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(54) Title: COMPOSITIONS FOR IMPROVED NRF2 ACTIVATION AND METHODS OF THEIR USE

(57) Abstract: Disclosed here are compositions and methods for preventing or treating certain health conditions associated with inflammation or oxidative stress. These compositions are prepared from ingredients containing phytochemicals that activate the Nrf2 pathways. Synergistic effects of the different phytochemicals are also disclosed.



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## COMPOSITIONS FOR IMPROVED NRF2 ACTIVATION AND METHODS OF THEIR USE

### RELATED APPLICATIONS

[0001] This application claims priority to U.S. Patent application 62/214,175 filed September 3, 2015, and U.S. Patent application 62/355,810 filed June 28, 2016, the entire content of which is hereby incorporated by reference into this application.

### BACKGROUND

#### I. Field of the Invention

[0002] The present disclosure relates to methods and compositions for preventing or treating certain health conditions. More particularly, the present disclosure relates to compositions and methods for preventing or treating certain health conditions associated with inflammation and/or oxidative stress.

#### II. Description of the Related Art

[0003] Nuclear factor-erythroid 2 related factor 2 (Nrf2) is a transcription factor that is regulated by Kelch-like ECH-Associated Protein 1 (Keap1). Nrf2 regulates gene expression of a wide variety of cytoprotective phase II detoxification enzymes and antioxidant enzymes through an enhancer sequence known as the antioxidant-responsive element (ARE) (Maher and Yamamoto 2010, Satoh, Moriguchi et al. 2010). Relevant to oxidative stress, the ARE is a promoter element found in many antioxidant enzymes, including superoxide dismutase (SOD), peroxiredoxins, thioredoxins, catalase, glutathione peroxidase, and heme oxygenase-1 (HO-1). Nrf2 plays a pivotal role in the ARE-driven cellular defense system against oxidative stress. *See*, Kensler, Wakabayashi et al. 2010; Hybertson and Gao 2014, Bocci and Valacchi 2015, Huang, Li et al. 2015, Johnson and Johnson 2015, Moon and Giaccia 2015, Petiwala and Johnson 2015, Sekhar and Freeman 2015, Suzuki and Yamamoto 2015.

### SUMMARY

[0004] The presently disclosed instrumentalities advance the art by providing combinations of agents that activate the Nrf2 cell signaling pathway. In one

embodiment, the combinations of agents may activate the Nrf2 pathway more effectively than individual agents. . In another embodiment, the combinations of agents may activate the Nrf2 pathway synergistically.

**[0005]** In one embodiment, combinations of more than one ingredients are disclosed here. In one aspect, each ingredient may contain one or more phytochemicals. In another aspect, these phytochemicals may be found in rosemary (*Rosmarinus officinalis*), ginger (*Zingiber officinale*), luteolin (from *Sophora Japonica*), milk thistle (*Silybum marianum*), and bacopa (*Bacopa monnieri*). In another aspect, the phytochemicals components are carnosol, shogaol, luteolin, silymarin, and bacosides, which may be found in rosemary, ginger, luteolin, milk thistle, and bacopa, respectively. In another aspect, , the disclosed compositions induce ARE-regulated antioxidant genes by the Nrf2-dependent pathway.

**[0006]** In another embodiment, specific combinations of rosemary, ashwagandha, and luteolin (referred to herein as PB125), specific combinations of rosemary, ginger, luteolin, and silymarin (referred to herein as PB127), and specific combinations of rosemary, ginger, luteolin, silymarin, and bacopa (referred to herein as PB129) are disclosed. In another embodiment, the combination of these agents may result in a synergistic Nrf2 activation, greater than simply the sum of their individual Nrf2 activation contributions. The active agents or combinations of the agents may be candidates for possible drug development. *See, e.g.,* Koehn and Carter 2005, Lee 2010.

**[0007]** In another embodiment, the disclosed compositions may contain rosemary (carnosol), ginger (6-shogaol and 6-gingerol), ashwagandha (withaferin A), milk thistle (silymarin), bacopa monnieri (bacosides) and luteolin.

**[0008]** In one aspect, the compositions may be administered orally, for example in the form of a tablet, capsule, softgel, syrup, aqueous solution or suspension, alcohol-extract, or powder. In another aspect, the synergistic compositions may be administered in the form of aerosol, for example to the lungs in the form of a fine aerosol mist or powder which is inhaled and partially deposited within the lung airways. In another aspect, the disclosed compositions may be administered by local administration, for example, by applying to the skin in the form

of a lotion, gel, ointment, aqueous spray, or within a bandage applied to the skin or to a wound.

**[0009]** In another embodiment, the disclosed composition may contain a combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), and luteolin (specified at 95-98% luteolin), in the mass ratio of 10:5:1, respectively. This formula is also referred to as PB123 in this disclosure.

**[0010]** In another embodiment, the disclosed composition may contain a combination of rosemary extract (specified at 5 to 10% carnosol), ashwagandha extract (specified at 1-3% withaferin A), and luteolin (specified at 95-98% luteolin), in the mass ratio of 30:10:4, respectively. This formula is also referred to as PB125 in this disclosure.

**[0011]** In another embodiment, the disclosed composition may contain a combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin), and milk thistle extract (specified at 50-90% silymarin), in the mass ratio of 10:5:1:30, respectively. This formula is also referred to as PB127 in this disclosure.

**[0012]** In another embodiment, the disclosed composition may contain a combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin), milk thistle extract (specified at 50-90% silymarin), and bacopa monnieri extract (specified at 10-60% bacosides) in the mass ratio of 10:5:1:30:48, respectively. This formula is also referred to as PB129 in this disclosure.

**[0013]** In another embodiment, the disclosed composition may contain a combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin), and bacopa monnieri extract (specified at 10-60% bacosides) in the mass ratio of 10:5:1:48, respectively. This formula is also referred to as PB131 in this disclosure.

**[0014]** In another embodiment, PB123 may be administered at 10 to 1000 mg per day as an oral administration to a human. For example, it may be administered

as a pill, softgel, or capsule to induce Nrf2 activation, and/or to reduce inflammation and oxidative stress, and/or to improve overall health and wellness.

**[0015]** In another embodiment, PB123 may be administered at 10 to 1000 mg per day as an oral administration to a human to improve protein homeostasis, and/or to prevent aging-related problems associated with protein homeostasis and/or autophagy in humans.

**[0016]** In another embodiment, PB125 or PB127 or PB129 or PB131 may be administered at 10 to 1000 mg per day as an oral administration to a human. For example, it may be administered as a pill, softgel, or capsule to induce Nrf2 activation, and/or to reduce inflammation and oxidative stress, and/or to improve overall health and wellness.

**[0017]** In another embodiment, PB125 or PB127 or PB129 or PB131 may be administered at 10 to 1000 mg per day as an oral administration to a human to improve protein homeostasis, and/or to prevent aging-related problems associated with protein homeostasis and/or autophagy in humans.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

**[0018]** Figure 1 shows the Nrf2 activation pathways and control points.

**[0019]** Figure 2 shows the “Shutdown Pathway”-Fyn-dependent deactivation of nuclear Nrf2.

**[0020]** Figure 3 shows the “Positive Feedback Loop”-Keap1 degradation by Nrf2-induced gene products.

**[0021]** Figure 4 shows Nrf2 activation induced by PB123, PB125, PB127, PB129, and PB131 in a transfected breast cancer cell line.

**[0022]** Figure 5 shows Nrf2 activation induced by PB123, PB125, PB127, PB129, and PB131 in a transfected liver cancer cell line.

**[0023]** Figure 6A-6C shows the synergistic effect of Nrf2 activation induced by PB129 in HepG2 (human liver, Fig. 6A), MCF7 (human breast, Fig. 6B), and A172 (human brain, Fig. 6C) cancer cell lines.

**[0024]** Figure 7A-7C shows the synergistic effect of Nrf2 activation induced by PB127 in HepG2 (human liver, Fig. 7A), MCF7 (human breast, Fig. 7B), and A172 (human brain, Fig. 7C) cancer cell lines.

[0025] Figure 8 shows increase of Mouse Liver HMOX1 gene expression in vivo.

[0026] Figure 9 shows Liver Catalase Activity Induced by PB125 in diet.

[0027] Figure 10 shows overlay of relative light units (RLU) observed with added luciferin after ARE-driven luciferase gene expression was induced by treatment with PB125 in stably transfected HepG2 (human liver), AREc32 (human breast), MCF7 (human breast), A549 (human lung), 293T (human kidney), and A172 (human brain) cancer cell lines. Strong Nrf2 activation was observed in liver, kidney, and breast cell lines by 5, 10, 15, 20, and 25 micrograms of PB125 per mL of culture solution.

[0028] Figure 11 shows that PB125 decreases LPS-induced expression of inflammatory genes.

[0029] Figure 12 shows that PB125 decreases LPS-induced expression of IL-6.

[0030] Figure 13 shows higher GCLM gene expression as a result of PB125 administration.

#### DETAILED DESCRIPTION

[0031] The Nrf2/ARE pathway has been implicated in the control of oxidative stress (Eggler, Gay et al. 2008, Cho and Kleeberger 2010, Huang, Li et al. 2015, Johnson and Johnson 2015). Certain agents and combinations of such agents (e.g., PB125) that target the Nrf2/ARE pathway may have beneficial effects on cellular function and survival. In one embodiment, these agents and combinations thereof may alleviate inflammatory responses and oxidative stress, and may have beneficial effects on health and wellness.

[0032] Prior studies have failed to demonstrate the therapeutic potential of direct antioxidant vitamins or supplements such as vitamins C and E, carotenoids, N-acetylcysteine, and other compounds that react stoichiometrically with reactive oxygen species (ROS) such as superoxide and hydrogen peroxide. Here, an improved antioxidant defenses is demonstrated by using Nrf2 activating combinations (Koehn 2006, Eggler, Gay et al. 2008, Boutten, Goven et al. 2010, Cho and Kleeberger 2010).

**[0033]** In the present disclosure, a multiplicity of agents were combined in a novel way, i.e., by acting at different control points in the Nrf2 activation pathway. Figure 1 shows Nrf2 activation pathways and control points A, B, C, D, and E at which low concentrations of agents that act at those control points work together to effect desired Nrf2-dependent gene expression by combinations such as PB125, PB127, and PB129. In the basal state Nrf2 is sequestered and kept inactive by Kelch-like ECH-associated protein 1 (Keap1), which targets Nrf2 for polyubiquitination and degradation by the proteasome. A. Nrf2 activation involves oxidation of specific thiol residues of Keap1, causing it to Nrf2 to be released from Keap1. B. Nrf2 phosphorylation may play a role in targeting it for nuclear import. C. Nrf2 translocation into the nucleus enables Nrf2 to bind promoters containing the Antioxidant Response Element (ARE), initiating transcription of cytoprotective programming. D. Inactive cytosolic Fyn may be phosphorylated by GSK3 $\beta$ , and this now active p-Fyn translocates to the nucleus, where it can phosphorylate Nrf2 at a second site resulting in nuclear export and degradation. E. A “positive feedback loop” involves SESN2, SQSTM1 and ULK1, gene products induced by Nrf2. SESN2, SQSTM1 and ULK1 collaborate to activate autophagy of Keap1, liberating more Nrf2, which induces more of these gene products, tending to maintain Nrf2 activation once this positive feedback loop has been triggered.

**[0034]** Also in the present disclosure, the combinations of agents gave surprisingly high Nrf2 activation levels compared to what would be predicted based on the prior art and also based on concurrent experiments examining the Nrf2 activating properties of each agent alone and what would be predicted based on adding them together. The Nrf2 activation by the combination of the agents show a synergistic effect. See, e.g., Figures 6 and 7.

**[0035]** An embodiment of the present disclosure comprises combinations of dietary agents - such as in the PB125, PB127, and PB129 combinations - that act on Nrf2 activation by engagement of different, specific control points so that the combination of agents that synergistically activate the Nrf2 pathway. Thus the new combinations of agents that act on different control points of the Nrf2 signaling pathway to increase expression of Nrf2-dependent genes are novel.

**[0036]** By way of example, a number of embodiments of the present disclosure are listed below:

**[0037]** Item 1. A composition comprising two or more phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, said one or more phytochemicals being present in the composition in an amount effective to activate the Nrf2 (Nuclear factor-erythroid 2 related factor 2) pathway.

**[0038]** Item 2. The composition of Item 1, wherein the two or more phytochemicals exert their effects on at least two different control points of the Nrf2 activation pathway when administered to a mammal, said control points being selected from the group consisting of control points A, B, C, D and E. In one embodiment, at least one of the phytochemicals exerts its effects on one control point, while at least another phytochemical exerts its effects on a different control point of the Nrf2 activation pathway as depicted in Fig. 1.

**[0039]** Item 3. The composition of any of the preceding Items, wherein the two or more phytochemicals have a synergistic effect on Nrf2 activation when administered to a mammal.

**[0040]** Item 4. The composition of any of the preceding Items, wherein the composition comprises at least two ingredients selected from the group consisting of rosemary, ginger, luteolin, and ashwagandha.

**[0041]** Item 5. The composition of any of the preceding Items, wherein the composition also comprises one or more phytochemicals selected from the group consisting of milk thistle and bacopa.

**[0042]** Item 6. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ginger extract, and luteolin, said rosemary extract being specified at 5-10% carnosol, said ginger extract being specified at 10-20% 6-shogaol, said luteolin being specified at 95-99% luteolin, wherein the ratio between rosemary extract, ginger extract, and luteolin in the composition is approximately 10:5:1 (w/w).

**[0043]** Item 7. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, said rosemary extract being specified at 5-10% carnosol, said ashwagandha extract being specified at 1-3% withaferin A, said luteolin being specified at 95-99% luteolin,



wherein the ratio between said rosemary extract, ashwagandha extract, and luteolin in the composition is approximately 30:10:4 (w/w).

**[0044]** Item 8. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ginger extract, and luteolin, and wherein the ratio between said rosemary extract, ginger extract, and luteolin is approximately 10:5:1 (w/w).

**[0045]** Item 9. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, the ratio between said rosemary extract, ashwagandha extract, and luteolin being approximately 30:10:4 (w/w).

**[0046]** Item 10. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ginger extract, luteolin and milk thistle extract, the ratio between said rosemary extract, ginger extract, luteolin and milk thistle extract being approximately 10:5:1:30 (w/w).

**[0047]** Item 11. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ginger extract, luteolin, milk thistle extract, and bacopa monnieri extract, the ratio between said rosemary extract, ginger extract, luteolin, milk thistle extract and bacopa monnieri extract being approximately 10:5:1:30:48 (w/w).

**[0048]** Item 12. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ginger extract, luteolin, and bacopa monnieri extract, the ratio between said rosemary extract, ginger extract, luteolin, and bacopa monnieri extract being approximately 10:5:1:48 (w/w).

**[0049]** Item 13. The composition of any of the preceding Items, wherein the composition is used to prevent and/or treat a disease or a condition selected from the group consisting of oxidative stress, detoxification, inflammation, cancer, or a related disease or condition.

**[0050]** Item 14. The composition of any of the preceding Items, wherein the composition is used as a nutritional supplement.

**[0051]** Item 15. The composition of any of the preceding Items, wherein the composition is in the form of a tablet, a capsule, a soft gel, a liquid, a lotion, a gel, a powder, an ointment, or an aerosol.

**[0052]** Item 16. A method of treating and/or preventing a disease or condition, comprising the step of administering a composition to a mammal, the composition comprising one or more phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, said one or more phytochemicals being present in the composition in an amount effective to activate the Nrf2 (NF-E2 related factor 2) pathway.

**[0053]** Item 17. The method of any of the preceding Items, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, wherein the rosemary extract is specified at 5-10% carnosol, the ashwagandha extract is specified at 1-3% withaferin A, and the luteolin is specified at 95-99% luteolin, the ratio between said rosemary extract, ashwagandha extract, and luteolin being approximately 30:10:4 (w/w).

**[0054]** Item 18. The method of Item 17, wherein the composition comprises rosemary extract, ginger extract, and luteolin, wherein the rosemary extract is specified at 5-10% carnosol, the ginger extract is specified at 10-20% 6-shogaol, and the luteolin is specified at 95-99% luteolin, the ratio between said rosemary extract, ginger extract, and luteolin being approximately 10:5:1 (w/w).

**[0055]** Item 19. The method of any of Items 17-18, wherein the composition is administered orally to a human at 10-1000 mg per day.

**[0056]** Item 20. The method of any of Items 17-19, wherein the composition comprises at least two phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, wherein the at least two phytochemicals exert their effects on at least two different control points of the Nrf2 activation pathway, said control points being selected from the group consisting of control points A, B, C, D and E.

**[0057]** It will be readily apparent to those skilled in the art that the compositions and methods described herein may be modified and substitutions may be made using suitable equivalents without departing from the scope of the embodiments disclosed herein. Having now described certain embodiments in detail,

the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting.

### **Examples**

#### **Example 1    Effects on Nrf2 action pathways**

[0058] The different agents, PB123, PB125, PB127, PB129, and PB131, each exhibit strong, potent Nrf2 activation as demonstrated in vitro by using these combinations to treat cell lines that have been stably transfected with a promoter/reporter construct containing a known Nrf2-binding antioxidant response element inserted in to drive production of the readily detectable luciferase gene such that Nrf2 activation results in luciferase production which is detected by luciferin-dependent chemiluminescence. As shown in the Figures 4 and 5, potent Nrf2 activation is induced by the PB123, PB125, PB127, PB129, and PB131 combinations in transfected cancer cell lines independent of tissue type (breast and liver cell data are shown).

[0059] These control points include, but are not limited to, Control point A: release of Nrf2 from binding and inhibition by Keap1; Control point B: action on Nrf2 by enzymes such as kinases that phosphorylate and activate Nrf2; Control point C: activation of other transcription factors that improve the gene expression profile; Control point D: action on mechanisms such as Fyn that control the export of Nrf2 from the nucleus; and Control point E: degradation of Keap1 and mTOR inhibition by SESN2/SQSTM1/ULK1. See Figure 1. For example the PB125 combination that includes rosemary (carnosol), ashwagandha (withaferin A), and luteolin acts at multiple control points in the Nrf2 activation pathway. In HepG2 cells stably transfected with an ARE-driven luciferase reporter gene we inhibited Fyn (with 5 µg/ml saracatinib; AZD0530, a Src family kinase inhibitor (Kaufman, Salazar et al. 2015)) and showed that the inhibition of Fyn increased Nrf2 activation caused by another dietary supplement Nrf2 activator (Protandim) by up to 9-fold. In contrast Fyn inhibition did not further increase PB125-induced Nrf2 activation, confirming that while other dietary Nrf2 activators such as Protandim allow the “shutdown pathway” to remain active, PB125 appears to block the pathway, permitting Nrf2 activation by a smaller amount of the PB125 dietary supplement combination.

[0060] By acting on more than one of the control points, a combination of agents such as PB123 or PB125, along with related combinations based on the core Nrf2 activator triads in PB123 or PB125, such as PB127, PB129, or PB131 give an improved Nrf2 activation and gene regulation response and do so at lower doses than would be predicted based on known properties of the active agents in the combinations and based on what is taught by the prior art. The active ingredients in PB123, 125, PB127, PB129, and PB131 act together in a synergistic fashion, whereby the amount of Nrf2 activation and Nrf2-dependent gene expression is higher for the combined ingredients than would be predicted based on the sum of their individual activities on Nrf2 at the same concentrations, even in different cell types (Figures 6 and 7). One of the surprising findings was that relatively small amounts of luteolin added to the other ingredients gave a larger than expected increase in Nrf2 activation and gene regulation.

[0061] A rosemary (6.7% carnosol), ashwagandha (1% withaferin A), and luteolin (98% luteolin) combination of PB125 (at 30:10:4 rosemary:ashwagandha:luteolin) increased Nrf2-dependent gene expression in mice fed 35 days of PB125 added to mouse chow. See Figures 8 and 9.

[0062] The PB125 phytochemical components are standardized, with rosemary extract (specified at 6% carnosol), ashwagandha extract (specified at 1% withaferin A), and luteolin (specified at 98% purity), so 100 ppm equates to  $6.83 \times 10^{-5}$  mg rosemary extract,  $2.27 \times 10^{-5}$  mg ashwagandha extract, and  $9.43 \times 10^{-6}$  mg luteolin per g of diet. PB125 in mouse diet activates the Nrf2 pathway (e.g., increased hmox1 gene expression in mouse liver) and increases catalase activity. The PB125 dosages were well tolerated by mice as evidenced by no change compared to control diet in weight stability, consistent food intake, and no noticeable GI distress or changes in behavior. The 100 ppm PB125 diet produced significant increases in liver hmox1 gene expression in mice (measured after 35 days of diet consumption)(Figure 8).

[0063] The individual ingredients in PB125, PB127, and PB129 have a long history of human consumption and proven safety in both humans and in animal studies (Saller, Meier et al. 2001, Roodenrys, Booth et al. 2002, Aggarwal, Takada et al. 2004, Boon and Wong 2004, Anadon, Martinez-Larranaga et al. 2008, Zick, Djuric et al. 2008, Johnson 2011, Chandrasekhar, Kapoor et al. 2012, Theoharides, Asadi et

al. 2012, Taliou, Zintzaras et al. 2013, Zhang, Gan et al. 2013, Gonzalez-Vallinas, Reglero et al. 2015, Kumar, Srivastava et al. 2015, Nabavi, Braidy et al. 2015, Petiwala and Johnson 2015). Rosemary, ashwagandha, ginger, milk thistle, bacopa monnieri, and luteolin have been extensively studied in various diseases and have an extensive record of safe use (Mishra, Singh et al. 2000, Roodenrys, Booth et al. 2002, Aggarwal, Takada et al. 2004, Boon and Wong 2004). Rosemary (*Rosmarinus officinalis*) is a common Mediterranean herb widely consumed in foods as a spice and flavoring agent. Also, rosemary has a long history of use in traditional therapies for the treatment of a variety of disorders [1], with emphasis on anti-inflammatory (Emami, Ali-Beig et al. 2013), antioxidant (Klancnik, Guzej et al. 2009, Raskovic, Milanovic et al. 2014, Ortuno, Serrano et al. 2015), and antimicrobial benefits (Del Campo, Amiot et al. 2000, Bozin, Mimica-Dukic et al. 2007). Ashwagandha (*Withania somnifera*, also known as Indian winter cherry or Indian ginseng) is a member of the Solanaceae family of flowering plants. It has been utilized for centuries in South Asia in traditional therapies, with historical and current emphasis on immunomodulatory (Khan, Subramanayaan et al. 2015), anti-tumor (Rai, Jogee et al. 2016), neurological (Raghavan and Shah 2015), anti-inflammatory (Kumar, Srivastava et al. 2015), antioxidant (Priyandoko, Ishii et al. 2011), and other benefits (Wankhede, Langade et al. 2015). Ginger has a long history of safe usage for pain, GI, and aging-related conditions, with evidence of benefit against oxidative stress (Wang, Zhang et al. 2014, Lakhan, Ford et al. 2015, Wilson 2015). Silymarin has a good safety profile (Saller, Meier et al. 2001, Jacobs, Dennehy et al. 2002) even in those with cirrhosis, and even at high doses (up to 900mg a day) that are much higher than used in PB127 or PB129. Bacopa monnieri has proven to be safe in human studies of memory loss at doses higher than used in PB129, and animal studies have not demonstrated any adverse toxicities for any of its components (Mishra, Singh et al. 2000, Roodenrys, Booth et al. 2002). Luteolin is a bioflavonoid flavone compound commonly consumed in the human diet from multiple food sources (e.g., onions, tea, apples, broccoli, olives, celery, spinach, oranges, oregano, etc.), resulting in a typical dietary intake of approximate 1 mg/day from normal food sources (Chun, Chung et al. 2007, Seelinger, Merfort et al. 2008, Jun, Shin et al. 2015, Kim, Park et al. 2015, Nabavi, Braidy et al. 2015). Luteolin is frequently utilized as a dietary supplement with emphasis on its antioxidant (Sun, Sun et al. 2012), neurological (Xu, Wang et al.

2014), and anti-inflammatory benefits (Seelinger, Merfort et al. 2008, Taliou, Zintzaras et al. 2013, Paredes-Gonzalez, Fuentes et al. 2015).

**[0064]** As an example of properties of PB125, we cultured cell lines that had been stably transfected with constructs of the luciferase gene driven in its promoter region by copies of the ARE Nrf2-binding sequence, known as promoter-reporter constructs (Simmons, Fan et al. 2011, Shukla, Huang et al. 2012). Briefly, the stably transfected cells of types HepG2 (human liver), AREc32 (human breast), MCF7 (human breast), A549 (human lung), 293T (human kidney), and A172 (human brain) were seeded at low density in 24-well plates and incubated at 37°C with 10% CO<sub>2</sub>. After 24 h various concentrations of PB125 were added to the cells. After an additional 18 h of incubation, the cells were lysed in their wells with 100 µl of a lysing buffer that contains 3.5 mM sodium pyrophosphate to stabilize light output by luciferase. A 20 µl aliquot of cell lysate was added to a small test tube, placed in a BD Monolight 3010 luminometer for background luminescence, and then 50 µl of 1 mM luciferin was injected into the tube. Relative Light Units integrated for 10 sec were measured for each sample. The liver, breast, brain, and kidney cell types tested exhibited Nrf2 gene activation and luciferase expression by treatment with PB100-series combinations with (Figure 10).

**[0065]** As an example of the cell protective mechanisms induced by PB125 treatment, we examined the gene upregulation in cells treated with PB125. Briefly, cultured HepG2 liver cells were treated with PB125 at 8 micrograms/mL concentration for 18 hours, then total RNA was extracted from the HepG2 cells by using the RNeasy Total RNA Isolation Kit (QIAGEN Inc. Valencia, California, USA). The concentration of each sample was determined based on the absorbance at 260 nm (A<sub>260</sub>). The purity of each sample was determined based on the ratio of A<sub>260</sub> to A<sub>280</sub>. A range of 1.9-2.1 was considered adequately pure. The integrity of Total RNA samples was verified by Agilent 2200 Tape Station. Total RNA (250ng) was converted to double-stranded cDNA (ds-cDNA) by using the cDNA synthesis kit (Affymetrix). An oligo-dT primer containing a T7 RNA polymerase promoter was utilized. The ds-cDNA was then purified and recovered by using purification beads (Affymetrix). Next, in vitro transcription was performed to generate biotin-labeled cRNA using a RNA Transcript Labeling Kit (Affymetrix). Biotin-labeled cRNA was purified using an RNeasy affinity column (Qiagen). To ensure optimal hybridization

to the oligonucleotide array, the cRNA was fragmented. Fragmentation was performed such that the cRNA fragments are between 50-200 bases in length by incubating the cRNA at 94°C for 35 min in a fragmentation buffer. The sample was then added to a hybridization solution containing 100 mM MES, 1 M Na<sup>+</sup>, and 20 mM EDTA in the presence of 0.01% Tween 20. The final concentration of the fragmented cRNA was 0.05 µg/µL. Hybridization was performed by incubating 200 µL of the sample to the Affymetrix GeneChip® PrimeView™ human gene expression array (Affymetrix Inc., Santa Clara, California, USA) at 45 °C for 16 hours using a GeneChip® Hybridization Oven 640 (Affymetrix). After hybridization, the hybridization solutions were removed and the arrays were washed and stained with Streptavidin-phycoerythrin using a GeneChip® Fluidics Station 450 (Affymetrix). Arrays were read at a resolution of 2.5 to 3 microns using the GeneChip Scanner 3000 (Affymetrix). Each gene was represented by the use of ~11 probes per transcript and many control probes. The Command Console GeneChip software program was used to determine the intensity of expression for all genes on the array. For this experiment, fold-induction of genes by PB125 treatment of HepG2 cells was calculated compared to the average intensity observed in control HepG2 cells in culture solution without any added stimulus such as PB125. As depicted in Table 1, genes upregulated by PB125 included a variety of Nrf2-regulated antioxidant, anti-inflammatory, cell stress response and other protective genes. These genes include, for example, genes involved in GSH production and regeneration, iron sequestration, GSH utilization, thioredoxin (TXN) production, regeneration and utilization, etc. Table 1 lists relevant example genes that are upregulated by PB125. In summary, this example supports that the mechanism of cellular protection by PB125 involves activation of the Nrf2 cell signaling pathway.

Table 1            Gene Microarray analysis revealed that PB125 regulates numerous Nrf2 associated genes and genes associated with antioxidant, anti-inflammatory, and other cell protective effects.

Probe Set ID	HepG2 (Control)	Fold Induction by BP125	Representative Public ID	Gene Title	Gene Symbol
11715650_a_at	45.53	10.10	AF208018.1	thioredoxin reductase 1	TXNRD1
11756634_a_at	414.69	2.81	CR597200.1	glutathione reductase	GSR
11750770_a_at	1005.93	2.37	AK304288.1	glutamate-cysteine ligase, catalytic subunit	GCLC
11759710_at	199.19	2.04	BC024223.2	thioredoxin domain containing 9	TXNDC9
11744680_a_at	231.18	7.72	AB040875.1	solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11	SLC7A11
11756634_a_at	414.69	2.81	CR597200.1	glutathione reductase	GSR
11716939_a_at	1217.99	8.63	NM_002133.1	heme oxygenase (decycling) 1	HMOX1
11725496_a_at	488.83	8.87	NM_032717.3	1-acylglycerol-3-phosphate O-acyltransferase 9	AGPAT9
11752577_at	771.67	3.62	AY258285.1	ferritin, heavy polypeptide 1	FTH1
11715649_s_at	3236.76	4.73	NM_003330.2	thioredoxin reductase 1	TXNRD1
11716950_s_at	1908.04	5.45	NM_080725.1	sulfiredoxin 1	SRXN1
11752843_x_at	1202.52	4.54	AK304877.1	sequestosome 1	SQSTM1
11750416_a_at	69.07	9.41	AK293322.1	thioredoxin reductase 1	TXNRD1
11756585_a_at	86.47	6.47	CR614710.1	aquaporin 3 (Gill blood group)	AQP3
11735676_a_at	231.82	3.98	NM_182980.2	oxidative stress induced growth inhibitor 1	OSGIN1
11753445_a_at	244.58	10.37	BT019785.1	heme oxygenase (decycling) 1	HMOX1
11723490_at	1195.87	6.07	BC041809.1	glutamate-cysteine ligase, modifier subunit	GCLM
11756915_a_at	63.77	8.33	AL833940.1	cytochrome P450, family 4, subfamily F, polypeptide 11	CYP4F11
11736655_a_at	499.98	7.20	NM_012212.3	prostaglandin reductase 1	PTGR1
11719171_a_at	2722.97	6.99	NM_001353.5	aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase)	AKR1C1
11742378_a_at	1112.08	4.32	NM_001080538.1	aldo-keto reductase family 1, member B10 (aldose reductase) /// aldo-keto reductase family 1, member B15	AKR1B10 /// AKR1B15
11729101_a_at	2435.26	6.95	NM_205845.1	aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III) /// aldo-keto reductase family 1 member C2-like	AKR1C2 /// LOC100653286
11757882_x_at	59.22	2.02	BU784580	glutathione S-transferase alpha 1 /// glutathione S-transferase alpha 2	GSTA1 /// GSTA2

**[0066]** As an example of the anti-inflammatory mechanisms induced by PB125 treatment, we examined cytokine levels in primary cells treated with PB125 and stimulated with bacterial lipopolysaccharide endotoxin (LPS). Mouse peritoneal macrophages were obtained after treatment with thioglycollate into the peritoneal cavity for 1 week followed by lavage recovery of approximately 7 million macrophages. Aliquots of cells were plated and treated with ethanol control (0.1% to match PB125) or PB125 (5 ug/mL) for 16 h, then stimulated with lipopolysaccharide (100 ng/mL) or vehicle (negative control) for 5 h. Total RNA was isolated from the cells for quantitative PCR analysis to measure TNF $\alpha$  (tumor necrosis factor- $\alpha$ ) and IL-1 $\beta$  (interleukin-1 beta) gene expression, normalized to 18s levels. Notably, PB125 treatment caused a dramatic decrease in LPS-induced expression of the pro-inflammatory cytokines TNF $\alpha$  and IL-1 $\beta$ . See Figure 11.

**[0067]** A rosemary (6.7% carnosol), ashwagandha (1% withaferin A), and luteolin (98% luteolin) combination of PB125 (at 30:10:4 rosemary:ashwagandha:luteolin) increased Nrf2-dependent gene expression of the GCLM gene in buccal cell samples from a human subject taking 60 mg of PB125 daily p.o., compare to buccal cell samples two normal control subjects (assayed by



quantitative RT-PCR on purified RNA, using human GCLM specific primers (Forward Primer: TTGCCTCCTGCTGTGTGATG (SEQ ID NO. 1), Reverse Primer: GTGCGCTTGAATGTCAGGAA) (SEQ ID NO. 2), normalized to GAPDH, with relative fold change calculated by the  $2^{-(\Delta\Delta C_t)}$  method. See Figure 13.

**[0068]** As additional data supporting the invention, we found surprising amounts of synergy between the Rosemary, Ginger, Ashwagandha, and Luteolin ingredients. For example, low concentrations of Luteolin synergized with combinations of Rosemary extracts and Ginger extracts to activate Nrf2. In the present invention, other agents can be added to the Nrf2-activating combinations provided they do not interfere with the Nrf2 activating functionality. We found that the silymarin and bacosides ingredients did not antagonize the Nrf2 activation of the Rosemary, Ginger, Ashwagandha, and Luteolin ingredients.

**[0069]** Following up on this experiment in another way, luciferase RLU measured 17, 24, 41, and 48 hours after treatment of HepG2 cells in which the PB125 treatment at 0-10 ug/mL and 0-50 ug/mL ranges was washed off after 2 hours of exposure time and replaced by fresh cell culture media showed that Nrf2-driven production of luciferase was highest at 17 h, then rapidly decreased to approximately baseline levels by 48 hours after treatment.

**[0070]** Repeating treatments on cultured HepG2 cells with 2 hour exposures once every 24 hours, then read 24 hours later showed that the Nrf2 activation by PB125 wore off between 24 and 48 hours and the cells could still be activated again if treated again with PB125.

**[0071]** As an example of the anti-inflammatory mechanisms induced by PB123 or PB125 treatment, we examined gene expression and cytokine levels in primary human pulmonary artery endothelial cells (HPAEC) treated with PB123 or PB125 and stimulated with bacterial lipopolysaccharide endotoxin (LPS). LPS stimulation induced the expression of inflammation-related genes, and this upregulation was attenuated by treatment with PB123 or PB125. Table 2 shows the 40 genes most highly upregulated by LPS treatment, and shows that both PB123 treatment and PB125 treatment attenuated LPS-induced gene expression. LPS stimulation increased the release of pro-inflammatory interleukin-6 (IL6) protein from

the HPAEC cells, and this increase was attenuated by treatment with PB125. See Figure 12.

Table 2 Gene Microarray analysis revealed that PB123 and PB125 exhibited anti-inflammatory effects. Both PB123 and PB125 lowered the LPS-induced expression signals of the 40 genes that were the most highly up-regulated by LPS.

Gene Symbol	Control	LPS	LPS+PB123	LPS+PB125	Gene Title	Gene Symbol	LPS/LPS +PB123	LPS/LPS +PB125
CXCL3	33	1441	492	225	chemokine (C-X-C motif) ligand 3	CXCL3	2.9	6.4
CCL20	196	4776	2055	1034	chemokine (C-C motif) ligand 20	CCL20	2.3	4.6
CXCL2	292	5407	2956	2669	chemokine (C-X-C motif) ligand 2	CXCL2	1.8	2.0
CSF2	41	621	132	133	colony stimulating factor 2 (granulocyte-macrophage)	CSF2	4.7	4.7
TNFAIP6	33	390	91	60	tumor necrosis factor, alpha-induced protein 6	TNFAIP6	4.3	6.5
IL8	590	6750	5571	4257	interleukin 8	IL8	1.2	1.6
TNFAIP2	285	3089	798	512	tumor necrosis factor, alpha-induced protein 2	TNFAIP2	3.9	6.0
CXCL10	67	668	47	31	chemokine (C-X-C motif) ligand 10	CXCL10	14.3	21.3
CXCL1	1195	11398	7858	7819	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	CXCL1	1.5	1.5
CX3CL1	386	3618	444	288	chemokine (C-X3-C motif) ligand 1	CX3CL1	8.2	12.5
BIRC3	86	798	349	190	baculoviral IAP repeat containing 3	BIRC3	2.3	4.2
CD69	36	333	111	45	CD69 molecule	CD69	3.0	7.3
TNFAIP3	94	814	309	190	tumor necrosis factor, alpha-induced protein 3	TNFAIP3	2.6	4.3
SELE	1465	12425	5605	2612	selectin E	SELE	2.2	4.8
CXCL6	246	1683	458	178	chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	CXCL6	3.7	9.5
NKX3-1	60	398	141	125	NK3 homeobox 1	NKX3-1	2.8	3.2
CSF3	92	592	272	290	colony stimulating factor 3 (granulocyte)	CSF3	2.2	2.0
RND1	98	601	224	236	Rho family GTPase 1	RND1	2.7	2.5
LTB	244	1478	374	314	lymphotoxin beta (TNF superfamily, member 3)	LTB	3.9	4.7
FAM101A /// 3	63	329	70	78	family with sequence similarity 101, member A /// protein FAM101A	FAM101A /// ZNF664	4.7	4.2
CXCL5	163	844	127	63	chemokine (C-X-C motif) ligand 5	CXCL5	6.7	13.3
CEBPD	183	947	493	489	CCAAT/enhancer binding protein (C/EBP), delta	CEBPD	1.9	1.9
MAP3K8	26	128	75	45	mitogen-activated protein kinase kinase kinase 8	MAP3K8	1.7	2.9
TRAF1	158	730	421	328	TNF receptor-associated factor 1	TRAF1	1.7	2.2
IL6	429	1967	1165	1105	interleukin 6 (interferon, beta 2)	IL6	1.7	1.8
VCAM1	1315	5963	2065	1116	vascular cell adhesion molecule 1	VCAM1	2.9	5.3
ICAM1	288	1290	543	416	intercellular adhesion molecule 1	ICAM1	2.4	3.1
SLC7A2	355	1592	660	583	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2	SLC7A2	2.4	4.2
CXCR7	291	1286	660	521	chemokine (C-X-C motif) receptor 7	CXCR7	1.9	2.5
NCOA7	132	561	212	137	nuclear receptor coactivator 7	NCOA7	2.6	4.1
IRF1	240	1014	579	489	interferon regulatory factor 1	IRF1	1.8	2.1
BCL2A1	31	130	39	18	BCL2-related protein A1	BCL2A1	3.3	7.0
TNFRSF9	32	124	33	30	tumor necrosis factor receptor superfamily, member 9	TNFRSF9	3.7	4.1
IL1A	236	888	589	561	interleukin 1, alpha	IL1A	1.5	1.6
MT1G	36	134	116	163	metallothionein 1G	MT1G	1.2	0.8
TIFA	81	293	175	147	TRAF-interacting protein with forkhead-associated domain	TIFA	1.7	2.0
CCL5	95	330	95	83	chemokine (C-C motif) ligand 5	CCL5	3.5	4.0
CAB39	26	91	48	43	calcium binding protein 39	CAB39	1.9	2.1
SOC51	29	95	73	76	suppressor of cytokine signaling 1	SOC51	1.3	1.2
IL1B	52	170	58	66	interleukin 1, beta	IL1B	2.9	2.6

## Example 2 PB125

[0072] One embodiment of the present disclosure is a combination of rosemary extract (specified at 5 to 50% carnosol), ashwagandha extract (specified at 0.5-10% withaferin A), and luteolin (specified at 10-100% luteolin), in the mass ratios of 30:10:6, 30:10:5, 30:10:4, or 30:10:1 with a daily human dose of the combination ranging from 42 to 1050 mg as shown in Table 3.

Table 3. Composition with specifications for the ingredients and the daily dose ranges of PB125 for human

Ingredient:	Rosemary	Ashwagandha	Luteolin
Spec range:	5-50% carnosol or 10-100% diterpenes	0.5-10% withaferin A	10-100% luteolin
Preferred spec range:	5-10% carnosol	1-3% withaferin A	95-99% luteolin
Daily dose range:	30-750 mg	10-250 mg	2-50 mg
Composition range:	30-90%	10-30%	2-8%
Preferred mass ratio	30	10	6
Preferred mass ratio	30	10	5
Preferred mass ratio	30	10	4
Preferred mass ratio	30	10	1

### Example 3 PB127

[0073] Another embodiment of the present disclosure is a PB127 combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin), and milk thistle extract (specified at 50-90% silymarin), in the mass ratio of 10:5:1:30, respectively, with a daily human dose of the combination ranging from 46 to 920 mg as shown in Table 4.

Table 4. Composition with specifications for the ingredients and the daily dose ranges of PB127 for human

Ingredient:	Rosemary	Ginger	Luteolin	Milk Thistle
Spec range:	5-50% carnosol or 10-100% diterpenes	0.5-20% 6-shogaol or 6-gingerol	10-100% luteolin	10-100% silymarin
Preferred spec range:	5-10% carnosol	10-20% 6-shogaol	95-99% luteolin	75-100% silymarin
Daily dose range:	10-200 mg	5-100 mg	1-20 mg	30-600 mg
Composition range:	10-30%	5-15%	1-3%	25-75%

Preferred mass ratio	10	5	1	30
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#### Example 4 PB129

**[0074]** Another embodiment of the present disclosure is a PB129 combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin), milk thistle extract (specified at 50-90% silymarin), and bacopa monnieri extract (specified at 10-60% bacosides) in the mass ratio of 10:5:1:30:48, respectively, with a daily human dose of the combination ranging from 94 to 1820 mg as shown in Table 5.

Table 5. Composition with specifications for the ingredients and the daily dose ranges of PB129 for human

Ingredient:	Rosemary	Ginger	Luteolin	Milk Thistle	Bacopa
Spec range:	5-50% carnosol or 10-100% diterpenes	0.5-20% 6-shogaol or 6-gingerol	10-100% luteolin	10-100% silymarin	10-80% bacosides
Preferred spec range:	5-10% carnosol	10-20% 6-shogaol	95-99% luteolin	75-100% silymarin	20-60% bacosides
Daily dose range:	10-200 mg	5-100 mg	1-20 mg	30-600 mg	48-900 mg
Composition range:	5-15%	2.5-7.5%	0.5-1.5%	12.5-37.5%	25-75%
Preferred mass ratio	10	5	1	30	48

#### Example 5 PB123

**[0075]** Another embodiment of the present disclosure is a PB123 combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin) in the mass ratio of 10:5:1, respectively, with a daily human dose of the combination ranging from 16 to 320 mg as shown in Table 6.

Table 6. Composition with specifications for the ingredients and the daily dose ranges of PB123 for human

Ingredient:	Rosemary	Ginger	Luteolin
Spec range:	5-50% carnosol or 10-100% diterpenes	0.5-20% 6- shogaol or 6-gingerol	10-100% luteolin
Preferred spec range:	5-10% carnosol	10-20% 6-shogaol	95-99% luteolin
Daily dose range:	10-200 mg	5-100 mg	1-20 mg
Composition range:	10-30%	5-15%	1-3%
Preferred mass ratio	10	5	1

### Example 6 PB131

[0076] Another embodiment of the present invention is a PB131 combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin) and bacopa monnieri extract (specified at 10-60% bacosides) in the mass ratio of 10:5:1:48, respectively, with a daily human dose of the combination ranging from 64 to 1220 mg as shown in Table 7.

Table 7. Composition with specifications for the ingredients and the daily dose ranges of PB131 for human

Ingredient:	Rosemary	Ginger	Luteolin	Bacopa
Spec range:	5-50% carnosol or 10-100% diterpenes	0.5-20% 6- shogaol or 6-gingerol	10-100% luteolin	10-80% bacosides
Preferred spec range:	5-10% carnosol	10-20% 6-shogaol	95-99% luteolin	20-60% bacosides
Daily dose range:	10-200 mg	5-100 mg	1-20 mg	48-900 mg
Composition range:	5-15%	2.5-7.5%	0.5-1.5%	25-75%
Preferred mass ratio	10	5	1	48

[0077] The contents of all cited references (including literature references, patents, patent applications, and websites) that may be cited throughout this application or listed below are hereby expressly incorporated by reference in their entirety for any purpose into the present disclosure. The disclosure may employ, unless otherwise indicated, conventional techniques of microbiology, molecular biology and cell biology, which are well known in the art.

[0078] The disclosed methods and systems may be modified without departing from the scope hereof. It should be noted that the matter contained in the above description or shown in the accompanying drawings should be interpreted as illustrative and not in a limiting sense.

### List of References

[0079] The following references, patents and publication of patent applications are either cited in this disclosure or are of relevance to the present disclosure. All documents listed below, along with other papers, patents and publication of patent applications cited throughout this disclosures, are hereby incorporated by reference as if the full contents are reproduced herein.

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## CLAIMS

What is claimed is:

1. A composition comprising two or more phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, said two or more phytochemicals being present in the composition in an amount effective to activate the Nrf2 (Nuclear factor-erythroid 2 related factor 2) pathway.
2. The composition of claim 1, wherein the two or more phytochemicals exert their effects on at least two different control points of the Nrf2 activation pathway when administered to a mammal, said control points being selected from the group consisting of control points A, B, C, D and E.
3. The composition of claim 1, wherein the two or more phytochemicals have a synergistic effect on Nrf2 activation when administered to a mammal.
4. The composition of claim 1, wherein the composition comprises at least two ingredients selected from the group consisting of rosemary, ginger, luteolin, and ashwagandha.
5. The composition of claim 4, wherein the composition further comprises one or more phytochemicals selected from the group consisting of milk thistle and bacopa.
6. The composition of claim 4, wherein the composition comprises rosemary extract, ginger extract, and luteolin, said rosemary extract being specified at 5-10% carnosol, said ginger extract being specified at 10-20% 6-shogaol, said luteolin being specified at 95-99% luteolin, wherein the ratio between rosemary extract, ginger extract, and luteolin in the composition is approximately 10:5:1 (w/w).
7. The composition of claim 4, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, said rosemary extract being specified at 5-10% carnosol, said ashwagandha extract being specified at 1-3% withaferin A, said luteolin being specified at 95-99% luteolin, wherein the ratio between said rosemary extract, ashwagandha extract, and luteolin in the composition is approximately 30:10:4 (w/w).
8. The composition of claim 1, wherein the composition comprises rosemary extract, ginger extract, and luteolin, and wherein the ratio between said rosemary extract, ginger extract, and luteolin is approximately 10:5:1 (w/w).



9. The composition of claim 1, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, the ratio between said rosemary extract, ashwagandha extract, and luteolin being approximately 30:10:4 (w/w).
10. The composition of claim 5, wherein the composition comprises rosemary extract, ginger extract, luteolin and milk thistle extract, the ratio between said rosemary extract, ginger extract, luteolin and milk thistle extract being approximately 10:5:1:30 (w/w).
11. The composition of claim 5, wherein the composition comprises rosemary extract, ginger extract, luteolin, milk thistle extract, and bacopa monnieri extract, the ratio between said rosemary extract, ginger extract, luteolin, milk thistle extract and bacopa monnieri extract being approximately 10:5:1:30:48 (w/w).
12. The composition of claim 5, wherein the composition comprises rosemary extract, ginger extract, luteolin, and bacopa monnieri extract, the ratio between said rosemary extract, ginger extract, luteolin, and bacopa monnieri extract being approximately 10:5:1:48 (w/w).
13. The composition of claim 1, wherein the composition is used to prevent and/or treat a disease or a condition selected from the group consisting of oxidative stress, detoxification, inflammation, cancer, or a related disease or condition.
14. The composition of claim 1, wherein the composition is used as a nutritional supplement.
15. The composition of claim 1, wherein the composition is in the form of a tablet, a capsule, a soft gel, a liquid, a lotion, a gel, a powder, an ointment, or an aerosol.
16. A method of treating and/or preventing a disease or condition, comprising the step of administering a composition to a mammal, the composition comprising one or more phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, said one or more phytochemicals being present in the composition in an amount effective to activate the Nrf2 (NF-E2 related factor 2) pathway.
17. The method of claim 16, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, wherein the rosemary extract is specified at 5-10% carnosol, the ashwagandha extract is specified at 1-3% withaferin A, and the luteolin is specified at 95-99% luteolin, the ratio between said rosemary extract, ashwagandha extract, and luteolin being approximately 30:10:4 (w/w).

18. The method of claim 16, wherein the composition comprises rosemary extract, ginger extract, and luteolin, wherein the rosemary extract is specified at 5-10% carnosol, the ginger extract is specified at 10-20% 6-shogaol, and the luteolin is specified at 95-99% luteolin, the ratio between said rosemary extract, ginger extract, and luteolin being approximately 10:5:1 (w/w).
19. The method of claim 18, wherein the composition is administered orally to a human at 10-1000 mg per day.
20. The method of claim 18, wherein the composition comprises at least two phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, wherein the at least two phytochemicals exert their effects on at least two different control points of the Nrf2 activation pathway, said control points being selected from the group consisting of control points A, B, C, D and E.

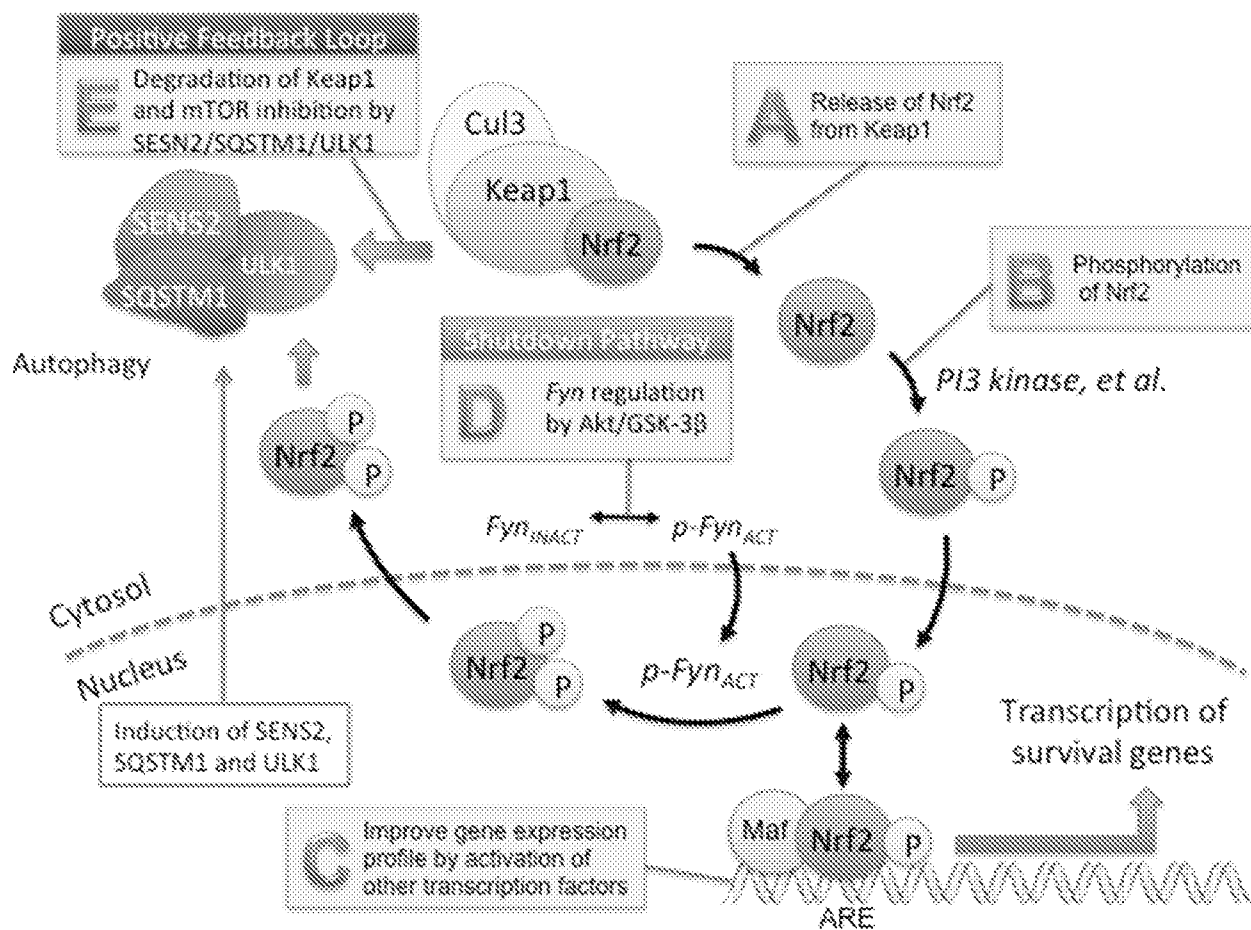


FIG. 1

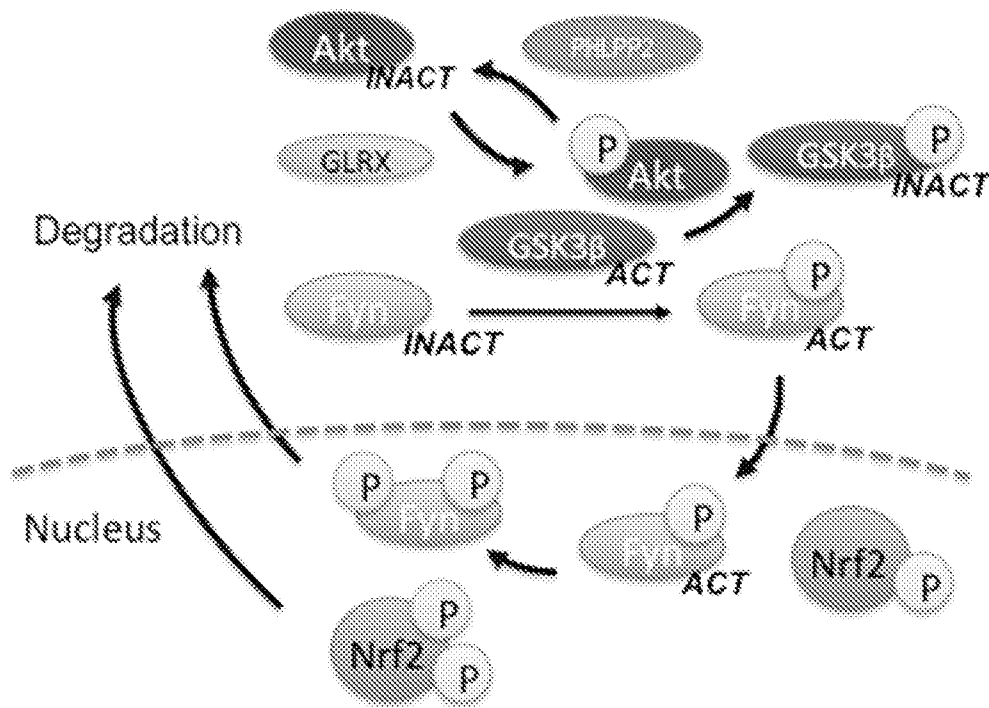
The Akt1/PHLPP2/GSK3 $\beta$ /Fyn "Shutdown" Pathway

FIG. 2

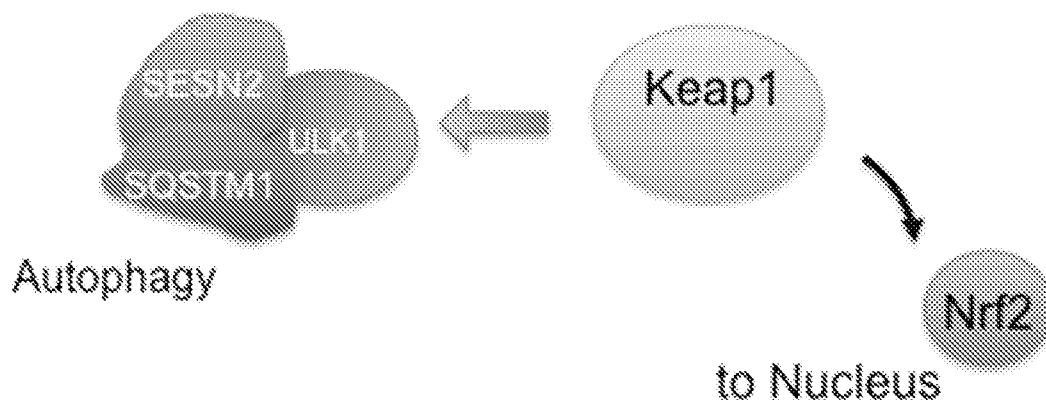


FIG. 3

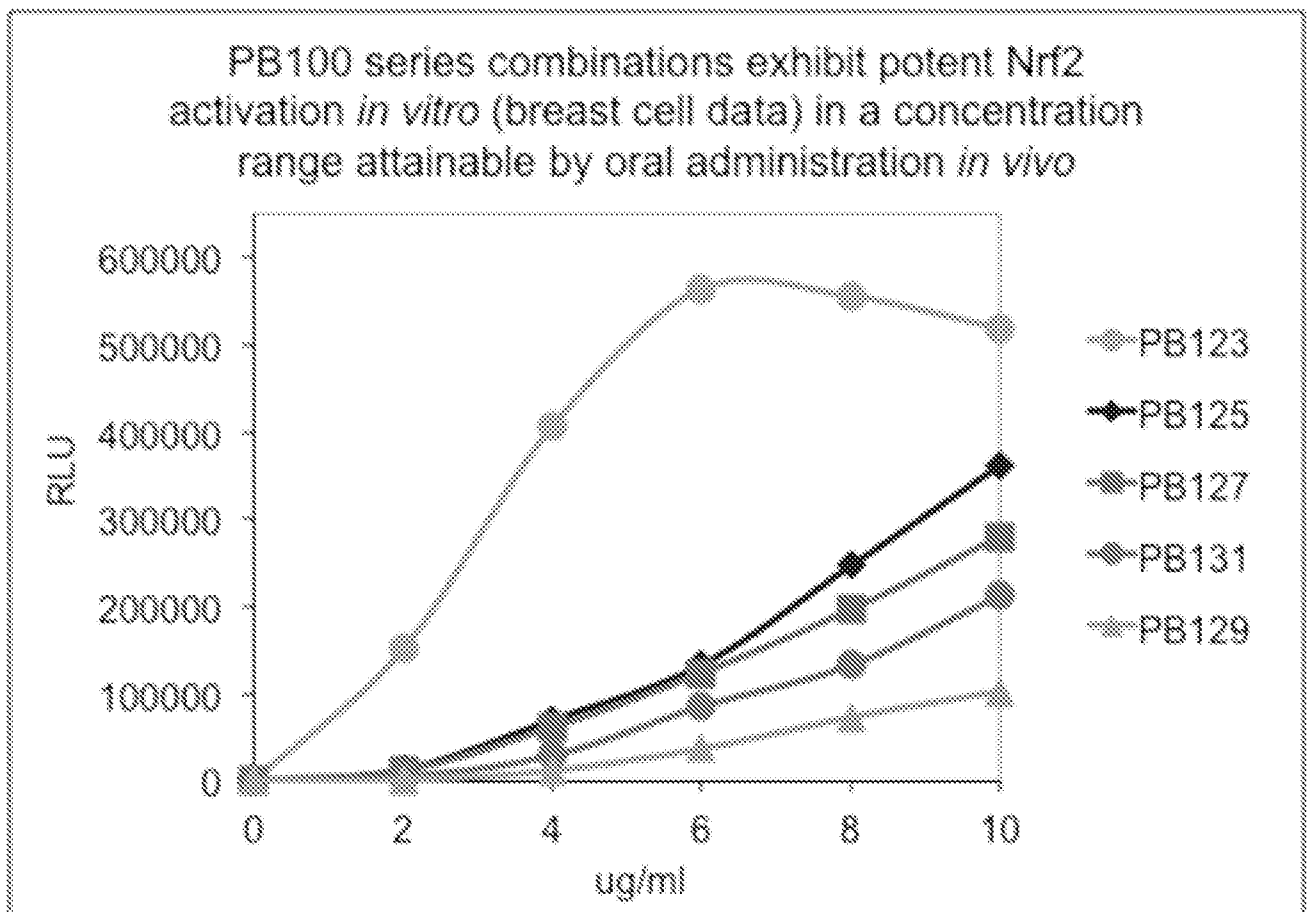


FIG. 4

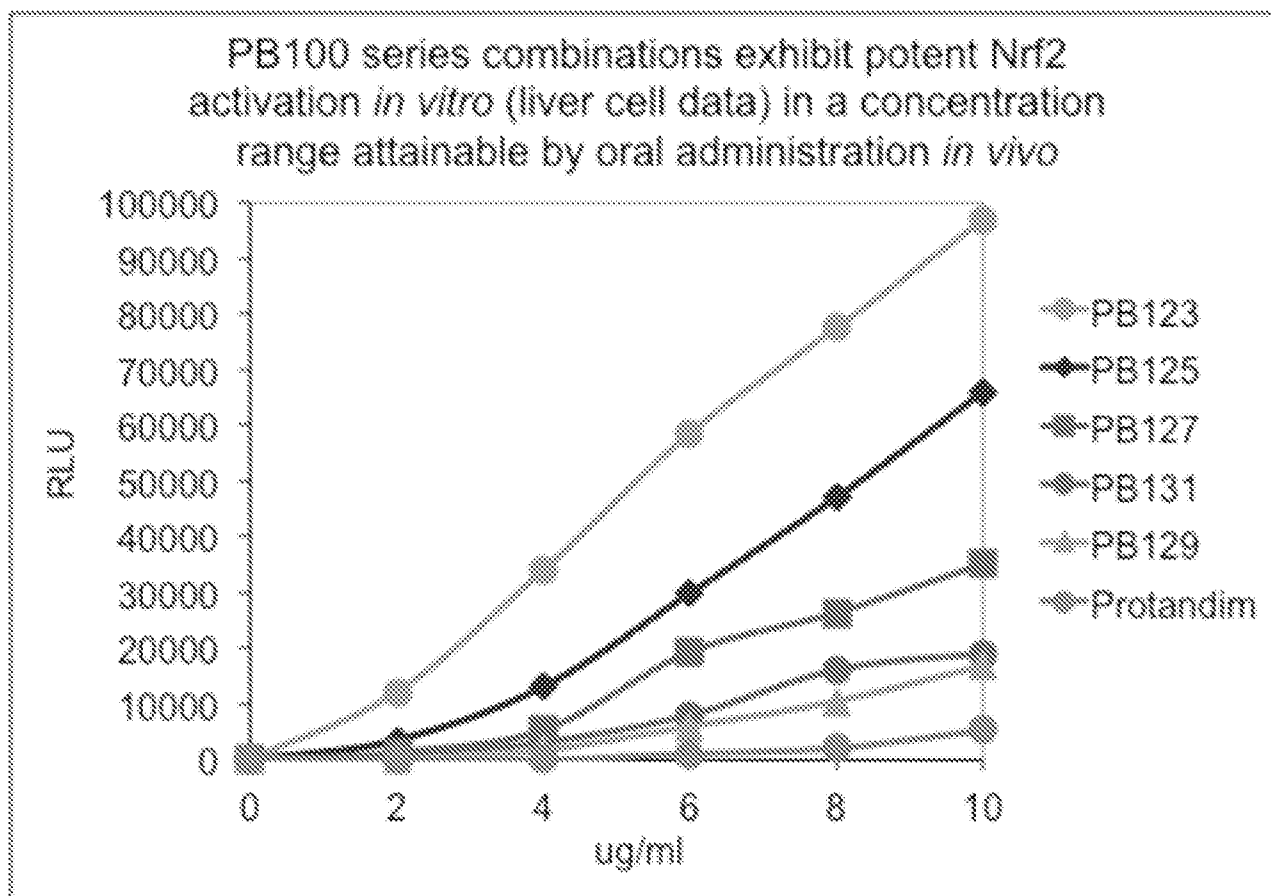


FIG. 5

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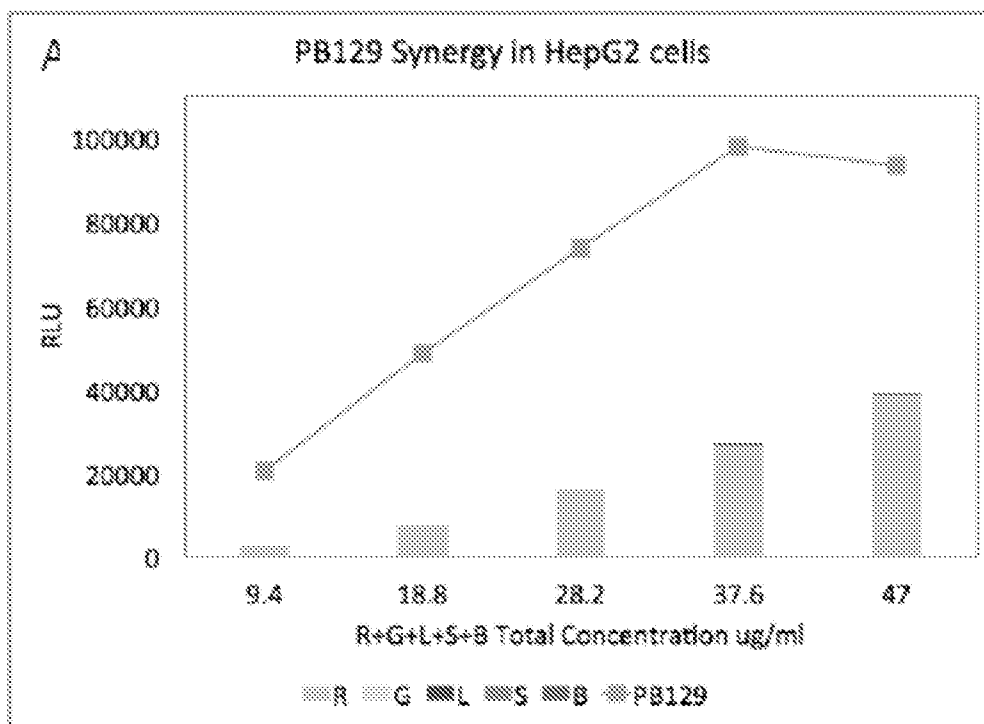


FIG. 6A

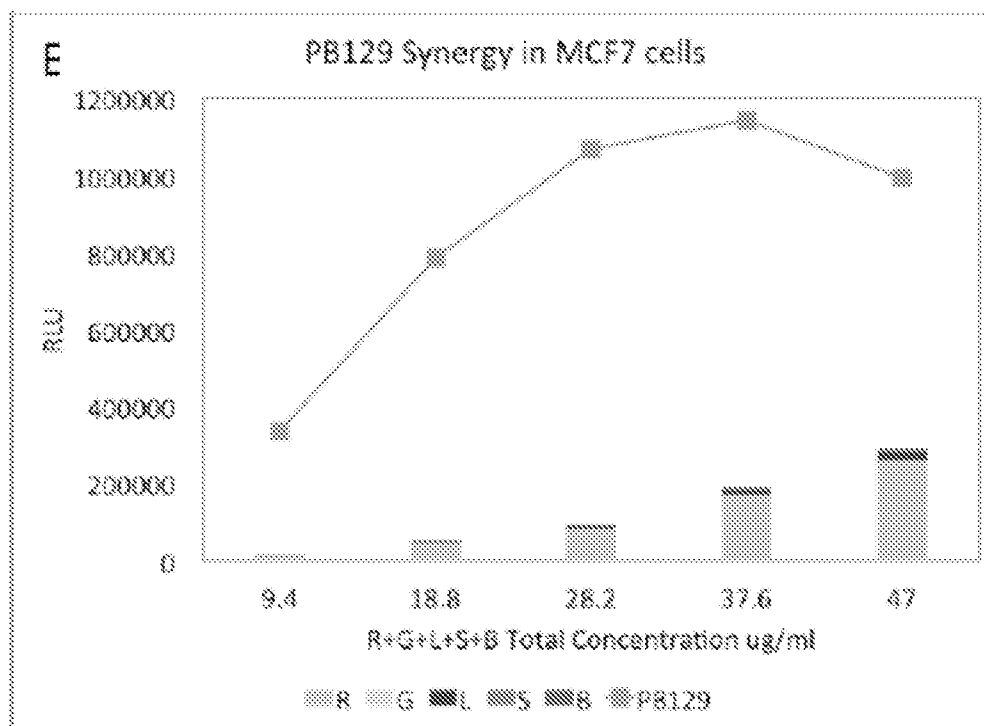


FIG. 6B

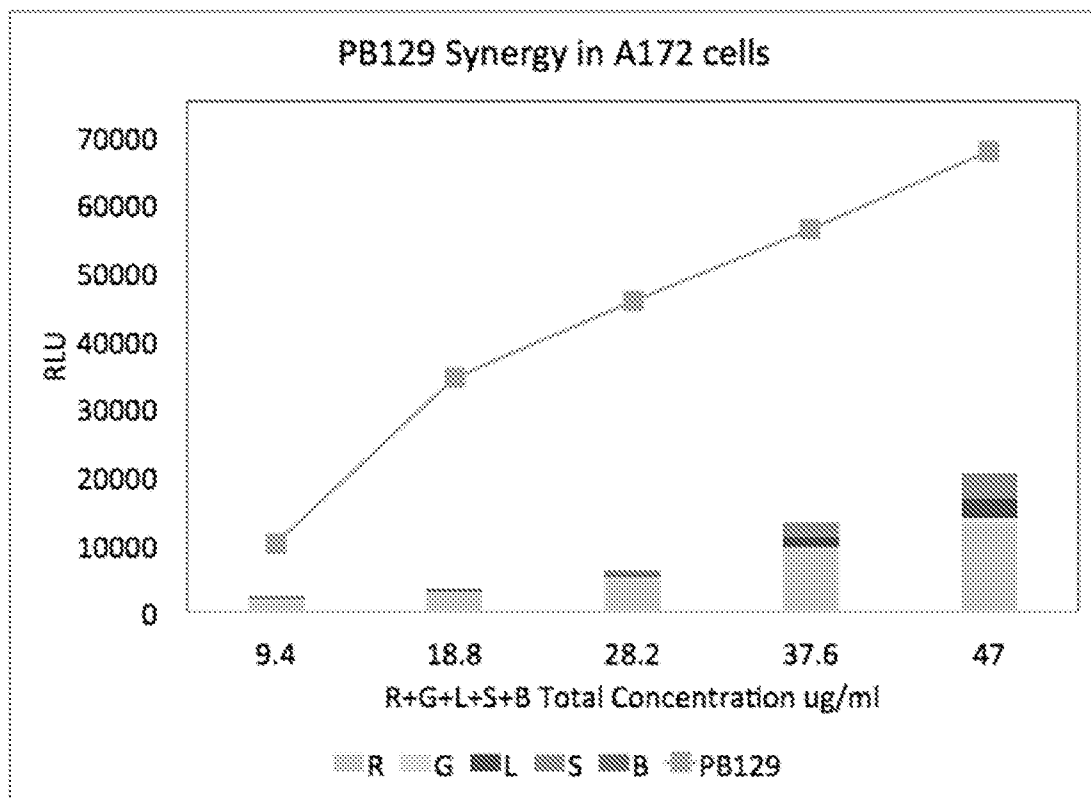


FIG. 6C

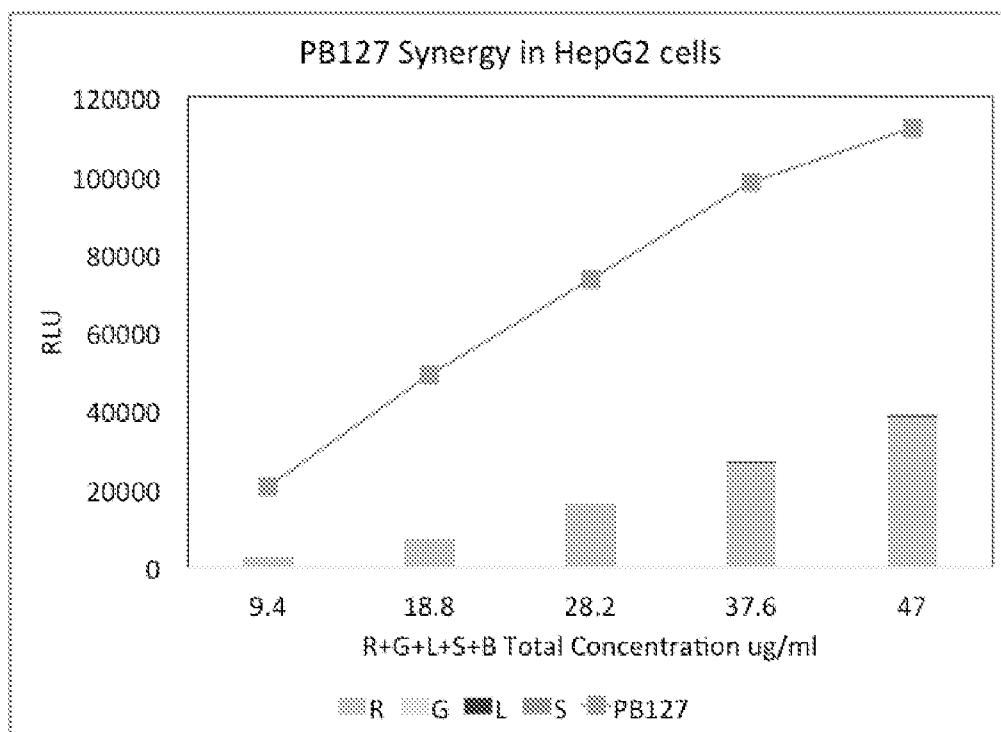


FIG. 7A



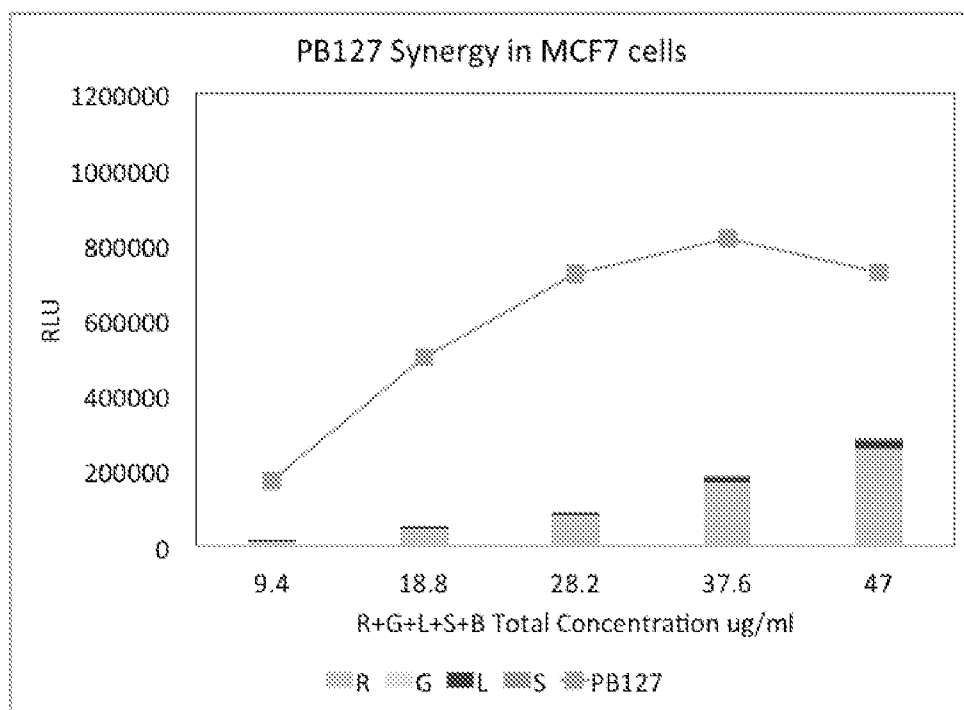
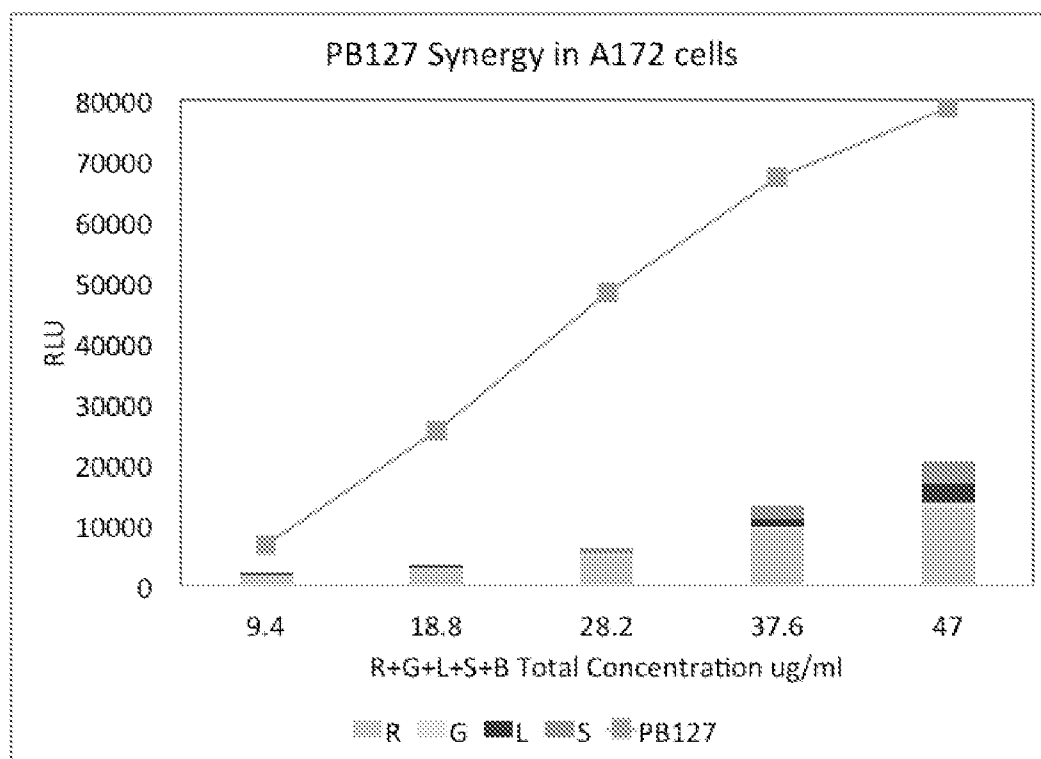


FIG. 7B



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FIG. 7C

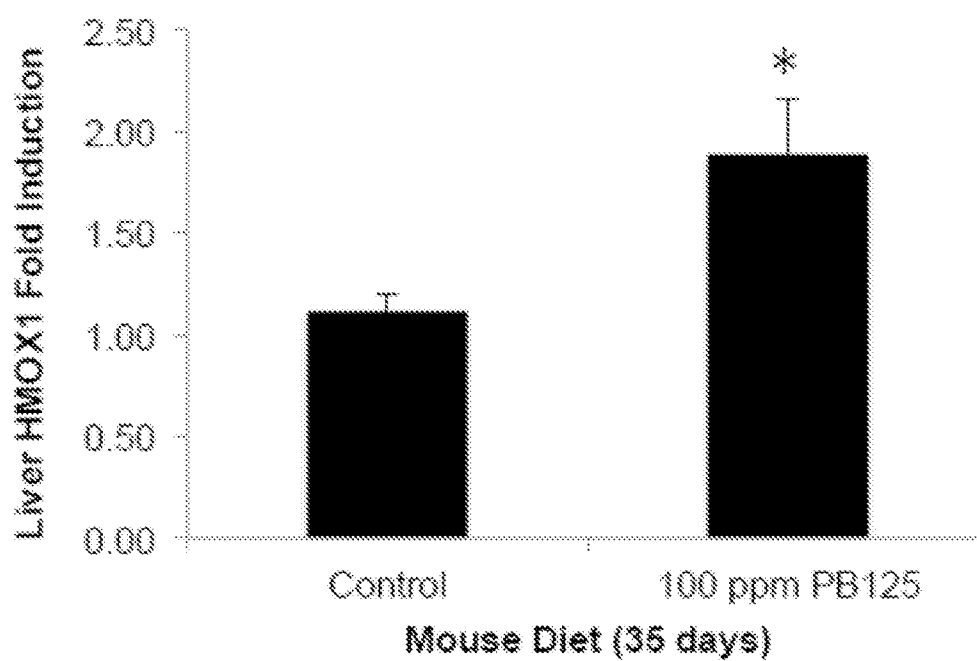


FIG. 8

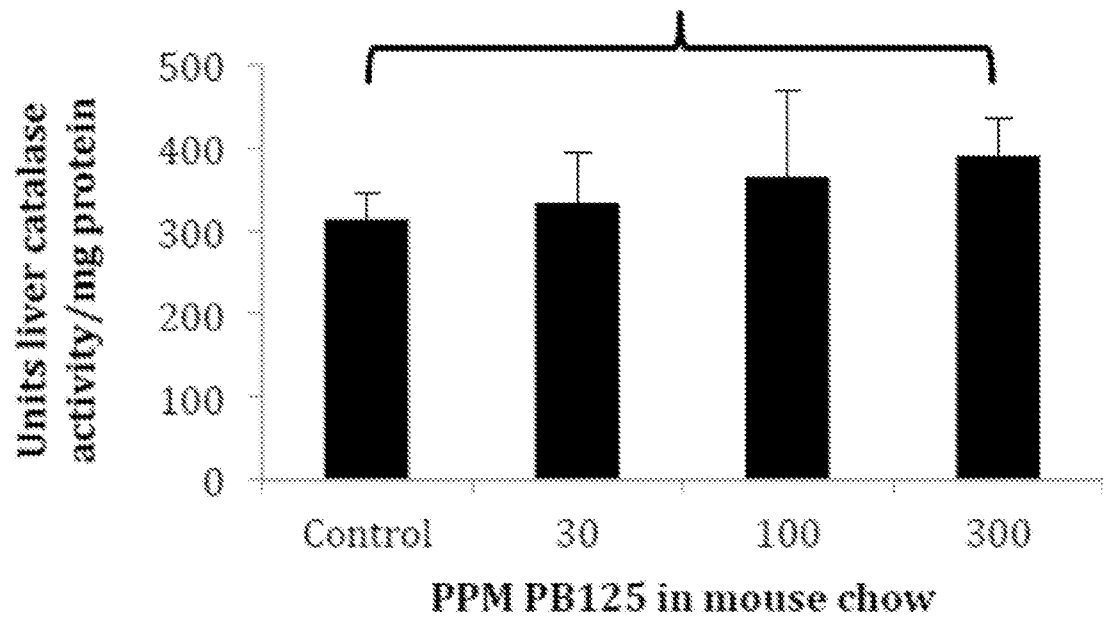


FIG. 9

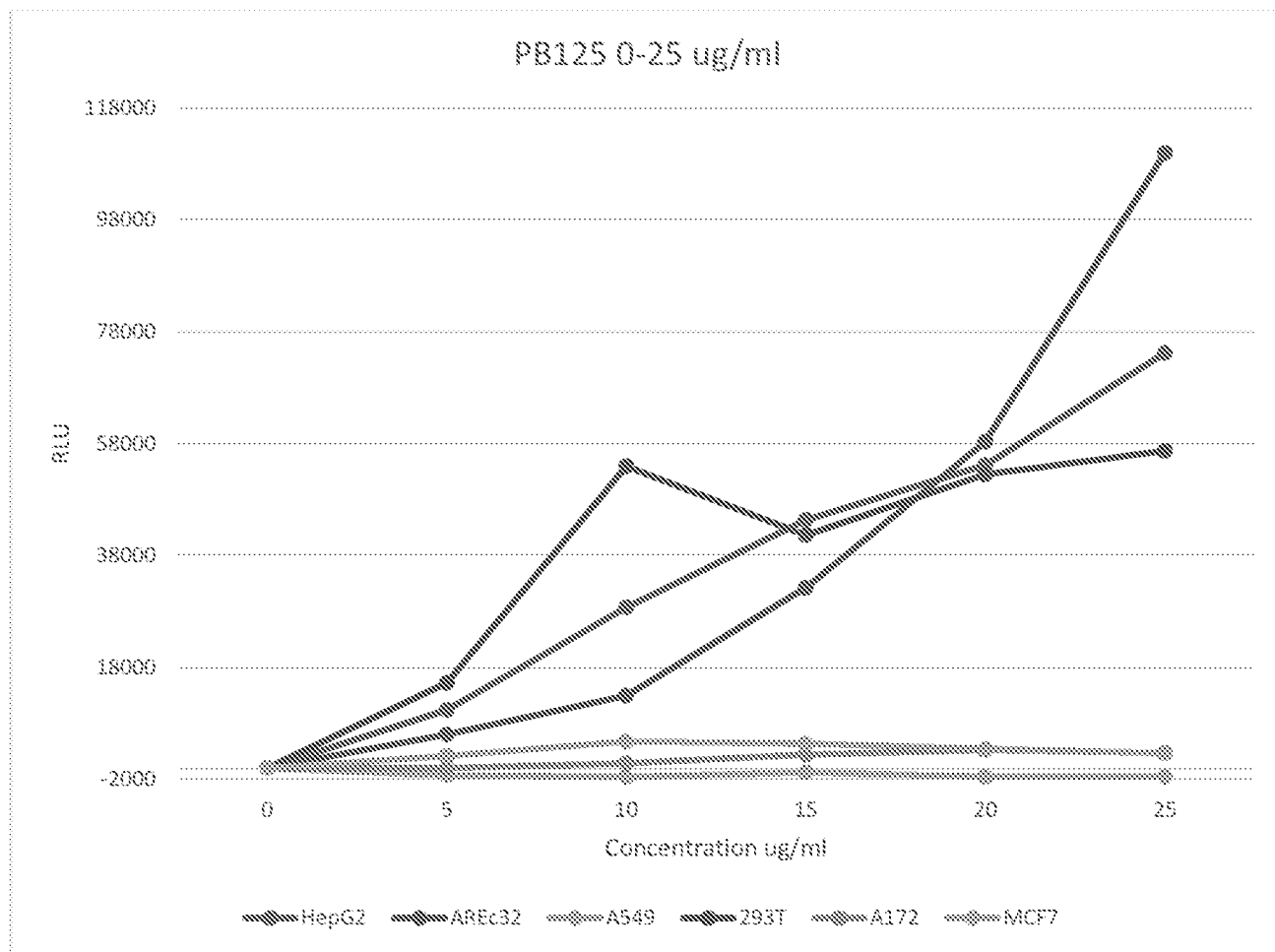


FIG. 10

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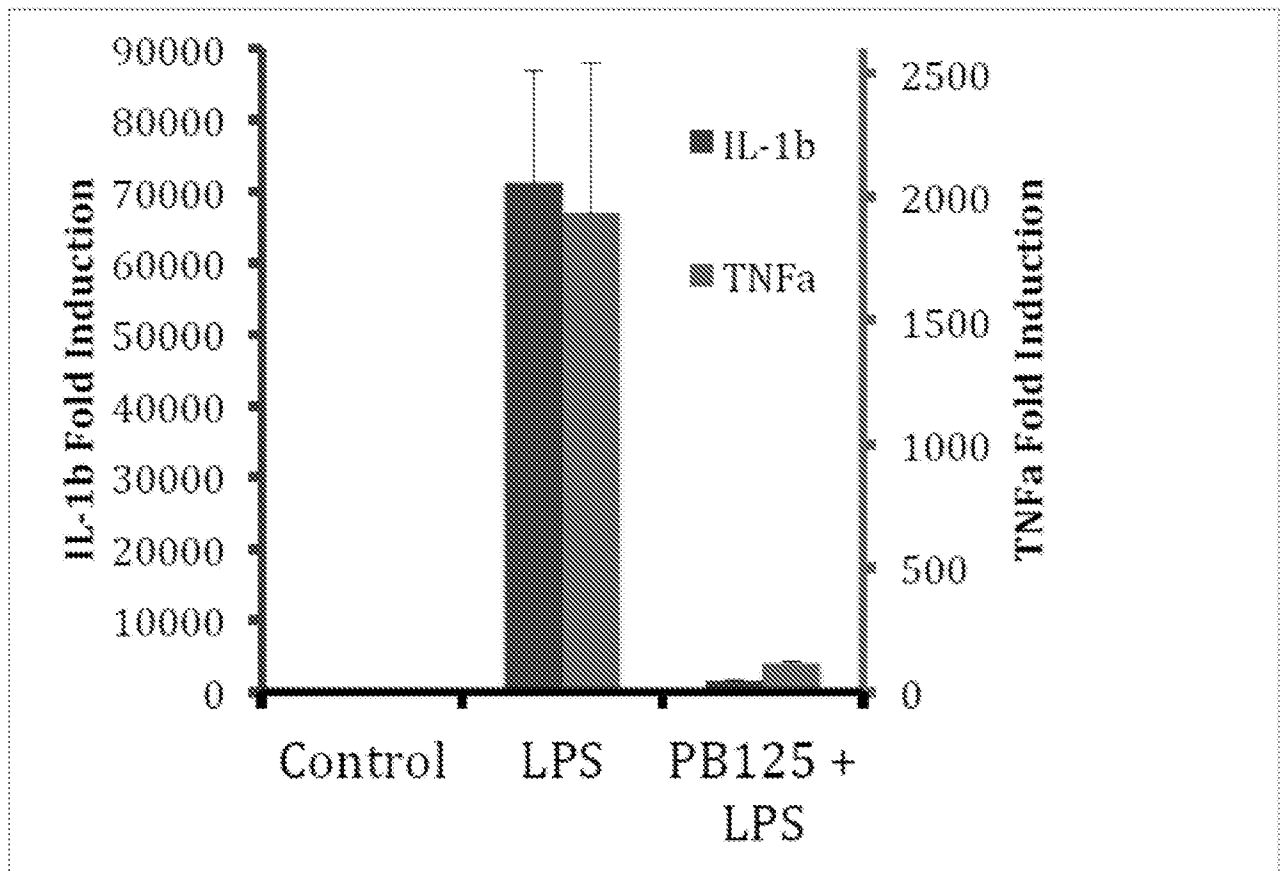


FIG. 11

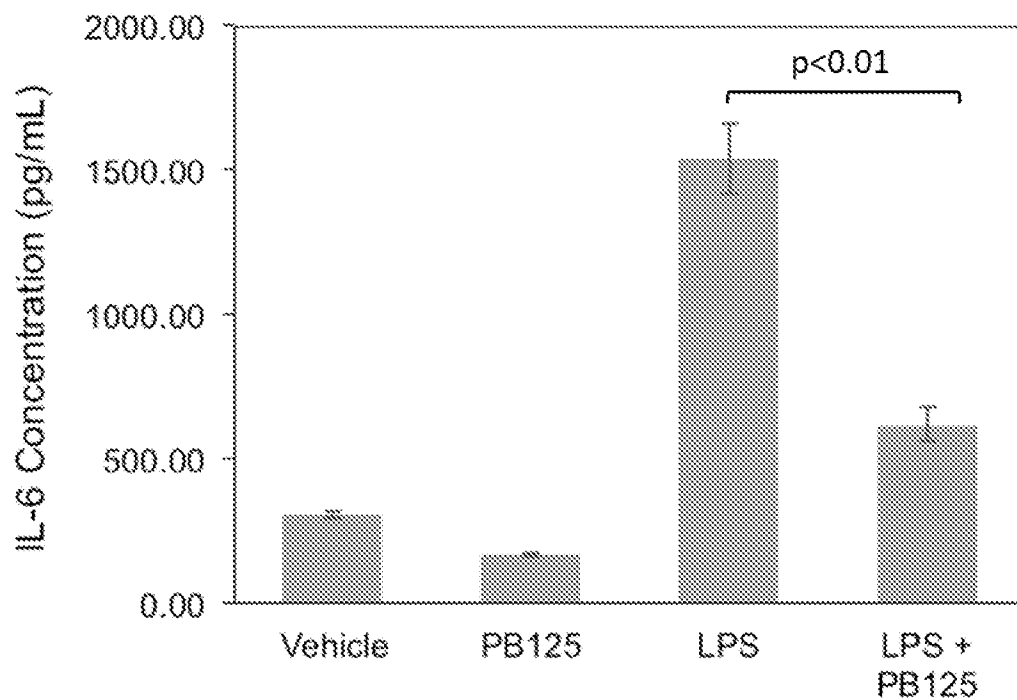


FIG. 12

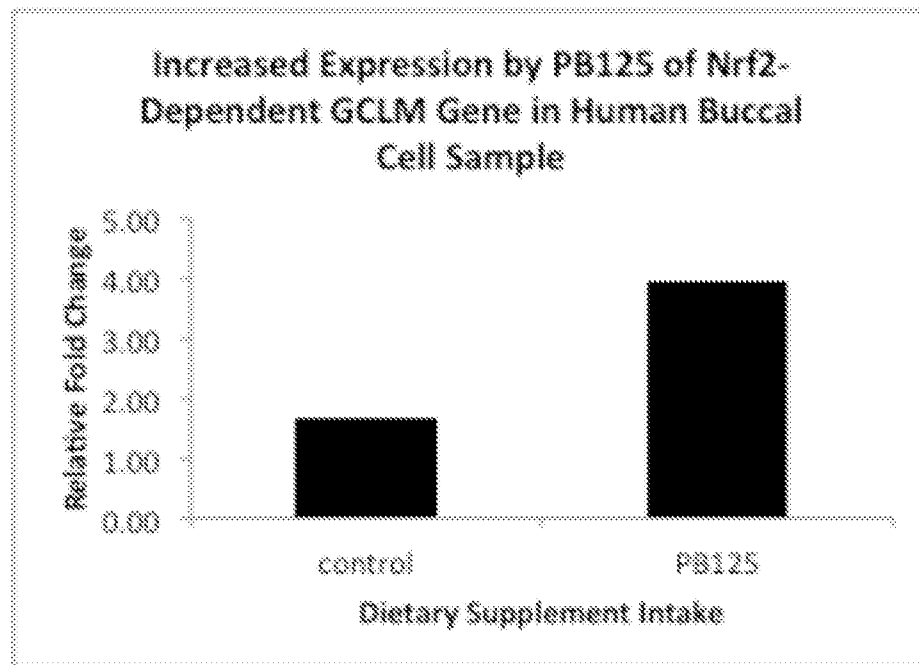


FIG. 13

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/50292

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61P 25/00; A61K 31/426; A61K 31/4439 (2016.01)

CPC - A61K 31/19; A61K 31/26; A61K 31/12

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)

CPC - A61K 31/19; A61K 31/26; A61K 31/12

IPC (8): A61P 25/00; A61K 31/426; A61K 31/4439 (2016.01)

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

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

PatBase, PubWest, ProQuest Dialog, Google

Search Terms: Nrf2, Nuclear factor-erythroid 2 related factor 2, pathway, carnosol, carnosic acid, shogaol, gingerol, luteolin, withaforin  
A, control point, rosemary, ginger, luteolin, ashwagandha, milk thistle, bacopa

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2014/0271944 A1 (McCord et al.) 18 Septmber 2014 (18.09.2014) Title, para [0113], [0116], [0159], [0232], [0281], [0326], [0345], Fig. 1, 34A,	16 ----- 2-3, 5-13, 17-20
X --- Y	US 2009/0304823 A1 (Offord Cavin et al.) 10 December 2009 (10.12.2009) para [0047], [0052], [0076], [0114], [0118], Table 1C,	1, 4, 14-15 ----- 2-3, 5-13, 17-20
X --- Y	WO 2014/151891 A1 (Walley) 25 Septmber 2014 (25.09.2014) abstract, para [0017], [0076]	16 ----- 1
X --- Y	US 2014/0287071 A1 (Barnett, III) 25 Septmber 2014 (25.09.2014) abstarct, para [0005]	16 ----- 1
X,P	WO 2016/037971 A1 (BIOS LINE S.P.A.) 17 March 2016 (17.03.2016) abstract	1, 16

☐ Further documents are listed in the continuation of Box C.

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"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

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Date of the actual completion of the international search

26 October 2016

Date of mailing of the international search report

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