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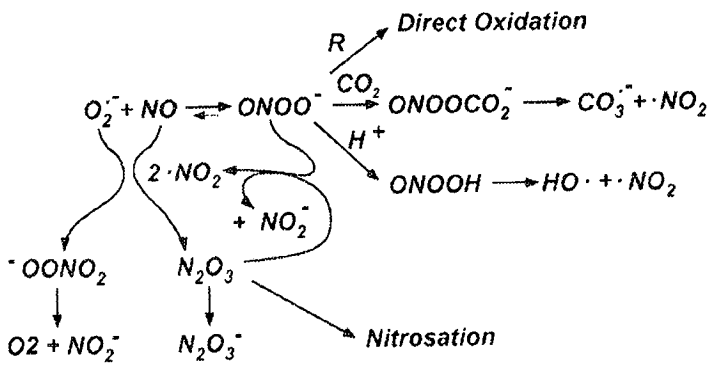

FIG. 1

(57) Abstract: Compositions comprising apple, grape, green tea, and olive extracts are presented herein. This synergistic formulations apple, grape, green tea, and olive extract are in amounts that provide a greater antioxidant activity or protein kinase modulating activity than provided by an equivalent amount of any one extract or a sum of the extracts. Further presented are methods of regulating oxidative stress, disease-associated protein kinase activity, and enhancing the therapeutic effect of a primary therapeutic agent. Also presented are methods of making an activity enhancing composition for regulating oxidative stress, disease-associated protein kinase activity, and enhancing the therapeutic effect of a primary therapeutic agent.

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PHYTOCOMPLEXES EXHIBITING MULTIPLE, SYNERGISTIC ANTIOXIDANT ACTIVITIES USEFUL IN FOODS, DIETARY SUPPLEMENTS, COSMETICS AND PHARMACEUTICAL PREPARATIONS

PRIORITY DATA

This application claims the benefit to United States Provisional Patent Application Serial No. 62/133,945 filed on March 16, 2015, which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

Oxidative stress influences a number of in-vivo metabolic pathways and is implicated in many pathophysiological conditions including disorders associated with tissue-specific modulation of protein kinase activity stimulated through the propagation of reactive species of oxygen and nitrogen. Oxidative stress related pathologies and metabolic disorders can include metabolic syndrome, type I and type II diabetes, obesity, high cholesterol levels accompanied by increased oxidized LDL cholesterol, atherosclerosis, arterial hypertension and various forms of inflammation to name just a few.

BRIEF DESCRIPTION OF THE DRAWINGS

Features and advantages of the invention will be apparent from the detailed description that follows, and which taken in conjunction with the accompanying figures, together illustrate features of the invention. It is understood that the figures merely depict exemplary embodiments and are, therefore, not to be considered limiting in scope.

Fig. 1 schematically displays the interplay of nitric oxide, superoxide, peroxynitrite and nitrogen dioxide in a cell;

Fig. 2 schematically displays the roles played by macrophage (M) during early lesion development within an atherosclerotic plaques of the vascular endothelium; and

Fig. 3 schematically displays the mechanisms of cardiovascular dysfunction in diabetes illustrating the role of superoxide, peroxynitrite and specific kinase signalling cascades.

DESCRIPTION OF EMBODIMENTS

Before invention embodiments are disclosed and described, it is to be understood that no limitation to the particular structures, process steps, or materials disclosed herein is

intended, but also includes equivalents thereof as would be recognized by those ordinarily skilled in the relevant arts. It should also be understood that terminology employed herein is used to describe particular examples only and is not intended to be limiting. The same reference numerals in different drawings represent the same element. Numbers provided in flow charts and processes are provided for clarity in illustrating steps and operations and do not necessarily indicate a particular order or sequence. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise.

As used in this specification, the singular forms "a," "an," and "the" specifically also plural referents, unless the content clearly dictates otherwise. For example, "an excipient" refers to one or more excipients.

Additionally, as used herein, unless specifically indicated otherwise, the word "or" is used in the "inclusive" sense of "and/or" and not the "exclusive" sense of "either/or."

The term "about" is used herein refers to a degree of deviation. It means approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. It is understood that support in this specification for numerical values used in connection with the term "about" is also provided for the exact numerical value itself as though "about" were not used

Concentrations, amounts, and other numerical data may be expressed or presented herein in a range format. It is to be understood that such a range format is used merely for convenience and brevity and thus should be interpreted flexibly to include not only the numerical values explicitly recited as the limits or endpoints of the range, but also to include all the individual numerical values and/or sub-ranges encompassed within that range as if each numerical value (including fractions) and sub-range is explicitly recited. As an illustration, a numerical range of "about 1 to about 5" should be interpreted to include not only the explicitly recited values of about 1 to about 5, but also include individual values and sub-ranges within the indicated range. Thus, included in this numerical range are individual values such as 2, 2.6, 3, 3.8, and 4 and sub-ranges such as from 1-3, from 2-4, and from 3-5, etc., as well as 1, 2, 3, 4, and 5, individually.

Formulation or compositional ingredients included or recited herein are to be presumed to be in wt% unless specifically stated otherwise. In addition, ingredient

amounts presented in the form of ratios are to be presumed to be in wt% (e.g. %w/w) ratios. As such, a composition containing four ingredients at a 1:1:1:1 ratio would indicate that each ingredient is present in an amount of 25 wt%. Accordingly, in some aspects, the amount of an ingredient in a composition or formulation in terms of wt% can be derived from a numerical ratio value.

As used herein, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean "includes," "including," and the like, and are generally interpreted to be open ended terms. The terms "consisting of" or "consists of" are closed terms, and include only the components, structures, steps, or the like specifically listed in conjunction with such terms, as well as that which is in accordance with U.S. Patent law. "Consisting essentially of" or "consists essentially of" have the meaning generally ascribed to them by U.S. Patent law. In particular, such terms are generally closed terms, with the exception of allowing inclusion of additional items, materials, components, steps, or elements, that do not materially affect the basic and novel characteristics or function of the item(s) used in connection therewith. For example, trace elements present in a composition, but not affecting the composition's nature or characteristics would be permissible if present under the "consisting essentially of" language, even though not expressly recited in a list of items following such terminology. When using an open ended term in the specification, like "comprising" or "including," it is understood that direct support should be afforded also to "consisting essentially of" language as well as "consisting of" language as if stated explicitly and vice versa.

As used herein, "substantial" or "substantially" when used in reference to a quantity or amount of a material, or a specific characteristic thereof, refers to an amount that is sufficient to provide an effect that the material or characteristic was intended to provide. The exact degree of deviation allowable may in some cases depend on the specific context. Similarly, "substantially free of" or the like refers to the lack of an identified element or agent in a composition. Particularly, elements that are identified as being "substantially free of" are either completely absent from the composition, or are included only in amounts which are small enough so as to have no measurable effect on the composition.

As used herein, "active agent," "active agent," and the like refer to a molecule, compound, mixture, or ingredient that has a measurable physiologic effect on a subject when administered thereto in an appreciable amount, such as an effective, or

therapeutically effective amount. Like terms such as "active fraction," "active component," and "active constituent" can be used interchangeable therewith. When the activity of an "active agent" exerts or otherwise results in a therapeutic effect of benefit in a subject to which the agent has been administered, the "active agent" can be referred to as a "therapeutic agent".

"Bergamot" refers to bergamot orange (*Citrus bergamia* Risso). This citrus species, grows abundantly in the Calabria region of southern Italy, and has been used in Calabrian folk medicine to treat cardiovascular ailments for centuries. Bergamot comprises two 3-hydroxymethylglutaryl (HMG) derivatives of naturally occurring flavonoid glycosides brutieridin and melitidin. These glycosides are the HMG derivatives of glucosylated hesperetin and naringenin, respectively, and have a structural similarity to the commercially available HMG-CoA reductase inhibitors known as the statins. As used herein bergamot can be used interchangeably to refer to the fruit and/or the extract.

As used herein a "concentrate" refers to dried powder derived from a component that does not include the use of any solvents during the concentration process.

The term "dosage unit" is understood to mean a unitary, *i.e.* a single dose which is capable of being administered to a subject or patient, and that may be readily handled and packed, remaining as a physically and chemically stable unit dose comprising either the active ingredient as such or a mixture of it with solid or liquid pharmaceutical vehicle materials. Dosages can be oral, nasal, enteral, parenteral, transdermal, transmucosal, etc.

The term "extract" refers to those substances prepared using a solvent, e.g., ethanol, water, steam, superheated water, methanol, hexane, chloroform liquid, liquid CO_2, liquid N_2, propane, supercritical CO_2 or any combination thereof. Extracts, as used herein, can refer to an extract in a liquid form, or can refer to a product obtained from further processing of the liquid form, such as a dried powder or other solid form. Extracts may take many forms including but not limited to: solid, liquid, particulate, chopped, distillate, etc. and may be performed by any number of procedures or protocols, such as chopping, grinding, pulverizing, boiling, steaming, soaking, steeping, infusing, applying a gas, etc., and may employ any suitable reagents, such as water, alcohol, steam, or other organic materials. Extracts typically have a given purity percentage and can be relatively to highly pure. In some embodiments, extracts can be phytoextracts made from specific parts of a source, such as the skin, pulp, leaves, flowers, fruits of a plant etc., or can be made from the whole source. In some aspects an extract may include one or more active fractions or active agents. In some extracts, maltodextrin can be added as a carrier. In

some aspects, the purity of an extract can be controlled by, or be a function of the extraction process or protocol.

As used herein, "formulation" and "composition" can be used interchangeably and refer to a combination of at least two ingredients. In some embodiments, at least one ingredient may be an active agent or otherwise have properties that exert physiologic activity when administered to a subject.

As used herein, "increased or decreased concentration, secretion or biosynthesis," means an appreciable increase or decrease in amount (e.g. by at least 3%), concentration, rate of secretion or amount of biosynthesis of the referent compound.

As used herein, "linear inhibitory effect" or "dose-response" refers to a linear decrease in secretion or biosynthesis resulting from all concentrations of the inhibiting material over a dose-response curve. For example, inhibition at low concentrations followed by a failure of inhibition or increased secretion at higher concentrations represents a lack of a linear inhibitory effect.

As used herein, "Leaky Gut Syndrome (LGS)" is an increase in permeability of the intestinal mucosa to luminal macromolecules, antigens and toxins associated with inflammatory degenerative and/or atrophic mucosal damage. LGS can lead to any number of seemingly unrelated symptoms affecting every organ system in the body. LGS has also been linked with having a causative role in a large number of distinct illnesses. Many of these are autoimmune diseases, which means the immune system attacks the body's own cells. LGS plays a role in these types of illness because it increases immune reactions to food particles and then cross reactivity may occur meaning that the immune system attacks body tissues that are chemically similar to the foods to which it has become sensitized. A sampling of the many diseases in which leaky gut syndrome may have a role includes: rheumatoid arthritis, osteoarthritis, asthma, multiple sclerosis, vasculitis, Crohn's Disease, colitis, Addison's disease, lupus, thyroiditis, chronic fatigue syndrome, and fibromyalgia.

As used herein, "pharmaceutically acceptable" refers generally to materials which are suitable for administration to a subject in connection with an active agent or ingredient. For example, a "pharmaceutically acceptable carrier" can be any substance or material that can be suitably combined with an active agent to provide a composition or formulation suitable for administration to a subject. Excipients, diluents, and other ingredients used in or used to prepare a formulation or composition for administration to a subject can be used with such term.

As used herein the term "primary therapeutic agent" designates the presence of a therapeutic agent in a composition at an amount *greater than* the total combined amount of the extracts providing a synergistic effect in the composition.

The term "prevent" and its variants refer to prophylaxis against a particular undesirable physiological condition. The prophylaxis may be partial or complete. Partial prophylaxis may result in the delayed onset of a physiological condition. The person skilled in the art will recognize the desirability of delaying onset of a physiological condition, and will know to administer the compositions of the invention to subjects who are at risk for certain physiological conditions in order to delay the onset of those conditions. For example, the person skilled in the art will recognize that obese subjects are at elevated risk for coronary artery disease. Thus, the person skilled in the art can administer compositions to increase insulin sensitivity in an obese subject, whereby the onset of diabetes mellitus or dyslipemia may be prevented entirely or delayed.

As used herein, "oxidative stress" refers to an imbalance between the manifestations of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates. ROS result in the formation of free radicals. Free radicals (e.g. hydroxyl, nitric acid, superoxide) or the non-radicals (e.g. hydrogen peroxide, lipid peroxide) lead to damage (called oxidative damage) specific molecules with consequential injury to cells or tissue. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. While short term oxidative stress can be beneficial; over time oxidative stress can be involved in the etiology of many conditions and diseases. Increased production of ROS occurs as a result of fungal or viral infection, inflammation, aging, U.V. radiation, pollution, excessive alcohol consumption, cigarette smoking, etc. Removal or neutralization of ROS is achieved with antioxidants, endogenous (e.g. catalase, glutathione, superoxide dismutase) or exogenous (e.g. vitamins A, C, E, bioflavonoids, carotenoids).

As used herein "oxidative stress-associated pathologies" defines any condition that increases the cellular oxidation state to produce an oxidative stress response preceding a disease state. This generally results from increasing the production of reactive oxygen or reactive nitrogen species (ROS and RNS, respectively) (superoxide, hydrogen peroxide, hydroxyl radical, peroxynitrite, singlet oxygen) relative to cellular antioxidant defenses (antioxidants, antioxidant enzymes). Although an oxidative stress response does not necessarily result in disease, it is a critical component in the mechanism of many diseases.

A non-limiting example of such disease includes metabolic syndrome, obesity, atherosclerosis, arterial hypertension, diabetes (types 1, 2 and 3), diminished exercise capacity, premature ejaculation, congestive cardiac failure, cardiovascular disease including cardiac arrest and myocardial infarction, motor dysfunctions, cataracts, cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, Fragile X syndrome pancreatitis, various forms of inflammation including osteoarthritis, rheumatoid arthritis, inflammatory bowel disease, colitis, leaky gut syndrome, renal diseases and hemodialysis, shock, trauma, ischemia, Parkinson's disease, drug reactions, Crohn's disease, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia, many cancers including prostate and breast cancers, enhanced cancer chemotherapy, diseases associated with skin such as slow wound healing, wrinkles, and premature signs of aging.

As used herein the term "secondary therapeutic agent" designates the presence of a therapeutic agent in a composition at an amount *less than* the total combined amount of the extracts providing a synergistic effect in the composition.

The term, "subject," "subjects," or "subjects in need thereof" include humans as well as non-human subjects, particularly domesticated and farm animals. It will be understood that the subject to which a compound of the invention is administered need not suffer from a specific traumatic state. Indeed, the compounds of the invention may be administered prophylactically, prior to any development of symptoms. The term "therapeutic," "therapeutically," and the like are used to encompass therapeutic, palliative as well as prophylactic uses.

As used herein, the term "solvent" refers to a liquid of gaseous, aqueous or organic nature possessing the necessary characteristics to extract solid material from a plant product. Examples of solvents would include, but not limited to, water, steam, superheated water, methanol, ethanol, ethyl acetate, hexane, chloroform, liquid CO_2, liquid N_2, propane, or any combinations of such materials.

As used herein, "synergistic" means more than the additive effect of the individual components against a mechanism of action. For example if F1 produces response X, F2 produces response Y, then the combination of F1 + F2 > X + Y. In some situations F2 produces no response and the value for Y is equal to zero.

The phrase "effective amount," "therapeutically effective amount," or "therapeutically effective rate(s)" of an active ingredient refers to a non-toxic, but sufficient amount or delivery rates of the active ingredient, to achieve therapeutic results

in treating a disease or condition for which the ingredient is being delivered. It is understood that various biological factors may affect the ability of a substance to perform its intended task. Therefore, an "effective amount," "therapeutically effective amount, "or "therapeutically effective rate(s)" may be dependent in some instances on such biological factors. Further, while the achievement of therapeutic effects may be measured by a physician or other qualified medical personnel using evaluations known in the art, it is recognized that individual variation and response to treatments may make the achievement of therapeutic effects a subjective decision. The determination of a therapeutically effective amount or delivery rate is well within the ordinary skill in the art of pharmaceutical sciences and medicine.

The terms "treat," "treating," or "treatment" as used herein and as well understood in the art, mean an approach for obtaining beneficial or desired results, including without limitation clinical results in a subject being treated. Beneficial or desired results can include, but are not limited to, alleviation or amelioration of one or more signs or symptoms of a condition, diminishment of extent of disease, stabilizing (i.e. not worsening) the state of a disease or condition, delaying or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission (whether partial or total), whether detectable or undetectable. For example, where the physiological state is poor glucose tolerance, "treatment" refers to improving the glucose tolerance of a treated subject. As another example, where the physiological state is obesity, the term "treatment" refers to reducing the body fat mass, improving the body mass or improving the body fat ratio of a subject. Treatment of diabetes means improvement of blood glucose control. Treatment of inflammatory diseases means reducing the inflammatory response either systemically or locally within the body. "Treat," "treating," and "treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment and can be prophylactic. Such prophylactic treatment can also be referred to as prevention or prophylaxis of a disease or condition. The prophylaxis may be partial or complete. Partial prophylaxis may result in the delayed onset of a physiological condition. The person skilled in the art will recognize that treatment may, but need not always, include remission or cure.

As used herein, "compounds" may be identified either by their chemical structure, chemical name, or common name. When the chemical structure, chemical name, or common name conflict, the chemical structure is determinative of the identity of the compound. The compounds described herein may contain one or more chiral centers

and/or double bonds and therefore, may exist as stereoisomers, such as double-bond isomers (*i.e.*, geometric isomers), enantiomers or diastereomers. Accordingly, the chemical structures depicted herein encompass all possible enantiomers and stereoisomers of the illustrated or identified compounds including the stereoisomerically pure form (*e.g.*, geometrically pure, enantiomerically pure or diastereomerically pure) and enantiomeric and stereoisomeric mixtures. Enantiomeric and stereoisomeric mixtures can be resolved into their component enantiomers or stereoisomers using separation techniques or chiral synthesis techniques well known to the skilled artisan. The compounds may also exist in several tautomeric forms including the enol form, the keto form and mixtures thereof. Accordingly, the chemical structures encompass all possible tautomeric forms of the illustrated or identified compounds. The compounds described also encompass isotopically labeled compounds where one or more atoms have an atomic mass different from the atomic mass conventionally found in nature. Examples of isotopes that may be incorporated into the compounds of the invention include, but are not limited to, ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, etc. Compounds may exist in un-solvated forms as well as solvated forms, including hydrated forms and as N-oxides. In general, compounds may be hydrated, solvated or N-oxides. Certain compounds may exist in multiple crystalline or amorphous forms. Also contemplated are congeners, analogs, hydrolysis products, metabolites and precursor or prodrugs of the compound. In general, all physical forms are equivalent for the uses contemplated herein and are intended to be within the scope of the present disclosure.

Comparative terms such as "more effectively," "greater than," "improved," "enhanced," and like terms can be used to state a result achieved or property present in a formulation or process that has a measurably better or more positive outcome than the thing to which comparison is made. In some instances comparison may be made to the prior art.

Reference is made hereinafter in detail to specific embodiments of the invention. While the invention will be described in conjunction with these specific embodiments, it will be understood that it is not intended to limit the invention to such specific embodiments. On the contrary, it is intended to cover alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims. In the following description, numerous specific details are set forth in order to provide a thorough understanding of the present invention. The present invention may be practiced without some or all of these specific details. In other instances,

well known process operations have not been described in detail, in order not to unnecessarily obscure the present invention.

All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA.

In humans, oxidative stress is involved in the etiology of many diseases. For example, oxidative stress is implicated in metabolic syndrome, obesity, atherosclerosis, arterial hypertension, diabetes (types I, II, and III), diminished exercise capacity, premature ejaculation, congestive cardiac failure, cardiovascular disease including cardiac arrest and myocardial infarction, motor dysfunctions, cataracts, cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, Fragile X syndrome pancreatitis, various forms of inflammation including osteoarthritis, rheumatoid arthritis, inflammatory bowel disease, colitis, leaky gut syndrome, renal diseases and hemodialysis, shock, trauma, ischemia, Parkinson's disease, drug reactions, Crohn's disease, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia, many cancers including prostate and breast cancers, enhanced cancer chemotherapy, diseases associated with skin such as slow wound healing, wrinkles, premature signs of aging, as well as others.

Reactive oxygen species (ROS), reactive nitrogen species (RNS), other free radicals, and oxidant sources (collectively i.e. O_2^-, $1O_2$, HO^-, NO^-, $ONOO^-$, HOCl, $RO(O)^-$, $O(O)^-$), can cause severe damage to cells of the body. For example, this damage can be to the DNA, proteins, and other macromolecules and forms the basis for a wide variety of inflammation-based diseases.

Experimental evidence directly or indirectly suggests that there are six *major* reactive oxygen species that cause oxidative damage in the human body. These species include superoxide anion (O^-), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO^-), hydroxyl radical (HO^-), singlet oxygen ($1O_2$), and peroxynitrite ($ONOO^-$). In order to combat this damage, antioxidants inhibit the oxidation and prevent the formation of free radicals. Within biological systems, there are at least four general sources of antioxidants: (1) enzymes, (i.e. superoxide dismutase, glutathione peroxidase, and catalase); (2) large molecules (i.e. albumin, ceruloplasmin, ferritin, other proteins); (3) small molecules,

(ascorbic acid, glutathione, uric acid, tocopherol, carotenoids, (poly) phenols); and (4) some hormones (estrogen, angiotensin, melatonin, etc.).

Oxidants and antioxidants can have different chemical and physical characteristics. Individual antioxidants can act by multiple mechanisms in a single system or by a different single mechanism and can respond in a different manner to different radicals or oxidant sources. For example, carotenoids are not particularly good quenchers of peroxyl radicals relative to phenolics and other antioxidants; however, carotenoids are exceptional in quenching singlet oxygen, at which most other phenolics and antioxidants are relatively ineffective. Singlet oxygen is not a radical and does not react via radical mechanisms but reacts mostly by the addition to double bonds, forming endo- peroxides that can be reduced to alkoxyl radicals that initiate radical chain reactions. Due to the multiple reaction characteristics and mechanisms as well as different phase localizations are usually involved, no single assay will accurately reflect all of the radical sources or all antioxidants in a mixed or complex system.

Living cells have a biological defense system composed of enzymatic antioxidants that convert ROS/RNS to harmless species. For example, H_2O_2 can be converted to water and oxygen by catalase. In another example, $O^{\cdot}$ is converted to oxygen and hydrogen peroxide by superoxide dismutase (SOD) or reacts with nitric oxide ($NO^{\cdot}$) to form peroxynitrite. When nitric oxide and superoxide are both present, they may also react with nitrogen dioxide to form N_2O_3 and peroxynitrate (*See* Figure 1). Peroxynitrate decomposes to give nitrite and oxygen, while N_2O_3 can react with thiols to give nitrosothiols or with hydroxide anion to give nitrite. Peroxynitrate also reacts at a diffusion-limited rate with peroxynitrite to yield two molecules of nitrogen dioxide and one of nitrite. This creates a cycle to generate more nitrogen dioxide when bolus additions of peroxynitrite are added at neutral pH and substantially increases the number of potential reactions occurring. These same reactions will also occur in vivo, particularly when nitric oxide is produced faster than superoxide.

Peroxynitrite can also be naturally produced by alveolar macrophages (*See* Figure 2). When alveolar macrophages are stimulated to produce both superoxide and nitric oxide, peroxynitrite is quantitatively produced as evidenced by the amount of nitric oxide and superoxide produced and the amount of oxygen consumed. Extracellular addition of superoxide dismutase (SOD) in high concentrations does not significantly reduce the amount of peroxynitrite formed and instead serves as a catalyst of tyrosine nitration. This suggests that superoxide produced at the membrane surface and nitric oxide diffusing

through the membrane react at the membrane interface so quickly that SOD in the bulk phase cannot compete.

Nitric oxide (NO) and peroxynitrite play significant roles in cardiovascular pathophysiology. NO can activate the soluble guanylate cyclase (sGC)-cGMP signal transduction pathway which mediates various physiological/beneficial effects in the cardiovascular system including vasodilatation, inhibition of platelet aggregation, anti-inflammatory, anti-remodeling, and anti-apoptotic effects. Under pathological conditions associated with increased oxidative stress and inflammation (myocardial infarction, ischemic heart disease, myocarditis, cardiomyopathy, hypertension, etc.), NO and superoxide (O_2^-) react to form peroxynitrite ($ONOO^-$) which induces cell damage via lipid peroxidation, inactivation of enzymes and other proteins by oxidation and nitration, and also activation of stress signaling, matrix metalloproteinases (MMPs) among others.

Peroxynitrite also triggers the release of proapoptotic factors such as cytochrome c and apoptosis-inducing factor (AIF) from the mitochondria, which mediate caspase-dependent and -independent apoptotic death pathways. Moreover, peroxynitrite, in concert with other oxidants, causes stand breaks in DNA, activating the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1). Mild damage of DNA activates the DNA repair machinery. In contrast, once excessive oxidative and nitrosative stress-induced DNA damage occurs, like in various forms of myocardial reperfusion injury and heart failure, over activated PARP initiates an energy-consuming cycle by transferring ADP-ribose units from nicotinamide adenine dinucleotide (NAD+) to nuclear proteins, resulting in rapid depletion of the intracellular NAD^+ and ATP pools, slowing the rate of glycolysis and mitochondrial respiration, eventually leading to cellular dysfunction and death. Poly(ADP-ribose) glycohydrolase (PARG) degrades poly(ADP-ribose) (PAR) polymers, generating free PAR polymer and ADP-ribose. Over activated PARP also facilitates the expression of a variety of inflammatory genes leading to increased inflammation and associated oxidative stress, thus facilitating the progression of cardiovascular dysfunction and heart.

Peroxynitrite also functions to amplify the inflammatory signaling in chronic inflammatory conditions. Moreover, inflammation is triggered by the activation of multiple signaling cascades culminating in the up regulated production of an array of pro-inflammatory cytokines and chemokines. Those initiate a more complex inflammatory reaction characterized by the activation of inflammatory cells and the stimulated activity of enzymes, including inducible NO synthase (iNOS), which produces high amounts of

NO, and the superoxide (O_2^-) producing enzymes NADPH oxidase (NADPHox) and xanthine oxidase (XO). The simultaneous production of NO and O_2^- results in the generation of peroxynitrite ($ONOO^-$), which in turn damages target molecules including proteins, glutathione (GSH), mitochondria, and DNA. DNA damage can initiate apoptotic cell death and is also the obligatory trigger for the activation of poly(ADP-ribose) polymerase (PARP), which may induce cell necrosis by ATP depletion. Both $ONOO^-$ and PARP further participate to the up regulation of pro-inflammatory signal transduction pathways, thereby producing a self- amplifying cycle of inflammatory cell injury.

Superoxide and peroxynitrite also coordinate cardiovascular dysfunction in diabetes (*See* Figure 3). Hyperglycemia induces increased superoxide anion (O_2^-) production via activation of multiple pathways including xanthine and NAD(P)H oxidases, cyclooxygenase, uncoupled nitric oxide synthase (NOS), glucose autoxidation, mitochondrial respiratory chain, polyol pathway, and formation of advanced glycation end products (AGE). Superoxide activates AGE, protein kinase C (PKC), polyol (sorbitol), hexosamine, and stress-signaling pathways leading to increased expression of inflammatory cytokines, angiotensin II (Ang II), endothelin-1 (ET-1), and NAD(P)H oxidases, which in turn generate more superoxide via multiple mechanisms. Hyperglycemia-induced increased superoxide generation may also favor an increased expression of nitric oxide synthases (NOS) through the activation of NFκB, which may increase the generation of nitric oxide (NO). Superoxide anion may quench NO, thereby reducing the efficacy of a potent endothelium-derived vasodilator system. Superoxide can also be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) and interact with NO to form a reactive oxidant peroxynitrite ($ONOO^-$), which induces cell damage via lipid peroxidation, inactivation of enzymes and other proteins by oxidation and nitration, and activation of matrix metalloproteinases (MMPs) among others.

Peroxynitrite can also act on mitochondria [decreasing the membrane potential (Ψ)], triggering the release of proapoptotic factors such as cytochrome c (Cyt c) and apoptosis-inducing factor (AIF). These factors mediate caspase-dependent and caspase-independent apoptotic death pathways.

Peroxynitrite, in concert with other oxidants (e.g., H_2O_2), can cause strand breaks in DNA, activating the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1). Mild damage to DNA activates the DNA repair machinery. Once excessive oxidative and nitrosative stress-induced DNA damage occurs, overactivated PARP-1 initiates an energy-consuming cycle by transferring ADP-ribose units (small red spheres) from NAD^+ to

nuclear proteins, resulting in rapid depletion of the intracellular NAD^+ and ATP pools, slowing the rate of glycolysis and mitochondrial respiration, and eventually leading to cellular dysfunction and death. Poly(ADP-ribose) glycohydrolase (PARG) degrades poly(ADP-ribose) (PAR) polymers, generating free PAR polymer and ADP-ribose, which may signal to the mitochondria to induce AIF release. PARP-1 activation also leads to the inhibition of cellular glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity, which in turn favors the activation of PKC, AGE, and hexosamine pathway leading to increased superoxide generation. PARP-1 also regulates the expression of a variety of inflammatory mediators, which might facilitate the progression of diabetic cardiovascular complications.

Additional conditions in which the reaction products of peroxynitrite have been detected and in which pharmacological inhibition of its formation or its decomposition have been shown to be of benefit include vascular diseases, ischaemia–reperfusion injury, circulatory shock, pain and neurodegeneration.

No enzymatic action is presently known that scavenges $ROO^{\cdot}$, $HO^{\cdot}$, $1O_2$, and $ONOO^{\cdot}$. Therefore, the burden of defense relies on a variety of non-enzymatic antioxidants such as vitamins C and E and many phytochemicals that have the property of scavenging oxidants and free radicals. To comprehensively evaluate the oxidant-scavenging capacity of a food sample, assays have to be designed to include these ROS (i.e. ORAC (Oxygen Radical Absorbance Capacity) Assay).

At the cellular level, signal transduction refers to the movement of a signal or signaling moiety from outside of the cell to the cell interior. The signal, upon reaching its receptor target, may initiate ligand-receptor interactions requisite to many cellular events, some of which may further act as a subsequent signal. Such interactions serve to not only as a series cascade but moreover an intricate interacting network or web of signal events capable of providing fine-tuned control of homeostatic processes. This network however can become dysregulated, thereby resulting in an alteration in cellular activity and changes in the program of genes expressed within the responding cell.

Signal transducing receptors are generally classified into three classes. The first class of receptors are receptors that penetrate the plasma membrane and have some intrinsic enzymatic activity. Representative receptors that have intrinsic enzymatic activity include: tyrosine kinases (*e.g.* PDGF, insulin, EGF and FGF receptors), tyrosine phosphatases (*e.g.* CD45 [*cluster determinant-45*] protein of T cells and macrophages), guanylate cyclases (*e.g.* natriuretic peptide receptors) and serine/threonine kinases (*e.g.*

activin and TGF-β receptors). Receptors with intrinsic tyrosine kinase activity are capable of autophosphorylation as well as phosphorylation of other substrates.

Receptors of the second class are those that are coupled, inside the cell, to GTP-binding and hydrolyzing proteins (termed G-proteins). Receptors of this class that interact with G-proteins have a structure that is characterized by seven trans-membrane spanning domains. These receptors are termed *serpentine* receptors. Examples of this class are the adrenergic receptors, odorant receptors, and certain hormone receptors (*e.g.* glucagon, angiotensin, vasopressin and bradykinin).

The third class of receptors may be described as receptors that are found intracellularly and upon ligand binding, migrate to the nucleus where the ligand-receptor complex directly affects gene transcription.

The proteins which encode for receptor tyrosine kinases (RTK) contain four major domains, those being: a) a transmembrane domain, b) an extracellular ligand binding domain, c) an intracellular regulatory domain, and d) an intracellular tyrosine kinase domain. The amino acid sequences of RTKs are highly conserved with those of cAMP-dependent protein kinase (within the ATP and substrate binding regions). RTK proteins are classified into families based upon structural features in their extracellular portions which include the cysteine rich domains, immunoglobulin-like domains, cadherin domains, leucine-rich domains, Kringle domains, acidic domains, fibronectin type III repeats, discoidin I-like domains, and EGF-like domains. Based upon the presence of these various extracellular domains the RTKs have been sub-divided into at least 14 different families.

Many receptors have intrinsic tyrosine kinase activity upon phosphorylation and can interact with other proteins of the signaling cascade. These other proteins contain a domain of amino acid sequences that are homologous to a domain first identified in the c-Src proto-oncogene; these domains are termed SH2 domains. The interactions of SH2 domain containing proteins with RTKs or receptor associated tyrosine kinases leads to tyrosine phosphorylation of the SH2 containing proteins. The resultant phosphorylation produces an alteration (either positively or negatively) in that activity. Several SH2 containing proteins that have intrinsic enzymatic activity include phospholipase C-γ (PLC-γ), the proto-oncogene c-Ras associated GTPase activating protein (rasGAP), phosphatidylinositol-3-kinase (PI-3K), protein tyrosine phosphatase-1C (PTP1C), as well as members of the Src family of protein tyrosine kinases (PTKs).

Non-receptor protein tyrosine kinases (PTK) by and large couple to cellular receptors that lack enzymatic activity themselves. An example of receptor-signaling through protein interaction involves the insulin receptor (IR). This receptor has intrinsic tyrosine kinase activity but does not directly interact, following autophosphorylation, with enzymatically active proteins containing SH2 domains (*e.g.* PI-3K or PLC-γ). Instead, the principal IR substrate is a protein termed IRS-1.

The receptors for the TGF-β superfamily represent the prototypical receptor serine/threonine kinase (RSTK). Multifunctional proteins of the TGF- β superfamily include the activins, inhibins and the bone morphogenetic proteins (BMPs). These proteins can induce and/or inhibit cellular proliferation or differentiation and regulate migration and adhesion of various cell types. One major effect of TGF-β is a regulation of progression through the cell cycle. Additionally, one nuclear protein involved in the responses of cells to TGF-β is c-Myc, which directly affects the expression of genes harboring Myc-binding elements. PKA, PKC, and MAP kinases represent three major classes of non-receptor serine/threonine kinases.

There can be a relationship between kinase activity and disease states. Such relationships can be either causative of the disease itself or intimately related to the expression and progression of disease-associated symptomology and pathology. For example, kinase activity is thought to be implicated in cognitive disorders, including Alzheimer's disease, congestive cardiac failure, pulmonary hypertension, cardiomyopathies, motor dysfunction, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, various forms of inflammation including osteoarthritis, rheumatoid arthritis, type I and type II diabetes, metabolic syndrome, obesity, inflammatory bowel disease, Crohn's disease, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, and fibromyalgia among others.

Autoimmune diseases result from a dysfunction of the immune system in which the body produces auto-antibodies that attack its own organs, tissues and cells – a process mediated via protein phosphorylation. Over 80 clinically distinct autoimmune diseases have been identified and collectively afflict approximately 24 million people in the U.S. Autoimmune diseases can affect any tissue or organ of the body. Because of this variability, they can cause a wide range of symptoms and organ injuries, depending upon the site of autoimmune attack. Although treatments exist for many autoimmune diseases, there are no definitive cures for any of them. Treatments to reduce the severity often have adverse side effects.

The etiology and pathogenesis of autoimmune diseases in humans is still poorly understood, but is viewed to progress in three phases, an initation phase, an effector phase, and an activation phase. In the initiation phase, dendritic cells present self-antigens to autoreactive T cells. The T cells activate autoreactive B cells via cytokines resulting in the production of autoantibodies, which in turn form immune complexes in joints. In the effector phase, the immune complexes bind Fcf receptors on macrophages and mast cells, resulting in release of cytokines and chemokines, inflammation and pain. In the activation phase, the final phase, cytokines and chemokines activate and recruit synovial fibroblasts, osteoclasts and polymorphonuclear neutrophils that release proteases, acids, and ROS such as $O_2^{\cdot}$, resulting in irreversible cartilage and bone destruction. B cell activation signals through spleen tyrosine kinase (Syk) and phosphoinositide 3-kinase (PI3K) following antigen receptor triggering. After the engagement of antigen receptors on B cells, Syk is phosphorylated on three tyrosines. Syk is a 72-kDa protein-tyrosine kinase that plays a central role in coupling immune recognition receptors to multiple downstream signaling pathways. This function is a property of both its catalytic activity and its ability to participate in interactions with effector proteins containing SH2 domains. Phosphorylation of Tyr-317, -342, and -346 create docking sites for multiple SH2 domain containing proteins. Association of the 72-kDa protein-tyrosine kinase Ptk72 with the B-cell antigen receptor. In one invention aspect, there is provided a safe, long-term treatment approach for pain relief in these patients suffering from autoimmune disorders. Since inducers of COX-2 and iNOS synthesis signal through the Syk, PI3K, p38, ERK1/2, and NF-kB dependent pathways, inhibitors of these pathways may be therapeutic in autoimmune conditions and in particular in the inflamed and degenerating joints of rheumatoid arthritis (RA) or osteoarthritis (OA) patients.

Syk has been shown to be required for the activation of PI3K in response to a variety of signals including engagement of the B cell antigen receptor (BCR) and macrophage or neutrophil Fc receptors. In B cells, the BCR-stimulated activation of PI3K can be accomplished through the phosphorylation of adaptor proteins such as BCAP, CD19, or Gab1, which creates binding sites for the p85 regulatory subunit of PI3K. Signals transmitted by many IgG receptors require the activities of both Syk and PI3K and their recruitment to the site of the clustered receptor. In neutrophils and monocytes, a direct association of PI3K with phosphorylated immuno-receptor tyrosine based activation motif sequences on FcgRIIA was proposed as a mechanism for the recruitment of PI3K to the receptor. A direct molecular interaction between Syk and PI3K has been reported.

The cdc-like kinase CLK1 is involved in cell proliferation as a dual-specificity kinase acting on both serine/threonine and tyrosine-containing substrates; it phosphorylates serine- and arginine-rich proteins of the spliceosomal complex and may be a constituent of a network of regulatory mechanisms that enable SR proteins to control RNA splicing. The Clks also regulate the alternative splicing of microtubule-associated protein tau and are implicated in frontotemporal dementia and Parkinson's disease through the phosphorylation of splicing factors. Inhibitors of Clk isoforms may alter these events and could prove to be useful agents in disease phenotypes characterized by abnormal splicing.

The signaling pathways identified for the insulin receptor (IR) include G-protein coupled receptor signaling pathways, activation of MAPK activity, activation of protein kinase B activity, carbohydrate metabolic process, cellular response to growth factor stimulus, exocrine pancreas development, glucose homeostasis, positive regulation of glucose import, positive regulation of glycogen biosynthetic process, and positive regulation of glycolysis.

Glycogen synthase kinase 3 (GSK3) was initially described as a key enzyme involved in glycogen metabolism, but is now known to regulate a diverse array of cell functions. Two forms of the enzyme, GSK-3a and GSK-3b, have been previously identified. Small molecules inhibitors of GSK-3 may, therefore, have several therapeutic uses, including the treatment of neurodegenerative diseases, diabetes type II, bipolar disorders, stroke, cancer, and chronic inflammatory disease. Glycogen synthase kinase 3 (GSK-3) inhibitors as new promising drugs for diabetes, neurodegeneration, cancer, and inflammation.

AMP-activated protein kinase (AMPK) plays a key role as a master regulator of cellular energy homeostasis. The kinase is activated in response to stresses that deplete cellular ATP supplies such as low glucose, hypoxia, ischemia, and heat shock. Due to its role as a central regulator of both lipid and glucose metabolism, AMPK is considered to be a potential therapeutic target for the treatment of type II diabetes mellitus, obesity, and cancer. AMPK has also been implicated in a number of species as a critical modulator of aging through its interactions with mTOR and sirtuins.

In one invention embodiment, there is presented oxidative stress modulating compositions and associated methods. An exemplary composition can comprise a combination of apple, grape, green tea, and olive extracts in amounts that provide a greater antioxidant activity than provided by an equivalent amount of any one extract or a sum of

the extracts. In one example, the apple, grape, green tea, and olive extracts can comprise extracts formulated from the leaves, skin, rind, pulp, juice, seeds, or combinations of these raw materials.

In one example, the apple extract can comprise an extract derived from a member selected from the group consisting of *Malus domestica, Malus sieversii, Malus sylvestris, Malus pumila,* and combinations thereof. In one example the apple extract can be derived from the species *Malus pumila.* In one example the apple extract can be derived from a combination of *Malus domestica* and *Malus pumila.* In some embodiments the apple extract can comprise any or all parts of the apple, including but not limited to the skin, flesh/fruit (exocarp, mesocarp, and/or endocarp), seed, stalk, stem, leaf, or a combination thereof. In one example, the apple extract comprises the skin and fruit of the apple. In some embodiments, the extract can be derived from immature apples. In one embodiment, an extraction solvent can be ethanol.

In one example, the grape extract can comprise a member selected from the group consisting of *Vitis vinifera, Vitis labrusca, Vitis riparia, Vitis rotundifolia, Vitis rupestris, Vitis aestivalis, Vitis mustangensis*, and combinations thereof. In one example, the grape extract can be derived from *Vitis vinifera.* In some embodiments, the grape extract can comprise any or all parts of the grape including but not limited to the skin, flesh/fruit, seed, vascular bundles, vine, leaves, or combination thereof. In one embodiment, the grape extract can be derived from the seeds. In another embodiment, the grape extract can be derived from the skin. In yet another embodiment, the grape extract can be derived from the seeds and skin of the grape. In some embodiments, the grape extract comprises from about 75 wt% to about 95 wt% phenolics on a dry weight basis. In other embodiments, the grape extract can comprise from about 80 wt% to 97 wt% phenolics on a dry weight basis. In one example, the extraction solvent can be ethanol, water, or a mixture thereof.

In one example, the green tea extract can be derived from *Camellia sinensis.* In some embodiments, the green tea extract can comprise any or all parts of the tea including but not limited to the leaf, seed, stem, flower, or combination thereof. In one embodiment, the green tea extract can be derived from the leaves. In another example, the extract solvent can be water, ethanol, ethyl acetate, or combinations thereof.

In one example, the olive extract comprises a subspecies of *Olea europea* selected from the group consisting of the subspecies *europea, cuspidiata, guanchica, cerasiformis, maroccana, laperrinei, cerasiformis,* or a combination thereof. In some embodiments, the olive extract can comprise any or all parts of the olive including but not limited to the leaf,

seed, pulp, fruit, stem, or combination thereof. In one embodiment, the olive extract can be derived from the leaves. In another example, the extraction solvent can be an ethanol and water solution.

In some embodiments, the plant or herb to extract ratio can range from about 1 to about 10. In other examples, the raw plant or herb to extract ratio can be from about 2 to about 5, from about 4 to about 7, or from about 8 to about 10. Moreover, the ratio of the extracts can be present in the formulation at any ratio to the other extracts that provides a greater antioxidant activity than provided by an equivalent amount of any one extract or a sum of the extracts. In one example, at least one of the extracts in the composition can be present in a different amount than the other extracts. In another example, the extracts can all be present in the composition at the same amount.

By way of example, in some embodiments, each extract can be present at a ratio of from about 1 to about 50 times the amount of another extract. This applies to each and every extract in the formulation, including those listed in the examples below. In one aspect, the apple extract can be present in the formulation at a ratio of from 1 to 50 times the amount of a grape, green tea, or olive extract. In another aspect, the apple extract can be present in the formulation at a ratio of from about 1 to 25 times the amount of a grape, green tea, or olive extract. In a further aspect, the apple extract in the formulation can be present at a ratio of from 1 to 10 times the amount of a grape, green tea, or olive extract. In an additional aspect, the apple extract can be present in at a ratio of from 1 to 5 times the amount of a grape, green tea, or olive extract. In yet another aspect, the apple extract can be present in the formulation at a ratio of 1 times the amount of a grape, green tea, or olive extract. Any specific numerical value within the numerical range is included. In fact, each of the apple, grape, green tea, and olive extracts may be present in a ratio of anywhere between 1 to 50 times and 1 times the amount of the other extracts. For example, the amount of apple extract to grape extract to green tea extract to olive extract may in some embodiments be 1-25:1-25:1-25:1-25 respectively. As such, any number given specific ratio that yields a synergistic effect as recited herein can be used, for example 25:1:1:1 or 1:25:1:1, or 1:1:25:1, 1:1:1:25. When considered in terms of wt%, this would equate to one ingredient being present in an amount of 89.28 wt% and the other three ingredients being present in amounts of 3.57 wt%. This can be considered either in terms of the formulation as a whole (when only these four ingredients are present), or in terms of the synergistic extract or enhancer portion of the formulation only. For example, in a formulation containing only these four extracts, at a 1:1:1:1 ratio the relative amount

of each would be 25 wt% each (i.e. 100/4= 25). However, in a formulation where the amount of apple, grape, green tea an olive extracts was only 20 wt% of the total formulation and the extracts of each were present in a 1:1:1:1 ratio respectively, it can be considered that each extract is present in the overall formulation in an amount of 5 wt% each (25 x 20 x .001 = 5wt%).

As mentioned, any number of ratios of one extract to another that results in a synergistic effect (i.e. more than an equal amount of activity provided by any one extract, or more than the simple sum of the activity provided by the total extracts) can be used. For example, keeping in the order of the extracts listed above, in one embodiment, the ratios can be any number within the range of 1-50 for each extract, such as 1-10:1-10:1-10:1-10, which would include without limitation, 1:1:1:1, 1:2:1:2, 1:5:6:1, 10:1:5:2, 3:7:2:4, and 1:3:5:8 for example. Additionally, these ratios and ranges of ratios can be applied to not only the four ingredients of apple, grape, green tea, and olive extracts, but also to the other extracts listed in the examples below as PC 4.1, 4.2, 8, 9, or 10. In some embodiments, the extracts can all be present at a weight ratio of about 1:1:1:1. In other embodiments, the apple, grape, green tea, and olive extracts can be present at a weight ratio of about 6:1:3:1.

In some embodiments, the composition further comprises a primary or a secondary therapeutic agent. In one embodiment, the primary or secondary therapeutic agent can comprise a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, aminogen®, biotin, black bean powder, copper citrate, ferrous fumarate, fructose, garbanzo bean, gum arabic, magnesium oxide, manganese citrate, medium chain triglycerides, pea fiber, pea protein isolate, potassium citrate, vitamin B6, riboflavin, rice bran, rice protein, sodium citrate, sodium selenate, thiamin HCl, vitamin D2, vitamin E, zinc citrate, adzuki bean, D-calcium pantothenate, lycopene, polyphenols, ascorbic acid, β-glucans, lutein, blueberry, borage oil, broccoli flowers, carrot root, cranberry fruit, chromium nicotinate, cyanocobalamin, flax seed/linum usitatissimum, folic acid, lo han extract, niacinamide, pomegranate fruit, vitamin A, carotentiods, vitamin E, phytosterols, lignin, CoQ10, glutathione, and combinations thereof.

In another embodiment, the primary or secondary therapeutic agent can comprise a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10,

vitamins, fiber, inulin, and combinations thereof. In one embodiment, the primary or secondary therapeutic agent can be bergamot extract. In another example, the primary or secondary therapeutic agent can be mangosteen extract. The mangosteen extract can be a mangosteen fruit extract and/or mangosteen pericarp extract. In a further embodiment, the primary or secondary therapeutic agent can comprise a mangosteen pericarp extract and a bergamot extract. In yet another example, the primary or secondary therapeutic agent can comprise berberine. In another example, the primary or secondary therapeutic agent can comprise arginine and beet or citrulline and beet. In a further embodiment, the primary or secondary therapeutic agent can comprise phytosterols. In another embodiment, the primary or secondary therapeutic agent can comprise protein. The protein can be a whey protein, soy protein, pea protein, a calcium caseinate protein, or a combination thereof. In yet another example the primary or secondary therapeutic agent comprises curcumin. In a further embodiment, the primary or secondary therapeutic agent comprises a fiber source and/or inulin.

In one embodiment, the bergamot extract can be derived from *Citrus bergamia Risso*. In one embodiment, the mangosteen extract can be derived from *Garcinia mangosana*. In one example, the mangosteen extract can be an extract of the fruit, the pericarp, or both the fruit and the pericarp. The fruit extract can be derived from any part of the fruit including but not limited to the pulp, the rind, the seeds, or a combination thereof. In one embodiment the mangosteen pericarp extract is derived solely from the rind of the fruit.

In some embodiments, the apple, grape, green tea, and olive extract composition further comprises blueberry extract/concentrate, capsicum extract, and turmeric extract. In one example, the blueberry extract/concentrate can be obtained from *Vaccinium angustifolium*. In one example the blueberry concentrate can be a dried powder created without the use of a solvent. In one embodiment, it can take about 5 kg, about 8 kg, about 10 kg, or about 12 kg of blueberries to obtain 1 kg of dried powder. In one embodiment, the capsicum extract can be obtained from *Capsicum annuum*. In some embodiments, capsicum extract can be derived from powdered dried ripe fruits. In one example the turmeric extract can be obtained from *Curcuma longa*. In some embodiments, the turmeric extract can comprise an extract of the root, the rhizome, or a combination thereof. In another embodiment, the turmeric extract can be derived from a turmeric powder. In on embodiment, the turmeric powder can have from about 1 to about 10% curcuminoids, from about 3 to about 5% curcuminoids, from about 2% to about 8% curcuminoids, or

from about 4% to about 12% curcuminoids. In some embodiments, the grape extract can comprise an extract of a grape skin and a grape seed extract. In one embodiment, the composition comprising the blueberry concentrate, capsicum extract, and turmeric extract in addition to the apple, grape, green tea, and olive extract can further comprise a mangosteen extract. The mangosteen extract can comprise a *Garcinia mangostana* extract and can be a fruit extract, a pericarp extract, or a combination thereof. In other embodiments, the composition comprising the blueberry concentrate, capsicum extract, and turmeric extract in addition to the apple, grape, green tea, and olive extract can further comprise a bergamot extract. In another embodiment, the composition comprising the blueberry concentrate, capsicum extract, and turmeric extract in addition to the apple, grape, green tea, and olive extract can further comprise a mangosteen extract and a bergamot extract.

The antioxidant compositions can have a variety of mechanisms of action. In one embodiment the antioxidant composition can quench free radicals. In another embodiment, the antioxidant composition can modulate peroxynitrate formation. In one example, the antioxidant composition modulates stress signaling enzymes such as matrix metalloproteinases and myeloperoxidase.

The antioxidant compositions can be used for modulating oxidative stress in a mammal in need thereof. Oxidative stress related conditions can include, but without limitation, metabolic syndrome, type I, type II and type III diabetes, obesity, high cholesterol levels accompanied by increased oxidized LDL cholesterol, various forms of inflammation including osteoarthritis, rheumatoid arthritis, endotoxemia, inflammatory bowel disease, leaky gut, Crohn's disease, prostate hyperplasia, lower urinary tract symptoms (LUTS), pulmonary arterial hypertension, diminished exercise capacity, premature ejaculation, low female sex drive, congestive disorders, cardiac failure, pulmonary hypertension, various cardiovascular diseases, motor dysfunction, cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia skin disorders such as wrinkles, discolorations and sagging, as well as cancers arising from oxidized damage to DNA, and other disorders associated with tissue-specific modulation of protein kinase activity stimulated through the propagation of reactive species of oxygen and nitrogen. In one example, the mammal in need thereof has increased oxidized LDL(oxLDL) cholesterol. In another example, the mammal in need thereof has at least one of metabolic syndrome, type I, II, or II diabetes.

In another example, the mammal in need thereof has at least one of leaky gut, endotoxemia, or inflammatory bowel disease.

The antioxidant compositions can be useful for treating a spectrum of physiological disorders in which oxidative stress participates through etiology, expression, or progression. The phytocomplexes and various combinations thereof that may be used to regulate oxidative stress to treat numerous disease-related signs and symptoms with a concomitant increase in the quality of life. The resulting compositions can be consumed as a dietary supplement to address the risk factors associated with oxidative stress, benign prostate hyperplasia, obesity, metabolic syndrome, diabetes, increasing exercise endurance or other inflammatory-based pathologies.

In some embodiments, the antioxidant activity can have a greater antioxidant activity than provided by an equivalent amount of any one extract or a sum of the extracts. The synergistic antioxidant activity can be greater than or about 1.1 times, about 1.2 times, about 1.3 times, about 1.5, about 1.75 times, about 2 times, about 2.5 times or about 3 times greater than the antioxidant activity provided by an equivalent amount of any one extract or a sum of the extracts. In one embodiment a composition comprising blueberry concentrate, capsicum, turmeric, apple, grape, green tea, olive and mangosteen fruit extract can have equal to or greater than 1.2 times antioxidant activity provided by an equivalent amount of any one extract or a sum of the extracts. In some embodiments this composition can have greater than 1.5 times the antioxidant activity provided by an equivalent amount of any one extract or a sum of the extracts. In another embodiment a composition comprising blueberry concentrate, capsicum, turmeric, apple, grape, green tea, olive and mangosteen pericarp extract can have equal to or greater than 1.3 times antioxidant activity provided by an equivalent amount of any one extract or a sum of the extracts. In yet another embodiment, a composition comprising blueberry concentrate, capsicum, turmeric, apple, grape, green tea, olive, mangosteen fruit, and bergamot extract can have equal to or greater than 2.5 times antioxidant activity provided by an equivalent amount of any one extract or a sum of the extracts. In a further embodiment, a composition comprising blueberry concentrate, capsicum, turmeric, apple, grape, green tea, olive, mangosteen pericarp, and bergamot extract can have equal to or greater than 1.5 times antioxidant activity provided by an equivalent amount of any one extract or a sum of the extracts.

Also presented herein is a protein kinase modulating composition, comprising a combination of apple, grape, green tea, and olive extracts in amounts that provide a greater

protein kinase modulating activity than provided by an equivalent amount of any one extract or a sum of the extracts. The composition can further comprise the additional extracts, primary therapeutic agent, and/or secondary therapeutic agents as identified above. The extracts, amounts of the components, primary therapeutic agents, and/or secondary therapeutic agents can be as identified above.

The protein kinase modulating composition can dramatically and synergistically modulate kinase signaling of any of the protein kinases shown in Table 14. In one example the protein kinase modulating composition modulates the expression of Abl, ACK1, ALK, Aurora, AMPK, CaMKII, EGFR, EphA, FAK, FGFR, GSK3, IGF-1(activated), IKK, IR, MAPK1, Met, MTOR, NEK1/2/6, PAK1/4/5/6, PDGFR, PI3K, PKC, ROCKI/II, RSK1/2/34, SRC, Syk and combinations thereof. In one embodiment, the protein kinase modulating activity can comprise PI3 kinase. In another embodiment, the protein kinase modulating activity can comprise MET kinase. In yet another embodiment, the protein kinase modulating activity can comprise at least one of the Aurora kinases, Aurora-A, Aurora-B, and Aurora-C.

In some embodiments, the protein kinase activity can be modulated in selected target tissue for treating signs and symptoms associated with diseases or conditions selected from the group consisting of prostate cancer, LUTS, pulmonary arterial hypertension, diminished exercise capacity, congestive cardiac failure, pulmonary hypertension, various cardiovascular diseases, motor dysfunction, cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, various forms of inflammation including osteoarthritis, rheumatoid arthritis, type I and type II diabetes, metabolic syndrome, obesity, endotoxemia, inflammatory bowel disease, leaky gut, Crohn's disease, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia and other disorders associated with tissue-specific modulation of protein kinase activity.

The modulation of the protein kinase can reduce, minimize, or inhibit production or presence of oxidized LDL (oxLDL) cholesterol in the subject, can ameliorate at least one of metabolic syndrome, type I diabetes, type II diabetes, or type III diabetes, can ameliorates at least one of leaky gut, endotoxemia, or inflammatory bowel disease, can ameliorates at least one of obesity, inflammation conditions including osteoarthritis and rheumatoid arthritis, Crohn's disease, prostate hyperplasia, lower urinary tract symptoms, pulmonary arterial hypertension, diminished exercise capacity, premature ejaculation, low female sex drive, congestive disorders, cardiac failure, pulmonary hypertension,

cardiovascular diseases, motor dysfunction, cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia and skin disorders including skin wrinkles, skin discolorations and skin sagging, or can results at least one of in stimulation of skeletal muscle fatty acid oxidation and muscle glucose uptake, hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, and modulation of insulin secretion by pancreatic beta-cells.

In one embodiment, the modulation of the protein kinases can ameliorate at least one of obesity, inflammation conditions including osteoarthritis and rheumatoid arthritis, Crohn's disease, prostate hyperplasia, lower urinary tract symptoms, pulmonary arterial hypertension, diminished exercise capacity, premature ejaculation, low female sex drive, congestive disorders, cardiac failure, pulmonary hypertension, cardiovascular diseases, motor dysfunction, cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia and skin disorders including skin wrinkles, skin discolorations and skin sagging. In another embodiment, the modulation of the protein kinases can ameliorate at least one of leaky gut, endotoxemia, and inflammatory bowel disease. In a further embodiment, the modulation of the protein kinases can ameliorates at least one of at least one of metabolic syndrome, type I diabetes, type II diabetes, and type III diabetes. In another embodiment, the modulation of the protein kinase reduces, minimizes, or inhibits production or presence of oxidized LDL (oxLDL) cholesterol in the subject.

In some embodiments, the protein kinase modulating activity can have a greater protein kinase modulating activity than provided by an equivalent amount of any one extract or a sum of the extracts. The synergistic protein kinase modulating activity can be greater than or about 1.1 times, about 1.2 times, about 1.3 times, about 1.5, about 1.75 times, about 2 times, about 2.5 times or about 3 times greater than the protein kinase modulating activity provided by an equivalent amount of any one extract or a sum of the extracts.

Further presented herein are therapeutic compositions comprising a primary therapeutic agent and a combination of apple, grape, green tea, and olive extracts in amounts that increase a therapeutic effect of the primary therapeutic agent more than an increase in therapeutic effect provided by an equivalent amount of any one extract. The

compositions can further comprise the additional extracts, and/or secondary therapeutic agents as identified above. The extracts, amounts, and secondary therapeutic agents can be as identified above.

The primary therapeutic agent can be any antioxidant, metabolic agent, or kinase pathway signal transducer. In one embodiment, the primary therapeutic agent can be an antioxidant. In another embodiment the primary therapeutic agent can be a metabolic agent. In yet another embodiment the primary therapeutic agent can be a kinase pathway signal transducer. In other embodiments the primary therapeutic agent can be an agent that enhances NO production. In one embodiment, the primary therapeutic agent can comprise a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, aminogen®, biotin, black bean powder, copper citrate, ferrous fumarate, fructose, garbanzo bean, gum arabic, magnesium oxide, manganese citrate, medium chain triglycerides, pea fiber, pea protein isolate, potassium citrate, vitamin B6, riboflavin, rice bran, rice protein, sodium citrate, sodium selenate, thiamin HCl, vitamin D2, vitamin E, zinc citrate, adzuki bean, D-calcium pantothenate, lycopene, polyphenols, ascorbic acid, β-glucans, lutein, blueberry, borage oil, broccoli flowers, carrot root, cranberry fruit, chromium nicotinate, cyanocobalamin, flax seed/linum usitatissimum, folic acid, lo han extract, niacinamide, pomegranate fruit, vitamin A, carotentiods, vitamin E, phytosterols, lignin, CoQ10, glutathione, and combinations thereof.

In one example the primary therapeutic agent regulates metabolic dysfunction. In some embodiments, the primary therapeutic agent which regulates metabolic dysfunction comprises a member selected from the group consisting of aminogen®, biotin, black bean powder, copper citrate, ferrous fumarate, fructose, garbanzo bean, gum arabic, inulin, magnesium oxide, manganese citrate, medium chain triglycerides, pea fiber, pea protein isolate, potassium citrate, vitamin B6, riboflavin, rice bran, rice protein, sodium citrate, sodium selenate, thiamin HCl, vitamin D2, vitamin E, zinc citrate, adzuki bean, D-calcium pantothenate, and combinations thereof. In another embodiment, the therapeutic agent can be aminogen®. In another example, the primary therapeutic agent can be inulin.

In another example, the primary therapeutic agent can be an antioxidant. In some embodiments the antioxidant comprises a member selected from the group consisting of lycopene, polyphenols, ascorbic acid, β-glucans, lutein, blueberry, borage oil, broccoli flowers, carrot root, cranberry fruit, chromium nicotinate, cyanocobalamin, flax

seed/linum usitatissimum, folic acid, lo han extract, niacinamide, pomegranate fruit, vitamin A, carotentiods, vitamin E, zinc, sodium selenate, phytosterols, lignin, CoQ10, glutathione, and combinations thereof. In one embodiment, the antioxidant comprises a member selected from the group consisting of ascorbic acid, β-glucans, blueberry, borage oil, broccoli flowers, carrot root, cranberry fruit, chromium nicotinate, cyanocobalamin, flax seed/linum usitatissimum, folic acid, lo han extract, niacinamide, pomegranate fruit, vitamin A, phytosterols, and combinations thereof.

In one example, the primary therapeutic agent can comprise fish oil. In one example, the fish oil can lower TG, LDLc, oxLDLc, raise HDLc, or any combination thereof. The extracts when combined with the fish oil can enhance the TG lowering properties of the fish oil, improve NO formation, and lower blood pressure. In one example the fish oil can be included in the composition at between 1-5 grams.

In another example, the primary therapeutic agent can be berberine. In one example the berberine can influence endotoxemia and inflammation in the body. In one example, this formulation can inhibit or down-regulate MPO an enzyme known to in turn inhibit or remove eNOS and NO production. Endotexemia and inflammation are initiators of metabolic dysfunction/CVD and obesity. The therapeutic composition comprising berberine as the primary therapeutic agent can be used to combat metabolic dysfunction.

The oxidative stress modulating compositions, protein kinase modulating compositions, and/or the therapeutic compositions discussed above can be provided in any convenient form. These compositions can be provided as dietary supplement in capsule or tablet form. They can be formulated into a food or drink, and provided, for example, as a snack bar, a cereal, a drink, a gum, or in any other easily ingested form. They can also be provided as a cream or lotion for topical application. In one example, the composition can be an oral composition in the form of discrete units as capsules, sachets, tablets, soft gels or lozenges, each containing a predetermined amount of the active ingredient; in the form of a powder or granules; in the form of a solution or a suspension in an aqueous liquid or non-aqueous liquid, such as ethanol or glycerol; or in the form of an oil-in-water emulsion or a water-in-oil emulsion. Such oils may be edible oils, such as *e.g.* cottonseed oil, sesame oil, coconut oil, sunflower oil, or peanut oil. Suitable dispersing or suspending agents for aqueous suspensions include synthetic or natural gums such as tragacanth, alginate, gum arabic, dextran, sodium carboxymethylcellulose, gelatin, methylcellulose and polyvinylpyrrolidone. In another embodiment, the oral dosage form can comprise a capsule, a tablet, a powder, a beverage, a syrup, a suspension, or a food.

In addition, the compositions can be formulated as a depot preparation. Such long-acting compositions may be administered by implantation (*e.g.* subcutaneously, intra-abdominally, or intramuscularly) or by intramuscular injection. Thus, for example, the active ingredient may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in a pharmaceutically acceptable oil), or an ion exchange resin.

In some embodiments, the oxidative stress modulating compositions, protein kinase modulating compositions, and/or the therapeutic compositions discussed above can further comprise a pharmaceutically acceptable carrier. In some embodiments, the formulations can comprise pharmaceutically acceptable excipients. Exemplary pharmaceutically acceptable excipients can be selected from the group consisting of coatings, isotonic and absorption delaying agents, binders, adhesives, lubricants, disintergrants, coloring agents, flavoring agents, sweetening agents, absorbents, detergents, and emulsifying agents.

When the formulation includes an emulsifying agent, the emulsifiers can be added to improve the stability of the final product. Exemplary emulsifiers include, but are not limited to, lecithin (e.g., from egg or soy), or mono- and di-glycerides. Other emulsifiers are readily apparent to the skilled artisan and selection of suitable emulsifier(s) will depend, in part, upon the formulation and final product.

The formulation can further include flavorings, coloring agents, spices, nuts, preservatives, antioxidants, vitamins, minerals, proteins, fats, and/or carbohydrates. The amount of other ingredients can vary based on the particular design, intended dosage, and method of administration. The total amount of other ingredients can also depend, in part, upon the condition and weight of the subject.

Flavors, coloring agents, spices, nuts and the like can be incorporated into the product. Flavorings can be in the form of flavored extracts, volatile oils, chocolate flavorings (e.g., non-caffeinated cocoa or chocolate, chocolate substitutes such as carob), peanut butter flavoring, cookie crumbs, crisp rice, vanilla or any commercially available flavoring. Flavorings can be protected with mixed tocopherols. Examples of useful flavorings include but are not limited to pure anise extract, imitation banana extract, imitation cherry extract, chocolate extract, pure lemon extract, pure orange extract, pure peppermint extract, imitation pineapple extract, imitation rum extract, imitation strawberry extract, or pure vanilla extract; or volatile oils, such as balm oil, bay oil, bergamot oil, cedarwood oil, cherry oil, walnut oil, cinnamon oil, clove oil, or peppermint oil; peanut butter, chocolate flavoring, vanilla cookie crumb, butterscotch or toffee. In one

embodiment, the formulation can contain berry or other fruit flavors. The food compositions may further be coated, for example with a yogurt coating.

Preservatives or stabilizers can be added to the formulation to extend the shelf life of the product. Exemplary preservatives include potassium sorbate, sodium sorbate, potassium benzoate, sodium benzoate, or calcium disodium EDTA.

The formulation can also include natural or artificial sweeteners. In one embodiment, the potential sweeteners can include glucose, sucrose, fructose, saccharides, cyclamates, aspartamine, sucralose, aspartame, acesulfame K, sorbitol, or a combination thereof.

The formulation can further include pharmaceutically acceptable forms of vitamins, minerals, and other nutrients. The nutrients chosen for inclusion in the formulation can vary depending on the particular design, intended dosage, method of administration, and condition of the subject. Individuals skilled in the art are aware of vitamins, minerals, and other nutrients that can be incorporated into formulations and how to incorporate these.

The components in the formulation can be included as salts. In particular, pharmaceutically acceptable salts of the components are contemplated. A "pharmaceutically acceptable salt" is a combination of a compound and either an acid or a base that forms a salt (such as, for example, the magnesium salt, denoted herein as "Mg" or "Mag") with the compound. Pharmaceutically acceptable salts can be tolerated by a subject under therapeutic conditions. In general, a pharmaceutically acceptable salt of a compound will have a therapeutic index (the ratio of the lowest toxic dose to the lowest therapeutically effective dose) of 1 or greater. Those skilled in the art recognize that the lowest therapeutically effective dose will vary from subject to subject and from indication to indication, and will thus adjust the formulation accordingly.

In addition, polymers may be added according to standard methodologies in the art for sustained release of a given compound.

Any compositions used to treat a disease or condition will use a pharmaceutical grade compound and that the composition will further comprise a pharmaceutically acceptable carrier. It is further contemplated that these compositions of the invention may be prepared in unit dosage forms appropriate to both the route of administration and the disease and patient to be treated. The compositions may conveniently be presented in dosage unit form be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient into association with the

vehicle that constitutes one or more auxiliary constituents. In general, the compositions are prepared by uniformly and intimately bringing the active ingredient into association with a liquid vehicle or a finely divided solid vehicle or both, and then, if necessary, shaping the product into the desired composition.

Also presented herein are methods of regulating oxidative stress in a subject. The method can comprise administering to the subject a therapeutically effective combination of apple, grape, green tea, and olive extracts, in amounts that provide a combined antioxidant activity that is greater than an antioxidant activity provided by an equivalent amount of any one extract or a sum of the extracts. In one example, the method can further comprise administering to the subject at least one secondary therapeutic agent. The secondary therapeutic agent can be co-administered in a single formulation, administered separately, or administered sequentially with the administration of the apple, grape, green tea, and olive extracts. The method can comprise administering any of the additional extracts, and/or secondary therapeutic agents as identified above. The extracts, amounts, and secondary therapeutic agents can be as identified above.

The method can regulate stress related pathologies and metabolic disorders comprise at least one member selected from the group consisting of metabolic syndrome, type 1 diabetes, type 2 diabetes, obesity, high cholesterol levels, oxidized LDL cholesterol, inflammation, osteoarthritis, rheumatoid arthritis, endotoxemia, inflammatory bowel disease, leaky gut, Crohn's disease, prostate hyperplasia, lower urinary tract symptoms, pulmonary arterial hypertension, diminished exercise capacity, premature ejaculation, low female sex drive, congestive disorders, cardiac failure, pulmonary hypertension, various cardiovascular diseases, motor dysfunction, cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia skin disorders, wrinkles, skin discoloration, sagging skin, cancers arising from oxidized damage to DNA, and combinations thereof.

Further presented herein is a method of regulating disease-associated protein kinase activity in a subject comprising administering to the subject a therapeutically effective combination of an apple extract, a grape extract, a green tea extract, and an olive extract, in amounts that provide a combined kinase regulating activity that is greater than an a kinase regulating activity provided by an equivalent amount of any one extract or a sum of the extracts. In one example the method can further comprise administering to the subject at least one secondary therapeutic agent. The secondary therapeutic agent can be

co-administered in a single formulation, administered separately, or administered sequentially with the administration of the apple, grape, green tea, and olive extracts. The method can comprise administering any of the additional extracts, and/or secondary therapeutic agents as identified above. The extracts, amounts, and secondary therapeutic agents can be as identified above.

The method can modulate activity of protein kinases of any of the protein kinases shown in Table 14. In one example the method modulates the expression of: Abl, ACK1, ALK, Aurora, AMPK, CaMKII, EGFR, EphA, FAK, FGFR, GSK3, IGF-1(activated), IKK, IR, MAPK1, Met, MTOR, NEK1/2/6, PAK1/4/5/6, PDGFR, PI3K, PKC, ROCKI/II, RSK1/2/34, SRC, Syk and combinations thereof. In one embodiment the protein kinase modulating activity can comprise PI3 kinase. In another embodiment, the protein kinase modulating activity can comprise MET kinase. In yet another embodiment, the protein kinase modulating activity can comprise at least one of the Aurora kinases, Aurora-A, Aurora-B, and Aurora-C.

The administration step of the method of regulating oxidative stress and method of regulating disease-associated protein kinase activity can be administered to a subject in need of such activity. The formulation in the method can be administered in the form of an oral, transdermal, transmucosal, rectal, ophthalmic (including intravitreal or intracameral), nasal, nasal by inhalation, topical (including buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, and intratracheal), by implantation, or intramuscularly. In one exemplary embodiment the method administers the formulation orally.

The amount administered can coincide with the recommended daily amounts of each ingredient. The actual amount of each ingredient per unit dosage will depend upon the number of units administered daily to the individual in need thereof. This is a matter of product design and is well within the skill of the nutritional supplement formulator.

Additionally presented are methods of enhancing the therapeutic effect of a primary therapeutic agent. In one example, the method can comprise combining a primary therapeutic agent with apple, grape, green tea, and olive extracts in amounts that increase the therapeutic effect of the primary therapeutic agent more than an increase provided by an equivalent amount of any one extract. In one example, the method can further comprise administering the formulation to a subject in need thereof. In one embodiment the method additionally comprises administering a secondary therapeutic agent can be co-administered in a single formulation, administered separately, or administered sequentially

with the administration of the apple, grape, green tea, and olive extracts. The method can comprise administering any of the additional extracts, and/or secondary therapeutic agents as identified above. The extracts, amounts, primary therapeutic agents, and secondary therapeutic agents can be as identified above.

Further presented herein are methods of making an activity-enhanced compositions for regulating oxidative stress, methods of making protein kinase modulating compositions, and making therapeutic, including therapeutic-specific compositions. The methods of making the oxidative stress modulating compositions and protein kinase modulating compositions can comprise combining apple, grape, green tea, and olive extracts in amounts that provide a greater antioxidant activity than provided by an equivalent amount of any one extract or a sum of the extracts. The method of making the therapeutic composition further comprises providing a primary therapeutic agent and combining the primary therapeutic agent with apple, grape, green tea, and olive extracts.

In any or all of the methods above, the extracts can be created from the raw ingredients. When formulation from raw ingredients, the apple, grape, green tea and olive extracts can be extracted using an extraction solvent selected from the group consisting of water, ethanol, ethyl acetate, and combinations thereof. In an example, the extraction process can include forming a pulp concentrate of the raw material, extracting the raw materials, purifying the raw materials, eluting the raw materials, collecting the eluted material, concentrating the material, and spray drying material. In another example, the extraction process can further comprise filtering the material.

In one example, the method can further comprise combining at least one secondary therapeutic agent to the apple, grape, green tea, and olive extracts. The extracts, amounts, primary therapeutic agents, and secondary therapeutic agents can be as identified above.

In some embodiments the compositions, methods of use, and methods of making the oxidative stress modulating compositions, protein kinase modulating compositions, and/or the therapeutic compositions discussed above can include formulating the compositions in the form of a pharmaceutical pack or kit. The pharmaceutical pack or kit can comprise one or more containers filled with one or more of the ingredients of the compositions of the invention (e.g., nutritional supplement in the form of a powder and capsules). Optionally associated with such container(s) can be a notice in the form prescribed by a government agency regulating the manufacture, use or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use of sale for human administration. The pack or kit can be labeled with information

regarding mode of administration, sequence of administration (e.g., separately, sequentially or concurrently), or the like. The pack or kit may also include means for reminding the patient to take the therapy. The pack or kit can be a single unit dosage of the combination therapy or it can be a plurality of unit dosages. In particular, the agents can be separated, mixed together in any combination, present in a formulation or tablet.

Embodiments

In one embodiment there is provided, an oxidative stress modulating composition comprising a combination of apple, grape, green tea, and olive extract in amounts that provide a greater antioxidant activity than provided by an equivalent amount of any one extract or a sum of the extracts.

In one embodiment of the oxidative stress modulating composition, the apple extract comprises an extract of a species *Malus pumila*.

In one embodiment of the oxidative stress modulating composition, the apple extract in the composition comprises both a skin and a fruit of the *Malus pumila*.

In one embodiment of the oxidative stress modulating composition, the grape extract comprises an extract of a species *Vitis vinifera*.

In one embodiment of the oxidative stress modulating composition, the grape extract in the composition comprises seeds of the *Vitis vinifera*.

In one embodiment of the oxidative stress modulating composition, the grape extract in the composition comprises from about 75 wt% to about 95 wt% phenolics on a dry weight basis.

In one embodiment of the oxidative stress modulating composition, the green tea extract comprises an extract of leaves of a species *Camellia sinensis*.

In one embodiment of the oxidative stress modulating composition, the olive extract comprises an extract of a subspecies *Olea europea europaea*.

In one embodiment of the oxidative stress modulating composition, the olive extract in the composition comprises leaves of the *Olea europea europea*.

In one embodiment of the oxidative stress modulating composition, at least one of the extracts in the composition is present in a different amount than an amount of at least one of another extract.

In one embodiment of the oxidative stress modulating composition, the apple, grape, green tea, and olive extracts are present in the composition at a weight ratio of about 1:1:1:1.

In one embodiment of the oxidative stress modulating composition, the apple, grape, green tea, and olive extracts are present in the composition at a weight ratio of about 6:1:3:1

In one embodiment of the oxidative stress modulating composition, the apple, grape, green tea, and olive extracts comprise extracts of the leaves, skin, rind, pulp, juice, seeds, or combinations thereof.

In one embodiment, the oxidative stress modulating composition further comprises at least one primary or secondary therapeutic agent.

In one embodiment of the oxidative stress modulating composition, the at least one primary or secondary therapeutic agent comprises a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, and combinations thereof.

In one embodiment of the oxidative stress modulating composition, the at least one primary or secondary therapeutic agent comprises bergamot.

In one embodiment of the oxidative stress modulating composition, the at least one primary or secondary therapeutic agent comprises mangosteen.

In one embodiment of the oxidative stress modulating composition, the at least one primary or secondary therapeutic agent comprises berberine.

In one embodiment of the oxidative stress modulating composition, the composition comprising a combination of apple, grape, green tea, and olive extract further comprises at least one secondary therapeutic agent.

In one embodiment of the oxidative stress modulating composition, the at least one primary or secondary therapeutic agent comprises arginine and beet.

In one embodiment of the oxidative stress modulating composition, the at least one primary or secondary therapeutic agent comprises citrulline and beet.

In one embodiment of the oxidative stress modulating composition, the at least one primary or secondary therapeutic agent comprises phytosterols.

In one embodiment of the oxidative stress modulating composition, the at least one primary or secondary therapeutic agent comprises protein.

In one embodiment of the oxidative stress modulating composition, the protein comprises at least one member selected from the group consisting of whey protein, soy protein, pea protein, calcium caseinate protein, and combinations thereof.

In one embodiment of the oxidative stress modulating composition, the at least one primary or secondary therapeutic agent comprises curcumin.

In one embodiment of the oxidative stress modulating composition, the at least one primary or secondary therapeutic agent comprises a fiber source and inulin.

In one embodiment of the oxidative stress modulating composition, the composition further comprises a pharmaceutically acceptable carrier.

In one embodiment of the oxidative stress modulating composition, the composition is an oral dosage formulation.

In one embodiment of the oxidative stress modulating composition, the oral dosage form comprises a capsule, a tablet, a powder, a beverage, a syrup, a suspension, or a food.

In one embodiment of the oxidative stress modulating composition, the antioxidant activity modulates stress related pathologies and metabolic disorders.

In one embodiment of the oxidative stress modulating composition, the composition the antioxidant activity of the oxidative stress modulating composition modulates stress related pathologies and metabolic disorders.

In one embodiment of the oxidative stress modulating composition, the stress related pathologies and metabolic disorders comprise at least one member selected from the group consisting of metabolic syndrome, type 1 diabetes, type 2 diabetes, obesity, high cholesterol levels, oxidized LDL cholesterol, inflammation, osteoarthritis, rheumatoid arthritis, endotoxemia, inflammatory bowel disease, leaky gut, Crohn's disease, prostate hyperplasia, lower urinary tract symptoms, pulmonary arterial hypertension, diminished exercise capacity, premature ejaculation, low female sex drive, congestive disorders, cardiac failure, pulmonary hypertension, various cardiovascular diseases, motor dysfunction, cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia skin disorders, wrinkles, skin discoloration, sagging skin, cancers arising from oxidized damage to DNA, and combinations thereof.

In one embodiment of the oxidative stress modulating composition, the antioxidant activity modulates oxidized LDL.

In one embodiment of the oxidative stress modulating composition, the antioxidant activity modulates at least one of metabolic syndrome, type I diabetes, type II diabetes, or type III diabetes.

In one embodiment of the oxidative stress modulating composition, the antioxidant activity modulates at least one of leaky gut, endotoxemia, or inflammatory bowel disease.

In one embodiment of the oxidative stress modulating composition, the oxidative stress modulating composition further comprises blueberry concentrate, capsicum extract, and turmeric extract.

In one embodiment of the oxidative stress modulating composition further comprising blueberry concentrate, capsicum extract, and turmeric extract, the blueberry concentrate comprises *Vaccinium angustifolium*, the capsicum extract comprises *Capsicum annuum*, and the turmeric extract comprises *Curcuma longa*.

In one embodiment of the oxidative stress modulating composition, the grape extract comprises grape skin and grape seed from *Vitis vinifera* in the oxidative stress modulating composition further comprises blueberry concentrate, capsicum extract, and turmeric extract.

In one embodiment of the oxidative stress modulating composition, the oxidative stress modulating composition further comprises blueberry concentrate, capsicum extract, turmeric extract, and mangosteen fruit extract.

In the embodiment of the oxidative stress modulating composition above, the composition comprises greater than 1.5 times the antioxidant activity of an equivalent amount of any one extract or concentrate or a sum of the extracts and concentrate.

In one embodiment of the oxidative stress modulating composition, the oxidative stress modulating composition further comprises blueberry concentrate, capsicum extract, turmeric extract, and bergamot extract.

In the embodiment of the oxidative stress modulating composition above, the composition comprises greater than 1.5 times the antioxidant activity of an equivalent amount of any one extract or concentrate or a sum of the extracts and concentrate.

In one embodiment of the oxidative stress modulating composition the bergamont extract comprises *Citrus bergamia Risso*.

In one embodiment of the oxidative stress modulating composition, the oxidative stress modulating composition further comprises blueberry concentrate, capsicum extract, turmeric extract, and mangosteen pericarp extract.

In the embodiment of the oxidative stress modulating composition above, the composition comprises greater than 1.25 times the antioxidant activity of an equivalent amount of any one extract or concentrate or a sum of the extracts and concentrate.

In one embodiment of the oxidative stress modulating composition, the oxidative stress modulating composition further comprises blueberry concentrate, capsicum extract, turmeric extract, mangosteen pericarp extract, and bergamot extract.

In one embodiment, the oxidative stress modulating composition further comprises blueberry concentrate, capsicum extract, turmeric extract, mangosteen pericarp extract and bergamot extract modulates oxidized LDL.

In one embodiment, a protein kinase modulating composition comprising a combination of apple, grape, green tea, and olive extracts in amounts that provide a greater protein kinase modulating activity than provided by an equivalent amount of any one extract or a sum of the extracts.

In one embodiment of the protein kinase modulating composition, the apple extract comprises an extract of a species *Malus pumila.*

In one embodiment of the protein kinase modulating composition, the apple extract comprises both skin and fruit of the *Malus pumila.*

In one embodiment of the protein kinase modulating composition, the grape extract comprises an extract of a species *Vitis vinifera.*

In one embodiment of the protein kinase modulating composition, the grape extract comprises seeds of the *Vitis vinifera.*

In one embodiment of the protein kinase modulating composition, the grape extract comprises from about 75 wt% to about 95 wt% phenolics on a dry weight basis.

In one embodiment of the protein kinase modulating composition, the green tea extract comprises an extract of leaves of a species *Camellia sinensis.*

In one embodiment of the protein kinase modulating composition, the olive extract comprises an extract of a subspecies *Olea europea europaea.*

In one embodiment of the protein kinase modulating composition, the olive extract comprises leaves of the *Olea europea europea.*

In one embodiment of the protein kinase modulating composition, at least one of the extracts in the composition is present in a different amount than an amount of at least one of another extract.

In one embodiment of the protein kinase modulating composition, the apple, grape, green tea, and olive extracts are present at a weight ratio of about 1:1:1:1.

In one embodiment of the protein kinase modulating composition, the apple, grape, green tea, and olive extracts are present at a weight ratio of about 6:1:3:1

In one embodiment of the protein kinase modulating composition, the apple, grape, green tea, and olive extracts comprise leaves, skin, rind, pulp, juice, seeds, or combinations thereof.

In one embodiment, the protein kinase modulating composition further comprises at least one primary or secondary therapeutic agent.

In one embodiment of the protein kinase modulating composition, the at least one primary or secondary therapeutic agent comprises a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, beet, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, and combinations thereof.

In one embodiment of the protein kinase modulating composition, the at least one primary or secondary therapeutic agent comprises bergamot.

In one embodiment of the protein kinase modulating composition, the at least one primary or secondary therapeutic agent comprises mangosteen.

In one embodiment of the protein kinase modulating composition, the at least one primary or secondary therapeutic agent comprises berberine.

In one embodiment of the protein kinase modulating composition, the at least one primary or secondary therapeutic agent comprises arginine and beet.

In one embodiment of the protein kinase modulating composition, the at least one primary or secondary therapeutic agent comprises citrulline and beet.

In one embodiment of the protein kinase modulating composition, the at least one primary or secondary therapeutic agent comprises phytosterols.

In one embodiment of the protein kinase modulating composition, the at least one primary or secondary therapeutic agent comprises protein.

In one embodiment of the protein kinase modulating composition, the protein comprises at least one member selected from the group consisting of whey protein, soy protein, pea protein, calcium caseinate protein, and combinations thereof.

In one embodiment of the protein kinase modulating composition, the at least one primary or secondary therapeutic agent comprises curcumin.

In one embodiment of the protein kinase modulating composition, the at least one primary or secondary therapeutic agent comprises a fiber source and inulin.

In one embodiment, the protein kinase modulating composition further comprises a pharmaceutically acceptable carrier.

In one embodiment, the protein kinase modulating composition is an oral dosage formulation.

In one embodiment of the protein kinase modulating composition, the oral dosage form comprises a capsule, a tablet, a powder, a beverage, a syrup, a suspension, or a food.

In one embodiment of the protein kinase modulating composition, the protein kinase modulating activity is a member selected from the group consisting of: Abl, ACK1, ALK, Aurora, AMPK, CaMKII, EGFR, EphA, FAK, FGFR, GSK3, IGF-1(activated), IKK, IR, MAPK1, Met, MTOR, NEK1/2/6, PAK1/4/5/6, PDGFR, PI3K, PKC, ROCKI/II, RSK1/2/34, SRC, Syk and combinations thereof.

In one embodiment of the protein kinase modulating composition, the modulation of the protein kinase ameliorates at least one of obesity, inflammation conditions including osteoarthritis and rheumatoid arthritis, Crohn's disease, prostate hyperplasia, lower urinary tract symptoms, pulmonary arterial hypertension, diminished exercise capacity, premature ejaculation, low female sex drive, congestive disorders, cardiac failure, pulmonary hypertension, cardiovascular diseases, motor dysfunction, cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia and skin disorders including skin wrinkles, skin discolorations and skin sagging.

In one embodiment of the protein kinase modulating composition, the modulation of the protein kinase ameliorates at least one of leaky gut, endotoxemia, and inflammatory bowel disease.

In one embodiment of the protein kinase modulating composition, the modulation of the protein kinase results ameliorates at least one of metabolic syndrome, type I diabetes, type II diabetes, and type III diabetes.

In one embodiment of the protein kinase modulating composition, the modulation of the protein kinase reduces, minimizes, or inhibits production or presence of oxidized LDL (oxLDL) cholesterol in the subject.

In one embodiment, the protein kinase modulating composition, further comprises blueberry concentrate, capsicum extract, and turmeric extract.

In one embodiment, the protein kinase modulating composition further comprises blueberry concentrate, capsicum extract, and turmeric extract, the blueberry concentrate

comprises *Vaccinium angustifolium*, the capsicum extract comprises *Capsicum annuum*, and the turmeric extract comprises *Curcuma longa*.

In one embodiment, the protein kinase modulating composition further comprises blueberry concentrate, capsicum extract, and turmeric extract, the grape extract comprises grape skin and grape seed from *Vitis vinifera*.

In one embodiment, the protein kinase modulating composition further comprises blueberry concentrate, capsicum extract, turmeric extract, and a mangosteen fruit extract.

In one embodiment, the protein kinase modulating composition further comprises blueberry concentrate, capsicum extract, turmeric extract, and bergamot extract *Citrus bergamia Risso*.

In one embodiment, the protein kinase modulating composition further comprises blueberry concentrate, capsicum extract, turmeric extract, and a mangosteen pericarp extract.

In one embodiment, the protein kinase modulating composition further comprises blueberry concentrate, capsicum extract, turmeric extract, mangosteen pericarp extract, and bergamot extract *Citrus bergamia Risso*.

In one embodiment, the protein kinase modulating composition further comprising blueberry concentrate, capsicum extract, turmeric extract, mangosteen pericarp extract, and bergamot extract *Citrus bergamia Risso*, has a protein kinase modulating activity comprising a member selected from the group consisting of: Abl, ACK1, ALK, Aurora, AMPK, CaMKII, EGFR, EphA, FAK, FGFR, GSK3, IGF-1(activated), IKK, IR, MAPK1, Met, MTOR, NEK1/2/6, PAK1/4/5/6, PDGFR, PI3K, PKC, ROCKI/II, RSK1/2/34, SRC, Syk and combinations thereof.

In one embodiment, the protein kinase modulating composition further comprising blueberry concentrate, capsicum extract, turmeric extract, mangosteen pericarp extract, and bergamot extract *Citrus bergamia Risso*, has a protein kinase modulating activity comprising modulating PI3 kinase.

In one embodiment, the protein kinase modulating composition further comprising blueberry concentrate, capsicum extract, turmeric extract, mangosteen pericarp extract, and bergamot extract *Citrus bergamia Risso*, has a protein kinase modulating activity comprising modulating MET kinase.

In one embodiment, the protein kinase modulating composition further comprising blueberry concentrate, capsicum extract, turmeric extract, mangosteen pericarp extract, and bergamot extract *Citrus bergamia Risso*, has a protein kinase modulating activity

comprising modulating comprises at least one of the Aurora kinases, Aurora-A, Aurora-B, and Aurora-C.

In one embodiment presented herein is a therapeutic composition comprising a primary therapeutic agent and a combination of apple, grape, green tea, and olive extracts in amounts that increase a therapeutic effect of the primary therapeutic agent more than an increase in therapeutic effect provided by an equivalent amount of any one extract.

In one embodiment of the therapeutic composition, the apple, grape, green tea, and olive extracts are present in the composition at a weight ratio of about 1:1:1:1.

In one embodiment of the therapeutic composition, the apple, grape, green tea, and olive extracts are present in the composition at a weight ratio of about 6:1:3:1

In one embodiment the therapeutic composition, further comprises at least one secondary therapeutic agent.

In one embodiment of the therapeutic composition, the at least one secondary therapeutic agent comprises a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, and combinations thereof.

In one embodiment of the therapeutic composition, the at least one secondary therapeutic agent comprises mangosteen and the mangosteen comprises a mangosteen fruit extract, a mangosteen pericarp extract, or a combination thereof.

In one embodiment of the therapeutic composition, the at least one secondary therapeutic agent comprises bergamot.

In one embodiment of the therapeutic composition, the composition further comprise arginine and citrulline.

In one embodiment of the therapeutic composition, the therapeutic agent regulates metabolic dysfunction.

In one embodiment of the therapeutic composition, the therapeutic agent comprises a member selected from the group consisting of aminogen®, biotin, black bean powder, copper citrate, ferrous fumarate, fructose, garbanzo bean, gum arabic, inulin, magnesium oxide, manganese citrate, medium chain triglycerides, pea fiber, pea protein isolate, potassium citrate, vitamin B6, riboflavin, rice bran, rice protein, sodium citrate, sodium selenate, thiamin HCl, vitamin D2, vitamin E, zinc citrate, adzuki bean, D-calcium pantothenate, and combinations thereof.

In one embodiment of the therapeutic composition, the therapeutic agent comprises an antioxidant.

In one embodiment of the therapeutic composition, the therapeutic agent comprises an antioxidant selected from the group consisting of ascorbic acid, β-glucans, blueberry, borage oil, broccoli flowers, carrot root, cranberry fruit, chromium nicotinate, cyanocobalamin, flax seed/linum usitatissimum, folic acid, lo han extract, niacinamide, pomegranate fruit, vitamin A, phytosterols, and combinations thereof.

In one embodiment presented herein is a method of regulating oxidative stress in a subject comprising, administering to the subject a therapeutically effective combination of apple, grape, green tea, and olive extracts, in amounts that provide a combined antioxidant activity that is greater than an antioxidant activity provided by an equivalent amount of any one extract or a sum of the extracts.

In one embodiment of the method of regulating oxidative stress in a subject, the apple extract comprises an extract of skin and fruit of *Malus pumila;* the grape extract comprises an extract of seeds of *Vitis vinifera*; the green tea extract comprises an extract of leaves of *Camellia sinensis; and* the olive extract comprises leaves of *Olea europea europaea.*

In one embodiment of the method of regulating oxidative stress in a subject, the apple, grape, green tea, and olive extracts are present at a weight ratio of about 1:1:1:1.

In one embodiment of the method of regulating oxidative stress in a subject, the apple, grape, green tea, and olive extracts are present at a weight ratio of about 6:1:3:1

In one embodiment of the method of regulating oxidative stress in a subject, the method further comprises administering to the subject at least one primary or secondary therapeutic agent.

In one embodiment of the method of regulating oxidative stress in a subject, the at least one primary or secondary therapeutic agent is co-administered to the subject with the therapeutically effective combination of apple, grape, green tea, and olive extracts.

In one embodiment of the method of regulating oxidative stress in a subject, the at least one primary or secondary therapeutic agent comprises a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, and combinations thereof.

In one embodiment of the method of regulating oxidative stress in a subject, the antioxidant activity modulates stress related pathologies and metabolic disorders.

In one embodiment of the method of regulating oxidative stress in a subject, the antioxidant activity modulates stress related pathologies and metabolic disorders and the

stress related pathologies and metabolic disorders comprise at least one member selected from the group consisting of metabolic syndrome, type 1 diabetes, type 2 diabetes, obesity, high cholesterol levels, oxidized LDL cholesterol, inflammation, osteoarthritis, rheumatoid arthritis, endotoxemia, inflammatory bowel disease, leaky gut, Crohn's disease, prostate hyperplasia, lower urinary tract symptoms, pulmonary arterial hypertension, diminished exercise capacity, premature ejaculation, low female sex drive, congestive disorders, cardiac failure, pulmonary hypertension, various cardiovascular diseases, motor dysfunction, cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia skin disorders, wrinkles, skin discoloration, sagging skin, cancers arising from oxidized damage to DNA, and combinations thereof.

In one embodiment of the method of regulating oxidative stress in a subject, the method further comprises administering to the subject a blueberry concentrate, a capsicum extract, and a turmeric extract.

In one embodiment of the method of regulating oxidative stress in a subject, the method further comprises administering to the subject mangosteen fruit extract.

In one embodiment of the method of regulating oxidative stress in a subject, the method further comprises administering to the subject bergamot extract.

In one embodiment of the method of regulating oxidative stress in a subject, the method further comprises administering to the subject a mangosteen pericarp extract.

In one embodiment of the method of regulating oxidative stress in a subject, the method further comprises administering to the subject mangosteen pericarp extract and bergamot extract.

In one embodiment presented herein is a method of regulating disease-associated protein kinase activity in a subject comprising: administering to the subject a therapeutically effective combination of an apple extract, a grape extract, a green tea extract, and an olive extract, in amounts that provide a combined kinase regulating activity that is greater than an a kinase regulating activity provided by an equivalent amount of any one extract or a sum of the extracts.

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the apple extract comprises an extract of skin and fruit of *Malus pumila;* the grape extract comprises an extract of seeds of *Vitis vinifera*; the green tea

extract comprises an extract of leaves of *Camellia sinensis; and* the olive extract comprises leaves of *Olea europea europaea.*

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the apple, grape, green tea, and olive extracts are present at a weight ratio of about 1:1:1:1.

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the apple, grape, green tea, and olive extracts are present at a weight ratio of about 6:1:3:1

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the method further comprises administering to the subject at least one primary or secondary therapeutic agent.

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the at least one primary or secondary therapeutic agent is co-administered to the subject with the therapeutically effective combination of apple, grape, green tea, and olive extracts.

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the at least one primary or secondary therapeutic agent comprises a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, and combinations thereof.

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the protein kinase modulating activity is a member selected from the group consisting of: Abl, ACK1, ALK, Aurora, AMPK, CaMKII, EGFR, EphA, FAK, FGFR, GSK3, IGF-1(activated), IKK, IR, MAPK1, Met, MTOR, NEK1/2/6, PAK1/4/5/6, PDGFR, PI3K, PKC, ROCKI/II, RSK1/2/34, SRC, Syk and combinations thereof.

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the method further comprises administering to the subject a blueberry concentrate, a capsicum extract, and a turmeric extract.

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the method further comprises administering to the subject mangosteen fruit extract.

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the method further comprises administering to the subject bergamot extract.

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the method further comprises administering to the subject a mangosteen pericarp extract.

In one embodiment of the method of regulating disease-associated protein kinase activity in a subject, the method further comprises administering to the subject mangosteen pericarp extract and bergamot extract.

In one embodiment presented herein is a method of enhancing a therapeutic effect provided by a primary therapeutic agent comprising: combining the primary therapeutic agent with apple, grape, green tea, and olive extracts in amounts that increase the therapeutic effect of the primary therapeutic agent more than an increase provided by an equivalent amount of any one extract.

In one embodiment of the method of enhancing a therapeutic effect provided by a primary therapeutic agent, the apple extract comprises an extract of skin and fruit of *Malus pumila;* the grape extract comprises an extract of seeds of *Vitis vinifera*; the green tea extract comprises an extract of leaves of *Camellia sinensis; and* the olive extract comprises leaves of *Olea europea europaea.*

In one embodiment of the method of enhancing a therapeutic effect provided by a primary therapeutic agent, the apple, grape, green tea, and olive extracts are combined at a weight ratio of about 1:1:1:1.

In one embodiment of the method of enhancing a therapeutic effect provided by a primary therapeutic agent, the apple, grape, green tea, and olive extracts are combined at a weight ratio of about 6:1:3:1.

In one embodiment of the method of enhancing a therapeutic effect provided by a primary therapeutic agent, the method further comprises combining the primary therapeutic agent and the apple, grape, green tea, and olive extracts with at least one secondary therapeutic agent.

In one embodiment of the method of enhancing a therapeutic effect provided by a primary therapeutic agent, wherein the at least one secondary therapeutic agent comprises a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, and combinations thereof.

In one embodiment of the method of enhancing a therapeutic effect provided by a primary therapeutic agent, the method further comprises combining the primary

therapeutic agent and the apple, grape, green tea, and olive extracts with a blueberry concentrate, a capsicum extract, and a turmeric extract.

In one embodiment of the method of enhancing a therapeutic effect provided by a primary therapeutic agent, the method further comprises combining the primary therapeutic agent, the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a mangosteen fruit extract.

In one embodiment of the method of enhancing a therapeutic effect provided by a primary therapeutic agent, the method further comprises combining the primary therapeutic agent, the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a bergamot extract.

In one embodiment of the method of enhancing a therapeutic effect provided by a primary therapeutic agent, the method further comprises combining the primary therapeutic agent, the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a mangosteen pericarp extract.

In one embodiment of the method of enhancing a therapeutic effect provided by a primary therapeutic agent, the method further comprises combining the primary therapeutic agent, the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with mangosteen pericarp extract and bergamot extract.

In one embodiment presented herein is a method of making an activity-enhanced composition for regulating oxidative stress in a subject, comprising: combining apple, grape, green tea, and olive extracts in amounts that provide a greater antioxidant activity than provided by an equivalent amount of any one extract or a sum of the extracts.

In one embodiment of the method of making an activity-enhanced composition for regulating oxidative stress in a subject, the apple, grape, green tea and olive extracts are extracted using an extraction solvent selected from the group consisting of water, ethanol, ethyl acetate, and combinations thereof.

In one embodiment of the method of making an activity-enhanced composition for regulating oxidative stress in a subject, the apple, grape, green tea, and olive extracts are present combined at a weight ratio of about 1:1:1:1.

In one embodiment of the method of making an activity-enhanced composition for regulating oxidative stress in a subject, the apple, grape, green tea, and olive extracts are combined at a weight ratio of about 6:1:3:1.

In one embodiment of the method of making an activity-enhanced composition for regulating oxidative stress in a subject, the method further comprises combining the apple,

grape, green tea, and olive extracts with at least one primary or secondary therapeutic agent.

In one embodiment of the method of making an activity-enhanced composition for regulating oxidative stress in a subject, the at least one primary or secondary therapeutic agent comprises a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, and combinations thereof.

In one embodiment of the method of making an activity-enhanced composition for regulating oxidative stress in a subject, the method further comprises combining the apple, grape, green tea, and olive extracts with a blueberry concentrate, a capsicum extract, and a turmeric extract.

In one embodiment of the method of making an activity-enhanced composition for regulating oxidative stress in a subject, the method further comprises combining the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a mangosteen fruit extract.

In one embodiment of the method of making an activity-enhanced composition for regulating oxidative stress in a subject, the method further comprises combining the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a bergamot extract.

In one embodiment of the method of making an activity-enhanced composition for regulating oxidative stress in a subject, the method further comprises combining the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a mangosteen pericarp extract.

In one embodiment of the method of making an activity-enhanced composition for regulating oxidative stress in a subject, the method further comprises combining the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with mangosteen pericarp extract and bergamot extract.

In one embodiment presented herein is a method of making an activity-enhanced composition for regulating disease-associated protein kinase activity in a subject, comprising: combining apple, grape, green tea, and olive extracts in amounts that provide a greater protein kinase regulating activity than provided by an equivalent amount of any one extract or a sum of the extracts.

In one embodiment of the method of making an activity-enhanced composition for regulating disease-associated protein kinase activity in a subject, the apple, grape, green

tea and olive extracts are extracted using an extraction solvent selected from the group consisting of water, ethanol, ethyl acetate, and combinations thereof.

In one embodiment of the method of making an activity-enhanced composition for regulating disease-associated protein kinase activity in a subject, the apple, grape, green tea, and olive extracts are present combined at a weight ratio of about 1:1:1:1.

In one embodiment of the method of making an activity-enhanced composition for regulating disease-associated protein kinase activity in a subject, the apple, grape, green tea, and olive extracts are combined at a weight ratio of about 6:1:3:1.

In one embodiment of the method of making an activity-enhanced composition for regulating disease-associated protein kinase activity in a subject, the method further comprises combining the apple, grape, green tea, and olive extracts with at least one secondary therapeutic agent.

In one embodiment of the method of making an activity-enhanced composition for regulating disease-associated protein kinase activity in a subject, the at least one secondary therapeutic agent comprises a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, and combinations thereof.

In one embodiment of the method of making an activity-enhanced composition for regulating disease-associated protein kinase activity in a subject, the method further comprises combining the apple, grape, green tea, and olive extracts with a blueberry concentrate, a capsicum extract, and a turmeric extract.

In one embodiment of the method of making an activity-enhanced composition for regulating disease-associated protein kinase activity in a subject, the method further comprises combining the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a mangosteen fruit extract.

In one embodiment of the method of making an activity-enhanced composition for regulating disease-associated protein kinase activity in a subject, the method further comprises combining the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a bergamot extract.

In one embodiment of the method of making an activity-enhanced composition for regulating disease-associated protein kinase activity in a subject, the method further comprises combining the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a mangosteen pericarp extract.

In one embodiment of the method of making an activity-enhanced composition for regulating disease-associated protein kinase activity in a subject, the method further comprises combining the blueberry concentrate and the apple, grape, green tea, olive, capsicum, and turmeric extracts with mangosteen pericarp extract and bergamot extract.

In one embodiment presented herein is a method of making a therapeutic composition comprising: providing a primary therapeutic agent; and combining apple, grape, green tea, and olive extracts with the primary therapeutic agent in amounts that increase a therapeutic effect of the primary therapeutic agent more than an increase in therapeutic effect provided by an equivalent amount of any one of the extracts alone.

In one embodiment of the method of making a therapeutic composition, the method further comprises first extracting the apple, grape, green tea and olive extracts using an extraction solvent selected from the group consisting of water, ethanol, ethyl acetate, and combinations thereof.

In one embodiment of the method of making a therapeutic composition, the apple, grape, green tea, and olive extracts are present combined at a weight ratio of about 1:1:1:1.

In one embodiment of the method of making a therapeutic composition,the apple, grape, green tea, and olive extracts are combined at a weight ratio of about 6:1:3:1.

In one embodiment of the method of making a therapeutic composition, the method further comprises combining the apple, grape, green tea, and olive extracts with at least one secondary therapeutic agent.

In one embodiment of the method of making a therapeutic composition, the at least one secondary therapeutic agent comprises a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, and combinations thereof.

In one embodiment of the method of making a therapeutic composition, the method further comprises combining the primary therapeutic agent, the apple, grape, green tea, and olive extracts with a blueberry concentrate, a capsicum extract, and a turmeric extract.

In one embodiment of the method of making a therapeutic composition, the method further comprises combining the primary therapeutic agent, the blueberry concentrate, and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a mangosteen fruit extract.

In one embodiment of the method of making a therapeutic composition, the method further comprises combining the primary therapeutic agent, the blueberry concentrate, and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a bergamot extract.

In one embodiment of the method of making a therapeutic composition, the method further comprises combining the primary therapeutic agent, the blueberry concentrate, and the apple, grape, green tea, olive, capsicum, and turmeric extracts with a mangosteen pericarp extract.

In one embodiment of the method of making a therapeutic composition, the method further comprises combining the primary therapeutic agent, the blueberry concentrate, and the apple, grape, green tea, olive, capsicum, and turmeric extracts with mangosteen pericarp extract and bergamot extract.

Embodiments of the present disclosure will be described with reference to the following examples, which are provided for illustrative purposes only and should not be used to limit the scope of or construe the invention.

EXAMPLES

Example 1

A Ten-component Phytocomplex ("PC10") Exhibits Synergy in Oxygen Radical Absorbance Capacity

A 10-component phytocomplex (PC10) that exhibits synergy in its ability to absorb oxygen radicals using the oxygen radical absorbance capacity (ORAC) assay is prepared as set forth below.

Chemicals – All chemicals were purchased from standard chemical suppliers (e.g. Sigma, St. Louis, MO) and were of the highest purity commercially available. Reagents used included 75mM potassium phosphate (KH2PO4) (pH=7.4); 0.64M AAPH (2'2'-Azobis (2-amidino-propane)dihydrochloride);10 mM Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid); 4.4 x10-6 M stock fluorescein, sodium salt; and 1:1 acetone/water.

PC10 Materials - Ten commercial samples apple fruit extract (R11309), bergamot fruit extract (R 13216), blueberry fruit concentrate (R10990), capsicum fruit (R11505), grape seed extract (R13545), grape skin extract (R13555), green tea leaf extract (R13568), mangosteen pericarp extract (R26699), olive leaf extract (R15020), and turmeric root & rhizome extract (R17065) were tested individually and in various combinations for their oxygen radical scavenging activity.

Sample preparation - Samples were ground to fine powder and mixed thoroughly. Fifty mg (accurate to 0.1 mg) of sample was transferred into a 35 ml centrifuge tube and mixed with 25 ml of acetone/water (50:50, v/v) extraction solution. Samples were then sonicated for 60 min (shaken from 20 to 40 min) and centrifuged at 3.5K rpm for 10 min.

The PC10 material was formulated by combining apple fruit extract, bergamot fruit extract, blueberry fruit concentrate, capsicum fruit, grape seed extract, grape skin extract, green tea leaf extract, mangosteen pericarp extract, olive leaf extract, and turmeric root & rhizome extract in a number of ratios beginning with 1:1:1:1:1:1:1:1:1:1 and increasing or decreasing the relative amount of a component based upon ORAC activity and cost of ingredient.

Assay methodology – Cuvettes for blank, standard and samples were placed in dry bath at 28 ± 1 °C. and 100 µL 8.8 x 10-8 M fluorescein and 2.50 ml buffer were added into each cuvette and 50 µL buffer was added for the blank. Fifty µL sample solution was added to the sample cuvette. Cuvettes were capped and mixed briefly. Cuvettes were placed into the holder of an RF-150 Spectrofluorophotometer and the initial florescence was recorded as f0. One-hundred µL AAPH was added to each cuvette at time t=0. The cuvette was capped and vortexed briefly. Fluorescence (RFU) was measured at five minute intervals until the fluorescence decay ceased or the value of fluorescence was < 5% of the initial fluorescence reading. RFU were recorded as f1, f2, etc. Fluorescence decay was complete in 60 min.

Calculations - The median inhibitory concentration (IC_{50}) for oxygen radical scavenging activity in this example was calculated by interpolating the concentration required for the inhibition of the fluorescence decay by 50 percent within 20 minutes. Synergy of test components was then quantified using the combination index (CI) parameter. This

parameter defines only the additive effect rather than synergism or antagonism. Synergy, however, was defined as a more than expected additive effect (CI >1), and antagonism as a less than expected additive effect (CI<1) as described below.

Expected median inhibitory concentrations of any multi-component combination were estimated using the relationship:

$$[1/\text{Expected } IC_{50}] = [Fa/IC_{50A}] + [Fb/IC_{50B}] + \ldots + [Fn/IC_{50N}]$$
$$\text{and } Fa + Fb + \ldots + Fn = 1$$

where Fa = mole fraction of component A in the combination and Fn = the mole fraction of the n^{th} component combination and IC_{50A} = the observed IC_{50} of the component A. The CI was then calculated thusly, CI = Expected [IC_{50}]/Observed [IC_{50}]. Using the designation of CI=1 as the additive effect, for mutually exclusive compounds that have the same mode of action or for mutually non-exclusive drugs that have totally independent modes of action the following relationships are defined: CI < 1,= 1, and >1 indicating antagonism, additivity and synergy, respectively.

Results – As seen in Table 1, the observed IC_{50} of the ten-component phytocomplex was 18.6 mg/L, while the calculated, expected IC_{50} value was 26.5 mg/L resulting in a CI=1.43. This level of difference was unexpected and constitutes a novel, unexpected finding for the PC10 formulation oxygen radical scavenging activity.

Table 1: Determining Combination Index for Oxygen Radical Absorbance Capacity of an Ten-component Phytocomplex (PC10)

Test Material	Observed IC_{50} [mg/L]	Relative Amount [F]	$Fn/[IC_{50}]$ $[\mu g/mL]^{-1}$
Apple fruit†	6.8	0.085	0.0124
Bergamot fruit†	46.3	0.704	0.0152
Blueberry fruit*	1,283	0.014	0.000011
Capsicum fruit†	887	0.014	0.000016
Grape seed*	10.2	0.014	0.0014
Grape skin†	373	0.014	0.000038
Green tea leaf†	67.4	0.042	0.00063
Mangosteen pericarp†	49.6	0.014	0.00028
Olive leaf†	101	0.014	0.000140

Turmeric root†	11.2	0.085	0.0076
Phytocomplex(10)**	**18.6**	**1.000**	**0.0377**

† extract /* concentrate/** Phytocomplex (10) contains relative amounts of each of the ten test ingredients; Expected IC50 for PC10 = 1/[0.0377] = 26.5 µg/mL.

Conclusion –The ten-component phytocomplex (PC10) in the ratios of about 6:50:1:1:1:1:3:1:1:6 exhibited an unexpected increase of 1.42-times the oxygen radical scavenging activity relative to the sum of its individual components.

Example 2

An Eight-component Phytocomplex Exhibits Synergy in Oxygen Radical Absorbance Capacity

A nine-component phytocomplex relative to the sum of the expected contributions of its components in the standard ORAC Assay is prepared as set forth below.

Chemicals – All chemicals used in this example were those previously described in Example 1.

PC8 Materials – With the exclusion of bergamot and mangosteen pericarp, the test materials used in this example included those previously described for Example 1.

Assay methodology – The methodology was generally as described in Example 1 with the exception that assays were performed in 96-well microplates fluorimeter at wavelength 493 nm (excitation)/520 nm (emission) every 2 minute for 20 minutes.

Calculations - The median inhibitory concentration (IC50) for oxygen radical scavenging activity was calculated using CalcuSyn (BIOSOFT, Ferguson, MO). This statistical package performs multiple drug dose-effect calculations using the median effect methods described by T-C Chou and P. Talaly [(1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22, 27-55.] hereby incorporated by reference.

Briefly, the analysis correlates the "Dose" and the "Effect" in the simplest possible form: fa/fu = (C/Cm)m, where C is the concentration or dose of the compound and Cm is the median-effective dose signifying the potency. Cm is determined from the x-intercept of the median-effect plot. The fraction affected by the concentration of the test material is fa and the fraction unaffected by the concentration is fu (fu = 1 – fa). The exponent m is the parameter signifying the sigmoidicity or shape of the dose-effect curve; it is estimated by the slope of the median-effect plot.

The median-effect plot is a graph of x= log(C) vs y = log(fa/fu) and is based on the logarithmic form of Chou's median-effect equation. The goodness of fit for the data to the median-effect equation is represented by the linear correlation coefficient r of the median-effect plot. Usually, the experimental data from enzyme or receptor systems have an r > 0.96, from tissue culture an r > 0.90 and from animal systems r > 0.85.

Synergy of test components was quantified using the combination index (CI) parameter as defined in Example 1.

Expected median inhibitory concentrations of any multi-component combination were estimated using the relationship:

$$[1/\text{Expected } IC_{50}] = [Fa/IC_{50A}] + [Fb/IC_{50B}] + \ldots + [Fn/IC_{50N}] \text{ and}$$
$$\text{and } Fa + Fb + \ldots + Fn = 1$$

where Fa = mole fraction of component A in the combination and Fn = the mole fraction of the n^{th} component combination and IC_{50A} = the observed IC_{50} of the component A. The CI was then calculated thusly, CI = Expected [IC_{50}]/Observed [IC_{50}].

Using the designation of CI = 1 as the additive effect, we obtain for mutually exclusive compounds that have the same mode of action or for mutually non-exclusive drugs that have totally independent modes of action the following relationships: CI < 1, = 1, and > 1 indicating antagonism, additivity and synergy, respectively.

Results – The median inhibitory concentration (IC_{50}) of PC8 was 0.0350 µg/mL, while the calculated, expected IC_{50} was 0.0436 µg/mL resulting in a CI = 1.31. Thus, PC8 synergistically produced 1.3-times the oxygen radical scavenging activity than expected from the sum of the activity of its components.

Table 2: Determining Combination Index for Oxygen Radical Absorbance Capacity of an Eight-component Phytocomplex (PC8)

Test Material	Observed IC_{50} [μg/mL]	Relative Amount [F]	Fn/[IC_{50}] [μg/mL]$^{-1}$
Apple fruit†	0.02	0.300	15
Blueberry fruit*	25.55	0.050	0.002
Capsicum fruit†	111	0.050	0.000450
Grape seed*	0.0413	0.050	1.212
Grape skin†	0.233	0.050	0.215
Green tea leaf†	0.04	0.150	3.75
Olive leaf†	0.08	0.050	0.625
Turmeric root†	0.43	0.300	0.698
Phytocomplex(PC8)**	**0.0350**	**1.000**	**21.58**

†extract /*concentrate/** Phytocomplex PC8 contains relative amounts [F] of each of the eight test materials; Expected IC_{50} for PC8 = 1/[21.58] = 0.046 μg/mL.

Conclusion – With CI = 1.31, PC8 unexpectedly produced 1.31-times the oxygen radical scavenging activity than expected from the sum of its components.

Example 3

A Nine-component Phytocomplex Containing Mangosteen Fruit ("PC9f") Exhibits Synergy in Oxygen Radical Absorbance Capacity

A nine-component phytocomplex relative to the sum of the expected contributions of the relative components in the standard ORAC Assay is prepared as set forth below.

All Chemicals, Methods and Calculations were performed as described in Example 2.

PC9f Material – The test material used in this example contained the ingredients and relative amounts listed in Table 3.

Results – The IC_{50} of PC9f was 0.0410 μg/mL, while the calculated, expected IC_{50} was 0.0.049 μg/mL resulting in a CI = 1.20. Thus, PC9f synergistically produced 1.20-times the oxygen radical scavenging activity than expected from the activity of the sum of its components.

Table 3: Determining Combination Index for Oxygen Radical Absorbance Capacity of a Nine-component Phytocomplex Containing Mangosteen Fruit (PC9f)

Test Material	Observed IC_{50} [µg/mL]	Relative Amount [F]	Fn/[IC_{50}] [µg/mL]$^{-1}$
Apple fruit†	0.02	0.286	14.3
Blueberry fruit*	25.55	0.0476	0.002
Capsicum fruit†	111	0.0476	0.000432
Grape seed*	0.0413	0.0476	1.19
Grape skin†	0.233	0.0476	0.238
Green tea leaf†	0.04	0.1429	3.573
Mangosteen fruit†	3.99	0.0476	0.012
Olive leaf†	0.08	0.0476	0.595
Turmeric root & rhizome†	0.43	0.286	0.664
Phytocomplex (PC9f)	**0.0410**	**1.000**	**20.56**

†extract /*concentrate/** Phytocomplex PC9f contains relative amounts [F] of each of the nine test materials: Expected IC_{50} for PC9f = 1/[20.56] = 0.049 µg/mL.

Conclusion - With CI = 1.20, PC9f unexpectedly produced 1.2-times the oxygen radical scavenging activity than expected from the sum of its components.

Example 4

An Eight-component Phytocomplex (PC8) Exhibits Synergy in Free Radical Quenching

A eight-component phytocomplex relative to the sum of the expected contributions of its components to scavenge free radicals is prepared as set forth below.

Methodology The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was used to assess scavenging of free radicals by the test materials. This assay is based on the theory that a hydrogen donor is an antioxidant. The assay measures compounds that are radical scavengers. The stable free radical DPPH* accepts hydrogen from an antioxidant. The antioxidant effect is proportional to the disappearance of DPPH$^{\cdot}$ in test samples. The procedure as described by Dudonne was used with the modification that the assay was conducted in 96-well, microtiter plates [Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., and Merillon, J. M. (2009) Comparative Study of Antioxidant Properties and Total

Phenolic Content of 30 Plant Extracts of Industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC Assays, J Agric Food Chem 57, 1768-1774].

PC8 Material – The test material used in this study were as described in Example 2 and Tables 2 and 4.

Calculations - Calculations were performed as described in Example 2.

Results – The median inhibitory concentration (IC_{50}) of PC8 was 15.6 µg/mL, while the calculated, expected IC_{50} was 17.9 µg/mL resulting in a CI = 1.15. Thus, PC8 synergistically produced 1.2-times the free radical quenching activity of the sum of its components.

Table 4: Determining Combination Index for Free Radical Quenching Capacity of an Eight-component Phytocomplex (PC8)

Test Material	Observed IC_{50} [µg/mL]	Relative Amount [F]	Fn/[IC_{50}] [µg/mL]$^{-1}$
Apple fruit†	14.1	0.300	0.0213
Blueberry fruit*	2713	0.050	0.0000184
Capsicum fruit†	7225	0.050	0.00000692
Grape seed*	5.52	0.050	0.00906
Grape skin†	107	0.050	0.000469
Green tea leaf†	6.89	0.150	0.0218
Olive leaf†	50.3	0.050	0.000993
Turmeric root†	137	0.300	0.00218
Phytocomplex(PC8)**	**15.6**	**1.000**	**0.0558**

†extract /*concentrate/** Phytocomplex PC8 contains relative amounts [F] of each of the eight test materials; Expected IC_{50} for PC8 = 1/[0.0558] = 17.92 µg/mL.

Conclusion – With CI = 1.15, PC8 unexpectedly produced 1.2-times the free radical quenching activity than the sum of its components.

Example 5

An Nine-component Phytocomplex Containing Mangosteen Fruit ("PC9f") Exhibits Synergy in Free Radical Quenching

A nine-component phytocomplex relative to the sum of the expected contribution of its components to scavenge free radicals is prepared as set forth below.

All *Chemicals* and *Calculations* were as described in Example 2 and the *Methods* were as previously presented in Example 4.

PC9f Material – the test material used in this example contained the ingredients and relative amounts listed in Tables 3 and 5.

Results – The IC_{50} of PC9f was 16.2 µg/mL, while the calculated, expected IC_{50} was 18.8 µg/mL resulting in a CI = 1.16. Thus, PC9f synergistically produced 1.8-times the oxygen radical scavenging activity than expected from the activity of the sum of its components.

Table 5: Determining Combination Index for Free Radical Quenching Capacity of a Nine-component Phytocomplex Containing Mangosteen Fruit (PC9f)

Test Material	Observed IC_{50} [µg/mL]	Relative Amount [F]	Fn/[IC_{50}] [µg/mL]$^{-1}$
Apple fruit†	14.1	0.286	0.0202664
Blueberry fruit*	2713	0.048	0.0000175
Capsicum fruit†	7225	0.048	0.00000659
Grape seed†	5.52	0.048	0.0086269
Grape skin†	106.6	0.048	0.0004465
Green tea leaf†	6.89	0.143	0.0207460
Mangosteen fruit†	3527	0.048	0.0000135
Olive leaf†	50.3	0.048	0.0009455
Turmeric root & rhizome†	137	0.286	0.0020804
Phytocomplex (PC9f)	**16.2**	**1.000**	**0.0531**

†extract /*concentrate/** Phytocomplex PC9f contains relative amounts [F] of each of the nine test materials; Expected IC_{50} for PC9f = 1/[0.0531] = 18.8 µg/mL.

Conclusion - With CI = 1.16, PC9f unexpectedly produced 1.2-times the oxygen radical scavenging activity than expected from the sum of its components.

Example 6

A Nine-component Phytocomplex Containing Mangosteen Pericarp ("PC9p") Exhibits Synergy in Free Radical Quenching

A nine-component phytocomplex relative to the sum of the expected contribution of its components to scavenge free radicals is prepared as set forth below.

Chemicals and *Methods* were as previously presented in Example 4, while *Calculations* were as described in Example 2.

PC9p Material – the test material used in this example contained the ingredients and relative amounts described in Table 6.

Table 6: Determining Combination Index for Free Radical Quenching Capacity of a Nine-component Phytocomplex Containing Mangosteen Pericarp (PC9p)

Test Material	Observed IC_{50} [µg/mL]	Relative Amount [F]	Fn/$[IC_{50}]$ $[\mu g/mL]^{-1}$
Apple fruit†	14.1	0.286	0.0203
Blueberry fruit*	2713	0.0476	0.0000175
Capsicum fruit†	7225	0.0476	0.0000066
Grape seed†	5.52	0.0476	0.0086
Grape skin†	106.6	0.0476	0.000447
Green tea leaf†	6.89	0.143	0.0207
Mangosteen pericarp†	26.7	0.0476	0.00178
Olive leaf†	50.3	0.0476	0.000945
Turmeric root & rhizome†	137	0.286	0.0021
Phytocomplex (PC9p)	**13.4**	**1.000**	**0.0549**

†extract /*concentrate/** Phytocomplex PC9p contains relative amounts [F] of each of the nine test materials; Expected IC_{50} for PC9p = 1/[0.0549] = 18.2 µg/mL.

Results – The IC_{50} of PC9p was 013.4 µg/mL, while the calculated, expected IC_{50} was 18.2 µg/mL resulting in a CI = 1.36. Thus, PC9p synergistically produced 1.4-times the free radical quenching activity than expected from the activity of the sum of its components.

Conclusion - With CI = 1.36, PC9p unexpectedly produced 1.4-times the free radical quenching activity than the sum of its components.

Example 7

A Nine-component Phytocomplex ("PC9b") Exhibits Synergy in Peroxynitrite ($ONOO^-$) Scavenging Capacity

The potential for synergy of a nine-component phytocomplex relative to the sum of the expected contributions of its components in scavenging peroxynitrite is prepared as set forth below.

Methodology - Peroxynitrite ($ONOO^-$) scavenging capacity was measured according to the procedure described by Kim et al. [Kim, J. Y., Kim, H. S., Kang, H. S., Choi, J. S., Yokozawa, T., and Chung, H. Y. (2008) Antioxidant potential of dimethyl lithospermate isolated from Salvia miltiorrhiza (red sage) against peroxynitrite, J Med Food 11, 21-28] with the modification that assays were conducted in 96-well microtiter plates instead of cuvettes.

Calculations were as described in Example 2.

PC9b Material – In addition to the bergamot extract as described in Example 1, the remaining test material used in this study were as described in Example 2 and Tables 2 and 4 as PC8.

The IC_{50} of PC9b was 0.974 µg/mL, while the calculated, expected IC_{50} was 1.67 µg/mL resulting in a CI = 1.71. Thus, PC9b synergistically produced 1.7-times the peroxynitrite-scavenging capacity than expected from the sum of its components.

Table 7: Determining Combination Index for Peroxynitrite ($ONOO^-$) Scavenging Capacity of a Nine-component Phytocomplex (PC9b) Containing Bergamot

Test Material	Observed IC_{50} [µg/mL]	Relative Amount [F]	$Fn/[IC_{50}]$ $[µg/mL]^{-1}$
Apple fruit†	0.875	0.0857	0.0979
Bergamot	3.34	0.714	0.214
Blueberry fruit*	35.7	0.0143	0.000401
Capsicum fruit†	31.0	0.0143	0.000461

Grape seed*	0.472	0.0143	0.0303
Grape skin†	3.11	0.0143	0.00460
Green tea leaf†	0.185	0.0429	0.232
Olive leaf†	1.17	0.0143	0.0122
Turmeric root†	10.3	0.0857	0.00834
Phytocomplex(PC9b)**	**0.974**	**1.000**	**0.600**

†extract /*concentrate/** Phytocomplex PC9b contains relative amounts [F] of each of the nine test materials; Expected IC_{50} for PC9b = 1/[0.600] = 1.67 µg/mL.

Conclusion – With CI = 1.71, PC9b unexpectedly produced 1.7-times the peroxynitrite-scavenging capacity than expected from the sum of its components.

Example 8

A Nine-component Phytocomplex ("PC9f") Exhibits Synergy in Peroxynitrite ($ONOO^-$) Scavenging Capacity

The potential for synergy of a novel, nine-component phytocomplex containing mangosteen fruit relative to the sum of the expected contributions of its components in scavenging peroxynitrite was evaluated.

Methodology Methods and Calculations were as previously described in Example 7, while *Calculations* were as described in Example 2.

PC9f Material – PC9f and components as described in Example 3 and Tables 3 and 8 were the test materials in this example. The IC_{50} of PC9f was 0.486 µg/mL, while the calculated, expected IC_{50} was 0.777 µg/mL resulting in a CI = 1.60. Thus, PC9f synergistically produced 1.6-times the peroxynitrite-scavenging capacity than expected from the sum of its components.

Table 8: Determining Combination Index for Peroxynitrite ($ONOO^-$) Scavenging Capacity of a Nine-component Phytocomplex (PC9f)

Test Material	Observed IC_{50} [µg/mL]	Relative Amount [F]	$Fn/[IC_{50}]$ $[\mu g/mL]^{-1}$
Apple fruit†	0.875	0.286	0.326
Blueberry fruit*	35.7	0.0476	0.001

Capsicum fruit†	31.0	0.0476	0.002
Grape seed†	0.472	0.0476	0.101
Grape skin†	3.11	0.0476	0.015
Green tea leaf†	0.185	0.143	0.773
Mangosteen fruit†	68.3	0.0476	0.001
Olive leaf†	1.17	0.0476	0.041
Turmeric root & rhizome†	10.3	0.286	0.028
Phytocomplex (PC9f)	**0.486**	**1.000**	**1.29**

†extract /*concentrate/** Phytocomplex PC9f contains relative amounts [F] of each of the nine test materials; Expected IC_{50} for PC9f = 1/[1.29] = 0.777 µg/mL.

Conclusion – With CI = 1.60, PC9f unexpectedly produced 1.6-times the peroxynitrite-scavenging capacity than expected from the sum of its components.

Example 9

A Nine-component Phytocomplex (PC9f) Plus Bergamot Exhibits Synergy in Peroxynitrite ($ONOO^-$) Scavenging Capacity

The potential for synergy of a novel, nine-component phytocomplex containing mangosteen fruit and bergamot relative to the sum of the expected contributions of its components in scavenging peroxynitrite was evaluated.

Methodology - Methods and Calculations were as previously described in Examples 7 and 2, respectively.

PC9f Plus Bergamot Material – PC9f and components as described in Example 3 and Tables 3 and 9 were the test materials in this example along with the bergamot extract as described in Example 1. The IC_{50} of PC9f plus bergamot fruit extract was 0.556 µg/mL, while the calculated, expected IC_{50} was 1.69 µg/mL resulting in a CI = 3.04. Thus, PC9f plus bergamot fruit extract synergistically produced 3.0-times the peroxynitrite-scavenging capacity than expected from the sum of its components.

Table 9: Determining Combination Index for Peroxynitrite ($ONOO^-$) Scavenging Capacity of a Nine-component Phytocomplex (PC9f) plus Bergamot Fruit Extract

Test Material	Observed IC_{50} [µg/mL]	Relative Amount [F]	Fn/[IC_{50}] [µg/mL]$^{-1}$
Apple fruit†	0.875	0.0845	0.097
Bergamot fruit†	3.34	0.7042	0.211
Blueberry fruit*	35.7	0.0141	0.000395
Capsicum fruit†	31.0	0.0141	0.000455
Grape seed†	0.472	0.0141	0.0299
Grape skin†	3.11	0.0141	0.00454
Green tea leaf†	0.185	0.0423	0.229
Mangosteen fruit†	68.3	0.0141	0.000206
Olive leaf†	1.17	0.0141	0.0121
Turmeric root & rhizome†	10.3	0.0845	0.00822
Phytocomplex (PC9f) + Bergamot	**0.556**	**1.000**	**0.592**

†extract /*concentrate/** Phytocomplex PC9f + Bergamot contains relative amounts [F] of each of the nine test materials; Expected IC_{50} for PC9f +Bergamot = 1/[0.592] = 1.69 µg/mL.

Conclusion – With CI = 3.04, PC9f + bergamot fruit extract unexpectedly produced 3.0-times the peroxynitrite-scavenging capacity than expected from the sum of its components

Example 10

A Nine-component Phytocomplex (PC9p) Exhibits Synergy in Peroxynitrite ($ONOO^-$) Scavenging Capacity

The potential for synergy of a novel, nine-component phytocomplex containing mangosteen pericarp (PC9p) relative to the sum of the expected contributions of its components in scavenging peroxynitrite was evaluated.

Methodology Methods and Calculations were as previously described in Examples 7 and 2, respectively.

PC9p Material – PC9p and components as described in Table 10 were the test materials in this example. The IC_{50} of PC0p was 0.523 µg/mL, while the calculated, expected IC_{50} was 0.768 µg/mL resulting in a CI = 1.47. Thus, PC9p synergistically produced 1.5-times the peroxynitrite-scavenging capacity than expected from the sum of its components.

Table 10: Determining Combination Index for Peroxynitrite ($ONOO^-$) Scavenging Capacity of a Nine-component Phytocomplex (PC9p)

Test Material	Observed IC_{50} [µg/mL]	Relative Amount [F]	$Fn/[IC_{50}]$ $[µg/mL]^{-1}$
Apple fruit†	0.875	0.286	0.326
Blueberry fruit*	35.7	0.048	0.00133
Capsicum fruit†	31.0	0.048	0.00154
Grape seed†	0.472	0.048	0.101
Grape skin†	3.11	0.048	0.0153
Green tea leaf†	0.185	0.143	0.773
Mangosteen pericarp†	3.26	0.048	0.0146
Olive leaf†	1.17	0.048	0.0408
Turmeric root & rhizome†	10.3	0.286	0.0278
Phytocomplex (PC9p)	**0.523**	**1.00**	**1.30**

†extract /*concentrate/** Phytocomplex PC9p + Bergamot contains relative amounts [F] of each of the nine test materials; Expected IC_{50} for PC9p +Bergamot = 1/[1.30] = 0.768 µg/mL.

Conclusion – With CI = 1.47, PC9p unexpectedly produced 1.5-times the peroxynitrite-scavenging capacity than expected from the sum of its components.

Example 11

A Ten-component Phytocomplex (PC10) Exhibits Synergy in Peroxynitrite ($ONOO^-$) Scavenging Capacity

The potential for synergy of a novel, ten-component phytocomplex (PC10) relative to the sum of the expected contributions of its components in scavenging peroxynitrite was evaluated.

Methodology Methods and Calculations were as previously described in Examples 7 and 2, respectively.

PC10 Material – PC10 and components as described in Example 1 and Table 1 were the test materials in this example.

Conclusion - The IC_{50} of PC10 was 1.02 μg/mL, while the calculated, expected IC_{50} was 1.68 μg/mL resulting in a CI = 1.64. Thus, PC10 synergistically produced 1.6-times the peroxynitrite-scavenging capacity than expected from the sum of its components.

Table 11: Determining Combination Index for Peroxynitrite ($ONOO^-$) Scavenging Capacity of a Ten-component Phytocomplex (PC10)

Test Material	Observed IC_{50} [μg/mL]	Relative Amount [F]	Fn/[IC_{50}] [μg/mL]$^{-1}$
Apple fruit†	0.875	0.085	0.0965
Bergamot fruit†	3.34	0.704	0.211
Blueberry fruit*	35.7	0.0141	0.000395
Capsicum fruit†	31.0	0.0141	0.000455
Grape seed†	0.472	0.0141	0.0299
Grape skin†	3.11	0.0141	0.00454
Green tea leaf†	0.185	0.0423	0.229
Mangosteen pericarp†	3.26	0.0141	0.00432
Olive leaf†	1.17	0.0141	0.0121
Turmeric root & rhizome†	10.3	0.0845	0.00822
Phytocomplex (PC10)	**1.02**	**1.00**	**0.596**

†extract /*concentrate/** Phytocomplex PC10 contains relative amounts [F] of each of the ten test materials; Expected IC_{50} for PC10 = 1/[0.596] = 1.68 μg/mL.

Conclusion – With CI = 1.64, PC10 unexpectedly produced 1.6-times the peroxynitrite-scavenging capacity than expected from the sum of its components

Example 12

Two Formulations of a Four-component Phytocomplex (PC4) Exhibit Synergy in Peroxynitrite ($ONOO^-$) Scavenging Capacity

The potential for synergy of a novel, four-component phytocomplex (PC4) relative to the sum of the expected contributions of its components in scavenging peroxynitrite when tested at two formulations was evaluated.

Methodology – Methods and Calculations were as previously described in Examples 7 and 2, respectively.

PC4 Material – PC4 consisted of components listed in Tables 12 and 13 and as described in Example 1. Relative amounts of the individual four components were 1:1:1:1 = PC4.1 (Table 13) and 6:3:1:1 = PC4.2.

Conclusion - The IC_{50} of PC4.1 was 0.216 µg/mL, while the calculated, expected IC_{50} was 0.420 µg/mL resulting in a CI = 1.95. Thus, PC4.1 synergistically produced 2.0-times the peroxynitrite-scavenging capacity than expected from the sum of its components.

Table 12: Determining Combination Index for Peroxynitrite ($ONOO^-$) Scavenging Capacity of a Four-component Phytocomplex (PC4.1)

Test Material	Observed IC_{50} [µg/mL]	Relative Amount [F]	Fn/[IC_{50}] [µg/mL]$^{-1}$
Apple fruit†	0.875	0.250	0.286
Grape seed†	0.472	0.250	0.530
Green tea leaf†	0.185	0.250	1.352
Olive leaf†	1.17	0.250	0.214
Phytocomplex (PC4.1)	**0.216**	**1.00**	**2.382**

†extract /*concentrate/** Phytocomplex PC4.1 contains relative amounts [F] of each of the four test materials; Expected IC_{50} for PC4.1 = 1/[2.382] = 0.420 µg/mL.

Conclusion – With CI = 1.95, PC4.1 unexpectedly produced 2.0-times the peroxynitrite-scavenging capacity than expected from the sum of its components

Table 13: Determining Combination Index for Peroxynitrite ($ONOO^-$) Scavenging Capacity of a Four-component Phytocomplex (PC4.2)

Test Material	Observed IC_{50} [µg/mL]	Relative Amount [F]	Fn/[IC_{50}] [µg/mL]$^{-1}$
Apple fruit†	0.875	0.545	0.623
Grape seed†	0.472	0.0909	0.193
Green tea leaf†	0.185	0.273	1.475
Olive leaf†	1.17	0.0909	0.078
Phytocomplex (PC4.2)	**0.201**	**1.000**	**2.369**

†extract /*concentrate/** Phytocomplex PC4.2 contains relative amounts [F] of each of the four test materials; Expected IC_{50} for PC4.2 = 1/[2.369] = 0.422 µg/mL.

Results - The IC_{50} of PC4.2 was 0.201 µg/mL, while the calculated, expected IC_{50} was 0.422 µg/mL resulting in a CI = 2.10. Thus, PC4.2 synergistically produced 2.0-times the peroxynitrite-scavenging capacity than expected from the sum of its components.

Conclusion – With CI = 2.10, PC4.2 unexpectedly produced 2.0-times the peroxynitrite-scavenging capacity than expected from the sum of its components

Example 13

Synergistic Interactions of PC9p and Bergamot (PC10) on 295 Protein Kinases

Protein kinases represent a transferase class of enzymes that are able to transfer a phosphate group from a donor molecule (usually ATP) to an amino acid residue of a protein (usually threonine, serine or tyrosine). Kinases are used in signal transduction for the regulation of enzymes, *i.e.*, they can inhibit or activate the activity of an enzyme, such as in cholesterol biosynthesis, amino acid transformations, or glycogen turnover. While most kinases are specialized to a single kind of amino acid residue, some kinases exhibit dual activity in that they can phosphorylate two different kinds of amino acids.

Methods - The inhibitory effect of the PC9p, bergamot and the combination of PC9p and bergamot (the PC10 formulation) were tested individually on human kinase activity in a panel of 295 kinases in the KinaseProfiler™ Assay (Millipore UK Ltd. Dundee, United Kingdom). The assay protocols for the specific kinases listed in Table 14 are summarized at http://www.millipore.com/techpublications/tech1/pf3036.

Median inhibitory concentrations (IC_{50}) were determined for the PC9p, bergamot, and PC10 by simple interpolation when a dose-response was observed over the three concentrations that captured the median effective concentration. The estimated IC_{50} for the PC10 formulation was computed as described in Example 2 using the observed IC_{50s} for PC9p and PC10 in the ratio of 3:7.

Synergy of test components was then quantified using the combination index (CI) parameter. This parameter defines only the additive effect rather than synergism or antagonism. Synergy, however, was defined as a more than expected additive effect (CI >1), and antagonism as a less than expected additive effect (CI<1) as described below.

Expected median inhibitory concentrations of any multi-component combination were estimated using the relationship:

$$[1/\text{Expected } IC_{50}] = [0.3/IC_{50}PC9p] + [0.7/IC_{50}\text{Bergamot}]$$

The CI was then calculated thusly, CI = Expected [IC_{50}]/Observed [IC_{50}]. Using the designation of CI=1 as the additive effect, for mutually exclusive compounds that have the same mode of action or for mutually non-exclusive drugs that have totally independent modes of action the following relationships are defined: CI<1, = 1, and >1 indicating antagonism, additivity and synergy respectively. In these studies, CI >1.10 were considered evidence of synergy between the formulations.

Table 14: Combination Index for the Interaction of PC9f, Bergamot, and PC10 on 295 Kinases

Kinase	PC9f IC_{50} [µg/mL]	Bergamot IC_{50} [µg/mL]	PC10 IC_{50} [µg/mL]	Est PC10 IC_{50}† [µg/mL]	CI
PI3 Kinase (p110a/p85a)	1068.73	5000	7.9	2377	002
PI3 Kinase (p110a(H1047R)/p85a)	5000.00	5000	25	5000	200
Met(Y1248D) HGFR	1.979	5000	0.05	6.59	132
TrkC	0.01	625	0.0002	0.02	86.1
BTK(R28H)	93.20	2.6	0.05	3.63	72.6
PKCβII	5000	5000	70	5000	71.9
Fms	4.18	9.0	0.12	6.70	54.0
Met(Y1248H)	0.73	5000	0.05	2.43	48.6
Met(M1268T) HGFR	0.500	187	0.05	1.66	33.2
GRK6 (clotting)	882.39	5000	63	2083	33.0
ACK1 (also TNK2)	0.05	65	0.01	0.166	24.9
Met(D1246N) (HGFR)	0.04	5000	0.005	0.133	24.4
PRAK (stress induced)	0.17	192	0.05	0.579	11.6
CK2	46	0.07	0.01	0.100	11.4
cKit(V654A)	4.5	9.74	0.735	7.20	9.80
PI3 Kinase (p110b/p85a)	20.91	5000	7.2	69.0	9.65
Rse (Toll-like receptors/NFKß)	24	5000	8.5	80.2	9.41
PI3 Kinase (p120g)	377.22	5000	120.29	1069	8.89
PIP5K1g(h)	0.02	5000	0.01	0.0780	8.33
EGFR	1.8	484	0.8	5.79	7.43

PKCμ(h)	163.46	5000	69.92	506	7.24
PEK(h)	3.3	5000	1.71	10.9	6.37
GCK(h)	0.01	20	0.01	0.0429	5.99
IR activated	6.35	197	3.54	19.7	5.56
MSSK1(h)	0.25	5000	0.16	0.848	5.42
cKit(D816H)	0.034	80	0.022	0.113	5.26
PrKX(h)	28.68	5000	18.65	94.3	5.06
Pim-1(h)	5.4	49	2.92	14.4	4.93
DRAK1(h)	21	131	11	50.7	4.63
GSK3β(h)	8.4	443	6.7	26.8	4.01
Mnk2(h)	0.51	19	0.41	1.60	3.93
TAK1(h)	316	1754	194	741	3.82
MELK(h)	0.78	55	0.66	2.51	3.80
PRK2(h)	11	5000	10	36.5	3.78
CK1γ3(h)	4.86	332	4.19	15.7	3.74
JNK3(h)	26	5000	23	84.1	3.73
NEK11(h)	1.6	5000	1.5	5.42	3.62
cKit	11.6	10	2.92	10.5	3.59
Lyn(h)	5.2	561	4.8	17.1	3.58
PKCδ(h)	50	5000	45	162	3.58
CSK(h)	2.3	411	2.17	7.62	3.51
PhKγ2(h)	0.9	5000	0.85	2.94	3.44
LOK(h)	6.3	5000	6.27	21.0	3.35
TBK1(h)	5.0	625	4.89	16.4	3.35
PKCζ(h)	5.0	5000	4.98	16.6	3.34
Met(D1246H)(h)	0.05	59744	0.050	0.167	3.33
Met(Y1248C)(h)	0.05	4356	0.050	0.167	3.33
Syk(h)	0.05	231	0.050	0.167	3.33
CLK1(h)	0.05	37.7	0.050	0.166	3.32
Flt4(h)	0.05	26.3	0.050	0.166	3.32
PDGFRα(D842V)(h)	0.05	18.4	0.050	0.166	3.31
DYRK2(h)	0.05	14.1	0.050	0.165	3.31
RIPK2(h)	0.05	13.5	0.050	0.165	3.30
MLK1(h)	0.05	7.64	0.050	0.164	3.28
PKCα(h)	175	5000	165	539	3.27
BrSK1(h)	11.7	130	9.9	32.2	3.25
EphA4	4.36	5000	4.50	14.51	3.22
AMPKα2	15.8	5000	16.6	52.3	3.16
CHK2(R145W)(h)	2.99	689	3.16	9.86	3.13

cEF-2K(h)	24.4	5000	25.8	80.3	3.12
Flt3(D835Y)(h)	0.05	1.33	0.050	0.153	3.06
LKB1(h)	101	5000	105	321	3.06
SRPK2(h)	5.46	5000	5.98	18.1	3.03
Flt3(h)	0.05	0.83	0.050	0.146	2.92
HIPK2(h)	4.85	25.0	3.85	11.1	2.89
MSK2(h)	12.9	5000	15.0	42.6	2.85
BrSK2(h)	17.3	43	10.8	29.6	2.74
CaMKI(h)	30.7	5000	37.4	101	2.70
Ros(h)	19.4	5000	24.9	64.2	2.57
FGFR1(h)	20.3	434	23.8	61.0	2.56
EphB1	12.6	625	16.0	40.2	2.50
Fyn(h)	0.655	328	0.872	2.17	2.49
mTOR(h)	27.9	5000	37.7	91.9	2.44
Pim-2(h)	5.72	63	6.57	15.7	2.39
cSRC(h)	21.5	866	28.6	67.7	2.37
CDK1/cyclinB(h)	6.57	5000	9.45	21.8	2.31
IGF-1R(h), activated	19.8	391	25.8	59.1	2.29
CHK2(I157T)(h)	3.89	4412	5.7	12.9	2.26
CK1γ1(h)	16.9	5000	24.7	55.8	2.26
PKBβ(h)	23.6	5000	34.6	77.8	2.24
CDK3/cyclinE(h)	20.1	5000	29.7	66.4	2.24
AMPKα1	6.88	1769	10.2	22.7	2.23
EphB3	26.9	5000	39.9	88.5	2.22
PAR-1Bα(h)	15.4	5000	23.1	51.0	2.20
Tec(h) activated	5.04	5000	7.6	16.8	2.19
DAPK2(h)	22.9	123	24	53.2	2.19
IRAK4(h)	7.67	764	12	25.0	2.16
PAK5(h)	19.7	5000	30	65.1	2.16
TAO1(h)	13.2	5000	20	43.6	2.15
IRAK1(h)	14.5	3679	22	47.9	2.15
ULK2(h)	151	5000	219	469	2.15
CDK2/cyclinE(h)	19.0	5000	29.2	62.7	2.14
MuSK(h)	15.5	5000	24.0	51.4	2.14
MAPK1(h)	19.4	5000	29.9	64.0	2.14
NEK2(h)	21.5	5000	33.5	71.0	2.12
PDGFRα(h)	35.2	2843	53.9	114	2.11
PAK1(h)	37.6	5000	58.4	123	2.11
Tie2(Y897S)(h)	32.2	5000	50.6	106	2.09

PDGFRβ(h)	24.7	5000	39.0	81.5	2.09
SRPK1(h)	3.65	5000	5.85	12.2	2.08
EphA7	44.3	5000	70.3	145	2.06
Pyk2(h)	30.8	5000	49.3	101	2.05
Tie2 (h)	26.8	5000	43.3	88.3	2.04
MINK(h)	8.85	5000	14.4	29.4	2.04
CHK1(h)	29.2	5000	47.2	96.0	2.03
Arg(h)	9.13	3417	15.1	30.3	2.01
ULK3(h)	19.4	5000	32.0	64.0	2.00
cKit(D816V)	7.43	5000	12.4	24.7	1.98
FGFR4(h)	3.52	5000	5.98	11.7	1.96
Aurora-A	11.1	91	14.8	28.9	1.95
MST3(h)	24.4	2027	40.7	79.2	1.95
Mer(h)	10.9	140	15.8	30.8	1.94
CDK2/cyclinA(h)	38.0	5000	64.1	125	1.94
PASK(h)	8.70	5000	14.9	28.9	1.94
CaMKIIβ(h)	16.8	1519	28.2	54.7	1.94
Abl(T315I)	5.47	801	9.26	17.9	1.94
WNK2(h)	13.7	5000	23.6	45.4	1.93
CaMKIIγ(h)	7.13	102	10.6	20.4	1.92
Axl(h)	17.4	5000	30.0	57.6	1.92
KDR(h)	1.94	5000	3.38	6.47	1.92
PDK1(h)	19.9	5000	34.3	65.8	1.92
MST4(h)	35.0	5000	60.2	115	1.91
Rsk1(h)	3.27	2483	5.72	10.9	1.90
SGK3(h)	29.5	5000	51.1	96.9	1.89
MAPKAP-K2(h)	29.8	5000	52.1	97.9	1.88
PAK4(h)	24.6	5000	43.3	81.2	1.88
CDK6/cyclinD3(h)	38.1	5000	66.5	125	1.88
SGK(h)	32.9	5000	57.9	108	1.87
MKK7β(h)	35.0	5000	61.7	115	1.86
p70S6K(h)	1.12	743	2.00	3.72	1.86
TAO3(h)	16.7	5000	29.8	55.3	1.86
MST2(h)	18.9	5000	33.7	62.4	1.85
FGFR2(h)	25.6	5000	45.6	84.5	1.85
Fer(h)	40.2	5000	71.5	132	1.84
ZAP-70(h)	11.7	5000	21.4	38.9	1.82
Fgr(h)	12.8	105	18.3	33.2	1.82
EphA3	24.7	5000	44.9	81.4	1.81

CDK5/p25(h)	13.2	5578	24.5	43.8	1.79
PKBα(h)	33.4	5000	61.3	110	1.79
IKKβ	35.3	5000	65.5	116	1.77
NLK(h)	25.0	5000	46.7	82.4	1.77
JAK3(h)	18.6	5000	34.8	61.4	1.76
WNK3(h)	17.6	5000	33.0	58.1	1.76
BTK(h)	7.09	5000	13.5	23.6	1.75
PKG1α(h)	25.6	5000	48.2	84.2	1.75
Ron(h)	8.97	5000	17.1	29.8	1.74
MARK1(h)	6.66	5000	12.7	22.1	1.74
Lck(h)	24.0	444	41.0	71.1	1.74
ROCK-I(h)	55.9	5000	105	182	1.74
TAO2(h)	14.7	5000	28.2	48.7	1.73
PKG1β(h)	19.3	5000	37.0	63.8	1.73
c-RAF(h)	21.2	5000	40.5	69.9	1.72
Fes(h)	24.2	5000	46.4	79.7	1.72
SGK2(h)	28.3	5000	54.4	93.2	1.71
DAPK1(h)	83.1	5000	156	267	1.71
LRRK2(h)	36.6	327	56.5	96.7	1.71
GSK3α(h)	5.67	60.0	9.09	15.5	1.70
ErbB4	27.2	5000	52.6	89.5	1.70
EGFR(T790M)	33.8	231	50.0	84.1	1.68
Hck(h)	17.3	97.1	24.3	40.8	1.68
PKCγ(h)	14.4	5000	28.6	47.8	1.67
CLK4(h)	0.0181	34.7	0.0363	0.0604	1.66
ROCK-II(h)	18.5	5000	36.7	61.0	1.66
HIPK1(h)	27.3	117	35.6	59.0	1.66
Bmx(h)	5.46	721	10.8	17.9	1.65
TSSK2(h)	30.2	5000	60.1	99.2	1.65
MLCK(h)	22.5	5000	45.3	74.2	1.64
FGFR3(h)	10.7	5000	21.6	35.4	1.64
MSK1(h)	52.3	5000	105	170	1.63
TSSK1(h)	21.5	5000	44.4	à	1.60
GRK7	42.0	5000	86.4	137	1.59
FGFR2(N549H)(h)	22.4	388	41.4	65.9	1.59
EphA5	32.4	5000	67.4	106	1.58
Met(h)	53.7	2538	108	171	1.57
PKBγ(h)	31.2	5000	65.2	102	1.57
EGFR(T790M,L858R)	16.9	60.1	21.8	34.1	1.56

TYK2(h)	31.7	82.7	35.8	55.8	1.56
PAK6(h)	29.3	5000	62.3	96.3	1.55
IKKε	37.2	5611	79.2	122	1.54
ZIPK(h)	16.8	5000	36.1	55.6	1.54
EGFR(L858R)	43.1	5000	92.3	141	1.53
ALK4(insulin receptor family)	136	5000	282	427	1.52
PKC0(h)	28.6	5000	62.1	94.0	1.51
EphA2	30.7	2613	65.9	100	1.51
SIK(h)	13.5	59.7	19.6	29.4	1.50
IKKα	46.2	5000	100	151	1.50
ASK1(h)	21.3	132	34.6	51.5	1.49
SAPK4(h)	75.8	5000	164	244	1.49
CLK2(h)	0.050	67.1	0.113	0.166	1.48
NEK6(h)	22.9	5000	51.2	75.4	1.47
EphA1	20.6	76.2	28.9	42.1	1.46
JAK1(h)	11.1	165	22.0	31.9	1.45
CHK2(h)	1.98	468	4.50	6.53	1.45
CDK9/cyclin T1(h)	11.4	27.2	13.3	19.2	1.45
TrkB(h)	6.33	357	14.0	20.3	1.45
CaMK1δ(h)	34.8	5000	79.0	114	1.44
Tie2(R849W)(h)	17.2	5000	39.5	56.8	1.44
MAPKAP-K3(h)	50.6	5000	115	165	1.44
HIPK3(h)	7.53	51.4	13.1	18.7	1.43
ALK(insulin receptor family)	7.74	5000	18.0	25.7	1.43
CDK7/cyclinH/MAT1(h)	41.7	5000	96.0	136	1.42
cKit(V560G)	3.12	0.05	0.0500	0.0709	1.42
LIMK1(h)	28.0	72	34.4	48.8	1.42
IR	49.0	5128	113	160	1.41
STK25(h)	44.6	5000	104	146	1.41
PTK5(h)	33.5	60	34.8	48.6	1.40
Fms(Y969C)(h)	23.4	18	14.0	19.4	1.39
mTOR/FKBP12(h)	39.5	5000	92.9	129	1.39
MRCKβ(h)	31.2	5000	73.9	102	1.39
EGFR(L861Q)	17.4	77	27.6	37.9	1.37
SAPK3(h)	123	5000	284	389	1.37
FAK(h)	64.9	5000	153	210	1.37
Ret(V804M)(h)	3.13	2320	7.61	10.4	1.37
EphB2	0.91	89	2.19	2.96	1.35
Abl (H396P)	5.15	127	11.7	15.7	1.34

Txk(h)	8.00	168	18.0	24.0	1.33
CK1γ2(h)	3.14	111	7.4	9.8	1.33
MRCKα(h)	52.2	5000	129	170	1.32
FGFR1(V561M)(h)	17.7	668	42.5	55.4	1.31
EphA8	51.5	5000	129	168	1.30
Src(1-530)(h)	7.60	37	13.2	17.2	1.30
Wee1(h)	52.9	5000	133	172	1.29
PKCι(h)	14.6	5000	38.2	48.4	1.27
CDK5/p35(h)	14.4	5000	37.6	47.5	1.26
CK1δ(h)	19.0	5000	50.9	62.8	1.23
Aurora-B	0.0181	10	0.0500	0.0602	1.20
PKA(h)	44.3	5000	121	145	1.20
CaMKIIδ(h)	2.53	59	6.40	7.66	1.20
B-Raf(h)	41.2	5000	114	135	1.18
DDR2(h)	32.9	5000	91.9	108	1.18
Itk(h)	0.967	5000	2.78	3.22	1.16
PI3 Kinase (p110a(E542K)/p85a)(h)	25.0	5000	71.5	82.4	1.15
CaMK1V(h)	50.7	5000	144	165	1.14
Abl(Y253F)	4.28	93.4	11.4	12.9	1.13
Abl	5.24	4975	15.5	17.4	1.12
MST1(h)	27.8	5000	81.7	91.6	1.12
Aurora-C	48.3	13.4	15.4	17.1	1.11
Rsk4(h)	7.68	5000	23.5	25.5	1.08
MEK1(h)	42.3	5000	130	138	1.06
Flt1	0.00683	119	0.0216	0.0228	1.06
PKCη(h)	330	5000	908	952	1.05
TLK2(h)	5128	5000	5000	5038	1.01
Abl (Q252H)	4.59	143	14.2	14.2	1.00
DMPK	5000	5000	5000	5000	1.00
JNK1α1(h)	5000	5000	5000	5000	1.00
JNK2α2(h)	5000	5000	5000	5000	1.00
PIP5K1a(h)	5000	5000	5000	5000	1.00
PKCβI(h)	5000	5000	5000	5000	1.00
PKCε(h)	5000	5000	5000	5000	1.00
PKD2(h)	5000	5000	5000	5000	1.00
Plk1(h)	5000	5000	5000	5000	1.00
Plk3(h)	5000	5000	5000	5000	1.00
TGFBR1(h)	5000	5000	5000	5000	1.00

VRK2(h)	5000	5000	5000	5000	1.00
JAK2(h)	145	5000	451	451	1.00
EphB4	0.140	91.3	0.472	0.465	0.985
Ret(h)	0.006	64.7	0.022	0.020	0.931
Ret (V804L)(h)	0.436	118	1.55	1.44	0.927
NEK3(h)	10.7	5000	38.8	35.6	0.917
Lck(h) activated	9.7	47.4	24.1	21.9	0.911
B-Raf(V599E)(h)	28.4	1364	100	90.4	0.903
Hck(h) activated	15.0	45.8	31.8	28.3	0.891
Pim-3(h)	55.5	24.5	33.3	29.4	0.884
Blk(h)	0.122	100	0.466	0.406	0.870
Rsk2(h)	0.753	966	2.91	2.50	0.860
Abl (M351T)	3.59	272	13.5	11.6	0.860
SAPK2b(h)	50.3	5000	201	164	0.814
STK33(h)	0.0121	5000	0.050	0.040	0.804
MAPK2(h)	53.3	5000	218	173	0.794
GRK5(h)	229	5000	874	689	0.789
TrkA(h)	0.414	103	1.83	1.37	0.746
Snk(h)	456	5000	1688	1253	0.742
PAK2(h)	39.5	5000	178	129	0.725
Rsk3(h)	0.66	1759	3.16	2.18	0.692
PIP4K2a(h)	14.0	5000	71.5	46.5	0.650
PDGFRα(V561D)(h)	1.44	0.0227	0.050	0.032	0.645
Src(T341M)(h)	29.1	31.7	58.0	30.9	0.533
BRK(h)	0.850	5000	5.45	2.83	0.519
NEK7(h)	64.4	5000	456	208	0.457
CK2α2(h)	22.2	0.0121	0.050	0.017	0.344
Haspin(h)	0.605	250	6.51	2.00	0.308
ARK5(h)	0.477	247	5.85	1.58	0.271
PI3 Kinase (p110a(E545K)/p85a)(h)	42.7	5000	559	140	0.250
SAPK2a(h)	366	5000	5000	1041	0.208
MKK6(h)	361	5000	5085	1029	0.202
DCAMKL2(h)	304	5000	5000	887	0.177
SAPK2a(T106M)(h)	234	5000	5000	703	0.141
PI3 Kinase (p110a/p65a)(h)	98.0	5000	2825	312	0.111
PI3 Kinase (p110d/p85a)(h)	141	5000	5000	442	0.088
Yes(h)	0.0359	60.2	1.66	0.120	0.072
PI3KC2g(h)	300	58.3	1609	76.8	0.048

CLK3(h)	0.0171	5000	3.61	0.0572	0.016
IGF-1R(h)	79.0	0.191	78.4	0.273	0.003
IRR(h)	9.69	0.050	25.1	0.0713	0.0028
PI3KC2a(h)	0.050	5000	5000	0.167	0.00003

†Estimated using the equation [1/Expected IC_{50}] = [0.3/IC_{50PC9p}] + [0.7/$IC_{50Bergamot}$]

The kinases identified above can all be modulated by the protein kinase modulating composition provided herein.

The following paragraphs briefly summarize the cellular functioning of those kinases most affected by the synergistic interaction of the materials as evidenced by CI >1.05. The grouping is provided solely to underscore the primary signaling pathways in which the kinase functions and is not meant to be comprehensive.

PC10 dramatically and synergistically modulates kinase signaling of Abl, ACK1, ALK, Aurora, AMPK, CaMKII, EGFR, EphA, FAK, FGFR, GSK3, IGF-1(activated), IKK, IR, MAPK1, Met, MTOR, NEK1/2/6, PAK1/4/5/6, PDGFR, PI3K, PKC, ROCKI/II, RSK1/2/34, SRC, and Syk implying stimulation of skeletal muscle fatty acid oxidation and muscle glucose uptake, hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, and modulation of insulin secretion by pancreatic beta-cells.

Interestingly, a number of kinases involved in glucose metabolism were affected synergistically by the PC10 formulation including PI3 and MET kinase as well as the insulin receptor (IR) itself. Both isoforms of AMPK were also inhibited synergistically by the PC10 formulation.

PC10 synergistically inhibited all seven MET kinases. In adults, MET participates in wound healing as well as organ regeneration, tissue remodeling and certain aspects of brain development. The MET pathway also regulates the immune response and the development and repair of the gastrointestinal tract.

MET is a receptor tyrosine kinase that transduces signals from the extracellular matrix into the cytoplasm by binding to hepatocyte growth factor/HGF ligand. It regulates many physiological processes including proliferation, scattering, morphogenesis and survival. Ligand binding at the cell surface induces autophosphorylation of MET on its intracellular domain that provides docking sites for downstream signaling molecules.

Following activation by ligand, interacts with the PI3-kinase subunit PIK3R1, PLCG1, SRC, GRB2, STAT3 or the adapter GAB1.

All three Aurora kinases were inhibited synergistically by PC10. Most human cancer cells are characterized by changes in the amount or organization of DNA resulting in chromosome instability and aneuploidy. Several mitotic kinases, Aurora kinases amongst others, regulate the progression of the cell through mitosis. So far three Aurora kinases have been identified in man: Aurora-A, Aurora-B and Aurora-C. Aurora kinases were recently identified as a potential target in anticancer therapy, and various Aurora-A and Aurora-B kinase inhibitors are in development [Kitzen, J. J., de Jonge, M. J., and Verweij, J. (2010) Aurora kinase inhibitors, Crit Rev Oncol Hematol 73, 99-110]. In cancerous cells, over-expression of these enzymes causes unequal distribution of genetic information, creating aneuploid cells, a hallmark of cancer.

Four of the six isoforms of Abl were inhibited synergistically by PC10. Abl is a non-receptor tyrosine-protein kinase that plays a role in many key processes linked to cell growth and survival such as cytoskeleton remodeling in response to extracellular stimuli, cell motility and adhesion, receptorendocytosis, autophagy, DNA damage response and apoptosis.

Example 14

Clinical Assessment of PC10 in Normal and Pre-Diabetic Subjects

The clinical safety and efficacy of the PC10 formula, above was studied in an open-label, observational clinical trial. The study population included males and females between the ages of 18 to 72 inclusive exhibiting the following lipid variables: serum triglycerides ≥150 mg/dl and/or serum low density lipoprotein cholesterol (calculated) ≥ 150 mg/dl. During the 12-week study, subjects were assigned to one of three groups to receive, respectively, 500, 750, or 1000 mg of PC10 to be taken as 2, 3 or 4 capsules taken once daily with the evening meal.

Subjects were instructed to maintain their current lifestyles including diet, exercise, and mind body spirit practices without change during trial participation. Subjects were also instructed not to make changes to their current prescription, non-prescription medications, medical foods or nutritional supplements while on the study.

At one, two and three months, blood was drawn for analysis including complete blood count (CBC), complete metabolic panel (CMP), fasting lipid panel including total cholesterol, triglycerides, HDLc, LDLc, oxLDL, MPO, PAI-1, and HbA1c.

During this 12-week trial, there were no reported adverse events related to the PC10 in the 500, 750, or 1000 mg/day groups (N=35). Efficacy was assessed only for the potential commercial formulation of 500 mg PC10 (n-11).

Table 15 summarizes the changes (Initial – 3 Months) in median lipid variables in subjects consuming 500 mg daily of the PC10 formulation over three months. Statistically significant changes were noted in Total Cholesterol, Total Cholesterol/HDL ration, LDL-c (calculated), Apo B and non-HDL for the group of eleven. The change in Total Cholesterol of 7% and LDL-c (calculated) of 10% are considered clinically meaningful.

Additionally, a subgroup analysis was conducted for subjects with a HbA1C ≥ 5.5% (all of whom were insulin resistant with HOMA scores greater than 2). Statistically significant changes were noted in Total Cholesterol, Total Cholesterol/HDL ration, LDL-c (calculated), Apo B, LDL-c (calculated)/HDL ratio, oxLDL, oxLDL/HDL ratio, non-HDL, Triglycerides, TG/HDL ratio and plasminogen activator inhibitor-1 (PAI-1) in this group of eight subjects. The change in Total Cholesterol of 10%, LDL-c (calculated) of 10%, oxLDL of 19%, TG of 27% and PAI-1 of 37% are clinically meaningful and demonstrate the broad spectrum of action of the PC10 formulation.

Table 15: Median changes in lipid biomarkers in all subjects and subset of subjects with elevated HbA1c consuming 500 mg daily of the PC10 formulation over three months

	Total (n=11)		**HbA1c >5.4 (n=8)†**	
Variable	**Median Change (% Change)**	**P ***	**Median Change (% Change)**	**P***
Weight	0.0 (0.0)	NS	1.0 (0.0)	NS
Total Cholesterol	-20 (-7.0)	0.003	-23 (-10)	0.008
HDL	-10 (-3.0)	NS	4.0 (8.0)	NS
Cholesterol/HDL	-2.0 (-26)	0.024	-2.5 (-45)	0.016
LDL	-19 (-10)	0.012	-21 (-10)	0.031
oxLDL	**-6.0 (-10)**	**NS**	**-14 (-19)**	**0.047**
APOB	-4.0 (-3.0)	0.037	-7.0 (-4.0)	0.016
oxLDL/HDL	**-0.2 (-17)**	**NS**	**-0.3 (-25)**	**0.039**
Non-HDL	-16 (-7.0)	0.007	-21 (-11)	0.008
Triglycerides	**-24 (-9.0)**	**NS**	**-35 (-27)**	**0.039**

LDL/HDL	**-0.3 (-7.0)**	**NS**	**-0.4 (-10)**	**0.031**
PAI-1	**-4.0 (-20)**	**NS**	**-7.0 (-37)**	**0.047**

*P-values were computed using the log-normal distribution of the ratio of change from baseline to 12 weeks using the Wilcoxon Signed Rank test of the median. The Null Hypothesis assumed a mean change from baseline of zero. NS = nonsignificant (P>0.05)

† Subgroup of subjects selected with HbA1c greater than 5.4; bolding highlights subgroup differences.

Of particular interest in both groups was the effect of PC10 on oxLDL levels, which is considered by many to be the most significant risk factor for development of atherosclerosis [Johnston, N., Jernberg, T., Lagerqvist, B., Siegbahn, A., and Wallentin, L. (2006) Improved identification of patients with coronary artery disease by the use of new lipid and lipoprotein biomarkers, Am J Cardiol 97, 640-645]. For the overall group, there was a 10% reduction that nearly placed subjects at completion in the low risk group. The subgroup began the trial at moderate risk and had improved with a fall to the low risk group by completion. This reduction in an important risk factor for the development of coronary artery disease offers an additional opportunity to promote healthy aging. The antioxidant components of the formula function to assist in lowering oxLDL levels and promote a healthy cholesterol metabolism to offer organ system protection.

This is the first clinical demonstration of a formulation containing Bergamot reducing oxLDL. Moreover, the remaining fruit and herb components of PC10 have not been shown to reduce oxLDL at the doses used in this study – a further indication of the coordinated synergy built into the development of the PC10 and related formulations.

Example 15

Synergistic Interactions of PC8 and Bergamot (PC10) on Ex Vivo Inhibition of LDL Oxidation

Objective - The objective of this example is to demonstrate the synergistic interaction between various forms of the phytocomplex and bergamot on the ex vivo inhibition of LDL oxidation.

Method - This method artificially induces autoxidation of linoleic acid or LDL by either Cu(II) or an azo initiator as reported by Pryor and co-workers [Pryor, W. A.; Cornicelli, J. A.; Devall, L. J.; Tait, B.; Trivedi, B. K.; Witiak, D. T.; Wu, M. A rapid screening test to

determine the antioxidants potencies of natural and synthetic antioxidants. J. Org. Chem. 1993, 58, 3521-3532]. The progress of autoxidation is monitored by UV absorbance at 234 nm.

Materials – PC8, PC9f, PC9p and bergamot are used in this example and there compositions are as previously described in earlier examples.

Calculations – Median inhibitory concentrations (IC_{50}) are calculated from a minimum of four concentrations evenly surrounding the median effect. The Combination Index (CI) is computed as previously described.

Conclusion – CI for PC8 plus bergamot, PC9f plus bergamot and PC9f plus bergamot are all greater than 3.0 indicating a high degree of synergy in the prevention of ex vivo oxidation of LDL. These results support the clinical findings of Example 14 and underscore the novelty of the inhibition of oxidized LDL for the combinations.

Thus, among the various formulations taught there have been disclosed novel methods and compositions of extracts of fruits and herbs that exhibited synergistic antioxidant activity toward differing oxidants over several configurations.

Example 16

Phytocomplex (PC4) Through Inhibition of Peroxynitrite Formation

This example describes formulae for the enhancement of function of formulations targeted for metabolic disorders associated with oxidative stress as previously described with the addition of PC4.1 or PC4.2 functioning as synergistic inhibitors of macrophage $ONOO^-$ production (See Figure 2). Under pathological conditions associated with increased oxidative stress and inflammation (myocardial infarction, ischemic heart disease, myocarditis, cardiomyopathy, hypertension, obesity, chronic intoxication, etc.), NO and superoxide (O_2^-) react to form $ONOO^-$ that induces cell damage via lipid peroxidation, inactivation of enzymes and other proteins by oxidation and nitration, and also activation of stress signaling enzymes such as matrix metalloproteinases and myeloperoxidase among others. Such stress signaling results in the attenuation of many products originally designed to address these conditions

The administration of therapeutic amounts of PC4.1 or PC4.2 in combination with specific product formulations would function to relieve the oxidative stress and improve product performance. Examples of formulations in which PC4.1 or PC4.2 would be useful for their enhanced performance can be found in the following tables. However, it is noted that the other formulations noted above as PC8, PC9, and PC10 could in some embodiments also be used.

Table 16: PC4.1 Formulation with Arginine and Watermelon Extract

Item Description	Amount [mg]
L-Arginine [granular]	2500
Citric Acid	2500
Red Beet Root	3000
Natural Citrus Sweetener [CitriSweet(TM)]	430
Malic Acid	400
Pomegranate Fruit Juice Concentrate	375
Xylitol [bulk]	500
Silicon Dioxide [Syloid® 244]	170
Thiamin (B1) (thiamine mononitrate) [91% B1]	110
Calcium Ascorbate [83% vit C, 9% Ca]	95
Citrus Blend Natural Flavor [WONF]	85
Huckleberry Natural Flavor	85
Magnesium Oxide [60% Mg, powder]	84
Stevia Leaf Extract	66
Apple Fruit Extract [75% polyphenols]	30
Watermelon Whole Fruit Extract [20% Citrulline]	23
Vitamin D3 (cholecalciferol) [100,000 IU/g, 100 SD/S]	16
Green Tea Leaf Extract [80%, decaffeinated]	15
D-Ribose	10
Grape Skin Extract	5
Red Grape Polyphenol Extract [ExGrape(TM) red wine extract]	5
Grape Seed Extract [MegaNatural®]	5
Olive Leaf Extract [12%, 7:1]	5
Folic Acid [10%, trituration]	3
Vitamin B6 (pyridoxine hydrochloride) [82% B6]	3
Vitamin B12 (cyanocobalamin) [1%, trituration]	0.9
Inulin (chicory root extract) [HD food grade]	2500

L-Glutamine	1000

Table 17: PC4.1 Formulation with Arginine and Citrulline for Enhanced Production of Nitric Oxide

Item Description	Amount [mg]
L-Arginine [granular]	20
Citric Acid	1000
Red Beet Root	3000
Natural Citrus Sweetener [CitriSweet(TM)]	300
Malic Acid	400
Silicon Dioxide [Syloid® 244]	170
Thiamin (B1) (thiamine mononitrate) [91% B1]	110
Calcium Ascorbate [83% vit C, 9% Ca]	95
Citrus Blend Natural Flavor [WONF]	85
Huckleberry Natural Flavor	85
Magnesium Oxide [60% Mg, powder]	84
Stevia Leaf Extract	50
Apple Fruit Extract [75% polyphenols]	30
Vitamin D3 (cholecalciferol) [100,000 IU/g, 100 SD/S]	7.5
Green Tea Leaf Extract [80%, decaffeinated]	15
Grape Skin Extract	5
Red Grape Polyphenol Extract [ExGrape(TM) red wine extract]	5
Grape Seed Extract [MegaNatural®]	5
Olive Leaf Extract [12%, 7:1]	5
Folic Acid [10%, trituration]	1
Vitamin B6 (pyridoxine hydrochloride) [82% B6]	3
Vitamin B12 (cyanocobalamin) [1%, trituration]	0.9
L-Citrulline	2500

Table 18: PC4.1 Formulation with Arginine for Enhanced Production of Nitric Oxide

Item Description	Amount [mg]
L-Arginine [granular]	5100
Citric Acid	2000
Red Beet Root	2000
Natural Citrus Sweetener [CitriSweet(TM)]	430

Malic Acid	400
Pomegranate Fruit Juice Concentrate	375
Xylitol [bulk]	250
Silicon Dioxide [Syloid® 244]	170
Thiamin (B1) (thiamine mononitrate) [91% B1]	110
Calcium Ascorbate [83% vit C, 9% Ca]	95
Citrus Blend Natural Flavor [WONF]	85
Huckleberry Natural Flavor	85
Magnesium Oxide [60% Mg, powder]	84
Stevia Leaf Extract	66
Apple Fruit Extract [75% polyphenols]	30
Watermelon Whole Fruit Extract [20% Citrulline]	23
Vitamin D3 (cholecalciferol) [100,000 IU/g, 100 SD/S]	16
Green Tea Leaf Extract [80%, decaffeinated]	15
D-Ribose	10
Grape Skin Extract	5
Red Grape Polyphenol Extract [ExGrape(TM) red wine extract]	5
Grape Seed Extract [MegaNatural®]	5
Olive Leaf Extract [12%, 7:1]	5
Folic Acid [10%, trituration]	3
Vitamin B6 (pyridoxine hydrochloride) [82% B6]	3
Vitamin B12 (cyanocobalamin) [1%, trituration]	0.9

Table 19: PC4.2 Formulation with Phytosterols

Item Description	**Amount [mg]**
Maltodextrin [M100 IP]	1453
Sucralose	30
Chocolate Natural Flavor	467
Cocoa [processed w/ alkali, 10-12% fat]	2333
Sunflower Oil Creamer (milk, soy)[RichmixSun50 HT, ALLERG]	2147
CLA (Conjugated Linoleic Acid) (milk) [Clarinol(TM), ALLERG]	156
Calcium Caseinate (contains milk) [ALLERGEN]	2213
Whey Protein Concentrate (milk, soy) [80% instantized ALLER]	15625
Whey Protein Isolate (milk) [instantized, ALLERGEN]	8203
Xanthan Gum [Keltrol(TM) Tf]	373
Apple Fruit Extract [75% polyphenols]	30
Green Tea Leaf Extract [80%, decaffeinated]	15

Grape Seed Extract [MegaNatural®] / Vitis vinifera	5
Olive Leaf Extract [12%, 7:1]	5
Phytosterols	2000

Table 20: PC4.2 Bergamot Fruit Extract Formulation for Enhanced Reduction of Oxidized LDL

Item Description	Amount [mg]
Bergamot orange fruit extract	250
Apple Fruit Extract [75% polyphenols]	30
Green Tea Leaf Extract [80%, decaffeinated]	15
Grape Seed Extract [MegaNatural®] / Vitis vinifera	5
Olive Leaf Extract [12%, 7:1]	5
Cellulose [hydroxypropyl cellulose, Klucel Nutra® D]	10
Magnesium Stearate (vegetable)	16.25
Cellulose [croscarmellose sodium, modified cellulose gum]	40
Silicon Dioxide [Syloid® 244]	3.12

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Table 21: PC4.2 Formulation for Enhanced Detoxification

Item Description	Amount [mg]
Broccoli Flowers / Brassica oleracea var. italica	18
Apple Fruit Extract [75% polyphenols] / Malus pumila	1.8
Cabbage Leaf / Brassica oleracea var. capitata	0.480
Carrot Root / Daucus carota	0.480
Flax Seed / Linum usitatissimum	36
Grape Seed Extract [MegaNatural®] / Vitis vinifera	0.300
Green Tea Leaf Extract [80%, decaffeinated] / Camellia sinensis	0.900
Guar Gum [Tico-LV] / Cyamopsis tetragonoloba	36
Olive Leaf Extract [12%, 7:1] / Olea europaea	0.300
Red Beet Root / Beta vulgaris	0.480
Rosemary Leaf / Rosmarinus officinalis	0.480
Stevia Leaf Extract / Stevia rebaudiana	1.8
Tomato Fruit / Solanum lycopersicum	0.480
Turmeric Rhizome / Curcuma longa	0.480
Psyllium Hulls [50] / Plantago ovata	187.920
Gum Arabic (Talha) / Acacia seyal	36
Apple Fruit Fiber [40 mesh]	108

Citric Acid	30
Citrus Blend Natural Flavor [WONF]	10.5
Fructose	60
L-Glutamine	150
Inulin (chicory root extract) [HD food grade]	150
Sodium Copper Chlorophyllin	6.0
Zinc Citrate [32% Zn, dihydrate]	0.240

Table 22: PC4.2 Formulation for Enhanced Detoxification

Item Description	Amount [mg]
Broccoli Flowers	1000
Cabbage Leaf	8
Carrot Root	8
Red Beet Root	8
Rosemary Leaf	8
Tomato Fruit	8
Turmeric Rhizome	1000
Flax Seed	600
Guar Gum [Tico-LV]	600
Stevia Leaf Extract	30
Psyllium Hulls [50]	3132
Gum Arabic (Talha)	600
Apple Fruit Fiber [40 mesh]	1800
L-Glutamine	2500
Fructose	1000
Inulin (chicory root extract) [HD food grade]	2500
Apple Fruit Extract [75% polyphenols]	30
Green Tea Leaf Extract [80%, decaffeinated]	15
Grape Seed Extract [MegaNatural®]	5
Olive Leaf Extract [12%, 7:1]	5
Zinc Citrate [32% Zn, dihydrate]	4
Citric Acid	750
Lemon Natural Flavor	500

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Table 23: PC4.2 Multivitamin Formulation for Enhanced Antioxidant Activity

Item Description	Amount [mg]
Biotin [1% trituration]	4.5
Chromium Chloride (2%)	2.0
Copper Gluconate [14% Cu]	3.9
Vitamin B12 (cyanocobalamin) [1%, trituration]	0.4428
Cellulose [MCC, Endurance(TM) VE-090]	105
Folic Acid [10%, trituration]	0.64
Vitamin B6 (pyridoxine hydrochloride) [82% B6]	5.4
Riboflavin (B2) [100%, type S]	6.0
Thiamin (B1) (thiamine mononitrate) [91% B1]	7.9
Vitamin A Palmitate [500,000 IU/gm]	2.7
Vitamin D3 (cholecalciferol) [100,000 IU/g, 100 SD/S]	2.0
Cellulose [hydroxypropyl cellulose, Klucel Nutra® D]	10
Lutein [5%, VG granules]	20
Cellulose [croscarmellose sodium, modified cellulose gum]	40
Dicalcium Phosphate [anhydrous]	382.5
Vitamin C (ascorbic acid) [97%, C97 SF]	155
Cellulose [Avicel® PH 200, microcrystalline]	130
Beta-Carotene [20%, CWD]	8.1
Pantothenic Acid (d-calcium pantothenate) [90%]	20.9
Vitamin E 700 IU, IP NON GMO (d-alpha tocopheryl acetate)	43
Stearic Acid (vegetable) [Hystrene® NF]	50
Magnesium Oxide [60% Mg, granular]	91
Magnesium Stearate (vegetable)	16.25
Niacinamide [97%, fine granular]	18
Sodium Copper Chlorophyllin	2.5
Silicon Dioxide [Syloid® 244]	3.12
Zinc Gluconate [13.8% Zn, fine granular]	65
Apple Fruit Extract [75% polyphenols]	30
Green Tea Leaf Extract [80%, decaffeinated]	15
Grape Seed Extract [MegaNatural®] / Vitis vinifera	5
Olive Leaf Extract [12%, 7:1]	5

Table 24: PC4.2 Meal Replacement Formula for Enhanced Weight Loss Activity

Item Description	Formula 1	Formula 2	Formula 3
Serving Size (g)	46	45	45
Calories	184	180	173
Fat (g)	5	3	5
Saturated Fat (g)	2	1	1
Trans Fat (g)	0	0	0
Cholesterol (mg)	32	0	0
Sodium (mg)	104	150	307
Potassium (mg)	368	95	187
Carbohydrate (g)	14	16	16
Dietary Fiber (g)	5	3	3
Sugars (g)	5	9	8
Protein (g)	20	20	20
Phytosterols (mg)	2000	2000	2000
Vitamin A (IU)	48	75	47
Vitamin C (mg)	48	75	47
Calcium (mg)	96	2	33
Iron (mg)	0	0	0
Vitamin A (IU)	48	75	47
Vitamin D (IU)	0	75	47
Vitamin E (IU)	48	0	47
Vitamin K (mcg)	0	0	0
Thiamin (mg)	48	75	47
Riboflavin (mg)	48	75	47
Niacin (mg)	48	75	47
Vitamin B6	48	75	47
Folate (as folic acid and L-5-methyltetrahydrofolate) (mcg)	104	75	47
Vitamin B12 (as cyanocobalamin) (mcg)	48	75	47
Biotin (mcg)	48	75	47
Pantothenic Acid (mg)	48	75	47
Apple Fruit Extract [75% polyphenols] (mg)	30	30	30
Green Tea Leaf Extract [80%, decaffeinated] (mg)	15	15	15
Grape Seed Extract [MegaNatural®] / Vitis vinifera (mg)	5	5	5
Olive Leaf Extract [12%, 7:1] (mg)	5	5	5
Phosphorus (mg)	48	0	20
Iodine (mcg)	0	75	47
Magnesium (mg)	72	0	33
Zinc (mg)	0	75	47

Selenium (mcg)	0	75	47
Copper (mg)	0	75	47
Manganese (mg)	0	75	47
Chromium (mcg)	176	75	47
Molybdenum	0	75	0
Chloride (mg)	0	0	0

Table 25: PC4.2 Curcumin Formulation for Enhanced Anti-inflammatory/Antioxidant Activity

Item Description	Amount [mg]
Tumeric rhizome extract (Curcuma longa) standardized to 95% curcuminoids	550
Apple Fruit Extract [75% polyphenols]	30
Green Tea Leaf Extract [80%, decaffeinated]	15
Grape Seed Extract [MegaNatural®] / Vitis vinifera	5
Olive Leaf Extract [12%, 7:1]	5
Cellulose [Avicel® PH 200, microcrystalline]	130
Magnesium Stearate (vegetable)	16.25
Silicon Dioxide [Syloid® 244]	3.12
Black pepper fruit extract (*Piper nigram*)	0.40

Table 26: PC4.2 Berberine Formulation for Enhanced Hypoglycemic/Anti-inflammatory Activity

Item Description	Amount [mg]
Berberine (*Berberis aristata*)	333
Apple Fruit Extract [75% polyphenols]	30
Green Tea Leaf Extract [80%, decaffeinated]	15
Grape Seed Extract [MegaNatural®] / Vitis vinifera	5
Olive Leaf Extract [12%, 7:1]	5
Cellulose [Avicel® PH 200, microcrystalline]	130
Magnesium Stearate (vegetable)	16.25
Silicon Dioxide [Syloid® 244]	3.12

Table 27: PC4.2 Fish Oil Soft Gel Formulation for Enhanced Lipid Lowering Activity

Item Description	Amount [mg]
Fish Oil ((380 mg EPA, 190 mg DHA)†	1028
Apple Fruit Extract [75% polyphenols]	30
Green Tea Leaf Extract [80%, decaffeinated]	15
Grape Seed Extract [MegaNatural®] / Vitis vinifera	5
Olive Leaf Extract [12%, 7:1]	5
Gelatin	300.6
Water	50.1
Natural lemon oil	29.01

†EPA= Eicosapentaenoic acid; DHA = Docosahexaenoic acid

Table 28: PCx CoQ10 Formulation for Enhanced Cardioprotective Activity

Item Description	Amount [mg]
Coenzyme Q10	100
Apple Fruit Extract [75% polyphenols]	30
Green Tea Leaf Extract [80%, decaffeinated]	15
Grape Seed Extract [MegaNatural®] / Vitis vinifera	5
Olive Leaf Extract [12%, 7:1]	5
Extra virgin olive oil	100
Beeswax	50
Gelatin	300.6
Water	50.1

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Exemplary Embodiments

The following exemplary invention embodiments pertain to further aspects of the disclosure.

10 In one example there is provided, a composition having a therapeutic effect against multiple biologically reactive forms of oxygen and nitrogen when administered to a subject comprising: an apple fruit extract, a grape seed extract, a green tea leaf extract, and an olive leaf extract, wherein the composition is more effective against the biologically active forms of oxygen and nitrogen than an equivalent amount of any single extract in the 15 composition.

In one example, the composition further comprises a blueberry fruit extract, a capsicum fruit extract, and a grape skin extract, wherein the composition is more effective against the biologically active forms of oxygen and nitrogen than an equivalent amount of any single extract in the composition.

In one example, the composition further comprises a bergamot fruit extract, a mangosteen fruit or pericarp extract, or a combination thereof, wherein the composition is more effective against the biologically active forms of oxygen and nitrogen than an equivalent amount of any single extract in the composition.

In one example, the composition further comprises a bergamot fruit extract.

In one example, the composition further comprises a mangosteen fruit extract.

In one example, the composition further comprises a mangosteen pericarp extract.

In one example, the composition further comprises a combination of a bergamot fruit extract and either a mangosteen fruit or mangosteen pericarp extract.

In one example, the extracts in the composition are each present in an equivalent amount.

In one example, the ratio is a 1:1 for all extracts in the composition.

In one example, at least one extract is present in a different amount than the other extracts.

In one example, a method for treating an oxidative stress related pathology in a subject comprising administering a therapeutically effective amount of a composition as recited in any of examples above to the subject.

In one example, the oxidative stress related pathology is increased oxidized LDL cholesterol (oxLDL).

In one example, the oxidative stress related pathology is any one of metabolic syndrome, type 1 diabetes, type 2 diabetes, or type 3 diabetes.

In one example, the oxidative stress related pathology is any one of leaky gut, endotoxemia, or inflammatory bowel disease.

In one example, the oxidative stress related pathology is any one of: obesity, an inflammation condition including osteoarthritis, rheumatoid arthritis, Crohn's disease, prostate hyperplasia, lower urinary tract symptoms, pulmonary arterial hypertension, diminished exercise capacity, premature ejaculation, low female sex drive, congestive disorders, cardiac failure, pulmonary hypertension, various cardiovascular diseases, motor dysfunction, cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, Addison's Disease,

lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia, skin disorders including skin wrinkles, skin discolorations and skin sagging, and cancers arising from oxidized damage to DNA.

In one example, a method of modulating disease-associated protein kinase activity in a subject in a manner beneficial to the subject's health comprising administering a therapeutically effective amount of a composition as recited in any one of the examples above to the subject.

In one example, the disease-associated protein kinase is a member selected from the group consisting of: Abl, ACK1, ALK, Aurora, AMPK, CaMKII, EGFR, EphA, FAK, FGFR, GSK3, IGF-1(activated), IKK, IR, MAPK1, Met, MTOR, NEK1/2/6, PAK1/4/5/6, PDGFR, PI3K, PKC, ROCKI/II, RSK1/2/34, SRC, Syk, and combinations thereof, and modulation of the protein kinase reduces, minimizes, or inhibits production or presence of oxidized LDL (oxLDL) cholesterol in the subject.

In one example, the disease-associated protein kinase is a member selected from the group consisting of: Abl, ACK1, ALK, Aurora, AMPK, CaMKII, EGFR, EphA, FAK, FGFR, GSK3, IGF-1(activated), IKK, IR, MAPK1, Met, MTOR, NEK1/2/6, PAK1/4/5/6, PDGFR, PI3K, PKC, ROCKI/II, RSK1/2/34, SRC, Syk, and combinations thereof and modulation of the protein kinase results ameliorates at least one of metabolic syndrome, type 1 diabetes, type 2 diabetes, or type 3 diabetes.

In one example, the disease-associated protein kinase is a member selected from the group consisting of Abl, ACK1, ALK, Aurora, AMPK, CaMKII, EGFR, EphA, FAK, FGFR, GSK3, IGF-1(activated), IKK, IR, MAPK1, Met, MTOR, NEK1/2/6, PAK1/4/5/6, PDGFR, PI3K, PKC, ROCKI/II, RSK1/2/34, SRC, Syk and combinations thereof and modulation of the protein kinase ameliorates at least one of leaky gut, endotoxemia, or inflammatory bowel disease.

In one example, the disease-associated protein kinase is a member selected from the group consisting of: Abl, ACK1, ALK, Aurora, AMPK, CaMKII, EGFR, EphA, FAK, FGFR, GSK3, IGF-1(activated), IKK, IR, MAPK1, Met, MTOR, NEK1/2/6, PAK1/4/5/6, PDGFR, PI3K, PKC, ROCKI/II, RSK1/2/34, SRC, Syk and combinations thereof and modulation of the protein kinase ameliorates at least one of obesity, inflammation conditions including osteoarthritis and rheumatoid arthritis, Crohn's disease, prostate hyperplasia, lower urinary tract symptoms, pulmonary arterial hypertension, diminished exercise capacity, premature ejaculation, low female sex drive, congestive disorders, cardiac failure, pulmonary hypertension, cardiovascular diseases, motor dysfunction,

cognitive disorders including Alzheimer's disease, Raynaud's phenomenon, essential hypertension, stroke, asthma, multiple sclerosis, vasculitis, Addison's Disease, lupus, thyroiditis, chronic fatigue syndrome, fibromyalgia and skin disorders including skin wrinkles, skin discolorations and skin sagging.

In one example, the disease associated protein kinase is a member selected from the group consisting of: Abl, ACK1, ALK, Aurora, AMPK, CaMKII, EGFR, EphA, FAK, FGFR, GSK3, IGF-1(activated), IKK, IR, MAPK1, Met, MTOR, NEK1/2/6, PAK1/4/5/6, PDGFR, PI3K, PKC, ROCKI/II, RSK1/2/34, SRC, Syk, and combinations thereof and modulation of the protein kinase results at least one of in stimulation of skeletal muscle fatty acid oxidation and muscle glucose uptake, hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, and modulation of insulin secretion by pancreatic beta-cells.

In one example, a method of treating a serum lipid disorder or condition in a subject comprising administering a therapeutically effective amount a composition as recited in any one of the example compositions above to the subject.

In one example, the lipid disorder or condition includes abnormally elevated LDL.

In one example, the LDL includes oxidized LDL (oxLDL).

In one example, the lipid disorder or condition includes abnormally low HDL.

In one example, a method of minimizing serum oxidized LDL (oxLDL) in a subject comprising administering a therapeutically effective amount of a composition as recited in any one of the example compositions above to the subject.

Thus, there have been disclosed novel compositions that synergistically l modulate oxidative stress and protein kinase activity; as well as, methods of regulating oxidative stress, disease associated protein kinase activity; and methods of making such activity enhancing compositions. It will be readily apparent to those skilled in the art, however that various changes and modifications of an obvious nature may be made without departing from the spirit of the disclosed invention embodiments, and all such changes and modifications are considered to fall within the scope of the invention as recited herein, including in the appended claims. Examples of such changes and modifications could include, but not be limited to, the incipient ingredients added to affect the capsule, tablet, powder, lotion, food or bar manufacturing process as well as vitamins, flavorings and carriers. Other examples of such changes or modifications could include the use of herbs

or other botanical products containing the combinations of the preferred embodiments disclosed above.

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CLAIMS

What is claimed is:

1. An oxidative stress modulating composition comprising:

a combination of apple, grape, green tea, and olive extract in amounts that provide a greater antioxidant activity than provided by an equivalent amount of any one extract or a sum of the extracts.

2. The composition of claim 1, wherein the apple extract comprises an extract of a species *Malus pumila*.

3. The composition of claim 1, wherein the grape extract comprises an extract of a species *Vitis vinifera*.

4. The composition of claim 1, wherein the green tea extract comprises an extract of leaves of a species *Camellia sinensis*.

5. The composition of claim 1, wherein the olive extract comprises an extract of a subspecies *Olea europea europaea*.

6. The composition of claim 1, wherein at least one of the extracts in the composition is present in a different amount than an amount of at least one of another extract.

7. The composition of claim 1, wherein the apple, grape, green tea, and olive extracts are present in the composition at a weight ratio of about 1:1:1:1.

8. The composition of claim 1, wherein the apple, grape, green tea, and olive extracts are present in the composition at a weight ratio of about 6:1:3:1

9. The composition of claim 1, wherein the apple, grape, green tea, and olive extracts comprise leaves, skin, rind, pulp, juice, seeds, or combinations thereof.

10. The composition of claim 1, further comprising at least one primary or secondary therapeutic agent.

11. The composition of claim 10, wherein the at least one primary or secondary therapeutic agent comprises a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, and combinations thereof.

12. The composition of claim 10, wherein the at least one primary or secondary therapeutic agent comprises bergamot.

13. The composition of claim 10, wherein the at least one primary or secondary therapeutic agent comprises mangosteen.

14. The composition of claim 10, wherein the at least one primary or secondary therapeutic agent comprises berberine.

15. The composition of claim 1, wherein the composition further comprises a pharmaceutically acceptable carrier.

16. The composition of claim 1, wherein the composition is an oral dosage formulation.

17. The composition of claim 16, wherein the oral dosage form comprises a capsule, a tablet, a powder, a beverage, a syrup, a suspension, or a food.

18. The composition of claim 1, wherein the antioxidant activity modulates stress related pathologies and metabolic disorders.

19. The composition of claim 1, wherein the antioxidant activity modulates oxidized LDL.

20. The composition of claim 1, further comprising blueberry concentrate, capsicum extract, and turmeric extract.

21. The composition of claim 20, wherein the blueberry concentrate comprises *Vaccinium angustifolium*, the capsicum extract comprises *Capsicum annuum*, and the turmeric extract comprises *Curcuma longa*.

22. The composition of claim 20, further comprising mangosteen fruit extract.

23. The composition of claim 22, wherein the composition comprises greater than 1.5 times the antioxidant activity of an equivalent amount of any one extract or concentrate or a sum of the extracts and concentrate.

24. The composition of claim 20, further comprising bergamot extract.

25. The composition of claim 24, wherein the bergamot extract comprises *Citrus bergamia Risso*.

26. The composition of claim 25, wherein the composition comprises greater than 1.5 times the antioxidant activity of an equivalent amount of any one extract or concentrate or a sum of the extracts and concentrate.

27. The composition of claim 20, further comprising a mangosteen pericarp extract.

28. The composition of claim 27, wherein the composition comprises greater than 1.25 times the antioxidant activity of an equivalent amount of any one extract or concentrate or a sum of the extracts and concentrate.

29. The composition of claim 20, further comprising mangosteen pericarp extract and bergamot extract.

30. A method of regulating oxidative stress in a subject comprising:

administering to the subject a therapeutically effective combination of apple, grape, green tea, and olive extracts, in amounts that provide a combined antioxidant activity that is greater than an antioxidant activity provided by an equivalent amount of any one extract or a sum of the extracts.

31. The method of claim 30, wherein;
the apple extract comprises an extract of skin and fruit of *Malus pumila;*
the grape extract comprises an extract of seeds of *Vitis vinifera*;
the green tea extract comprises an extract of leaves of *Camellia sinensis; and*
the olive extract comprises leaves of *Olea europea europaea.*

32. The method of claim 30, wherein the apple, grape, green tea, and olive extracts are present at a weight ratio of about 1:1:1:1.

33. The method of claim 30, wherein the apple, grape, green tea, and olive extracts are present at a weight ratio of about 6:1:3:1

34. The method of claim 30, further comprising administering to the subject at least one secondary therapeutic agent.

35. The method of claim 34, wherein the at least one secondary therapeutic agent is co-administered to the subject with the therapeutically effective combination of apple, grape, green tea, and olive extracts.

36. The method of claim 34, wherein the at least one secondary therapeutic agent comprises a member selected from the group consisting of bergamot, mangosteen, berberine, arginine, citrulline, glutamine, zinc, beet, loclo, protein, curcumin, phytosterols, fish oil, CoQ10, vitamins, fiber, inulin, and combinations thereof.

37. The method of claim 30, wherein the antioxidant activity modulates stress related pathologies and metabolic disorders.

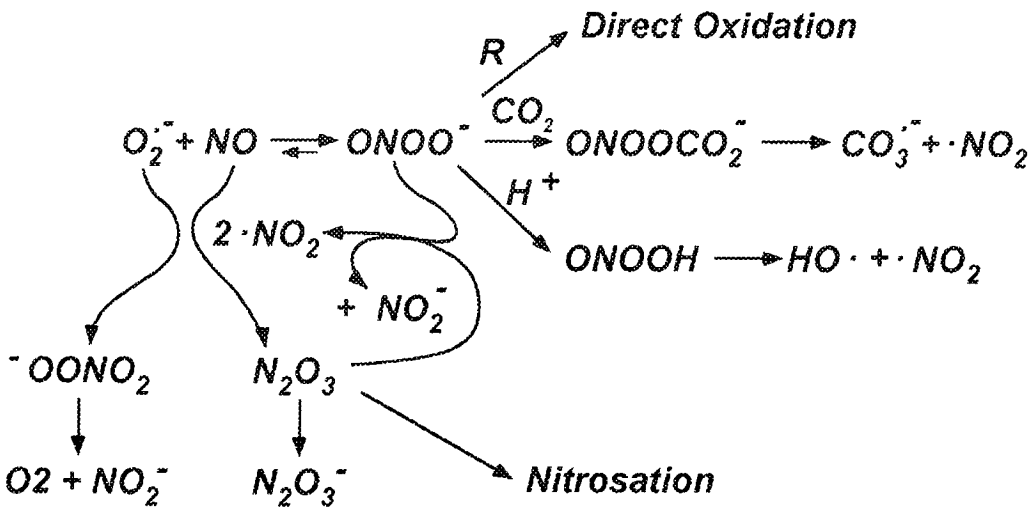
Direct Oxidation
R
CO_2
$O_2^{\cdot-}$ + NO
ONOO$^-$
ONOOCO$_2^-$
$CO_3^{\cdot-}$ + $\cdot NO_2$
H^+
$2 \cdot NO_2$
ONOOH
HO $\cdot$ + $\cdot NO_2$
+ NO_2^-
$^-$OONO$_2$
N_2O_3
O2 + NO_2^-
$N_2O_3^-$
Nitrosation

FIG. 1

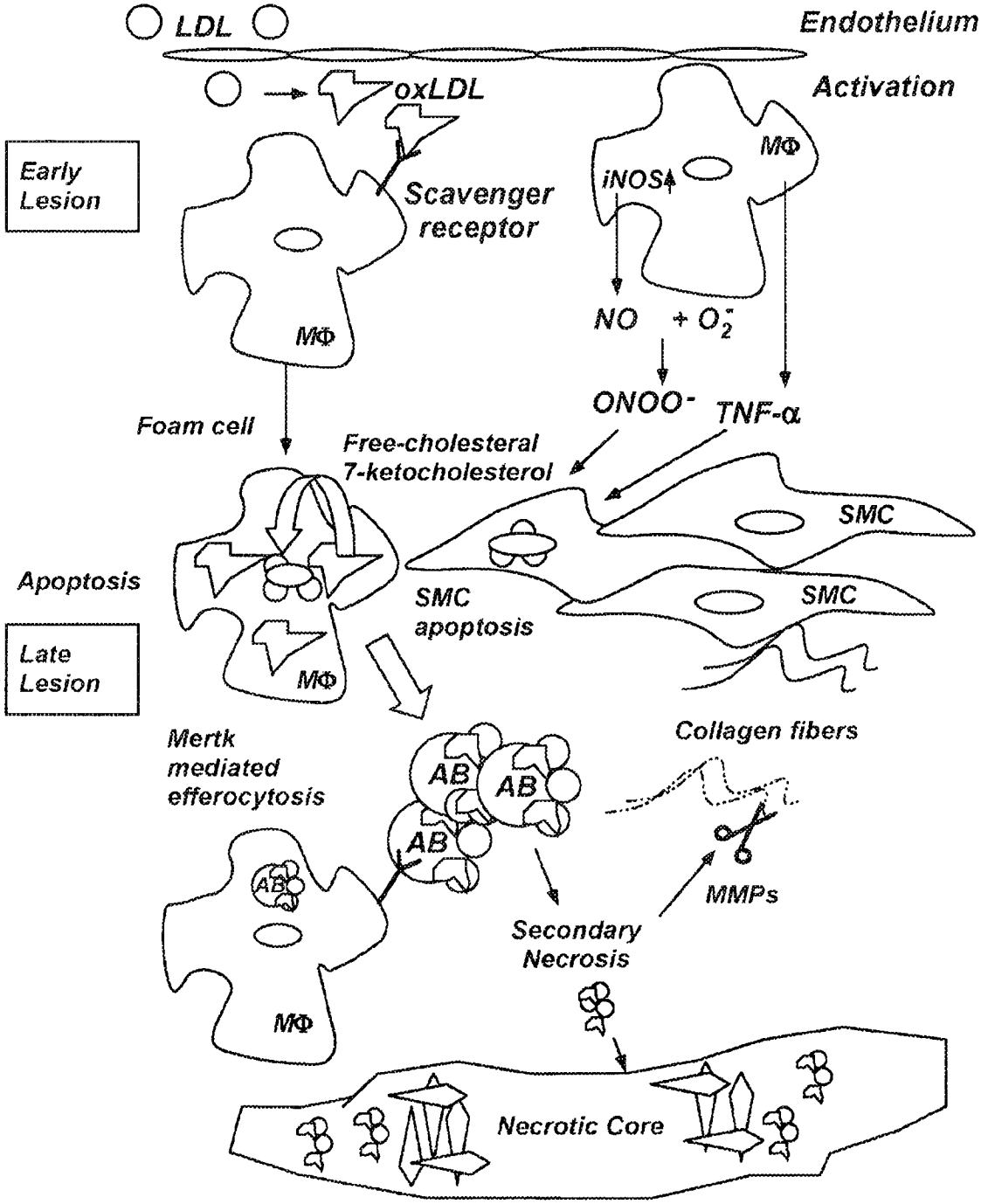
LDL
Endothelium
oxLDL
Activation
Early Lesion
Scavenger receptor
MΦ
iNOS
NO + O_2^-
ONOO-
TNF-α
Foam cell
Free-cholesteral
7-ketocholesterol
SMC
SMC
Apoptosis
SMC apoptosis
Late Lesion
MΦ
Mertk mediated efferocytosis
Collagen fibers
AB
MMPs
Secondary Necrosis
Necrotic Core

FIG. 2

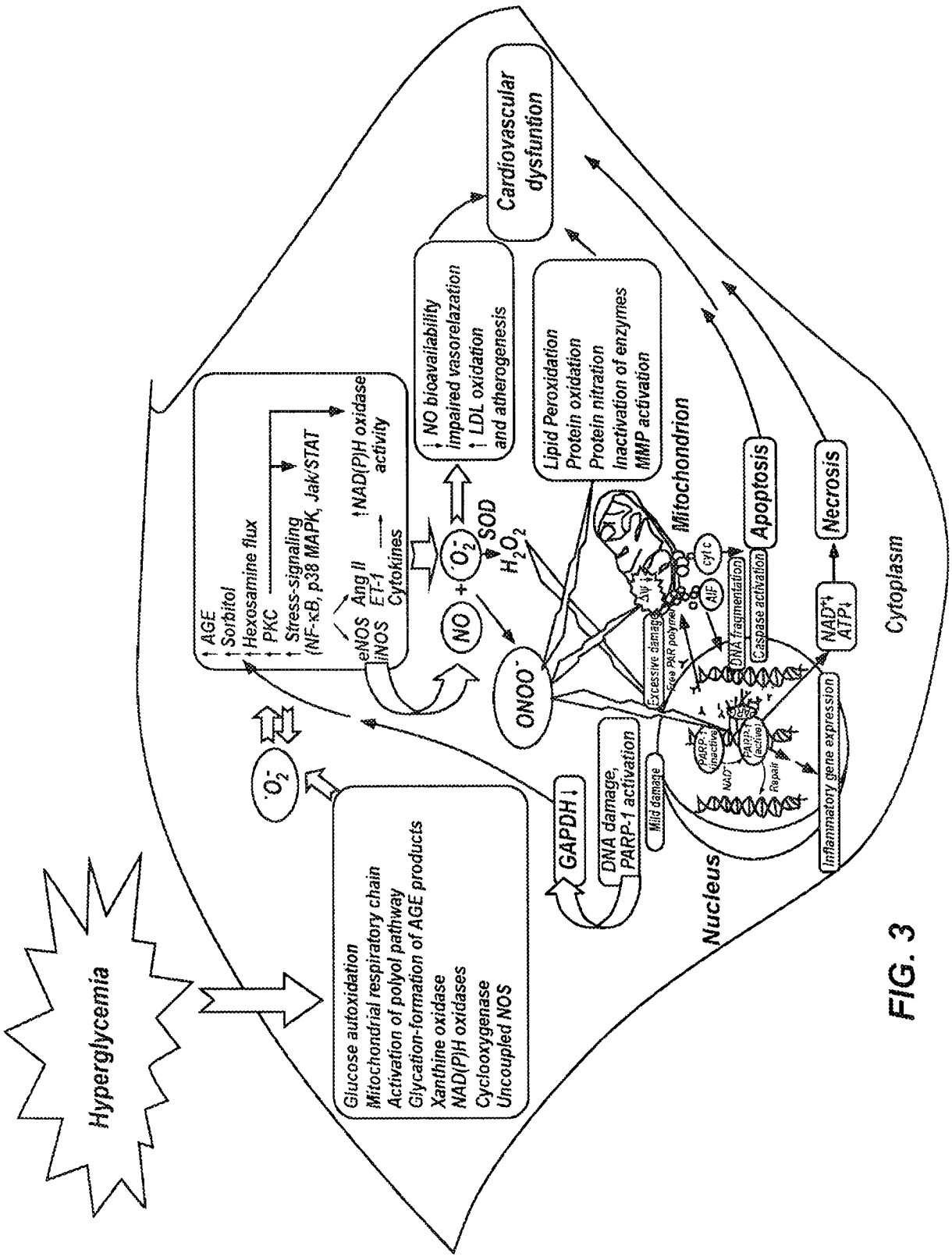
Hyperglycemia
Glucose autoxidation
Mitochondrial respiratory chain
Activation of polyol pathway
Glycation-formation of AGE products
Xanthine oxidase
NAD(P)H oxidases
Cyclooxygenase
Uncoupled NOS
↑ AGE
↑ Sorbitol
↑ Hexosamine flux
↑ PKC
↑ Stress-signaling
(NF-κB, p38 MAPK, Jak/STAT
eNOS Ang II ↑NAD(P)H oxidase
iNOS ET-1 activity
Cytokines
NO
SOD
H2O2
ONOO-
↓ NO bioavailability
impaired vasorelaxation
↑ LDL oxidation
and atherogenesis
Cardiovascular
dysfuntion
Lipid Peroxidation
Protein oxidation
Protein nitration
Inactivation of enzymes
MMP activation
Mitochondrion
Δψ↓
cyt c
AIF
Apoptosis
Necrosis
Excessive damage
Low PAR polymer
DNA fragmentation
Caspase activation
NAD+↓
ATP↓
GAPDH↓
DNA damage,
PARP-1 activation
Mild damage
PARP-1 inactive
NAD+
PARP-1 active
Repair
Inflammatory gene expression
Nucleus
Cytoplasm

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/22720

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23L 2/00; A61K 36/00; A61P 39/06 (2016.01)
CPC - A61K 36/87; A61K 36/63; A61K 36/82
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)
IPC(8): A23L 2/00; A61K 36/00; A61P 39/06 (2016.01)
CPC: A61K 36/87; A61K 36/63; A61K 36/82

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 426/599; 424/729; 424/766 (key word limited; see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar
Search terms used: phytocomplex, antioxidant/anti-oxidant, synergistic mixture/blend, grape, apple, green tea, olive extracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0215783 A1 (MCNEARY) 26 August 2010 (26.08.2010); para [0002]-[0003], [0009], [0024]-[0028], [0030]-[0031], [0033]-[0034], [0038], [0052]	1, 6-11, 13, 15-17, 20, 22-23, 30, 32-36
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Y		2-5, 12, 14, 18-19, 21, 24-29, 31, 37
Y	US 2013/0123207 A1 (SARDI) 16 May 2013 (16.05.2013); para [0029]-[0030], [0040], [0044]	2-3, 31
Y	WO 2014/088520 A1 (ERASLAN) 12 June 2014 (12.06.2014); pg 1 ln 10-11, 14-15; pg 2 ln 14; pg 3 ln 11-14	4-5, 31
Y	US 2015/0056255 A1 (RAGOT et al.) 26 February 2015 (26.02.2015); para [0076], [0088], [0192]	12, 24-26, 29
Y	US 2009/0136469 A1 (SENIN et al.) 28 May 2009 (28.05.2009); para [0011], [0013], [0024], [0035], [0056], [0060], [0067]	14, 18-19, 37
Y	WO 2012/129683 A1 (BIOPHARMACOPAE DESIGN INTERNATIONAL INC) 04 October 2012 (04.10.2012); pg 23 ln 11; pg 26 ln 19; pg 32 ln 4-5, 7	21
Y	US 2006/0088643 A1 (FUGAL et al.) 27 April 2006 (27.04.2006); para [0001], [0008]	27-29
A	US 2004/0023894 A1 (HASLER-NGUYEN et al.) 05 February 2004 (05.02.2004); entire document	1-37
A	US 2005/048143 A1 (MCANALLEY et al.) 03 March 2005 (03.03.2005); entire document	1-37

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

* 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 cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"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" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 May 2016 (13.05.2016)	21 JUN 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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代理人 王永伟 颜芳

(51)Int.Cl.
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A61K 36/00(2006.01)
A61P 39/06(2006.01)

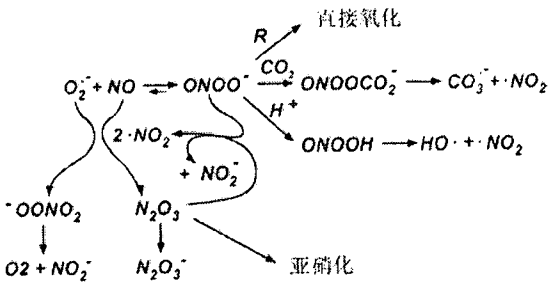
权利要求 12页 说明书 67页 附图3页

(54)发明名称

可用于食品、饮食补充剂、化妆品和药物制剂的呈现多种协同抗氧化剂活性的植物复合物

(57)摘要

本文提出了包括苹果、葡萄、绿茶和橄榄提取物的组合物。在该协同制剂中，苹果、葡萄、绿茶和橄榄提取物的用量是提供的抗氧化剂活性或蛋白激酶调节活性大于等量任一提取物或提取物总和所提供的抗氧化剂活性或蛋白激酶调节活性的用量。进一步提出了调控氧化应激、疾病相关的蛋白激酶活性和增强主要治疗剂的治疗效果的方法。还提出了制备用于调控氧化应激、疾病相关的蛋白激酶活性和增强主要治疗剂治疗效果的活性增强组合物的方法。



CN 107920561 A

1.氧化应激调节组合物，包括：

苹果、葡萄、绿茶和橄榄提取物的组合，所述苹果、葡萄、绿茶和橄榄提取物的用量是提供的抗氧化剂活性大于等量任一提取物或所述提取物总和所提供的抗氧化剂活性的用量。

2.权利要求1所述的组合物，其中所述苹果提取物包括物种Malus pumila的提取物。

3.权利要求1所述的组合物，其中所述葡萄提取物包括物种Vitis vinifera的提取物。

4.权利要求1所述的组合物，其中所述绿茶提取物包括物种Camellia sinensis的叶的提取物。

5.权利要求1所述的组合物，其中所述橄榄提取物包括亚种Olea europea europaea的提取物。

6.权利要求1所述的组合物，其中所述组合物中所述提取物中的至少一种的存在量不同于至少一种其它提取物的量。

7.权利要求1所述的组合物，其中所述苹果、葡萄、绿茶和橄榄提取物在所述组合物中以约1:1:1:1的重量比存在。

8.权利要求1所述的组合物，其中所述苹果、葡萄、绿茶和橄榄提取物在所述组合物中以约6:1:3:1的重量比存在。

9.权利要求1所述的组合物，其中所述苹果、葡萄、绿茶和橄榄提取物包括叶、表皮、皮层、浆、汁、种子或其组合。

10.权利要求1所述的组合物，进一步包括至少一种主要或次要治疗剂。

11.权利要求10所述的组合物，其中所述至少一种主要或次要治疗剂包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、Ioclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。

12.权利要求10所述的组合物，其中所述至少一种主要或次要治疗剂包括佛手柑。

13.权利要求10所述的组合物，其中所述至少一种主要或次要治疗剂包括山竹。

14.权利要求10所述的组合物，其中所述至少一种主要或次要治疗剂包括黄连素。

15.权利要求1所述的组合物，其中所述组合物进一步包括药学上可接受的载体。

16.权利要求1所述的组合物，其中所述组合物是口服剂制剂。

17.权利要求16所述的组合物，其中口服剂型包括胶囊、片剂、粉末、饮料、糖浆、悬浮液或食品。

18.权利要求1所述的组合物，其中所述抗氧化剂活性调节应激相关的病理和代谢障碍。

19.权利要求1所述的组合物，其中所述抗氧化剂活性调节氧化LDL。

20.权利要求1所述的组合物，进一步包括蓝莓浓缩物、辣椒提取物和姜黄提取物。

21.权利要求20所述的组合物，其中所述蓝莓浓缩物包括Vaccinium angustifolium，所述辣椒提取物包括Capsicum annuum，以及所述姜黄提取物包括Curcuma longa。

22.权利要求20所述的组合物，进一步包括山竹果实提取物。

23.权利要求22所述的组合物，其中所述组合物包括大于1.5倍的等量任一提取物或浓缩物或所述提取物和浓缩物总和的抗氧化剂活性。

24.权利要求20所述的组合物，进一步包括佛手柑提取物。

25.权利要求24所述的组合物，其中所述佛手柑提取物包括Citrus bergamia Risso。

26.权利要求25所述的组合物，其中所述组合物包括大于1.5倍的等量任一提取物或浓缩物或所述提取物和浓缩物总和的抗氧化剂活性。

27.权利要求20所述的组合物，进一步包括山竹果皮提取物。

28.权利要求27所述的组合物，其中所述组合物包括大于1.25倍的等量任一提取物或浓缩物或所述提取物和浓缩物总和的抗氧化剂活性。

29.权利要求20所述的组合物，进一步包括山竹果皮提取物和佛手柑提取物。

30.调控对象的氧化应激的方法，包括：

给予所述对象苹果、葡萄、绿茶和橄榄提取物的治疗有效组合，所述苹果、葡萄、绿茶和橄榄提取物的用量是提供的组合抗氧化剂活性大于等量任一提取物或所述提取物总和所提供的抗氧化剂活性的用量。

31.权利要求30所述的方法，其中；

所述苹果提取物包括Malus pumila的表皮和果实的提取物；

所述葡萄提取物包括Vitis vinifera的种子的提取物；

所述绿茶提取物包括Camellia sinensis的叶的提取物；和

所述橄榄提取物包括Olea europea europaea的叶。

32.权利要求30所述的方法，其中所述苹果、葡萄、绿茶和橄榄提取物以约1:1:1:1的重量比存在。

33.权利要求30所述的方法，其中所述苹果、葡萄、绿茶和橄榄提取物以约6:1:3:1的重量比存在。

34.权利要求30所述的方法，进一步包括给予所述对象至少一种次要治疗剂。

35.权利要求34所述的方法，其中所述至少一种次要治疗剂与所述苹果、葡萄、绿茶和橄榄提取物的治疗有效组合被共同给予所述对象。

36.权利要求34所述的方法，其中所述至少一种次要治疗剂包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、loclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。

37.权利要求30所述的方法，其中所述抗氧化剂活性调节应激相关的病理和代谢障碍。

可用于食品、饮食补充剂、化妆品和药物制剂的呈现多种协同抗氧化剂活性的植物复合物

[0001] 优先权资料

[0002] 本申请要求2015年3月16日提交的美国临时专利申请序号62/133,945的权益，其通过引用被并入本文。

[0003] 发明背景

[0004] 氧化应激影响多种体内代谢途径，并且涉及多种病理生理状况，包括通过氧和氮反应性物种的传播所刺激的与蛋白激酶活性的组织特异性调节相关的障碍。氧化应激相关的病理和代谢障碍可包括代谢综合征、I型和II型糖尿病、肥胖、高胆固醇水平伴随氧化型LDL胆固醇升高、动脉粥样硬化、动脉高血压和各种形式的炎症——仅举几例。

[0005] 附图简述

[0006] 本发明的特征和优点将通过下文详细描述而显而易见，下文详细描述结合附图示例了本发明的特征。要理解，附图仅描述示例性实施方式，因此不被认为对范围有限制性。

[0007] 图1示意性显示了细胞中氮氧基、超氧基、过氧亚硝酸根和二氧化氮的相互作用；

[0008] 图2示意性显示了巨噬细胞(M)在血管内皮动脉粥样硬化斑块内的早期损伤发展期间发挥的作用；和

[0009] 图3示意性显示了糖尿病中的心血管功能异常机制，示例了超氧基、过氧亚硝酸根和具体激酶信号级联的作用。

[0010] 实施方式描述

[0011] 在公开和描述本发明实施方式之前，要理解，没有受限于本文公开的具体结构、方法步骤或材料的意图，而是还包括相关领域普通技术人员所理解的其等同形式。还应理解，本文使用的术语仅用于描述具体实例，而非意图限制。在不同附图中相同的参考编号表示相同的元素。流程图和方法中所提供的编号被提供的目的是为了清楚示例步骤和操作，而不一定暗示特定顺序或次序。除非另外限定，本文使用的所有技术和科学术语均与本公开所属领域普通技术人员的普遍理解具有相同含义。在说明书和所附权利要求书中，除非上下文明确另外指定，单数形式包括复数指代。

[0012] 如本说明书所用，除非上下文明确另外指定，单数形式“一个(a或an)”、“一种(a或an)”和“所述(the)”还具体包括复数指代。例如，“一种赋形剂(an excipient)”指代一种或多种赋形剂。

[0013] 另外，如本文所用，除非具体另外指明，用词“或”以作为“和/或”的“包容性”意义使用，而非以作为“二选一/或”的“排他性”意义使用。

[0014] 术语“约”在本文中用于指代一定偏离程度。其意思是近似地、在该区域内、大致上地或周围左右。当术语“约”结合数值范围使用时，其通过在所示数值以上和以下扩展边界来修饰该范围。要理解，本说明书对于结合术语“约”所用的数值的支持同样提供给该确切数值本身，如同“约”未被使用。

[0015] 浓度、量(或用量)和其它数据在本文中可以范围形式表示或提出。要理解，采用这种范围形式仅仅是为了方便和简洁，因此其应被灵活地解释为不仅包括作为范围极限或端

点被明确记载的数值，而且还包括该范围内包括的所有个体数值和/或子范围，如同每个数值(包括分数)和子范围均被明确记载。作为示例，“约1至约5”的数值范围应被解释为不仅包括明确记载的约1至约5的值，而且还包括所示范围内的个体数值和子范围。因此，该数值范围包括各个个体数值如2、2.6、3、3.8和4，及子范围如1-3、2-4和3-5等，以及1、2、3、4和5。

[0016] 除非具体另外说明，推定本文包括或记载的配方或组成成分以wt%为单位。另外，推定以比例形式表示的成分含量为wt%(例如，%w/w)比例。由此，包含1:1:1:1比例的四种成分的组合物将表示每种成分的存在量为25wt%。因此，在一些方面，组合物或制剂中以wt%为单位的成分含量可源自于比例数值。

[0017] 如本文所用，“包括(comprises)”、“包含(comprising)”、“含有(containing)”和“具有(having)”和类似术语可具有美国专利法对其赋予的含义，可意为“包括(includes)”、“包含(including)”和类似含义，并且总体上被解释为开放型术语。术语“由……组成的(consisting of)”或“由……组成(consists of)”是封闭型术语，仅包括与该术语一起具体列举的组分、结构、步骤或类似物，以及符合美国专利法的组分、结构、步骤或类似物。“主要由……组成的(consisting essentially of)”或“主要由……组成(consistd essentially of)”总体上具有美国专利法对其赋予的含义。具体地，这种术语总体上是封闭型术语，除了允许包括不实质上影响与该术语联用的项目(一个或多个)的基本特性或功能以及新特性或功能的其它项目、材料、组分、步骤或元素。例如，在组合物中存在、但不影响组合物的属性或特性的微量元素，如果是在“主要由……组成”的用语下存在，则即使没有被明确记载在跟随该术语的项目陈列中，也是允许的。当说明书中使用开放型术语如“包含”或“包括”时，要理解，对于“主要由……组成”的用语以及“由……组成”用语将同样提供直接支持，如同已明确说明，并且反之亦然。

[0018] 如本文所用，“基本”或“基本上”在涉及材料的数量或量或其具体特征使用时指代足以提供该材料或特征意图提供的效果的量。可允许的确切偏离程度可在一些情况下取决于具体环境。类似地，"基本上无"或类似形式指代组合物中缺少指定元素或用剂。具体地，被指定为"基本上无"的元素完全不存在于组合物，或其含量足够小以至于对组合物不具有可测量的效果。

[0019] 如本文所用，“活性剂”、“活性剂”和类似小时指代在以可感知量如有效量或治疗有效量被给予对象时对于对象具有可测量的生理效果的分子、化合物、混合物或成分。类似的术语如"活性部分"、"活性组分"和"活性成分"可与之互换使用。在“活性剂”的活性在该用剂所给予的对象中发挥或以其它方式导致有益治疗效果时，该“活性剂”可被称为“治疗剂”。

[0020] “佛手柑”指代佛手柑橙(Citrus bergamia Risso)。此柑桔物种在意大利南部的卡拉布里亚地区大量生长，并且已被用于卡拉布里亚民间药物以治疗心血管疾病数百年。佛手柑包括天然存在的黄酮苷类的两种3-羟甲基戊二酰(HMG)衍生物brutieridin和melitidin。这些苷分别是糖基化橙皮素和柚皮素的HMG衍生物，并且与作为他汀类(statins)已知的市售HMG辅酶A还原酶抑制剂具有结构相似性。本文使用的佛手柑可互换使用指代果实和/或提取物。

[0021] 本文使用的“浓缩物”指代从不包括在浓缩过程中任何溶剂的使用的组分得到的干燥粉末。

[0022] 术语"剂量单位"被理解意为单位的——即，能够被给予对象或患者并且可容易操作和包装、保持作为包括活性成分本身或其与固体或液体药物介质材料的混合物的物理和化学稳定的单位剂量的单剂量。剂量可以是口服的、鼻的、肠的、胃肠外的、透皮的、跨粘膜的等。

[0023] 术语"提取物"指代利用溶剂——例如，乙醇、水、蒸汽、过热水、甲醇、己烷、氯仿液体、液体CO_2、液体N_2、丙烷、超临界CO_2或其任意组合——制备的那些物质。提取物，如本文所用，可指代液体形式的提取物，或可指代由液体形式的进一步处理获得的产物，如干燥粉末或其它固体形式。提取物可采取多种形式，包括但不限于：固体、液体、颗粒、切碎式、蒸馏物等，并且可通过多种程序或方案进行，如切剁、研磨、粉碎、煮沸、汽蒸、浸泡、浸渍、灌注、施加气体等，并且可利用任何适当的反应剂，如水、醇、蒸汽或其它有机材料。提取物一般具有给定的纯度百分比，并且可相对高纯。在一些实施方式中，提取物可以是植物提取物，由原料的具体部分制成，如植物的表皮、浆、叶、花、果实等，或可由整个原料制成。在一些方面，提取物可包括一种或多种活性部分或活性剂。在一些提取物中，麦芽糊精可作为载体被添加。在一些方面，提取物的纯度可通过提取方法或方案来控制或是提取方法或方案的函数。

[0024] 如本文所用，"制剂"和"组合物"可互换使用，并且指代至少两种成分的组合。在一些实施方式中，至少一种成分可以是活性剂或在被给予对象时具有发挥生理活性的特性。

[0025] 如本文所用，"增加或减少的浓度、分泌或生物合成"意为参考化合物的分泌量(例如，至少3%)、浓度、速率或生物合成的量的可感知的增加或减少。

[0026] 如本文所用，"线性抑制效果"或"剂量响应"指代在剂量响应曲线上所有浓度的抑制材料带来的分泌或生物合成线性减少。例如，在低浓度下的抑制后抑制失败或在较高浓度下的分泌增加表示不具有线性抑制效果。

[0027] 如本文所用，"漏肠综合征(Leaky Gut Syndrome，LGS)"是肠粘膜对于炎性退行性和/或萎缩性粘膜损伤相关的腔大分子、抗原和毒素的渗透性增加。LGS可导致多种看似无关的影响体内各器官系统的症状。LGS已与在多种不同的疾病中具有致病作用关联起来。其中多种是自身免疫性疾病——意味着免疫系统攻击身体自身的细胞。LGS在这些疾病类型中发挥作用，因为其增加对食品粒子的免疫反应，然后可发生交叉反应性，意味着免疫系统攻击与其敏感的食品在化学上相似的身体组织。其中漏肠综合征可占有一席的多种疾病的抽样包括：类风湿性关节炎、骨关节炎、哮喘、多发性硬化、血管炎、克罗恩病、结肠炎、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征和纤维肌痛。

[0028] 如本文所用，"药学上可接受的"总体上指代适于联合活性剂或成分给予对象的材料。例如，"药学上可接受的载体"可以是可与活性剂适当地组合以提供适于给予对象的组合物或制剂的任何物质或材料。用于给予对象的制剂或组合物或用于制备给予对象的制剂或组合物的赋形剂、稀释剂和其它成分可用以这种术语。

[0029] 本文使用的术语"主要治疗剂"表示组合物中治疗剂的存在量大于在组合物中提供协同效果的提取物的总组合量。

[0030] 术语"预防"及其变型指代针对具体的不期望的生理状况的预防。预防可以是部分的或完全的。部分预防可导致生理状况发作延迟。本领域技术人员将知道生理状况发作延迟的期望性，并且将知道要向具有某些生理状况风险的对象给予本发明的组合物以延迟那些状况发作。例如，本领域技术人员将知道，肥胖对象具有升高的冠状动脉疾病风险。因此，

本领域技术人员可给予组合物以增加肥胖对象的胰岛素敏感性，从而可完全预防或延迟糖尿病或血脂异常(dyslipemia)的发作。

[0031] 如本文所用，“氧化应激”指代反应性氧物种(ROS)的显示与生物系统容易使反应性中间体去毒的能力之间的失衡。ROS导致自由基形成。自由基(例如，羟基、硝酸、超氧基)或非自由基(例如，过氧化氢、脂质过氧化物)导致损伤(称作氧化性损伤)具体分子，进而损害细胞或组织。细胞正常氧化还原状态的扰乱可通过产生损伤细胞所有组分——包括蛋白质、脂质和DNA——的过氧化物和自由基而造成毒性效应。虽然短期氧化应激可以是有益的；但随时间推移的氧化应激可涉及多种状况和疾病的病因学。ROS产生增加因真菌或病毒感染、炎症、衰老、紫外线辐射、污染、过度饮酒、吸烟等而发生。ROS的去除或中和利用抗氧化剂——内源的(例如，过氧化氢酶、谷胱甘肽、超氧基歧化酶)或外源的(例如，维生素A、C、E、生物类黄酮、类胡萝卜素)——来实现。

[0032] 本文使用的“氧化应激相关的病理”限定增加细胞氧化状态以产生疾病状态前的氧化应激响应的任何状况。这总体上源于相对于细胞抗氧化剂防御(抗氧化剂、抗氧化剂酶)增加了反应性氧或反应性氮物种(分别为ROS和RNS)(超氧基、过氧化氢、羟基自由基、过氧亚硝酸根、单线态氧)的产生。虽然氧化应激响应不一定导致疾病，但其是多种疾病机制中的关键组分。这种疾病的非限制实例包括代谢综合征、肥胖、动脉粥样硬化、动脉高血压、糖尿病(1、2和3型)、运动能力下降、早泄、充血性心力衰竭、心血管疾病——包括心跳停止和心肌梗塞、运动功能异常、功能异常、认知障碍包括——阿尔茨海默病、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、脆性X综合征胰腺炎、各种形式的炎症——包括骨关节炎、类风湿性关节炎、炎性肠道疾病、结肠炎、漏肠综合征、肾脏疾病和血液透析、休克、创伤、缺血、帕金森病、药物反应、克罗恩病、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛、多种癌症——包括前列腺和乳腺癌、癌症化疗增强、表皮相关的疾病——如伤口愈合缓慢、皱纹和衰老征兆过早。

[0033] 本文使用的术语“次要治疗剂”表示组合物中治疗剂的存在量小于组合物中提供协同效果的提取物的总组合量。

[0034] 术语“对象”、“对象们”或“对其有需要的对象”包括人类以及非人类对象，具体地驯养和农场动物。将理解，本发明化合物所给予的对象至无需经历具体创伤状态。事实上，本发明的化合物可在症状任何发展之前被预防性地给予。术语“治疗性”、“治疗上”和类似形式用于涵盖治疗性、治标性以及预防性应用。

[0035] 如本文所用，术语“溶剂”指代具有从植物产品提取固体物所需的特征的气态、水性或有机属性的液体。溶剂实例包括但不限于水、蒸汽、过热水、甲醇、乙醇、乙酸乙酯、己烷、氯仿、液体CO_2、液体N_2、丙烷或这种材料的任意组合。

[0036] 如本文所用，“协同”意为相对于作用机制超过各个组分的叠加效应。例如，如果F1产生响应X，F2产生响应Y，则F1+F2的组合>X+Y。在一些情况下，F2不产生响应并且Y值等于零。

[0037] 短语活性成分的“有效量”、“治疗有效量”或“治疗有效率”指代活性成分的无毒但充足的量或递送率，以在治疗该成分所递送的疾病或状况时实现治疗效果。要理解，各种生物因素可影响物质执行其目标任务的能力。因此，“有效量”、“治疗有效量”或“治疗有效率”可在一些情况下取决于这种生物因素。进一步，虽然治疗效果的实现可由医师或其它适格

医务人员利用本领域已知的评价来测定，但公知个体变化和对治疗的响应可使得治疗效果的实现是主观决定。治疗有效量或递送率的确定完全属于药物科学和医药领域的普通技能。

[0038] 术语“治疗”(“treat”、“treating”或“treatment”)，如本文所用并且如本领域公知，意为获得有益的或期望的结果(非限制地包括被对象的临床结果)的方法。有益的或期望的结果可包括但不限于，缓和或改善状况的一种或多种征兆或症状、减轻疾病程度、稳定(即，不恶化)疾病或状况状态、延迟或减缓疾病进程、改善或缓和疾病状态、减轻疾病复发和缓解(部分或全部)——无论可检测到的还是不可检测到的。例如，在生理状态是葡萄糖耐受性不良的情况下，“治疗”指代提高被治疗对象的葡萄糖耐受性。作为另一实例，在生理状态是肥胖的情况下，术语“治疗”指代减少身体脂肪量，改善身体质量或改善对象的身体脂肪比例。糖尿病治疗意为提高血糖控制。炎性疾病治疗意为体内全身或局部减少炎性响应。“治疗”(“treat”、“treating”和“treatment”)还可意为与不接受治疗的预计生命期相比延长生命期，并且可以是预防性的。这种预防性治疗还可被称为预防或疾病或状况的预防。预防可以是部分的或完全的。部分预防可导致生理状况发作延迟。本领域技术人员将知道，治疗可以，但无需一定，包括缓和或治愈。

[0039] 如本文所用，“化合物”可通过其化学结构、化学名称或通用名称来鉴定。当化学结构、化学名称或通用名称冲突时，化学结构决定化合物的身份。本文所述的化合物可包含一个或多个手性中心和/或双键，因此可作为立体异构体存在，如双键异构体(即，几何异构体)、对映体或非对映体。因此，本文所示的化学结构包括示例或鉴定的化合物的所有可能的对映体和立体异构体，包括立体异构体纯形式(例如，几何纯、对映体纯或非对映体纯)和对映体和立体异构体混合物。利用技术人员公知的分离技术或手性合成技术，对映体和立体异构体混合物可被分解成其组分对映体或立体异构体。化合物还可以数种互变异构形式存在，包括烯醇形式、酮形式和其混合物。因此，化学结构包括示例或鉴定的化合物的所有可能的互变异构形式。所述化合物还包括同位素标记的化合物，其中一种或多种原子的原子质量不同于自然界中常见的原子质量。可并入本发明化合物的同位素的实例包括但不限于，^{2}H、^{3}H、^{13}C、^{14}C、^{15}N、^{18}O、^{17}O、等。化合物可以非溶剂化形式以及溶剂化形式——包括水合形式——和N-氧化物存在。总体上，化合物可以是水合的、溶剂化的或N-氧化物。某些化合物可以多种晶体或无定形形式存在。还考虑该化合物的同源物、类似物、水解产物、代谢产物和前体或前体药物。总体上，所有物理形式对于本文考虑的应用而言都是等同的，并且意图处于本公开的范围内。

[0040] 比较性术语如“更有效”、“大于”、“提高”、“增强”和类似术语可用来说明制剂或方法中实现的效果或存在的特性与被比较的对象相比具有可测量地更好或更积极的结果。在一些情况下，可与现有技术进行比较。

[0041] 下文详细参考本发明的具体实施方式。虽然将结合这些具体实施方式描述本发明，但将理解，这并非意图本发明限于这种具体实施方式。相反，意图本发明涵盖可包括在所附权利要求限定的本发明的精神和范围之内的替代形式、改进和等同形式。在下文描述中，为提供对本发明的全面理解而提出了大量具体细节。本发明可在不具有这些具体细节中的部分或全部的情况下实践。在其它情况下，为了不使本发明不必要地晦涩难懂，公知的过程操作未被详细描述。

[0042] 所有生命形式其细胞中都保持还原环境。这种还原环境由酶来保持，该酶通过代谢能量恒定输入来保持还原状态。这种正常氧化还原状态的扰乱可通过产生损伤细胞所有组分——包括蛋白质、脂质和DNA——的过氧化物和自由基而导致毒性效应。

[0043] 在人类中，氧化应激涉及多种疾病的病因学。例如，氧化应激涉及代谢综合征、肥胖、动脉粥样硬化、动脉高血压、糖尿病(I、II和III型)、运动能力下降、早泄、充血性心力衰竭、心血管疾病——包括心跳停止和心肌梗塞、运动功能异常、功能异常、认知障碍——包括阿尔茨海默病、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、脆性X综合征胰腺炎、各种形式的炎症——包括骨关节炎、类风湿性关节炎、炎性肠道疾病、结肠炎、漏肠综合征、肾脏疾病和血液透析、休克、创伤、缺血、帕金森病、药物反应、克罗恩病、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛、多种癌症——包括前列腺和乳腺癌、癌症化疗增强、表皮相关的疾病——如伤口愈合缓慢、皱纹、衰老征兆过早、以及其它。

[0044] 反应性氧物种(ROS)、反应性氮物种(RNS)、其它自由基和氧化剂来源(一并即，O_2^-、$1O_2$、HO^-、NO^-、$ONOO^-$、HOCl、$RO(O)^-$、$O(O)^-$)可导致对身体细胞的严重损伤。例如，这种损伤可以是对DNA、蛋白质和其它大分子的，并且形成多种炎症系疾病的基础。

[0045] 实验证据直接或间接表明，存在导致人体氧化性损伤的六个主要反应性氧物种。这些物种包括超氧阴离子(O^-)、过氧化氢(H_2O_2)、过氧基自由基(ROO^-)、羟基自由基(HO^-)、单线态氧($1O_2$)和过氧亚硝酸根($ONOO^-$)。为对抗这种损伤，抗氧化剂抑制氧化和预防自由基形成。在生物系统中，抗氧化剂有至少四种主要来源：(1)酶(即，超氧基歧化酶、谷胱甘肽过氧化物酶和过氧化氢酶)；(2)大分子(即，白蛋白、血浆铜蓝蛋白、铁蛋白、其它蛋白质)；(3)小分子(抗坏血酸、谷胱甘肽、尿酸、生育酚、类胡萝卜素、(多)酚)；和(4)一些激素(雌激素、血管紧张素、褪黑激素等)。

[0046] 氧化剂和抗氧化剂可具有不同化学和物理特征。各抗氧化剂可通过单一系统中的多种机制或通过不同的单一机制来发挥作用，并且可以不同方式响应不同自由基或氧化剂来源。例如，类胡萝卜素相对于酚类化合物和其它抗氧化剂不是特别好的过氧基自由基猝灭剂；然而，类胡萝卜素在猝灭单线态氧方面优秀，而在这点上多数其它酚类化合物和抗氧化剂相对低效。单线态氧不是自由基并且不通过自由基机制发生反应，而是主要通过添加至双键来发生反应，形成内部-过氧化物，该内部-过氧化物可被还原成烷氧基自由基，发动自由基链反应。由于通常涉及多种反应特征和机制以及不同的相定位(phase localizations)，没有单一测验会准确反映出混合或复合系统中的所有自由基来源或所有抗氧化剂。

[0047] 活细胞具有由使ROS/RNS转化成无害物质的酶抗氧化剂构成的生物防御系统。例如，H_2O_2可被过氧化氢酶转化成水和氧。在另一实例中，O^-被超氧基歧化酶(SOD)转化成氧和过氧化氢，或与氮氧基(NO^-)反应形成过氧亚硝酸根。当氮氧基和超氧基均存在时，其还可与二氧化氮反应形成N_2O_3和过氧硝酸根(参见图1)。过氧硝酸根分解给出亚硝酸根和氧，同时N_2O_3可与硫醇反应给出亚硝基硫醇或与氢氧根阴离子反应给出亚硝酸根。过氧硝酸根还在扩散限制速率下与过氧亚硝酸根反应生成两分子的二氧化氮和一分子的亚硝酸根。这建立了在中性pH下加入过氧亚硝酸根的弹丸式添加时生成较多二氧化氮的循环，并且显著增加了发生的潜在反应的数量。这些相同的反应也将在体内发生，特别在氮氧基的产生快于超氧基时。

[0048] 过氧亚硝酸根也可由肺泡巨噬细胞天然产生(参见图2)。当肺泡巨噬细胞被刺激产生超氧基和氮氧基时,过氧亚硝酸根定量产生——通过氮氧基和超氧基产生量和氧消耗量证明。胞外添加高浓度超氧基歧化酶(SOD)不显著减少过氧亚硝酸根形成量,并且反而充当酪氨酸硝化的催化剂。这表明,膜表面处产生的超氧基和扩散透过膜的氮氧基在膜界面处迅速发生反应,使得体相(bulk phase)中的SOD无法竞争。

[0049] 氮氧基(NO)和过氧亚硝酸根在心血管病理生理学中具有重要作用。NO可激活可溶性鸟苷酸环化酶(sGC)-cGMP信号转导途径,该途径在心血管系统中介导各种生理/有益效果,包括血管舒张、抑制血小板聚集、抗炎、抗重塑和抗凋亡效果。在氧化应激和炎症增加相关的病理状况(心肌梗塞、缺血性心脏病、心肌炎、心肌病、高血压、等)下,NO和超氧基(O_2^-)反应形成过氧亚硝酸根($ONOO^-$),过氧亚硝酸根通过脂质过氧化引起细胞损伤,通过氧化和硝化引起酶和其它蛋白质失活,以及引起应激信号传导、基质金属蛋白酶(MMP)激活等。

[0050] 过氧亚硝酸根还触发促凋亡因子如细胞色素和凋亡诱导因子(AIF)从线粒体释放,该促凋亡因子介导胱天蛋白酶依赖性和不依赖性凋亡死亡途径。此外,过氧亚硝酸根与其它氧化剂协作导致DNA的链断裂,激活核酶聚(ADP-核糖)聚合酶-1(PARP-1)。DNA的轻度损伤激活DNA修复机制。相比之下,在过度的氧化性和亚硝化性应激诱导的DNA损伤发生(如心肌再灌注损害和心力衰竭的各种形式)后,过度激活的PARP通过将ADP-核糖单位从烟酰胺腺嘌呤二核苷酸(NAD+)转移至核蛋白质而发动耗能循环,导致胞内NAD^+和ATP储量迅速耗竭,减缓糖酵解和线粒体1呼吸速率,最终导致细胞功能障碍和死亡。聚(ADP-核糖)糖水解酶(PARG)降解聚(ADP-核糖)(PAR)多聚体,生成游离的PAR多聚体和ADP-核糖。过度激活的PARP还促进多种炎性基因表达,导致炎症和相关的氧化应激增加,因此促进心血管功能异常和心脏的进程。

[0051] 过氧亚硝酸根还作用于在慢性炎性状况中放大炎性信号传导。此外,炎症通过如下触发:激活多种信号传导级联,最终一组促炎性细胞因子和趋化因子的产生上调。这些发动更复杂的炎性反应,该炎性反应的特征在于炎性细胞激活和酶活性被刺激,该酶包括诱导性NO合酶(iNOS)——产生大量NO和超氧基(O_2^-)生成酶——NADPH氧化酶(NADPHox)和黄嘌呤氧化酶(XO)。NO和O_2^-的同时产生导致过氧亚硝酸根($ONOO^-$)生成,过氧亚硝酸根进而损伤目标分子——包括蛋白质、谷胱甘肽(GSH)、线粒体和DNA。DNA损伤可发动凋亡细胞死亡,并且还是聚(ADP-核糖)聚合酶(PARP)激活的强制性触发事件,聚(ADP-核糖)聚合酶(PARP)可通过ATP耗竭而引起细胞坏死。$ONOO^-$和PARP均进一步参与促炎性信号转导途径的上调,从而产生炎性细胞损害的自放大循环。

[0052] 超氧基和过氧亚硝酸根还在糖尿病中协调心血管功能异常(参见图3)。高血糖引起超氧阴离子(O_2^-)产生增加——通过激活多种途径,包括黄嘌呤和NAD(P)H氧化酶、环氧化酶、未偶联的氮氧基合酶(NOS)、葡萄糖自动氧化、线粒体呼吸链、多元醇途径和形成晚期糖化终产物(AGE)。超氧基激活AGE、蛋白激酶C(PKC)、多元醇(山梨醇)、己糖胺和应激信号传导途径,导致炎性细胞因子、血管紧张素II(Ang II)、内皮素-1(ET-1)和NAD(P)H氧化酶的表达增加,进而通过多种机制生成更多超氧基。高血糖引起的超氧基生成增加还可通过NFκB激活而有利于氮氧基合酶(NOS)的表达增加,这可增加氮氧基(NO)的生成。超氧阴离子可猝灭NO,从而降低强效内皮衍生血管舒张剂系统的效力。超氧基也可被超氧基歧化酶(SOD)转化成过氧化氢(H_2O_2),并与NO相互作用形成反应性氧化剂过氧亚硝酸根($ONOO^-$),该反应

性氧化剂过氧亚硝酸根($ONOO^-$)通过脂质过氧化引起细胞损伤，通过氧化和硝化引起酶和其它蛋白质失活，和引起基质金属蛋白酶(MMP)激活等。

[0053] 过氧亚硝酸根还可作用于线粒体[降低膜电位(Ψ)]，触发促凋亡因子如细胞色素c(Cyt c)和凋亡诱导因子(AIF)的释放。这些因子介导胱天蛋白酶依赖性和胱天蛋白酶不依赖性凋亡死亡途径。

[0054] 过氧亚硝酸根，与其它氧化剂(例如，H_2O_2)协作，可导致DNA的链断裂，激活核酶聚(ADP-核糖)聚合酶-1(PARP-1)。DNA的轻度损伤激活DNA修复机制。在过度的氧化性和亚硝化性应激诱导性DNA损伤发生后，过度激活的PARP-1通过将ADP-核糖单位(小红球)从NAD^+转移至核蛋白质而发动耗能循环，导致胞内NAD^+和ATP储量迅速耗竭，减缓糖酵解和线粒体1呼吸速率，并最终导致细胞功能障碍和死亡。聚(ADP-核糖)糖水解酶(PARG)降解聚(ADP-核糖)(PAR)多聚体，生成游离的PAR多聚体和ADP-核糖，其可传导信号至线粒体以引起AIF释放。PARP-1激活还导致细胞甘油醛-3-磷酸脱氢酶(GAPDH)活性被抑制，进而有利于PKC、AGE和己糖胺途径激活，导致超氧基生成增加。PARP-1还调控可促进糖尿病心血管并发症的进程的多种炎性介导因子的表达。

[0055] 过氧亚硝酸根的反应产物已被检测到并且其形成或其分解的药理抑制已显示有益的其它状况包括血管疾病、缺血-再灌注损害、循环性休克、疼痛和神经变性。

[0056] 清除ROO^-、HO^-、$1O_2$和$ONOO^-$的酶促作用目前是未知的。因此，防御工作依赖于具有清除氧化剂和自由基的特性的多种非酶抗氧化剂如维生素C和E以及多种植物化学。为综合评价食品样本的氧化剂清除能力，必须设计包括这些ROS的测验(即，ORAC(氧自由基吸收能力)测验)。

[0057] 在细胞水平上，信号转导指代信号或信号传导部分从细胞外部移至细胞内部。信号，在到达其受体靶后，可发动多种细胞事件需要的配体-受体相互作用，其中一些可进一步充当后续信号。这种相互作用不仅作为系列级联，而且还作为能够提供稳态过程微调控制的信号事件的错综相互作用网络或网来发挥作用。然而，这种网络可失调，从而导致细胞活性改变和响应细胞中表达的基因的编码变化。

[0058] 信号转导受体总体上分成三类。第一类受体是穿透质膜并且具有一定固有酶活性的受体。具有固有酶活性的代表性受体包括：酪氨酸激酶(例如，PDGF、胰岛素、EGF和FGF受体)、酪氨酸磷酸酶(例如，T细胞和巨噬细胞的CD45[簇决定因子-45]蛋白)、鸟苷酸环化酶(例如，利尿钠肽受体)和丝氨酸/苏氨酸激酶(例如，激活素和TGF-β受体)。具有固有酪氨酸激酶活性的受体能够进行自磷酸化以及其它底物的磷酸化。

[0059] 第二类受体是在细胞中偶联至GTP结合和水解蛋白(称为G蛋白)的那些受体。这类与G蛋白相互作用的受体具有如下特征的结构：7个跨膜结构域。这些受体被称为蛇形受体。这类的实例是肾上腺素能受体、气味受体和某些激素受体(例如，高血糖素、血管紧张素、加压素和缓激肽)。

[0060] 第三类受体可被描述为这样的受体：在胞内被发现，并且在配体结合后迁移至核，在此配体-受体复合体直接影响基因转录。

[0061] 编码受体酪氨酸激酶(RTK)的蛋白质包含四个主要结构域，即：a)跨膜结构域、b)胞外配体结合结构域、c)胞内调控结构域和d)胞内酪氨酸激酶结构域。RTK的氨基酸序列与cAMP依赖性蛋白激酶的氨基酸序列是高度保守的(在ATP和底物结合区域内)。RTK蛋白基于

其胞外部分的结构特征而分成多个家族，该胞外部分包括：富半胱氨酸结构域、免疫球蛋白样结构域、钙粘素结构域、富亮氨酸结构域、Kringle结构域、酸性结构域、纤维连接素III型重复、盘菌素I样结构域和EGF样结构域。基于这些不同胞外结构域的存在，RTK已被再分成至少14个不同家族。

[0062] 多种受体在磷酸化后具有固有的酪氨酸激酶活性，并且可与信号传导级联的其它蛋白质相互作用。这些其它蛋白质包含这样的结构域：其氨基酸序列与首次在c-Src原癌基因中鉴定出的结构域同源；这些结构域被称为SH2结构域。含SH2结构域蛋白与RTK或受体相关酪氨酸激酶的相互作用导致含SH2蛋白的酪氨酸磷酸化。造成的磷酸化产生该活性的改变(积极或消极)。具有固有酶活性的几种含SH2蛋白包括磷脂酶C-γ(PLC-γ)、原癌基因c-Ras相关GTP酶激活蛋白(rasGAP)、磷脂酰肌醇-3-激酶(PI-3K)、蛋白酪氨酸磷酸酶-1C(PTP1C)、以及蛋白酪氨酸激酶(PTK)的Src家族成员。

[0063] 非受体蛋白酪氨酸激酶(PTK)总体上偶联至自身缺乏酶活性的细胞受体。通过蛋白质相互作用信号传导的受体的实例包括胰岛素受体(IR)。这种受体具有固有的酪氨酸激酶活性，但在自磷酸化后不与包含SH2结构域的酶活性蛋白(例如，PI-3K或PLC-γ)直接相互作用。反而主要IR底物是称为IRS-1的蛋白质。

[0064] TGF-β超家族的受体代表原型受体丝氨酸/苏氨酸激酶(RSTK)。TGF-β超家族的多功能蛋白质包括激活素、抑制素和骨形态发生蛋白(BMP)。这些蛋白质可诱导和/或抑制细胞增殖或分化和调控各种细胞型的迁移和附着。TGF-β的一个主要效果是通过细胞周期调控进程。另外，细胞对TGF-β的响应中涉及的一种核蛋白是c-Myc，其直接影响具有Myc结合元素的基因的表达。PKA、PKC和MAP激酶代表非受体丝氨酸/苏氨酸激酶的三种主要类型。

[0065] 激酶活性与疾病状态之间可存在关系。这种关系本身可引起疾病，或与疾病相关症状和病理的表达和进程密切相关。例如，认为激酶活性涉及认知障碍，包括阿尔茨海默病、充血性心力衰竭、肺高血压、心肌病、运动功能障碍、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、各种形式的炎症——包括骨关节炎、类风湿性关节炎、I型和II型糖尿病、代谢综合征、肥胖、炎性肠道疾病、克罗恩病、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征和纤维肌痛等。

[0066] 自身免疫性疾病源于免疫系统功能障碍，其中身体产生攻击自身器官、组织和细胞的自身抗体——通过蛋白质磷酸化介导的过程。超过80种临床上不同的自身免疫性疾病已被鉴定，并且一共使美国约2400万人遭受痛苦。自身免疫性疾病可影响身体的任何组织或器官。由于这种可变性，其可根据自身免疫攻击的位点而引起多种症状和器官损害。虽然存在多种自身免疫性疾病的治疗，但其中没有任何一例被确定治愈。降低严重程度的治疗通常具有不利的副作用。

[0067] 对人类的自身免疫性疾病的病因学和发病机理仍知之甚少，但其被认为以三个阶段进展，开始阶段、效应阶段和激活阶段。在开始阶段，树突状细胞将自身抗原呈递至自身反应性T细胞。T细胞通过细胞因子激活自身反应性B细胞，导致自身抗体产生，自身抗体进而在关节处(joints)形成免疫复合物。在效应阶段，免疫复合物结合巨噬细胞和肥大细胞上的Fcf受体，导致细胞因子和趋化因子释放、炎症和疼痛。在激活阶段——最后阶段，细胞因子和趋化因子激活和募集滑膜成纤维细胞、破骨细胞和多形核中性白细胞，其释放蛋白酶、酸和ROS如O_2^-，导致不可逆的软骨和骨损坏。B细胞激活在抗原受体触发后通过脾酪氨酸

激酶(Syk)和磷酸肌醇3-激酶(PI3K)传导信号。在抗原受体在B细胞上接合后,Syk在三个酪氨酸上进行磷酸化。Syk是在偶联免疫识别受体至多个下游信号传导途径中起核心作用的72-kDa蛋白-酪氨酸激酶。此功能是其催化活性和其参与与包含SH2结构域的效应蛋白相互作用的能力两者的特性。Tyr-317、-342和-346的磷酸化产生多种含SH2结构域蛋白的停泊位点。72-kDa蛋白-酪氨酸激酶Ptk72与B细胞抗原受体相关。一个发明方面,提供了用于这些罹患自身免疫障碍的患者的疼痛缓解的安全的长期治疗方法。由于COX-2和iNOS合成的诱导因子通过Syk、PI3K、p38、ERK1/2和NF-kB依赖性途径传导信号,这些途径的抑制剂在自身免疫状况中并且具体地在类风湿性关节炎(RA)或骨关节炎(OA)患者的发炎和退行性关节中可具有治疗性。

[0068] Syk已显示被PI3K激活需要——响应包括B细胞抗原受体(BCR)以及巨噬细胞或中性白细胞Fc受体接合在内的多种信号。在B细胞中,BCR刺激的PI3K激活可通过接头蛋白如BCAP、CD19或Gab1的磷酸化来实现,该接头蛋白建立PI3K的p85调控亚单位的结合位点。多种IgG受体传送的信号需要Syk和PI3K的活性以及其募集至成簇受体位点。在中性白细胞和单核细胞中,提出PI3K与FcgRIIA上磷酸化的免疫受体酪氨酸系激活基序序列的直接相关性作为PI3K募集至受体的机制。Syk与PI3K之间的直接分子相互作用已被报道。

[0069] cdc样激酶CLK1涉及细胞增殖,作为对包含丝氨酸/苏氨酸和酪氨酸的底物均发挥作用的双特异性激酶;其磷酸化剪接体复合物的富丝氨酸和富精氨酸蛋白,并且可以是能够使SR蛋白控制RNA剪接的调控机制网络的成分。Clk还通过磷酸化剪接因子来调控微管相关蛋白tau的可选剪接和涉及额颞痴呆和帕金森病。Clk同种型的抑制剂可改变这些事件并且可证实在以异常剪接为特点的疾病表型中是有用的用剂。

[0070] 胰岛素受体(IR)的确定信号传导途径包括G蛋白偶联的受体信号传导途径,MAPK活性激活、蛋白激酶B活性激活、碳水化合物代谢过程、细胞响应至生长因子刺激、外分泌胰腺发育、葡萄糖稳态、葡萄糖输入的正调控、糖原生物合成过程的正调控和糖酵解的正调控。

[0071] 糖原合酶激酶3(GSK3)最初被描述为糖原代谢中涉及的关键酶,但现已知调控多种细胞功能。两种酶形式,GSK-3a和GSK-3b,此前已被鉴定。GSK-3的小分子抑制剂因此具有若干治疗用途,包括治疗神经变性疾病、糖尿病II型、双相性精神障碍、中风、癌症和慢性炎性疾病。糖原合酶激酶3(GSK-3)抑制剂作为新的有前景药物用于糖尿病、神经变性、癌症和炎症。

[0072] AMP激活的蛋白激酶(AMPK)作为细胞能量稳态的主要调控因子起着关键作用。该激酶响应耗竭细胞ATP供源的应激如低葡萄糖、低氧、缺血和热休克而被激活。由于其作为脂质和葡萄糖代谢的核心调控因子的作用,认为AMPK是治疗II型糖尿病、肥胖和癌症的潜在治疗目标。AMPK还牵涉到多个物种,通过其与mTOR和长寿蛋白(sirtuins)的相互作用作为衰老的重要调节剂。

[0073] 在一个发明实施方式中,提出了氧化应激调节组合物和相关方法。示例性组合物可包括苹果、葡萄、绿茶和橄榄提取物的组合,该苹果、葡萄、绿茶和橄榄提取物的用量是提供的抗氧化剂活性大于等量任一提取物或提取物总和所提供的抗氧化剂活性的用量。在一个实例中,苹果、葡萄、绿茶和橄榄提取物可包括由这些原料的叶、表皮、皮层、浆、汁、种子或组合配制的提取物。

[0074] 在一个实例中，苹果提取物可包括源自选自下列的成员的提取物：Malus domestica、Malus sieversii、Malus sylvestris、Malus pumila和其组合。在一个实例中，苹果提取物可源自物种Malus pumila。在一个实例中，苹果提取物可源自Malus domestica和Malus pumila的组合。在一些实施方式中，苹果提取物可包括苹果的任何或所有部分，包括但不限于表皮、果肉/果实(外果皮、中果皮和/或内果皮)、种子、茎、干、叶或其组合。在一个实例中，苹果提取物包括苹果的表皮和果实。在一些实施方式中，提取物可源自未成熟的苹果。在一个实施方式中，提取溶剂可以是乙醇。

[0075] 在一个实例中，葡萄提取物可包括选自下列的成员：Vitis vinifera、Vitis labrusca、Vitis riparia、Vitis rotundifolia、Vitis rupestris、Vitis aestivalis、Vitis mustangensis和其组合。在一个实例中，葡萄提取物可源自Vitis vinifera。在一些实施方式中，葡萄提取物可包括葡萄的任何或所有部分，包括但不限于表皮、果肉/果实、种子、维管束、藤蔓、叶或其组合。在一个实施方式中，葡萄提取物可源自种子。在另一实施方式中，葡萄提取物可源自表皮。在再另一实施方式中，葡萄提取物可源自葡萄的种子和表皮。在一些实施方式中，葡萄提取物包括在干重基础上约75wt％至约95wt％的酚类化合物。在其它实施方式中，葡萄提取物可包括在干重基础上约80wt％至97wt％的酚类化合物。在一个实例中，提取溶剂可以是乙醇、水或其混合物。

[0076] 在一个实例中，绿茶提取物可源自Camellia sinensis。在一些实施方式中，绿茶提取物可包括该茶的任何或所有部分，包括但不限于叶、种子、干、花或其组合。在一个实施方式中，绿茶提取物可源自叶。在另一实例中，提取溶剂可以是水、乙醇、乙酸乙酯或其组合。

[0077] 在一个实例中，橄榄提取物包括选自下列亚种的齐墩果(Olea europea)亚种：europea、cuspidiata、guanchica、cerasiformis、maroccana、laperrinei、cerasiformis或其组合。在一些实施方式中，橄榄提取物可包括橄榄的任何或所有部分，包括但不限于叶、种子、浆、果实、干或其组合。在一个实施方式中，橄榄提取物可源自叶。在另一实例中，提取溶剂可以是乙醇和水溶液。

[0078] 在一些实施方式中，植物或草本与提取物的比例可在约1至约10范围内。在其它实例中，原料植物或草本与提取物的比例可以为约2至约5、约4至约7或约8至约10。此外，该比例的提取物可在制剂中相对于其它提取物以任何这样的比例存在：提供的抗氧化剂活性大于等量任一提取物或提取物总和所提供的抗氧化剂活性。在一个实例中，组合物中的提取物中的至少一种可以不同于其它提取物的量存在。在另一实例中，提取物可全部以相同量存在于组合物中。

[0079] 作为实例，在一些实施方式中，各提取物可以另一提取物量的约1至约50倍的比例存在。这适用于制剂中的每种提取物，包括下文实施例中列举的那些。一方面，苹果提取物可在制剂中以葡萄、绿茶或橄榄提取物量的1至50倍的比例存在。另一方面，苹果提取物可在制剂中以葡萄、绿茶或橄榄提取物量的约1至25倍的比例存在。在进一步方面，制剂中的苹果提取物可以葡萄、绿茶或橄榄提取物量的1至10倍的比例存在。在另外的方面，苹果提取物可以葡萄、绿茶或橄榄提取物量的1至5倍的比例存在。再另一方面，苹果提取物可在制剂中以葡萄、绿茶或橄榄提取物量的1倍的比例存在。该数值范围内的任何具体数值被包括在内。事实上，苹果、葡萄、绿茶和橄榄提取物中的每一种可以其它提取物量的1至50倍与1

倍之间的任意倍数的比例存在。例如，在一些实施方式中，苹果提取物量/葡萄提取物/绿茶提取物/橄榄提取物可以分别是1-25:1-25:1-25:1-25。由此，可采用本文记载的产生协同效果的任何数值给出的具体比例，例如25:1:1:1或1:25:1:1或1:1:25:1、1:1:1:25。就wt%而言考虑时，这相当于一种成分的存在量为89.28wt%，而其它三种成分的存在量为3.57wt%。这可就制剂整体(当仅这四种成分存在时)而言考虑，或仅就制剂的协同提取物或增强剂部分而言考虑。例如，在仅包含这四种提取物的制剂中，处于1:1:1:1比例的每种提取物的相对量各为25wt%(即，100/4=25)。然而，在其中苹果、葡萄、绿茶、橄榄提取物的含量仅为总制剂的20wt%并且各自的提取物分别以1:1:1:1的比例存在的制剂中，可认为各提取物在整体制剂中的存在量各为5wt%(25×20×.001=5wt%)。

[0080] 如述，可采用产生协同效果(即，大于任一提取物提供的活性等量或大于全部提取物提供的活性的简单加和)的任何数值的一种提取物与另一种提取物的比例。例如，维持上文列出的提取物的顺序不变，在一个实施方式中，各提取物的比例可以是1-50范围内的任何数值，如1-10:1-10:1-10:1-10，其非限制地包括例如1:1:1:1、1:2:1:2、1:5:6:1、10:1:5:2、3:7:2:4和1:3:5:8。另外，这些比例和比例范围可不仅适用于苹果、葡萄、绿茶和橄榄提取物四种成分，而且还适用于下文实施例以PC 4.1、4.2、8、9或10列举的其它提取物。在一些实施方式中，提取物可全部以约1:1:1:1的重量比存在。在其它实施方式中，苹果、葡萄、绿茶和橄榄提取物可以约6:1:3:1的重量比存在。

[0081] 在一些实施方式中，组合物进一步包括主要或次要治疗剂。在一个实施方式中，主要或次要治疗剂可包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、loclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉、aminogen®、生物素、黑豆粉末、柠檬酸铜、富马酸亚铁、果糖、鹰嘴豆、阿拉伯胶、氧化镁、柠檬酸锰、中链甘油三酯、豌豆纤维、豌豆蛋白分离物、柠檬酸钾、维生素B6、核黄素、米糠、大米蛋白、柠檬酸钠、硒酸钠、盐酸硫胺素、维生素D2、维生素E、柠檬酸锌、菜豆(adzuki bean)、D-泛酸钙、番茄红素、多酚、抗坏血酸、β-葡聚糖、叶黄素、蓝莓、琉璃苣油(borage oil)、花椰菜花、胡萝卜根、蔓越莓果实、烟酸铬、氰钴胺、亚麻种子/栽培亚麻、叶酸、罗汉果(lo han)提取物、烟酰胺、石榴果实、维生素A、类胡萝卜素、维生素E、植物甾醇、木质素、辅酶Q10、谷胱甘肽和其组合。

[0082] 在另一实施方式中，主要或次要治疗剂可包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、loclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。在一个实施方式中，主要或次要治疗剂可以是佛手柑提取物。在另一实例中，主要或次要治疗剂可以是山竹提取物。山竹提取物可以是山竹果实提取物和/或山竹果皮提取物。在进一步实施方式中，主要或次要治疗剂可包括山竹果皮提取物和佛手柑提取物。在再另一实例中，主要或次要治疗剂可包括黄连素。在另一实例中，主要或次要治疗剂可包括精氨酸和甜菜或瓜氨酸和甜菜。在进一步实施方式中，主要或次要治疗剂可包括植物甾醇。在另一实施方式中，主要或次要治疗剂可包括蛋白质。该蛋白质可以是乳清蛋白、大豆蛋白、豌豆蛋白、酪蛋白钙蛋白或其组合。在再另一实例中，主要或次要治疗剂包括姜黄素。在进一步实施方式中，主要或次要治疗剂包括纤维源和/或菊粉。

[0083] 在一个实施方式中，佛手柑提取物可源自Citrus bergamia Risso。在一个实施方式中，山竹提取物可源自Garcinia mangosana。在一个实例中，山竹提取物可以是果实、果

皮或果实和果皮二者的提取物。果实提取物可源自果实的任何部分，包括但不限于浆、皮层、种子或其组合。在一个实施方式中，山竹果皮提取物仅源自果实的皮层。

[0084] 在一些实施方式中，苹果、葡萄、绿茶和橄榄提取物组合物进一步包括蓝莓提取物/浓缩物、辣椒提取物和姜黄(turmeric)提取物。在一个实例中，蓝莓提取物/浓缩物可得自Vaccinium angustifolium。在一个实例中，蓝莓浓缩物可以是不使用溶剂制成的干燥粉末。在一个实施方式中，可用约5kg、约8kg、约10kg或约12kg蓝莓获得1kg干燥粉末。在一个实施方式中，辣椒提取物可得自Capsicum annuum。在一些实施方式中，辣椒提取物可源自粉末化的干燥成熟果实。在一个实例中，姜黄提取物可得自Curcuma longa。在一些实施方式中，姜黄提取物可包括根、根茎或其组合的提取物。在另一实施方式中，姜黄提取物可源自姜黄粉末。在一个实施方式中，姜黄粉末可具有约1至约10％的类姜黄素、约3至约5％的类姜黄素、约2％至约8％的类姜黄素或约4％至约12％的类姜黄素。在一些实施方式中，葡萄提取物可包括葡萄表皮提取物和葡萄种子提取物。在一个实施方式中，除苹果、葡萄、绿茶和橄榄提取物外还包括蓝莓浓缩物、辣椒提取物和姜黄提取物的组合物可进一步包括山竹提取物。山竹提取物可包括Garcinia mangostana提取物，并且可以是果实提取物、果皮提取物或其组合。在其它实施方式中，除苹果、葡萄、绿茶和橄榄提取物外还包括蓝莓浓缩物、辣椒提取物和姜黄提取物的组合物可进一步包括佛手柑提取物。在另一实施方式中，除苹果、葡萄、绿茶和橄榄提取物外还包括蓝莓浓缩物、辣椒提取物和姜黄提取物的组合物可进一步包括山竹提取物和佛手柑提取物。

[0085] 抗氧化剂组合物可具有多种作用机制。在一个实施方式中，抗氧化剂组合物可猝灭自由基。在另一实施方式中，抗氧化剂组合物可调节过氧硝酸根形成。在一个实例中，抗氧化剂组合物调节应激信号传导酶如基质金属蛋白酶和髓过氧化物酶。

[0086] 抗氧化剂组合物可用于调节对其有需要的哺乳动物的氧化应激。氧化应激相关状况可非限制地包括代谢综合征、I型、II型和III型糖尿病、肥胖、高胆固醇水平伴随氧化型LDL胆固醇升高、各种形式的炎症——包括骨关节炎、类风湿性关节炎、内毒素血症、炎性肠道疾病、漏肠、克罗恩病、前列腺增生、下尿路症状(LUTS)、肺动脉高血压、运动能力下降、早泄、低女性性欲、充血性障碍、心力衰竭、肺高血压、各种心血管疾病、运动功能障碍、认知障碍——包括阿尔茨海默病、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛皮肤障碍——如皱纹、变色和下垂、以及DNA氧化损伤导致的癌症和通过氧和氮的反应性物种的传播刺激的与蛋白激酶活性的组织特异性调节相关的其它障碍。在一个实例中，对其有需要的哺乳动物具有增加的氧化LDL(oxLDL)胆固醇。在另一实例中，对其有需要的哺乳动物具有代谢综合征，I、II或III型糖尿病中的至少一种。在另一实例中，对其有需要的哺乳动物具有漏肠、内毒素血症或炎性肠道疾病中的至少一种。

[0087] 抗氧化剂组合物可有效用于治疗多种其中氧化应激通过病因学参与表达或进程的生理障碍。植物复合物和其各种组合可用于调控氧化应激，以治疗多种疾病相关征兆和症状并且伴随生活质量提高。所得组合物可作为饮食补充剂食用以解决与氧化应激、良性前列腺增生、肥胖、代谢综合征、糖尿病、增加运动耐力或其它炎性型病理相关的风险因素。

[0088] 在一些实施方式中，抗氧化剂活性可具有大于等量任一提取物或提取物总和所提供的抗氧化剂活性的抗氧化剂活性。协同抗氧化剂活性可大于或约1.1倍、约1.2倍、约1.3

倍、约1.5、约1.75倍、约2倍、约2.5倍或约3倍大于等量任一提取物或提取物总和所提供的抗氧化剂活性。在一个实施方式中，包括蓝莓浓缩物、辣椒、姜黄、苹果、葡萄、绿茶、橄榄和山竹果实提取物的组合物可具有等于或大于1.2倍等量任一提取物或提取物总和所提供的抗氧化剂活性。在一些实施方式中，该组合物可具有大于1.5倍等量任一提取物或提取物总和所提供的抗氧化剂活性的。在另一实施方式中，包括蓝莓浓缩物、辣椒、姜黄、苹果、葡萄、绿茶、橄榄和山竹果皮提取物的组合物可具有等于或大于1.3倍等量任一提取物或提取物总和所提供的抗氧化剂活性。在再另一实施方式中，包括蓝莓浓缩物、辣椒、姜黄、苹果、葡萄、绿茶、橄榄、山竹果实和佛手柑提取物的组合物可具有等于或大于2.5倍等量任一提取物或提取物总和所提供的抗氧化剂活性。在进一步实施方式中，包括蓝莓浓缩物、辣椒、姜黄、苹果、葡萄、绿茶、橄榄、山竹果皮和佛手柑提取物的组合物可具有等于或大于1.5倍等量任一提取物或提取物总和所提供的抗氧化剂活性。

[0089] 本文还提出蛋白激酶调节组合物，其包括苹果、葡萄、绿茶和橄榄提取物的组合，该苹果、葡萄、绿茶和橄榄提取物的用量是提供的蛋白激酶调节活性大于等量任一提取物或提取物总和所以提供的蛋白激酶调节活性的用量。组合物可进一步包括如上所述的另外的提取物、主要治疗剂和/或次要治疗剂。提取物、组分量、主要治疗剂和/或次要治疗剂可如上所述。

[0090] 蛋白激酶调节组合物可显著并且协同调节表14所示的蛋白激酶中的任意种的激酶信号传导。在一个实例中，蛋白激酶调节组合物调节Abl、ACK1、ALK、Aurora、AMPK、CaMKII、EGFR、EphA、FAK、FGFR、GSK3、IGF-1(激活)、IKK、IR、MAPK1、Met、MTOR、NEK1/2/6、PAK1/4/5/6、PDGFR、PI3K、PKC、ROCKI/II、RSK1/2/34、SRC、Syk和其组合的表达。在一个实施方式中，蛋白激酶调节活性可包括PI3激酶。在另一实施方式中，蛋白激酶调节活性可包括MET激酶。在再另一实施方式中，蛋白激酶调节活性可包括Aurora激酶、Aurora-A、Aurora-B和Aurora-C中的至少一种。

[0091] 在一些实施方式中，可调节选定目标组织中的蛋白激酶活性以治疗选自下列的疾病或状况相关的征兆和症状：前列腺癌、LUTS、肺动脉高血压、运动能力下降、充血性心力衰竭、肺高血压、各种心血管疾病、运动功能障碍、认知障碍——包括阿尔茨海默病、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、各种形式的炎症——包括骨关节炎、类风湿性关节炎、I型和II型糖尿病、代谢综合征、肥胖、内毒素血症、炎性肠道疾病、漏肠、克罗恩病、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛和与蛋白激酶活性的组织特异性调节相关的其它障碍。

[0092] 调节蛋白激酶可减少、最小化或抑制对象中氧化LDL(oxLDL)胆固醇的产生或存在，可改善代谢综合征、I型糖尿病、II型糖尿病或III型糖尿病中的至少一种，可改善漏肠、内毒素血症或炎性肠道疾病中的至少一种，可改善肥胖、炎症状况——包括骨关节炎和类风湿性关节炎、克罗恩病、前列腺增生、下尿路症状、肺动脉高血压、运动能力下降、早泄、低女性性欲、充血性障碍、心力衰竭、肺高血压、心血管疾病、运动功能障碍、认知障碍——包括阿尔茨海默病、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛和皮肤障碍——包括皮肤皱纹、皮肤变色和皮肤下垂中的至少一种，或可导致骨骼肌脂肪酸氧化和肌肉葡萄糖摄取、肝脂肪酸氧化和酮生成被刺激、胆固醇合成、脂肪生成、甘油三酯合成被抑制、脂肪细胞脂解和脂肪生成被抑制

和胰β细胞的胰岛素分泌被调节中的至少一种。

[0093] 在一个实施方式中，调节蛋白激酶可改善肥胖、炎症状况——包括骨关节炎和类风湿性关节炎、克罗恩病、前列腺增生、下尿路症状、肺动脉高血压、运动能力下降、早泄、低女性性欲、充血性障碍、心力衰竭、肺高血压、心血管疾病、运动功能障碍、认知障碍——包括阿尔茨海默病、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛和皮肤障碍——包括皮肤皱纹、皮肤变色和皮肤下垂中的至少一种。在另一实施方式中，调节蛋白激酶可改善漏肠、内毒素血症和炎性肠道疾病中的至少一种。在进一步实施方式中，调节蛋白激酶可改善代谢综合征、I型糖尿病、II、型糖尿病和III型糖尿病中的至少一种。在另一实施方式中，调节蛋白激酶会减少、最小化或抑制对象中氧化LDL(oxLDL)胆固醇的产生或存在。

[0094] 在一些实施方式中，蛋白激酶调节活性可具有大于等量任一提取物或提取物总和所提供的蛋白激酶调节活性的蛋白激酶调节活性。协同蛋白激酶调节活性可大于或约1.1倍、约1.2倍、约1.3倍、约1.5、约1.75倍、约2倍、约2.5倍或约3倍大于等量任一提取物或提取物总和所提供的蛋白激酶调节活性。

[0095] 本文进一步提出治疗性组合物，其包括主要治疗剂和苹果、葡萄、绿茶和橄榄提取物的组合，该苹果、葡萄、绿茶和橄榄提取物的用量是增加的主要治疗剂的治疗效果大于等量任一提取物所提供的治疗效果增加的用量。组合物可进一步包括如上所述的另外的提取物和/或次要治疗剂。提取物、含量和次要治疗剂可如上所述。

[0096] 主要治疗剂可以是任何抗氧化剂、代谢剂或激酶途径信号转导剂。在一个实施方式中，主要治疗剂可以是抗氧化剂。在另一实施方式中，主要治疗剂可以是代谢剂。在再另一实施方式中，主要治疗剂可以是激酶途径信号转导剂。在其它实施方式中，主要治疗剂可以是增强NO产生的用剂。在一个实施方式中，主要治疗剂可包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、Ioclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉、aminogen®、生物素、黑豆粉末、柠檬酸铜、富马酸亚铁、果糖、鹰嘴豆、阿拉伯胶、氧化镁、柠檬酸锰、中链甘油三酯、豌豆纤维、豌豆蛋白分离物、柠檬酸钾、维生素B6、核黄素、米糠、大米蛋白、柠檬酸钠、硒酸钠、盐酸硫胺素、维生素D2、维生素E、柠檬酸锌、菜豆、D-泛酸钙、番茄红素、多酚、抗坏血酸、β-葡聚糖、叶黄素、蓝莓、琉璃苣油、花椰菜花、胡萝卜根、蔓越莓果实、烟酸铬、氰钴胺、亚麻种子/栽培亚麻、叶酸、罗汉果提取物、烟酰胺、石榴果实、维生素A、类胡萝卜素、维生素E、植物甾醇、木质素、辅酶Q10、谷胱甘肽和其组合。

[0097] 在一个实例中，主要治疗剂调控代谢功能障碍。在一些实施方式中，调控代谢功能障碍的主要治疗剂包括选自下列的成员：aminogen®、生物素、黑豆粉末、柠檬酸铜、富马酸亚铁、果糖、鹰嘴豆、阿拉伯胶、菊粉、氧化镁、柠檬酸锰、中链甘油三酯、豌豆纤维、豌豆蛋白分离物、柠檬酸钾、维生素B6、核黄素、米糠、大米蛋白、柠檬酸钠、硒酸钠、盐酸硫胺素、维生素D2、维生素E、柠檬酸锌、菜豆、D-泛酸钙和其组合。在另一实施方式中，治疗剂可以是aminogen®。在另一实例中，主要治疗剂可以是菊粉。

[0098] 在另一实例中，主要治疗剂可以是抗氧化剂。在一些实施方式中，抗氧化剂包括选自下列的成员：番茄红素、多酚、抗坏血酸、β-葡聚糖、叶黄素、蓝莓、琉璃苣油、花椰菜花、胡

萝卜根、蔓越莓果实、烟酸铬、氰钴胺、亚麻种子/栽培亚麻、叶酸、罗汉果提取物、烟酰胺、石榴果实、维生素A、类胡萝卜素、维生素E、锌、硒酸钠、植物甾醇、木质素、辅酶Q10、谷胱甘肽和其组合。在一个实施方式中，抗氧化剂包括选自下列的成员：抗坏血酸、β-葡聚糖、蓝莓、琉璃苣油、花椰菜花、胡萝卜根、蔓越莓果实、烟酸铬、氰钴胺、亚麻种子/栽培亚麻、叶酸、罗汉果提取物、烟酰胺、石榴果实、维生素A、植物甾醇和其组合。

[0099] 在一个实例中，主要治疗剂可包括鱼油。在一个实例中，鱼油可降低TG、LDLc、oxLDLc，升高HDLc，或其任意组合。提取物与鱼油组合时可增强TG，降低鱼油的特性，提高NO形成，和降低血压。在一个实例中，组合物可包括1-5克之间的鱼油。

[0100] 在另一实例中，主要治疗剂可以是黄连素。在一个实例中，黄连素可影响身体中的内毒素血症和炎症。在一个实例中，这种制剂可抑制或下调MPO——已知进而抑制或去除eNOS和NO的产生的酶。内毒素血症和炎症是代谢功能障碍/CVD和肥胖的引发因素。包括黄连素作为主要治疗剂的治疗性组合物可用于对抗代谢功能障碍。

[0101] 上述氧化应激调节组合物、蛋白激酶调节组合物和/或治疗性组合物可以任何便利的形式提供。这些组合物可作为胶囊或片剂形式的饮食补充剂提供。其可被配制到食品或饮品中，并且例如作为小吃、谷物、饮品、口香糖或以任何其它容易摄入的形式提供。其也可作为用于局部施用的乳膏或洗液提供。在一个实例中，组合物可以是下列形式的口服组合物：离散单位形式，如胶囊、囊剂、片剂、软凝胶或锭剂，每种均包含预定量的活性成分；粉末或颗粒形式；溶液或悬浮液形式，在含水液体或非含水液体中，如乙醇或甘油；或水包油乳液或油包水乳液形式。这种油可以是可食用油，如例如，棉籽油、芝麻油、椰子油、葵花籽油或花生油。适于含水悬浮液的分散剂或悬浮剂包括合成或天然胶质如黄蓍胶、海藻酸胶、阿拉伯胶、葡聚糖(dextran)、羧甲基纤维素钠、明胶、甲基纤维素和聚乙烯吡咯烷酮。在另一实施方式中，口服的剂型可包括胶囊、片剂、粉末、饮料、糖浆、悬浮液或食品。

[0102] 另外，组合物可被配制为贮存制剂。这种长效组合物可通过植入(例如，皮下、腹内或肌内)或通过肌内注射给予。因此，例如，活性成分可与适当的聚合物或疏水材料(例如，作为在药学上可接受的油中的乳液)或离子交换树脂一起配制。

[0103] 在一些实施方式中，上述氧化应激调节组合物、蛋白激酶调节组合物和/或治疗性组合物可进一步包括药学上可接受的载体。在一些实施方式中，制剂可包括药学上可接受的赋形剂。示例性药学上可接受的赋形剂可选自包衣、等渗剂和吸收延迟剂、粘结剂、粘合剂、润滑剂、崩解剂、着色剂、调味剂、甜味剂、吸收剂、洗涤剂和乳化剂。

[0104] 当制剂包乳化剂时，可添加乳化剂以提高终产物的稳定性。示例性乳化剂包括但不限于，卵磷脂(例如，来自鸡蛋或大豆)或单-和二-甘油酯。其它乳化剂对于技术人员而言显而易见，并且适当乳化剂(一种或多种)的选择将部分取决于制剂和终产物。

[0105] 制剂可进一步包括调味剂、着色剂、香料、坚果、防腐剂、抗氧化剂、维生素、矿物质、蛋白质、脂肪和/或碳水化合物。其它成分的含量可基于具体设计、预期剂量和给予方法而相异。其它成分的总量还可部分取决于对象的状况和体重。

[0106] 调味剂、着色剂、香料、坚果和类似物可被掺入产物。调味剂可以是调味提取物、挥发油、巧克力调味剂(例如、无咖啡因可可或巧克力、巧克力替代品如角豆胶)、花生酱调味剂、饼干碎、脆米、香草或任何市售调味剂的形式。调味剂可用混合生育酚来保护。可用调味剂的实例包括但不限于纯茴香提取物、仿(imitation)香蕉提取物、仿樱桃提取物、巧克力

提取物、纯柠檬提取物、纯橙提取物、纯薄荷提取物、仿菠萝提取物、仿朗姆酒提取物、仿草莓提取物或纯香草提取物；或挥发油，如蜂花油、月桂油、佛手柑油、雪松油、樱桃油、核桃油、肉桂油、丁香油或薄荷油；花生酱、巧克力调味剂、香草饼干碎、奶油糖果(butterscotch)或太妃糖。在一个实施方式中，制剂可包含浆果或其它果实调味剂。食品组合物可进一步被包覆，例如用酸乳酪包衣。

[0107] 防腐剂或稳定剂可被加入制剂以延长产物保质期。示例性防腐剂包括山梨酸钾、山梨酸钠、苯甲酸钾、苯甲酸钠或EDTA二钠钙。

[0108] 制剂还可包括天然或人工甜味剂。在一个实施方式中，可能的甜味剂可包括葡萄糖、蔗糖、果糖、糖类、环己氨磺酸盐、aspartamine、蔗糖素(sucralose)、阿斯帕坦、阿糖精、山梨醇或其组合。

[0109] 制剂可进一步包括维生素、矿物质和其它营养物的药学上可接受形式。制剂中选择包含的营养物可根据具体设计、预期剂量、给予方法和对象状况而相异。本领域技术人员知道可掺入制剂的维生素、矿物质和其它营养物以及如何掺入这些。

[0110] 制剂中的组分可作为盐被包括。具体地，考虑组分的药学上可接受的盐。“药学上可接受的盐”是化合物和与该化合物一起形成盐(如，例如，镁盐，在本文中示为“Mg”或“Mag”)的酸或碱的组合。在治疗条件下对象可耐受该药学上可接受的盐。总体上，化合物的药学上可接受的盐具有为1或更高的治疗指数(最低毒性剂量与最低治疗有效剂量的比例)。本领域技术人员知道最低治疗有效剂量将根据对象不同和适应症不同而相异，并且因此相应地调节制剂。

[0111] 另外，可按照本领域用于持续释放给定化合物的标准方法添加聚合物。

[0112] 用于治疗疾病或状况的任何组合物将采用药物级化合物，并且该组合物将进一步包括药学上可接受的载体。进一步考虑，本发明的这些组合物可以适于给予途径以及待治疗疾病和患者的单位剂型制备。组合物可便利地以通过制药领域公知的任何方法制备的剂量单位形式呈现。所有方法均包括使活性成分与构成一种或多种辅助成分的介质结合的步骤。总体上，组合物通过如下制备：使活性成分与液体介质或细碎固体介质或两者均匀并且紧密地结合，然后——如需——使产物成形为期望的组合物。

[0113] 本文还提出调控对象的氧化应激的方法。方法可包括给予对象苹果、葡萄、绿茶和橄榄提取物的治疗有效组合，其用量是提供的组合抗氧化剂活性大于等量任一提取物或提取物总和所提供的抗氧化剂活性的用量。在一个实例中，方法可进一步包括给予对象至少一种次要治疗剂。次要治疗剂可在单一制剂中被共同给予，被分开给予，或与苹果、葡萄、绿茶和橄榄提取物给予进行顺序给予。方法可包括给予任何如上所述的另外的提取物和/或次要治疗剂。提取物、用量和次要治疗剂可如上所述。

[0114] 方法可调控应激相关的病理和代谢障碍，包括选自下列的至少一个成员：代谢综合征、1型糖尿病、2型糖尿病、肥胖、高胆固醇水平、氧化LDL胆固醇、炎症、骨关节炎、类风湿性关节炎、内毒素血症、炎性肠道疾病、漏肠、克罗恩病、前列腺增生、下尿路症状、肺动脉高血压、运动能力下降、早泄、低女性性欲、充血性障碍、心力衰竭、肺高血压、各种心血管疾病、运动功能障碍、认知障碍——包括阿尔茨海默病、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛皮肤障碍、皱纹、皮肤变色、皮肤下垂、DNA氧化损伤引起的癌症和其组合。

[0115] 本文进一步提出调控对象的疾病相关蛋白激酶活性的方法，包括给予对象苹果提取物、葡萄提取物、绿茶提取物和橄榄提取物的治疗有效组合，其用量是提供的组合激酶调控活性大于等量任一提取物或提取物总和所提供的激酶调控活性的用量。在一个实例中，方法可进一步包括给予对象至少一种次要治疗剂。次要治疗剂可在单一制剂中被共同给予，被分开给予或与苹果、葡萄、绿茶和橄榄提取物给予进行顺序给予。方法可包括给予任何如上所述的另外的提取物和/或次要治疗剂。提取物、用量和次要治疗剂可如上所述。

[0116] 方法可调节表14所示的任何蛋白激酶的蛋白激酶活性。在一个实例中，方法调节下列的表达：Abl、ACK1、ALK、Aurora、AMPK、CaMKII、EGFR、EphA、FAK、FGFR、GSK3、IGF-1(激活)、IKK、IR、MAPK1、Met、MTOR、NEK1/2/6、PAK1/4/5/6、PDGFR、PI3K、PKC、ROCKI/II、RSK1/2/34、SRC、Syk和其组合。在一个实施方式中，蛋白激酶调节活性可包括PI3激酶。在另一实施方式中，蛋白激酶调节活性可包括MET激酶。在再另一实施方式中，蛋白激酶调节活性可包括Aurora激酶、Aurora-A、Aurora-B和Aurora-C中的至少一种。

[0117] 调控氧化应激的方法和调控疾病相关蛋白激酶活性的方法的给予步骤可被给予需要这种活性的对象。方法中的制剂可以口服、透皮、跨粘膜、直肠、眼用(包括玻璃体内或眼房内)、鼻用、通过吸入鼻用、局部(包括颊部和舌下)、阴道、胃肠外的(包括皮下、肌内、静脉内、皮内和气管内)、通过植入或肌内的形式给予。在一个示例性实施方式中，方法口服给予制剂。

[0118] 给予量可符合各成分的建议日用量。每单位剂量各成分的实际量将取决于每日给予对其有需要的个体的单位的数量。这与产物设计有关，并且完全在营养补充剂配方技术范围内。

[0119] 另外提出增强主要治疗剂的治疗效果的方法。在一个实例中，方法可包括组合主要治疗剂与苹果、葡萄、绿茶和橄榄提取物，该苹果、葡萄、绿茶和橄榄提取物的用量是增加的主要治疗剂的治疗效果大于等量任一提取物所提供的增加的用量。在一个实例中，方法可进一步包括向对其有需要的对象给予制剂。在一个实施方式中，方法还包括给予次要治疗剂，该次要治疗剂可在单一制剂中被共同给予，被分开给予，或与苹果、葡萄、绿茶和橄榄提取物给予进行顺序给予。方法可包括给予如上所述的另外的提取物和/或次要治疗剂中的任意种。提取物、用量、主要治疗剂和次要治疗剂可如上所述。

[0120] 本文进一步提出制备活性增强的氧化应激调控组合物的方法，制备蛋白激酶调节组合物的方法和制备治疗性——包括治疗剂特异性——组合物的方法。制备氧化应激调节组合物和蛋白激酶调节组合物的方法可包括组合苹果、葡萄、绿茶和橄榄提取物，该苹果、葡萄、绿茶和橄榄提取物的用量是提供的抗氧化剂活性大于等量任一提取物或提取物总和所提供的抗氧化剂活性的用量。制备治疗性组合物的方法进一步包括提供主要治疗剂和组合主要治疗剂与苹果、葡萄、绿茶和橄榄提取物。

[0121] 在任何或全部上述方法中，提取物可由原料成分产生。当由原料成分制备时，苹果、葡萄、绿茶和橄榄提取物可利用选自水、乙醇、乙酸乙酯和其组合的提取溶剂提取。在实例中，提取过程可包括形成原料的浓缩浆，提取原料，纯化原料，洗脱原料，收集洗脱的材料，浓缩材料，和将材料喷雾干燥。在另一实例中，提取过程可进一步包括滤材料。

[0122] 在一个实例中，方法可进一步包括组合至少一种次要治疗剂与苹果、葡萄、绿茶和橄榄提取物。提取物、用量、主要治疗剂和次要治疗剂可如上所述。

[0123] 在一些实施方式中，上述氧化应激调节组合物、蛋白激酶调节组合物和/或治疗性组合物的组成、应用方法和制备方法可包括以药物包装品或试剂盒形式配制组合物。药物包装品或试剂盒可包括填充有本发明的组合物的成分中的一种或多种的一个或多个容器(例如，粉末和胶囊形式的营养补充剂)。与这种容器(一个或多个)任选地结合的可以是管控药物产品的生产、使用或销售的政府机构所规定的形式的告知(notice)，该告知反映出该机构对用于人类给药的生产、使用或销售的批准。包装物或试剂盒可标记有关于给药方式、给药顺序(例如，分开、相继或同时)或类似事项的信息。包装物或试剂盒还可包括提醒患者实施治疗的装置。包装物或试剂盒可以是一个单位剂量的组合治疗，或其可以是多个单位剂量。具体地，各剂可——分开、以任何组合混合在一起——存在于制剂或片剂中。

[0124] 实施方式

[0125] 在一个实施方式中，提供了氧化应激调节组合物，其包括苹果、葡萄、绿茶和橄榄提取物的组合，该苹果、葡萄、绿茶和橄榄提取物的用量是提供的抗氧化剂活性大于等量任一提取物或提取物总和所提供的抗氧化剂活性的用量。

[0126] 在氧化应激调节组合物的一个实施方式中，苹果提取物包括物种Malus pumila的提取物。

[0127] 在氧化应激调节组合物的一个实施方式中，组合物中的苹果提取物包括Malus pumila的表皮和果实。

[0128] 在氧化应激调节组合物的一个实施方式中，葡萄提取物包括物种Vitis vinifera的提取物。

[0129] 在氧化应激调节组合物的一个实施方式中，组合物中的葡萄提取物包括Vitis vinifera的种子。

[0130] 在氧化应激调节组合物的一个实施方式中，组合物中的葡萄提取物包括在干重基础上约75wt％至约95wt％的酚类化合物。

[0131] 在氧化应激调节组合物的一个实施方式中，绿茶提取物包括物种Camellia sinensi的叶的提取物。

[0132] 在氧化应激调节组合物的一个实施方式中，橄榄提取物包括的亚种Olea europea europaea提取物。

[0133] 在氧化应激调节组合物的一个实施方式中，组合物中的橄榄提取物包括Olea europea europaea的叶。

[0134] 在氧化应激调节组合物的一个实施方式中，组合物中提取物中的至少一种的存在量不同于其它提取物中的至少一种的量。

[0135] 在氧化应激调节组合物的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物在组合物中以约1:1:1:1的重量比存在。

[0136] 在氧化应激调节组合物的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物在组合物中以约6:1:3:1的重量比存在。

[0137] 在氧化应激调节组合物的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物包括叶、表皮、皮层、浆、汁、种子或其组合的提取物。

[0138] 在一个实施方式中，氧化应激调节组合物进一步包括至少一种主要或次要治疗剂。

[0139] 在氧化应激调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、loclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。

[0140] 在氧化应激调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括佛手柑。

[0141] 在氧化应激调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括山竹。

[0142] 在氧化应激调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括黄连素。

[0143] 在氧化应激调节组合物的一个实施方式中，包括苹果、葡萄、绿茶和橄榄提取物的组合的组合物进一步包括至少一种次要治疗剂。

[0144] 在氧化应激调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括精氨酸和甜菜。

[0145] 在氧化应激调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括瓜氨酸和甜菜。

[0146] 在氧化应激调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括植物甾醇。

[0147] 在氧化应激调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括蛋白质。

[0148] 在氧化应激调节组合物的一个实施方式中，该蛋白质包括选自下列的至少一个成员：乳清蛋白、大豆蛋白、豌豆蛋白、酪蛋白钙蛋白和其组合。

[0149] 在氧化应激调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括姜黄素。

[0150] 在氧化应激调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括纤维源和菊粉。

[0151] 在氧化应激调节组合物的一个实施方式中，组合物进一步包括药学上可接受的载体。

[0152] 在氧化应激调节组合物的一个实施方式中，组合物是口服剂制剂。

[0153] 在氧化应激调节组合物的一个实施方式中，口服的剂型包括胶囊、片剂、粉末、饮料、糖浆、悬浮液或食品。

[0154] 在氧化应激调节组合物的一个实施方式中，抗氧化剂活性调节应激相关的病理和代谢障碍。

[0155] 在氧化应激调节组合物的一个实施方式中，氧化应激调节组合物的组合物抗氧化剂活性调节应激相关的病理和代谢障碍。

[0156] 在氧化应激调节组合物的一个实施方式中，应激相关的病理和代谢障碍包括选自下列的至少一个成员：代谢综合征、1型糖尿病、2型糖尿病、肥胖、高胆固醇水平、氧化LDL胆固醇、炎症、骨关节炎、类风湿性关节炎、内毒素血症、炎性肠道疾病、漏肠、克罗恩病、前列腺增生、下尿路症状、肺动脉高血压、运动能力下降、早泄、低女性性欲、充血性障碍、心力衰竭、肺高血压、各种心血管疾病、运动功能障碍、认知障碍——包括阿尔茨海默病、雷诺现

象、特发性高血压、中风、哮喘、多发性硬化、血管炎、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛皮肤障碍、皱纹、皮肤变色、皮肤下垂、DNA氧化损伤引起的癌症和其组合。

[0157] 在氧化应激调节组合物的一个实施方式中，抗氧化剂活性调节氧化LDL。

[0158] 在氧化应激调节组合物的一个实施方式中，抗氧化剂活性调节代谢综合征、I型糖尿病、II型糖尿病或III型糖尿病中的至少一种。

[0159] 在氧化应激调节组合物的一个实施方式中，抗氧化剂活性调节漏肠、内毒素血症或炎性肠道疾病中的至少一种。

[0160] 在氧化应激调节组合物的一个实施方式中，氧化应激调节组合物进一步包括蓝莓浓缩物、辣椒提取物和姜黄提取物。

[0161] 在进一步包括蓝莓浓缩物、辣椒提取物和姜黄提取物的氧化应激调节组合物的一个实施方式中，蓝莓浓缩物包括Vaccinium angustifolium，辣椒提取物包括Capsicum annuum，并且姜黄提取物包括Curcuma longa。

[0162] 在氧化应激调节组合物的一个实施方式中，葡萄提取物包括来自Vitis vinifera的葡萄表皮和葡萄种子，氧化应激调节组合物进一步包括蓝莓浓缩物、辣椒提取物和姜黄提取物。

[0163] 在氧化应激调节组合物的一个实施方式中，氧化应激调节组合物进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物和山竹果实提取物.

[0164] 在上述氧化应激调节组合物的实施方式中，组合物包括大于1.5倍等量任一提取物或浓缩物或提取物和浓缩物总和的抗氧化剂活性。

[0165] 在氧化应激调节组合物的一个实施方式中，氧化应激调节组合物进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物和佛手柑提取物.

[0166] 在上述氧化应激调节组合物的实施方式中，组合物包括大于1.5倍等量任一提取物或浓缩物或提取物和浓缩物总和的抗氧化剂活性。

[0167] 在氧化应激调节组合物的一个实施方式中，佛手柑提取物包括Citrus bergamia Risso。

[0168] 在氧化应激调节组合物的一个实施方式中，氧化应激调节组合物进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物和山竹果皮提取物.

[0169] 在上述氧化应激调节组合物的实施方式中，组合物包括大于1.25倍等量任一提取物或浓缩物或提取物和浓缩物总和的抗氧化剂活性。

[0170] 在氧化应激调节组合物的一个实施方式中，氧化应激调节组合物进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物、山竹果皮提取物和佛手柑提取物.

[0171] 在一个实施方式中，氧化应激调节组合物进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物、山竹果皮提取物和佛手柑提取物，调节氧化LDL。

[0172] 在一个实施方式中，蛋白激酶调节组合物包括苹果、葡萄、绿茶和橄榄提取物的组合，该苹果、葡萄、绿茶和橄榄提取物的用量是提供的蛋白激酶调节活性大于等量任一提取物或提取物总和所提供的蛋白激酶调节活性的用量。

[0173] 在蛋白激酶调节组合物的一个实施方式中，苹果提取物包括物种Malus pumila的提取物。

[0174] 在蛋白激酶调节组合物的一个实施方式中，苹果提取物包括Malus pumila的表皮

和果实。

[0175] 在蛋白激酶调节组合物的一个实施方式中，葡萄提取物包括物种Vitis vinifera的提取物。

[0176] 在蛋白激酶调节组合物的一个实施方式中，葡萄提取物包括Vitis vinifera的种子。

[0177] 在蛋白激酶调节组合物的一个实施方式中，葡萄提取物包括在干重基础上约75wt％至约95wt％的酚类化合物。

[0178] 在蛋白激酶调节组合物的一个实施方式中，绿茶提取物包括物种Camellia sinensis的叶的提取物。

[0179] 在蛋白激酶调节组合物的一个实施方式中，橄榄提取物包括亚种Olea europea europaea的提取物。

[0180] 在蛋白激酶调节组合物的一个实施方式中，橄榄提取物包括Olea europea europaea的叶。

[0181] 在蛋白激酶调节组合物的一个实施方式中，组合物中提取物中的至少一种的存在量不同于其它提取物中的至少一种的量。

[0182] 在蛋白激酶调节组合物的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约1:1:1:1的重量比存在。

[0183] 在蛋白激酶调节组合物的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约6:1:3:1的重量比存在。

[0184] 在蛋白激酶调节组合物的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物包括叶、表皮、皮层、浆、汁、种子或其组合。

[0185] 在一个实施方式中，蛋白激酶调节组合物进一步包括至少一种主要或次要治疗剂。

[0186] 在蛋白激酶调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、甜菜、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。

[0187] 在蛋白激酶调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括佛手柑。

[0188] 在蛋白激酶调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括山竹。

[0189] 在蛋白激酶调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括黄连素。

[0190] 在蛋白激酶调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括精氨酸和甜菜。

[0191] 在蛋白激酶调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括瓜氨酸和甜菜。

[0192] 在蛋白激酶调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括植物甾醇。

[0193] 在蛋白激酶调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括蛋白

质。

[0194] 在蛋白激酶调节组合物的一个实施方式中，该蛋白质包括选自下列的至少一个成员：乳清蛋白、大豆蛋白、豌豆蛋白、酪蛋白钙蛋白和其组合。

[0195] 在蛋白激酶调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括姜黄素。

[0196] 在蛋白激酶调节组合物的一个实施方式中，至少一种主要或次要治疗剂包括纤维源和菊粉。

[0197] 在一个实施方式中，蛋白激酶调节组合物进一步包括药学上可接受的载体。

[0198] 在一个实施方式中，蛋白激酶调节组合物是口服剂制剂。

[0199] 在蛋白激酶调节组合物的一个实施方式中，口服的剂型包括胶囊、片剂、粉末、饮料、糖浆、悬浮液或食品。

[0200] 在蛋白激酶调节组合物的一个实施方式中，蛋白激酶调节活性是选自下列的成员：Abl、ACK1、ALK、Aurora、AMPK、CaMKII、EGFR、EphA、FAK、FGFR、GSK3、IGF-1(激活)、IKK、IR、MAPK1、Met、MTOR、NEK1/2/6、PAK1/4/5/6、PDGFR、PI3K、PKC、ROCKI/II、RSK1/2/34、SRC、Syk和其组合。

[0201] 在蛋白激酶调节组合物的一个实施方式中，蛋白激酶的调节改善肥胖、炎症状况——包括骨关节炎和类风湿性关节炎、克罗恩病、前列腺增生、下尿路症状、肺动脉高血压、运动能力下降、早泄、低女性性欲、充血性障碍、心力衰竭、肺高血压、心血管疾病、运动功能障碍、认知障碍——包括阿尔茨海默病、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛和皮肤障碍——包括皮肤皱纹、皮肤变色和皮肤下垂中的至少一种。

[0202] 在蛋白激酶调节组合物的一个实施方式中，蛋白激酶的调节改善漏肠、内毒素血症和炎性肠道疾病中的至少一种。

[0203] 在蛋白激酶调节组合物的一个实施方式中，蛋白激酶的调节导致改善代谢综合征、I型糖尿病、II型糖尿病和III型糖尿病中的至少一种。

[0204] 在蛋白激酶调节组合物的一个实施方式中，蛋白激酶的调节减少、最小化或抑制对象中氧化LDL(oxLDL)胆固醇的产生或存在。

[0205] 在一个实施方式中，蛋白激酶调节组合物，进一步包括蓝莓浓缩物、辣椒提取物和姜黄提取物。

[0206] 在一个实施方式中，蛋白激酶调节组合物进一步包括蓝莓浓缩物、辣椒提取物和姜黄提取物，蓝莓浓缩物包括Vaccinium angustifolium，辣椒提取物包括Capsicum annuum，并且姜黄提取物包括Curcuma longa。

[0207] 在一个实施方式中，蛋白激酶调节组合物进一步包括蓝莓浓缩物、辣椒提取物和姜黄提取物，葡萄提取物包括来自Vitis vinifera的葡萄表皮和葡萄种子。

[0208] 在一个实施方式中，蛋白激酶调节组合物进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物和山竹果实提取物。

[0209] 在一个实施方式中，蛋白激酶调节组合物进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物和佛手柑提取物Citrus bergamia Risso。

[0210] 在一个实施方式中，蛋白激酶调节组合物进一步包括蓝莓浓缩物、辣椒提取物、姜

黄提取物和山竹果皮提取物。

[0211] 在一个实施方式中，蛋白激酶调节组合物进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物、山竹果皮提取物和佛手柑提取物Citrus bergamia Risso。

[0212] 在一个实施方式中，进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物、山竹果皮提取物和佛手柑提取物Citrus bergamia Risso的蛋白激酶调节组合物具有蛋白激酶调节活性，包括选自下列的成员：Abl、ACK1、ALK、Aurora、AMPK、CaMKII、EGFR、EphA、FAK、FGFR、GSK3、IGF-1(激活)、IKK、IR、MAPK1、Met、MTOR、NEK1/2/6、PAK1/4/5/6、PDGFR、PI3K、PKC、ROCKI/II、RSK1/2/34、SRC、Syk和其组合。

[0213] 在一个实施方式中，进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物、山竹果皮提取物和佛手柑提取物Citrus bergamia Risso的蛋白激酶调节组合物具有蛋白激酶调节活性，包括调节PI3激酶。

[0214] 在一个实施方式中，进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物、山竹果皮提取物和佛手柑提取物Citrus bergamia Risso的蛋白激酶调节组合物具有蛋白激酶调节活性，包括调节MET激酶。

[0215] 在一个实施方式中，进一步包括蓝莓浓缩物、辣椒提取物、姜黄提取物、山竹果皮提取物和佛手柑提取物Citrus bergamia Risso的蛋白激酶调节组合物具有蛋白激酶调节活性，包括调节Aurora激酶、Aurora-A、Aurora-B和Aurora-C中的至少一种。

[0216] 在一个实施方式中，本文提出治疗性组合物，其包括主要治疗剂和苹果、葡萄、绿茶和橄榄提取物的组合，该苹果、葡萄、绿茶和橄榄提取物的用量是增加的主要治疗剂的治疗效果大于等量任一提取物所提供的治疗效果增加的用量。

[0217] 在治疗性组合物的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物在组合物中以约1:1:1:1的重量比存在。

[0218] 在治疗性组合物的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物在组合物中以约6:1:3:1的重量比存在。

[0219] 在一个实施方式中，治疗性组合物进一步包括至少一种次要治疗剂。

[0220] 在治疗性组合物的一个实施方式中，至少一种次要治疗剂包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、Ioclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。

[0221] 在治疗性组合物的一个实施方式中，至少一种次要治疗剂包括山竹，并且山竹包括山竹果实提取物、山竹果皮提取物或其组合。

[0222] 在治疗性组合物的一个实施方式中，至少一种次要治疗剂包括佛手柑。

[0223] 在治疗性组合物的一个实施方式中，组合物进一步包括精氨酸和瓜氨酸。

[0224] 在治疗性组合物的一个实施方式中，治疗剂调控代谢功能障碍。

[0225] 在治疗性组合物的一个实施方式中，治疗剂包括选自下列的成员：aminogen®、生物素、黑豆粉末、柠檬酸铜、富马酸亚铁、果糖、鹰嘴豆、阿拉伯胶、菊粉、氧化镁、柠檬酸锰、中链甘油三酯、豌豆纤维、豌豆蛋白分离物、柠檬酸钾、维生素B6、核黄素、米糠、大米蛋白、柠檬酸钠、硒酸钠、盐酸硫胺素、维生素D2、维生素E、柠檬酸锌、菜豆、D-泛酸钙和其组合。

[0226] 在治疗性组合物的一个实施方式中，治疗剂包括抗氧化剂。

[0227] 在治疗性组合物的一个实施方式中，治疗剂包括选自下列的抗氧化剂：抗坏血酸、

β-葡聚糖、蓝莓、琉璃苣油、花椰菜花、胡萝卜根、蔓越莓果实、烟酸铬、氰钴胺、亚麻种子/栽培亚麻、叶酸、罗汉果提取物、烟酰胺、石榴果实、维生素A、植物甾醇和其组合。

[0228] 在一个实施方式中，本文提出调控对象的氧化应激的方法，包括给予对象苹果、葡萄、绿茶和橄榄提取物的治疗有效组合，该苹果、葡萄、绿茶和橄榄提取物的用量是提供的组合抗氧化剂活性大于等量任一提取物或提取物总和所提供的抗氧化剂活性的用量。

[0229] 在调控对象的氧化应激的方法的一个实施方式中，苹果提取物包括Malus pumila的表皮和果实的提取物；葡萄提取物包括Vitis vinifera的种子的提取物；绿茶提取物包括Camellia sinensis的叶的提取物；并且橄榄提取物包括Olea europea europaea的叶。

[0230] 在调控对象的氧化应激的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约1:1:1:1的重量比存在。

[0231] 在调控对象的氧化应激的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约6:1:3:1的重量比存在。

[0232] 在调控对象的氧化应激的方法的一个实施方式中，方法进一步包括给予对象至少一种主要或次要治疗剂。

[0233] 在调控对象的氧化应激的方法的一个实施方式中，至少一种主要或次要治疗剂与苹果、葡萄、绿茶和橄榄提取物的治疗有效组合被共同给予对象。

[0234] 在调控对象的氧化应激的方法的一个实施方式中，至少一种主要或次要治疗剂包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、loclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。

[0235] 在调控对象的氧化应激的方法的一个实施方式中，抗氧化剂活性调节应激相关的病理和代谢障碍。

[0236] 在调控对象的氧化应激的方法的一个实施方式中，抗氧化剂活性调节应激相关的病理和代谢障碍，并且应激相关的病理和代谢障碍包括选自下列的至少一个成员：代谢综合征、1型糖尿病、2型糖尿病、肥胖、高胆固醇水平、氧化LDL胆固醇、炎症、骨关节炎、类风湿性关节炎、内毒素血症、炎性肠道疾病、漏肠、克罗恩病、前列腺增生、下尿路症状、肺动脉高血压、运动能力下降、早泄、低女性性欲、充血性障碍、心力衰竭、肺高血压、各种心血管疾病、运动功能障碍、认知障碍——包括阿尔茨海默病、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛皮肤障碍、皱纹、皮肤变色、皮肤下垂、DNA氧化损伤引起的癌症和其组合。

[0237] 在调控对象的氧化应激的方法的一个实施方式中，方法进一步包括给予对象蓝莓浓缩物、辣椒提取物和姜黄提取物。

[0238] 在调控对象的氧化应激的方法的一个实施方式中，方法进一步包括给予对象山竹果实提取物。

[0239] 在调控对象的氧化应激的方法的一个实施方式中，方法进一步包括给予对象佛手柑提取物。

[0240] 在调控对象的氧化应激的方法的一个实施方式中，方法进一步包括给予对象山竹果皮提取物。

[0241] 在调控对象的氧化应激的方法的一个实施方式中，方法进一步包括给予对象山竹果皮提取物和佛手柑提取物。

[0242] 在一个实施方式中，本文提出调控对象的疾病相关的蛋白激酶活性的方法，包括：给予对象苹果提取物、葡萄提取物、绿茶提取物和橄榄提取物的治疗有效组合，该苹果提取物、葡萄提取物、绿茶提取物和橄榄提取物的用量是提供的组合激酶调控活性大于等量任一提取物或提取物总和所提供的激酶调控活性的用量。

[0243] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，苹果提取物包括Malus pumila的表皮和果实的提取物；葡萄提取物包括Vitis vinifera的种子的提取物；绿茶提取物包括Camellia sinensis的叶的提取物；和橄榄提取物包括Olea europea europaea的叶。

[0244] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约1:1:1:1的重量比存在。

[0245] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约6:1:3:1的重量比存在。

[0246] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，方法进一步包括给予对象至少一种主要或次要治疗剂。

[0247] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，至少一种主要或次要治疗剂与苹果、葡萄、绿茶和橄榄提取物的治疗有效组合被共同给予对象。

[0248] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，至少一种主要或次要治疗剂包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、loclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。

[0249] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，蛋白激酶调节活性是选自下列的成员：Abl、ACK1、ALK、Aurora、AMPK、CaMKII、EGFR、EphA、FAK、FGFR、GSK3、IGF-1(激活)、IKK、IR、MAPK1、Met、MTOR、NEK1/2/6、PAK1/4/5/6、PDGFR、PI3K、PKC、ROCK I/II、RSK1/2/34、SRC、Syk和其组合。

[0250] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，方法进一步包括给予对象蓝莓浓缩物、辣椒提取物和姜黄提取物。

[0251] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，方法进一步包括给予对象山竹果实提取物。

[0252] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，方法进一步包括给予对象佛手柑提取物。

[0253] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，方法进一步包括给予对象山竹果皮提取物。

[0254] 在调控对象的疾病相关的蛋白激酶活性的方法的一个实施方式中，方法进一步包括给予对象山竹果皮提取物和佛手柑提取物。

[0255] 在一个实施方式中，本文提出增强主要治疗剂提供的治疗效果的方法，包括：组合主要治疗剂与苹果、葡萄、绿茶和橄榄提取物，该苹果、葡萄、绿茶和橄榄提取物的用量是增加的主要治疗剂的治疗效果大于等量任一提取物所提供的增加的用量。

[0256] 在增强主要治疗剂提供的治疗效果的方法的一个实施方式中，苹果提取物包括Malus pumila的表皮和果实的提取物；葡萄提取物包括Vitis vinifera的种子的提取物；绿茶提取物包括Camellia sinensis的叶的提取物；和橄榄提取物包括Olea europea

europaea的叶。

[0257] 在增强主要治疗剂提供的治疗效果的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约1:1:1:1的重量比组合。

[0258] 在增强主要治疗剂提供的治疗效果的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约6:1:3:1的重量比组合。

[0259] 在增强主要治疗剂提供的治疗效果的方法的一个实施方式中，方法进一步包括组合主要治疗剂和苹果、葡萄、绿茶和橄榄提取物与至少一种次要治疗剂。

[0260] 在增强主要治疗剂提供的治疗效果的方法的一个实施方式中，其中至少一种次要治疗剂包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、loclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。

[0261] 在增强主要治疗剂提供的治疗效果的方法的一个实施方式中，方法进一步包括组合主要治疗剂和苹果、葡萄、绿茶和橄榄提取物与蓝莓浓缩物、辣椒提取物和姜黄提取物。

[0262] 在增强主要治疗剂提供的治疗效果的方法的一个实施方式中，方法进一步包括组合主要治疗剂、蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果实提取物。

[0263] 在增强主要治疗剂提供的治疗效果的方法的一个实施方式中，方法进一步包括组合主要治疗剂、蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与佛手柑提取物。

[0264] 在增强主要治疗剂提供的治疗效果的方法的一个实施方式中，方法进一步包括组合主要治疗剂、蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果皮提取物。

[0265] 在增强主要治疗剂提供的治疗效果的方法的一个实施方式中，方法进一步包括组合主要治疗剂、蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果皮提取物和佛手柑提取物。

[0266] 在一个实施方式中，本文提出制备活性增强的用于调控对象的氧化应激的组合物的方法，包括：组合苹果、葡萄、绿茶和橄榄提取物，该苹果、葡萄、绿茶和橄榄提取物的用量是提供的抗氧化剂活性大于等量任一提取物或提取物总和所提供的抗氧化剂活性的用量。

[0267] 在制备活性增强的用于调控对象的氧化应激的组合物的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物利用选自水、乙醇、乙酸乙酯和其组合的提取溶剂来提取。

[0268] 在制备活性增强的用于调控对象的氧化应激的组合物的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约1:1:1:1的重量比存在组合。

[0269] 在制备活性增强的用于调控对象的氧化应激的组合物的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约6:1:3:1的重量比组合。

[0270] 在制备活性增强的用于调控对象的氧化应激的组合物的方法的一个实施方式中，方法进一步包括组合苹果、葡萄、绿茶和橄榄提取物与至少一种主要或次要治疗剂。

[0271] 在制备活性增强的用于调控对象的氧化应激的组合物的方法的一个实施方式中，至少一种主要或次要治疗剂包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、loclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。

[0272] 在制备活性增强的用于调控对象的氧化应激的组合物的方法的一个实施方式中，方法进一步包括组合苹果、葡萄、绿茶和橄榄提取物与蓝莓浓缩物、辣椒提取物和姜黄提取物。

[0273] 在制备活性增强的用于调控对象的氧化应激的组合物的方法的一个实施方式中，方法进一步包括组合蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果实提取物。

[0274] 在制备活性增强的用于调控对象的氧化应激的组合物的方法的一个实施方式中，方法进一步包括组合蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与佛手柑提取物。

[0275] 在制备活性增强的用于调控对象的氧化应激的组合物的方法的一个实施方式中，方法进一步包括组合蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果皮提取物。

[0276] 在制备活性增强的用于调控对象的氧化应激的组合物的方法的一个实施方式中，方法进一步包括组合蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果皮提取物和佛手柑提取物。

[0277] 在一个实施方式中，本文提出制备活性增强的用于调控对象的疾病相关的蛋白激酶活性的组合物的方法，包括：组合苹果、葡萄、绿茶和橄榄提取物，该苹果、葡萄、绿茶和橄榄提取物的用量是提供的蛋白激酶调控活性大于等量任一提取物或提取物总和所提供的蛋白激酶调控活性的用量。

[0278] 在制备活性增强的用于调控对象的疾病相关的蛋白激酶活性的组合物的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物利用选自水、乙醇、乙酸乙酯和其组合的提取溶剂来提取。

[0279] 在制备活性增强的用于调控对象的疾病相关的蛋白激酶活性的组合物的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约1:1:1:1的重量比存在组合。

[0280] 在制备活性增强的用于调控对象的疾病相关的蛋白激酶活性的组合物的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约6:1:3:1的重量比组合。

[0281] 在制备活性增强的用于调控对象的疾病相关的蛋白激酶活性的组合物的方法的一个实施方式中，方法进一步包括组合苹果、葡萄、绿茶和橄榄提取物与至少一种次要治疗剂。

[0282] 在制备活性增强的用于调控对象的疾病相关的蛋白激酶活性的组合物的方法的一个实施方式中，至少一种次要治疗剂包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、loclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。

[0283] 在制备活性增强的用于调控对象的疾病相关的蛋白激酶活性的组合物的方法的一个实施方式中，方法进一步包括组合苹果、葡萄、绿茶和橄榄提取物与蓝莓浓缩物、辣椒提取物和姜黄提取物。

[0284] 在制备活性增强的用于调控对象的疾病相关的蛋白激酶活性的组合物的方法的一个实施方式中，方法进一步包括组合蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果实提取物。

[0285] 在制备活性增强的用于调控对象的疾病相关的蛋白激酶活性的组合物的方法的一个实施方式中，方法进一步包括组合蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与佛手柑提取物。

[0286] 在制备活性增强的用于调控对象的疾病相关的蛋白激酶活性的组合物的方法的一个实施方式中，方法进一步包括组合蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果皮提取物。

[0287] 在制备活性增强的用于调控对象的疾病相关的蛋白激酶活性的组合物的方法的一个实施方式中，方法进一步包括组合蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果皮提取物和佛手柑提取物。

[0288] 在一个实施方式中，本文提出制备治疗性组合物的方法，包括：提供主要治疗剂；和组合苹果、葡萄、绿茶和橄榄提取物与主要治疗剂，该苹果、葡萄、绿茶和橄榄提取物的用量是增加的主要治疗剂的治疗效果大于等量任一提取物单独提供的治疗效果增加的用量。

[0289] 在制备治疗性组合物的方法的一个实施方式中，方法进一步包括首先利用选自水、乙醇、乙酸乙酯和其组合的提取溶剂提取苹果、葡萄、绿茶和橄榄提取物。

[0290] 在制备治疗性组合物的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约1:1:1:1的重量比存在组合。

[0291] 在制备治疗性组合物的方法的一个实施方式中，苹果、葡萄、绿茶和橄榄提取物以约6:1:3:1的重量比组合。

[0292] 在制备治疗性组合物的方法的一个实施方式中，方法进一步包括组合苹果、葡萄、绿茶和橄榄提取物与至少一种次要治疗剂。

[0293] 在制备治疗性组合物的方法的一个实施方式中，至少一种次要治疗剂包括选自下列的成员：佛手柑、山竹、黄连素、精氨酸、瓜氨酸、谷氨酰胺、锌、甜菜、Ioclo、蛋白质、姜黄素、植物甾醇、鱼油、辅酶Q10、维生素、纤维、菊粉和其组合。

[0294] 在制备治疗性组合物的方法的一个实施方式中，方法进一步包括组合主要治疗剂、苹果、葡萄、绿茶和橄榄提取物与蓝莓浓缩物、辣椒提取物和姜黄提取物。

[0295] 在制备治疗性组合物的方法的一个实施方式中，方法进一步包括组合主要治疗剂、蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果实提取物。

[0296] 在制备治疗性组合物的方法的一个实施方式中，方法进一步包括组合主要治疗剂、蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与佛手柑提取物。

[0297] 在制备治疗性组合物的方法的一个实施方式中，方法进一步包括组合主要治疗剂、蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果皮提取物。

[0298] 在制备治疗性组合物的方法的一个实施方式中，方法进一步包括组合主要治疗剂、蓝莓浓缩物以及苹果、葡萄、绿茶、橄榄、辣椒和姜黄提取物与山竹果皮提取物和佛手柑提取物。

[0299] 参考下文实施例来描述本公开的实施方式，实施例仅以示例目的提供，不应用于限制本发明的范围或解释本发明。

实施例

[0300] 实施例1

[0301] 10组分植物复合物("PC10")在氧自由基吸收能力方面呈现协同作用

[0302] 如下所述制备在其吸收氧自由基的能力方面呈现协同作用(利用氧自由基吸收能力(ORAC)测验)的10组分植物复合物(PC10)。

[0303] 化学剂——所有化学剂购自标准化学剂供应商(例如,Sigma,St.Louis,MO),并且具有市售最高纯度。所用反应剂包括75mM磷酸钾(KH2PO4)(pH=7.4);0.64M AAPH(2'2'-偶氮双(2-脒基-丙烷二盐酸盐));10mM Trolox(6-羟基-2,5,7,8-四甲基色原烷-2-羧酸);4.4x10-6M原荧光素(stock荧光素),钠盐;和1:1丙酮/水。

[0304] PC10材料——10个商品样本,苹果果实提取物(R11309)、佛手柑果实提取物(R13216)、蓝莓果实浓缩物(R10990)、辣椒果实(R11505)、葡萄种子提取物(R13545)、葡萄表皮提取物(R13555)、绿茶叶提取物(R13568)、山竹果皮提取物(R26699)、橄榄叶提取物(R15020)和姜黄根&根茎提取物(R17065)分别和以不同组合测试其氧自由基清除活性。

[0305] 样本制备——样本研磨成细粉并充分混合。将50mg(精确至0.1mg)样本转移到35ml离心管中并与25ml丙酮/水(50:50,v/v)提取液混合。然后将样本声波处理60min(振荡20至40min),并在3.5K rpm下离心10min。

[0306] 通过如下配制PC10材料:以多种比例组合苹果果实提取物、佛手柑果实提取物、蓝莓果实浓缩物,辣椒果实,葡萄种子提取物、葡萄表皮提取物、绿茶叶提取物、山竹果皮提取物、橄榄叶提取物和姜黄根&根茎提取物,该比例始于1:1:1:1:1:1:1:1:1:1,并且基于ORAC活性和成分成本增加或减少组分相对量。

[0307] 测验方法——将空白、标准和样本的比色皿置于28±1℃的干浴(dry bath)中,并将100μL 8.8x 10-8M荧光素和2.50ml缓冲剂加入各比色皿,空白中添加50μL缓冲剂。在样本比色皿中加入50μL样本溶液。将比色皿加盖并简短混合。将比色皿置于RF-150荧光分光光度计的固定器中,并记录初始荧光作为f0。在时间t=0,将100μL AAPH加入各比色皿。将比色皿加盖并简短涡旋。以5分钟间隔测量荧光(RFU),直到荧光衰减停止或荧光值<初始荧光读数的5%。记录RFU作为f1、f2等。荧光衰减在60min内完成。

[0308] 计算——通过内插20分钟内抑制荧光衰减50%所需的浓度,计算本实施例中氧自由基清除活性的中值抑制浓度(IC_{50})。然后利用联合指数(combination index,CI)参数来定量测试组分的协同作用。该参数仅定义叠加效应,而非协同或对抗。然而,协同被定义为大于预期叠加效应(CI>1),而对抗被定义为小于预期叠加效应(CI<1),如下文描述。

[0309] 任何多组分组合的预期中值抑制浓度都利用下列关系来估测:

[0310] $[1/预期IC_{50}]=[Fa/IC_{50A}]+[Fb/IC_{50B}]+\cdots+[Fn/IC_{50N}]$

[0311] 并且Fa+Fb+…+Fn=1

[0312] 其中Fa=组合中组分A的摩尔分数,Fn=组合中第n组分的摩尔分数,IC_{50A}=组分A的观测IC_{50}。CI然后由此计算,CI=预期[IC_{50}]/观测[IC_{50}]。利用指定CI=1作为叠加效应,对于具有相同作用模式的互斥性化合物或具有完全独立作用模式的非互斥性药物,

[0313] 限定下列关系:CI<1、=1和>1分别表示对抗、叠加和协同。

[0314] 结果——在表1中可见,10组分植物复合物的观测IC_{50}为18.6mg/L,而计算预期IC_{50}值为26.5mg/L,导致CI=1.43。该差异水平是未预料到的,并且构成PC10制剂氧自由基清除活性的预料不到的新发现。

[0315] 表1:确定10组分植物复合物(PC10)的氧自由基吸收能力的联合指数

[0316]

测试材料	观测 IC_{50}	相对量	$Fn/[IC_{50}]$

[0317]

	[mg/L]	[F]	$[\mu g/mL]^{-1}$
苹果果实†	6.8	0.085	0.0124
佛手柑果实†	46.3	0.704	0.0152
蓝莓果实*	1,283	0.014	0.000011
辣椒果实†	887	0.014	0.000016
葡萄种子*	10.2	0.014	0.0014
葡萄表皮†	373	0.014	0.000038
绿茶叶†	67.4	0.042	0.00063
山竹果皮†	49.6	0.014	0.00028
橄榄叶†	101	0.014	0.000140
姜黄根†	11.2	0.085	0.0076
植物复合物（10）**	**18.6**	**1.000**	**0.0377**

[0318] †提取物/*浓缩物/**植物复合物(10)包含10种测试成分中每一种的相对量;PC10的预期IC50＝1/[0.0377]＝26.5μg/mL。

[0319] 结论——约6:50:1:1:1:1:3:1:1:6比例的10组分植物复合物(PC10)呈现预料不到的相对于其各组分的总和1.42倍氧自由基清除活性的增加。

[0320] 实施例2

[0321] 8组分植物复合物在氧自由基吸收能力方面呈现协同作用

[0322] 如下所述制备9组分植物复合物(相对于标准ORAC测验中其组分的预期贡献总和)。

[0323] 化学剂——本实施例用到的所有化学剂均是此前在实施例1中描述的那些。

[0324] PC8材料——除佛手柑和山竹果皮外,本实施例用到的测试材料包括此前在实施例1中描述的那些。

[0325] 测验方法——方法总体上如实施例1所述,除了测验每2分钟在96孔微板荧光计中在波长493nm(激发)/520nm(发射)下进行20分钟。

[0326] 计算——利用CalcuSyn(BIOSOFT,Ferguson,MO)计算氧自由基清除活性的中值抑制浓度(IC50)。此统计包进行多次药物剂量-效果计算——利用T-C Chou和P.Talaly[(1984)Quantitative analysis of dose-effect relationships:the combined effects of multiple drugs or enzyme inhibitors.Adv Enzyme Regul 22,27-55](通过引用并入本文)描述的中值效应法。

[0327] 简言之,该分析将"剂量"和"效果"以最简单的可能形式:fa/fu＝(C/Cm)m关联起来,其中C是化合物的浓度或剂量,并且Cm是表示效力的中值-有效剂量。Cm由中值-效果作图(median-effect plot)的x-截距来确定。受测试材料浓度影响的分数是fa和不受该浓度影响的分数为fu(fu＝1-fa)。指数m是参数表示剂量-效果曲线的S形或形状;其通过中值-

效果作图的斜率来估测。

[0328] 中值-效果作图是x＝log(C) vs y＝log(fa/fu)的图，并且基于Chou中值-效果方程式的对数形式。数据与中值-效果方程式的拟合优度由中值-效果作图的线性相关系数r表示。通常，来自酶或受体系统的实验数据的r>0.96，来自组织培养的实验数据的r>0.90，并且来自动物系统的实验数据的r>0.85。

[0329] 测试组分的协同作用利用实施例1限定的联合指数(CI)参数来定量。

[0330] 任何多组分组合的预期中值抑制浓度利用下列关系来估测：

[0331] $[1/预期IC_{50}]=[Fa/IC_{50A}]+[Fb/IC_{50B}]+\cdots+[Fn/IC_{50N}]$

[0332] 并且Fa+Fb+…+Fn＝1

[0333] 其中Fa＝组合中组分A的摩尔分数，Fn＝组合中第n组分的摩尔分数，并且IC_{50A}＝组分A的观测IC_{50}。CI然后由此计算，CI＝预期[IC_{50}]/观测[IC_{50}]。

[0334] 利用指定CI＝1作为叠加效应，获得了具有相同作用模式的互斥性化合物或具有完全独立作用模式的非互斥性药物的下列关系：CI<1、＝1和>1分别表示对抗、相加和协同。

[0335] 结果——PC8的中值抑制浓度(IC_{50})为0.0350μg/mL，而计算预期IC_{50}为0.0436μg/mL导致CI＝1.31。因此，PC8协同地产生相对于其组分活性总和的预期1.3倍的氧自由基清除活性。

[0336] 表2：确定8组分植物复合物(PC8)的氧自由基吸收能力的联合指数

[0337]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	Fn/[IC_{50}] [μg/mL]$^{-1}$
苹果果实†	0.02	0.300	15
蓝莓果实*	25.55	0.050	0.002
辣椒果实†	111	0.050	0.000450
葡萄种子*	0.0413	0.050	1.212
葡萄表皮†	0.233	0.050	0.215
绿茶叶†	0.04	0.150	3.75
橄榄叶†	0.08	0.050	0.625
姜黄根†	0.43	0.300	0.698

[0338]

植物复合物（PC8）**	**0.0350**	**1.000**	**21.58**

[0339] †提取物/*浓缩物/**植物复合物PC8包含8种测试材料中的每一种的相对量[F]；PC8的预期IC_{50}＝1/[21.58]＝0.046μg/mL。

[0340] 结论——在CI＝1.31的情况下，PC8预料不到地产生相对于其组分总和的预期1.31倍的氧自由基清除活性。

[0341] 实施例3

[0342] 含有山竹果实的9组分植物复合物(“PC9f”)在氧自由基吸收能力方面呈现协同作用

[0343] 如下所述制备9组分植物复合物——相对于标准ORAC测验中相对组分的预期贡献的总和。

[0344] 所有化学剂、方法和计算如实施例2所述进行。

[0345] PC9f材料——本实施例用到的测试材料包含表3列出的成分和相对量。

[0346] 结果——PC9f的IC_{50}为0.0410μg/mL，而计算预期IC_{50}为0.0.049μg/mL，导致CI＝1.20。因此，PC9f协同地产生相对于其组分总和的活性的预期1.20倍的氧自由基清除活性。

[0347] 表3：确定包含山竹果实的9组分植物复合物(PC9f)的氧自由基吸收能力的联合指数

[0348]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	$Fn/[IC_{50}]$ $[\mu g/mL]^{-1}$
苹果果实†	0.02	0.286	14.3
蓝莓果实*	25.55	0.0476	0.002
辣椒果实†	111	0.0476	0.000432
葡萄种子*	0.0413	0.0476	1.19
葡萄表皮†	0.233	0.0476	0.238
绿茶叶†	0.04	0.1429	3.573
山竹果实†	3.99	0.0476	0.012
橄榄叶†	0.08	0.0476	0.595
姜黄根 & 根茎†	0.43	0.286	0.664
植物复合物（PC9f）	**0.0410**	**1.000**	**20.56**

[0349] †提取物/*浓缩物/**植物复合物PC9f包含9种测试材料中每一种的相对量[F]；PC9f的预期IC_{50}＝1/[20.56]＝0.049μg/mL。

[0350] 结论——在CI＝1.20的情况下，PC9f预料不到地产生相对于其组分总和的预期1.2倍的氧自由基清除活性。

[0351] 实施例4

[0352] 8组分植物复合物(PC8)在自由基猝灭方面呈现协同作用

[0353] 如下所述制备8组分植物复合物——相对于其组分对于清除自由基的预期贡献的总和。

[0354] 方法——利用2,2-二苯基-1-苦基肼(DPPH)测验评估测试材料导致的自由基清除。此测验基于氢供体是抗氧化剂的原理。该测验测量作为自由基清除剂的化合物。稳定的自由基DPPH*接受来自抗氧化剂的氢。抗氧化剂效果与测试样本中DPPH的消失成比例。采用Dudonne描述的程序，并且具有测验在96孔微滴定板中进行的改动[Dudonne,S.,Vitrac,X.,Coutiere,P.,Woillez,M.,and Merillon,J.M.(2009)Comparative Study of Antioxidant Properties and Total Phenolic Content of 30Plant Extracts of Industrial Interest Using DPPH,ABTS,FRAP,SOD,and ORAC Assays,J Agric Food Chem 57,1768-1774]。

[0355] PC8材料——本研究用到的测试材料如实施例2以及表2和4所述。

[0356] 计算——计算如实施例2所述进行。

[0357] 结果——PC8的中值抑制浓度(IC_{50})为15.6μg/mL，而计算预期IC_{50}为17.9μg/mL，导致CI＝1.15。因此，PC8协同地产生其组分总和的1.2倍自由基猝灭活性。

[0358] 表4：确定8组分植物复合物(PC8)的自由基猝灭能力的联合指数

[0359]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	$Fn/[IC_{50}]$ $[\mu g/mL]^{-1}$
苹果果实†	14.1	0.300	0.0213
蓝莓果实*	2713	0.050	0.0000184
辣椒果实†	7225	0.050	0.00000692
葡萄种子*	5.52	0.050	0.00906
葡萄表皮†	107	0.050	0.000469
绿茶叶†	6.89	0.150	0.0218
橄榄叶†	50.3	0.050	0.000993
姜黄根†	137	0.300	0.00218

[0360]

植物复合物（PC8）**	**15.6**	**1.000**	**0.0558**

[0361] †提取物/*浓缩物/**植物复合物PC8包含8种测试材料中每一种的相对量[F]；PC8的预期IC_{50}＝1/[0.0558]＝17.92μg/mL。

[0362] 结论——在CI＝1.15的情况下，PC8预料不到地产生相对于其组分总和1.2倍的自由基猝灭活性。

[0363] 实施例5

[0364] 包含山竹果实的9组分植物复合物("PC9f")在自由基猝灭方面呈现协同作用

[0365] 如下所述制备9组分植物复合物——相对于其组分清除自由基的预期贡献的总和。

[0366] 所有化学剂和计算如实施例2所述，并且方法如之前实施例4所述。

[0367] PC9f材料——本实施例用到的测试材料包含表3和5列出的成分和相对量。

[0368] 结果——PC9f的IC_{50}为16.2μg/mL，而计算预期IC_{50}为18.8μg/mL，导致CI＝1.16。因此，PC9f协同地产生相对于其组分总和的活性的预期1.8倍的氧自由基清除活性。

[0369] 表5：确定包含山竹果实的9组分植物复合物(PC9f)的自由基猝灭能力的联合指数

[0370]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	$Fn/[IC_{50}]$ $[\mu g/mL]^{-1}$
苹果果实†	14.1	0.286	0.0202664
蓝莓果实*	2713	0.048	0.0000175
辣椒果实†	7225	0.048	0.00000659
葡萄种子†	5.52	0.048	0.0086269
葡萄表皮†	106.6	0.048	0.0004465
绿茶叶†	6.89	0.143	0.0207460
山竹果实†	3527	0.048	0.0000135
橄榄叶†	50.3	0.048	0.0009455
姜黄根 & 根茎†	137	0.286	0.0020804
植物复合物（PC9f）	**16.2**	**1.000**	**0.0531**

[0371] †提取物/*浓缩物/**植物复合物PC9f包含9种测试材料中每一种的相对量[F]；预期PC9f的IC_{50}=1/[0.0531]=18.8μg/mL。

[0372] 结论——在CI=1.16的情况下，PC9f预料不到地产生相对于其组分总和的预期1.2倍的氧自由基清除活性。

[0373] 实施例6

[0374] 含有山竹果皮的9组分植物复合物（“PC9p”）在自由基猝灭方面呈现协同作用

[0375] 如下所述制备9组分植物复合物——相对于其组分清除自由基的的预期贡献的总和。

[0376] 化学剂和方法如之前实施例4所述，而计算如实施例2所述。

[0377] PC9p材料——本实施例用到的测试材料包含表6描述的成分和相对量。

[0378] 表6：确定包含山竹果皮的9组分植物复合物(PC9p)的自由基猝灭能力的联合指数

[0379]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	$Fn/[IC_{50}]$ $[\mu g/mL]^{-1}$
苹果果实†	14.1	0.286	0.0203
蓝莓果实*	2713	0.0476	0.0000175
辣椒果实†	7225	0.0476	0.0000066
葡萄种子†	5.52	0.0476	0.0086
葡萄表皮†	106.6	0.0476	0.000447
绿茶叶†	6.89	0.143	0.0207
山竹果皮†	26.7	0.0476	0.00178
橄榄叶†	50.3	0.0476	0.000945
姜黄根 & 根茎†	137	0.286	0.0021
植物复合物（PC9p）	**13.4**	**1.000**	**0.0549**

[0380] †提取物/*浓缩物/**植物复合物PC9p包含9种测试材料中每一种的相对量[F]；PC9p的预期$IC_{50}=1/[0.0549]=18.2\mu g/mL$。

[0381] 结果——PC9p的IC_{50}为013.4μg/mL，而计算预期IC_{50}为18.2μg/mL，导致CI＝1.36。因此，PC9p协同地产生相对于其组分总和的活性的预期1.4倍的自由基猝灭活性。

[0382] 结论——在CI＝1.36的情况下，PC9p预料不到地产生相对于其组分总和1.4倍的自由基猝灭活性。

[0383] 实施例7

[0384] 9组分植物复合物（“PC9b”）在过氧亚硝酸根（$ONOO^-$）清除能力方面呈现协同作用

[0385] 如下所述制备9组分植物复合物相对于其组分的预期贡献总和在清除过氧亚硝酸根方面的协同作用潜力。

[0386] 方法——过氧亚硝酸根（$ONOO^-$）清除能力按照Kim等[Kim,J.Y.,Kim,H.S.,Kang,H.S.,Choi,J.S.,Yokozawa,T.,and Chung,H.Y.(2008)Antioxidant potential of dimethyl lithospermate isolated from Salvia miltiorrhiza(red sage)against peroxynitrite,J Med Food 11,21-28]描述的程序来测量，其中改动是测验在替代比色皿的96孔微滴定板中进行。

[0387] 计算如实施例2所述。

[0388] PC9b材料——除实施例1所述的佛手柑提取物外，本研究用到的其余测试材料如实施例2以及表2和4作为PC8所述。

[0389] PC9b的IC_{50}为0.974μg/mL，而计算预期IC_{50}为1.67μg/mL，导致CI＝1.71。因此，PC9b协同地产生相对于其组分总和的预期1.7倍的过氧亚硝酸根清除能力。

[0390] 表7：确定包含佛手柑的9组分植物复合物（PC9b）的过氧亚硝酸根（$ONOO^-$）清除能力的联合指数

[0391]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	$Fn/[IC_{50}]$ $[\mu g/mL]^{-1}$
苹果果实†	0.875	0.0857	0.0979
佛手柑	3.34	0.714	0.214
蓝莓果实*	35.7	0.0143	0.000401
辣椒果实†	31.0	0.0143	0.000461
葡萄种子*	0.472	0.0143	0.0303
葡萄表皮†	3.11	0.0143	0.00460
绿茶叶†	0.185	0.0429	0.232
橄榄叶†	1.17	0.0143	0.0122
姜黄根†	10.3	0.0857	0.00834
植物复合物（PC9b）**	**0.974**	**1.000**	**0.600**

[0392] †提取物/*浓缩物/**植物复合物PC9b包含9种测试材料中每一种的相对量[F]；PC9b的预期IC_{50}=1/[0.600]=1.67μg/mL。

[0393] 结论——在CI=1.71的情况下，PC9b预料不到地产生相对于其组分总和的预期1.7倍的过氧亚硝酸根清除能力。

[0394] 实施例8

[0395] 9组分植物复合物（"PC9f"）在过氧亚硝酸根（$ONOO^-$）清除能力方面呈现协同作用

[0396] 评价包含山竹果实的新型9组分植物复合物相对于其组分的预期贡献总和在清除过氧亚硝酸根方面的协同作用潜力。

[0397] 方法——方法和计算如之前实施例7所述，而计算如实施例2所述。

[0398] PC9f材料——实施例3以及表3和8所述的PC9f和组分是本实施例中的测试材料。PC9f的IC_{50}为0.486μg/mL，而计算预期IC_{50}为0.777μg/mL，导致CI=1.60。因此，PC9f协同地产生相对于其组分总和的预期1.6倍的过氧亚硝酸根清除能力。

[0399] 表8：确定9组分植物复合物（PC9f）的过氧亚硝酸根（$ONOO^-$）清除能力的联合指数

[0400]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	$Fn/[IC_{50}]$ $[\mu g/mL]^{-1}$
苹果果实†	0.875	0.286	0.326
蓝莓果实*	35.7	0.0476	0.001
辣椒果实†	31.0	0.0476	0.002
葡萄种子†	0.472	0.0476	0.101
葡萄表皮†	3.11	0.0476	0.015
绿茶叶†	0.185	0.143	0.773
山竹果实†	68.3	0.0476	0.001
橄榄叶†	1.17	0.0476	0.041
姜黄根 & 根茎†	10.3	0.286	0.028
植物复合物（PC9f）	**0.486**	**1.000**	**1.29**

[0401] †提取物/*浓缩物/**植物复合物PC9f包含9种测试材料中每一种的相对量[F]；PC9f的预期IC_{50}=1/[1.29]=0.777μg/mL。

[0402] 结论——在CI=1.60的情况下，PC9f预料不到地产生相对于其组分总和的预期1.6倍的过氧亚硝酸根清除能力。

[0403] 实施例9

[0404] 9组分植物复合物(PC9f)+佛手柑在过氧亚硝酸根($ONOO^-$)清除能力方面呈现协同作用

[0405] 评价包含山竹果实和佛手柑的新型9组分植物复合物相对于其组分的预期贡献总和在清除过氧亚硝酸根方面的协同作用潜力。

[0406] 方法——方法和计算分别如之前实施例7和2所述。

[0407] PC9f+佛手柑材料——实施例3以及表3和9所述的PC9f和组分和实施例1所述的佛手柑提取物是本实施例中的测试材料。PC9f+佛手柑果实提取物的IC_{50}为0.556μg/mL，而计算预期IC_{50}为1.69μg/mL，导致CI=3.04。因此，PC9f+佛手柑果实提取物协同地产生相对于其组分总和的预期3.0倍的过氧亚硝酸根清除能力。

[0408] 表9：确定9组分植物复合物(PC9f)+佛手柑果实提取物过氧亚硝酸根(ONOO-)清除能力的联合指数

[0409]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	$Fn/[IC_{50}]$ $[\mu g/mL]^{-1}$
苹果果实†	0.875	0.0845	0.097
佛手柑果实†	3.34	0.7042	0.211
蓝莓果实*	35.7	0.0141	0.000395
辣椒果实†	31.0	0.0141	0.000455
葡萄种子†	0.472	0.0141	0.0299
葡萄表皮†	3.11	0.0141	0.00454
绿茶叶†	0.185	0.0423	0.229
山竹果实†	68.3	0.0141	0.000206
橄榄叶†	1.17	0.0141	0.0121
姜黄根 & 根茎†	10.3	0.0845	0.00822
植物复合物（PC9f）+ 佛手柑	**0.556**	**1.000**	**0.592**

[0410] †提取物/*浓缩物/**植物复合物PC9f+佛手柑包含9种测试材料中每一种的相对量[F]；PC9f+佛手柑的预期IC_{50}=1/[0.592]=1.69μg/mL。

[0411] 结论——在CI=3.04的情况下，PC9f+佛手柑果实提取物预料不到地产生相对于其组分总和的预期3.0倍的过氧亚硝酸根清除能力

[0412] 实施例10

[0413] 9组分植物复合物(PC9p)在过氧亚硝酸根($ONOO^-$)清除能力方面呈现协同作用

[0414] 评价包含山竹果皮(PC9p)的新型9组分植物复合物相对于其组分的预期贡献总和在清除过氧亚硝酸根方面的协同作用潜力。

[0415] 方法——方法和计算分别如之前实施例7和2所述。

[0416] PC9p材料——表10描述的PC9p和组分是本实施例中的测试材料。PC0p的IC_{50}为0.523μg/mL，而计算预期IC_{50}为0.768μg/mL，导致CI=1.47。因此，PC9p协同地产生相对于其组分总和的预期1.5倍的过氧亚硝酸根清除能力。

[0417] 表10：确定9组分植物复合物(PC9p)的过氧亚硝酸根($ONOO^-$)清除能力的联合指数

[0418]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	Fn/[IC_{50}] [μg/mL]$^{-1}$
苹果果实†	0.875	0.286	0.326
蓝莓果实*	35.7	0.048	0.00133
辣椒果实†	31.0	0.048	0.00154
葡萄种子†	0.472	0.048	0.101
葡萄表皮†	3.11	0.048	0.0153
绿茶叶†	0.185	0.143	0.773
山竹果皮†	3.26	0.048	0.0146
橄榄叶†	1.17	0.048	0.0408
姜黄根 & 根茎†	10.3	0.286	0.0278
植物复合物（PC9p）	**0.523**	**1.00**	**1.30**

[0419] †提取物/*浓缩物/**植物复合物PC9p+佛手柑包含9种测试材料中每一种的相对量[F]；PC9p+佛手柑的预期IC_{50}=1/[1.30]=0.768μg/mL。

[0420] 结论——在CI=1.47的情况下，PC9p预料不到地产生相对于其组分总和的预期1.5倍的过氧亚硝酸根清除能力。

[0421] 实施例11

[0422] 10组分植物复合物(PC10)在过氧亚硝酸根($ONOO^-$)清除能力方面呈现协同作用

[0423] 评价新型10组分植物复合物(PC10)相对于其组分的预期贡献总和在清除过氧亚硝酸根方面的协同作用潜力。

[0424] 方法——方法和计算分别如之前实施例7和2所述。

[0425] PC10材料——实施例1和表1所述的PC10和组分是本实施例中的测试材料。

[0426] 结论——PC10的IC_{50}为1.02μg/mL，而计算预期IC_{50}为1.68μg/mL，导致CI=1.64。因此，PC10协同地产生相对于其组分总和的预期1.6倍的过氧亚硝酸根清除能力。

[0427] 表11：确定10组分植物复合物(PC10)的过氧亚硝酸根($ONOO^-$)清除能力的联合指数

[0428]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	Fn/[IC_{50}] [μg/mL]$^{-1}$
苹果果实†	0.875	0.085	0.0965

[0429]

佛手柑果实†	3.34	0.704	0.211
蓝莓果实*	35.7	0.0141	0.000395
辣椒果实†	31.0	0.0141	0.000455
葡萄种子†	0.472	0.0141	0.0299
葡萄表皮†	3.11	0.0141	0.00454
绿茶叶†	0.185	0.0423	0.229
山竹果皮†	3.26	0.0141	0.00432
橄榄叶†	1.17	0.0141	0.0121
姜黄根 & 根茎†	10.3	0.0845	0.00822
植物复合物（PC10）	**1.02**	**1.00**	**0.596**

[0430] †提取物/*浓缩物/**植物复合物PC10包含10种测试材料中每一种的相对量[F]；PC10的预期IC_{50}=1/[0.596]=1.68μg/mL。

[0431] 结论——在CI=1.64的情况下，PC10预料不到地产生相对于其组分总和的预期1.6倍的过氧亚硝酸根清除能力。

[0432] 实施例12

[0433] 4组分植物复合物(PC4)的两种制剂在过氧亚硝酸根($ONOO^-$)清除能力方面呈现协同作用

[0434] 在以两种制剂测试时，评价新型4组分植物复合物(PC4)相对于其组分的预期贡献总和在清除过氧亚硝酸根方面的协同作用潜力。

[0435] 方法——方法和计算分别如之前实施例7和2所述。

[0436] PC4材料——PC4由表12和13列举和实施例1描述的组分组成。四种组分各自的相对量为1:1:1:1=PC4.1(表13)和6:3:1:1=PC4.2。

[0437] 结论——PC4.1的IC_{50}为0.216μg/mL，而计算预期IC_{50}为0.420μg/mL，导致CI=1.95。因此，PC4.1协同地产生相对于其组分总和的预期2.0倍的过氧亚硝酸根清除能力。

[0438] 表12：确定4组分植物复合物(PC4.1)的过氧亚硝酸根($ONOO^-$)清除能力的联合指数

[0439]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	$Fn/[IC_{50}]$ $[\mu g/mL]^{-1}$
苹果果实†	0.875	0.250	0.286

[0440]

葡萄种子†	0.472	0.250	0.530
绿茶叶†	0.185	0.250	1.352
橄榄叶†	1.17	0.250	0.214
植物复合物（PC4.1）	**0.216**	**1.00**	**2.382**

[0441] †提取物/*浓缩物/**植物复合物PC4.1包含4种测试材料中每一种的相对量[F]；PC4.1的预期IC_{50}=1/[2.382]=0.420μg/mL。

[0442] 结论——在CI=1.95的情况下，PC4.1预料不到地产生相对于其组分总和的预期2.0倍的过氧亚硝酸根清除能力。

[0443] 表13：确定4组分植物复合物(PC4.2)的过氧亚硝酸根($ONOO^-$)清除能力的联合指数

[0444]

测试材料	观测 IC_{50} [μg/mL]	相对量 [F]	$Fn/[IC_{50}]$ $[\mu g/mL]^{-1}$
苹果果实†	0.875	0.545	0.623
葡萄种子†	0.472	0.0909	0.193
绿茶叶†	0.185	0.273	1.475
橄榄叶†	1.17	0.0909	0.078
植物复合物（PC4.2）	**0.201**	**1.000**	**2.369**

[0445] †提取物/*浓缩物/**植物复合物PC4.2包含4种测试材料中每一种的相对量[F]；PC4.2的预期IC_{50}=1/[2.369]=0.422μg/mL。

[0446] 结果——PC4.2的IC_{50}为0.201μg/mL，而计算预期IC_{50}为0.422μg/mL，导致CI=2.10。因此，PC4.2协同地产生相对于其组分总和的预期2.0倍的过氧亚硝酸根清除能力。

[0447] 结论——在CI=2.10的情况下，PC4.2预料不到地产生相对于其组分总和的预期2.0倍的过氧亚硝酸根清除能力。

[0448] 实施例13

[0449] 对于295种蛋白激酶的PC9p和佛手柑(PC10)的协同相互作用

[0450] 蛋白激酶是酶中能够将磷酸基从供体分子(通常ATP)转移至蛋白质的氨基酸残基(通常苏氨酸、丝氨酸或酪氨酸)的转移酶类。激酶用于酶调控的信号转导，即，其可抑制或激活酶活性，如在胆固醇生物合成、氨基酸转化或糖原转换中。虽然多数激酶针对单一种类的氨基酸残基，但一些激酶呈现双重活性，因为其可磷酸化两个不同种类的氨基酸。

[0451] 方法——在KinaseProfilerTM测验(Millipore UK Ltd。Dundee，英国)中，分别测试PC9p、佛手柑以及PC9p和佛手柑组合(PC10制剂)对一组295种激酶的人激酶活性的抑制效果。表14列出的具体激酶的测验方案概述于http://www.millipore.com/techpublications/tech1/pf3036。

[0452] 在经过捕获中值有效浓度的三个浓度观察到剂量响应时，通过简单内插法确定PC9p、佛手柑和PC10的中值抑制浓度(IC_{50})。利用比例为3:7的PC9p和PC10的观测IC_{50s}，如实施例2所述计算PC10制剂的估测IC_{50}。

[0453] 然后利用联合指数(CI)参数，定量测试组分的协同作用。此参数仅限定叠加效应，而不限定协同或对抗效应。然而，协同作用被定义为大于预期的叠加效应(CI>1)，并且对抗效应被定义为小于预期的叠加效应(CI<1)，如下所述。

[0454] 任何多组分组合的预期中值抑制浓度均利用下列关系来估测：

[0455] [1/预期IC_{50}]=[0.3/IC_{50}PC9p]+[0.7/IC_{50}佛手柑]

[0456] 然后由此计算CI，CI＝预期[IC_{50}]/观测[IC_{50}]。利用指定CI＝1为叠加效应，对于具有相同作用模式的互斥性化合物或对于具有完全独立作用模式的非互斥性药物，限定下列关系：CI<1、＝1和>1，分别表明对抗、叠加和协同。在这些研究中，CI>1.10被认为证明了制剂之间的协同作用。

[0457] 表14：PC9f、佛手柑和PC10对于295种激酶的相互作用的联合指数

[0458]

激酶	PC9f IC_{50} [μg/mL]	佛手柑 IC_{50} [μg/mL]	PC10 IC_{50} [μg/mL]	Est PC10 IC_{50}† [μg/mL]	CI
PI3 激酶（p110a/p85a）	1068.73	5000	7.9	2377	002
PI3 激酶（p110a（H1047R）/p85a）	5000.00	5000	25	5000	200
Met（Y1248D）HGFR	1.979	5000	0.05	6.59	132
TrkC	0.01	625	0.0002	0.02	86.1
BTK（R28H）	93.20	2.6	0.05	3.63	72.6
PKCβII	5000	5000	70	5000	71.9
Fms	4.18	9.0	0.12	6.70	54.0
Met（Y1248H）	0.73	5000	0.05	2.43	48.6
Met（M1268T）HGFR	0.500	187	0.05	1.66	33.2
GRK6（凝固）	882.39	5000	63	2083	33.0
ACK1（以及 TNK2）	0.05	65	0.01	0.166	24.9
Met（D1246N）（HGFR）	0.04	5000	0.005	0.133	24.4
PRAK（引起应激）	0.17	192	0.05	0.579	11.6

[0459]

CK2	46	0.07	0.01	0.100	11.4
cKit（V654A）	4.5	9.74	0.735	7.20	9.80
PI3 激酶（p110b/p85a）	20.91	5000	7.2	69.0	9.65
Rse（Toll 样受体/NFKß）	24	5000	8.5	80.2	9.41
PI3 激酶（p120g）	377.22	5000	120.29	1069	8.89
PIP5K1g（h）	0.02	5000	0.01	0.0780	8.33
EGFR	1.8	484	0.8	5.79	7.43
PKCμ（h）	163.46	5000	69.92	506	7.24
PEK（h）	3.3	5000	1.71	10.9	6.37
GCK（h）	0.01	20	0.01	0.0429	5.99
IR 激活	6.35	197	3.54	19.7	5.56
MSSK1（h）	0.25	5000	0.16	0.848	5.42
cKit（D816H）	0.034	80	0.022	0.113	5.26
PrKX（h）	28.68	5000	18.65	94.3	5.06
Pim-1（h）	5.4	49	2.92	14.4	4.93
DRAK1（h）	21	131	11	50.7	4.63
GSK3β（h）	8.4	443	6.7	26.8	4.01
Mnk2（h）	0.51	19	0.41	1.60	3.93
TAK1（h）	316	1754	194	741	3.82
MELK（h）	0.78	55	0.66	2.51	3.80
PRK2（h）	11	5000	10	36.5	3.78
CK1γ3（h）	4.86	332	4.19	15.7	3.74
JNK3（h）	26	5000	23	84.1	3.73
NEK11（h）	1.6	5000	1.5	5.42	3.62
cKit	11.6	10	2.92	10.5	3.59
Lyn（h）	5.2	561	4.8	17.1	3.58
PKCδ（h）	50	5000	45	162	3.58
CSK（h）	2.3	411	2.17	7.62	3.51
PhKγ2（h）	0.9	5000	0.85	2.94	3.44
LOK（h）	6.3	5000	6.27	21.0	3.35
TBK1（h）	5.0	625	4.89	16.4	3.35
PKCζ（h）	5.0	5000	4.98	16.6	3.34
Met（D1246H）（h）	0.05	59744	0.050	0.167	3.33
Met（Y1248C）（h）	0.05	4356	0.050	0.167	3.33
Syk（h）	0.05	231	0.050	0.167	3.33

[0460]

CLK1 (h)	0.05	37.7	0.050	0.166	3.32
Flt4 (h)	0.05	26.3	0.050	0.166	3.32
PDGFRα (D842V) (h)	0.05	18.4	0.050	0.166	3.31
DYRK2 (h)	0.05	14.1	0.050	0.165	3.31
RIPK2 (h)	0.05	13.5	0.050	0.165	3.30
MLK1 (h)	0.05	7.64	0.050	0.164	3.28
PKCα (h)	175	5000	165	539	3.27
BrSK1 (h)	11.7	130	9.9	32.2	3.25
EphA4	4.36	5000	4.50	14.51	3.22
AMPKα2	15.8	5000	16.6	52.3	3.16
CHK2 (R145W) (h)	2.99	689	3.16	9.86	3.13
eEF-2K (h)	24.4	5000	25.8	80.3	3.12
Flt3 (D835Y) (h)	0.05	1.33	0.050	0.153	3.06
LKB1 (h)	101	5000	105	321	3.06
SRPK2 (h)	5.46	5000	5.98	18.1	3.03
Flt3 (h)	0.05	0.83	0.050	0.146	2.92
HIPK2 (h)	4.85	25.0	3.85	11.1	2.89
MSK2 (h)	12.9	5000	15.0	42.6	2.85
BrSK2 (h)	17.3	43	10.8	29.6	2.74
CaMKI (h)	30.7	5000	37.4	101	2.70
Ros (h)	19.4	5000	24.9	64.2	2.57
FGFR1 (h)	20.3	434	23.8	61.0	2.56
EphB1	12.6	625	16.0	40.2	2.50
Fyn (h)	0.655	328	0.872	2.17	2.49
mTOR (h)	27.9	5000	37.7	91.9	2.44
Pim-2 (h)	5.72	63	6.57	15.7	2.39
cSRC (h)	21.5	866	28.6	67.7	2.37
CDK1/细胞周期蛋白 B (h)	6.57	5000	9.45	21.8	2.31
IGF-1R (h), 激活	19.8	391	25.8	59.1	2.29
CHK2 (I157T) (h)	3.89	4412	5.7	12.9	2.26
CK1γ1 (h)	16.9	5000	24.7	55.8	2.26
PKBβ (h)	23.6	5000	34.6	77.8	2.24
CDK3/细胞周期蛋白 E (h)	20.1	5000	29.7	66.4	2.24
AMPKα1	6.88	1769	10.2	22.7	2.23
EphB3	26.9	5000	39.9	88.5	2.22
PAR-1Bα (h)	15.4	5000	23.1	51.0	2.20

[0461]

Tec (h) 激活	5.04	5000	7.6	16.8	2.19
DAPK2 (h)	22.9	123	24	53.2	2.19
IRAK4 (h)	7.67	764	12	25.0	2.16
PAK5 (h)	19.7	5000	30	65.1	2.16
TAO1 (h)	13.2	5000	20	43.6	2.15
IRAK1 (h)	14.5	3679	22	47.9	2.15
ULK2 (h)	151	5000	219	469	2.15
CDK2/细胞周期蛋白 E (h)	19.0	5000	29.2	62.7	2.14
MuSK (h)	15.5	5000	24.0	51.4	2.14
MAPK1 (h)	19.4	5000	29.9	64.0	2.14
NEK2 (h)	21.5	5000	33.5	71.0	2.12
PDGFRα (h)	35.2	2843	53.9	114	2.11
PAK1 (h)	37.6	5000	58.4	123	2.11
Tie2 (Y897S) (h)	32.2	5000	50.6	106	2.09
PDGFRβ (h)	24.7	5000	39.0	81.5	2.09
SRPK1 (h)	3.65	5000	5.85	12.2	2.08
EphA7	44.3	5000	70.3	145	2.06
Pyk2 (h)	30.8	5000	49.3	101	2.05
Tie2 (h)	26.8	5000	43.3	88.3	2.04
MINK (h)	8.85	5000	14.4	29.4	2.04
CHK1 (h)	29.2	5000	47.2	96.0	2.03
Arg (h)	9.13	3417	15.1	30.3	2.01
ULK3 (h)	19.4	5000	32.0	64.0	2.00
cKit (D816V)	7.43	5000	12.4	24.7	1.98
FGFR4 (h)	3.52	5000	5.98	11.7	1.96
Aurora-A	11.1	91	14.8	28.9	1.95
MST3 (h)	24.4	2027	40.7	79.2	1.95
Mer (h)	10.9	140	15.8	30.8	1.94
CDK2/细胞周期蛋白 A (h)	38.0	5000	64.1	125	1.94
PASK (h)	8.70	5000	14.9	28.9	1.94
CaMKIIβ (h)	16.8	1519	28.2	54.7	1.94
Abl (T315I)	5.47	801	9.26	17.9	1.94
WNK2 (h)	13.7	5000	23.6	45.4	1.93
CaMKIIγ (h)	7.13	102	10.6	20.4	1.92
Axl (h)	17.4	5000	30.0	57.6	1.92

[0462]

KDR (h)	1.94	5000	3.38	6.47	1.92
PDK1 (h)	19.9	5000	34.3	65.8	1.92
MST4 (h)	35.0	5000	60.2	115	1.91
Rsk1 (h)	3.27	2483	5.72	10.9	1.90
SGK3 (h)	29.5	5000	51.1	96.9	1.89
MAPKAP-K2 (h)	29.8	5000	52.1	97.9	1.88
PAK4 (h)	24.6	5000	43.3	81.2	1.88
CDK6/细胞周期蛋白 D3 (h)	38.1	5000	66.5	125	1.88
SGK (h)	32.9	5000	57.9	108	1.87
MKK7β (h)	35.0	5000	61.7	115	1.86
p70S6K (h)	1.12	743	2.00	3.72	1.86
TAO3 (h)	16.7	5000	29.8	55.3	1.86
MST2 (h)	18.9	5000	33.7	62.4	1.85
FGFR2 (h)	25.6	5000	45.6	84.5	1.85
Fer (h)	40.2	5000	71.5	132	1.84
ZAP-70 (h)	11.7	5000	21.4	38.9	1.82
Fgr (h)	12.8	105	18.3	33.2	1.82
EphA3	24.7	5000	44.9	81.4	1.81
CDK5/p25 (h)	13.2	5578	24.5	43.8	1.79
PKBα (h)	33.4	5000	61.3	110	1.79
IKKβ	35.3	5000	65.5	116	1.77
NLK (h)	25.0	5000	46.7	82.4	1.77
JAK3 (h)	18.6	5000	34.8	61.4	1.76
WNK3 (h)	17.6	5000	33.0	58.1	1.76
BTK (h)	7.09	5000	13.5	23.6	1.75
PKG1α (h)	25.6	5000	48.2	84.2	1.75
Ron (h)	8.97	5000	17.1	29.8	1.74
MARK1 (h)	6.66	5000	12.7	22.1	1.74
Lck (h)	24.0	444	41.0	71.1	1.74
ROCK-I (h)	55.9	5000	105	182	1.74
TAO2 (h)	14.7	5000	28.2	48.7	1.73
PKG1β (h)	19.3	5000	37.0	63.8	1.73
c-RAF (h)	21.2	5000	40.5	69.9	1.72
Fes (h)	24.2	5000	46.4	79.7	1.72
SGK2 (h)	28.3	5000	54.4	93.2	1.71

[0463]

DAPK1 (h)	83.1	5000	156	267	1.71
LRRK2 (h)	36.6	327	56.5	96.7	1.71
GSK3α (h)	5.67	60.0	9.09	15.5	1.70
ErbB4	27.2	5000	52.6	89.5	1.70
EGFR (T790M)	33.8	231	50.0	84.1	1.68
Hck (h)	17.3	97.1	24.3	40.8	1.68
PKCγ (h)	14.4	5000	28.6	47.8	1.67
CLK4 (h)	0.0181	34.7	0.0363	0.0604	1.66
ROCK-II (h)	18.5	5000	36.7	61.0	1.66
HIPK1 (h)	27.3	117	35.6	59.0	1.66
Bmx (h)	5.46	721	10.8	17.9	1.65
TSSK2 (h)	30.2	5000	60.1	99.2	1.65
MLCK (h)	22.5	5000	45.3	74.2	1.64
FGFR3 (h)	10.7	5000	21.6	35.4	1.64
MSK1 (h)	52.3	5000	105	170	1.63
TSSK1 (h)	21.5	5000	44.4	å	1.60
GRK7	42.0	5000	86.4	137	1.59
FGFR2 (N549H) (h)	22.4	388	41.4	65.9	1.59
EphA5	32.4	5000	67.4	106	1.58
Met (h)	53.7	2538	108	171	1.57
PKBγ (h)	31.2	5000	65.2	102	1.57
EGFR (T790M,L858R)	16.9	60.1	21.8	34.1	1.56
TYK2 (h)	31.7	82.7	35.8	55.8	1.56
PAK6 (h)	29.3	5000	62.3	96.3	1.55
IKKε	37.2	5611	79.2	122	1.54
ZIPK (h)	16.8	5000	36.1	55.6	1.54
EGFR (L858R)	43.1	5000	92.3	141	1.53
ALK4 (胰岛素受体家族)	136	5000	282	427	1.52
PKCθ (h)	28.6	5000	62.1	94.0	1.51
EphA2	30.7	2613	65.9	100	1.51
SIK (h)	13.5	59.7	19.6	29.4	1.50
IKKα	46.2	5000	100	151	1.50
ASK1 (h)	21.3	132	34.6	51.5	1.49
SAPK4 (h)	75.8	5000	164	244	1.49
CLK2 (h)	0.050	67.1	0.113	0.166	1.48
NEK6 (h)	22.9	5000	51.2	75.4	1.47

[0464]

EphA1	20.6	76.2	28.9	42.1	1.46
JAK1 (h)	11.1	165	22.0	31.9	1.45
CHK2 (h)	1.98	468	4.50	6.53	1.45
CDK9/细胞周期蛋白 T1 (h)	11.4	27.2	13.3	19.2	1.45
TrkB (h)	6.33	357	14.0	20.3	1.45
CaMKIδ (h)	34.8	5000	79.0	114	1.44
Tie2 (R849W) (h)	17.2	5000	39.5	56.8	1.44
MAPKAP-K3 (h)	50.6	5000	115	165	1.44
HIPK3 (h)	7.53	51.4	13.1	18.7	1.43
ALK (胰岛素受体家族)	7.74	5000	18.0	25.7	1.43
CDK7/细胞周期蛋白 H/MAT1(h)	41.7	5000	96.0	136	1.42
cKit (V560G)	3.12	0.05	0.0500	0.0709	1.42
LIMK1 (h)	28.0	72	34.4	48.8	1.42
IR	49.0	5128	113	160	1.41
STK25 (h)	44.6	5000	104	146	1.41
PTK5 (h)	33.5	60	34.8	48.6	1.40
Fms (Y969C) (h)	23.4	18	14.0	19.4	1.39
mTOR/FKBP12 (h)	39.5	5000	92.9	129	1.39
MRCKβ (h)	31.2	5000	73.9	102	1.39
EGFR (L861Q)	17.4	77	27.6	37.9	1.37
SAPK3 (h)	123	5000	284	389	1.37
FAK (h)	64.9	5000	153	210	1.37
Ret (V804M) (h)	3.13	2320	7.61	10.4	1.37
EphB2	0.91	89	2.19	2.96	1.35
Abl (H396P)	5.15	127	11.7	15.7	1.34
Txk (h)	8.00	168	18.0	24.0	1.33
CK1γ2 (h)	3.14	111	7.4	9.8	1.33
MRCKα (h)	52.2	5000	129	170	1.32
FGFR1 (V561M) (h)	17.7	668	42.5	55.4	1.31
EphA8	51.5	5000	129	168	1.30
Src (1-530) (h)	7.60	37	13.2	17.2	1.30
Wee1 (h)	52.9	5000	133	172	1.29
PKCι (h)	14.6	5000	38.2	48.4	1.27
CDK5/p35 (h)	14.4	5000	37.6	47.5	1.26
CK1δ (h)	19.0	5000	50.9	62.8	1.23
Aurora-B	0.0181	10	0.0500	0.0602	1.20

[0465]

PKA (h)	44.3	5000	121	145	1.20
CaMKIIδ (h)	2.53	59	6.40	7.66	1.20
B-Raf (h)	41.2	5000	114	135	1.18
DDR2 (h)	32.9	5000	91.9	108	1.18
Itk (h)	0.967	5000	2.78	3.22	1.16
PI3 激酶 (p110a (E542K) /p85a) (h)	25.0	5000	71.5	82.4	1.15
CaMKIV (h)	50.7	5000	144	165	1.14
Abl (Y253F)	4.28	93.4	11.4	12.9	1.13
Abl	5.24	4975	15.5	17.4	1.12
MST1 (h)	27.8	5000	81.7	91.6	1.12
Aurora-C	48.3	13.4	15.4	17.1	1.11
Rsk4 (h)	7.68	5000	23.5	25.5	1.08
MEK1 (h)	42.3	5000	130	138	1.06
Flt1	0.00683	119	0.0216	0.0228	1.06
PKCη (h)	330	5000	908	952	1.05
TLK2 (h)	5128	5000	5000	5038	1.01
Abl (Q252H)	4.59	143	14.2	14.2	1.00
DMPK	5000	5000	5000	5000	1.00
JNK1α1 (h)	5000	5000	5000	5000	1.00
JNK2α2 (h)	5000	5000	5000	5000	1.00
PIP5K1a (h)	5000	5000	5000	5000	1.00
PKCβI (h)	5000	5000	5000	5000	1.00
PKCε (h)	5000	5000	5000	5000	1.00
PKD2 (h)	5000	5000	5000	5000	1.00
Plk1 (h)	5000	5000	5000	5000	1.00
Plk3 (h)	5000	5000	5000	5000	1.00
TGFBR1 (h)	5000	5000	5000	5000	1.00
VRK2 (h)	5000	5000	5000	5000	1.00
JAK2 (h)	145	5000	451	451	1.00
EphB4	0.140	91.3	0.472	0.465	0.985
Ret (h)	0.006	64.7	0.022	0.020	0.931
Ret (V804L) (h)	0.436	118	1.55	1.44	0.927
NEK3 (h)	10.7	5000	38.8	35.6	0.917
Lck (h) 激活	9.7	47.4	24.1	21.9	0.911
B-Raf (V599E) (h)	28.4	1364	100	90.4	0.903

[0466]

Hck (h) 激活	15.0	45.8	31.8	28.3	0.891
Pim-3 (h)	55.5	24.5	33.3	29.4	0.884
Blk (h)	0.122	100	0.466	0.406	0.870
Rsk2 (h)	0.753	966	2.91	2.50	0.860
Abl (M351T)	3.59	272	13.5	11.6	0.860
SAPK2b (h)	50.3	5000	201	164	0.814
STK33 (h)	0.0121	5000	0.050	0.040	0.804
MAPK2 (h)	53.3	5000	218	173	0.794
GRK5 (h)	229	5000	874	689	0.789
TrkA (h)	0.414	103	1.83	1.37	0.746
Snk (h)	456	5000	1688	1253	0.742
PAK2 (h)	39.5	5000	178	129	0.725
Rsk3 (h)	0.66	1759	3.16	2.18	0.692
PIP4K2a (h)	14.0	5000	71.5	46.5	0.650
PDGFRα (V561D) (h)	1.44	0.0227	0.050	0.032	0.645
Src (T341M) (h)	29.1	31.7	58.0	30.9	0.533
BRK (h)	0.850	5000	5.45	2.83	0.519
NEK7 (h)	64.4	5000	456	208	0.457
CK2α2 (h)	22.2	0.0121	0.050	0.017	0.344
Haspin (h)	0.605	250	6.51	2.00	0.308
ARK5 (h)	0.477	247	5.85	1.58	0.271
PI3 激酶 (p110a (E545K) /p85a) (h)	42.7	5000	559	140	0.250
SAPK2a (h)	366	5000	5000	1041	0.208
MKK6 (h)	361	5000	5085	1029	0.202
DCAMKL2 (h)	304	5000	5000	887	0.177
SAPK2a (T106M) (h)	234	5000	5000	703	0.141
PI3 激酶 (p110a/p65a) (h)	98.0	5000	2825	312	0.111
PI3 激酶 (p110d/p85a) (h)	141	5000	5000	442	0.088
Yes (h)	0.0359	60.2	1.66	0.120	0.072
PI3KC2g (h)	300	58.3	1609	76.8	0.048
CLK3 (h)	0.0171	5000	3.61	0.0572	0.016
IGF-1R (h)	79.0	0.191	78.4	0.273	0.003
IRR (h)	9.69	0.050	25.1	0.0713	0.0028
PI3KC2a (h)	0.050	5000	5000	0.167	0.00003

[0467] †利用方程式[1/预期IC_{50}]=[0.3/[IC_{50PC9p}]+[0.7/[IC_{50}所引用]估测

[0468] 上述激酶全部可被本文提供的蛋白激酶调节组合物调节。

[0469] 下文段落简要总结了通过CI>1.05证明最受材料协同相互作用影响的那些激酶的细胞功能。提供分组仅仅是为了突显激酶发挥作用的主要信号传导途径，而非意为是全面的。

[0470] PC10显著并且协同调节Abl、ACK1、ALK、Aurora、AMPK、CaMKII、EGFR、EphA、FAK、FGFR、GSK3、IGF-1(激活)、IKK、IR、MAPK1、Met、MTOR、NEK1/2/6、PAK1/4/5/6、PDGFR、PI3K、PKC、ROCK I/II、RSK1/2/34、SRC和Syk的激酶信号传导，意味着骨骼肌脂肪酸氧化和肌肉葡萄糖摄取、肝脂肪酸氧化和酮生成被刺激、胆固醇合成、脂肪生成和甘油三酯合成被抑制、脂肪细胞脂解和脂肪生成被抑制和胰β细胞的胰岛素分泌被调节。

[0471] 值得注意，包括PI3和MET激酶的PC10制剂以及胰岛素受体(IR)本身协同影响葡萄糖代谢中涉及的多种激酶。AMPK的两种同种型也均被PC10制剂协同抑制。

[0472] PC10协同抑制全部7种MET激酶。在成年者体内，MET参与伤口愈合以及器官再生，组织重塑和脑发育的某些方面。MET途径还调控免疫响应以及胃肠道的发育和修复。

[0473] MET是通过结合至肝细胞生长因子/HGF配体将信号从胞外基质转导到细胞质中的受体酪氨酸激酶。其调控多种生理过程，包括增殖、散射(scattering)、形态发生和生存。细胞表面的配体结合引起其胞内结构域上的MET自磷酸化，提供下游信号传导分子的停泊位点。在通过配体激活后，与PI3-激酶亚单位PIK3R1、PLCG1、SRC、GRB2、STAT3或衔接分子GAB1的相互作用。

[0474] 三种Aurora激酶全部被PC10协同抑制。多数人类癌细胞的特征在于DNA的量或组织(organization)发生变化，导致染色体不稳定性和非整倍性。几种有丝分裂激酶，Aurora激酶等，通过有丝分裂调控细胞进程。目前为止，三种Aurora激酶在人体中已被鉴定出来：Aurora-A、Aurora-B和Aurora-C。最近Aurora激酶被鉴定为抗癌治疗中的潜在目标，并且各种Aurora-A和Aurora-B激酶抑制剂正在研发中[Kitzen,J.J.,de Jonge,M.J.,and Verweij,J.(2010)Aurora kinase inhibitors,Crit Rev Oncol Hematol 73,99-110]。在癌细胞中，这些酶的过表达导致遗传信息分布不均，产生非整倍体细胞——癌症标志。

[0475] Abl的六种同种型中的四种被PC10协同抑制。Abl是非受体酪氨酸-蛋白激酶，其在细胞生长和生存相关的多个关键过程——如响应胞外刺激的细胞骨架重塑、细胞运动和附着、受体内吞，自噬，DNA损伤响应和凋亡——中发挥作用。

[0476] 实施例14

[0477] 在正常对象和糖尿病前期对象中PC10的临床评估

[0478] 在开放标签的观察性临床试验中研究上述PC10配方的临床安全性和效力。研究群体包括呈现下列脂质变量的18至72岁之间的男性和女性：血清甘油三酯≥150mg/dl和/或血清低密度脂蛋白胆固醇(计算)≥150mg/dl。在12周研究期间，对象被分配到三组中的一组，分别接受500、750或1000mg的PC10，以2、3或4粒胶囊服用，一日一次随晚餐服用。

[0479] 指示对象保持其当前的生活方式，包括饮食、运动和心灵精神实践，在试验参与期间不做改变。还指示对象在处于研究时不对其当前的处方、非处方药物、医疗食品或营养补充剂做出改变。

[0480] 在1、2和3个月时，采集血液用于分析，分析包括全血计数(CBC)，代谢全套(complete metabolic panel,CMP)，禁食脂质套组(fasting lipid panel)——包括总胆

固醇、甘油三酯、HDLc、LDLc、oxLDL、MPO、PAI-1和HbA1c。

[0481] 在此12周试验期间，500、750或1000mg/天小组中没有PC10相关的不利事件报告(N=35)。仅对潜在商品制剂500mg PC10(n-11)评估效力。

[0482] 表15总结了在三个月摄入每日500mg PC10制剂的对象的的变化中值脂质变量(初始-3个月)。标注了11员小组的总胆固醇、总胆固醇/HDL定量、LDL-c(计算)、Apo B和非HDL的统计学显著变化。临床认为7%的总胆固醇变化和10%的LDL-c(计算)变化是有意义的。

[0483] 另外，对HbA1C≥5.5%的对象(其全部具有胰岛素耐受性，并且HOMA值大于2)进行亚组分析。标注了此8对象小组的总胆固醇、总胆固醇/HDL定量、LDL-c(计算)、Apo B、LDL-c(计算)/HDL比例、oxLDL、oxLDL/HDL比例、非HDL、甘油三酯、TG/HDL比例和纤溶酶原激活物抑制剂-1(PAI-1)的统计学显著变化。10%的总胆固醇、10%的LDL-c(计算)、19%的oxLDL、27%的TG和37%的PAI-1的变化是有临床意义的，并且证明了PC10制剂的宽范作用。

[0484] 表15:在三个月摄入每日500mg PC10制剂的、HbA1c升高的所有对象和对象子组中的脂质生物标记物的中值变化

[0485]

	总计（n=11）		HbA1c >5.4（n=8）†	
变量	**中值变化（%变化）**	**P***	**中值变化（%变化）**	**P***
重量	0.0 (0.0)	NS	1.0 (0.0)	NS
总胆固醇	-20 (-7.0)	0.003	-23 (-10)	0.008
HDL	-10 (-3.0)	NS	4.0 (8.0)	NS
胆固醇/HDL	-2.0 (-26)	0.024	-2.5 (-45)	0.016
LDL	-19 (-10)	0.012	-21 (-10)	0.031
oxLDL	**-6.0 (-10)**	**NS**	**-14 (-19)**	**0.047**
APOB	-4.0 (-3.0)	0.037	-7.0 (-4.0)	0.016

[0486]

oxLDL/HDL	**-0.2 (-17)**	**NS**	**-0.3 (-25)**	**0.039**
非HDL	-16 (-7.0)	0.007	-21 (-11)	0.008
甘油三酯	**-24 (-9.0)**	**NS**	**-35 (-27)**	**0.039**
LDL/HDL	**-0.3 (-7.0)**	**NS**	**-0.4 (-10)**	**0.031**
PAI-1	**-4.0 (-20)**	**NS**	**-7.0 (-37)**	**0.047**

[0487] *P值利用中值的Wilcoxon Signed Rank检验、利用从基线至12周的变化比例的对数正态分布来计算。零假设(Null Hypothesis)假定从基线为零起的平均变化。NS=不显著(P>0.05)

[0488] †选定的HbA1c大于5.4的对象亚组；粗体突出了亚组差异。

[0489] 在两组中均特别注意PC10对oxLDL水平的影响，oxLDL水平被很多学者认为是动脉粥样硬化发展的最显著风险因子[Johnston,N.,Jernberg,T.,Lagerqvist,B.,Siegbahn,A.,and Wallentin,L.(2006)Improved identification of patients with coronary artery disease by the use of new lipid and lipoprotein biomarkers,Am J

Cardiol 97,640-645]。对于整个组，有10％的降低，这几乎使对象归在完成时为低风险组。亚组在中度风险下开始试验，并且得到改善——到完成时降至低风险组。冠状动脉疾病发展的重要风险因子的这种降低提供了额外的促进健康衰老的可能。配方中的抗氧化剂组分作用于协助降低oxLDL水平和促进健康胆固醇代谢以提供器官系统保护。

[0490] 这是包含佛手柑的制剂降低oxLDL的首次临床证明。此外，PC10的其余果实和草本组分在本研究所用的剂量下还未被证实降低oxLDL——进一步表明协调的协同作用被并入到PC10和相关制剂的研发中。

[0491] 实施例15

[0492] 对于LDL氧化的离体抑制的PC8和佛手柑(PC10)协同相互作用

[0493] 目的——本实施例的目的是证明对于LDL氧化的离体抑制的各种形式的植物复合物和佛手柑之间的协同相互作用。

[0494] 方法——本方法通过Cu(II)或Pryor和同事[Pryor,W.A.;Cornicelli,J.A.;Devall,L.J.;Tait,B.;Trivedi,B.K.;Witiak,D.T.;Wu,M.A rapid screening test to determine the antioxidants potencies of natural and synthetic antioxidants.J.Org.Chem.1993,58,3521-3532]报道的偶氮引发剂来人工诱导亚油酸或LDL的自氧化。通过234nm的UV吸收监测自氧化的进展。

[0495] 材料——PC8、PC9f、PC9p和佛手柑用于本实施例，并且其组合物如前文以上实施例所述。

[0496] 计算——由均匀围绕中值效果的四个浓度中的最小值计算中值抑制浓度(IC_{50})。如前所述计算联合指数(CI)。

[0497] 结论——PC8+佛手柑、PC9f+佛手柑和PC9f+佛手柑的CI全部大于3.0，表明在预防LDL离体氧化方面的高度协同。这些结果支持实施例14的临床发现，并且突显了该组合的氧化LDL抑制的新颖性。

[0498] 因此，在各种教导制剂中，公开了基于几种配置呈现对于不同氧化剂的协同抗氧化剂活性的果实和草本提取物的新颖方法和组合物。

[0499] 实施例16

[0500] 通过抑制过氧亚硝酸根形成的植物复合物(PC4)

[0501] 本实施例描述了增强如前所述用于氧化应激相关代谢障碍的制剂的功能的配方，通过添加充当巨噬细胞$ONOO^-$产生的协同抑制剂的PC4.1或PC4.2(参见图2)。在氧化应激和炎症增加相关的病理状况(心肌梗塞、缺血性心脏病、心肌炎、心肌病、高血压、肥胖、慢性中毒等)下，NO与超氧基(O_2^-)反应形成$ONOO^-$，$ONOO^-$通过脂质的过氧化、酶和其它蛋白质通过氧化和硝化而失活、以及应激信号传导酶如基质金属蛋白酶和髓过氧化物酶的激活等引起细胞损伤。这种应激信号传导导致原先被设计处理这些状况的多种产品弱化。

[0502] 治疗量PC4.1或PC4.2与具体产品制剂的组合给予作用以缓解氧化应激和提高产品性能。下表中可找到PC4.1或PC4.2以其增强性能有效应用的的制剂实施例。然而，注意，也可在一些实施方式中使用上文以PC8、PC9和PC10描述的其它制剂。

[0503] 表16:具有精氨酸和西瓜提取物的PC4.1制剂

[0504]

项目描述	用量 [mg]
L-精氨酸[粒状]	2500
柠檬酸	2500
红甜菜根	3000
天然柑桔甜味剂[CitriSweet（TM）]	430
苹果酸	400
石榴果实汁浓缩物	375
木糖醇[bulk]	500
二氧化硅[Syloid® 244]	170

[0505]

硫胺素（B1）（一硝酸硫胺素）[91% B1]	110
抗坏血酸钙[83%维生素 C，9% Ca]	95
柑桔掺混物天然调味剂[WONF]	85
Huckle 浆果天然调味剂	85
氧化镁[60% Mg，粉末]	84
甜菊叶提取物	66
苹果果实提取物[75%多酚]	30
西瓜全果实提取物[20%瓜氨酸]	23
维生素 D3（胆钙化醇）[100,000 IU/g，100 SD/S]	16
绿茶叶提取物[80%，无咖啡因]	15
D-核糖	10
葡萄表皮提取物	5
红葡萄多酚提取物[ExGrape（TM）红酒提取物]	5
葡萄种子提取物[MegaNatural®]	5
橄榄叶提取物[12%，7：1]	5
叶酸[10%，磨碎]	3
维生素 B6（盐酸吡哆醇）[82% B6]	3
维生素 B12（氰钴胺）[1%，磨碎]	0.9
菊粉（菊苣根提取物）[HD 食品级]	2500
L-谷氨酰胺	1000

[0506] 表17：用于增加氮氧基产生的具有精氨酸和瓜氨酸的PC4.1制剂

[0507]

项目描述	用量 [mg]
L-精氨酸[粒状]	20
柠檬酸	1000
红甜菜根	3000
天然柑桔甜味剂[CitriSweet（TM）]	300
苹果酸	400
二氧化硅[Syloid® 244]	170
硫胺素（B1）（一硝酸硫胺素）[91% B1]	110
抗坏血酸钙[83%维生素 C，9% Ca]	95
柑桔掺混物天然调味剂[WONF]	85
Huckle 浆果天然调味剂	85

[0508]

氧化镁[60% Mg，粉末]	84
甜菊叶提取物	50
苹果果实提取物[75%多酚]	30
维生素 D3（胆钙化醇）[100,000 IU/g，100 SD/S]	7.5
绿茶叶提取物[80%，无咖啡因]	15
葡萄表皮提取物	5
红葡萄多酚提取物[ExGrape（TM）红酒提取物]	5
葡萄种子提取物[MegaNatural®]	5
橄榄叶提取物[12%，7：1]	5
叶酸[10%，磨碎]	1
维生素 B6（盐酸吡哆醇）[82% B6]	3
维生素 B12（氰钴胺）[1%，磨碎]	0.9
L-瓜氨酸	2500

[0509] 表18:用于增加氮氧基产生的具有精氨酸的PC4.1制剂

[0510]

项目描述	用量 [mg]
L-精氨酸[粒状]	5100
柠檬酸	2000
红甜菜根	2000
天然柑桔甜味剂[CitriSweet（TM）]	430
苹果酸	400
石榴果实汁浓缩物	375
木糖醇[bulk]	250
二氧化硅[Syloid® 244]	170
硫胺素（B1）（一硝酸硫胺素）[91% B1]	110
抗坏血酸钙[83%维生素 C，9% Ca]	95
柑桔掺混物天然调味剂[WONF]	85
Huckle 浆果天然调味剂	85
氧化镁[60% Mg，粉末]	84
甜菊叶提取物	66
苹果果实提取物[75%多酚]	30
西瓜全果实提取物[20%瓜氨酸]	23
维生素 D3（胆钙化醇）[100,000 IU/g，100 SD/S]	16

[0511]

绿茶叶提取物[80%，无咖啡因]	15
D-核糖	10
葡萄表皮提取物	5
红葡萄多酚提取物[ExGrape（TM）红酒提取物]	5
葡萄种子提取物[MegaNatural®]	5
橄榄叶提取物[12%，7：1]	5
叶酸[10%，磨碎]	3
维生素 B6（盐酸吡哆醇）[82% B6]	3
维生素 B12（氰钴胺）[1%，磨碎]	0.9

[0512] 表19:具有植物甾醇的PC4.2制剂

[0513]

项目描述	用量 [mg]
麦芽糊精[M100 IP]	1453
蔗糖素	30
巧克力天然调味剂	467
可可[加工 w/碱，10-12%脂肪]	2333
葵花籽油奶精（乳汁、大豆）[RichmixSun50 HT，ALLERG]	2147
CLA（共轭亚油酸）（乳汁）[Clarinol（TM），ALLERG]	156
酪蛋白钙（包含乳汁）[ALLERGEN]	2213
乳清蛋白浓缩物（乳汁，大豆）[80%速溶化 ALLER]	15625
乳清蛋白分离物（乳汁）[速溶化，ALLERGEN]	8203
黄原胶[Keltrol（TM）Tf]	373
苹果果实提取物[75%多酚]	30
绿茶叶提取物[80%，无咖啡因]	15
葡萄种子提取物[MegaNatural®] / Vitis vinifera	5
橄榄叶提取物[12%，7：1]	5
植物甾醇	2000

[0514] 表20：用于增加氧化LDL还原的PC4.2佛手柑果实提取物制剂

[0515]

项目描述	用量 [mg]
佛手柑橙果实提取物	250
苹果果实提取物[75%多酚]	30

[0516]

绿茶叶提取物[80%，无咖啡因]	15
葡萄种子提取物[MegaNatural®] / Vitis vinifera	5
橄榄叶提取物[12%，7：1]	5
纤维素[羟丙基纤维素，Klucel Nutra® D]	10
硬脂酸镁（植物）	16.25
纤维素[交联羧甲纤维素钠，改性纤维素胶]	40
二氧化硅[Syloid® 244]	3.12

[0517] 表21：用于增强去毒的PC4.2制剂

[0518]

项目描述	用量 [mg]
花椰菜花/ Brassica oleracea var. italica	18
苹果果实提取物[75%多酚] / Malus pumila	1.8
卷心菜叶/ Brassica oleracea var。capitata	0.480
胡萝卜根/ Daucus carota	0.480
亚麻种子/栽培亚麻	36
葡萄种子提取物[MegaNatural®] / Vitis vinifera	0.300
绿茶叶提取物[80%，无咖啡因] / Camellia sinensis	0.900
瓜尔胶[Tico-LV] / Cyamopsis tetragonoloba	36
橄榄叶提取物[12%，7：1] / Olea europaea	0.300
红甜菜根/ Beta vulgaris	0.480
迷迭香叶/ Rosmarinus officinalis	0.480
甜菊叶提取物/ Stevia rebaudiana	1.8
番茄果实/ Solanum lycopersicum	0.480
姜黄根茎/ Curcuma longa	0.480
车前子壳[50] / Plantago ovata	187.920
阿拉伯胶（Talha）/ Acacia seyal	36
苹果果实纤维[40 目]	108
柠檬酸	30
柑桔掺混物天然调味剂[WONF]	10.5
果糖	60
L-谷氨酰胺	150
菊粉（菊苣根提取物）[HD 食品级]	150
叶绿素铜钠	6.0

[0519]

柠檬酸锌[32% Zn，二水合物]	0.240

[0520] 表22：用于增强去毒的PC4.2制剂

[0521]

项目描述	用量 [mg]
花椰菜花	1000
卷心菜叶	8
胡萝卜根	8
红甜菜根	8
迷迭香叶	8
蕃茄果实	8
姜黄根茎	1000
亚麻种子	600
瓜尔胶[Tico-LV]	600
甜菊叶提取物	30
车前子壳[50]	3132
阿拉伯胶（Talha）	600
苹果果实纤维[40 目]	1800
L-谷氨酰胺	2500
果糖	1000
菊粉（菊苣根提取物）[HD 食品级]	2500
苹果果实提取物[75%多酚]	30
绿茶叶提取物[80%，无咖啡因]	15
葡萄种子提取物[MegaNatural®]	5
橄榄叶提取物[12%，7：1]	5
柠檬酸锌[32% Zn，二水合物]	4
柠檬酸	750
柠檬天然调味剂	500

[0522] 表23:用于增强抗氧化剂活性的PC4.2多维生素制剂

[0523]

项目描述	用量 [mg]
生物素[1%磨碎]	4.5
氯化铬（2%）	2.0
葡萄糖酸铜[14% Cu]	3.9
维生素 B12（氰钴胺）[1%，磨碎]	0.4428
纤维素[MCC，Endurance（TM）VE-090]	105
叶酸[10%，磨碎]	0.64
维生素 B6（盐酸吡哆醇）[82% B6]	5.4
核黄素（B2）[100%，S 型]	6.0
硫胺素（B1）（一硝酸硫胺素）[91% B1]	7.9
棕榈酸维生素 A[500,000 IU/gm]	2.7
维生素 D3（胆钙化醇）[100,000 IU/g，100 SD/S]	2.0
纤维素[羟丙基纤维素，Klucel Nutra® D]	10
叶黄素[5%，VG 颗粒]	20
纤维素[交联羧甲纤维素钠，改性纤维素胶]	40
磷酸二钙[无水]	382.5
维生素 C（抗坏血酸）[97%，C97 SF]	155
纤维素[Avicel® PH 200，微晶]	130
B 胡萝卜素[20%，CWD]	8.1
泛酸（D-泛酸钙）[90%]	20.9
维生素 E 700 IU，IP NON GMO（d-α-生育酚乙酸酯）	43
硬脂酸（植物）[Hystrene® NF]	50
氧化镁[60% Mg，粒状]	91
硬脂酸镁（植物）	16.25
烟酰胺[97%，细粒状]	18
叶绿素铜钠	2.5
二氧化硅[Syloid® 244]	3.12
葡萄糖酸锌[13.8% Zn，细粒状]	65
苹果果实提取物[75%多酚]	30
绿茶叶提取物[80%，无咖啡因]	15
葡萄种子提取物[MegaNatural®] / Vitis vinifera	5
橄榄叶提取物[12%，7：1]	5

[0524] 表24：用于增强重量减轻活性的PC4.2膳食替代配方

[0525]

项目描述	配方 1	配方 2	配方 3
份量（g）	46	45	45
卡路里	184	180	173
脂肪（g）	5	3	5
饱和脂肪（g）	2	1	1
反式脂肪（g）	0	0	0
胆固醇（mg）	32	0	0
钠（mg）	104	150	307
钾（mg）	368	95	187
碳水化合物（g）	14	16	16
膳食纤维（g）	5	3	3
糖类（g）	5	9	8
蛋白质（g）	20	20	20
植物甾醇（mg）	2000	2000	2000
维生素 A（IU）	48	75	47
维生素 C（mg）	48	75	47
钙（mg）	96	2	33
铁（mg）	0	0	0
维生素 A（IU）	48	75	47
维生素 D（IU）	0	75	47
维生素 E（IU）	48	0	47
维生素 K（mcg）	0	0	0
硫胺素（mg）	48	75	47
核黄素（mg）	48	75	47
烟酸（mg）	48	75	47
维生素 B6	48	75	47
叶酸盐/酯（作为叶酸和 L-5-甲基四氢叶酸盐/酯）（mcg）	104	75	47
维生素 B12（作为氰钴胺）（mcg）	48	75	47
生物素（mcg）	48	75	47
泛酸（mg）	48	75	47
苹果果实提取物[75%多酚]（mg）	30	30	30
绿茶叶提取物[80%，无咖啡因]（mg）	15	15	15
葡萄种子提取物[MegaNatural®] / Vitis vinifera（mg）	5	5	5

[0526]

橄榄叶提取物[12%，7：1]（mg）	5	5	5
磷（mg）	48	0	20
碘（mcg）	0	75	47
镁（mg）	72	0	33
锌（mg）	0	75	47
硒（mcg）	0	75	47
铜（mg）	0	75	47
锰（mg）	0	75	47
铬（mcg）	176	75	47
钼	0	75	0
氯离子（mg）	0	0	0

[0527] 表25：用于增强抗炎/抗氧化剂活性的PC4.2姜黄素制剂

[0528]

项目描述	用量 [mg]
姜黄根茎提取物（Curcuma longa），相对于95%类姜黄素标准化	550
苹果果实提取物[75%多酚]	30
绿茶叶提取物[80%，无咖啡因]	15
葡萄种子提取物[MegaNatural®] / Vitis vinifera	5
橄榄叶提取物[12%，7：1]	5
纤维素[Avicel® PH 200，微晶]	130
硬脂酸镁（植物）	16.25
二氧化硅[Syloid® 244]	3.12
黑胡椒果实提取物（*Piper nigram*）	0.40

[0529] 表26：用于增强降血糖/抗炎性活性的PC4.2黄连素制剂

[0530]

项目描述	用量 [mg]
黄连素（*Berberis aristata*）	333
苹果果实提取物[75%多酚]	30
绿茶叶提取物[80%，无咖啡因]	15
葡萄种子提取物[MegaNatural®] / Vitis vinifera	5
橄榄叶提取物[12%，7：1]	5
纤维素[Avicel® PH 200，微晶]	130
硬脂酸镁（植物）	16.25

[0531]

二氧化硅[Syloid® 244]	3.12

[0532] 表27：用于增强降脂质活性的PC4.2鱼油软凝胶制剂

[0533]

项目描述	用量 [mg]
鱼油（(380 mg EPA，190 mg DHA）†	1028
苹果果实提取物[75%多酚]	30
绿茶叶提取物[80%，无咖啡因]	15
葡萄种子提取物[MegaNatural®] / Vitis vinifera	5
橄榄叶提取物[12%，7：1]	5
明胶	300.6
水	50.1
天然柠檬油	29.01

[0534] †EPA＝二十碳五烯酸；DHA＝二十二碳六烯酸

[0535] 表28：用于增强心脏保护活性的PCx辅酶Q10制剂

[0536]

项目描述	量 [mg]
辅酶Q10	100
苹果果实提取物[75%多酚]	30
绿茶叶提取物[80%，无咖啡因]	15
葡萄种子提取物[MegaNatural®] / Vitis vinifera	5
橄榄叶提取物[12%，7：1]	5
特级初榨橄榄油	100
蜂蜡	50
明胶	300.6
水	50.1

[0537] 示例性实施方式

[0538] 下列示例性发明实施方式涉及本公开的进一步方面。

[0539] 在一个实施例中，提供了在被给予对象时针对氧和氮的多种生物反应性形式具有治疗效果的组合物，其包括：苹果果实提取物、葡萄种子提取物、绿茶叶提取物和橄榄叶提取物，其中该组合物比等量的、该组合物中任何单个提取物更加有效针对氧和氮的生物活性形式。

[0540] 在一个实施例中，组合物进一步包括蓝莓果实提取物、辣椒果实提取物和葡萄表皮提取物，其中该组合物比等量的、该组合物中任何单个提取物更加有效针对氧和氮的生物活性形式。

[0541] 在一个实施例中，组合物进一步包括佛手柑果实提取物、山竹果实或果皮提取物或其组合，其中该组合物比等量的、该组合物中任何单个提取物更加有效针对氧和氮的生物活性形式。

[0542] 在一个实施例中，组合物进一步包括佛手柑果实提取物。

[0543] 在一个实施例中，组合物进一步包括山竹果实提取物。

[0544] 在一个实施例中，组合物进一步包括山竹果皮提取物。

[0545] 在一个实施例中，组合物进一步包括佛手柑果实提取物与山竹果实提取物或山竹果皮提取物的组合。

[0546] 在一个实施例中，组合物中的提取物每种均等量存在。

[0547] 在一个实施例中，组合物中所有提取物的比例是1:1。

[0548] 在一个实施例中，至少一种提取物的存在量不同于其它提取物。

[0549] 在一个实施例中，治疗对象的氧化应激相关病理的方法包括向对象给予治疗有效量的、上述任何实施例中记载的组合物。

[0550] 在一个实施例中，氧化应激相关病理是氧化LDL胆固醇(oxLDL)增加。

[0551] 在一个实施例中，氧化应激相关病理是代谢综合征、1型糖尿病、2型糖尿病或3型糖尿病中的任一种。

[0552] 在一个实施例中，氧化应激相关病理是漏肠、内毒素血症或炎性肠道疾病中的任

一种。

[0553] 在一个实施例中，氧化应激相关病理是下列中的任一种：肥胖、炎症状况——包括骨关节炎、类风湿性关节炎、克罗恩病、前列腺增生、下尿路症状、肺动脉高血压、运动能力下降、早泄、低女性性欲、充血性障碍、心力衰竭、肺高血压、各种心血管疾病、运动功能障碍、认知障碍——包括阿尔茨海默病、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛、皮肤障碍——包括皮肤皱纹、皮肤变色和皮肤下垂，和DNA氧化损伤引起的癌症。

[0554] 在一个实施例中，以有益于对象的健康的方式调节对象中的疾病相关的蛋白激酶活性的方法包括向对象给予治疗有效量的、上述任一实施例记载的组合物。

[0555] 在一个实施例中，疾病相关的蛋白激酶是选自下列的成员：Abl、ACK1、ALK、Aurora、AMPK、CaMKII、EGFR、EphA、FAK、FGFR、GSK3、IGF-1(激活)、IKK、IR、MAPK1、Met、MTOR、NEK1/2/6、PAK1/4/5/6、PDGFR、PI3K、PKC、ROCKI/II、RSK1/2/34、SRC、Syk和其组合，并且蛋白激酶的调节减少、最小化或抑制对象中氧化LDL(oxLDL)胆固醇的产生或存在。

[0556] 在一个实施例中，疾病相关的蛋白激酶是选自下列的成员：Abl、ACK1、ALK、Aurora、AMPK、CaMKII、EGFR、EphA、FAK、FGFR、GSK3、IGF-1(激活)、IKK、IR、MAPK1、Met、MTOR、NEK1/2/6、PAK1/4/5/6、PDGFR、PI3K、PKC、ROCKI/II、RSK1/2/34、SRC、Syk和其组合，并且蛋白激酶的调节改善代谢综合征、1型糖尿病、2型糖尿病或3型糖尿病中的至少一种。

[0557] 在一个实施例中，疾病相关的蛋白激酶是选自下列的成员：Abl、ACK1、ALK、Aurora、AMPK、CaMKII、EGFR、EphA、FAK、FGFR、GSK3、IGF-1(激活)、IKK、IR、MAPK1、Met、MTOR、NEK1/2/6、PAK1/4/5/6、PDGFR、PI3K、PKC、ROCKI/II、RSK1/2/34、SRC、Syk和其组合，并且蛋白激酶的调节改善漏肠、内毒素血症或炎性肠道疾病中的至少一种。

[0558] 在一个实施例中，疾病相关的蛋白激酶是选自下列的成员：Abl、ACK1、ALK、Aurora、AMPK、CaMKII、EGFR、EphA、FAK、FGFR、GSK3、IGF-1(激活)、IKK、IR、MAPK1、Met、MTOR、NEK1/2/6、PAK1/4/5/6、PDGFR、PI3K、PKC、ROCKI/II、RSK1/2/34、SRC、Syk和其组合，并且蛋白激酶的调节改善下列中的至少一种：肥胖、炎症状况——包括骨关节炎和类风湿性关节炎、克罗恩病、前列腺增生、下尿路症状、肺动脉高血压、运动能力下降、早泄、低女性性欲、充血性障碍、心力衰竭、肺高血压、心血管疾病、运动功能障碍、认知障碍——包括阿尔茨海默病、雷诺现象、特发性高血压、中风、哮喘、多发性硬化、血管炎、阿狄森病、狼疮、甲状腺炎、慢性疲劳综合征、纤维肌痛和皮肤障碍——包括皮肤皱纹、皮肤变色和皮肤下垂。

[0559] 在一个实施例中，疾病相关蛋白激酶是选自下列的成员：Abl、ACK1、ALK、Aurora、AMPK、CaMKII、EGFR、EphA、FAK、FGFR、GSK3、IGF-1(激活)、IKK、IR、MAPK1、Met、MTOR、NEK1/2/6、PAK1/4/5/6、PDGFR、PI3K、PKC、ROCKI/II、RSK1/2/34、SRC、Syk和其组合，并且蛋白激酶的调节导致下列中的至少一种：骨骼肌脂肪酸氧化和肌肉葡萄糖摄取被刺激、肝脂肪酸氧化和酮生成、胆固醇合成、脂肪生成、甘油三酯合成被抑制、脂肪细胞脂解和脂肪生成被抑制和胰β细胞的胰岛素分泌被调节。

[0560] 在一个实施例中，治疗对象的血清脂质障碍或状况的方法包括向对象给予治疗有效量的、上述任一实施例组合物记载的组合物。

[0561] 在一个实施例中，脂质障碍或状况包括LDL异常升高。

[0562] 在一个实施例中，LDL包括氧化LDL(oxLDL)。

[0563] 在一个实施例中，脂质障碍或状况包括HDL异常低。

[0564] 在一个实施例中，最小化对象的血清氧化LDL(oxLDL)的方法包括向对象给予治疗有效量的、上述任一实施例组合物中记载的组合物。

[0565] 因此，公开了协同调节氧化应激和蛋白激酶活性的新型组合物；以及调控氧化应激、疾病相关蛋白激酶活性的方法；和制备这种活性增强组合物的方法。然而，对于本领域技术人员显而易见的是，在没有脱离本公开发明实施方式的精神的情况下，可做出明显性质的各种改变和改动，并且所有这种改变和改动均被认为落入包括所附权利要求书在内的本文限定的本发明的范围。这种改变和改动的实例可包括但不限于，被加入以影响胶囊、片剂、粉末、洗液、食品或小吃制造方法的的初期成分以及维生素、调味剂和载体。这种改变或改动的其它实例包括使用包含上文公开的优选实施方式的组合的草本或其它植物性产品。

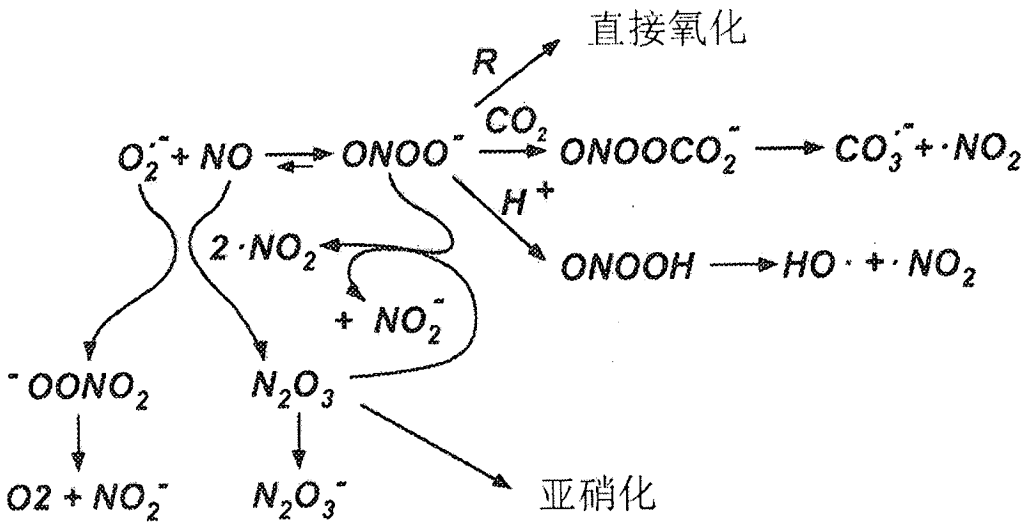

直接氧化
R
CO2
O2·- + NO
ONOO-
ONOOCO2-
CO3·- + ·NO2
H+
2 ·NO2
ONOOH
HO· + ·NO2
+ NO2-
-OONO2
N2O3
O2 + NO2-
N2O3-
亚硝化

图1

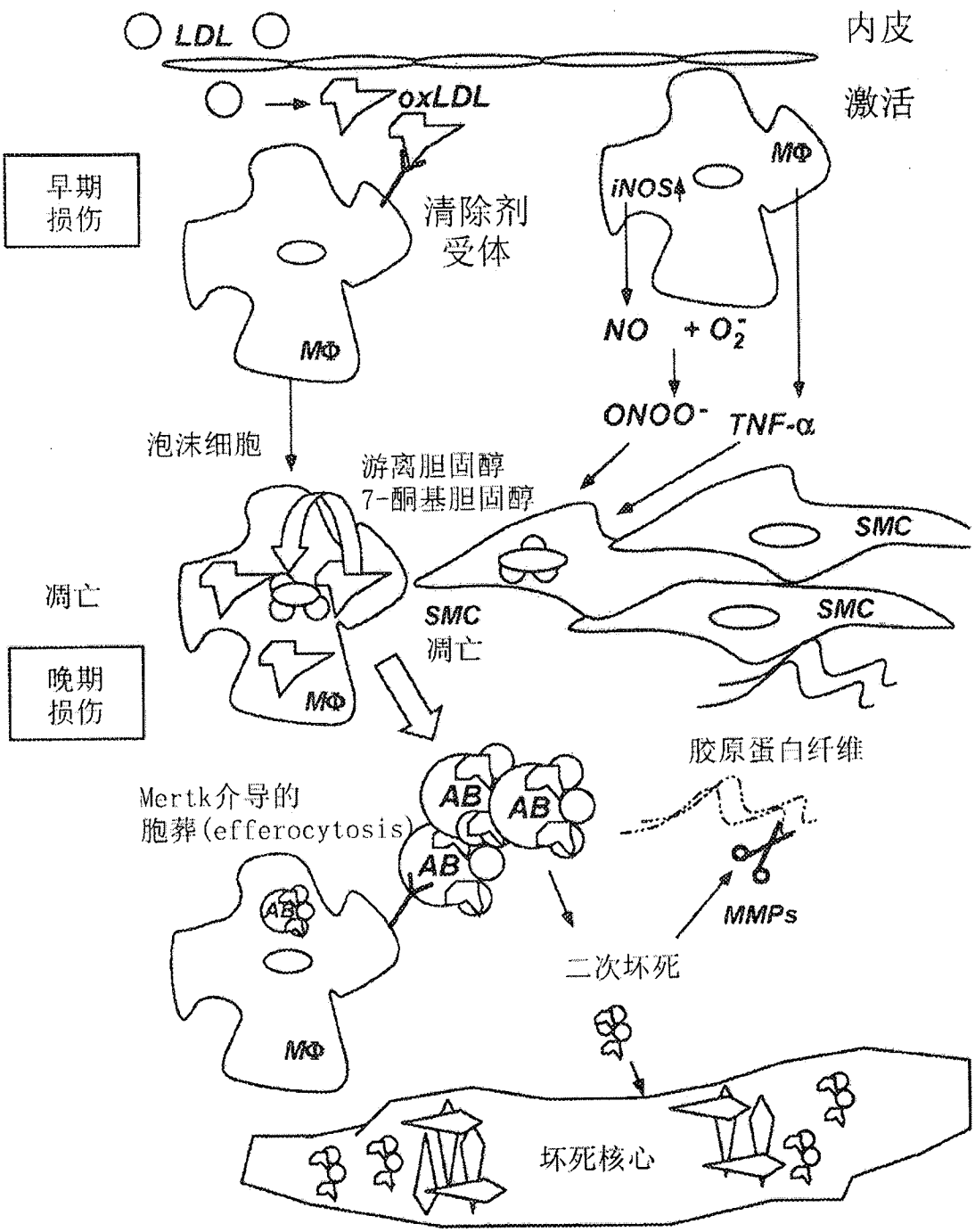

LDL
内皮
激活
oxLDL
早期
损伤
清除剂
受体
MΦ
iNOS
NO + O_2^-
ONOO⁻
TNF-α
泡沫细胞
游离胆固醇
7-酮基胆固醇
SMC
凋亡
SMC
凋亡
晚期
损伤
胶原蛋白纤维
Mertk介导的
胞葬(efferocytosis)
AB
MMPs
二次坏死
坏死核心

图2

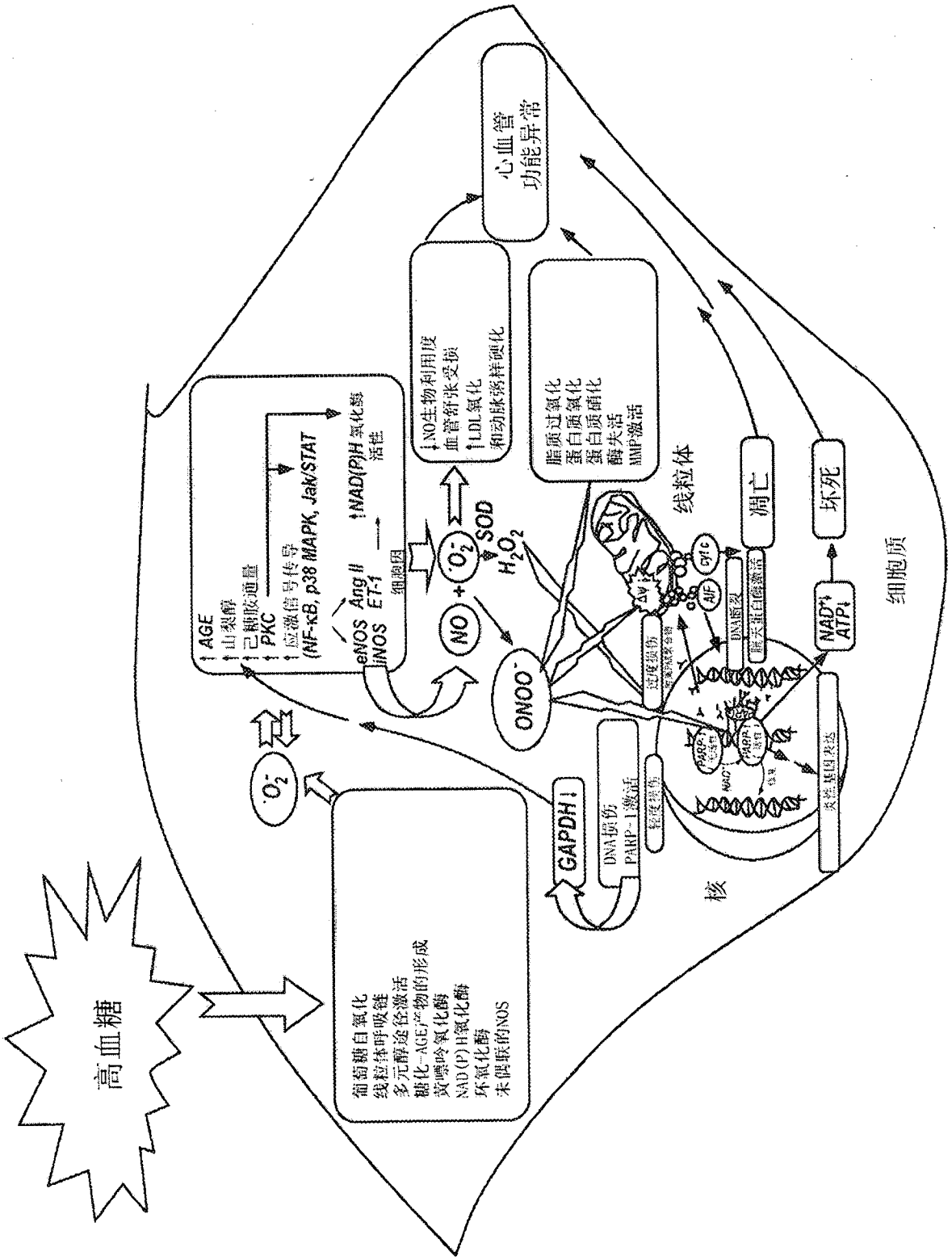

高血糖
葡萄糖自氧化
线粒体呼吸链
多元醇途径激活
糖化-AGE产物的形成
黄嘌呤氧化酶
NAD(P)H氧化酶
环氧化酶
未偶联的NOS
↑AGE
↑山梨醇
↑己糖胺通量
↑PKC
↑应激信号传导
(NF-κB, p38 MAPK, Jak/STAT
eNOS Ang II
iNOS ET-1
细胞因
↑NAD(P)H氧化酶活性
SOD
NO
ONOO⁻
↓NO生物利用度
血管舒张受损
↑LDL氧化
和动脉粥样硬化
心血管功能异常
脂质过氧化
蛋白质氧化
蛋白质硝化
酶失活
MMP激活
线粒体
GAPDH↓
DNA损伤
PARP-1激活
核
细胞质
DNA断裂
凋亡蛋白酶激活
凋亡
坏死
NAD⁺
ATP↓

图3

Abstract

PHYTOCOMPLEXES EXHIBITING MULTIPLE, SYNERGISTIC ANTIOXIDANT ACTIVITIES USEFUL IN FOODS, DIETARY SUPPLEMENTS, COSMETICS AND PHARMACEUTICAL PREPARATIONS

Compositions comprising apple, grape, green tea, and olive extracts are presented herein. This synergistic formulations apple, grape, green tea, and olive extract are in amounts that provide a greater antioxidant activity or protein kinase modulating activity than provided by an equivalent amount of any one extract or a sum of the extracts. Further presented are methods of regulating oxidative stress, disease-associated protein kinase activity, and enhancing the therapeutic effect of a primary therapeutic agent. Also presented are methods of making an activity enhancing composition for regulating oxidative stress, disease-associated protein kinase activity, and enhancing the therapeutic effect of a primary therapeutic agent.

摘 要

可用于食品、饮食补充剂、化妆品和药物制剂的呈现多种协同抗氧化剂活性的植物复合物

本文提出了包括苹果、葡萄、绿茶和橄榄提取物的组合物。在该协同制剂中，苹果、葡萄、绿茶和橄榄提取物的用量是提供的抗氧化剂活性或蛋白激酶调节活性大于等量任一提取物或提取物总和所提供的抗氧化剂活性或蛋白激酶调节活性的用量。进一步提出了调控氧化应激、疾病相关的蛋白激酶活性和增强主要治疗剂的治疗效果的方法。还提出了制备用于调控氧化应激、疾病相关的蛋白激酶活性和增强主要治疗剂治疗效果的活性增强组合物的方法。