecules and play important roles in homeostasis (D'Autréaux and Toledano, 2007). However, excessive production of ROS can lead to a situation known as oxidative stress, resulting in a significant damage to cell structures, a process known to be involved in ageing (Finkel and Holbrook, 2000). Oxidative stress plays a role in the pathogenesis of diseases such as diabetes, atherosclerosis, chronic inflammations or cancer (Crimi et al., 2006). In addition to act as a power house, mitochondria also function as signaling

platforms to regulate innate protective mechanisms (West et al, 2011). It is not known whether mitochondria activities, through ATP and ROS generation, also drive adaptation.

[0007] Toll-like-receptors and homeostasis: Toll-like receptors (TLRs) are evolutionary-conserved type I transmembrane proteins that act as key regulators of innate defense mechanisms essential to the protection and survival of the organism. Mucosal surfaces, exposed to food, represent critical physical and functional interfaces between the body's internal and external world. TLRs are abundantly expressed at mucosal surfaces, especially in the buccal cavity (Wang *et al*, 2009) and in villus and crypt intestinal epithelial cells at their apical poles (Didierlaurent *et al*, 2002; Ortega-Cava 2003). In the general intestinal mucosa, TLRs play a homeostatic role, maintaining the epithelial barrier and preventing responses to TLR agonists on luminal microorganisms (Backhed et al, 2005; Rakoff-Nahoum et al 2004; Abreu 2010). Indeed, deregulation of TLR signaling in the gut can result in chronic inflammatory and excessive and even destructive repair responses that may be associated with diseases like colon cancer and inflammatory bowel diseases (Abreu 2010, Cario 2010). Particularly, the role of mucosal TLR2 in homeostasis is important because it plays a role in gate keeping functions of intestinal epithelial cells (Chabot *et al*, 2006) and controls mucosal inflammation by regulating epithelial barrier function (Cario *et al*, 2007, 2008). Although there is evidence that TLR1/2/4 signaling induces mitochondrial ROS generation through TRAF6 and ECSIT interaction to augment macrophage bactericidal activity (West et al, 2011), it is not known whether TLRs also regulate mitochondria-driven homeostasis. A recent study of TLR evolution shows a clear signature of positive selection in their rates of substitution across primates, suggesting TLRs may also play a significant role in adaptation (Wlasiuk and Nachman, 2010)

[0008] Neurotransmission and homeostasis: Maintaining balanced levels of neurotransmitters contribute to homeostasis. Dopamine (DA) is the key neurotransmitter that regulates the reward, motivation and reinforcement center of the central nervous system (CNS), as well as the hypothalamic-pituitary-adrenal (HPA) axis, known to control stress responses. DA is involved in many motivational behaviors including rewarding, motivation, food intake, food reward, addiction and motor control. Striatum and nucleus accumbens (NAcc) are brain structures where DA is produced, and they are referred sites of control of food reward. It is well documented that DA production in striatum and NAcc is

excessive following binge intake of sugar and fat. There is also modification in Ach and opioids systems such as those observed in drug abuse. Binge eating, associated to obesity, afflicting a large proportion of the American adult population, is also associated with depression, anxiety and substance abuse. Serotonin, the neurotransmitter that plays a role in depression and anxiety through the regulation of mood, can also affect dopamine release in the MLDS brain regions.

Active ingredients

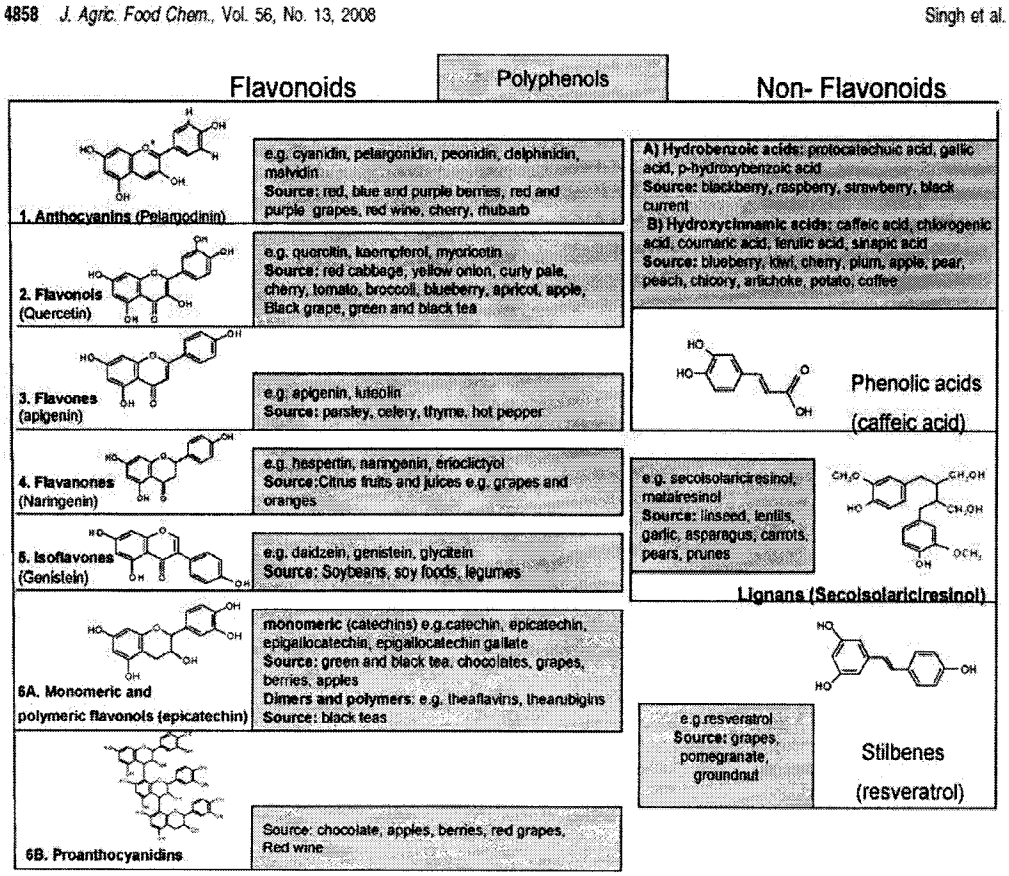
[0009] Adaptogens: The concept of "adaptogens" is thousands of years old, and was long considered an important feature of ancient medical systems in parts of Asia and in northern Europe. Although pharmacological evidence is still missing, herbalists claim that adaptogens derived from plants exert a normalizing effect on the body without any risk of creating an unbalanced state, by inducing healthy functions while decreasing unhealthy responses triggered by stress. For that reason, adaptogens are believed to increase resistance to stress, trauma, anxiety and fatigue. It is believed that many antioxidants are indeed adaptogenic. Studies demonstrated that some adaptogenic plants can modulate TLR activity. For example, it was recently shown that the polyphenol epigallocatechin-3-gallate isolated from green tea, have been shown to down regulate TLR4 signal transduction (Byun *et al* 2010). Also, it was shown that dioscorin, a glycoprotein from *Dioscorea alata,* is a novel TLR4 activator and induces macrophage activation via typical TLR4-signaling pathways (Fu *et al* 2006). Another study demonstrated that 9,10-Dihydro-2,5-dimethoxyphenanthrene-1,7-diol, a phenanthrene isolated from *Eulophia ochreata* of the *Orchidaceae* family, blocked TLR4-dependent NF-kappaB-regulated inflammatory cytokine production (Datla *et al*, 2010). It is not known whether plant adaptogens can affect mitochondrial activity through TLR modulation to promote homeostasis.

[0010] Antioxidants: Antioxidants are described as molecules capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent that can produce free radicals, which are toxic byproducts of cell metabolism that can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. Antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types

of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.. The human body naturally produces antioxidant molecules but this process is not totally effective and that effectiveness declines with age. The fundamental nutritional benefit of fruit and vegetables in the prevention of diseases - especially in the light of the current "anti-aging wave" has directed the attention of scientists and consumers to a variety of berry fruits and their constituents. Many berries have a long tradition in European and North American folk medicine. Based on these experiences and due to the growing interest the number of food supplements on the market containing fruit powders, juice concentrates or extracts of these fruits has increased considerably. Advertising for these products mainly focuses on the phenolic compounds, especially the anthocyanins and proanthocyanidins and their preventive effects. Most of the preparations are combinations, e.g. of extracts of different fruits with vitamins and trace elements, etc. which are labeled in a way which does not allow a comparison of the products (Krenn et al, 2007). Antioxidant-rich food include berries (blueberries, blackberries, raspberries, strawberries and cranberries), beans (small red, kidney, pinto and black beans), fruits (apples, avocados, cherries, pears, pineapple, oranges, plums, kiwi), vegetables (Artichoke, spinach, red cabbage, potatoes, broccoli), beverages (green tea, coffee, red wine, fruit juices), nuts (walnuts, pistachios, pecans, hazelnuts and almonds), many herbs and spices, grains, and dark chocolate. Diets deficient in antioxidants can accelerate cell death, mitochondrial decay contributing to the development of chronic diseases, and other dysfunction. Antioxidant deficiency increases oxidative stress, and consequently leads to mitochondrial dysfunction and age-associated diseases, including metabolic syndrome.

[0011] Polyphenols: Polyphenols occur ubiquitously in plant foods and structurally have variations in the C ring that characterizes the different types namely, flavonols, flavones, isoflavones, flavonones, flavanol and anthocyanins. Polyphenols are present in large amount of fruits and vegetables as secondary plants metabolites and have a significant impact in preventing many diseases in human (Heim, Tagliaferro, & Bobilya, 2002). Polyphenols are potential adaptogens. It is a widely distributed compound in plants and not only have proprieties associated with food quality such as color and aroma, but also may have potential health benefits, including reduction of cancer risk (Macheix, Fleuriet, & Billot, 1990). According to Thériault at al. (2005), maple syrup (Acer saccharum) contains

antioxidant and antiradical activities due to the presence of phenolic compounds. Cranberry polyphenolics, like other dietary polyphenolics, may induce activation mitochondrial apoptosis pathway. This anticancer property lead to tumor cells to apoptosis. The possible effects of cranberry on expression of genes controlling steps in the mitochondrial apoptosis pathway are currently under investigation. The main groups of polyphenols with their individual compounds and food sources are summarized in **Table 1**.



[0012] Anthocyanins: Anthocyanins are polyphenolic compounds that belongs to the flavonoid family. They are colored (blue-red) pigments abundant in many fruits, vegetables and flowers. They are known to have antioxidant activity (Zafra-Stone et al, 2007). It is easy to presume that anthocyanins are also potential adaptogens. They readily donate hydrogen to form relatively stable unpaired-electron structures, and chelate transition metals such as iron. In the context of the nervous system, anthocyanins have been shown to im*prove* motor and cognitive functions in experimental animals, and to prevent ROS-mediated apoptotic death in neurons (Levites et al., 2002). When

mitochondrial proteins integrity is threatened, this may contribute to neurodegenerative disorders along with other apoptogenic mitochondrial disorders. There is evidence that spoiled cytochrome c, a protein in the electron transport chain, can lead to these disorders, and increase oxidative stress, respiratory chain dysfunction, and apoptotic cell death. Moreover, dopamine oxidation in neurons can result in the production of ROS and increase oxidative stress, reduce cytochrome c properties, and, by interfering with normal cytochrome redox cycling, may contribute to dopaminergic neurodegeneration (Mazzio et al. 2004). Yao and Vieira (2007) show that anthocyanins from *Vaccinium* species are potent inhibitors of dopamine oxidative forms.

Antioxidants and prevention of diseases

[0013] Antioxidants are said to be extremely good for us in many ways, helping to prevent and keep under control some serious illnesses. Recent research has shown that antioxidants can help with human health and lifestyles in many ways. Enhancement of antioxidant defenses through dietary supplementation is a reasonable and practical approach to help the body fight off chronic diseases such as cancer, cardiovascular diseases, neurodegenerative diseases, chronic inflammatory diseases, and diabetes (Finkel and Holbrook, 2000). Antioxidants help prevent the physiopathology of these diseases which involves the excessive and chronic presence of inflammatory mediators, oxidative stress and cell death. Antioxidants also help slowing down the ageing process and the development of age-related diseases (Finkel and Holbrook, 2000).

The invention

[0014] It has now been found that the combination of antioxidant extracts can mutually potentiate their antioxidant potential. Combinations of antioxidant sweeteners such as maple syrup and a herbal extract (plants and/or herbs) and/or a plant-product extract (fruits and/or vegetables) having antioxidant properties have never been studied. The present invention demonstrates the synergistic effect of these combinations on antioxidant capacity of functional foods or beverages formulations.

[0015] It has further been found that combinations of antioxidant-rich extracts possess synergistic effects to modulate mechanisms regulating homeostasis, thereby helping to sustain homeostasis.

[0016] It is found that antioxidant-rich formulations developed by Applicant regulate TLR2-

dependent oxidative and energetic mitochondrial responses in oral mucosal cells to promote homeostasis, providing important scientific evidence for their role as regulators of adaptation.

SUMMARY OF THE INVENTION

[0017] In a first aspect, the invention relates to the potentiated antioxidant potential of a composition comprising the following ingredients: an antioxidant herbal extract, an antioxidant plant-product extract; and a natural sweetener having antioxidant properties. This combination creates synergy to potentiate the formulation's antioxidant potential.

[0018] Particularly, there is provided a composition having synergistic antioxidant properties, essentially consisting of: an antioxidant sweetener, an antioxidant plant-product extract and an antioxidant herbal extract, and optionally, a natural flavor or aroma.

[0019] More particularly, there is provided a composition having synergistic antioxidant properties, consisting of: an antioxidant sweetener, an antioxidant plant-product extract and an antioxidant herbal extract; and a natural flavor or aroma.

[0020] This invention proposes that the composition of a natural sweetener such as maple syrup, honey, corn syrup, stevia (or a steviol glycosides such as, for example, Stevioside or Rebaudioside A) or agave with an antioxidant herbal extract and an antioxidant plant-product extract potentiates the antioxidant potential of the extracts to produce a potent antioxidant functional food product.

[0021] In a further aspect of the invention, there is provided a method for maintaining / promoting or recovering homeostasis in a mammal comprising the step of administering a composition as defined above. In particular, oral ingestion is favored as a mode of administration. There is also provided the use of the composition as defined above for the maintenance / promotion or recovery of homeostasis in a mammal. Particularly, the mammal is a human.

[0022] In a further aspect of the invention, there is provided a method for the maintenance of homeostasis in a mammal, the method comprising the steps of: ingesting a composition as defined above that is a TLR modulator. Particularly, TLR modulator acts on TLR-expressing cells to release MIP3alpha, in turn, to affect at least one process involving: dopamine; ATP; inflammatory cytokines such as IL-6 and IL-10; or reduce reactive oxygen species or cell death.

[0023] Still, more particularly, with respect to the above-mentioned aspects, the composition is a liquid or a solid, more particularly an ingestible solid or liquid, most particularly a solid food or a liquid food (i.e. beverage).

[0024] In a further aspect of the invention, there is provided a method for preventing inflammation in a mammal comprising the steps of: ingesting a composition as defined above. There is also provided a use of the composition as defined above to prevent inflammation.

[0025] In a further aspect of the invention, there is provided a method for protecting or maintaining mucosal immunity in a mammal comprising the steps of: ingesting a composition as defined above. There is also provided the use of the composition as defined above for the protection or maintenance of mucosal immunity in a mammal.

[0026] In a further aspect of the invention, there is provided a method for the conservation of ATP levels in a cell of a mammal comprising the steps of: ingesting a composition as defined above. There is also provided the use of the composition as defined above for the conservation of ATP reserves in a cell of a mammal.

[0027] In a further aspect of the invention, there is provided a method for blocking energy release from a cell of a mammal comprising the steps of: ingesting a composition as defined above. The is also provided the use of the composition as defined above for blocking energy release from a cell of a mammal.

[0028] In a further aspect of the invention, there is provided a method for raising energy levels in a cell of a mammal comprising the steps of: ingesting a composition as defined above. There is also provided the use of a composition as defined above for increasing the energy levels of a cell in a mammal.

[0029] In a further aspect of the invention, there is provided a method for protecting a neural cell in a mammal comprising the steps of: ingesting a composition as defined above. There is also provided the use of the composition as defined above for the protection of a neural cell in a mammal.

[0030] In a further aspect of the invention, there is provided a method for the identification of a food or a food ingredient useful for the maintenance of homeostasis in a mammal, the method comprising the steps of:

- measuring the TLR activity of said food or food ingredient;

whereby said food or food ingredient is a TLR modulator when said TLR induces a release of one or more of: IL-10, IL-6, MIP3α, dopamine and ATP; or a decrease in cell death or reactive oxygen species (ROS).

[0031] In an further aspect of the invention, there is provided a transwell assay for the identification of a TLR2 modulator, the assay comprising:

a) in a top well, incubating a layer of TLR-expressing cells with a test agent;

b) in a bottom well in contact with said top chamber, incubating a layer of sub-epithelial cells sensitive to MIP3α;

c) measuring the presence of IL-6 or IL-10; or identifying an increase in ATP; or measuring a decrease in ROS; or a decrease in cell-death from cells in said bottom well ;

wherein the presence of one of said response from step c) is indicative that said test agent is a potential TLR2 modulator.

[0032] In a further aspect of the invention, there is provided a method for the identification of a synergistic antioxidant composition, the method comprising the steps of:

- measuring the antioxidant potential of each component individually;
- measuring the antioxidant potential of the components when combined; and
- comparing the antioxidant potential of the combination versus the individual components;

wherein said combination has synergistic antioxidant properties when the antioxidant potential of the combination is higher than the sum of the antioxidant potential of the individual components.

DETAILED DESCRIPTION OF THE INVENTION

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] Figure 1 shows the levels of polyphenols per dose present in various formulations.

[0034] Figure 2 shows levels of total polyphenols of each ingredient present in the amount used for one dose of formulation 1. Values are expressed as mean +/- SEM of n=3.

[0035] Figure 3 shows levels of total polyphenols of each ingredient present in the

amount used for one dose of formulation 2. Values are expressed as mean +/- SEM of n=3.

[0036] Figure 4 shows levels of total polyphenols of each ingredient present in the amount used for one dose of formulation 3. Values are expressed as mean +/- SEM of n=3.

[0037] Figure 5 shows levels of total polyphenols of each ingredient present in the amount used for one dose of formulation 4. Values are expressed as mean +/- SEM of n=3.

[0038] Figure 6 shows the amount of anthocyanins per dose present in various formulations.

[0039] Figure 7 shows that functional beverages developed by Applicant have high anti-oxidant chemical capacity when compared to various competitors as shown by oxygen-radical-antioxidant capacity (ORAC) value. ORAC values (mean ± SD of 3 independent experiments) are expressed as µmol Trolox equivalent (TE)/ml.

[0040] Figure 8 shows the synergistic effect of ingredients of Formulation 1 to enhance the antioxidant potential of beverages as shown by ORAC value. ORAC values (mean ± SD of 3 independent experiments) are expressed as µmol Trolox equivalent (TE)/ml.

[0041] Figure 9 shows the synergistic effect of ingredients of Formulation 2 to enhance the antioxidant potential of beverages as shown by ORAC value. ORAC values (mean ± SD of 3 independent experiments) are expressed as µmol Trolox equivalent (TE)/ml.

[0042] Figure 10 shows the synergistic effect of ingredients of Formulation 3 to enhance the antioxidant potential of beverages as shown by ORAC value. ORAC values (mean ± SD of 3 independent experiments) are expressed as µmol Trolox equivalent (TE)/ml.

[0043] Figure 11 shows the synergistic effect of ingredients of Formulation 4 to enhance the antioxidant potential of beverages as shown by ORAC value. ORAC values (mean ± SD of 3 independent experiments) are expressed as µmol Trolox equivalent (TE)/ml.

[0044] Figure 12 shows that the combination of three types of ingredients (herbal extract, fruit extract and maple syrup) provides the synergistic effect of Formulation 1. ORAC values are representative of two separate experiments and are expressed as µmol Trolox equivalent (TE)/ml.

[0045] Figure 13 shows that the combination of three types of ingredients (herbal extract, fruit extract and maple syrup) provides the synergistic effect of Formulation 2. ORAC values are representative of two separate experiments and are expressed as µmol Trolox equivalent (TE)/ml.

[0046] Figure 14 shows the antioxidant potential of sweeteners at the same molarity as shown by their ORAC value. ORAC values (n=5) were normalized to the respective sweetening power of each sweetener, and are expressed as µmol Trolox equivalent (TE)/ml.

[0047] Figure 15 shows that agave, another natural sweetener with antioxidant property, can also produce synergy when formulated with the herbal and fruit extracts. ORAC values are expressed as µmol Trolox equivalent (TE)/ml.

[0048] Figure 16 shows the anti-ROS potential in tongue epithelial cells of sweeteners at the same molarity. Values obtained from 3 separate experiments were normalized to the respective sweetening power of each sweetener tested and are expressed as Relative Fluorescence Unit (RFU).

[0049] Figure 17 shows that formulations, used as functional beverages, developed by Applicant have higher antioxidant biochemical potential than most competitors. Values of ROS production are expressed as mean +/- SEM of Relative Fluorescence Unit for n=3.

[0050] Figure 18 shows that formulations, used as functional beverages, developed by Applicant have high antioxidant biochemical potential compared to competitors products as shown by a reduction in ROS production in Cal27 cells. Values of ROS production were normalized to control (40AAPH in cells not exposed HBSS). This data are expressed as mean +/- SEM of n=3.

[0051] Figure 19 shows the antioxidant potential of functional beverages developed by Applicant. Values are representative of two separate experiments, and ROS production is expressed as Relative Fluorescence Unit (RFU).

[0052] Figure 20 shows the anti-ROS activity of various dilutions of functional beverages developed by Applicant and competitors in tongue epithelial cells (Cal27). Values normalized to control are mean+/-SEM of minimum n=3.

[0053] Figure 21 shows that ingredients of Formulation 1 act in synergy to modulate ROS

production artificially induced by AAPH in tongue epithelial CAL27 cells treated for up to 120 minutes. Values are representative of two separate experiments, and ROS production is expressed as Relative Fluorescence Unit (RFU).

[0054] Figure 22 shows that ingredients of Formulation 2 act in synergy to modulate ROS production artificially induced by AAPH in tongue epithelial CAL27 cells treated for 60 minutes. Values are representative of two separate experiments, and ROS production is expressed as Relative Fluorescence Unit (RFU).

[0055] Figure 23 shows that ingredients of Formulation 3 act in synergy to modulate ROS production artificially induced by AAPH in tongue epithelial CAL27 cells treated for 60 minutes. Values are representative of two separate experiments, and ROS production is expressed as Relative Fluorescence Unit (RFU).

[0056] Figure 24 shows that ingredients of Formulation 4 act in synergy to modulate ROS production artificially induced by AAPH in tongue epithelial CAL27 cells treated for 60 minutes. Values are representative of two separate experiments, and ROS production is expressed as Relative Fluorescence Unit (RFU).

[0057] Figure 25 shows the impact of various formulations on basal ROS production (without the addition of the ROS inducer AAPH) in Cal27 cells. Values of ROS production are expressed as mean +/- SEM of n=3.

[0058] Figure 26 shows the impact of various formulations on mitochondrial activity of Cal27 cells. MTT results measuring mitochondrial activity were normalized to control levels. Values are expressed as mean +/- SEM of n=3.

[0059] Figure 27 shows ATP responses of Cal27 cells exposed to various formulations. Extracellular levels of ATP were used as a measure of ATP secretion. Data from 3 separate experiments (n=3) were normalized to control, and values are expressed as mean of normalized ATP levels +/- SEM, where control equals 100%.

[0060] Figure 28 shows the overall energetic potential of Cal27 cells exposed to various formulations. Data were obtained for 3 separate experiments (n=3). Values are expressed as mean +/- SEM.

[0061] Figure 29 shows ATP responses in Cal27 cells undergoing oxidative stress (treated with 40mM AAPH), and exposed to various formulations. Extracellular ATP levels

were measured to determine levels of ATP secreted (dashed lines). Values were normalized to control levels, and are expressed as mean +/- SEM of normalized ATP levels of three separate experiments (n=3).

[0062] Figure 30 shows the overall energetic potential of Cal27 cells exposed to various formulations. Values of three separate experiments (n=3) were normalized to control levels, and are expressed as mean +/- SEM of normalized ATP levels produced.

[0063] Figure 31 shows that ingredients of formulation 2 act in synergy to block ATP secretion in Cal27 to cause a maintained state of energy conservation. Extracellular ATP levels were normalized to untreated control (100%). Values are mean +/- SEM of n=2.

[0064] Figure 32 demonstrates that ingredients of formulation 1 act in synergy to enhance mitochondrial activity of CAL27 cells undergoing high oxidative stress. Values are expressed as mean +/- SEM of n=3.

[0065] Figure 33 demonstrates that ingredients of formulation 2 act in synergy to enhance mitochondrial activity of CAL27 cells undergoing high oxidative stress. Values are expressed as mean +/- SEM of n=3.

[0066] Figure 34 shows the impact of TLR2 activation with TLR2 agonist Pam3Cys (500ng/ml) on ROS production, mitochondrial activity and ATP responses in Cal27 cells undergoing low or high oxidative stress upon treatment with 6.4 mM and 16mM AAPH respectively. Values are expressed as mean +/- SEM of n=3.

[0067] Figure 35 demonstrates the impact of various formulations on TLR2-dependent ROS production in Cal27 exposed for 30 minutes to various concentrations of AAPH. Data is representative of two separate experiments.

[0068] Figure 36 shows TLR2-specific ROS production in HEK-TLR2+ cells treated for 3 hours with Pam3Cys (500ng/ml). Values are expressed as mean +/- SEM of n=4.

[0069] Figure 37 shows ROS production by dopaminergic SH-SY5Y neurons exposed to supernatant of Cal27 cells exposed to various formulations and the impact of low TLR2 activation induced with 50ng/ml of Pam3Cys. Data are representative of 2 separate experiments.

[0070] Figure 38 shows that Ingredients of functional beverage developed by Applicant (Formulation 1) act in synergy to modulate the TLR2-dependent release of DC-attracting

chemokine MIP3α from HEK-TLR2+ cells (16 hours incubation). This data is representative of two separate experiments.

[0071] Figure 39 Ingredients of functional beverage developed by Applicant (Formulation 2) act in synergy to modulate the TLR2-dependent release of DC-attracting chemokine MIP3α from HEK-TLR2+ cells (16 hours incubation). This data is representative of two separate experiments.

[0072] Figure 40 shows that ingredients of Formulation 1 (SN) act in synergy to inhibit release of pro-inflammatory cytokine IL-6 in THP1-PMA cells treated with PAM3CSK4 for 22 hours. This data is representative of two separate experiments.

[0073] Figure 41 shows that ingredients of Formulation 1 act in synergy to enhance release of anti-inflammatory cytokine IL-10 in THP1-PMA cells at basal condition. Values are expressed as pg/ml of human IL10.

[0074] Figure 42 shows the levels of salivary dopamine detected 5 minutes after drinking 20 ml of various beverages in healthy human individuals. Values are expressed as Mean +/- SEM of salivary dopamine ng/ml of at least n=6.

[0075] Figure 43 is a schematic representation of the mechanism of action of Applicant's invention. Formulations developed by Applicant, delivered at oral mucosal surfaces, can regulate mucosal and sub mucosal responses to promote homeostasis.

[0076] Figure 44 is a schematic representation of results obtained with formulation 1.

[0077] Figure 45 is a table that summarizes results obtained for all formulations tested.

[0078] Figure 46 shows the antioxidant potential of maple syrup (SE), stevia, steviol glycosides (stevioside, rebaudioside A), aspartame and sucralose, individually or in combination. The results are representative of 3 separate experiments.

[0079] Figure 47 shows the levels of polyphenols for different anti-oxidant sweeteners The results are representative of 3 separate experiments.

ABBREVIATIONS

[0080] The term "ECSIT" means evolutionarily conserved signaling intermediate in Toll pathways

[0081] The term "ORAC" means Oxygen Radical Absorbance Capacity and is expressed

in ORAC value when measured in µmol Trolox equivalent (TE/ml).

[0082] The term "TRAF6" means TNF receptor associated factor (TRAF) 6.

DEFINITIONS

[0083] In the present description, a number of terms are extensively used. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

[0084] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one" but it is also consistent with the meaning of "one or more", "at least one", and "one or more than one".

[0085] Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. In general, the terminology "about" is meant to designate a possible variation of up to 10%. Therefore, a variation of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 % of a value is included in the term "about".

[0086] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, un-recited elements or method steps.

[0087] The term "concentrate" means a composition comprising substantially all the same ingredients as the native fruit/vegetable/herb but in at a higher concentration i.e. where a portion or all of the water/solvent has been removed.

[0088] The term "extract" are used herein means a type of concentrate where certain active compounds are enriched when compared to other compounds in the native fruit/vegetable/herb composition.

[0089] The term "subject" or "patient" as used herein refers to an animal, preferably a mammal, and most preferably a human who is the object of treatment, observation or experiment.

[0090] "Mammals" includes humans, domestic animals such as laboratory animals and

household pets, (e.g. cats, dogs, swine, cattle, sheep, goats, horses, rabbits), and non-domestic animals such as wildlife and the like.

[0091] The term "herbal extract" as used herein defines an extract that is obtained from a plant, or a herb, including flowers, stems, roots, seeds and leaves.

[0092] The term "plant-product" as used herein defines an extract that is obtained from the product of a plant such as, for example, a fruit or a vegetable, or a seed.

[0093] The compounds and extracts described herein can be formulated as ingestible compositions by formulation with additives such as physiologically acceptable excipients, physiologically acceptable carriers, and physiologically acceptable vehicles, or as nutraceutical or nutritional formulations with additives such as nutraceutically or nutritionally acceptable excipients, nutraceutically or nutritionally acceptable carriers, and nutraceutically or nutritionally acceptable vehicles. As used herein, the term "physiologically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar unwanted reaction, such as gastric upset, dizziness and the like, when administered to human. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compounds of the present invention may be administered. Sterile water or aqueous saline solutions and aqueous dextrose and glycerol solutions may be employed as carrier, particularly for injectable solutions.

[0094] The compounds, compositions and extracts of the present invention can be prepared as nutritional formulations such as foods, including medical or functional foods and dietary supplements. A "medical or functional food" is defined as being consumed as part of a usual diet but which has been demonstrated to have physiological benefits and/or to reduce the risk of a disease or condition such as a chronic disease, beyond basic nutritional functions. A "dietary supplement" is defined as a product that is intended to supplement the human diet and is typically provided in the form of a pill, capsule, tablet, or like formulation. By way of example, but not limitation, a dietary supplement may include one or more of the following ingredients: vitamins, minerals, herbs, botanicals, amino acids, dietary substances intended to supplement the diet by increasing total dietary intake, and concentrates, metabolites, constituents, extracts or combinations of any of the foregoing. Dietary supplements may also be incorporated into food stuffs, such as functional foods designed to promote health or to prevent disease or disorders. If

administered as a medicinal preparation, the composition can be administered, either as a prophylaxis or treatment, to a patient in any of a number of methods. The subject compositions may be administered alone or in combination with other pharmaceutical agents and can be combined with a physiologically acceptable carrier thereof. The effective amount and method of administration and aim of the particular formulation can vary based on the individual subject, the stage of the disease or condition, and other factors evident to one skilled in the art. In the case of a pharmaceutical formulation as well as a nutraceutical formulation, during the course of the treatment, the concentration of the subject compositions may be monitored (for example, blood plasma levels may be monitored) to insure that the desired level is maintained.

[0095] The term "nutraceutical" has been used to refer to any substance that is a food or a part of a food and provides medical or health benefits, including the prevention and treatment of disease or condition. Thus, a nutraceutical is a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with foods. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease. Hence, compositions falling under the label "nutraceutical" may range from isolated nutrients, dietary supplements and specific diets to genetically engineered designer foods, herbal products, and processed foods such as cereals, soups and beverages. In a more technical sense, the term has been used to refer to a product isolated or purified from foods, and generally sold in medicinal forms not usually associated with food and demonstrated to have a physiological benefit or provide protection against chronic disease. Suitable nutraceutically acceptable excipients may include liquid solutions such as a solution comprising a vegetable- and/or animal-and/or fish-derived oil.

[0096] As used herein, the terms "disease" and "disorder" may be used interchangeably or may be different in that the particular malady or condition may not have a known causative agent (so that etiology has not yet been worked out) and it is therefore not yet recognized as a disease but only as an undesirable condition or syndrome, wherein a more or less specific set of symptoms have been identified by clinicians.

[0097] The compounds, compositions and extracts of the present invention may be administered as either a food or a food supplement. For example, when provided as a food, the extracts of the present invention are combined with material primarily made up of

protein, carbohydrate and/or fat that is used in the body, preferably a human body, to sustain growth, repair, and vital processes, and to furnish energy. When provided as a food supplement, the compositions comprise selected substances such that they can be eaten at or about the same time as a food. The food supplements are generally eaten within about one hour before or after the food is eaten, typically within about one-half hour before or after the food is eaten, preferably within about 15 minutes of when the food is eaten, and further preferably within one to five minutes of the time the food is eaten. The food supplement can also be eaten at the same time as the food, or even with the food.

[0098] A "natural product" refers to naturally-occurring compounds that are end products of secondary metabolism; often, they are unique compounds for particular organisms or classes of organisms. An "all-natural product" refers to a product made with and/or from only natural compounds or products.

[0099] "Organic certification", "organic certifiable" or the like refers to a certification process for producers of organic food and other organic agricultural products. In general, any business directly involved in food production can be certified, including seed suppliers, farmers, food processors, retailers and restaurants. Requirements vary from country to country, and generally involve a set of production standards for growing, storage, processing, packaging and shipping that include, for example: avoidance of most synthetic chemical inputs (e.g. fertilizer, pesticides, antibiotics, food additives), genetically modified organisms, irradiation, and the use of sewage sludge; use of farmland that has been free from synthetic chemicals for a number of years (often, three or more); keeping detailed written production and sales records (audit trail); maintaining strict physical separation of organic products from non-certified products; and undergoing periodic on-site inspections. In some countries, certification is overseen by the government, and commercial use of the term organic is legally restricted.

[00100] An "organic food" refers to a food made with ingredients derived from crops obtained from organic farming and made in a way that limits or excludes the use of synthetic materials during production. Organic agricultural methods are internationally regulated and legally enforced based in large part on the standards set by the International Federation of Organic Agriculture Movements (IFOAM). For greater clarity, unless otherwise specified the use herein of the term "organic" preceding any plant, herb, animal or food product thereof refers to a product made with ingredients derived from

crops obtained from organic farming and made in a way that limits or excludes the use of synthetic materials during production.

[00101] "As used herein, the phrase "sweetening compounds", "sweetener compounds", "sweetener" or the like generally refers to a natural additive which increases the basic taste of sweetness of a product to be ingested and can be considered as a sugar substitute (with or without additional calories).

[00102] "Antioxidant compounds" refers to any molecules capable of slowing or preventing the oxidation of other molecules that may cause oxidative stress and may damage or kill cells. Oxidative stress is thought to be associated with many human diseases.

[00103] A "food additive" refers to any substance added to foods during processing thereof to improve characteristics such as color, texture, flavor, and/or conservation. A "food supplement", "dietary supplement" or "nutritional supplement", refers to a preparation intended to provide nutrients, such as vitamins, minerals, fiber, fatty acids or amino acids, that may be missing or may not consumed in sufficient quantities in an individual's diet.

[00104] "Medical food" refers to any food that is specially formulated and intended for the dietary management of a disease that has distinctive nutritional needs that cannot be met by normal di*et al*one.

[00105] A "functional food" is similar in appearance to, or may be, a conventional food that is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions, i.e. they contain a bioactive compound.

[00106] "Synergy" is the condition where a combination of ingredients generates a higher activity (chemical and/or biological) than the sum of their individual ingredients for that same activity.

DETAILED DESCRIPTION OF PARTICULAR EMBODIMENTS

[00107] Because maple syrup and other natural sweeteners of the present invention has sweetening potential with antioxidant activity, it can be of special interest to the food industry, for example, as a food additive, a food supplement, and/or a functional food.

[00108] Therefore, in a first aspect, the invention relates to the potentiated antioxidant potential of a natural health products formulated with a combination having the following main ingredients: at least one of an antioxidant herbal extract, at least one of an antioxidant fruit extract; and at least one of a natural sweetener having antioxidant properties. This combination creates synergy to potentiate the formulation's antioxidant potential. This invention proposes that the combination of a natural sweetener having antioxidant activity such as maple syrup, honey, corn syrup, coconut sugar, Stevia or steviol glycosides (such as, for example, stevioside or Rebaudioside A) or agave in combination with an antioxidant plant extract and an antioxidant fruit extract potentiates the anti-oxidant potential of the fruit and plant extracts to produce a potent antioxidant functional food product.

[00109] In a further aspect, there is provided a composition having synergistic antioxidant properties, comprising: an antioxidant sweetener, an antioxidant fruit extract and an antioxidant plant extract, in such proportions that each component potentiates the antioxidant effect of other component. Particularly, there is provided a composition having synergistic antioxidant properties, essentially consisting of: an antioxidant sweetener, an antioxidant fruit extract and an antioxidant plant extract, in such proportions that each component potentiates the antioxidant effect of other component. More particularly, there is provided, there is provided a composition having synergistic antioxidant properties, consisting of: an antioxidant sweetener, an antioxidant fruit extract and an antioxidant plant extract, in such proportions that each component potentiates the antioxidant effect of other component; and a flavor or aroma.

Food format

[00110] Particularly, with respect to the above-mentioned aspects, the functional food products to which this invention can apply to include functional ingredients is selected from the group consisting of: liquid or solid food, formulated for oral intake. Particularly, the liquid food can be in the form of a functional beverage or in the form of a solid in concentrated form (such as a tablet, capsule, caplet or a powder) to be dissolved in water (such as plain water, carbonized water, source water or mineral water) prior to consumption. Particularly, the solid food can be in the form of a capsule for direct ingestion, or a bar or may take a semi-solid form (such as a pudding or yogurt). In the latter case, the combination can be formulated as nanoparticles to stay in suspension and

be protected from the other ingredients.

[00111] Particularly, the composition may further comprise flavor or aroma, more particularly, the flavor or aroma is natural.

Antioxidant synergistic food combinations

[00112] In a further aspect of the invention, there is provided a method for the identification of synergistic antioxidant compositions, the method comprising the steps of:

- measuring the antioxidant potential of each component individually;
- measuring the antioxidant potential of the components when combined; and
- comparing the antioxidant potential of the combination versus the individual components;

wherein said combination has synergistic antioxidant properties when the antioxidant potential of the combination is higher than the sum of the antioxidant potential; of the individual components.

Antioxidant Fruit/vegetables extracts:

[00113] Anthocyanins contained in blueberry, bilberry, cranberry, elderberry, raspberry seeds and strawberry can reduce age-induced oxidative stress, modulate inflammatory responses, and cal also improve neuronal and cognitive brain functions, ocular health, and protect genomic DNA integrity (Zafra-Stone *et al*, 2007). Applicant's invention can involve any fruit/vegetable extract containing at least one fruit/vegetable extract with antioxidant property.

[00114] Fruits and vegetables and their extract that have high antioxidant properties may be selected from: Acai, apple, apricot, avocado, banana, bakeapple, blackberry, blueberry, cherry, chokeberry, cloudberry, cranberry, current, dates, elderberry, fig, dogberry, gooseberry, grapefruit, grape, guava, raspberry, strawberry, kiwi, lemon, lime, makiang, maloud, mango, mango steam, melon, nectarine, noni fruit, orange, papaya, peach, pear, pineapple, plum, pomegranate, prune, raisin, rosehip, tangerine, watermelon, alfalfa seed sprouted, artichoke, arrugula, asparagus, lima bean, bean, beet, broccoli, cabbage, carrot, cauliflower, celery, chive, coriander, corn, cucumber, eggplant, fennel, leek, lemon balm, lettuce, mushroom, pea, pepper, tomato, pumpkin, radish, soy bean, spinach, squash, sweet potato.

[00115] Red wine is considered herein to be a fruit extract since it comes from

grapes and contains many of the grape's polyphenols and other functional molecules.

[00116] This category also includes leguminous such as peas and lentil, particularly chick peas, cow peas, broad peas, and chocolate.

Plant/herbs and antioxidant:

[00117] The herbal extracts used in Applicant's functional drinks contain vitamins, polysaccharides, polyphenols (tannins, flavonoids), terpenes, alkaloides and organic acids (succinic, cafeic, chlorogenic, geranic). For details, refer to the table below or plant/herb file. Applicant's invention can involve any plant/herb extract containing at least one plant/herb extract with antioxidant property.

[00118] Antioxidant plant or herbal extracts or spices may be selected from: basil, dill weed, marjoram, oregano, peppermint, sage, savory, cardamon, chili, cinnamon, cloves, cumin, curry, garlic, ginger, Juniper plant, mustard, nutmeg, onion, paprika, parsley, pepper, poppy seed, rosemary, thyme, turmeric, vanilla beans, tarragon.

[00119] Teas are also included in the definition of herb/plant extract such as black tea, green tea, white tea, Labrador tea. Cereals can also be considered as plant/herb extract, such as, for example: rice, bran or sorghum.

Non-exhaustive list of herbal extracts:

English	***Latin***
Dandeleon root	*Taraxacum officinale*
Bilberry leaf	*Vaccinium myrtillus*
Galega leaf	*Galega officinalis*
Echinacea	*Echinacea purpurea*
Nettle	*Urtica dioica*
Sage	*Salvia officinalis*
Thyme	*Thymus vulgaris*
Lavender	*Lavandula angustifolia*
Hawthorn	*Cratagus oxycantha*
Verbena	*Lippia citriodora*
Rosemary	*Rosmarinus officinalis L.*
Lemon balm	*Melissa officinalis*
Peppermint leaf	*Mentha X piperita*
Oat	*Avena sativa*
Marshmellow root	*Athaea officinalis*
Red raspberry leaf	*Rubus idaeus*
Astragalus	*Astragalus menbranaceus*
Fennel seed	*Foeniculum vulgare*
Skullcaps	*Scutellaria laterifolia*
Yarrow	*Achillea millefolium*
Hyssop	*Hyssopus officinalis*

Motherwort	*Leonurus cardiaca*
Labrador tea	*Ledum palustre*
Garden angelica	*Angelica archangelica* L.
Winter Cherry	*Withania somnifera*
Fenugreek	*Trigonella foenum-graecum*

Anti-oxidant natural sweeteners:

[00120] There is an increasing interest for natural sweeteners. This interest stems partially from increasing consumer demand for natural products, but also from the rise of a variety of businesses selling natural products and requiring suppliers of such products to certify that natural ingredients are used in any products being supplied. Sweeteners are divided into two major categories : nutritive and non-nutritive sweeteners. Nutritive sweeteners include sugar cane, high fructose corn syrup (HFCS), agave syrup, honey, and maple syrup. Non-nutritive sweeteners provide no energy (zero-calorie) and are further divided into two separate groups. You can have artificial/synthetic or natural non-nutritive sweeteners. These non-nutritive sweeteners are used in diet food products as sugar alternatives. Artificial or synthetic sweeteners that will be used in this study are aspartame and sucralose. The only natural zero-calorie sweetener is stevia, a sweetening extract rich in stevioside and rebaudioside A derived from the plant *Stevia Rebaudiana*. Once absorbed by the body, stevioside and rebaudioside A from stevia extract is metabolized into steviol. The molecular composition of each sweetener is also shown which involves various levels of sucrose, fructose, glucose, sucralose or maltose and the presence of other molecules with antioxidant and anti-inflammatory properties such as polyphenols (see **Figure 47**). Since glucose is the main source of energy for brain cells, we will also use a glucose solution as the control of reference for sugar in our experiments.

[00121] Irrespective of the fact of whether a sweetener is nutritive or non-nutritive, or natural or artificial, it has been found by Applicant that only the following sweeteners have potent anti-oxidant activity that can synergize the anti-oxidant potential of other extracts such as from plants, vegetables or fruits. Such sweeteners are selected from: maple syrup, honey, coconut sugar, agave, corn syrup or stevia (or steviol glycosides such as stevioside or Rebaudioside A).

[00122] Particularly, the invention proposes that the composition comprises an anti-oxidant sweetener selected from the group consisting of: maple syrup, honey, agave or

stevia (or steviol glycosides such as stevioside or Rebaudioside A). More particularly, the composition comprises maple syrup, honey, stevia, stevioside or Rebaudioside A. Most particularly, the composition comprises maple syrup or honey.

Maple syrup:

[00123] Maple products obtained from the sap of maple tress (*Acer saccharum*) are typically viewed as sweeteners with energetic and sugar-containing nutritional potentials. However, maple syrup, native to North America, is much more than a concentrated sugar solution. It contains organic acids, amino acids, minerals, and a wide variety of unidentified compounds chemicals formed during the evaporation process that contribute to color and taste. It is non-obvious for the food industry to consider maple-derived ingredients for dietary products for individuals with diabetic and obesity problems.

Health benefits of maple syrup:

[00124] Functional properties of maple syrup are numerous. There is now evidence that it also has anti-cancer properties, possibly due to its antioxidant properties. Various studies related to maple sap and maple syrup from Quebec showed that phenolic compounds (see Table 2) interfere with three essential phenomena involved in the development of tumors: oxidation, inflammation and angiogenesis, the process of developing new blood vessels (Béliveau *et al*, 2006, Legault J. *et al*, 2007).

Table 2

Table 1. Typical Organic Components of Maple Sap

Component	Fraction of Total Organic Content[a,b]	Actual Concentration in Sap
Sucrose	98.0–100%	2–2.5%
Glucose	0–0.17%	0–0.004%
Phenolic compounds	0–4.55 ppm	0–0.1 ppm
Primary amines	0.5–36.1 ppm	0.01–0.9 ppm
Peptides	0.4–18.6 ppm	0.01–0.41 ppm
Amino acids	0–11.3 ppm	0–0.25 ppm
Protein	0–50.9 ppm	0–1.2 ppm
Other organic acids	0–45 ppm	0–1 ppm

[a]The total solids in the sap are 1.0–5.4% and the pH of the sap is 3.9 – 7.9. [b]The data are from ref 1, Appendix 2 and are used with permission.

[00125] Another study demonstrated that maple sap and syrup can inhibit nitric oxide overproduction and can have antiproliferative effect in cells *in vitro* (Legault *et al*, 2010). Recently, a research group identified 20 compounds, 13 of which were newly

discovered (Li *et al*, 2010). Several of these antioxidant compounds newly identified have also been reported to have anti-cancer, anti-bacterial, and anti-diabetic properties. Maple syrup contains important quantity of abcissic acid, a phytohormone that stimulates insulin release through pancreatic cells and that increases sensitivity of fat cells to insulin, which makes it a potent weapon against metabolic syndrome and diabetes according to researchers from Rhode Island University. There is evidence that dietary abcissic acid can help ameliorate glucose tolerance and obesity-related inflammation in mice fed with high fat diets, and that it may also play a role in reducing the risk of atherosclerosis and IBD by suppressing the inflammatory conditions related with these diseases (Guri *et al*, 2010). Anti-hyperglycemic activity of maples products has been proposed, because the compound acertannin, known to have that activity has been isolated from maple leaves.

Specific formulations

[00126] Particularly, there is provided a composition comprising: extract of Lemon balm, extract of Skullcaps *(Scutellaria)*, blueberry concentrate, cranberry concentrate and maple syrup. More particularly, there is provided to use of such a composition for the relief of nervousness or sleeplessness due to mental stress.

[00127] Particularly, there is provided a composition comprising: extract of Hawthorn, extract of Skullcaps, apple concentrate, cranberry concentrate and maple syrup. More particularly, there is provided to use of such a composition for supporting cardiovascular health in adults and helping relieve nervousness.

[00128] Particularly, there is provided a composition comprising: extract of Skullcaps, extract of Raspberry bush, Yarrow, apple concentrate, cranberry concentrate and maple syrup. More particularly, there is provided to use of such a composition for the maintenance of women's health, particularly for the relief of menstrual pain.

[00129] Particularly, there is provided a composition comprising: extract of Thyme, extract of Hyssop, apple concentrate, cranberry concentrate and maple syrup. More particularly, there is provided to use of such a composition for the maintenance of digestive health, particularly for the relief of flatulent dyspensia and/or colics.

Use for the maintenance of homeostasis

[00130] In a further aspect of the invention, there is provided a method for the maintenance of homeostasis in a mammal, the method comprising the steps of: ingesting

a food or a composition that is a TLR modulator. Particularly, the food or composition is as defined herein.

Method for the identification of foods useful for homeostasis

[00131] In a further aspect of the invention, there is provided a method for the identification of a food or a food ingredient useful for the maintenance of homeostasis in a mammal, the method comprising the steps of:

- measuring the TLR activity by said food or food ingredient;

whereby said food or food ingredient is a TLR modulator when said TLR modulates the release of one of: IL10, IL6, MIP3α, dopamine and ATP; or a decrease in cell death or reactive oxygen species (ROS).

Inflammation and homeostasis

Chronic inflammation:

[00132] Research by scientists at the University of California, San Diego, and Switzerland's University of Fribourg discovered that inflammation provoked by immune cells called macrophages leads to insulin resistance and then to type II diabetes. Their research also showed that obesity without inflammation doesn't result in insulin resistance. Neurodegenerative diseases are characterized by the loss of either specific neurons or the impairment of specific neurotransmission function. Parkinson's disease is characterized by the progressive loss of dopaminergic neurons in the substantia nigra. Neuroinflammation is believed to contribute to the pathogenesis of PD and neurodegeneration is associated with activated microglia. Alzheimer's disease (AD) is characterized by the accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles leading to dysfunction mainly to cholinergic neurons. Various cytokines (IL-1, IL-6 and TNF-alpha) are especially elevated in AD patients. Huntington's disease (HD) is characterized by the progressive loss of neurons of the basal ganglia region including striatal cells. The pathogenesis of HD appears to involve chronic ATP depletion, oxidative stress and mitochondrial dysfunction. These cell conditions are known to produce pro-inflammatory responses. Amyotrophic lateral sclerosis (ALS) is characterized by the progressive loss of motor neurons in the central nervous system (brain and spinal cord) that control voluntary muscle movement.

Use against inflammation:

[00133] In a further aspect of the invention, there is provided a method for preventing inflammation in a mammal, the method comprising the steps of: ingesting a food or a composition that is TLR modulator. Particularly, the food or composition is as defined herein.

Toll-like receptors (TLRs)

[00134] TLRs are evolutionary-conserved type I transmembrane proteins (TLR1-11) that can recognize specific patterns of various types of molecules of bacterial, viral, parasitic, fungal or host origin. Thus, TLRs are versatile but selective. TLR activation triggers signaling cascades that result in the transcription of a multitude of inflammatory and immunomodulatory genes such as cytokines, chemokines and co-stimulatory molecules critical in determining the fate of immune responses. For that reason, TLRs are often considered critical linkers of innate and adaptive immunity. TLRs are abundantly present in immune cells that have the capacity to present antigens to lymphocytes in order to control the fate of the immune responses. Immune TLR-expressing cells include dendritic cells, macrophages, NK cells, and microglia located in the central nervous system.

TLRs in mucosal epithelia:

[00135] TLRs are present on epithelial cells of mucosal surfaces. For example. TLRs are abundantly expressed at mucosal surfaces, in the buccal cavity in taste bud cells. Recent evidence shows that epithelial TLR2 and TLR4 are involved in gatekeeping functions of the mucosa (Chabot et al, 2006). They demonstrated that activation of TLR2 mediates mucosal uptake of particles by the follicle-associated epithelium of intestinal Peyer's patches, which results in enhanced numbers of subepithelial dendritic cells (DCs) located into the FAE (Chabot et al, 2006, 2007, 2008, Anasova et al, 2008), suggesting that TLR2 activation causes the release of signals that attracts DC to take up particles that are transported across the FAE. Interactions between epithelial cells (ECs) and DCs provide an important bridge critical in determining the fate of immune responses. ECs "educate" sub-epithelial DCs through mechanisms that include the release of soluble mediators, such as the DC-attracting chemokine MIP3alpha. The release of MIP3alpha is also induced upon TLR2 activation. Although villus and crypt epithelial cells express TLRs at their apical poles *in vivo*, the intestinal mucosa of healthy individuals nevertheless

coexists with the commensal microflora without chronic inflammation. It is thought that sensing of commensal bacteria through epithelial TLRs in vivo contributes to TLR-dependent intestinal homeostasis observed in vivo. Expression of TLR2, TLR3 and TLR4 has been shown in a human salivary gland cell line and their expressed was enhanced in labial salivary glands of patients with Sjögren's syndrome. Activation of intestinal epithelial TLR results in the release of IL-6, IL-10, and TNFalpha (de Kivit et al, 2011).

TLRs and homeostasis:

[00136] TLRs participate in maintenance of tissue homeostasis and responses to injury. In the gut, the role of TLR has been associated with intestinal development, epithelial cell homeostasis and gut repair mechanisms. Indeed, deregulation of TLR signaling in the gut can result in chronic inflammation and excessive and even destructive repair responses that may be associated with diseases like colon cancer and inflammatory bowel diseases. Several negative regulators of TLR signaling that include IRAK-M, Tollip, SIGIRR, A20, Nod and PPARgamma contribute to control their activation in the intestinal epithelium in order to avoid prolonged and excessive activation of TLRs leading to uncontrolled inflammation detrimental to the host. In the pancreas, TLR signaling through the adaptor protein Myd88 has been shown to mediate a homeostatic effect on beta cells primarily in the setting of injury. The role of TLR2 in mucosal homeostasis is of particular interest because it appears to play a specific role in gatekeeping functions of mucosal intestinal epithelial cells (Chabot *et al*, 2006). A recent study demonstrates that TLR2 act as a transporting receptor that triggers uptake of TLR ligand across intestinal epithelial cells from the apical side to the basolateral side of the epithelial barrier by exosome-associated transcellular transcytosis (Bu *et al*, 2010). Studies aimed to determine the functional relevance of TLR to control tight junction-associated intestinal epithelial barrier integrity to balance mucosal homeostasis against inflammatory stress-induced damage show that oral intake of TLR2 ligand significantly inhibited mucosal inflammation and apoptosis by impacting epithelial integrity *in vivo*. This study suggests that TLR2 controls mucosal inflammation by regulating epithelial barrier function (Cario *et al*, 2007, 2008). A recent study shows that TLR activation affect gap junctional intercellular communication by modulating connexin-43 synthesis, suggesting that TLR2 regulates epithelial barrier functions through this mechanism (Ey *et al*, 2009). The role of TLR2 in regulating mucosal homeostasis is further confirmed in interleukin-10

deficient mice that develop experimental colitis. These studies suggest that TLR2 contributes to the anti-inflammatory action of IL-10 since IL-10 deficient mice lack TGF-beta/Smad-mediated TLR2 degradation that inhibits proinflammatory gene expression in intestinal epithelial cells under chronic inflammatory conditions. The protective role of TLR2 is further demonstrated using TLR2-deficient mice to study the development of colitis-associated colorectal cancer. Indeed, TLR2-deficient developed more and larger colorectal tumors (Lowe *et al*, 2010). Finally, TLR2 plays a role in glucose homeostasis since it was shown to be involved in insulin resistance and pancreatic beta cell function. Data show that glucose tolerance, insulin sensitivity and insulin secretion were improved in TLR2-deficient mice under high-fat diet, while tissue inflammation is reduced (Ehses *et al*, 2010). This study further suggest that TLR2 plays a role in the regulation of glucose homeostasis and the regulation of tissue inflammation.

TLRs in diseases:

[00137] Among the TLRs, TLR2 and TLR4 play a critical role in the pathogenesis of diabetes, obesity and neurodegenerative diseases demonstrated in both clinical and experimental conditions.

Plant/herbs/Food and TLRs:

[00138] Plant/herb-derived molecules can impact the TLR system. Recent evidence supports the search for plant/herb-derived TLR antagonists, as potential anti-inflammatory agents. It has been shown that some plant/herb-derived compounds can activate both innate and adaptive immunity through toll-like receptors signaling pathways and particularly the toll-like receptor 4 (TLR4) signaling pathway. TLR4 is a promising molecular target for immune-modulating drugs, and TLR4 agonists are of therapeutic potential for treating immune diseases and cancers. Several medicinal herb-derived components have recently been reported to act via TLR4-dependent pathways, suggesting that medicinal plant/herbs are potential resources for identifying TLR4 activators. Interestingly, anticancer drug Paclitaxel from Pacific yew tree interacts with human MD-2 and activates dendritic cells through TLR4. In 2006, scientists have discovered that dioscorin, a glycoprotein from *Dioscorea alata,* is a novel TLR4 activator and induces macrophage activation via typical TLR4-signaling pathways (Fu *et al* 2006). Datia *et al* (2010) showed that 9,10-Dihydro-2,5-dimethoxyphenanthrene-1,7-diol, a phenanthrene isolated from *Eulophia ochreata*, one of the Orchidaceae family, blocked

signals generated by TLR4 activation, as shown by down-regulation of NF-kappaB-regulated inflammatory cytokines. It is known that the intake of glucose or a high-fat, high-carbohydrate meal induces an increase in inflammation and oxidative stress in circulating mononuclear cells (MNCs) of normal-weight subjects. A recent study has shown that the combination of glucose or water and the HFHC meal induced oxidative and inflammatory stress and an increase in TLR expression and plasma endotoxin concentrations. In contrast, orange juice intake with the HFHC meal prevented meal-induced oxidative and inflammatory stress, including the increase in endotoxin and TLR expression (Ghanim *et al.* 2010). Saponines from plant can suppress TLR4-mediated inflammatory cytokine production and NF-κB activation specifically by inhibiting TLR4 ligand binding to TLR4-MD-2. This is the case for saponine from *Glycyrrhizae Radix* (Honda *et al.* 2010). In 2009, Mirsha *et al* have shown that aqueous *rhodiola imbricata* rhizome extract induced TLR-4 expression and intracellular granzyme-B in treated splenocytes while the same extract stimulated IL-1beta, IL-6, and TNF-alpha in human peripheral blood mononuclear cells suggesting that aqueous rhodiola extract could be used in modulating the immune system of immunocompromised individuals. From a mode of action standpoint, to activate the downstream signaling pathways and subsequently induce immune responses, ligand-induced dimerization of TLR4 is required. A 2006 study implies that phytochemicals consumed on a daily basis can contribute to the activation of immune inflammatory responses by activating TLR receptors. This study showed that TLR 4 receptors are the molecular target of curcuma. Curcumin and sesquiterpene lactone inhibit both ligand-induced and ligand-independent dimerization of TLR4 (Young *et al.* 2006). Plant derived polyphenols, in particular the polyphenol epigallocatechin-3-gallate isolated from green tea have been shown to downregulateTLR4 signal transduction (Byun *et al* 2010).

TLRs and immune tolerance:

[00139] Immune tolerance is the process by which the immune system remains unresponsive to antigens that can be self or foreign in nature. There are 3 forms of immune tolerance: central, peripheral and acquired. Oral tolerance is the most important form of acquired tolerance and involves the suppression of specific immune responses to antigens administered orally that are not threatening to the organism, such as food or commensal flora. Dendritic cells (DCs), that act as the commander-in-chief of the immune system, can become tolerant to antigens and known as tolerogenic DCs. TLR2 activation

of tolerogenic DCs results in enhanced anti-inflammatory responses and TLR2 expression.

IL-6:

[00140] IL-6 is a pro-inflammatory cytokine. IL-6 is pleiotropic cytokine involved in the physiology of virtually every organ system. Recent studies suggest that IL a very important regulator of Treg/Th17 balance. Moreover, IL-6 has neuro-specific activities including the modulation of the hypothalamic-pituitary-adrenal (HPA) function that plays a major role in regulating stress. IL-6 affects the CNS in that it activates the HPA axis and increases brain tryptophan and serotonin metabolism. It has been proposed that IL-6 could be a biomarker for aging and that it could link inflammation, obesity, stress and coronary heart diseases. Preclinical animals models have provided evidence that specific blockade of IL-6 may be an effective treatment for chronic inflammatory and autoimmune disease.

IL-10:

[00141] IL-10 is an anti-inflammatory cytokine that regulates the inflammatory responses. The interaction of IL-10 with its receptor can have various outcomes such as the induction of a cellular anti-viral state, the inhibition of pro-inflammatory response, the induction of apoptosis, and the modulation of cell growth. Mice deficient in IL-10 develop a chronic inflammatory bowel disease (IBD) that predominates in the colon and shares histopathological features with human IBD and TLR4 appears to contribute to the development of this condition. Importantly, IL-10 plays a crucial role in peripheral immune tolerance and in the control of epithelial homeostasis. Interestingly, IL-10-deficient mice lacking also the serotonin reuptake transporter have even more severe intestinal inflammation compared to mice with the serotonin reuptake transporter, suggesting that IL-10 also plays a role in neuroimmune interactions. Boosting IL-10 production is thought to be an effective therapeutic approach for autoimmune diseases and chronic inflammatory diseases.

Methods to measure antioxidant potential

[00142] The antioxidant potential of any products can be measured using methods such as the chemical measure of high oxygen radical absorbance (ORAC) and the biochemical release of reactive oxygen species (ROS) from cells. (See materials and

methods).

Use for energy lift

Mitochondrial energy production:

[00143] Mitochondria provide energy for basic metabolic processes through cell respiratory. This process can extract energy from organic substances in oxygen presence. In order to use the food we eat, our body must degrade the food into smaller molecules. After this, the cells will use these molecules, either as a source of energy, or as materials to construct other molecules. The first stage of degradation, digestion takes place in our intestinal cells. The digestion in our intestinal cells is the first step of degradation. Through the action of enzymes, the larger ingested molecules are degraded in intestinal cells. Proteins are degraded into amino acids, the polysaccharides into carbohydrates, fats into fatty acids and glycerol. Subsequently, small molecules derived from food will enter into the cell's cytosol and will begin their progressive oxidation. Carbohydrates and fats are the main sources of energy for most organisms, including humans. In the cytosol, each glucose molecule undergoes a series of chemical reactions called glycolysis which then the result is a molecule of pyruvate. The pyruvate can enter in the Kreb's cycle in mitochondria. For this research, we will focus on reactions confined to the mitochondria. It is in the membranes of mitochondria that much energy is produced. More than 90% of our cellular energy is produce by mitochondria. Carbohydrates and fats are the main sources of energy for most organisms, including humans.Furthermore, mitochondria provide essential metabolism such as heme biosynthesis, function in calcium and iron homeostasis, and play a key role in programmed cell death.

Diet and mitochondrial decay:

[00144] Diets deficient in nutrients can accelerate mitochondrial decay and contribute to neurodegeneration, and other dysfunction. Nutrient deficiency increase ROS and oxidative stress, and consequently leading to mitochondrial dysfunction and age-associated diseases, including metabolic syndrome. Vitamins, minerals and other metabolites play a key role as cofactors for the synthesis of mitochondrial enzymes and support mitochondrial function, including ATP synthesis.

Mitochondrial nutrients:

[00145] Liu and al. (2009) ranked the «mitochondrial nutrients» in four groups

according their beneficial functions. Mitochondrial nutrients can perform a numbers of beneficial functions: 1) prevent oxidant production or scavenge free radicals to eliminate oxidative stress in mitochondria; 2) act as enzyme inducers, can enhance antioxidant 3) enhance mitochondrial metabolism, by repairing and degrading mitochondria, and by increasing mitochondrial biogenesis; 4) protect mitochondrial enzymes and/or stimulate mitochondrial enzyme activity by elevating substrate and cofactor levels. They also classified mitochondrial nutrients into the following three groups: 1) antioxidants, such as coenzyme Q, lipoic acid (LA), glutathione, and α-tocopherol; 2) energy enhancers and others, such as carnitine/acetyl-Lcarnitine, creatine, pyruvate, and choline; and 3) cofactors and their precursors, such as lipoic acid, coenzyme Q, and the B vitamins.

Calcium signaling and energy production:

[00146] Mitochondria play a key role in cellular Ca^{2+} homeostasis. Mitochondria have an important influence in regulation of intracellular Ca^{2+} in both normal and pathological cell function. This latter accumulate calcium and release them during certain cellular events. Ca^{2+} act as second messenger in neurotransmitters release from neurons, and is implicated in contraction of all muscle cell types. Extracellular Ca^{2+} maintains the potential difference across cell membranes. Many enzymes require also Ca^{2+} ions as a cofactor. In mitochondria, the calcium can directly up-regulate enzymes of Kreb's cycle and other OXPHOS enzymes, resulting in faster respiratory chain activity and higher ATP output. ATP increase allows meet the cellular ATP demand. Thus any perturbation in mitochondrial or cytosolic Ca^{2+} homeostasis will have heavy consequence for cell function, and the level of ATP synthesis. In the cell, there are also and important coordination between endoplasmic reticulum and mitochondria in order to regulate mitochondria morphology. Perturbation in this relation can lead to Ca^{2+} overload and mitochondria fission, fragmentation, and apoptosis. It seems that Ca^{2+} plays opposite roles intervening such a physiological stimulus ATP synthesis and can become a pathological stimulus for ROS generation, cytochrome c release, and apoptosis.

Energy conservation and homeostasis:

[00147] Meditation is used as an energy conservation technique. Highly experienced yogi have the capacity to meditate for very long periods of time without eating. Not much is known about the mechanisms underlying this enhanced capacity of energy conservation. If the body as a whole is functioning in a healthy and relaxed

manner, it has the resources to fight a specific illness. Since meditation, with its ability to restore and maintain a general state of homeostasis, acts as the command post, this may be all you need to turn around a serious illness. Meditation works because it restores the body to a state of homeostasis, in which the systems within the body are at rest or operating within sustainable limits. The body is quite capable of operating outside the state of balance. We can run a marathon, for example, or eat a massive meal, without suffering unduly. However, when this occurs, these systems in the body are stressed. If the body is stressed for too long, damage takes place and pathologies start to occur. In fact, sickness can be easily defined as a state of imbalance in one or more of the systems in the body. The body is always striving to return to homeostasis. Not only do systems work best when in balance, but this is also the optimum state for self-repair and growth.

[00148] Functional drinks that induce a meditative state have not been described. This invention provides the first evidence that a meditative state does not necessitate a first input from the "spirit", and that it can be induced via the intake of a functional drink designed to induce a state of energy conservation.

[00149] In a further aspect of the invention, there is provided a method to provide an energy lift in a mammal, the method comprising the steps of: ingesting a food or a composition as defined herein.

Use for neuroprotection

ATP and neurotransmission:

[00150] In addition to its energetic role, ATP serves an important function in neurotransmission. Indeed, ATP signalling is crucial for communication from taste bud cells and the gustatory nerves that are in contact with the taste bud cells of the tongue. These gustatory nerves communicate with the brain center to regions that are involved in reward mechanisms. The dopaminergic system is an important regulator of this reward center that involves in particular the ventral forebrain structures, such as the hypothalamus, the amygdala, and the nucleus accumbens.

[00151] In a further aspect of the invention, there is provided a method for the protection of the nervous system in a mammal, the method comprising the steps of: ingesting a food or a composition as defined herein.

Use for cardioprotection

[00152] In accordance with a further aspect of the invention, there is provided a composition useful for use in cardioprotection in a mammal, wherein the composition has anti-atherosclerotic activity, anti-hypertensive activity, and anti-arteriosclerosis activity. Particularly, due to the presence of berry anthocyanins, formulations 1 and 2 may act as cardioprotectant by maintaining vascular permeability, reducing inflammatory vascular responses, platelet aggregation and by offering superior vascular protection.

Use for homeostasis

[00153] In accordance with a further aspect of the invention, there is provided a composition useful for use in maintaining, preserving or recovering homeostasis in a mammal, wherein the composition has TLR2 activity. Particularly, due to the presence of plant/berry polyphenols and berry anthocyanins, formulations 1 and 2 may act as TLR2 inhibitors and homeostasis promoters.

[00154] The present invention is illustrated in further details by the following non-limiting examples.

EXAMPLES

Material and methods

Formulations:

[00155] **Table 3** describes the composition and associated usage of each formulations developed by Applicant. As indicated, Formulation 1 is an adaptogenic formulation used for the relief of nervousness or sleeplessness due to mental stress, while Formulation 2 is for the maintenance of cardiovascular functions.

[00156] **Table 4** described the ingredients of the formulations and competitors used in this study. Ingredients are listed in order of proportion, from the most abundant to least abundant.

[00157] Molecules that may act as potential active ingredient present in each formulation developed by Applicant and their reported beneficial effects are described in **Tables 5, 6, 7 and 8**. Note that other potential molecules may be present in trace or undetectable amount, and are not listed in the tables.

Table 3. Beverage Composition (per dose of ready-to-drink 300ml) and associated usage

Formulation 1	Formulation 2	Formulation 3	Formulation 4
Herbal mix containing: Limon balm 1-2g/L Scutellaria 0.5-1.5g Blueberry concentrate 5-15g Cranberry concentrate 1-5g Maple syrup 10-20g Natural aroma 0.01-0.1g	Herbal mix containing: Hawthorn 1-2g Scutellaria 0.3-1g Apple concentrate 10-20g Maple syrup 5-10g Cranberry concentrate 1-5g Natural aroma 0.01-0.1g	Herbal mix containing: Scutellaria 0.50-1.5g Raspberry bush 0.5-1.5g Yarrow 0.2-0.8g Apple concentrate 2-10g Cranberry concentrate 1-5g Maple syrup 10-20g Natural aroma 0.01-0.1g	Herbal mix containing: Thyme 1-2g Hysop 2-4g Apple concentrate 10-20 g Cranberry concentrate 1-5g Maple syrup 5-10g Natural aroma 0.01-0.1g
Use: Helps relieve nervousness, and sleep aid in case of restlessness or insomnia due to mental stress	**Use:** Helps support cardiovascular health in adults and helps relieve nervousness.	**Use:** Helps maintain women's health. Helps relieve menstruation pain.	**Use:** Digestive tonic. Helps relieve abdominal discomfort due to flatulent dyspepsia and colics.
Relaxation formula with energizing potential	Cardioprotective formula with energizing potential	Formula for hormonal balance, for women only	Formula used as an alternative to dairy-based probiotics

Table 4.

Ready–to-drink formulations from Applicant and competitors and their ingredients per dose

Formulations	*Formulation 1*	*Formulation 2*	*Competitor1*	*Competitor 2*	*Competitor 3*	*Competitor 8*	*Competitor 9*
Ingredients (listed in order of proportion from most abundant to least abundant)	Herbal extract containing: - lemon balm 1.21g (melissa officinalis) - skullcap 0.73g (scutellaria laterifolia) maple syrup blueberry concentrate cranberry concentrate natural aroma	Herbal extract containing: - hawthorn 1.25g (crategus oxyacantha) - skullcap 0.44g (scutellaria laterifolia) maple syrup cranberry concentrate apple concentrate natural aroma	Green tea (from real brewed green tea concentrate) **Raw sugar cane** Pomegranate juice from concentrate Natural flavor Citric acid Ascorbic acid (vitamin C) Sodium citrate	**Glucose-fructose** Citric acid Concentrated tea from tea leaves Potassium citrate Natural lemon flavor	**Fruit juice from concentrate - grape** -apple -pomegranate -cranberry -blueberry -lemon -elderberry -blackberry Natural flavour Ascorbic acid (Vitamin C)	**Glucose-fructose** Coca-cola mix Caramel colour Phosphoric acid Natural flavour caffeine	Taurine 1000mg Glucuronolactone 600mg Caffeine 80mg Niacin (niacinamide) 18mg Pantothenic acid (calcium d-pantothenate) 6mg Vitamine B6 (pyridoxine HCl) 2mg Riboflavine 1.65mg Vitmaine B12 (cyanocobalamine) 1mcg **Sucrose** **Glucose** Citric acid Inositol Natural aroma caramel

Table 5. Formulation 1 ingredients

Ingredients	Potential detectable molecules	Reported Beneficial Effects
Herbal infusion - *Melissa officinalis* - *Scutellaria laterifolia*	*Melissa* extract ➢Polyphenols –**Flavonoids:** Apigenin (A) and luteolin (L) *Scutellaria* extract ➢Polyphenols – **Flavonoids:** Wogonin (W), Baicalein (B1), Baicalin (B2)	• Monoamine transporter activator (A, L) • Anti-cancer (A, L) • Autophagia (cellular dormancy/energy conservation) (A) • Inhibitor of cytochrome 450 (A, B1) • Prevent renal damage due to cyclosporin (A) • anxiolytic (A, W) • antioxidant (L) • anti-septic schock (L) • neuroprotective in MS (L) • anti-inflammatory (B1) • enhance liver health (W, B2) • muscle relaxant (W) • prolyl endopeptidase inhibitor, affect GABA receptors (B2)
Cranberry concentrate *Vaccinum macrocarpum*	➢ Polyphenols – **Flavonoids:** Proanthocyanins (P), Anthocyanins (AN)	• Antioxidant activity (AN) •Effect on urinary tract infections (P) •Dental health •Favorable Glycemic Response of Type 2 Diabetics
Blueberry concentrate *Vaccinum spp.*	➢ Polyphenols – **Flavonoids:** Anthocyanins (AN)	• anti-cancer (AN) • anti-hypercholesterolemia (AN) •anti-hyperglycemic (AN) • anti-diabetic (AN) • anti-inflammatory (AN) • anti-microbial (anti-fungal, anti-viral) (AN) • neuroprotective (reduction of cognitive decline) (AN) • Cardioprotection (AN)
Maple syrup *Acer saccharum*	➢ Phytohormones – **Abcisic acid (AB)**	- Ameliorate inflammatory bowel disease (AB) - ameliorates atherosclerosis (AB) - ameliorates glucose tolerance and diabetes (AB) - ameliorate obesity-related inflammation (AB)

Table 6. Formulation 2 ingredients

Ingredients	Potential detectable molecules	Reported Beneficial Effects
Herbal infusion *-Crataegus oxyacantha* *-Scutellaria lateriifolia*	*Crataegus* extract(CE) ➢Polyphenols – **Flavonoids (F):** Proanthocyanins (P), Quercetin (Q), Hyperoside (3-O-galactoside of quercetin, H), Rutin (flavonoid glycoside, R), Vitexin (apigenin flavone glucoside, V) *Scutellaria* extract ➢Polyphenols – **Flavonoids:** Wogonin (W), Baicalein (B1), Baicalin (B2)	•Hypotensive effect(CE) •Treatment for various cardiovascular conditions(F,H,V,Q) •Beneficial effect on blood lipids(CE) •Antioxidant activity(P,Q,H) •Inhibitor of cytochrome 450 (B1) •Anti-inflammatory (B1) • Enhance liver health (W, B2) • Muscle relaxant (W) • Prolyl endopeptidase inhibitor, affect GABA receptors (B2)
Apple concentrate	➢ Polyphenols – **Flavonoids:** Proanthocyanins (P)	•Beneficial effect on blood lipids (polyphenol in general)
Cranberry concentrate *Vaccinum macrocarpum*	➢ Polyphenols – **Flavonoids:** Proanthocyanins (P), Anthocyanins (AN) Flavonols (F), Quercetin (Q) – **Stilbenes:** Resveratrol (R) – **Phenolic acids:** Ellagic acid (EL)	•Antioxidant activity (P,AN,F,Q,R,EL) •Effect on urinary tract infections (P) •Anti-cancer activity (Q,EL) •Limit the development of vascular diseases(R) •Dental health •Anti-inflammatory (R) •Favorable Glycemic Response of Type 2 Diabetics
Maple syrup *Acer saccharum*	➢ Phytohormones – **Abcisic acid (AB)**	- Ameliorate inflammatory bowel disease (AB) - ameliorates atherosclerosis (AB) - ameliorates glucose tolerance and diabetes (AB) - ameliorate obesity-related inflammation (AB)

Table 7. Formulation 3 ingredients

Ingredients	Potential detectable molecules	Reported Beneficial Effects
Herbal infusion -*Achillea millefolium* -*Scutellaria lateriifolia* -*Rubus Idaeus*	*Achillea* extract(AE) ➢Polyphenols -**Flavonoids:** Luteolin (L) -**Tannins** (T) ➢Phenolic acids – **Salicylic acid (SA)** ➢Alkaloid – **Achilleine, betaine, trigonelline, betonicide, stachydrine, and moschatine** *Rubus* extract(RE) ➢Polyphenols -**Flavonoids:** Luteolin (L), Quercetin (Q), Kaempferol (K) -**Tannins** ➢Phenolic acids – **Salicylic acid (SA), Ellagic acid (EL), Gallic acid (GA)** *Scutellaria* extract ➢Polyphenols – **Flavonoids:** Wogonin (W), Baicalein (B1), Baicalin (B2)	•Anti-inflammatory (AE) •Antiangiogenic activity, cancer treatment(GA) •Relaxant activity (RE) •Antioxidant activity (Q) •Inhibitor of cytochrome 450 (B1) •anti-inflammatory (B1) • enhance liver health (W, B2) • muscle relaxant (W) • prolyl endopeptidase inhibitor, affect GABA receptors (B2)
Apple concentrate	➢ Undetectable Polyphenols	•Beneficial effect on blood lipids (phenolic compounds)
Cranberry concentrate *Vaccinum macrocarpum*	➢ Undetectable Polyphenols –	•Favorable Glycemic Response of Type 2 Diabetics
Maple syrup *Acer saccharum*	➢ Phytohormones – **Abcisic acid (AB)**	- Ameliorate inflammatory bowel disease (AB) - ameliorates atherosclerosis (AB) - ameliorates glucose tolerance and diabetes (AB,) - ameliorate obesity-related inflammation (AB)

Table 8. Formulation 4 ingredients

Ingredients	Potential detectable molecules	Reported Beneficial Effects
Herbal infusion *-Thymus vulgaris* *-Hyssopus officinalis*	*Thymus* extract (TE) ➢Polyphenols –**Flavonoids:** Luteolin (L), Hispidulin (H) ➢Phenolic acids –**Caffeic acid and rosmarinic acid** *Hyssopus* extract(HE) –**Flavonoids:** apigenin (A), quercetin (Q), diosmin (D), luteolin (L) ➢Phenolic acids –**Chlorogenic, protocatechuic, ferulic, syringic, p-hydroxybenzoic and caffeic acids**	•Antioxidant activity(PA,H,F) •Antitussive action (TE) •antifungal and anti-bacterial properties(HE) •Anti-diabetes(reduced hyperglycemia) •Prevents the ascorbic acid oxidation(F) •Anti-cancer (H)
Apple concentrate	➢ Polyphenols – **Flavonoids:** Proanthocyanins (P), Quercetin (Q) exclusively in peel, Catechin (C), Epicatechin (C), Cyanidin-3-galactoside-C3 (C3) ➢ Phenolic acids – **Chlorogenic acid (CH), Coumaric acid (CO), Gallic acid (GA)** ➢ Organic acids	•Reduced risk for cancer, especially lung cancer(F) •Positively associated with general pulmonary health(F) •Reduced risk of cardiovascular disease(F) •Reduced risk of Type II diabetes(Q) •Antioxidant activity(F,VC,C3,CA,CH,GA) •Beneficial effect on blood lipids(phenolic compounds)
Cranberry concentrate *Vaccinum macrocarpum*	➢ Undetectable Polyphenols –	•Dental health •Favorable Glycemic Response of Type 2 Diabetics
Maple syrup *Acer saccharum*	➢ Phytohormones – **Abcisic acid (AB)**	- Ameliorate inflammatory bowel disease (AB) - ameliorates atherosclerosis (AB) - ameliorates glucose tolerance and diabetes (AB) - ameliorate obesity-related inflammation (AB)

Polyphenol detection

[00158] Total amount of polyphenols were measured by spectrophotometry using an adaptation of a protocol already described by Grubesic et al, 2005. Extraction of polyphenols was done by acidified methanol. 50% Folin (Sigma-Aldrich) was added to extracts, and absorbance was measured at 760nm. Galic acid (GA) was used as positive control and results are expressed as mg equivalent GA pas 100ml of extract.

Anthocyanin detection

[00159] Anthocyanin levels were measured by HPLC and were performed by an independent laboratory.

Determination of mitochondrial activity:

[00160] Cell lines (CAL27, SH-SY5Y) used for this assay were plated in plaque assays at a density of 50,000-100,000 cells per well. Colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was dissolved in HBSS buffer to obtain a final concentration of 0.5mg/ml per well. Cell plates were incubated for 2 hours at 37°C. Conditioned medium was then removed, and acid-isopropanol (0.04N HCl in isopropanol) was added to all wells. HCL was used as positive control to induce cell death. Plates were read at 530nm/630nm in a Synergy Biotek machine. The amount of dark blue crystals determined by spectrophotometry serves as an estimate for the number of mitochondria and hence the number of living cells in the test sample. This method is an adaptation of a previously described assay (Mossman, 1983).

Determination of the antioxidant capacity:

[00161] The antioxidant capacity of formulations and beverages was determined with the ORAC (Oxygen Radical Absorbance Capacity) test. ORAC assays were performed as previously described (Prior et al, 2005). This assay determines the ability of a sample to block the oxidation of fluorescein (40nM) induced by 20mM of [2,2-Azobis (2-methylpropionamide) dihydrochloride (AAPH), based on the oxidation of fluorescein by peroxyl radicals revealed by a reduction of emitted fluorescence over time. These free radicals are generated by the exposition of flurorescein to AAPH. A delay of this AAPH-induced oxidation reveals an antioxidant property. The oxidation process is a kinetic reaction that is measured fluorescence levels (excitation: 485nm, emission: 528nm) over time using a *Synergy HT* (Biotek) microplate reader. Area under the curves (AUC) from

each compound tested are calculated and compared to the blank and the control of reference the vitamine E analog 6-Hydroxy-2,5,7,8 Teramethylchromane-2-carboxylic acid (Trolox). ORAC values are expressed as µmol of Trolox equivalent per ml (µmol TE/ml) of compound tested.

Cell lines, cultures and reagents:

[00162] All cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, US). **Table 9** below describes the cell lines used and their culture condition. In some experiments, cells were transferred to a 96 wells plate and treated with different concentration of TLR2 agonist synthetic triacylated lipoprotein PAM3CSK4 (InvivoGen) starting by 500ng/ml final concentration to activate TLR2.

Table 9: Cells lines and culture conditions

Human cell line	*Cell type*	*Culture condition*
CAL27	Tongue squamous epithelial cell carcinoma (ATCC CRL-2095)	Cells were grown in DMEM high-glucose 4.5g/L culture medium containing 2mM L-glutamine, 1mM sodium pyruvate, 10% fetal bovine serum, and 100ug/ml Pen/Strep.
SY-SH5T	Neuroblastoma that exhibit moderate levels of dopamine beta hydroxylase activity (ATCC CRL-2266)	Cells were grown in EMEM/F12 culture medium containing 10% fetal bovine serum.
HEK 293	HEK-null: Primary embryonal kidney cells transformed by sheared human adenovirus type 5 DNA transfected with plasmid pUNO-mcs (Invivogen), HEK-TLR2: Primary embryonal kidney cells transformed by sheared human adenovirus type 5 DNA cotransfected with hTLR2 and CD14 genes (Invivogen). HEK-TLR4: Primary embryonal kidney cells transformed by sheared human adenovirus type 5 DNA cotransfected with hTLR4A, MD2, and CD14 genes (Invivogen).	Cells were grown in DMEM high-glucose 4.5g/L culture medium containing 2mM L-glutamine, 1mM sodium pyruvate, 10% fetal bovine serum, 50ug/ml Pen/Strep, 100ug/ml Normocin and 10ug/ml blasticidin. For HEK-TLR2, 50ug/ml HygroGold was also added.

Measurement of ROS production:

[00163] Cellular ROS levels were obtained by measuring the oxidation of CM-H_2DCFDA (5-(and6)-chlromethyl-20,70-dichlorodihydrofluresceindiacetate; Invitrogen) a cell-permeant indicator. Cal27, HEK 293 and SH-SY5Y were plated in 96 well plates at a cell density of 25000 cells per well. Cells were treated for 1 hour with 5µM CM-H_2DCFDA.

After removing the CDFDA solution, cells were treated with various adaptogenic formulations prepared with ROS buffer (HBSS containing 2% FBS). After 30min of exposure with samples, a first reading was taken using the *synergy HT* (Biotek) plate reader. Various concentrations of AAPH (100mM, 40mM, 16mM,6,4mM and 2,5 mM) were then added, and readings (excitation: 485nm, emission: 530nm) were taken every 30 minutes for 2 hours. The fluorescence intensity is an indicator of H_2O_2 intracellular level so values are expressed in Relative Fluorescence Unit (RFU).

Detection of ATP:

[00164] ATP intracellular levels were measured from whole cell lysates obtained through a freeze-thaw cycle, whereas ATP secreted from cells were measured from conditioned cell culture supernatants. Both intracellular and secreted ATP using measured using an ATP determination kit purchased from Invitrogen (Molecular Probes). ATP levels were detected at luminescence 560nm. ATP levels were normalized to untreated control and were expressed as % ATP levels.

Sample collection (saliva):

[00165] Saliva samples were collected from fasting 25-55 year old healthy volunteer individuals (men and women) who have the following profile: do not smoke, have no addictions, normal weight, active, exercizing at least 2-3 times a week. Volunteer were given 20ml-size samples of various formulations that they had to drink on separate days, first thing in the morning. Before swallowing, sample formulations were kept in the mouth for at 15-20 seconds, after which saliva was collected over the next 5 minutes. Saliva samples were frozen immediately.

Detection of salivary dopamine

[00166] Levels of dopamine in human saliva samples were measured using a "Dopamine ELISA" kit by following the instructions provided by the company (Genway Biotech, CA).

Statistical analysis

[00167] Statistical analysis was performed using GRAPH PRISM software. Experiments were done in triplicate. All data are presented as mean ±SEM. Statistical analysis was done by using a Dunett comparison multiple tests, One-way ANOVA.

Results were considered significant when $p \leq 0.05$.

Examples of various formulations of the functional food products for the present invention

[00168] **Table 3** shows the constitution of functional formulations developed by Applicant. The four beverages are composed of three main natural ingredients: antioxidant herbal extract, antioxidant fruit extract and maple syrup. Traces of natural aroma are also present in each beverage. Formulations are 100% natural.

Formulation 1

[00169] This formulation described in **Table 5** targets specifically the nervous system. Ingredients of this drink have been selected based on traditional uses reported in herbalism literature. Both scientific data and herbal medicine data provide evidence that this beverage may promote a relaxed state of the nervous system and the body in general. This beverage can be viewed as an anti-stress formula.

Formulation 2

[00170] This formulation described in **Table 6** specifically targets the maintenance of cardiovascular functions. Ingredients of this drink have been selected based on traditional uses reported in herbalism literature. Both scientific data and herbal medicine data provide evidence that this beverage may have beneficial actions on the heart and blood vessel, helps in soothing nervous tension, and stimulates the immune system.

Formulation 3

Physiological impact

[00171] This formulation described in **Table 7** specifically targets women health. Ingredients of this drink have been selected based on traditional uses reported in herbalism literature. Both scientific data and herbal medicine data provide evidence that this beverage may have the ability to contribute to the wellness of women from puberty to post-menopause. Here is a literature review summarizing the herbalism and scientific review of the potential health benefits of the plants and fruits included in the beverage.

Formulation 4

[00172] This formulation described in **Table 8** specifically targets the maintenance of a healthy digestive system. Ingredients of this drink have been selected based on traditional uses reported in herbalism literature. Both scientific data and herbal medicine data provide evidence that this beverage may have the ability to stimulate digestion and to

help soothing digestive problems.

Results

[00173] Data presented in **Figure 1** shows that Formulation 1 and Formulation 4 developed by Applicant have the highest levels of polyphenols, followed by competitor 3. Formulation 2, formulation 3, and competitor 4 had comparable levels of polyphenols. Competitor 1 and 2 had low levels of polyphenols where as competitor 5 and competitor 6 had no significant levels of polyphenols. Values are expressed as mean mg/dose +/- SEM of n=6. Gallic acid was used to make the standard curve.

[00174] **Figure 2** shows that the herbal mix and blueberry concentrate of formulation 1 contained significant levels of polyphenols, whereas maple syrup and cranberry concentrate did not. **Figure 3** shows that maple syrup and apple concentrate of formulation 2 do not contain significant levels of polyphenols. **Figure 4** shows levels of total polyphenols of each ingredient present in the amount used for one dose of formulation 3. **Figure 5** shows levels of total polyphenols of each ingredient present in the amount used for one dose of formulation 4. The herbal mix and apple concentrate contained significant levels of polyphenols, whereas maple syrup and cranberry concentrate did not. For each of the formulations presented in **Figures 2-5**, the ingredients, when combined together, do not cause the formation of additional polyphenols since the amount of polyphenols present in formulation 1 is not greater than the sum of ingredients.

[00175] **Figure 6** shows the amount of anthocyanins per dose present in various formulations. Formulation 1 developed by Applicant and competitor 3 had the highest levels of anthocyanins. Formulation 2 had low, but detectable levels of anthocyanins.

[00176] **Figure 7** shows that functional beverages developed by Applicant have high anti-oxidant chemical capacity when compared to various competitors as shown by oxygen-radical-antioxidant capacity (ORAC) value. Formulation 1, formulation 4 and competitor 3 have the highest antioxidant potential compared to the other formulations tested. Formulation 2, formulation 3 and competitor 4 have high antioxydant potential, whereas competitors 1, 2, 5, 6 and 7 had low to undetectable antioxydant potentials. Interestingly, the ORAC profile was comparable to that of the polyphenol profile (**Figure 1**), suggesting that polyphenols are mainly responsible for this property.

[00177] **Figures 8, 9, 10 and 11** show the synergistic effect of ingredients of Formulations 1, 2, 3 and 4 respectively to enhance the antioxidant potential of beverages as shown by ORAC value.

[00178] **Figures 12 and 13** show that the combination of three types of ingredients (herbal extract, fruit extract and maple syrup) provides the synergistic effect of Formulations 1 and 2 respectively.

[00179] **Figure 14** shows the antioxidant potential of different sweeteners when assessed at the same molarity [0.2M] as shown by their ORAC value. The nutritive natural sweeteners with antioxidant properties include maple syrup, agave, honey and cord syrup. Given this data, formulations developed by Applicant could be composed of any of these 4 antioxidant sweeteners.

[00180] **Figure 15** shows that agave, another natural sweetener with antioxidant property, can also produce synergy when formulated with the herbal and fruit extracts.

[00181] **Figure 16** shows the anti-ROS potential in tongue epithelial cells of sweeteners when assessed at the same molarity [0.2M]. Data show that honey, agave, maple syrup and corn syrup have high biochemical antioxidant potential when compared to other sweeteners as shown by a reduction in ROS levels after 60 minutes exposed to various concentrations of AAPH. This data suggests the use of sweeteners with high antioxidant potential could be used in the formulations proposed.

[00182] **Figure 17** shows that formulations, used as functional beverages, developed by Applicant have higher antioxidant biochemical potential than most competitors, except for competitor 1, 3 and 4, as shown by a reduction in ROS production after 120 minutes in THP1 cells in suspension using various concentrations of ROS inducer AAPH. Formulation 1 had the highest antioxidant potential. Since whole formulations never come in contact with monocytic cells, THP-1 cells were only used as model of ROS production.

[00183] **Figure 18** shows that formulations, used as functional beverages, developed by Applicant have high antioxidant biochemical potential compared to competitors products as shown by a reduction in ROS production in Cal27 cells using various concentrations of AAPH. Of all formulations tested, formulation 1 was the best formulation at inhibiting ROS production in oral mucosal cells. HBSS buffer, used as sample diluent, was used as control.

[00184] **Figure 19** shows the antioxidant potential that functional beverages developed by Applicant, formulations 1, 3, and 2 have high antioxidant biochemical potential when compared to competitors as shown by a reduction in ROS production induced with a sus-maximal dose of AAPH (40mM) in THP-1 cells in suspension (30 minutes). Since whole formulations never come in contact with monocytic cells, THP-1 cells were only used as model of ROS production. Thus, results obtained from this assay should be considered physiologically relevant. Of all formulations tested, formulation 1 has the highest anti-ROS activity. Various concentrations of samples were tested with a fixed dose of AAPH.

[00185] **Figure 20** shows the anti-ROS activity of various dilutions of functional beverages developed by Applicant and competitors in tongue epithelial cells (Cal27). Since whole formulations never come in contact with oral epithelium, results from this assay are applicable physiologically. Data show that formulations 1 has the highest antioxidant potential when compared to all other formulations tested, including the 4 competitors, as shown by a reduction in ROS production induced with a high dose of AAPH (40mM) for 60 minutes in Cal27 cells.

[00186] **Figure 21** shows that ingredients of Formulation 1 act in synergy to modulate ROS production artificially induced by AAPH in tongue epithelial CAL27 cells treated for up to 120 minutes. Ingredients act in synergy to promote homeostasis by maintaining non-damaging levels of ROS, this effect can last more than 2 hours after mucosal exposure.

[00187] **Figure 22** shows that ingredients of Formulation 2 act in synergy to modulate ROS production artificially induced by AAPH in tongue epithelial CAL27 cells treated for 60 minutes. Ingredients act in synergy to promote homeostasis by maintaining non-damaging levels of ROS.

[00188] **Figure 23** shows that ingredients of Formulation 3 act in synergy to modulate ROS production artificially induced by AAPH in tongue epithelial CAL27 cells treated for 60 minutes.

[00189] **Figure 24** shows that ingredients of Formulation 4 act in synergy to modulate ROS production artificially induced by AAPH in tongue epithelial CAL27 cells treated for 60 minutes.

[00190] Data from **Figure 25** demonstrate that most formulations, except competitor 9, will have little or no impact on basal ROS production, maintaining cells within homeostasis

ROS levels. Formulation 1 and 2 did not cause mucosal toxicity by inducing high levels of ROS. High production of ROS by competitor 9 suggests that the formulation is toxic for mucosal cells, and that the formulation can create oxidative stress in a dose-dependent manner by increasing after each application.

[00191] Of all products tested, as shown in **Figure 26,** formulations developed by Application were best at increasing mitochondria activity in oral epithelium Cal27 cells, and this increase is most pronounced upon a third application of the formulation. Competitor 1 increased mitochondria activity only after the third application of the beverage suggesting this formulation act more slowly than formulations 1 and 2 or that a higher dose of this drink is required to enhance mitochondria activity. Competitor 3 and 8 both increased mitochondria activity of Cal27, but this increase is not maintained after the second and third application. These data suggest that formulations may have a mucosal protective effect by increasing mitochondria activity. Competitor 9 is the only formulation tested that had the opposite effect by inhibiting mitochondria activity in Cal27 cells, suggesting that this formulation causes mucosal toxicity.

[00192] For the assay presented in **Figure 27**, ATP levels were measured from the conditioned culture media collected following exposure to the different formulations tested. Results show that all formulations, except Competitor 9, inhibit ATP secretion from Cal27 cells in a dose-dependent manner as shown by the inhibition getting stronger upon exposures, suggesting that the retention of ATP is favored for energy conservation purposes. Formulation 1 developed by Applicant was the only formulation tested to block ATP secretion upon the first exposure, suggesting that it is the strongest blocker of ATP secretion. Interestingly, the strong inhibition of ATP secretion by formulation 1 and competitor 3 (the two strongest) correlates with their anthocyanin levels detected in these beverages, suggesting that anthocyanins may be responsible for the energy conservation induced by these formulations. By inducing ATP secretion from Cal27, application of Competitor 9 depletes mucosal cells from their energy fuel that could be required for intracellular reactions. Intracellular levels of ATP from whole cell lysates were used as a measure of ATP synthesis. Again, Formulation 1 was better than other formulation at increasing ATP synthesis of Cal27, while Formulation 2 and Competitor 1 caused a lower increase in ATP synthesis. Competitor 3 and 8 did not cause an energy lift in these cells. Competitor 9 was the only formulation to cause an inhibition of ATP synthesis after the

fifth exposure, suggesting that it can act as an energy blocker upon repeated exposures.

[00193] By measuring the ratio between intracellular versus extracellular ATP generated upon mucosal exposures to the various formulations, data from **Figure 28** confirm that Formulation 1 developed by Applicant is the best formulation at augmenting the energetic potential of mucosal cells causing an energy lift, while Competitor 9 is the worst of all causing a energy depletion because more ATP is being secreted than produced causing the drainage of cellular fuel. Formulation 2, Competitor 1, 3 and 8 also enhance the energetic potential of mucosal cells. Intracellular ATP levels (ATPi) were divided by extracellular ATP levels (ATPs) to obtain ATPi/ATPs ratio.

[00194] Like in normal Cal27 cells (see Figure 21), **Figure 29** shows data indicating that formulation 1 as well as competitors 1 and 3 inhibit ATP secretion in stressed Cal27. However, in contrast to what was observed in normal cells (Figure 21), ATP secretion was induced upon the first application of competitor 8 and formulation 2 developed by Applicant, suggesting they can provide a rapid energy boost in stressed conditions. Like in normal cells (figure 21), competitor 9 induced ATP secretion from Cal27 cells. Intracellular ATP produced (full lines) upon exposure to various formulations were measured from whole cell lysates. Formulation 1 and 2 slightly, but significantly, induced ATP production in stressed Cal27 cells. Others formulations did not, and competitor 3 even caused its inhibition after 3 exposures.

[00195] By measuring the ratio between intracellular versus extracellular ATP generated upon mucosal exposures to the various formulations, **Figure 30** shows data confirming that formulation 1 developed by Applicant was best at inducing a strong energy lift in stressed Cal27 cells, and ATPi/ATPs ratio were much higher (100X more) than those observed in normal cells (Figure 22) indicating that formulation is better at inducing an energy lift in stressed cells than normal cells. Formulation 2, competitor 1 and competitor 8 also induced ATP production in stressed Cal27 cells causing a significant energy lift, but in contrast to what was observed in normal cells (Figure 22), competitor 3 did not induce a significant energy lift. Like in normal cells, competitor 9 inhibited the production of ATP causing an energy depleted state.

[00196] **Figure 31** shows that ingredients of formulation 2 act in synergy to block ATP secretion in Cal27 to cause a maintained state of energy conservation. The synergy can be observed 30 minutes after exposure with whole formulations, when compared to

individual ingredients.

[00197] **Figure 32** demonstrates that ingredients of formulation 1 act in synergy to enhance mitochondrial activity of CAL27 cells undergoing high oxidative stress. Cal27 were treated with 100mM AAPH for 120 minutes.

[00198] **Figure 33** demonstrates that ingredients of formulation 2 act in synergy to enhance mitochondrial activity of CAL27 cells undergoing high oxidative stress. Cal27 were treated with 100mM AAPH for 120 minutes.

[00199] **Figure 34** shows the impact of TLR2 activation with TLR2 agonist Pam3Cys (500ng/ml) on ROS production, mitochondrial activity and ATP responses in Cal27 cells undergoing low or high oxidative stress upon treatment with 6.4 mM and 16mM AAPH respectively. ROS production increases upon AAPH treatment while mitochondrial activity decreases, and TLR2 agonist had no significant impact on these responses. ATP secretion and ATP production were not affected by low or high AAPH treatment, TLR2 agonist slightly increased ATP secretion in cells without AAPH, while it increases ATP production in cell undergoing high oxidative stress.

[00200] **Figure 35** demonstrates the impact of various formulations on TLR2-dependent ROS production in Cal27 exposed for 30 minutes to various concentrations of AAPH. TO obtain TLR2-specific ROS production, ROS values of Pam3Cys treated cells were divided by that of untreated cells to obtain the ratio shown in the graph. As expected, in control cells, TLR2-dependent ROS production increased in a dose-dependent manner. However, when cells were exposed to formulation 1, formulation 2, competitor 3, and to a lesser extent competitor 1, TLR2-dependent ROS production was significantly reduced to maintain homeostasis levels of ROS. Competitor 8 and 9 did not significantly affect TLR2-dependent ROS production.

[00201] **Figure 36** shows TLR2-specific ROS production in HEK-TLR2+ cells treated for 3 hours with Pam3Cys (500ng/ml). Cells were then exposed to various formulations with or without ROS inducer (40mM AAPH) for 30 minutes. TLR2-specific ROS production was inhibited in AAPH-treated Cal27 by formulation 1, formulation 2, and competitor 3 providing evidence that they can act as TLR2 inhibitors when cells are undergoing high oxidative stress. This TLR2 inhibition was not observed in normal cells. Other formulations tested had no impact on TLR2-specific ROS production in cells undergoing oxidative

stress. One should note that formulation 2 had no longer TLR2-inhibitory effect after 60 minutes, while formulation 1 and competitor 3 conserved its TLR2-inhibitory potential as long as 120 minutes (data not shown).

[00202] **Figure 37** shows ROS production by dopaminergic SH-SY5Y neurons exposed to supernatant of Cal27 cells exposed to various formulations and the impact of low TLR2 activation induced with 50ng/ml of Pam3Cys. This assay mimics the impact of mucosal cells on subepithelial neuronal cells. Results demonstrate that TLR2 activation of Cal27 cells can reduce ROS production by dopaminergic neurons, suggesting a neuroprotective role for mucosal TLR2. One should note that higher dose of Pam3Cys (250 or 500ng/ml) did not have this neuroprotective role, suggesting that mucosal TLR2 can only be neuroprotective under low activity. Exposure to formulation 1, formulation 2, and competitor 3 had a neuroprotective effect, inhibiting ROS production in dopaminergic cells, while competitor 8 and competitor 9 did not. Competitor 1 had a low neuroprotective potential compared to formulation 1.

[00203] **Figure 38** shows that Ingredients of functional beverage developed by Applicant (Formulation 1) act in synergy to modulate the TLR2-dependent release of DC-attracting chemokine MIP3α from HEK-TLR2+ cells (16 hours incubation), suggesting that formulation 1 can cause the recruitment of DCs via TLR2 modulation to promote the activation of innate immune responses.

[00204] **Figure 39** Ingredients of functional beverage developed by Applicant (Formulation 2) act in synergy to modulate the TLR2-dependent release of DC-attracting chemokine MIP3α from HEK-TLR2+ cells (16 hours incubation), suggesting that formulation 2 can cause the recruitment of DCs via TLR2 modulation to modulate innate immune responses.

[00205] **Figure 40** shows that ingredients of Formulation 1 (SN) act in synergy to inhibit release of pro-inflammatory cytokine IL-6 in THP1-PMA cells treated with PAM3CSK4 for 22 hours, suggesting that formulation 1 can have an impact on pain regulation via an impact on IL-6 release.

[00206] **Figure 41** shows that ingredients of Formulation 1 act in synergy to enhance release of anti-inflammatory cytokine IL-10 in THP1-PMA cells at basal condition, suggesting that formulation 1 can promote a tolerant response in normal condition and via

TLR2 modulation.

[00207] **Figure 42** shows the levels of salivary dopamine detected 5 minutes after drinking 20 ml of various beverages in healthy human individuals. Competitor 9 significantly enhanced levels of salivary dopamine compared to control (water), whereas all other formulations tested did not. This data suggests that, in contrast to competitor 9, formulations developed by Applicant do not induce an addictive response.

[00208] **Figure 43** is a schematic representation of Applicant's invention. Formulations developed by Applicant, delivered at oral mucosal surfaces, can regulate mucosal and submucosal responses to promote homeostasis. The responses modulated by formulations developed by Applicant include oxidative stress, TLR2 activation, mitochondrial activity, ATP and inflammatory responses.

[00209] **Figure 44** is a schematic representation of results obtained with formulation 1. In summary, formulation 1 has the following properties: antioxidant, energizing, neuroprotective, anti-inflammatory, pro-tolerant (via IL-10) and a possible analgesics and anti-aging formulation.

[00210] **Figure 45** is a table that summarizes results obtained for all formulations tested.

[00211] The ORAC measures for different natural sweeteners shown in **Figure 46** were compared to the sum of the ORAC value of maple syrup added that of individual ingredients (SUM of SE + ingredient). Maple syrup combined with stevia-derived molecules (whole stevia extract or stevioside or Rebaudiside A) act in synergy to potentiate the antioxidant potential. Combination of maple syrup with aspartame and sucralose does not create this synergy. This result suggest that diet products could be formulated maple and stevia to obtain additional antioxidant activity.

Conclusions

[00212] Mucosal TLR2 regulates oxidative and energetic responses and adaptogenic formulations of the present invention act via this pathway to promote homeostasis.

[00213] Formulations developed using the adaptogen concept of herbalism according to the present invention promote homeostasis. This finding provides scientific

evidence to support the concept of adaptogens.

[00214] Ingredients of adaptogenic formulations according to the present invention act in synergy to promote homeostasis. Synergy potentiates the antioxidant potential, mitochondrial activity and neuroprotective property of formulations.

[00215] Formulation 1 of the present invention developed by Applicant prevents increase of salivary dopamine levels in humans, while inducing an energy lift in mucosal cells, suggesting that the formulation can energize without causing addiction.

[00216] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

[00217] All patents, patent applications and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent, patent application, or publication was specifically and individually indicated to be incorporated by reference.

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CLAIMS:

1. A composition comprising: at least one of an antioxidant plant concentrate having an ORAC value in said composition of at least 2000, at least one of an antioxidant plant-product concentrate having an ORAC value in said composition of at least 200; and at least one of an antioxidant natural sweetener having an ORAC value in said composition of at least 20, wherein said composition has synergistic antioxidant properties having an ORAC value in said composition greater than the addition of ORAC values of each component.

2. A composition having synergistic antioxidant properties, essentially consisting of: an antioxidant sweetener having an ORAC value in said composition of at least 20, an antioxidant plant-product concentrate having an ORAC value in said composition of at least 200 and an antioxidant plant concentrate having an ORAC value in said composition of at least 2000, in such proportions that each component potentiates the antioxidant effect (ORAC value) of other components.

3. A composition having synergistic antioxidant properties, consisting of: an antioxidant sweetener having an ORAC value in said composition of at least 20, an antioxidant plant-product concentrate having an ORAC value in said composition of at least 200 and an antioxidant plant concentrate having an ORAC value in said composition of at least 2000, in such proportions that each component potentiates the antioxidant effect (ORAC value) of other components; and a natural flavor or aroma.

4. The composition according to claim 1, 2 or 3, wherein said plant concentrate is a herb extract.

5. The composition according to claim 4, wherein said herb is selected from the group consisting of: basil, dill weed, marjoram, oregano, peppermint, savory, cardamon, chili, cinnamon, cloves, cumin, curry, garlic, ginger, Juniper plant, mustard, nutmeg, onion, paprika, parsley, pepper, poppy seed, rosemary, turmeric, vanilla beans, tarragon, black tea, green tea, white tea, rice, bran, oat, sorghum, Dandelion root, Bilberry leaf, Galega leaf, Echinacea, Nettle; Sage, Thyme, Lavender, Hawthorn, Verbena, Rosemary, Lemon balm, Peppermint leaf, Marshmellow root, Red raspberry leaf, Astragalus, Fennel seed, Skullcaps, Yarrow, Hyssop, Motherwort, Labrador tea, Garden angelica, Winter Cherry and Fenugreek.

6. The composition according to claim 5, wherein said herb is selected from the group consisting of: dandeleon root, Bilberry leaf, Galega leaf, Echinacea, Nettle, Sage, Thyme, Lavender, Hawthorn, Verbena, Rosemary, Lemon balm, Peppermint leaf, Oat, Marshmellow root, Red raspberry leaf, Astragalus, Fennel seed, Skullcaps, Yarrow, Hyssop, Motherwort, Labrador tea, Garden angelica, Winter Cherry, Fenugreek and Juniper plant.

7. The composition according to claim 6, wherein said herb is selected from the group consisting of: Thyme, Hawthorn, Lemon balm, Red raspberry leaf, Skullcaps, Yarrow and Hyssop.

8. The composition according to claim 1, 2 or 3, wherein said plant-product concentrate is a fruit or vegetable concentrate or extract.

9. The composition according to claim 8, wherein said fruit is selected from the group consisting of: Acai, apple, apricot, avocado, banana, bakeapple, blackberry, blueberry, cherry, chokeberry, cloudberry, cranberry, current, dates, elderberry, fig, dogberry, gooseberry, grapefruit, grape, guava, raspberry, Saskatoon berry, strawberry, juniper berry, kiwi fruit, lemon, lime, makiang, maloud, mango, mango steam, melon, nectarine, noni fruit, orange, papaya, peach, pear, pineapple, plum, pomegranate, rosehip, tangerine, watermelon, wild cherry, and red wine.

10. The composition according to claim 9, wherein said fruit is selected from the group consisting of: Acai, apple, blackberry, blueberry, cherry, chokeberry, cloudberry, cranberry, current, elderberry, dogberry, gooseberry, grape, raspberry, juniper berry, strawberry, plum, pomegranate, rosehip, Saskatoon berry and wild cherry.

11. The composition according to claim 10, wherein said fruit is selected from the group consisting of: apple, blueberry and cranberry.

12. The composition according to claim 8, wherein said vegetable is selected from the group consisting of: alfalfa seed sprouted, artichoke, arrugula, asparagus, beet, broccoli, cabbage, carrot, cauliflower, celery, chive, corn, cucumber, eggplant, fennel, leek, lettuce, mushroom, pea, pepper, tomato, pumpkin, radish, spinach, squash, sweet potato, soy bean, lima bean, bean, peas and lentil, particularly chick peas, cow peas, broad peas, and chocolate.

13. The composition according to claim 1, 2 or 3, wherein said natural sweetener is

selected from the group consisting of: maple syrup, honey, corn syrup, coconut sugar, agave, stevia, stevioside and Rebaudioside A.

14. The composition according to claim 12, wherein said natural sweetener is selected from the group consisting of: maple syrup, honey, corn syrup, agave, stevia, stevioside and Rebaudioside A.

15. The composition according to claim 14, wherein said natural sweetener is maple syrup, honey, stevia, stevioside or Rebaudioside A.

16. The composition according to claim 15, wherein said natural sweetener is maple syrup or honey.

17. The composition according to claim 16, wherein said natural sweetener is maple syrup.

18. The composition according to any one of claims 1, 2 or 3 comprising: Lemon balm; Skullcaps; blueberry concentrate; cranberry concentrate and maple syrup.

19. The composition according to claim 18 essentially consisting of: Lemon balm; Skullcaps; blueberry concentrate; cranberry concentrate and maple syrup.

20. The composition according to claim 19 consisting of: Lemon balm; Skullcaps; blueberry concentrate; cranberry concentrate and maple syrup, and a natural aroma.

21. The composition according to any one of claims 1, 2 or 3 comprising Hawthorn; Skullcaps; apple concentrate; cranberry concentrate and maple syrup.

22. The composition according to claim 21 essentially consisting of: Hawthorn; Skullcaps; apple concentrate; cranberry concentrate and maple syrup.

23. The composition according to claim 22 consisting of: Hawthorn; Skullcaps; apple concentrate; cranberry concentrate and maple syrup, and a natural aroma.

24. The composition according to any one of claims 1, 2 or 3 comprising: Skullcaps; Raspberry leaf; Yarrow; apple concentrate; cranberry concentrate and maple syrup.

25. The composition according to claim 24 essentially consisting of: Skullcaps; Raspberry leaf; Yarrow; apple concentrate; cranberry concentrate and maple syrup.

26. The composition according to claim 25 consisting of: Skullcaps; Raspberry leaf; Yarrow; apple concentrate; cranberry concentrate and maple syrup, and optionally natural

aroma.

27. The composition according to any one of claims 1, 2 or 3 comprising: Thyme; Hyssop, apple concentrate; cranberry concentrate and maple syrup.

28. The composition according to claim 27, essentially consisting of: Thyme; Hyssop, apple concentrate; cranberry concentrate and maple syrup.

29. The composition according to claim 28 consisting of: Thyme; Hyssop, apple concentrate; cranberry concentrate and maple syrup, and optionally a natural aroma.

30. The composition of any one of claims 1 to 29, in the form of a liquid or a solid food.

31. The composition according to claim 30, in the form of a drink for human consumption.

32. The composition according to claim 30, in the form of a powder dissolvable in water to form a drink for human consumption.

33. A method for the maintenance of homeostasis in a mammal, the method comprising the steps of: ingesting a food or a composition that is an activator of TLR2 as defined according to any one of claims 1 to 32.

34. A method for preventing inflammation comprising the steps of: administering a combination of foods having synergistic antioxidant properties as defined according to any one of claims 1 to 32.

35. Use of the composition as defined according to any one of claims 1 to 32, for the prevention of chronic inflammation in a mammal.

36. The use according to claim 35, wherein said composition is as defined in claim 18.

37. A method for stimulating, protecting or maintaining mucosal immunity in a mammal comprising the steps of: administering a composition according to any one of claims 1 to 32.

38. Use of the composition according to any one of claims 1 to 32 for the stimulation, protection or maintenance of mucosal immunity in a mammal.

39. The use according to claim 38, wherein said composition is as defined in claim 18 or 21.

40. A method for the conservation of ATP levels in a cell of a mammal comprising the steps of: administering a composition according to any one of claims 1 to 32.

41. Use of the composition according to any one of claims 1 to 32 for the conservation of ATP reserves in a cell of a mammal.

42. The use of claim 41, wherein said composition is as defined in claims 18 or 21.

43. A method for blocking energy release from a cell of a mammal comprising the steps of: administering a composition according to any one of claims 1 to 32.

44. Use of the composition according to any one of claims 1 to 32 for blocking energy release from a cell of a mammal.

45. The use of claim 44, wherein said composition is as defined in claims 18 or 21.

46. A method for raising energy levels in a cell of a mammal comprising the steps of: administering a composition according to any one of claims 1 to 32.

48. Use of a composition according to any one of claims 1 to 32 for increasing the energy levels of a cell in a mammal.

49. The use of claim 48, wherein said composition is as defined in claim 18 or 21.

50. A method for protecting a neural cell in a mammal comprising the steps of: administering a composition according to any one of claims 1 to 32.

51. Use of the composition according to any one of claims 1 to 32 for the protection of a neural cell in a mammal.

52. The use of claim 51, wherein said composition is as defined in claims 18 or 21.

53. A method for the identification of a food or a food ingredient useful for the maintenance of homeostasis in a mammal, the method comprising the steps of:

- measuring the TLR activity by said food or food ingredient;

whereby said food or food ingredient is a TLR modulator when said TLR modulates the release of one of: IL-10, IL-6, MIP3α, dopamine and ATP; or a decrease in cell death or reactive oxygen species (ROS).

54. A transwell assay for the identification of TLR modulators, the assay comprising:

a) in a top chamber, incubating a layer of TLR-expressing cells with a test agent;

b) in a bottom chamber in contact with said top chamber, incubating a layer of sub-

epithelial cells sensitive to MIP3α; and

c) measuring the presence of IL6 or IL10; identifying an increase in ATP; or measuring a decrease in ROS; or a decrease in cell-death from cells in said second well ;

wherein the presence of one of said condition from step c) is indicative that said test agent is a potential TLR modulator.

55. A method for the identification of composition having synergistic antioxidant activity, the method comprising the steps of:

- measuring the antioxidant potential of each component individually;
- measuring the antioxidant potential of the components when combined; and
- comparing the antioxidant potential of the combination versus the individual components;

wherein said combination has synergistic antioxidant properties when the antioxidant potential of the combination is higher than the sum of the antioxidant value as determined by the ORAC value of each individual component when assessed in the composition; of the individual components.

FIGURE 1:

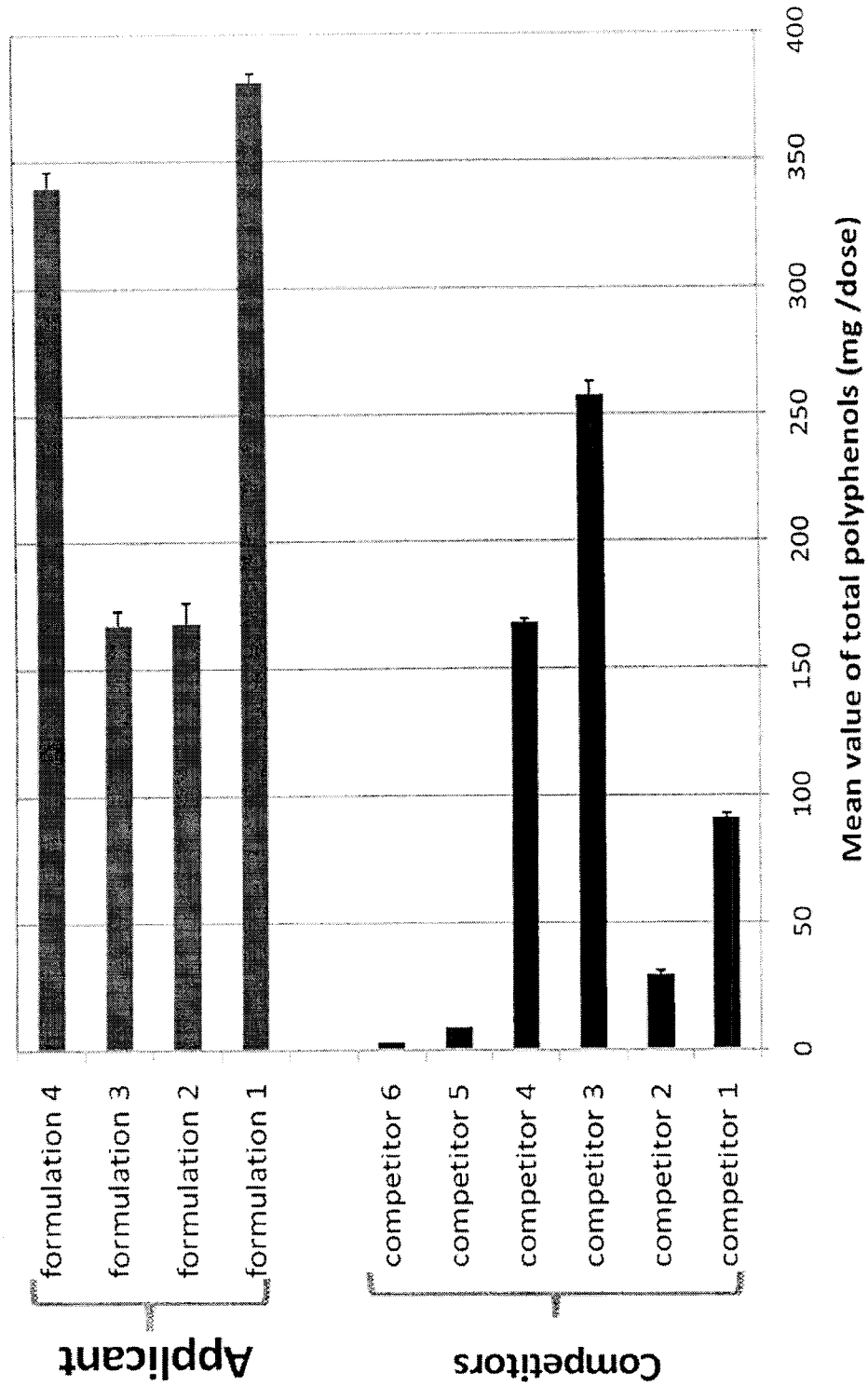

Mean value of total polyphenols (mg /dose)
0
50
100
150
200
250
300
350
400
Applicant
formulation 4
formulation 3
formulation 2
formulation 1
Competitors
competitor 6
competitor 5
competitor 4
competitor 3
competitor 2
competitor 1

FIGURE 2:

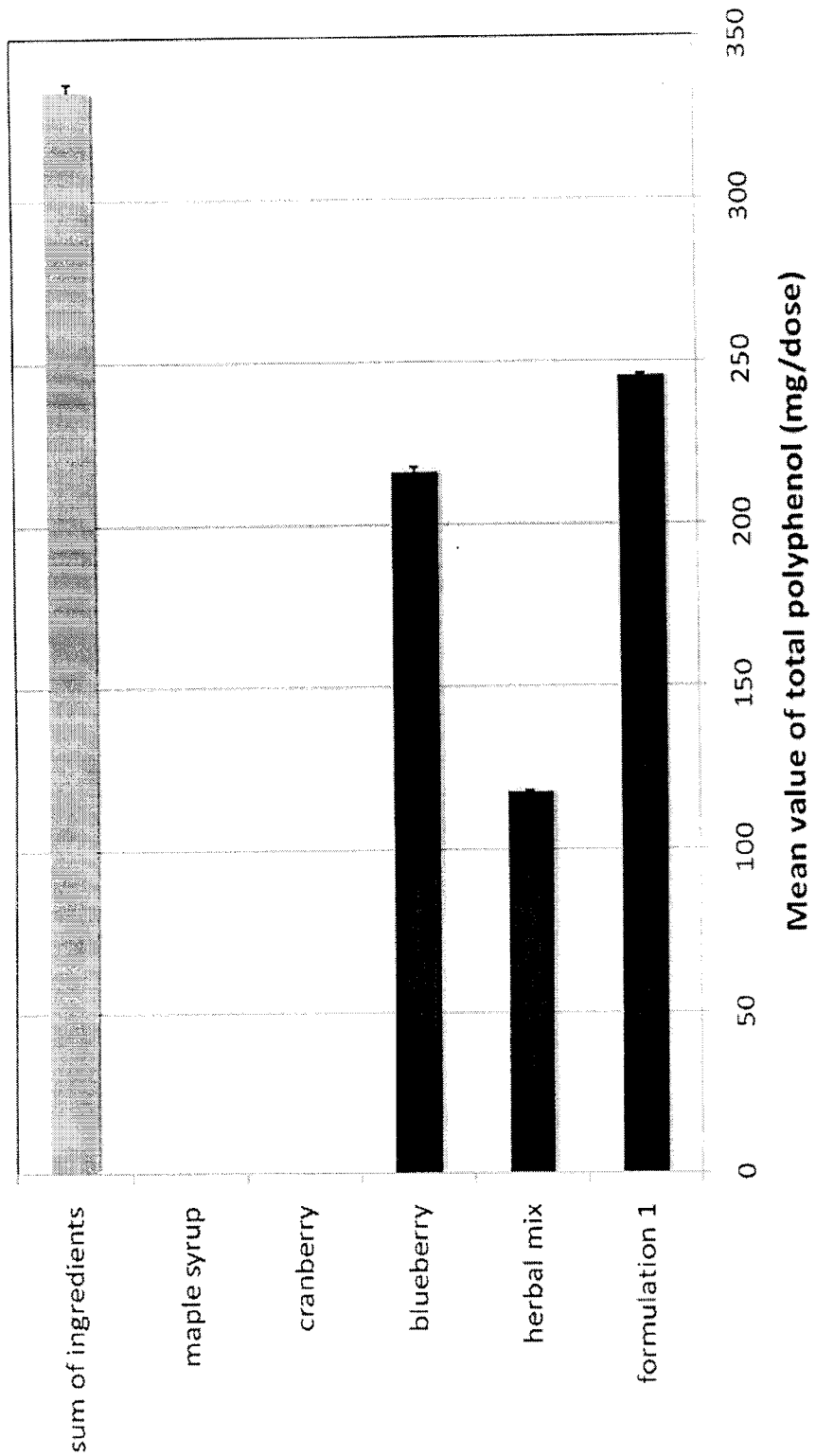

Mean value of total polyphenol (mg/dose)
0
50
100
150
200
250
300
350
sum of ingredients
maple syrup
cranberry
blueberry
herbal mix
formulation 1

FIGURE 3:

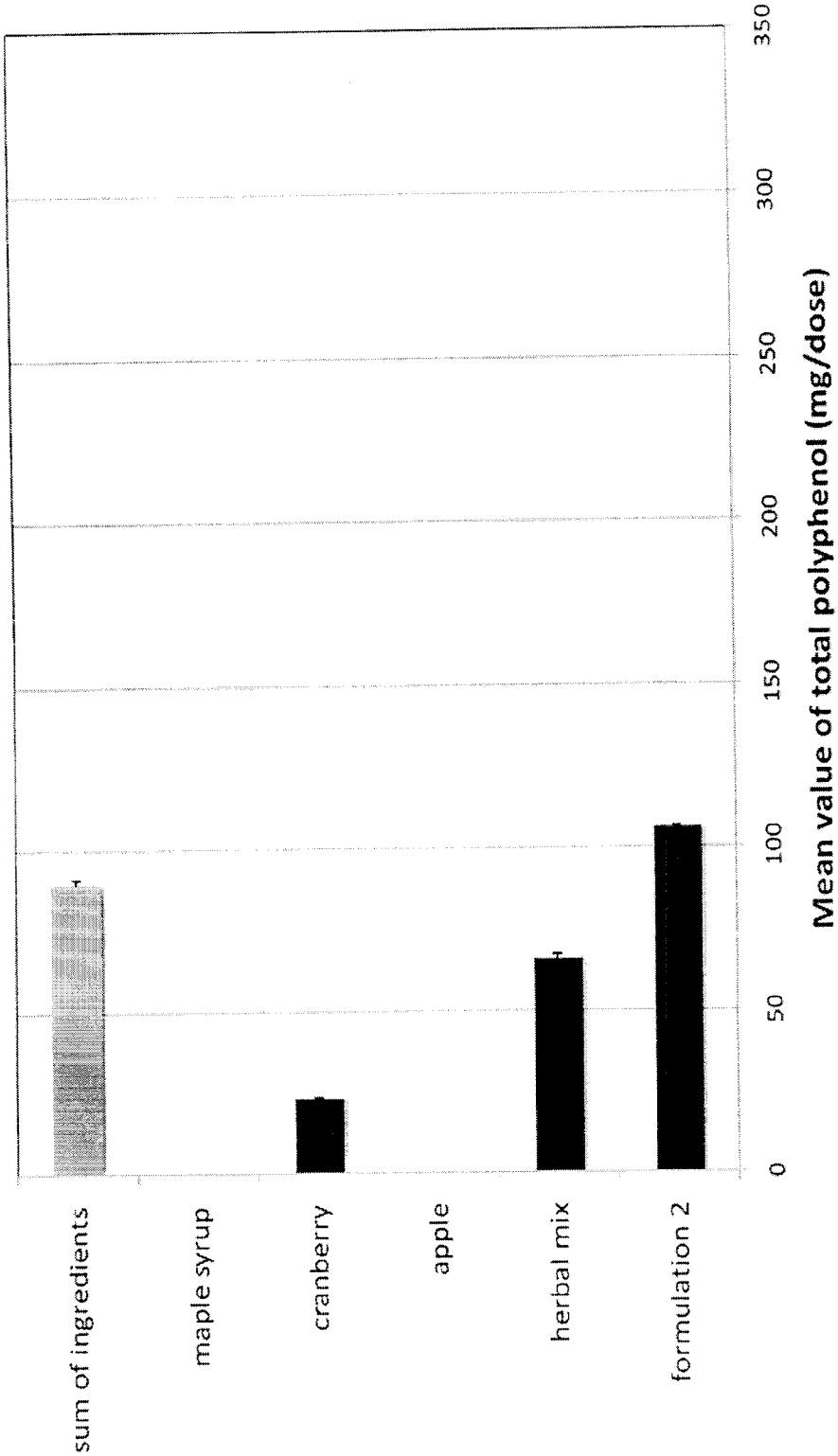

Mean value of total polyphenol (mg/dose)
0
50
100
150
200
250
300
350
sum of ingredients
maple syrup
cranberry
apple
herbal mix
formulation 2

FIGURE 4:

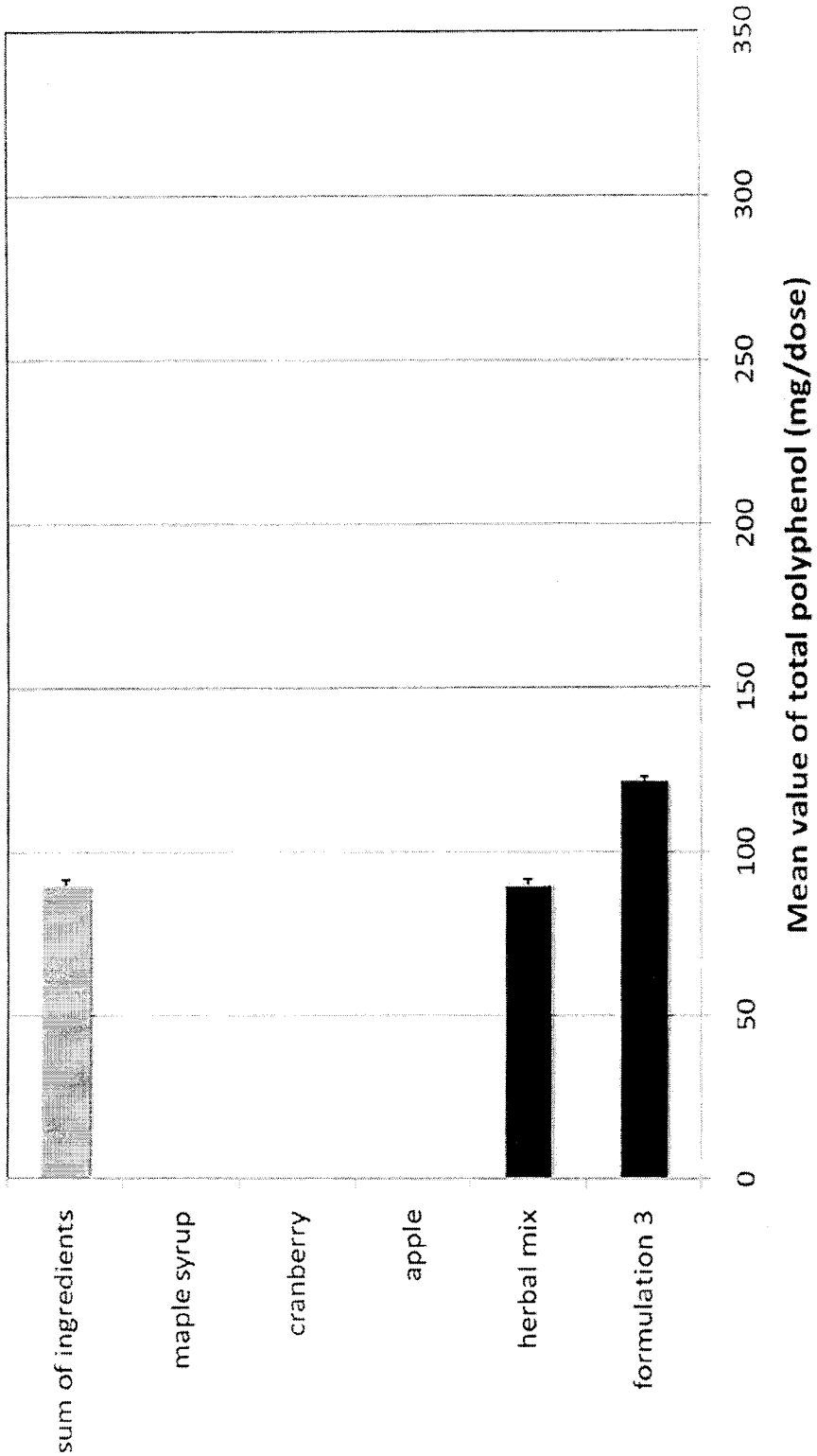

0
50
100
150
200
250
300
350
Mean value of total polyphenol (mg/dose)
sum of ingredients
maple syrup
cranberry
apple
herbal mix
formulation 3

FIGURE 5:

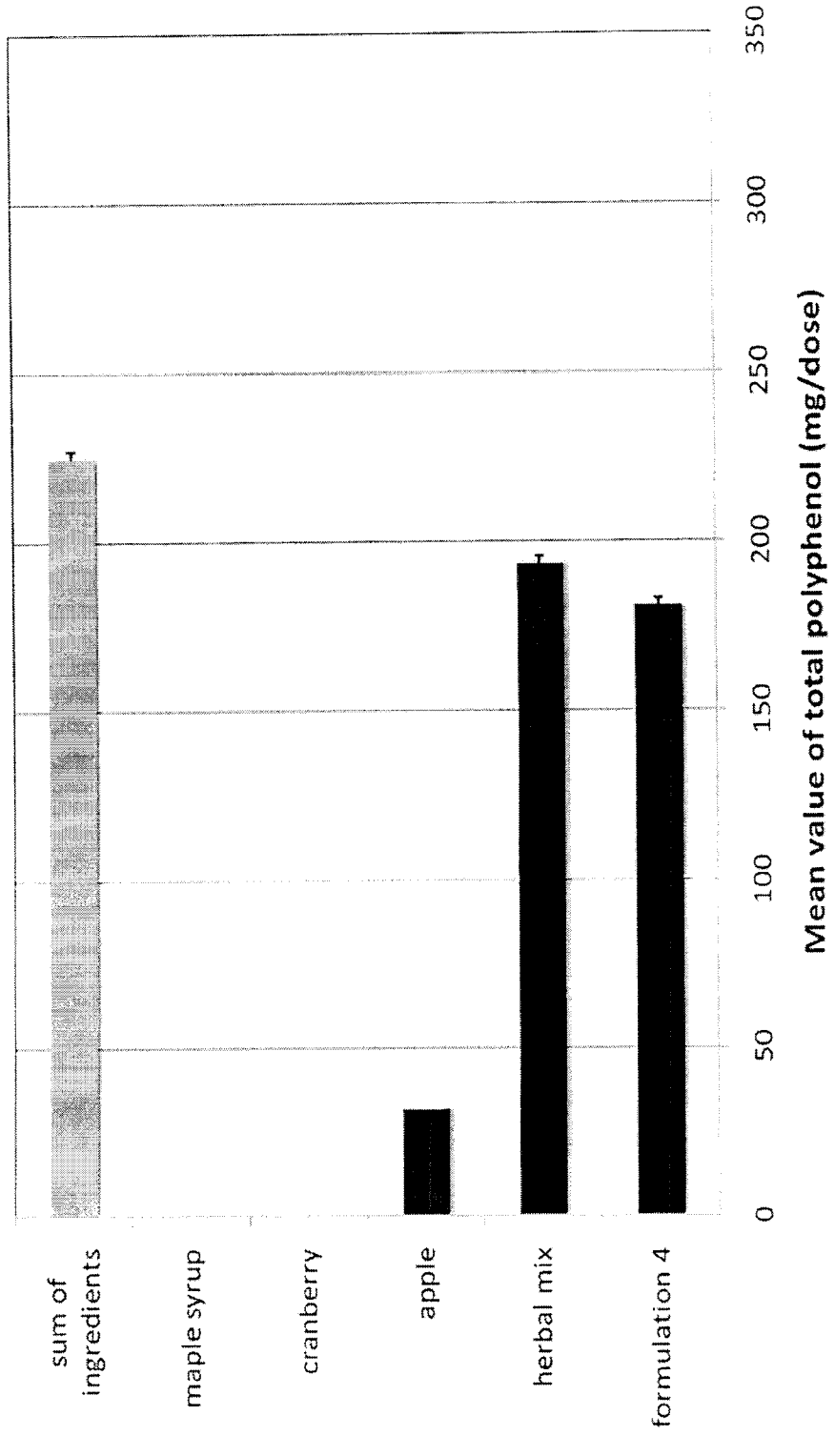

Mean value of total polyphenol (mg/dose)
0
50
100
150
200
250
300
350
sum of ingredients
maple syrup
cranberry
apple
herbal mix
formulation 4

FIGURE 6:

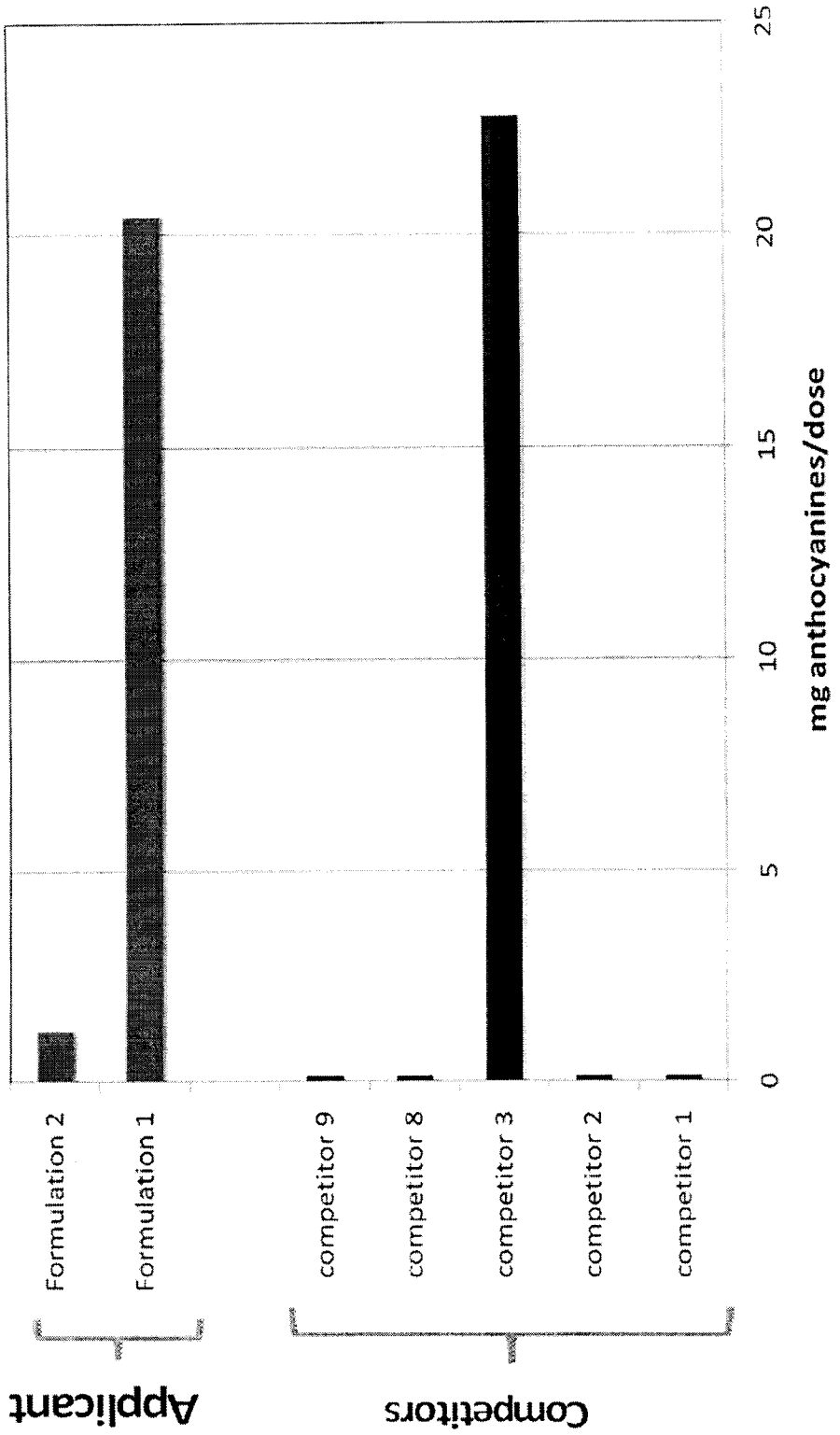

Applicant
Competitors
Formulation 2
Formulation 1
competitor 9
competitor 8
competitor 3
competitor 2
competitor 1
0
5
10
15
20
25
mg anthocyanines/dose

FIGURE 7:

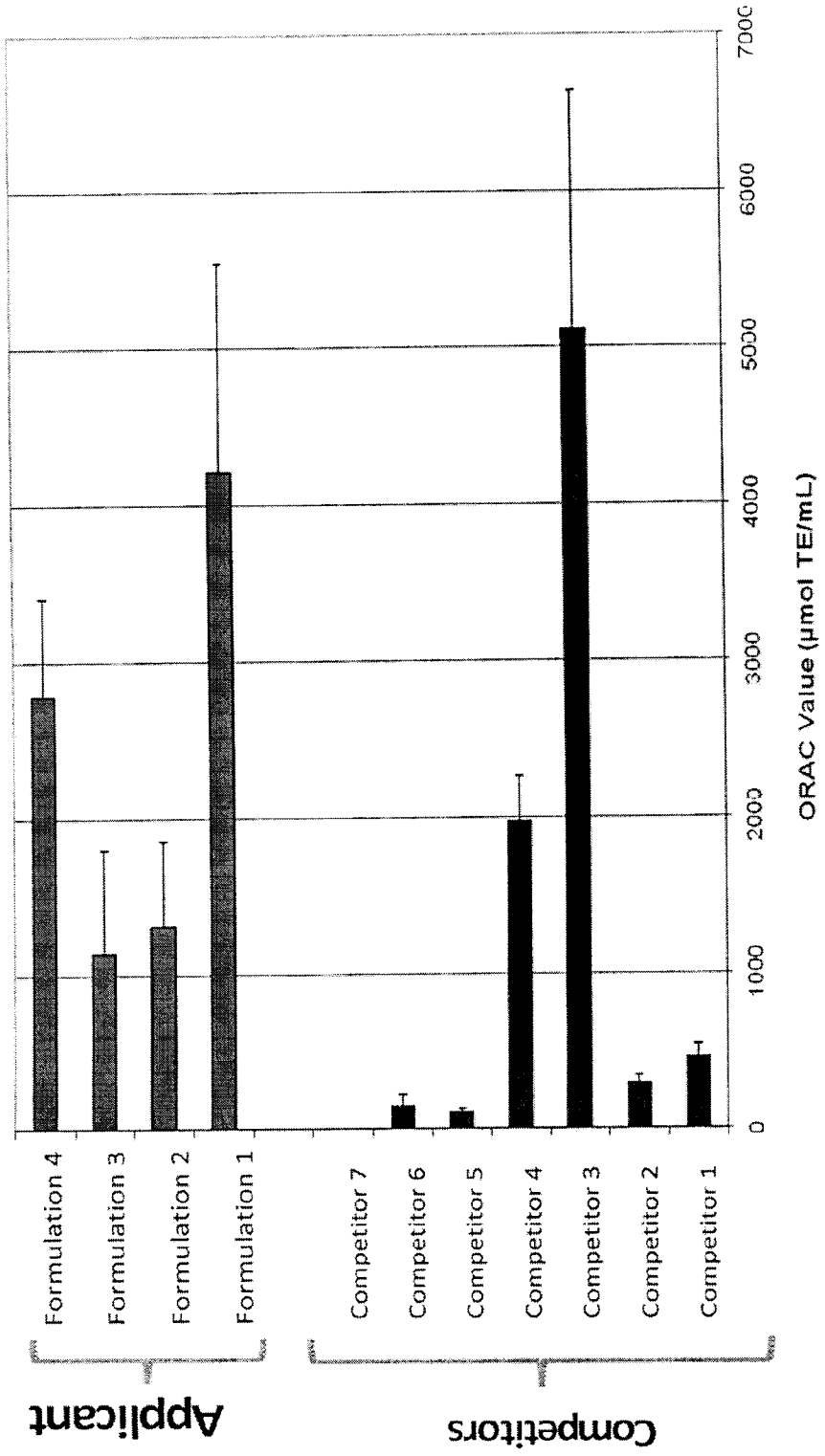

ORAC Value (µmol TE/mL)
0
1000
2000
3000
4000
5000
6000
7000
Formulation 4
Formulation 3
Formulation 2
Formulation 1
Competitor 7
Competitor 6
Competitor 5
Competitor 4
Competitor 3
Competitor 2
Competitor 1
Applicant
Competitors

FIGURE 8:

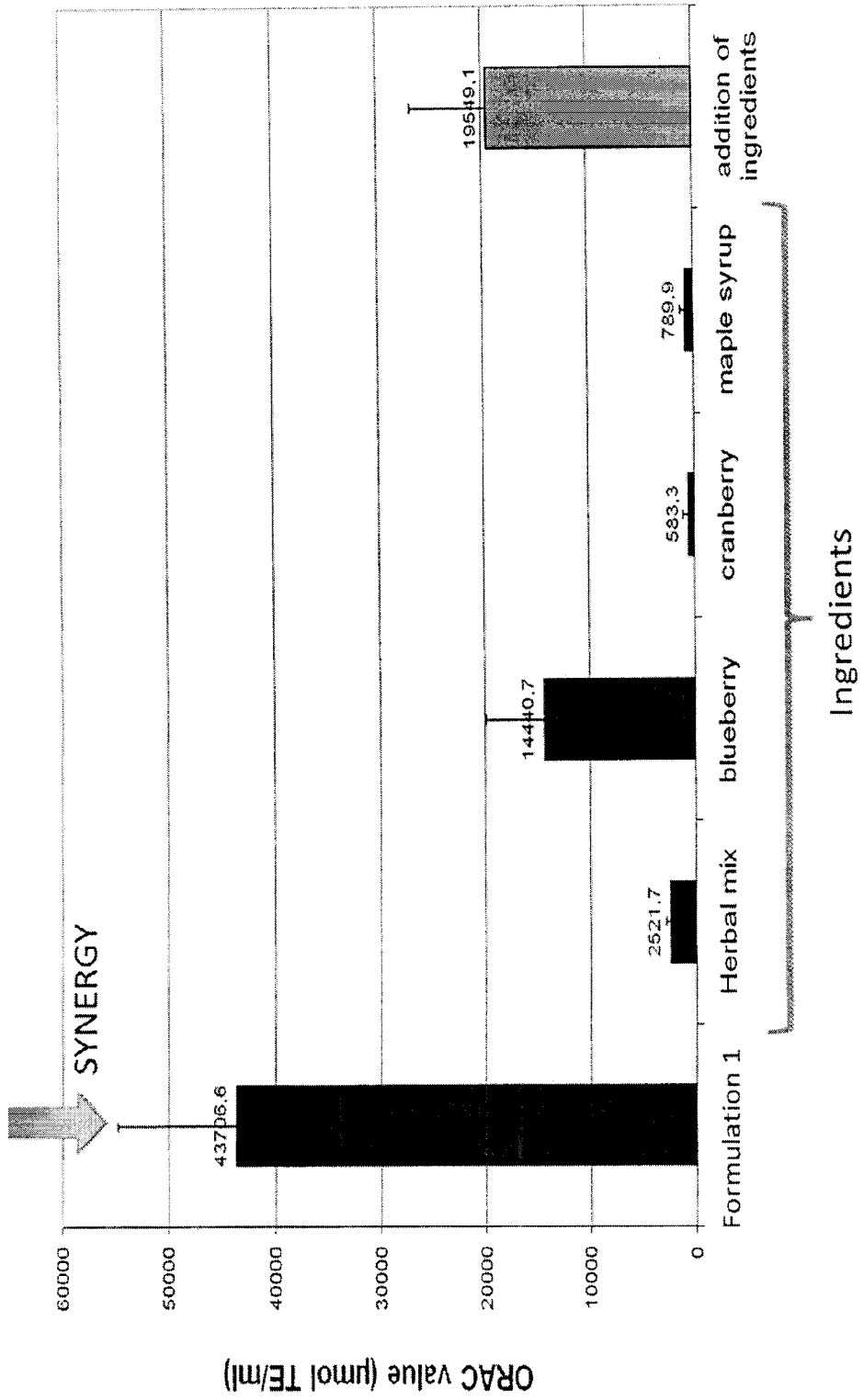

SYNERGY
ORAC value (µmol TE/ml)
60000
50000
40000
30000
20000
10000
0
43706.6
2521.7
14440.7
583.3
789.9
19549.1
Formulation 1
Herbal mix
blueberry
cranberry
maple syrup
addition of ingredients
Ingredients

FIGURE 9:

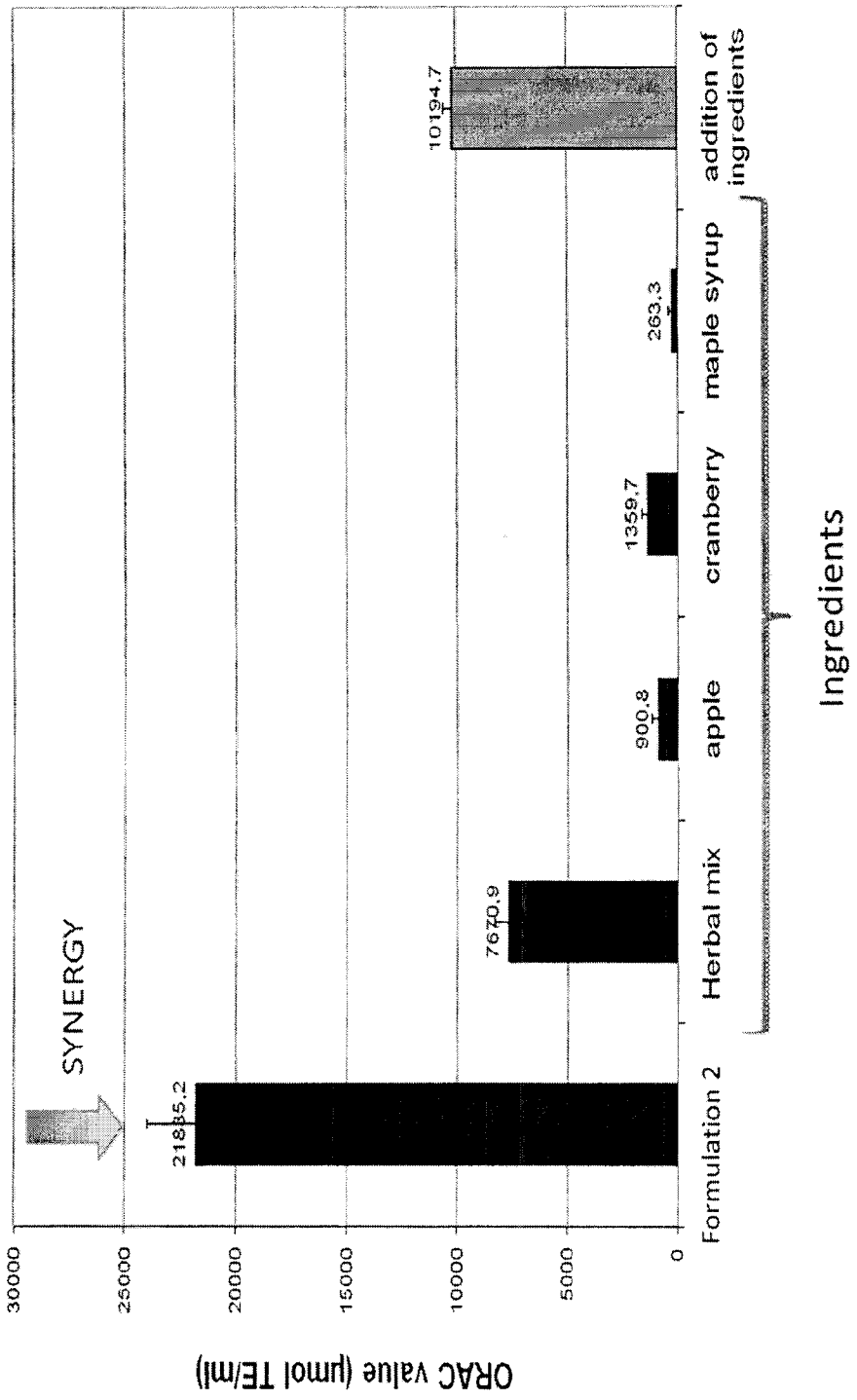

ORAC value (µmol TE/ml)
30000
25000
20000
15000
10000
5000
0
SYNERGY
21885.2
7670.9
900.8
1359.7
263.3
10194.7
Formulation 2
Herbal mix
apple
cranberry
maple syrup
addition of ingredients
Ingredients

FIGURE 10:

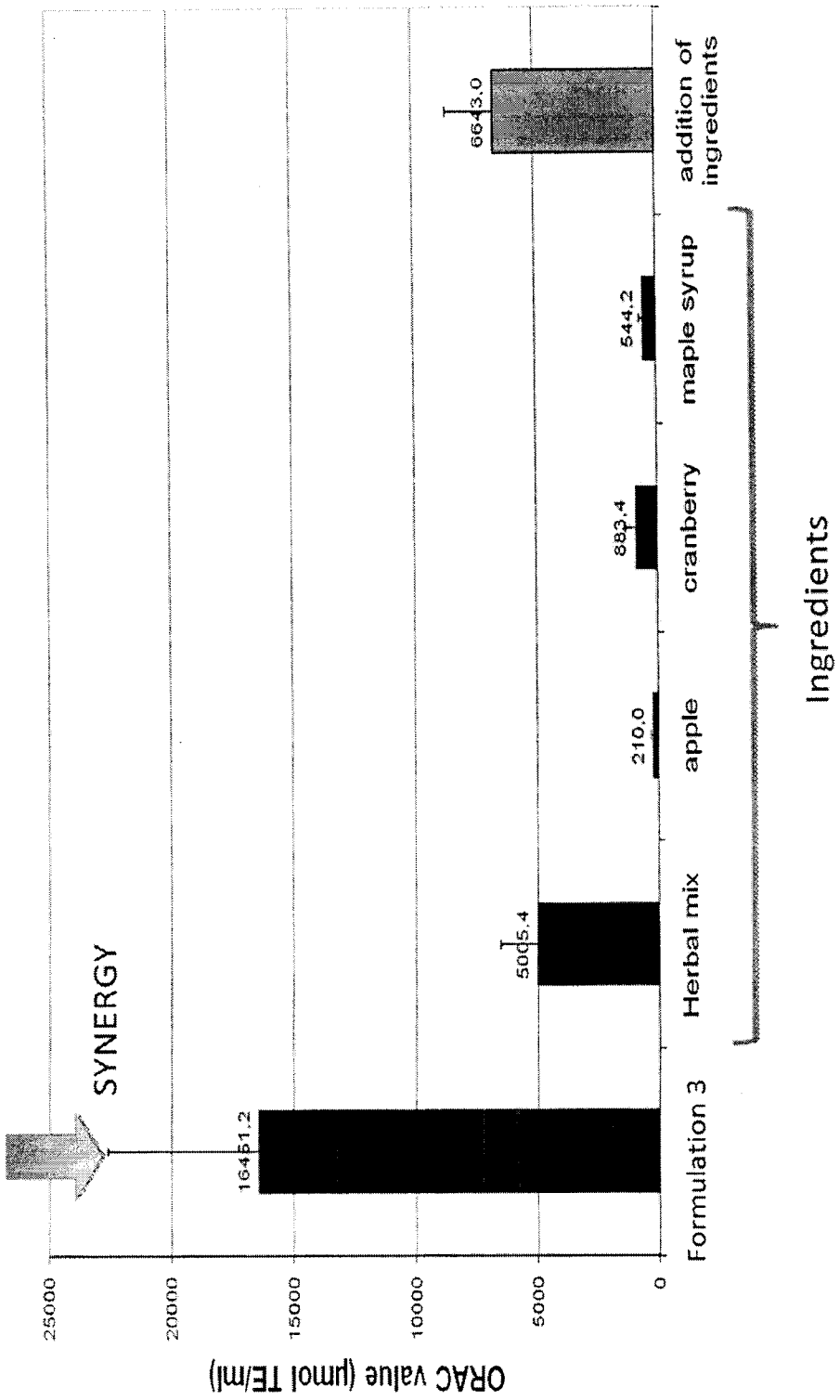

SYNERGY
ORAC value (µmol TE/ml)
25000
20000
15000
10000
5000
0
16451.2
5005.4
210.0
883.4
544.2
6643.0
Formulation 3
Herbal mix
apple
cranberry
maple syrup
addition of ingredients
Ingredients

FIGURE 11:

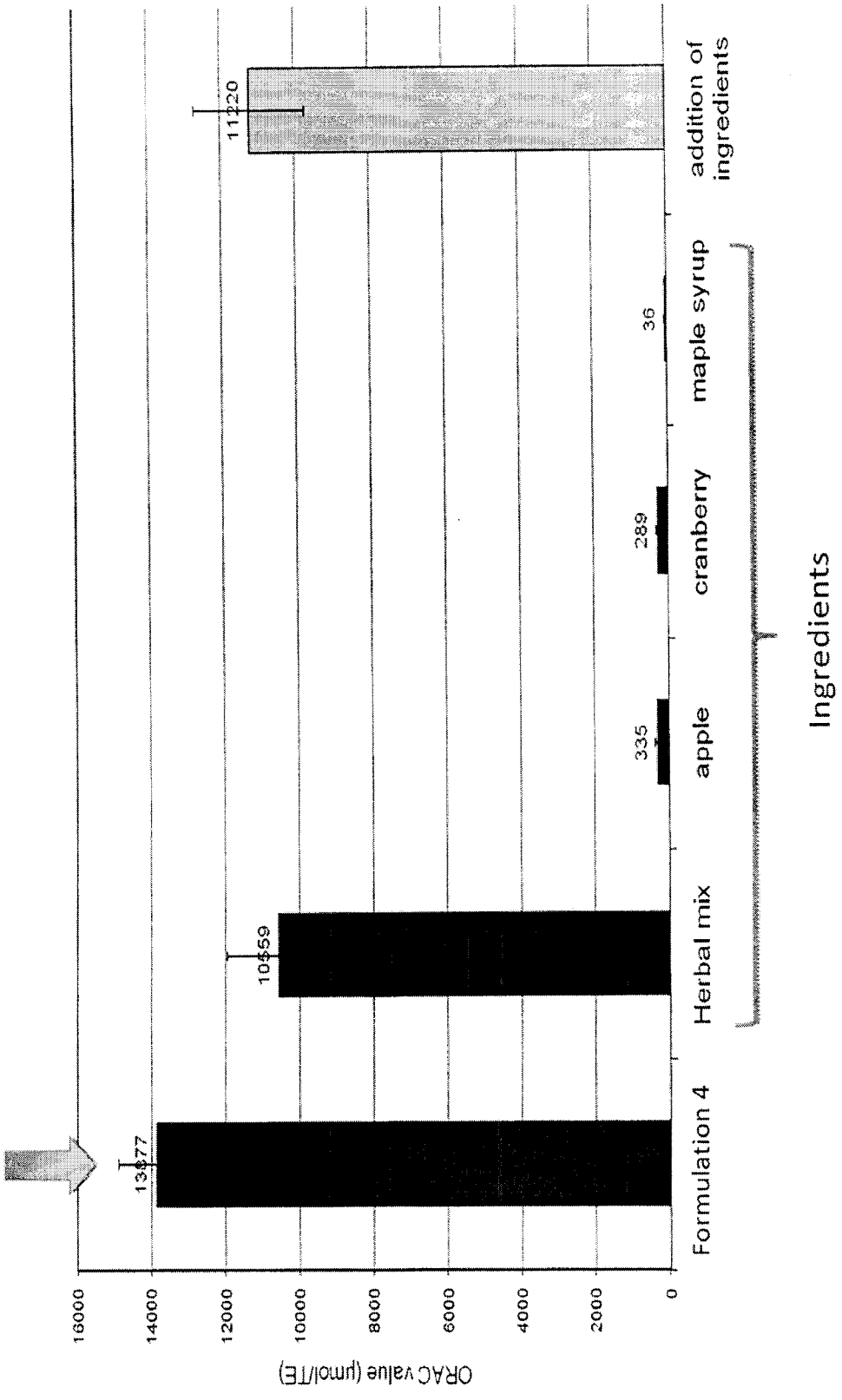

ORAC value (µmol/TE)
16000
14000
12000
10000
8000
6000
4000
2000
0
13477
10459
335
289
36
11220
Formulation 4
Herbal mix
apple
cranberry
maple syrup
addition of ingredients
Ingredients

FIGURE 12:

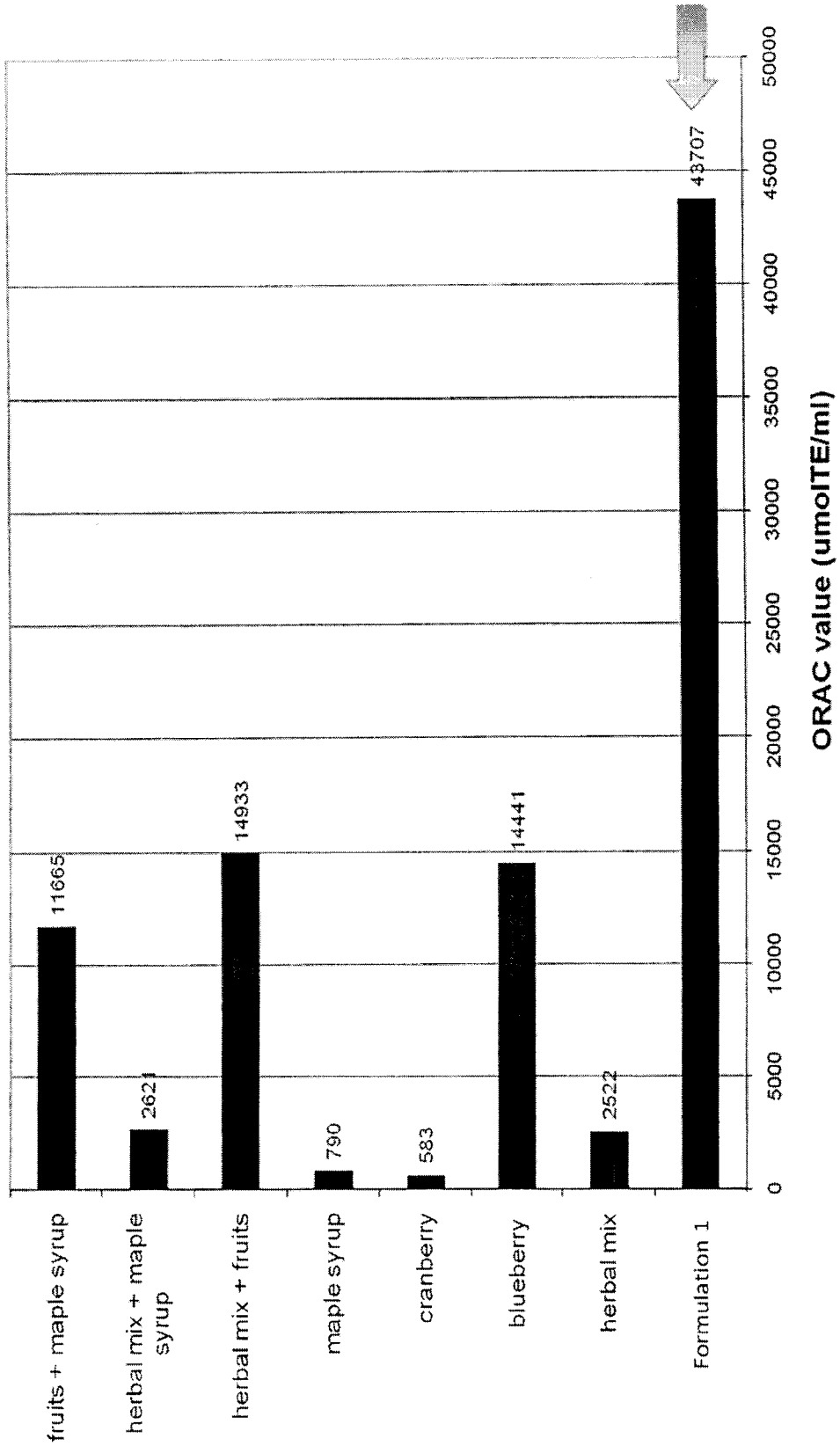

ORAC value (umolTE/ml)
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
11665
2621
14933
790
583
14441
2522
43707
fruits + maple syrup
herbal mix + maple syrup
herbal mix + fruits
maple syrup
cranberry
blueberry
herbal mix
Formulation 1

FIGURE 13:

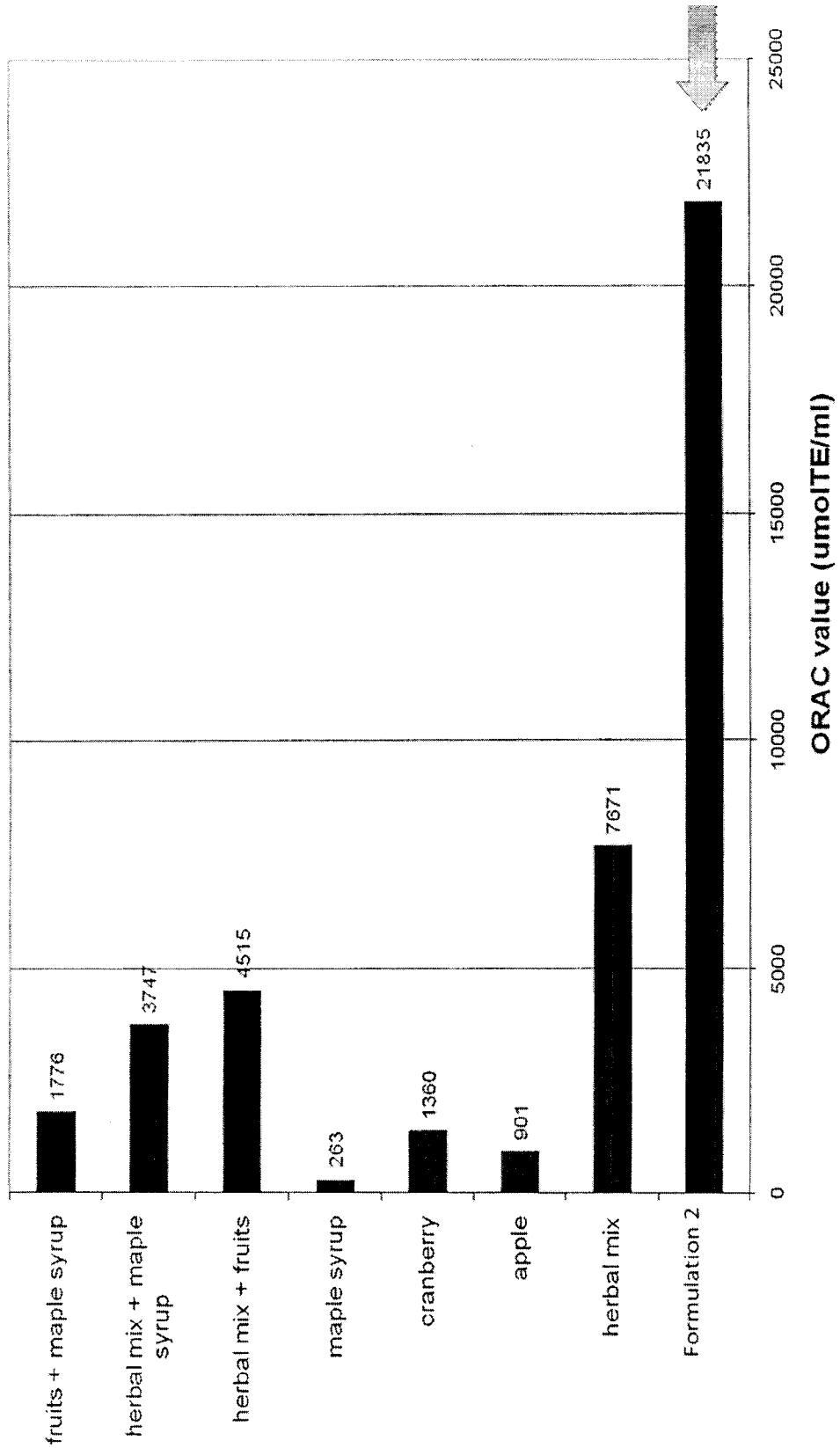

fruits + maple syrup
herbal mix + maple syrup
herbal mix + fruits
maple syrup
cranberry
apple
herbal mix
Formulation 2
1776
3747
4515
263
1360
901
7671
21835
0
5000
10000
15000
20000
25000
ORAC value (umolTE/ml)

FIGURE 14:

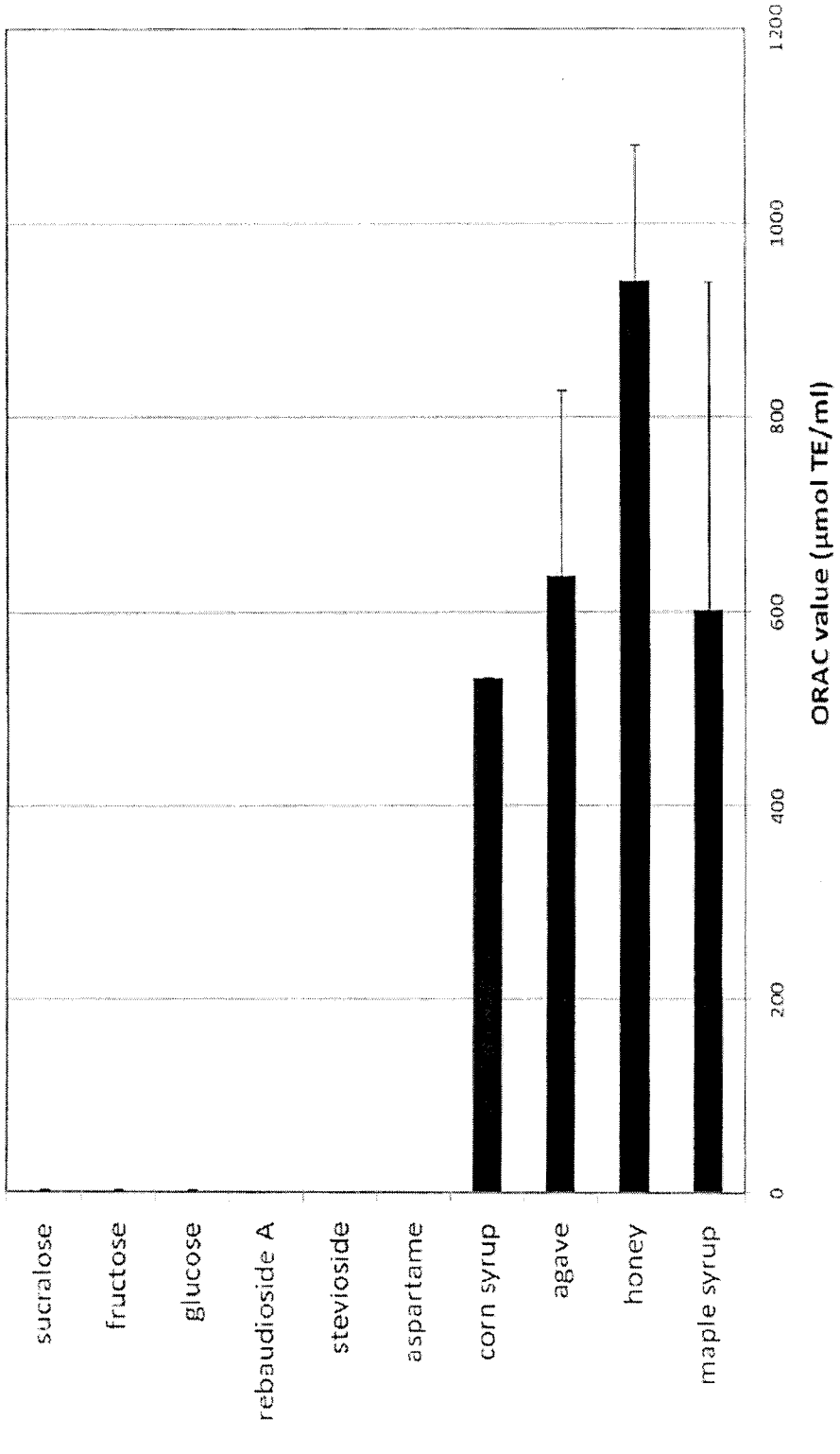

ORAC value (µmol TE/ml)
0
200
400
600
800
1000
1200
sucralose
fructose
glucose
rebaudioside A
stevioside
aspartame
corn syrup
agave
honey
maple syrup

FIGURE 15:

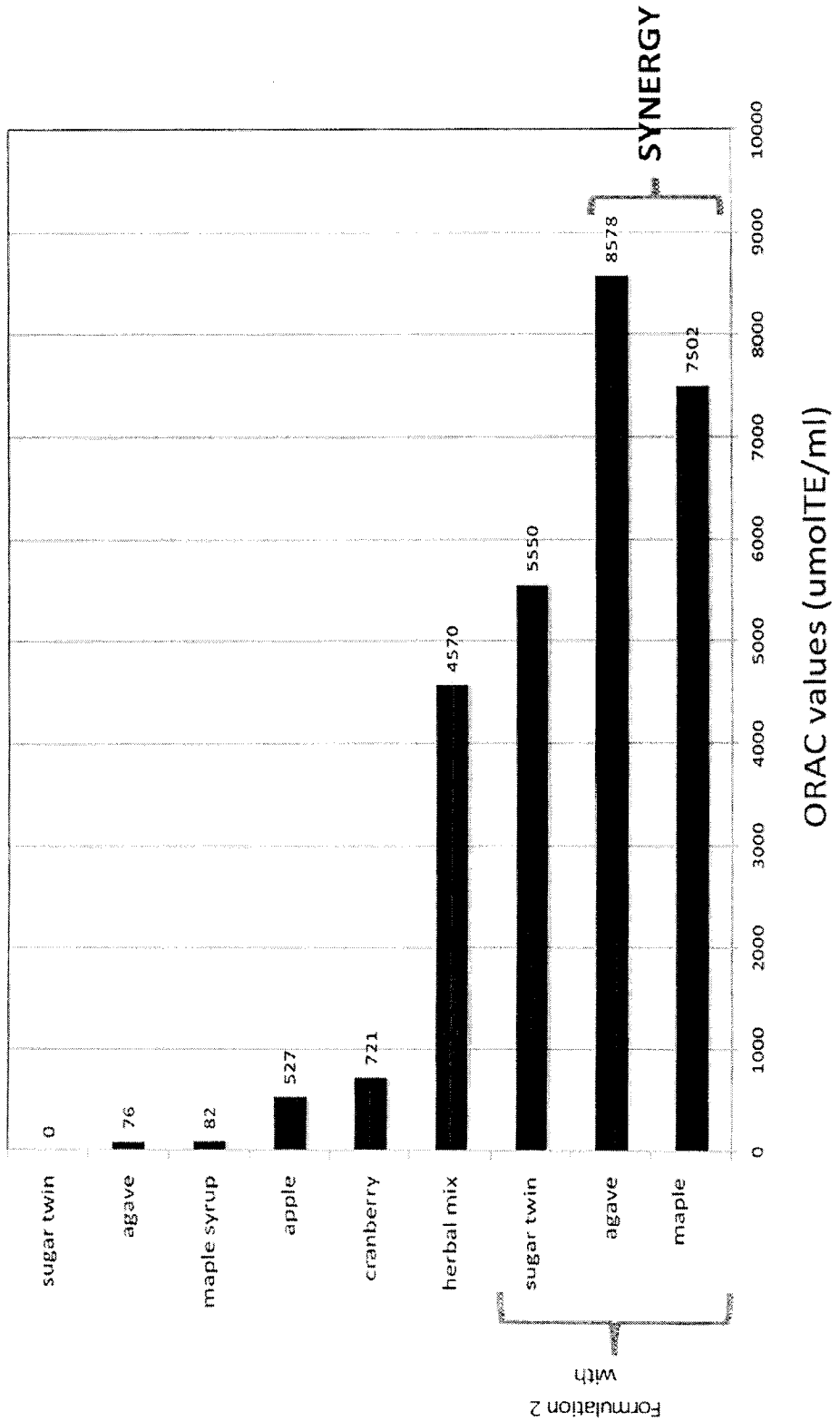

ORAC values (umolTE/ml)
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
0
76
82
527
721
4570
5550
8578
7502
SYNERGY
sugar twin
agave
maple syrup
apple
cranberry
herbal mix
sugar twin
agave
maple
Formulation 2
with

FIGURE 16:

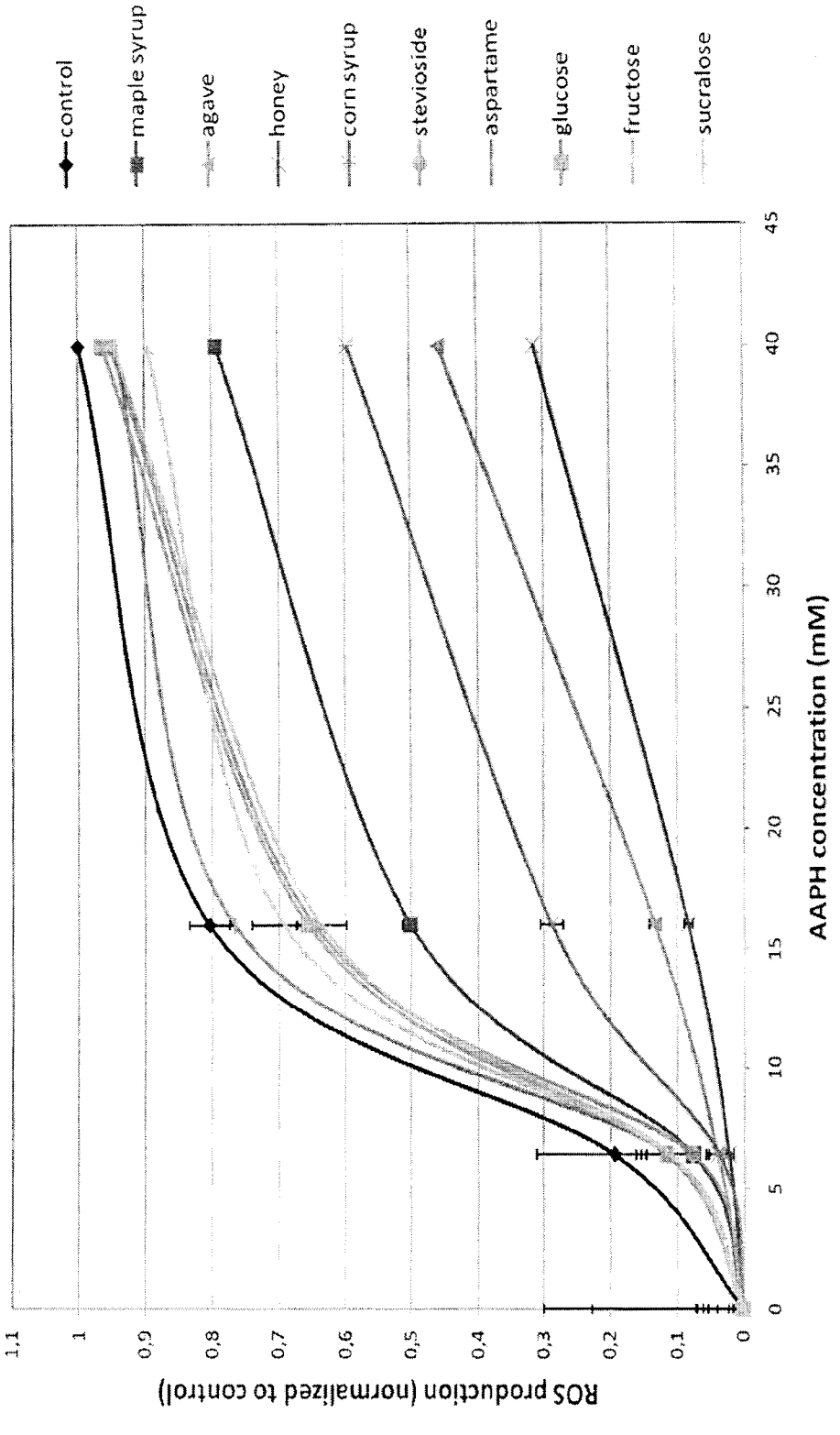

control
maple syrup
agave
honey
corn syrup
stevioside
aspartame
glucose
fructose
sucralose
ROS production (normalized to control)
AAPH concentration (mM)
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
1
1,1
0
5
10
15
20
25
30
35
40
45

FIGURE 17:

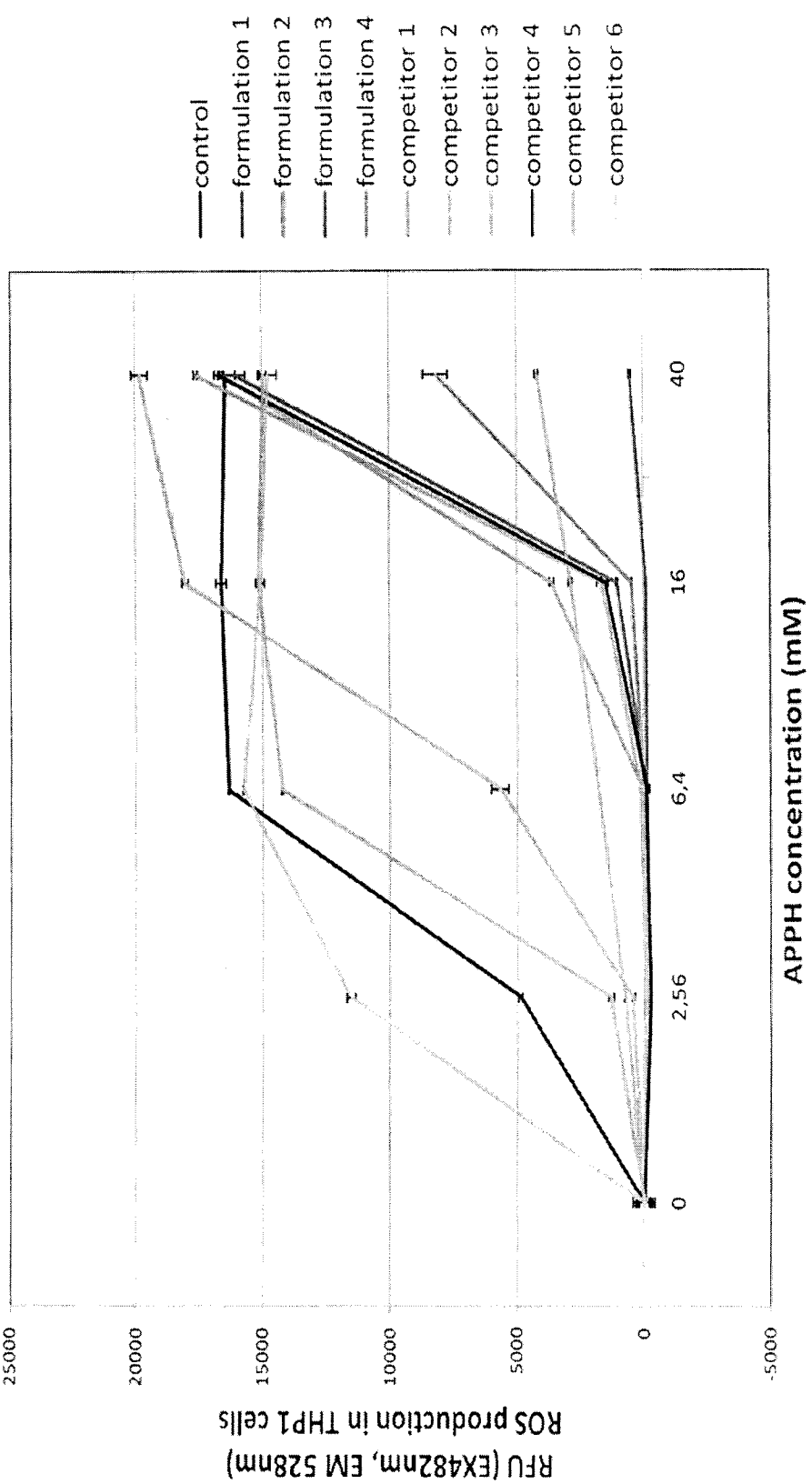

control
formulation 1
formulation 2
formulation 3
formulation 4
competitor 1
competitor 2
competitor 3
competitor 4
competitor 5
competitor 6
RFU (EX482nm, EM 528nm)
ROS production in THP1 cells
25000
20000
15000
10000
5000
0
-5000
0
2,56
6,4
16
40
APPH concentration (mM)

FIGURE 18:

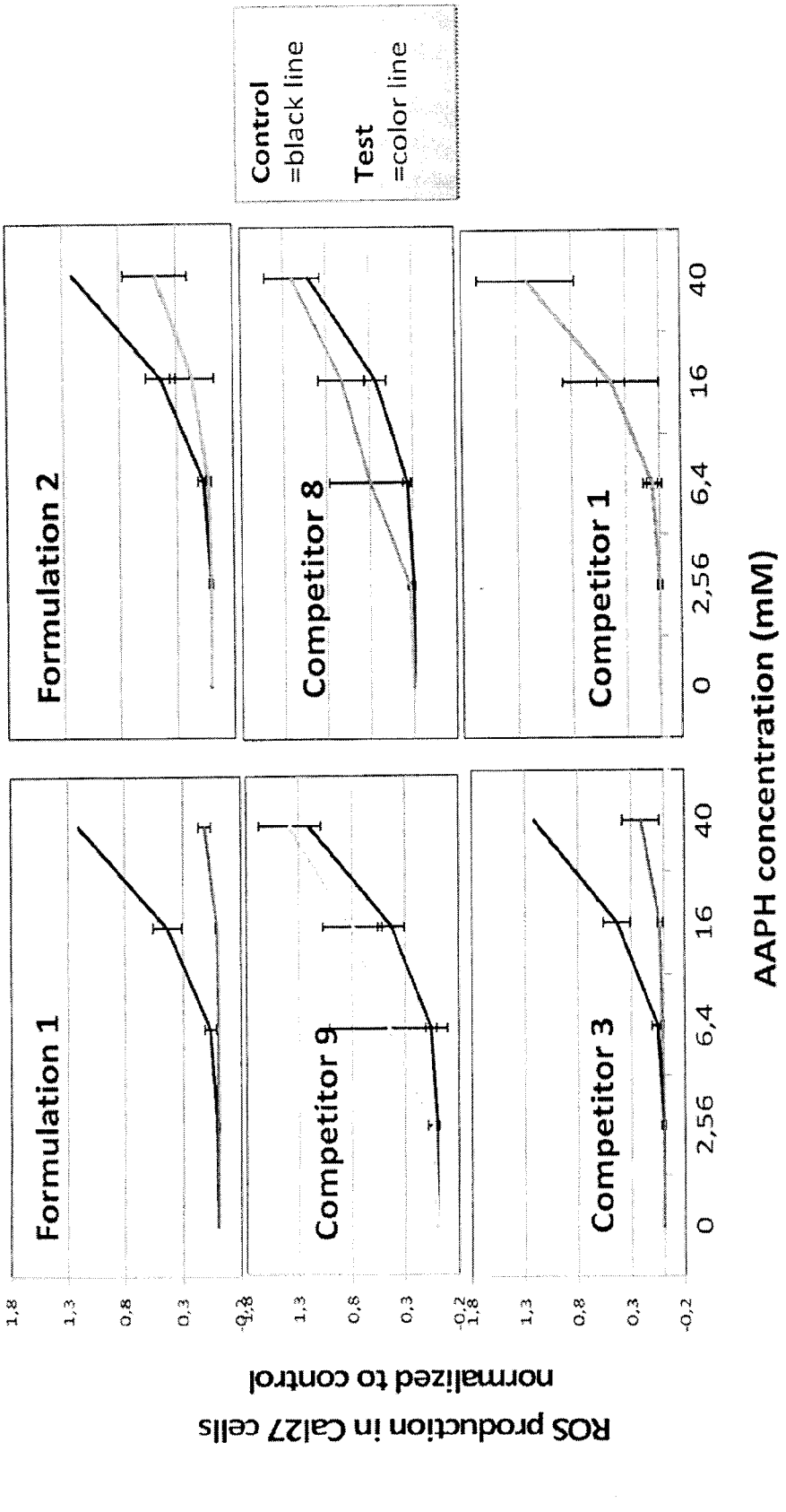

Control
=black line
Test
=color line
Formulation 1
Formulation 2
Competitor 9
Competitor 8
Competitor 3
Competitor 1
ROS production in Cal27 cells
normalized to control
AAPH concentration (mM)
0
2,56
6,4
16
40
1,8
1,3
0,8
0,3
-0,2

FIGURE 19:

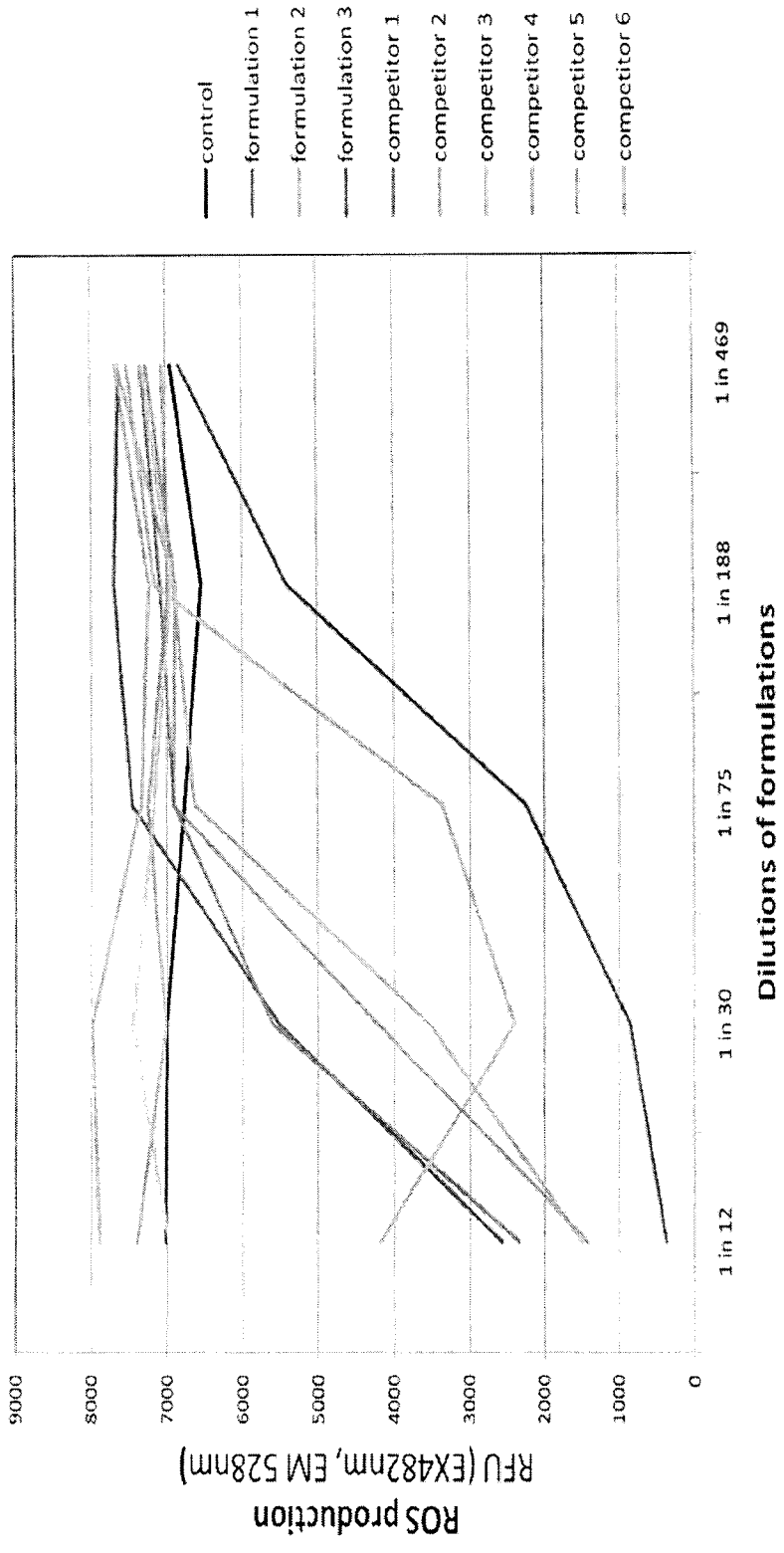

ROS production
RFU (EX482nm, EM 528nm)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
1 in 12
1 in 30
1 in 75
1 in 188
1 in 469
Dilutions of formulations
control
formulation 1
formulation 2
formulation 3
competitor 1
competitor 2
competitor 3
competitor 4
competitor 5
competitor 6

FIGURE 20:

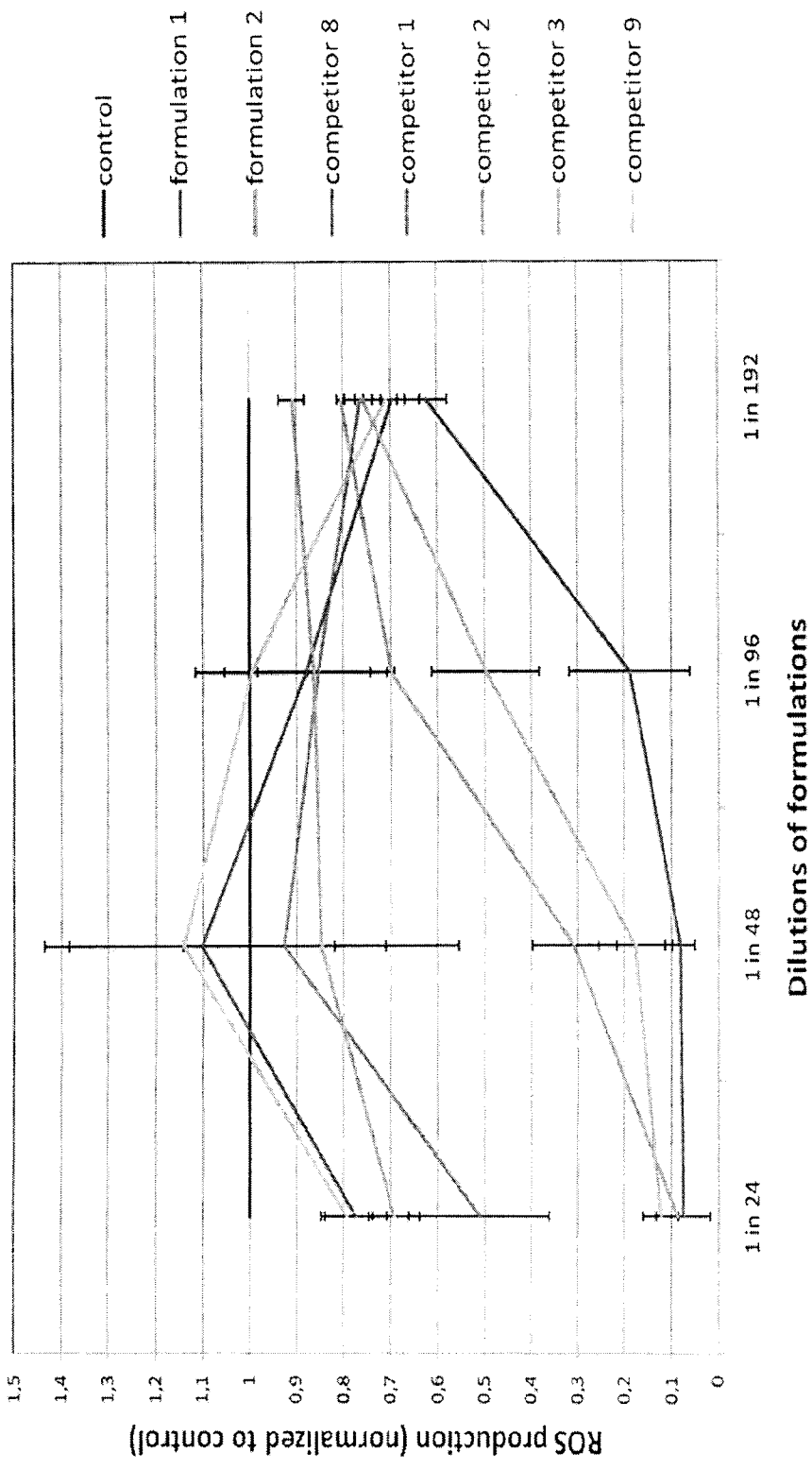

control
formulation 1
formulation 2
competitor 8
competitor 1
competitor 2
competitor 3
competitor 9
ROS production (normalized to control)
1,5
1,4
1,3
1,2
1,1
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
1 in 24
1 in 48
1 in 96
1 in 192
Dilutions of formulations

FIGURE 21:

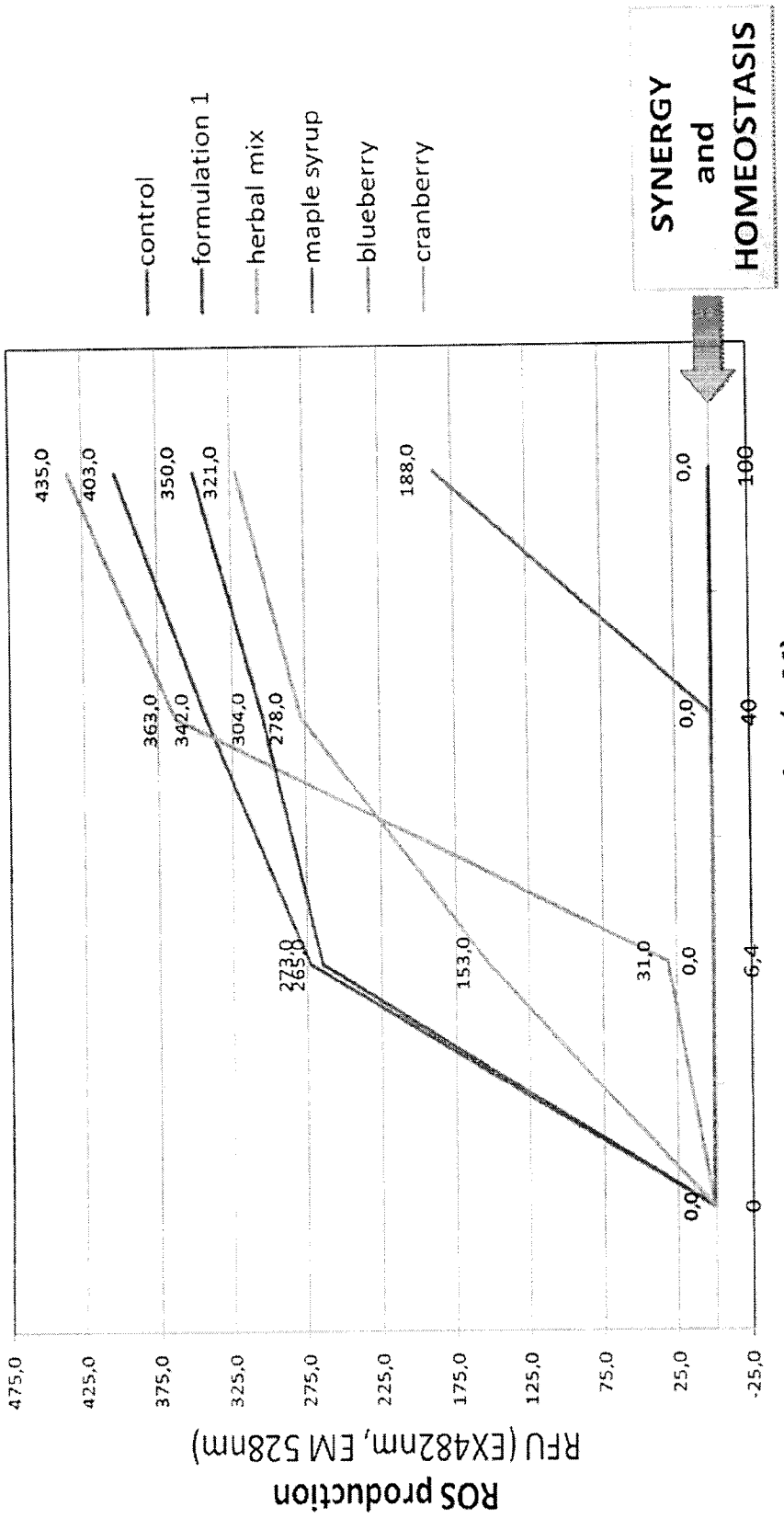

ROS production
RFU (EX482nm, EM 528nm)
475,0
425,0
375,0
325,0
275,0
225,0
175,0
125,0
75,0
25,0
-25,0
AAPH concentration (mM)
0
6,4
40
100
control
formulation 1
herbal mix
maple syrup
blueberry
cranberry
SYNERGY and HOMEOSTASIS
435,0
403,0
363,0
342,0
350,0
321,0
304,0
278,0
273,0
265,0
188,0
153,0
31,0
0,0
0,0
0,0
0,0
0,0

FIGURE 22:

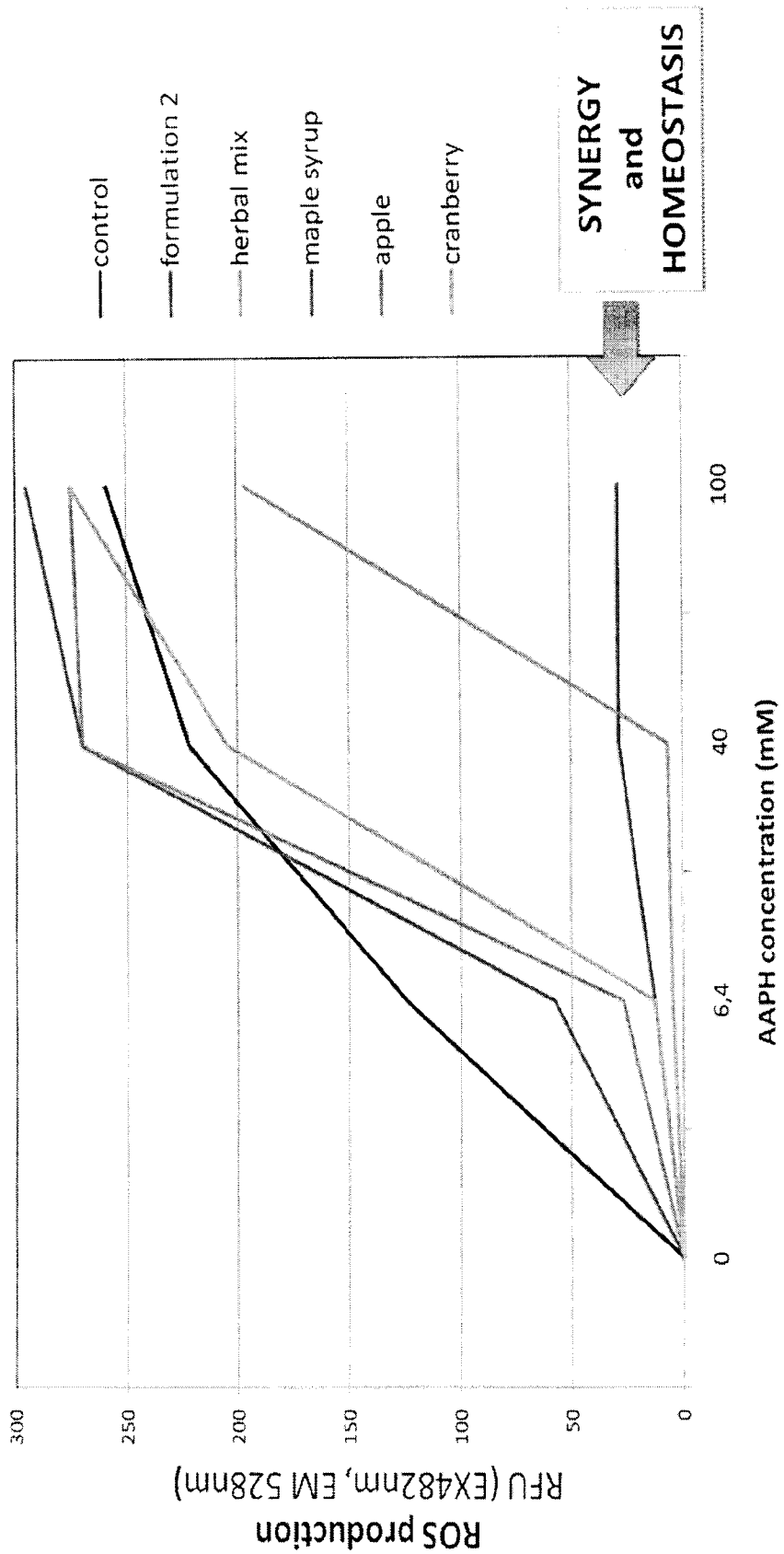

ROS production
RFU (EX482nm, EM528nm)
300
250
200
150
100
50
0
0
6,4
40
100
AAPH concentration (mM)
control
formulation 2
herbal mix
maple syrup
apple
cranberry
SYNERGY
and
HOMEOSTASIS

FIGURE 23:

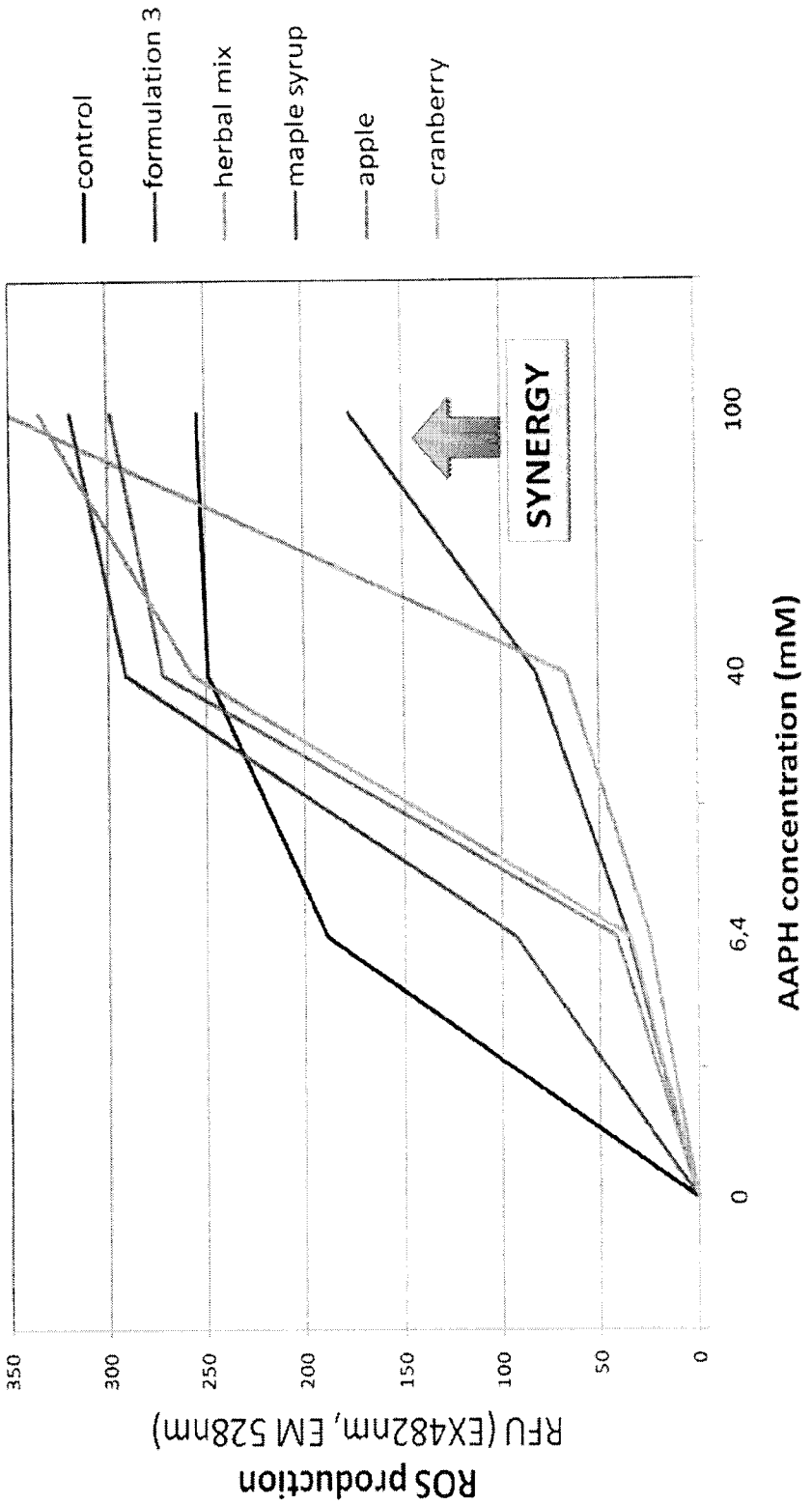

ROS production
RFU (EX482nm, EM 528nm)
350
300
250
200
150
100
50
0
0
6,4
40
100
AAPH concentration (mM)
SYNERGY
control
formulation 3
herbal mix
maple syrup
apple
cranberry

FIGURE 24:

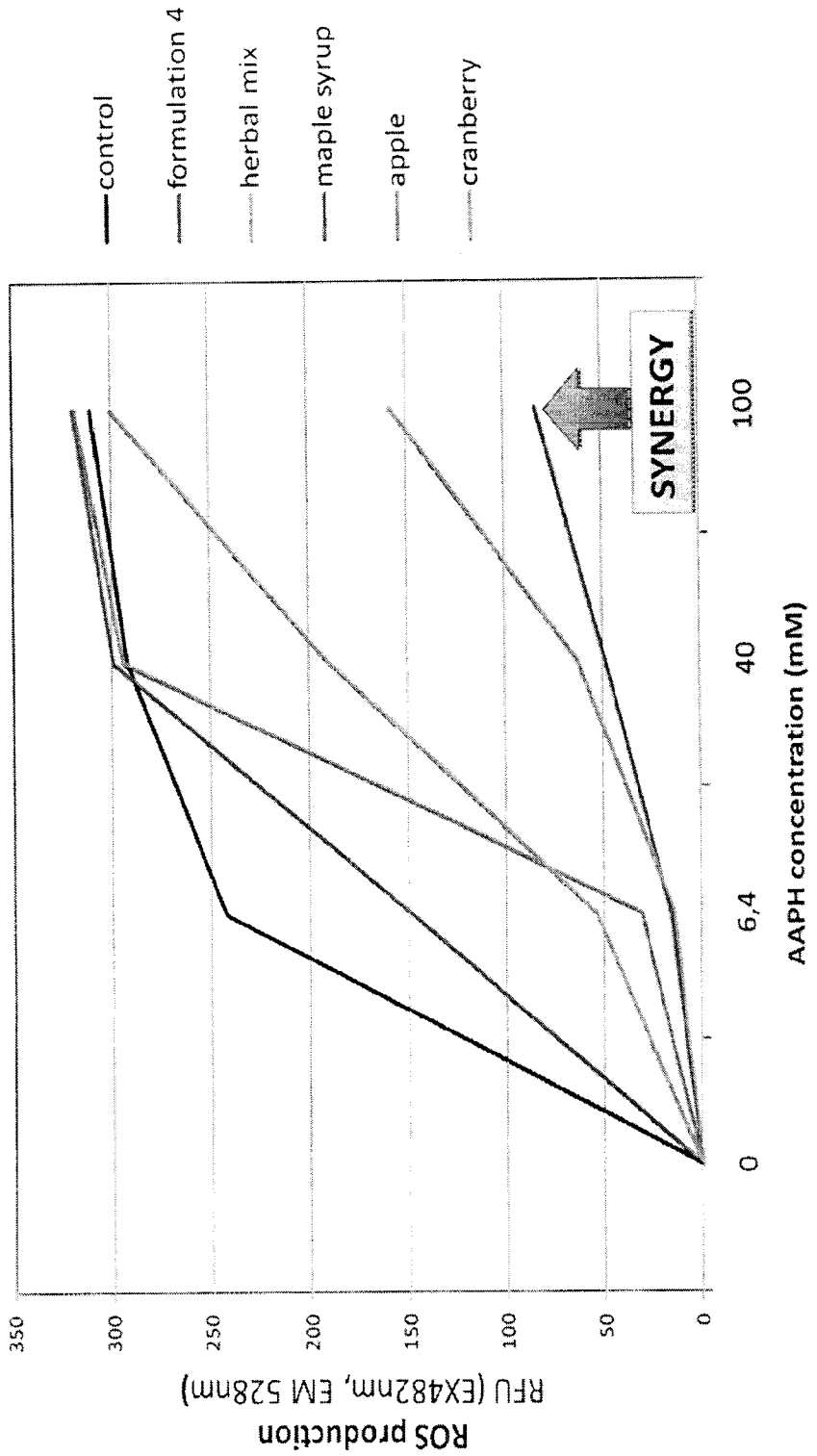

control
formulation 4
herbal mix
maple syrup
apple
cranberry
SYNERGY
ROS production
RFU (EX482nm, EM 528nm)
AAPH concentration (mM)
0
50
100
150
200
250
300
350
0
6,4
40
100

FIGURE 25:

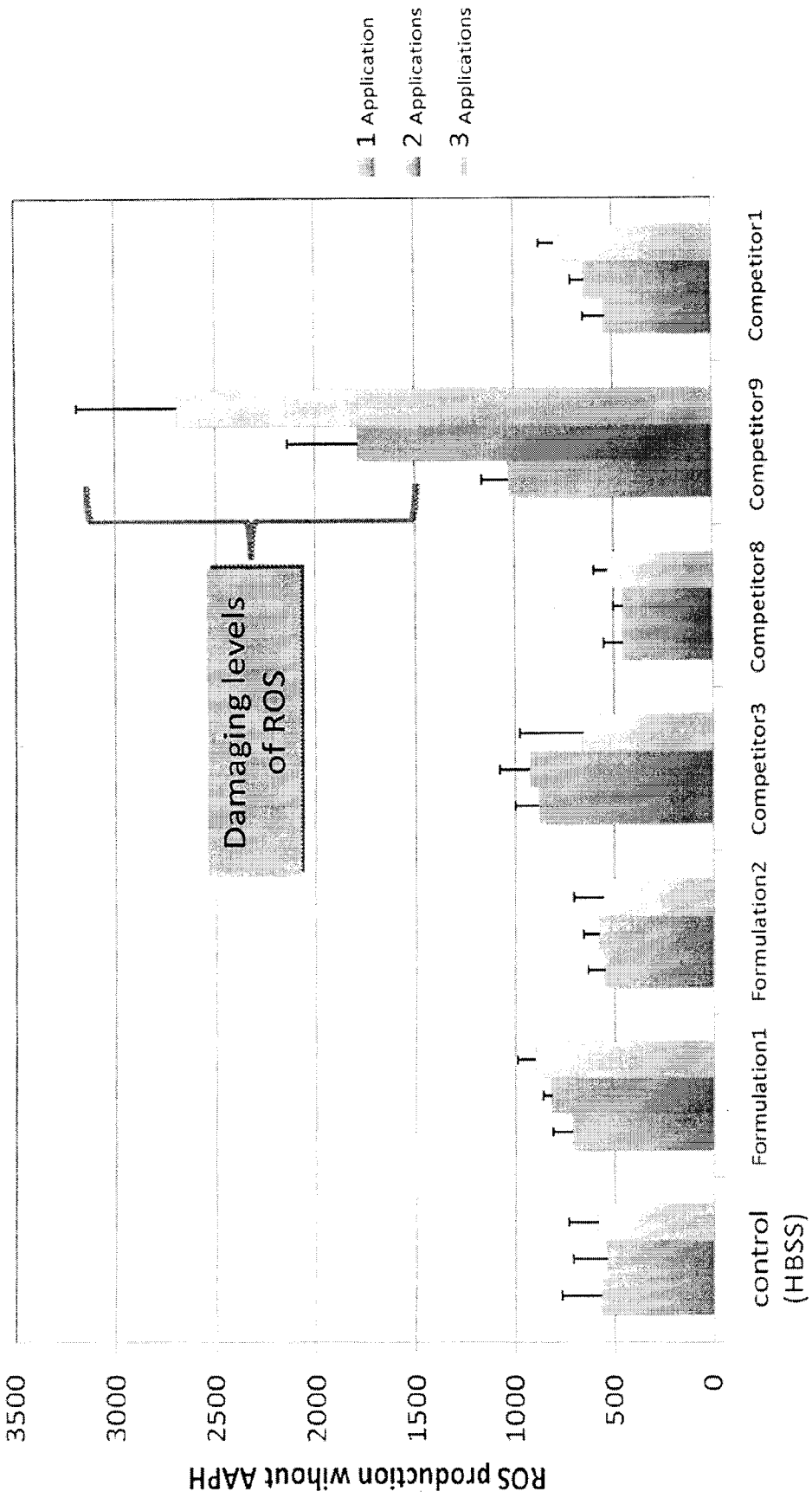

1 Application
2 Applications
3 Applications
Damaging levels of ROS
ROS production wihout AAPH
3500
3000
2500
2000
1500
1000
500
0
control (HBSS)
Formulation1
Formulation2
Competitor3
Competitor8
Competitor9
Competitor1

FIGURE 26:

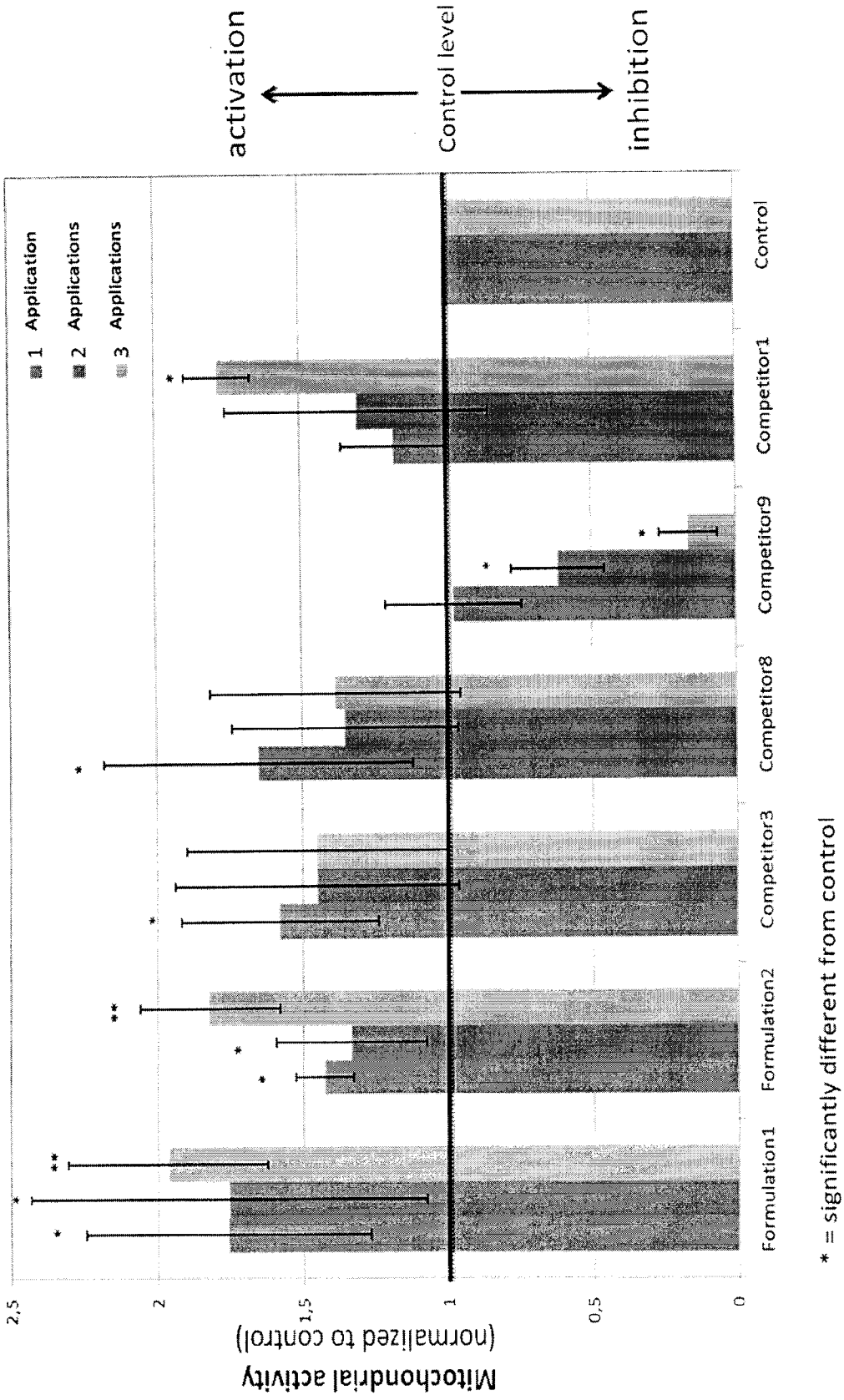

activation
Control level
inhibition
1 Application
2 Applications
3 Applications
Mitochondrial activity
(normalized to control)
2,5
2
1,5
1
0,5
0
Formulation1
Formulation2
Competitor3
Competitor8
Competitor9
Competitor1
Control
* = significantly different from control

FIGURE 27:

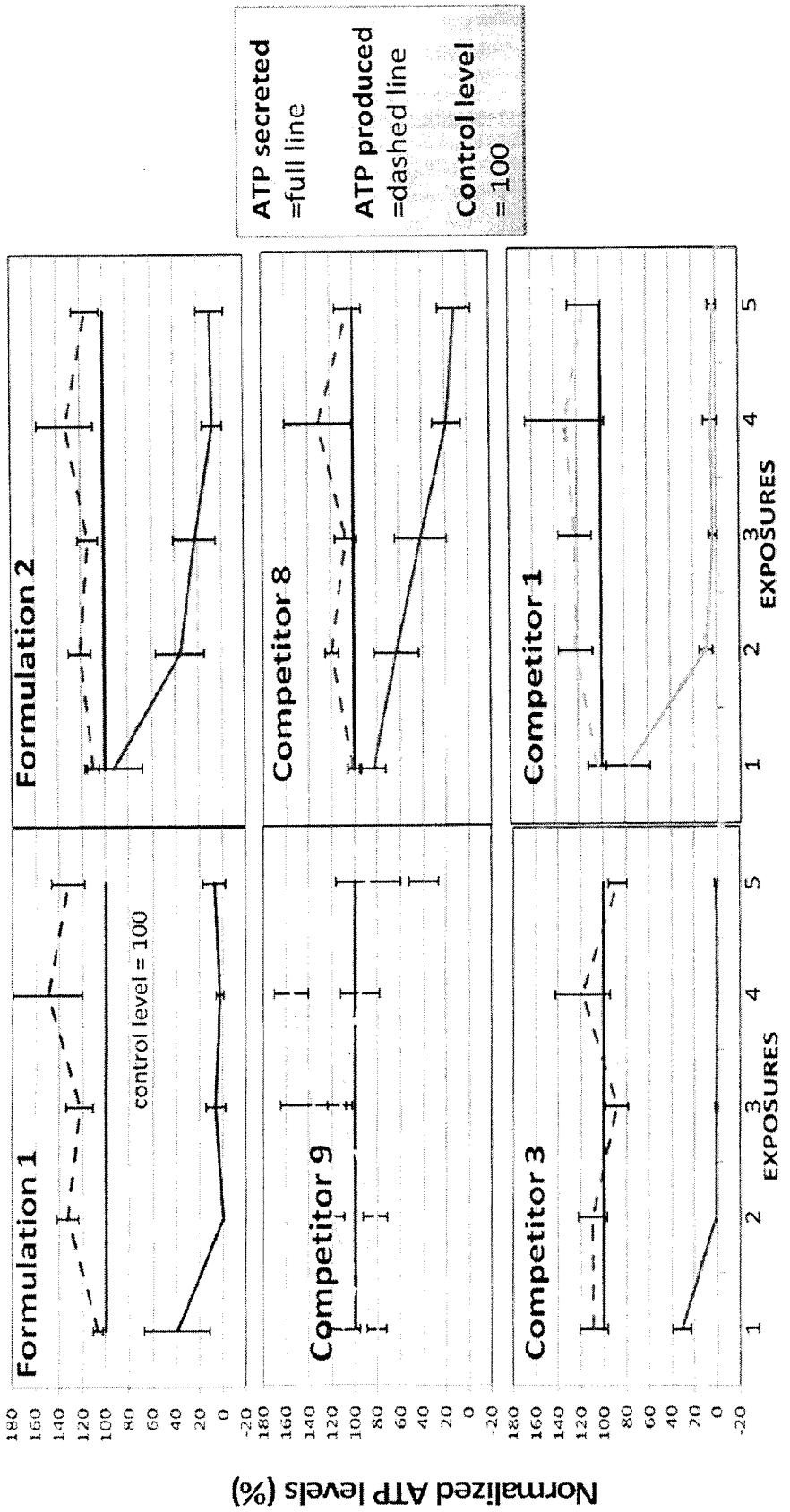

Formulation 1
Formulation 2
Competitor 9
Competitor 8
Competitor 3
Competitor 1
control level = 100
ATP secreted
=full line
ATP produced
=dashed line
Control level
= 100
Normalized ATP levels (%)
EXPOSURES
EXPOSURES

FIGURE 28:

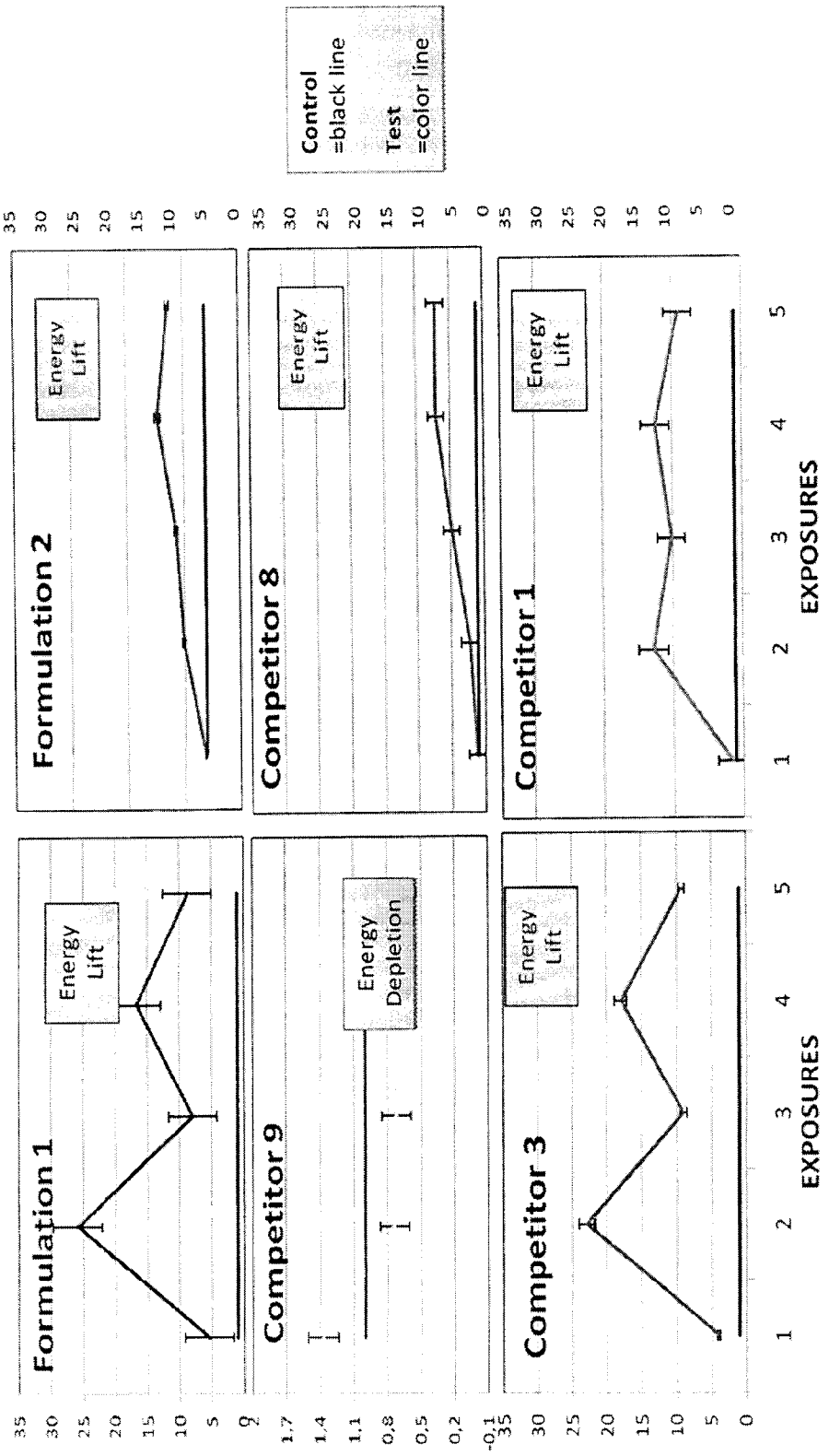

ATP intracellular (ATPi)/ATP secreted (ATPs)
Overall ENERGETIC POTENTIAL
Formulation 1
Energy Lift
Competitor 9
Energy Depletion
Competitor 3
Energy Lift
Formulation 2
Energy Lift
Competitor 8
Energy Lift
Competitor 1
Energy Lift
Control =black line
Test =color line
EXPOSURES
EXPOSURES

FIGURE 29:

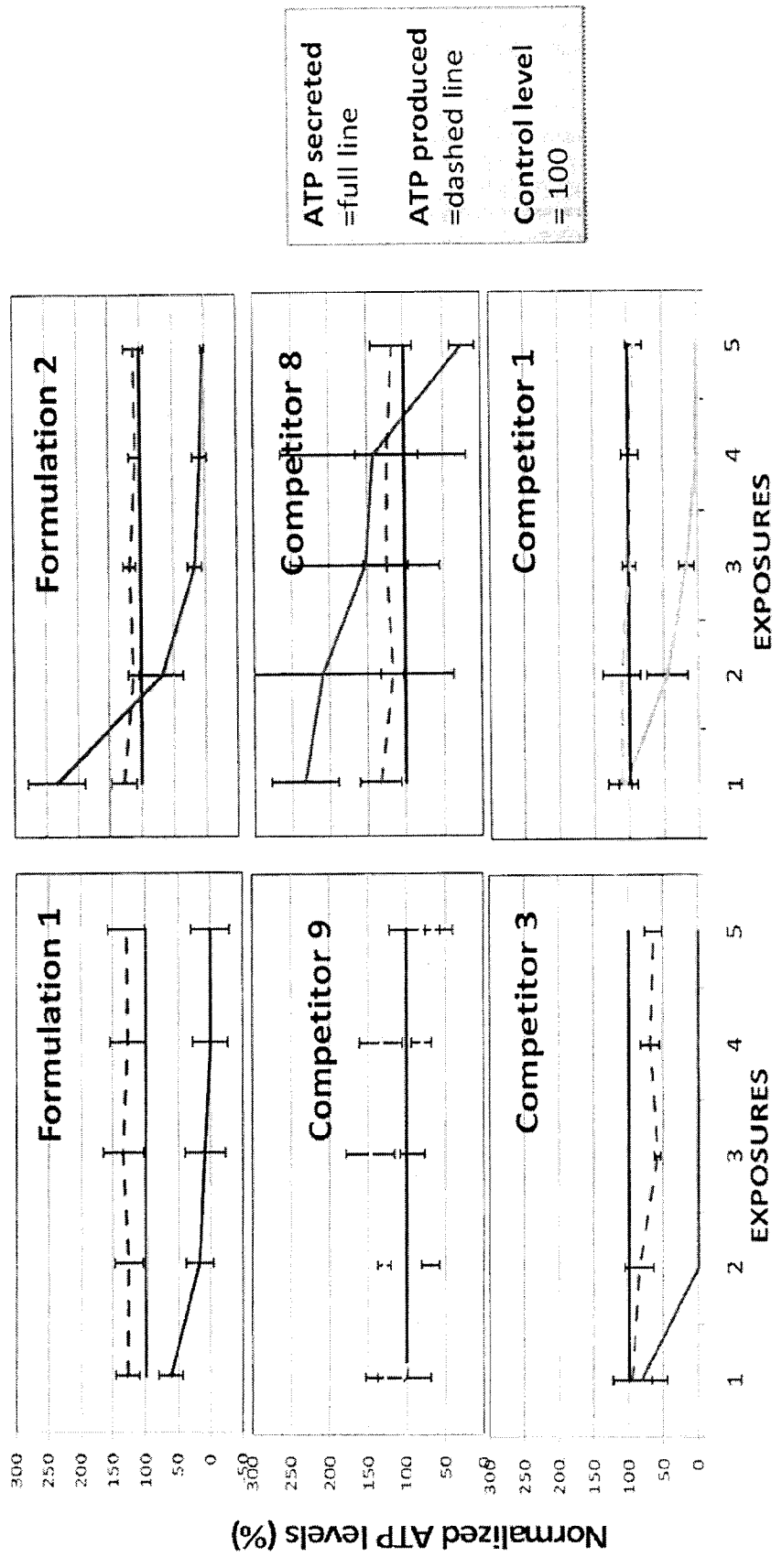

+ 40mM AAPH
Formulation 1
Formulation 2
Competitor 9
Competitor 8
Competitor 3
Competitor 1
Normalized ATP levels (%)
EXPOSURES
ATP secreted
=full line
ATP produced
=dashed line
Control level
=100

FIGURE 30:

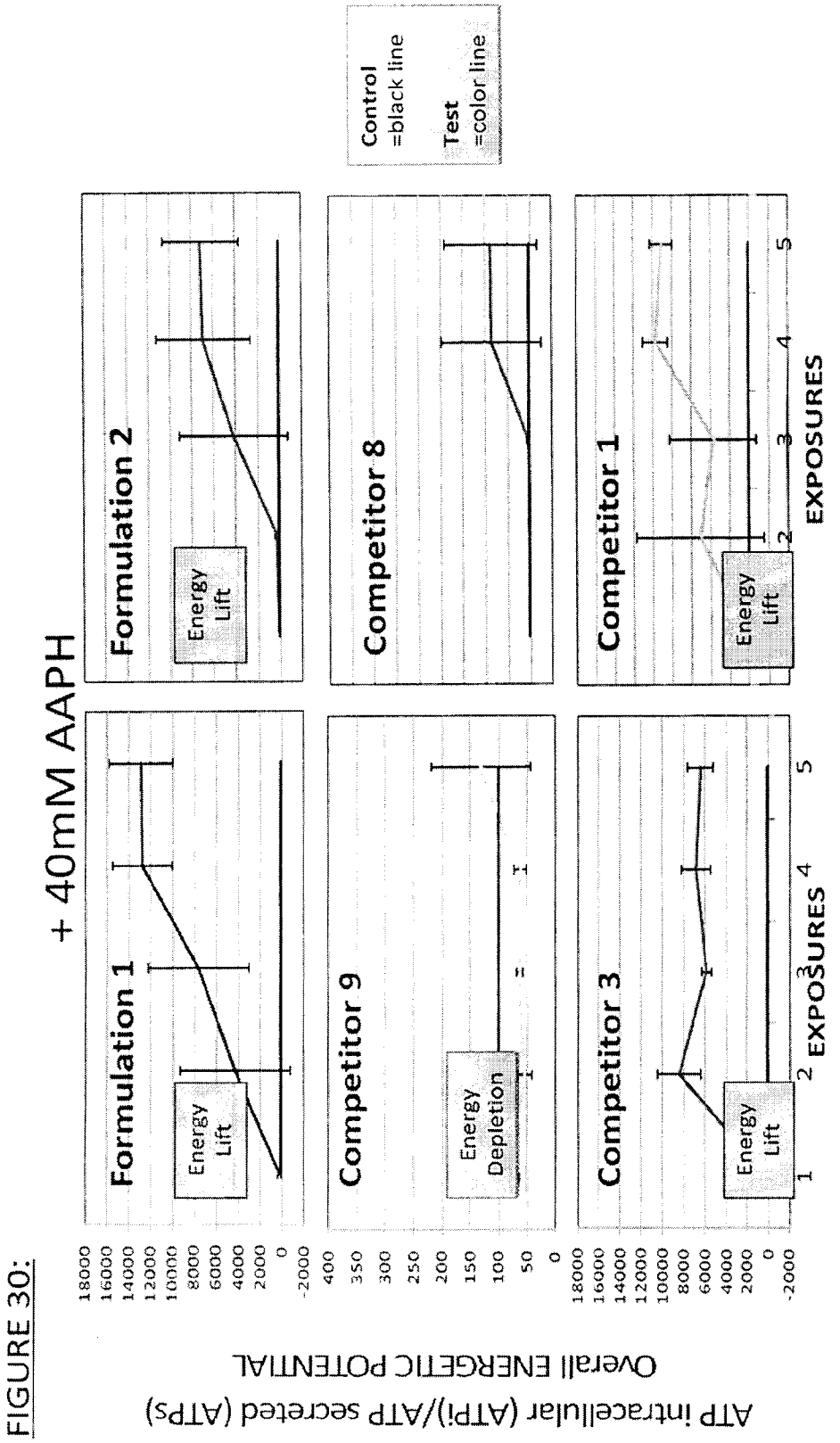

+ 40mM AAPH
Formulation 1
Formulation 2
Competitor 9
Competitor 8
Competitor 3
Competitor 1
Energy Lift
Energy Depletion
Control =black line
Test =color line
ATP intracellular (ATPi)/ATP secreted (ATPs)
Overall ENERGETIC POTENTIAL
EXPOSURES

FIGURE 31:

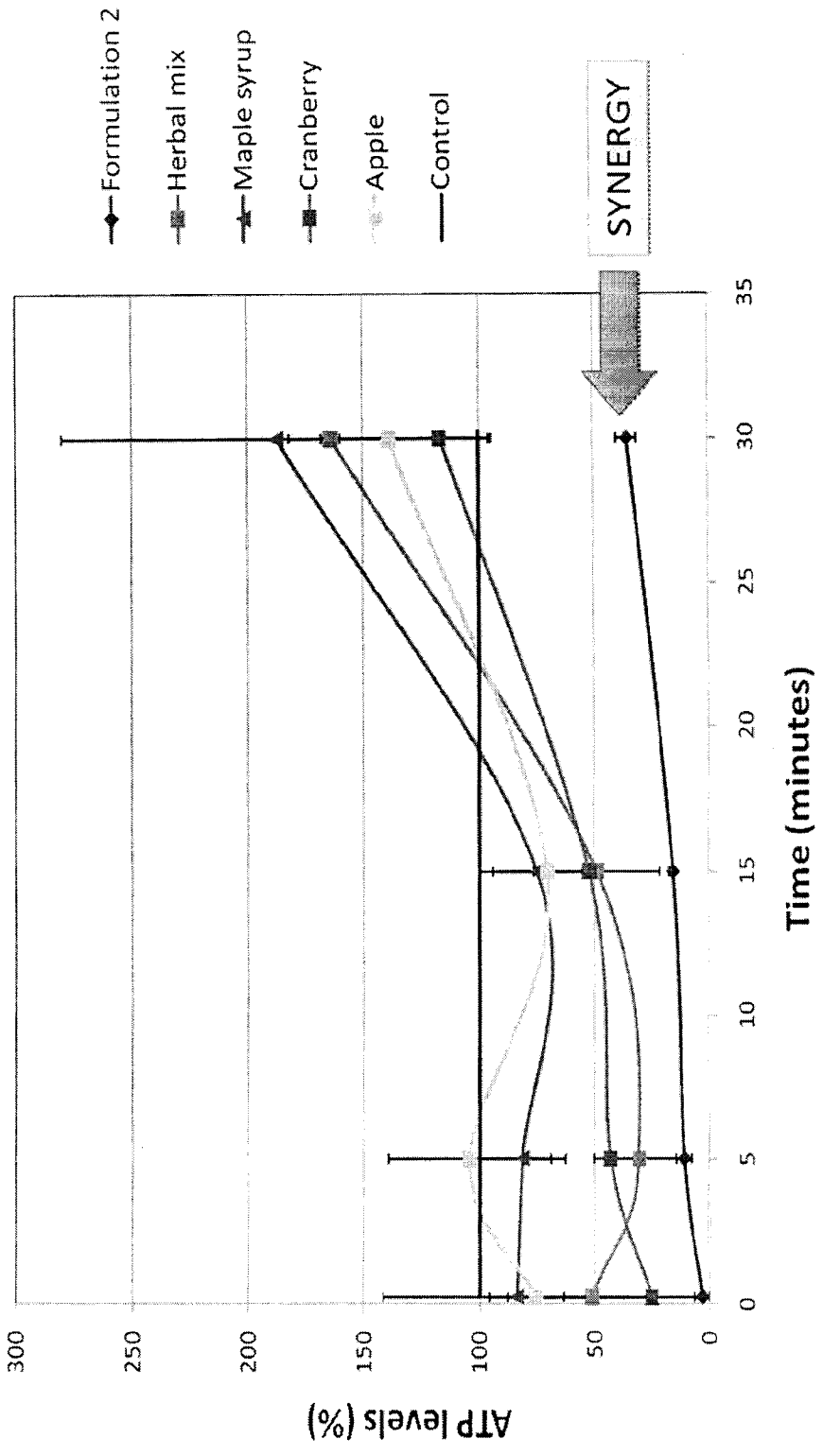

Formulation 2
Herbal mix
Maple syrup
Cranberry
Apple
Control
SYNERGY
ATP levels (%)
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
Time (minutes)

FIGURE 32:

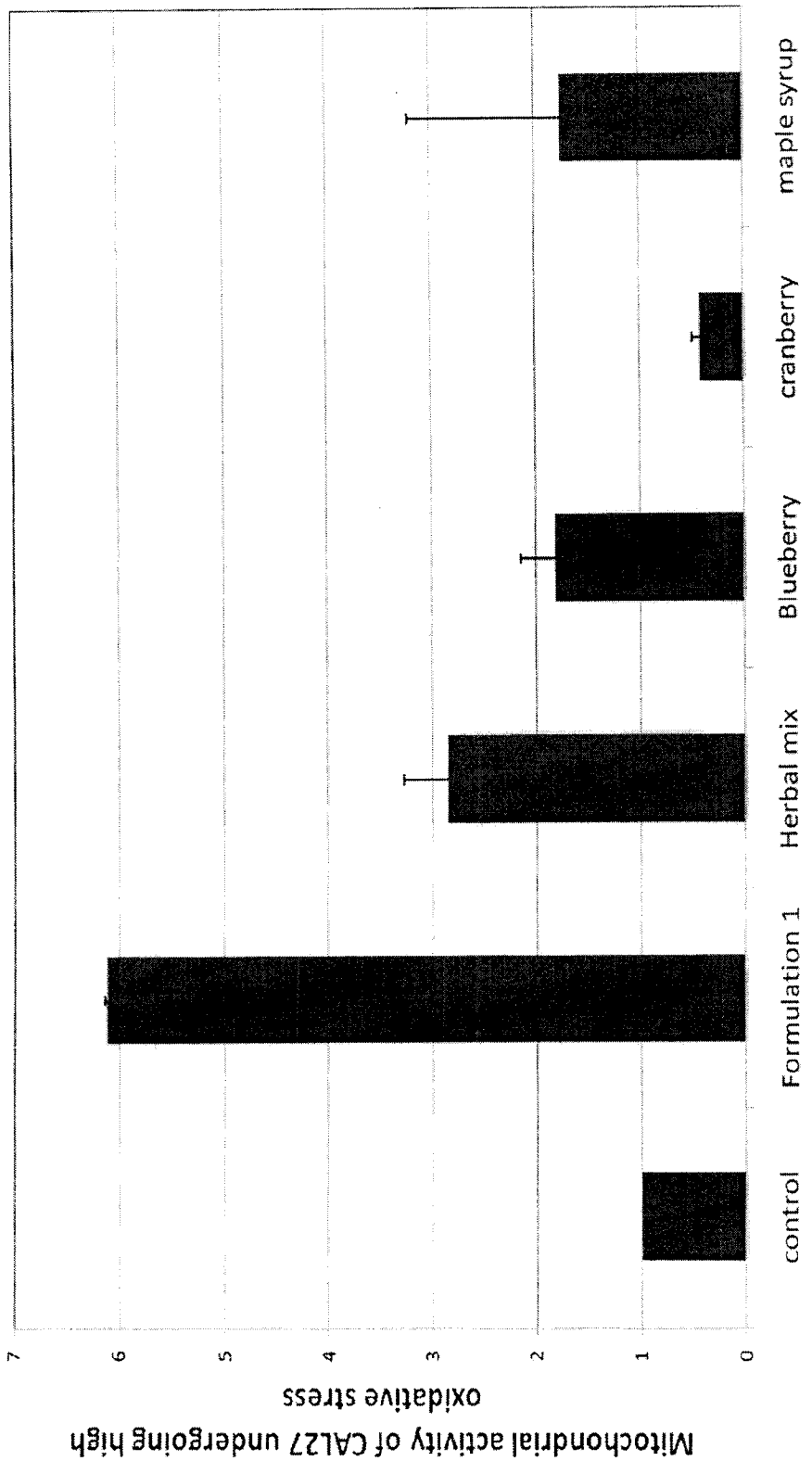

Mitochondrial activity of CAL27 undergoing high oxidative stress
0
1
2
3
4
5
6
7
control
Formulation 1
Herbal mix
Blueberry
cranberry
maple syrup

FIGURE 33:

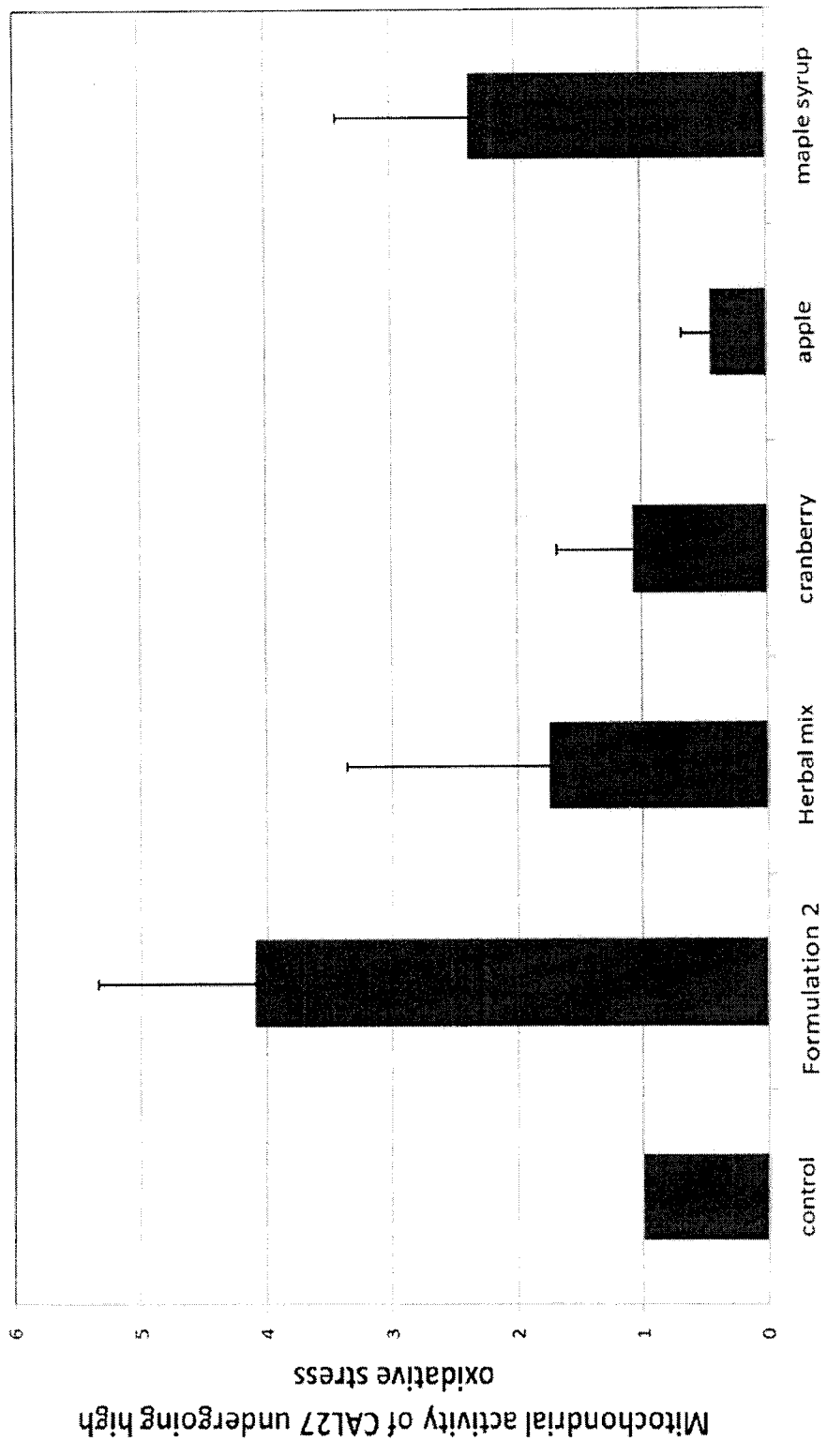

Mitochondrial activity of CAL27 undergoing high oxidative stress
0
1
2
3
4
5
6
control
Formulation 2
Herbal mix
cranberry
apple
maple syrup

FIGURE 34:

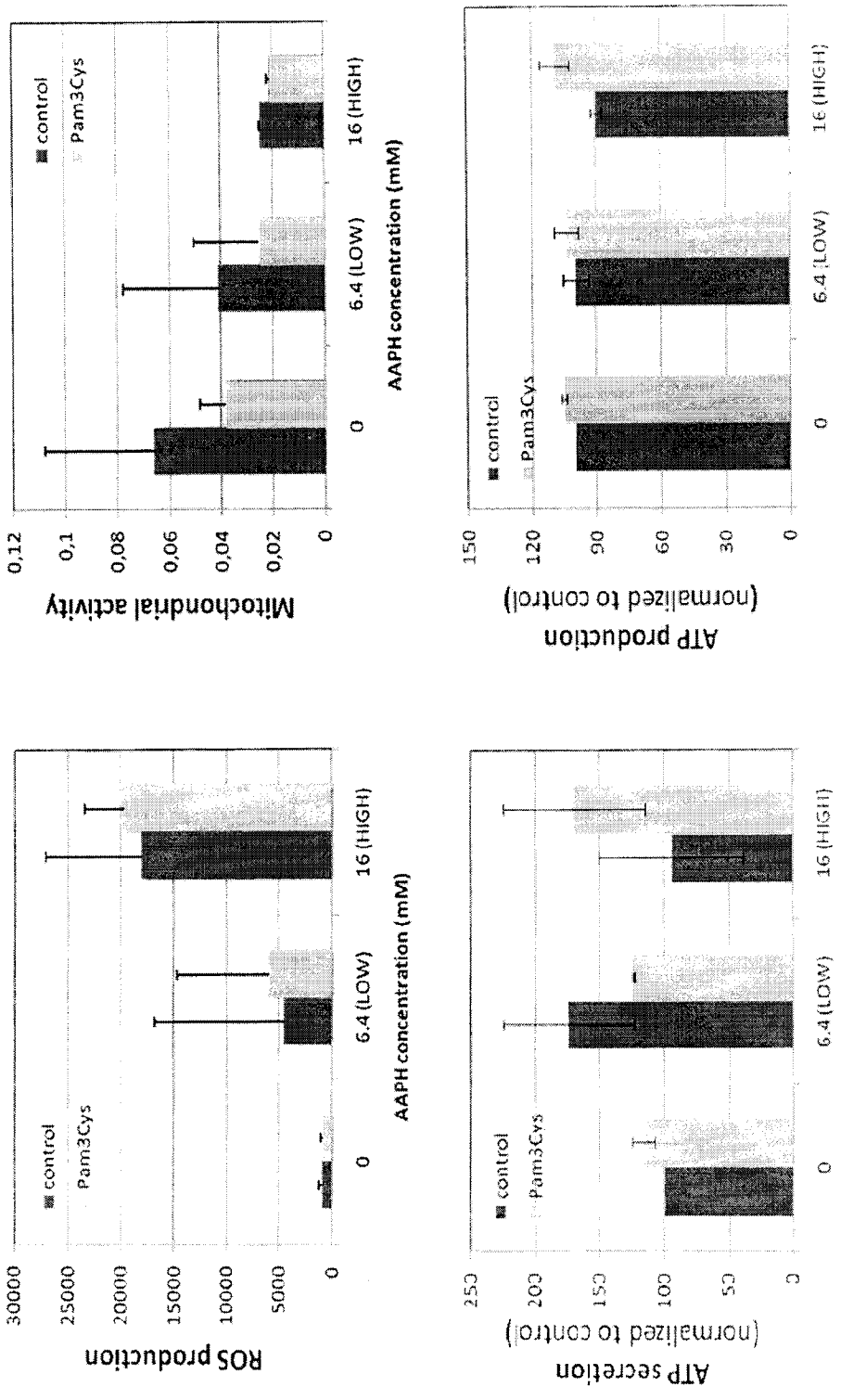

ROS production
ATP secretion (normalized to control)
Mitochondrial activity
ATP production (normalized to control)
control
Pam3Cys
AAPH concentration (mM)
0
6.4 (LOW)
16 (HIGH)

FIGURE 35:

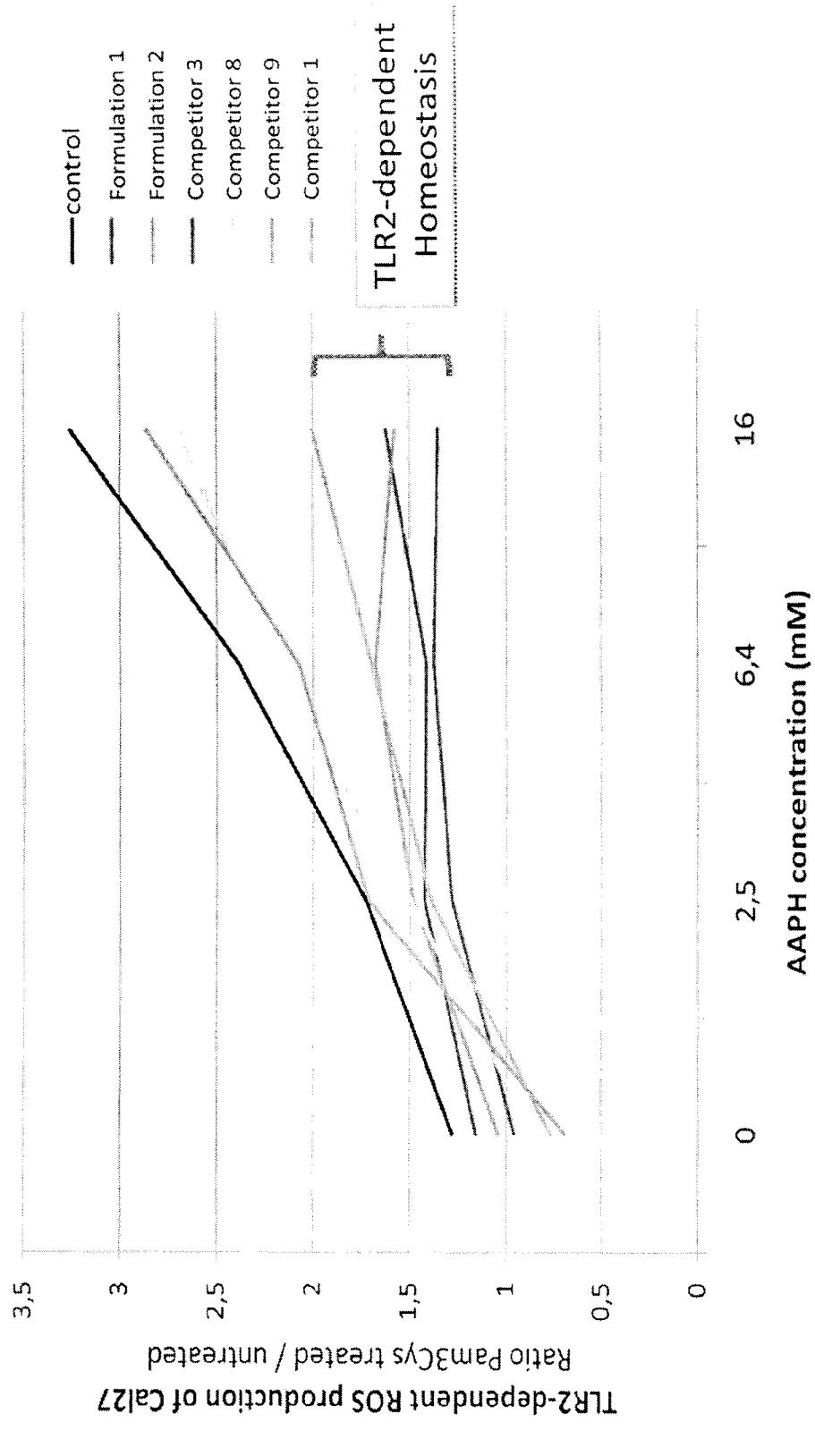

TLR2-dependent ROS production of Cal27
Ratio Pam3Cys treated / untreated
3,5
3
2,5
2
1,5
1
0,5
0
0
2,5
6,4
16
AAPH concentration (mM)
control
Formulation 1
Formulation 2
Competitor 3
Competitor 8
Competitor 9
Competitor 1
TLR2-dependent
Homeostasis

FIGURE 36:

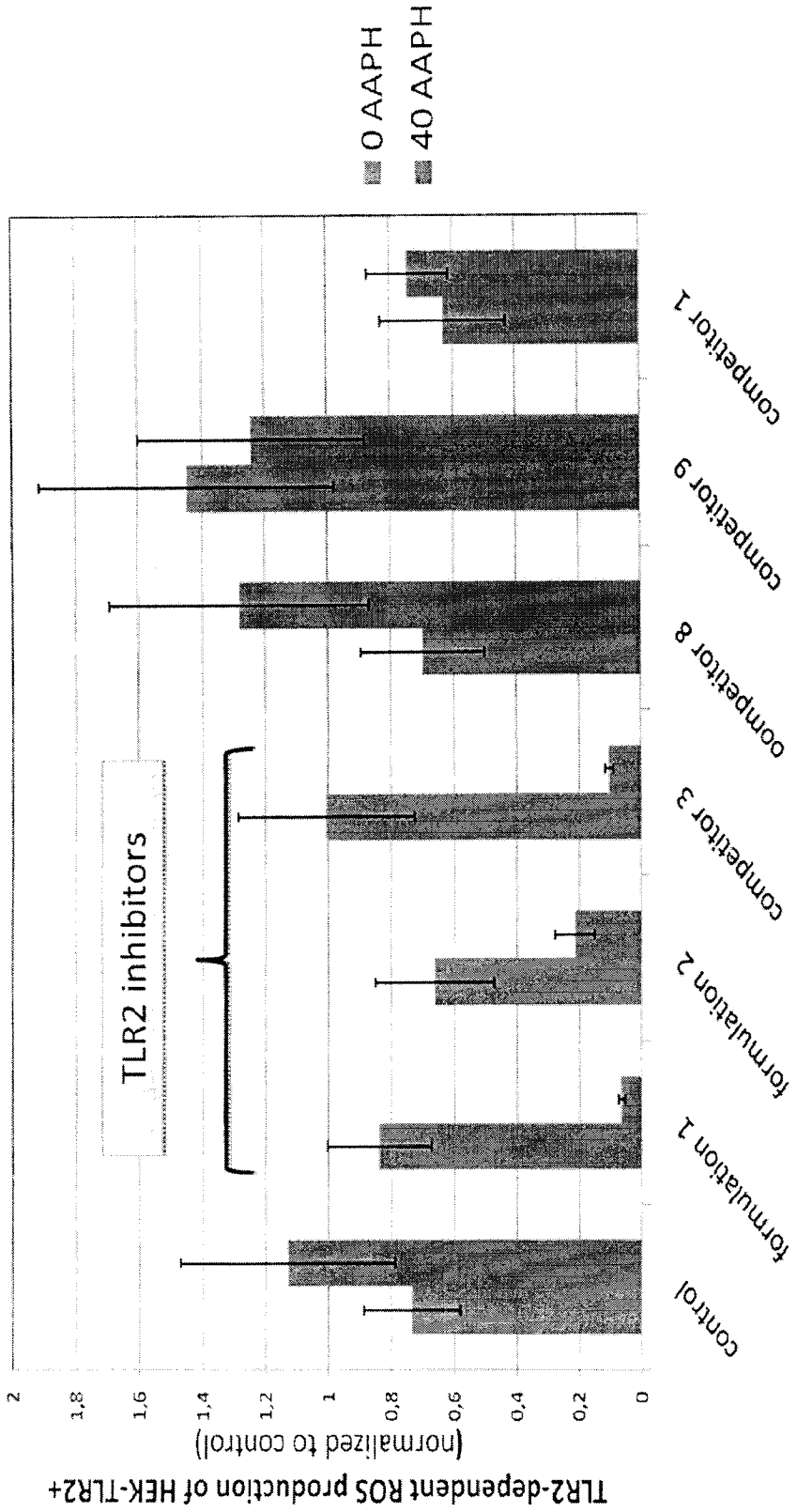

TLR2-dependent ROS production of HEK-TLR2+
(normalized to control)
2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0 AAPH
40 AAPH
TLR2 inhibitors
control
formulation 1
formulation 2
competitor 3
competitor 8
competitor 9
competitor 1

FIGURE 37:

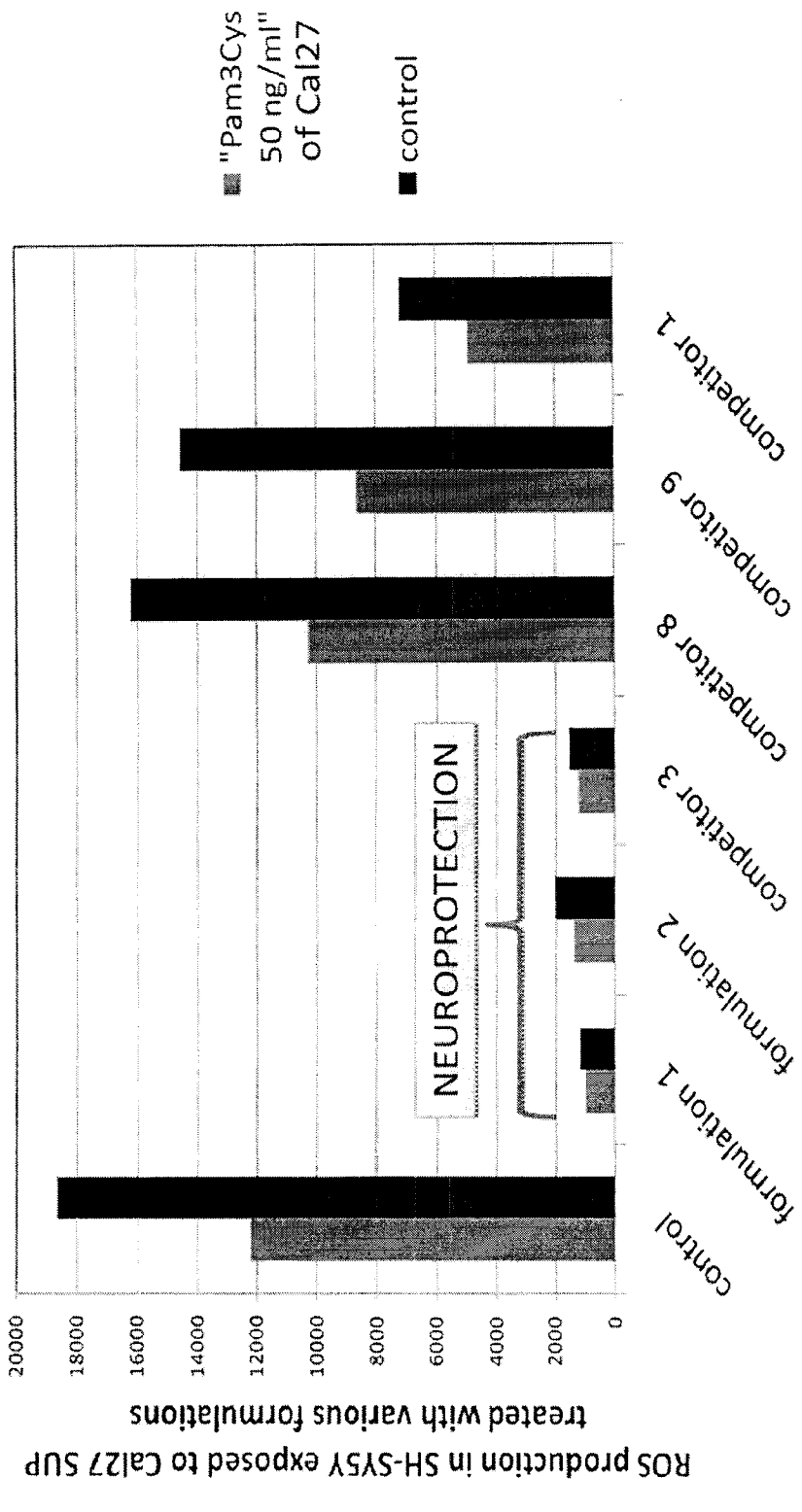

ROS production in SH-SY5Y exposed to Cal27 SUP
treated with various formulations
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
control
formulation 1
formulation 2
competitor 3
competitor 8
competitor 9
competitor 1
NEUROPROTECTION
control
"Pam3Cys 50 ng/ml" of Cal27

FIGURE 38:

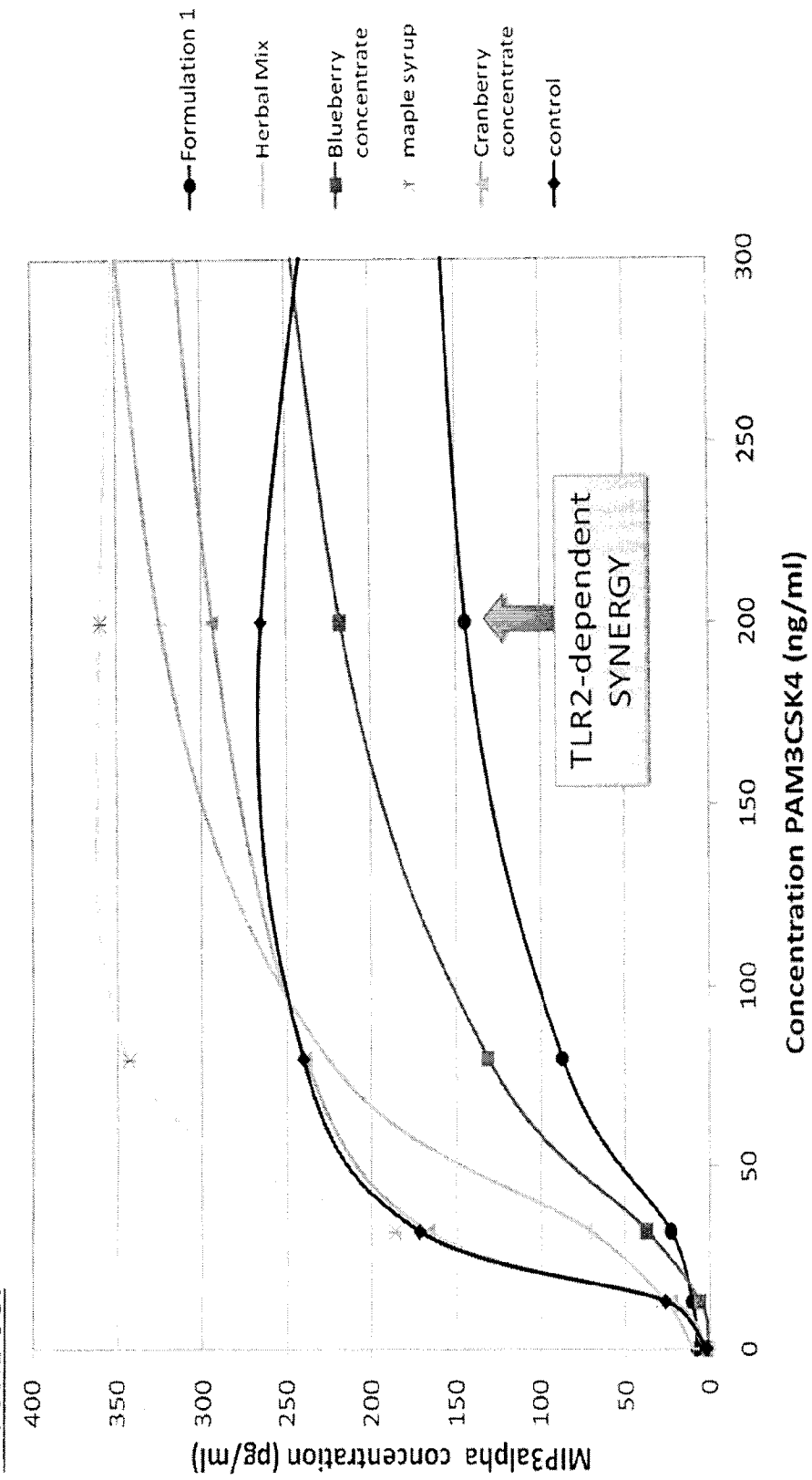

MIP3alpha concentration (pg/ml)
400
350
300
250
200
150
100
50
0
0
50
100
150
200
250
300
Concentration PAM3CSK4 (ng/ml)
Formulation 1
Herbal Mix
Blueberry concentrate
maple syrup
Cranberry concentrate
control
TLR2-dependent SYNERGY

FIGURE 39:

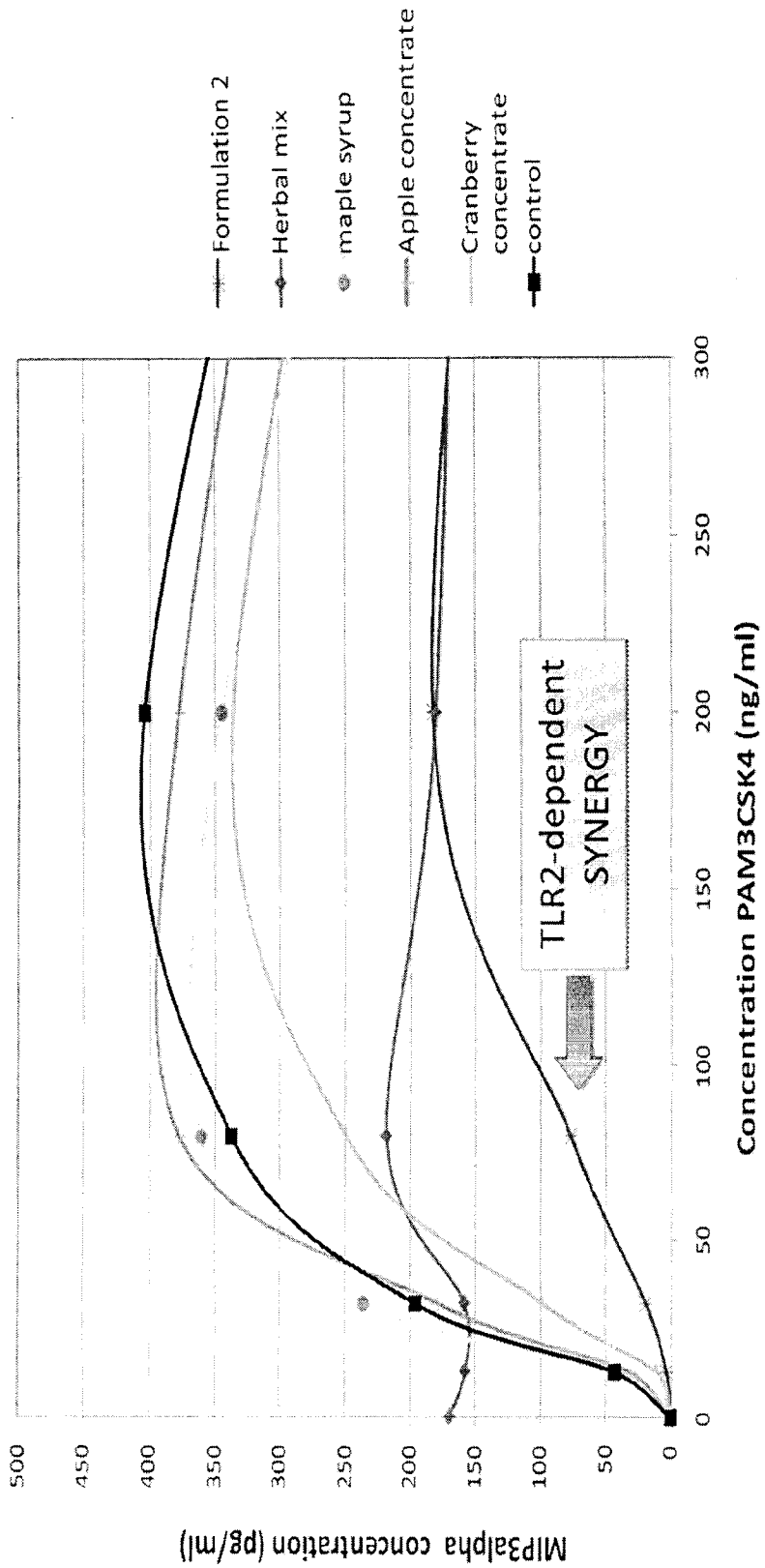

MIP3alpha concentration (pg/ml)
500
450
400
350
300
250
200
150
100
50
0
0
50
100
150
200
250
300
Concentration PAM3CSK4 (ng/ml)
TLR2-dependent SYNERGY
Formulation 2
Herbal mix
maple syrup
Apple concentrate
Cranberry concentrate
control

FIGURE 40:

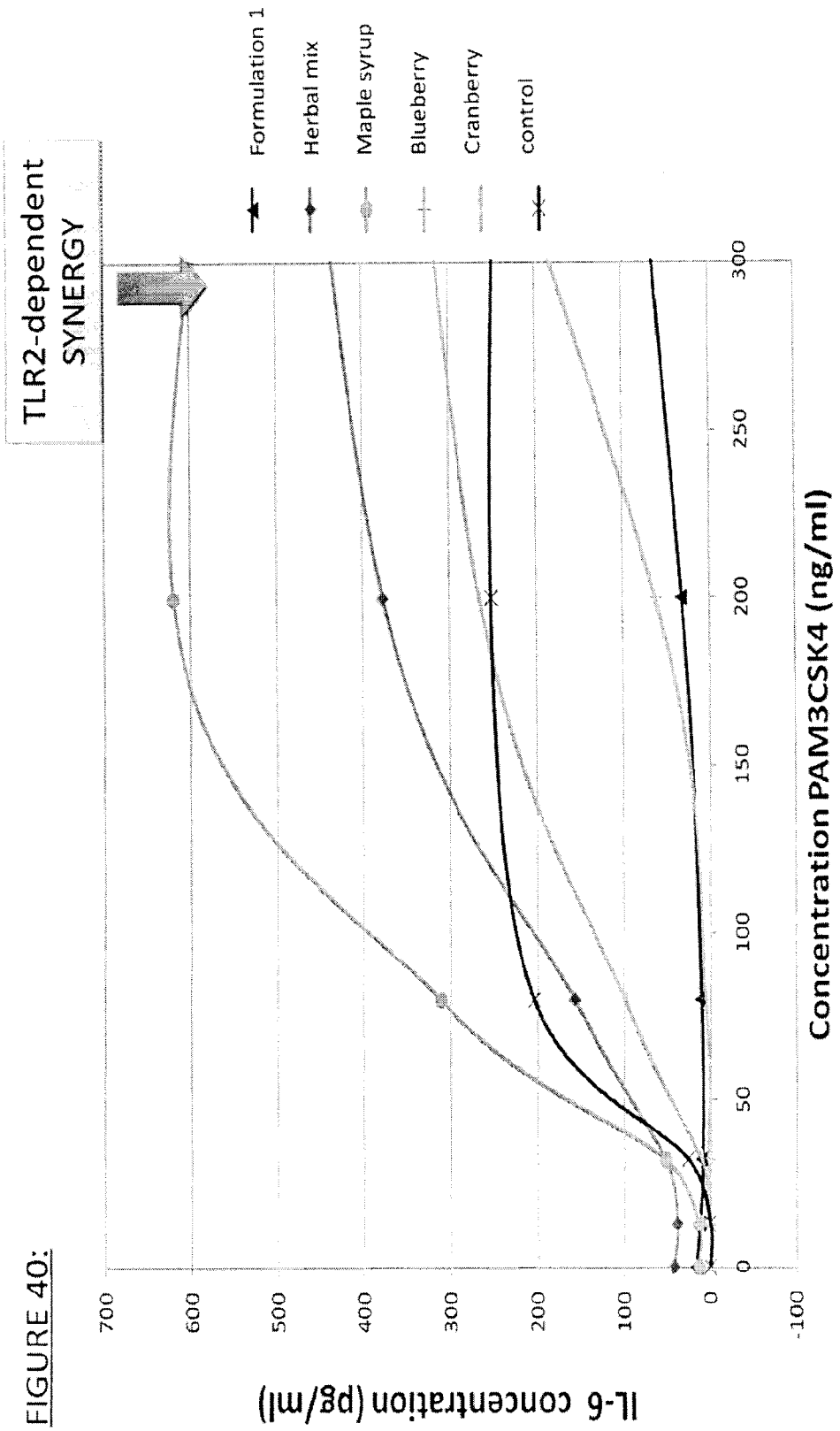

TLR2-dependent SYNERGY
Formulation 1
Herbal mix
Maple syrup
Blueberry
Cranberry
control
IL-6 concentration (pg/ml)
700
600
500
400
300
200
100
0
-100
0
50
100
150
200
250
300
Concentration PAM3CSK4 (ng/ml)

FIGURE 41:

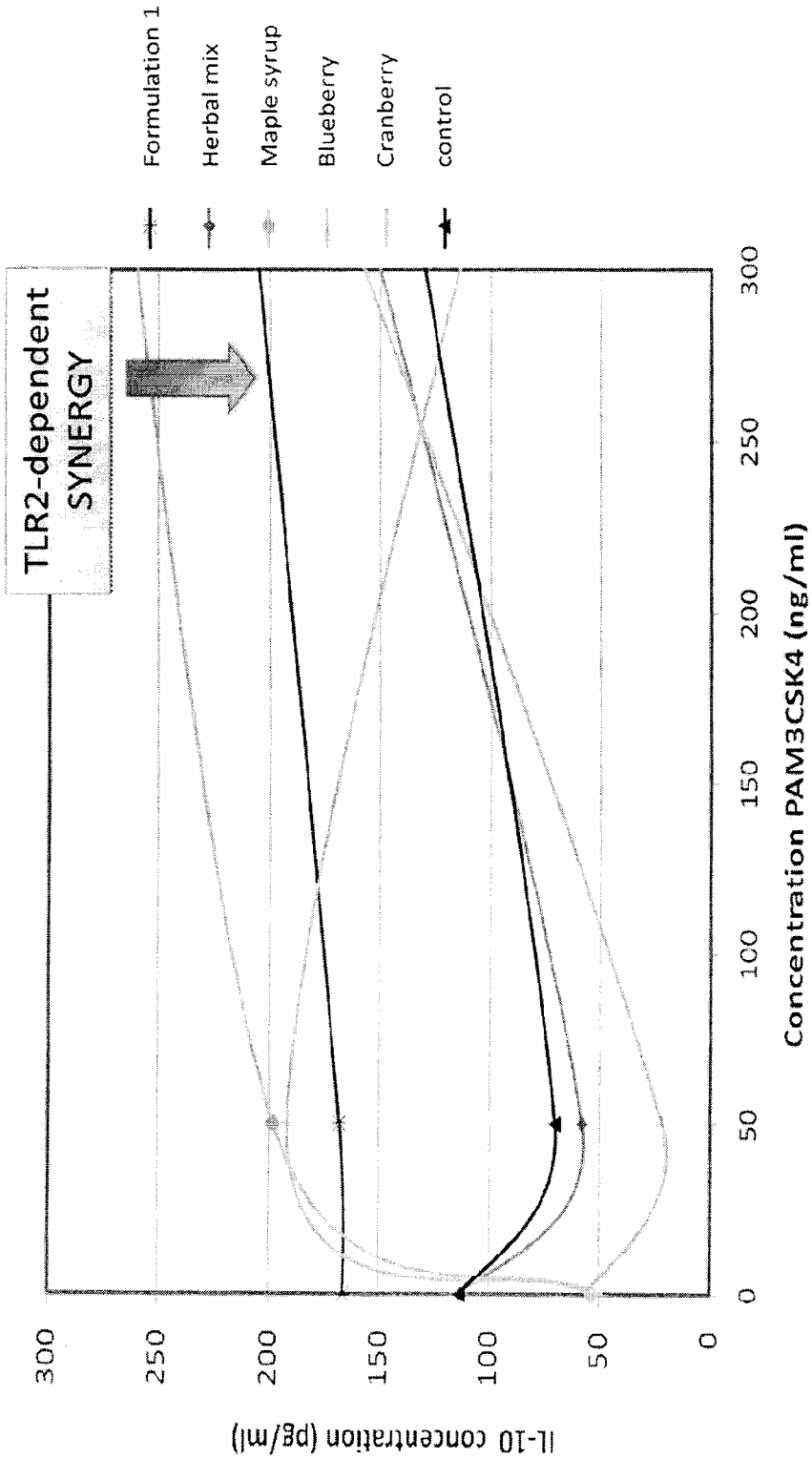
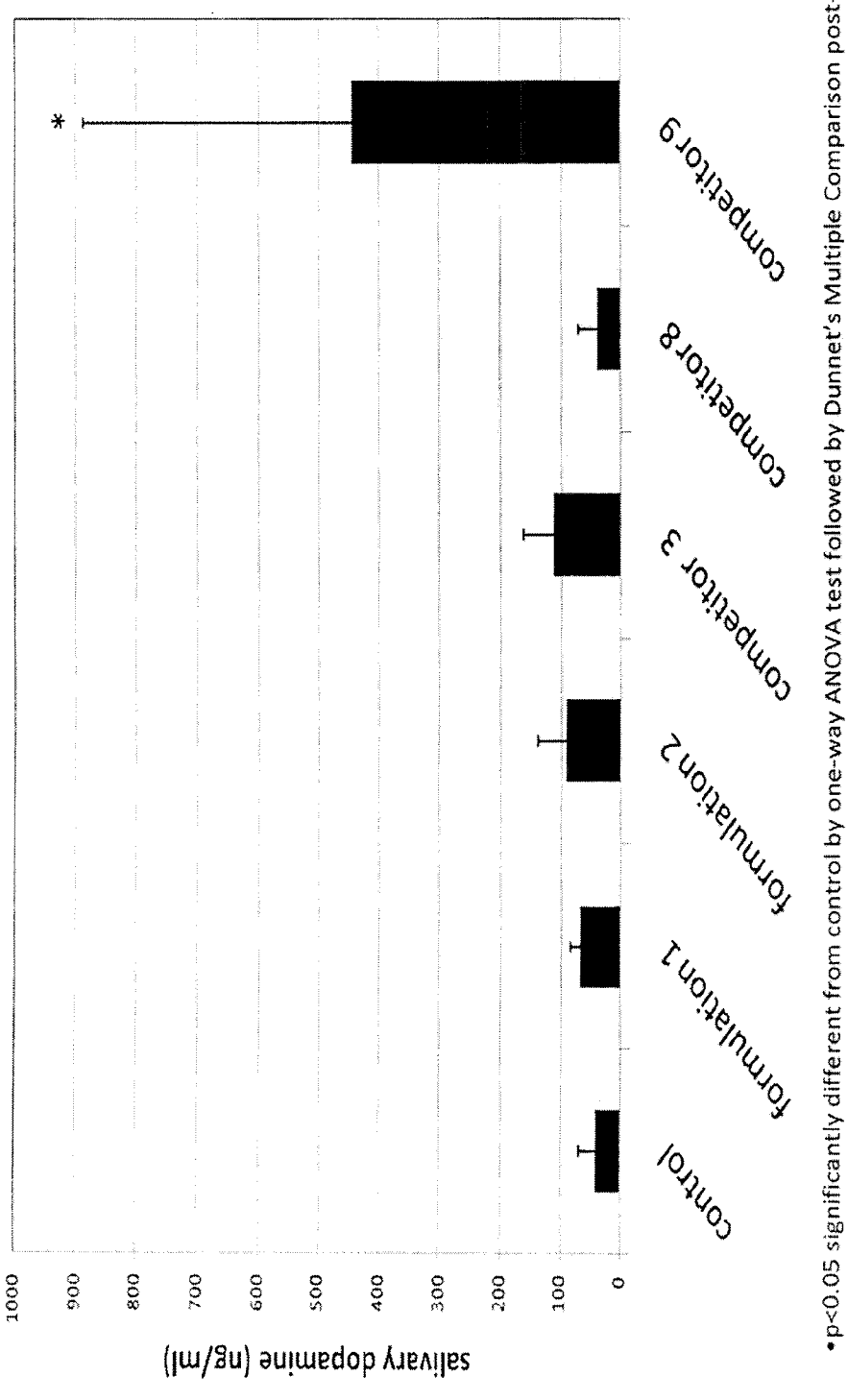

TLR2-dependent SYNERGY
Formulation 1
Herbal mix
Maple syrup
Blueberry
Cranberry
control
IL-10 concentration (pg/ml)
0
50
100
150
200
250
300
Concentration PAM3CSK4 (ng/ml)
0
50
100
150
200
250
300

FIGURE 42:


salivary dopamine (ng/ml)
1000
900
800
700
600
500
400
300
200
100
0
*
control
formulation 1
formulation 2
competitor 3
competitor 8
competitor 9
•p<0.05 significantly different from control by one-way ANOVA test followed by Dunnet's Multiple Comparison post-test

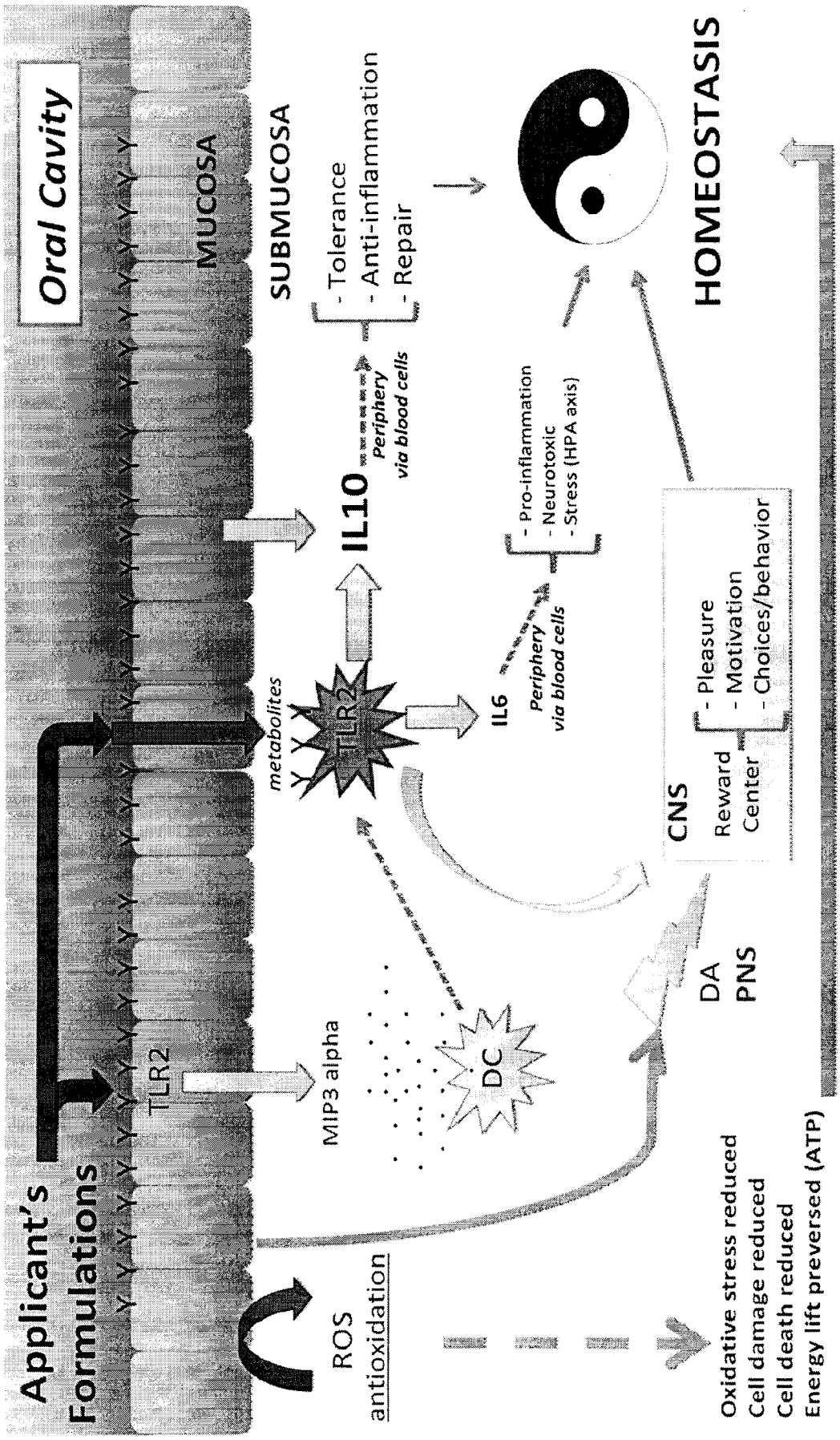

Oral Cavity
MUCOSA
SUBMUCOSA
Applicant's Formulations
TLR2
MIP3 alpha
DC
ROS
antioxidation
metabolites
TLR2
IL10
Periphery
via blood cells
- Tolerance
- Anti-inflammation
- Repair
IL6
Periphery
via blood cells
- Pro-inflammation
- Neurotoxic
- Stress (HPA axis)
CNS
Reward Center
- Pleasure
- Motivation
- Choices/behavior
DA
PNS
HOMEOSTASIS
Oxidative stress reduced
Cell damage reduced
Cell death reduced
Energy lift preversed (ATP)

FIGURE 43

FIGURE 44

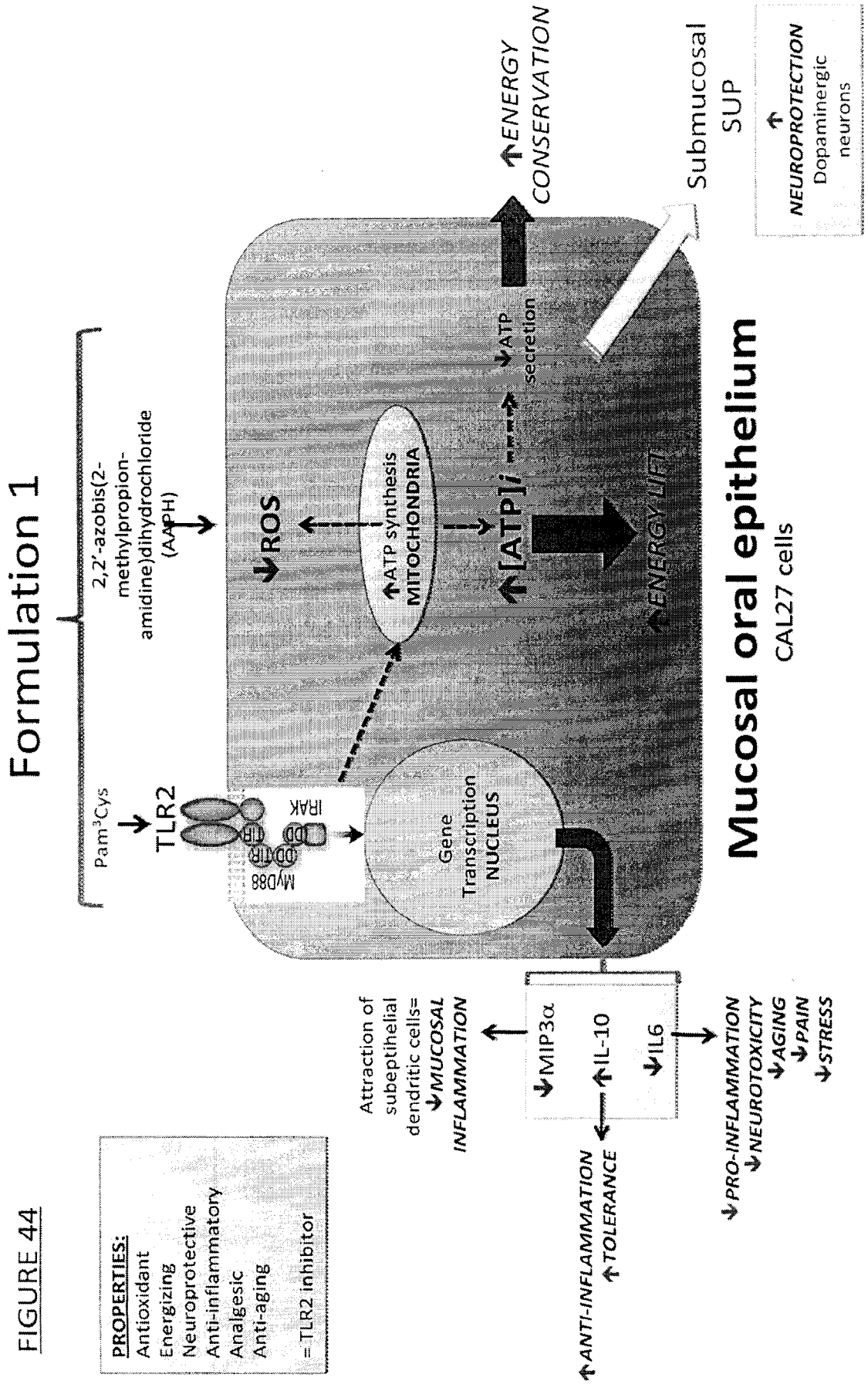

Formulation 1
2,2'-azobis(2-methylpropion-amidine)dihydrochloride (AAPH)
Pam3Cys
TLR2
MyD88
IRAK
ROS
ATP synthesis MITOCHONDRIA
[ATP]i
ATP secretion
ENERGY CONSERVATION
ENERGY LIFT
Gene Transcription NUCLEUS
Mucosal oral epithelium
CAL27 cells
Submucosal SUP
NEUROPROTECTION
Dopaminergic neurons
PROPERTIES:
Antioxidant
Energizing
Neuroprotective
Anti-inflammatory
Analgesic
Anti-aging
= TLR2 inhibitor
Attraction of subepithelial dendritic cells= MUCOSAL INFLAMMATION
MIP3α
IL-10
IL6
ANTI-INFLAMMATION
TOLERANCE
PRO-INFLAMMATION
NEUROTOXICITY
AGING
PAIN
STRESS

FIGURE 45

SUMMARY of RESULTS

BEVERAGE	ANTIOXYDANT (anti-ROS) 1=low antioxydant 10= high antioxydant	Energy LIFT	Energy DEPLETION	Energy CONSERVATION	Mucosal TLR2 regulation	NEURO-protection dopaminergic system	CALMING effect	Mucosal TOXICITY (Cell death)
Formulation 1	10 time and dose-dependent	yes	no	yes	yes	yes	yes	no
Formulation 2	7 time and dose-dependent	yes	no	yes	yes	yes	no	no
Competitor 8	2	no	no	yes	no	no	no	no
Competitor 9	1	no	yes	no	no	no	no	Yes
Competitor 3	8 Not time and dose-dependent	no	no	yes	yes	yes	no	no
Competitor 1	5	yes	no	yes	slight	no	no	no
Competitor 2	1	no	no	yes	no	no	no	no

+	-

FIGURE 46

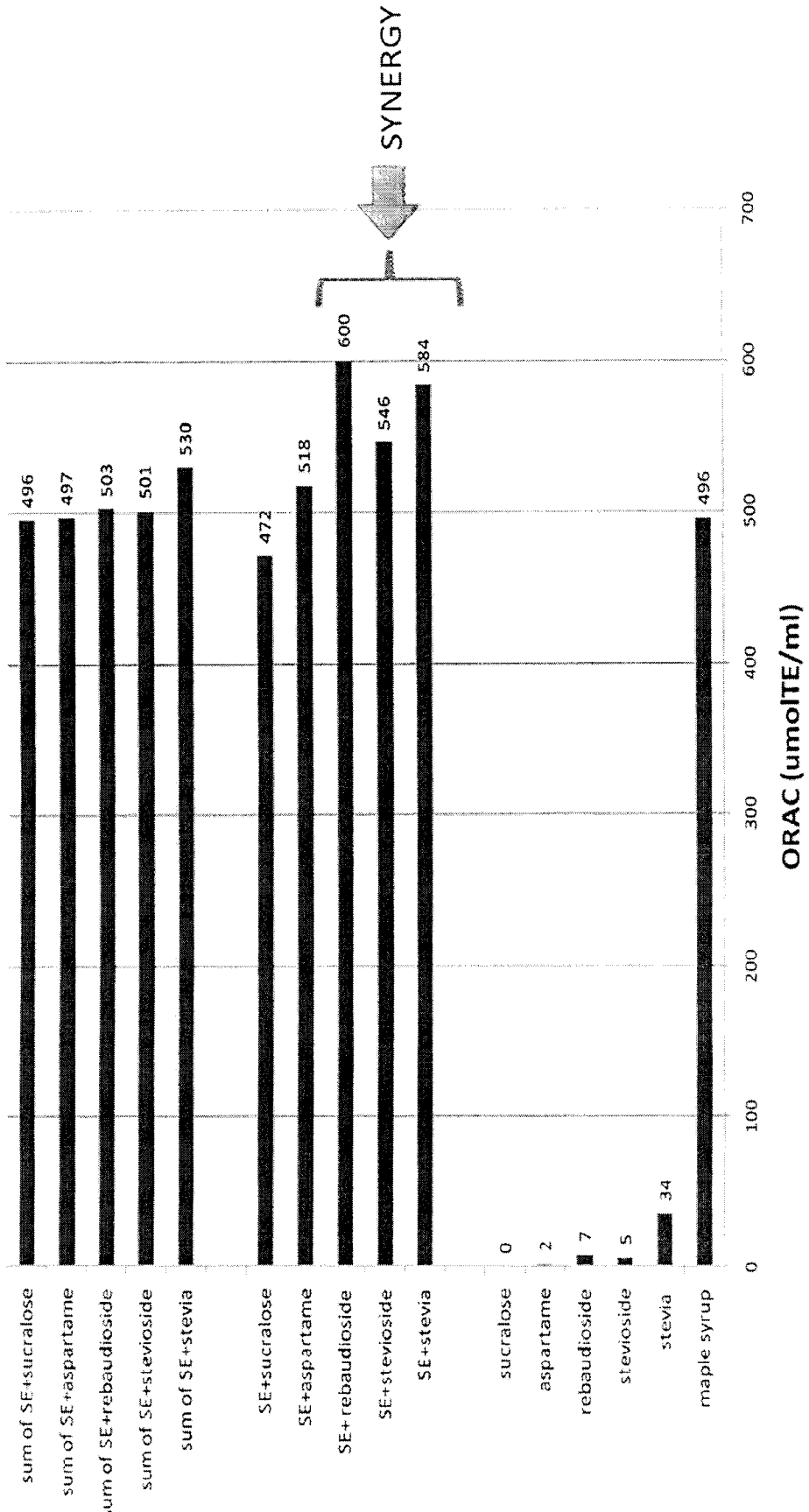
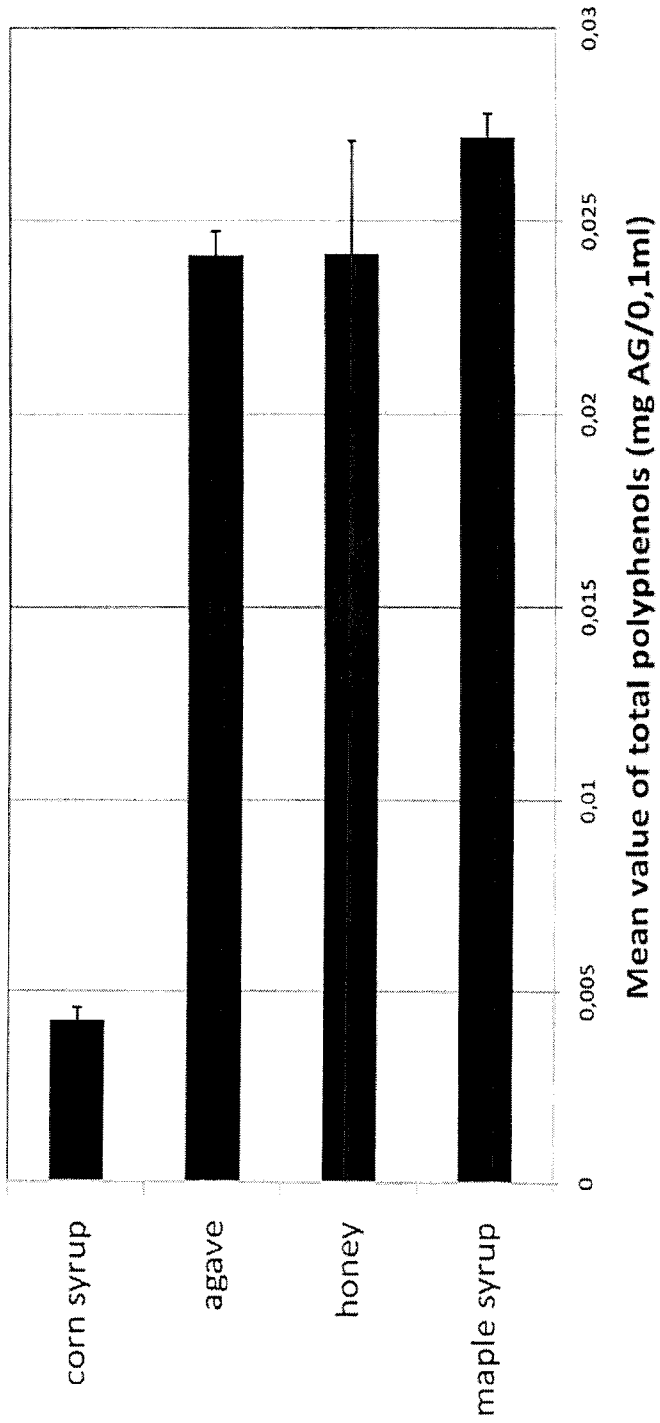

SYNERGY
ORAC (umolTE/ml)
0
100
200
300
400
500
600
700
sum of SE+sucralose
sum of SE+aspartame
sum of SE+rebaudioside
sum of SE+stevioside
sum of SE+stevia
SE+sucralose
SE+aspartame
SE+rebaudioside
SE+stevioside
SE+stevia
sucralose
aspartame
rebaudioside
stevioside
stevia
maple syrup
496
497
503
501
530
472
518
600
546
584
0
2
7
5
34
496

Figure 47


Mean value of total polyphenols (mg AG/0,1ml)
0
0,005
0,01
0,015
0,02
0,025
0,03
corn syrup
agave
honey
maple syrup

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2012/000090

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 36/77*** (2006.01) , ***A61K 36/00*** (2006.01) , ***A61K 36/28*** (2006.01) , ***A61K 36/45*** (2006.01) , ***A61K 36/53*** (2006.01) , ***A61K 36/539*** (2006.01) (more IPCs on extra sheet)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 36/77 (2006.01) , ***A61K 36/00*** (2006.01) , ***A61K 36/28*** (2006.01) , ***A61K 36/45*** (2006.01) , ***A61K 36/53*** (2006.01) , ***A61K 36/539*** (2006.01), ***A61K 36/73*** (2006.01) , ***A61K 36/734*** (2006.01) , ***A61P 29/00*** (2006.01) , ***A61P 3/02*** (2006.01) ,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

EPODOC, TotalPatent, SCIRUS, Pubmed, Canadian Patent Database. Keywords: (see extra sheet)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US2005/0048143 A1 (McAnalley BH et al.) 3 March 2005 (03-03-2005) *abstract, Example 2, Figures 1 & 4*	55 1-32, 35-36, 38-39 and 51-52
Y	US2010/0021533 A1 (Mazed MA et al.) 28 January 2011 (28-01-2011) *abstract; Example 5*	1-32
Y	US6299925 B1 (Xiong W et al.) 9 October 2001 (09-10-2001) *abstract; page 3, lines 6-12 and 25-28; Examples V-VIII*	1-32
X	WO2010/027344 A1 (Bauer JA et al.) 11 March 2010 (11-03-2010) *abstract; paragraphs [083-084]*	53-54

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

Date of the actual completion of the international search	Date of mailing of the international search report
May 29, 2012 (08-05-2012)	29 May 2012 (29-05-2012)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Sonja Kosuta (819) 994-9535

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2012/000090

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 33, 34, 37, 40, 43, 46 and 50

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 34, 37, 40, 43, 46 and 50 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search under Rule 39.1 (iv) of the PCT. However, a search has been carried out based on the alleged therapeutic effects of a composition having synergistic antioxidant properties.

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

Please see extra sheet.

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Leonarduzzi G et al. 21 August 2010 (21-08-2010) epub Targeting tissue oxidative damage by means of cell signaling modulators: the antioxidant concept revisited Pharmacol & Therapeutics 128(2):336-74, ISSN:0163-7258 *abstract*	35-36, 38-39 and 51-52
Y	Kanzler H et al. 3 May 2007 (03-05-2007) epub Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists Nature Medicine 13:552-559, EISSN:1546-170X *abstract*	35-36, 38-39 and 51-52

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2005048143A1	03 March 2005 (03-03-2005)	AU2004268233A1	10 March 2005 (10-03-2005)
		AU2004268233B2	22 July 2010 (22-07-2010)
		AU2006237559A1	26 October 2006 (26-10-2006)
		AU2006237559B2	11 August 2011 (11-08-2011)
		AU2006237559C1	15 December 2011 (15-12-2011)
		AU2009220028A1	15 October 2009 (15-10-2009)
		CA2533570A1	10 March 2005 (10-03-2005)
		CA2599759A1	26 October 2006 (26-10-2006)
		CA2741404A1	10 March 2005 (10-03-2005)
		CN101170999A	30 April 2008 (30-04-2008)
		DE10159748T1	19 May 2011 (19-05-2011)
		EP1664746A2	07 June 2006 (07-06-2006)
		EP1664746A4	21 January 2009 (21-01-2009)
		EP1868448A2	26 December 2007 (26-12-2007)
		EP2218453A1	18 August 2010 (18-08-2010)
		ES2355101T1	22 March 2011 (22-03-2011)
		IL185642D0	06 January 2008 (06-01-2008)
		JP2007504443A	01 March 2007 (01-03-2007)
		JP2008531728A	14 August 2008 (14-08-2008)
		JP2011067197A	07 April 2011 (07-04-2011)
		KR20070100390A	10 October 2007 (10-10-2007)
		KR100805474B1	20 February 2008 (20-02-2008)
		KR20060041313A	11 May 2006 (11-05-2006)
		KR100805521B1	20 February 2008 (20-02-2008)
		KR20070117630A	12 December 2007 (12-12-2007)
		MX2007010644A	13 March 2008 (13-03-2008)
		NO20060321A	24 March 2006 (24-03-2006)
		NZ545231A	29 January 2010 (29-01-2010)
		NZ561001A	31 March 2011 (31-03-2011)
		NZ577993A	25 February 2011 (25-02-2011)
		TWI344000B	21 June 2011 (21-06-2011)
		TW201102645A	16 January 2011 (16-01-2011)
		US7999003B2	16 August 2011 (16-08-2011)
		US2005087452A1	28 April 2005 (28-04-2005)
		US2005214413A1	29 September 2005 (29-09-2005)
		US2011256248A1	20 October 2011 (20-10-2011)
		WO2005022116A2	10 March 2005 (10-03-2005)
		WO2005022116A3	23 June 2005 (23-06-2005)
		WO2005022116A9	10 November 2005 (10-11-2005)
		WO2006112958A2	26 October 2006 (26-10-2006)
		WO2006112958A8	11 October 2007 (11-10-2007)
		WO2006112958A3	29 November 2007 (29-11-2007)
		ZA200601611A	30 May 2007 (30-05-2007)
		ZA200707439A	26 August 2009 (26-08-2009)
WO2010027344A1	11 March 2010 (11-03-2010)	AU2008361375A1	11 March 2010 (11-03-2010)
		CA2733249A1	11 March 2010 (11-03-2010)
		CN102143756A	03 August 2011 (03-08-2011)
		IL211041D0	28 April 2011 (28-04-2011)
		JP2012502026A	26 January 2012 (26-01-2012)
		MX2011002080A	29 March 2011 (29-03-2011)
US2010021533A1	28 January 2010 (28-01-2010)	US2010021533A1	28 January 2010 (28-01-2010)
		US8017147B2	13 September 2011 (13-09-2011)
		US2009252758A1	08 October 2009 (08-10-2009)
		US2009252796A1	08 October 2009 (08-10-2009)
		US2011274680A1	10 November 2011 (10-11-2011)
US6299925B1	09 October 2001 (09-10-2001)	AU5041000A	31 January 2001 (31-01-2001)
		CA2375979A1	04 January 2001 (04-01-2001)
		CN1358063A	10 July 2002 (10-07-2002)
		CN1217584C	07 September 2005 (07-09-2005)
		EP1196043A1	17 April 2002 (17-04-2002)
		EP1196043A4	05 November 2002 (05-11-2002)
		JP2003503324A	28 January 2003 (28-01-2003)
		WO0100038A1	04 January 2001 (04-01-2001)

Continued from Sheet 1, Box III:

The claims are directed to multiple inventions as follows:

Group 1 - Claims 1-52 are directed to a composition comprising two antioxidant plant concentrates with ORAC values of at least 2000 and 200, respectively, and an antioxidant natural sweetener with an ORAC value of at least 20, as well as therapeutic methods and uses comprising said composition;
Group 2 - Claim 53 is directed to a method of identifying a food or a food ingredient useful for maintaining homeostasis in a mammal comprising measuring the TLR activity of said food or food ingredient;
Group 3 - Claim 54 is directed to a transwell assay for the identification of TLR modulators; and
Group 4 - Claim 55 is directed to a method for the identification of a composition having synergistic antioxidant activity by comparing the antioxidant potential of each component individually with that of the composition.

The claims must be limited to one inventive concept as set out in Rule 13 of the PCT.

Continued from Sheet 2, Box A:

A61K 36/73 (2006.01) , ***A61K 36/734*** (2006.01) , ***A61P 29/00*** (2006.01) , ***A61P 3/02*** (2006.01) ,
A61P 39/06 (2006.01) , ***C12Q 1/02*** (2006.01) , ***G01N 33/48*** (2006.01)

Continued from Sheet 2, Box B:

key words: synergy, synergistically, homeostasis, functional food, antioxidant, ORAC, ORAC value, herbal extract, lemon balm, scutellaria, hawthorn, yarrow, thyme, hyssop, tea, extract, apple, blueberry, cranberry, raspberry, maple syrup, fruit extract, natural sweetener, honey, coconut sugar, agave, corn syrup, stevia, how to calculate ORAC values, TLR, immunity, inflammation, food, activity, modulat*

(((basil OR dill OR marjoram OR oregano OR peppermint OR savory OR cardamon OR chili OR cinnamon OR cloves OR cumin OR curry OR garlic OR ginger OR juniper OR mustard OR nutmeg OR onion OR paprika OR parsley OR pepper OR (poppy w seed) OR rosemary OR turmeric OR vanilla OR tarragon OR ((black OR green OR white OR labrador) w tea) OR rice OR bran OR oat OR sorghum OR dandelion OR bilberry OR galega OR echinacea OR nettle OR sage OR thyme OR lavender OR hawthorn OR verbena OR rosemary OR (lemon w balm) OR peppermint OR marshmellow OR raspberry OR astragalus OR fennel OR skullcaps OR yarrow OR hyssop OR motherwort OR angelica OR (winter w cherry) OR fenugreek)