Title: 7-HYDROXYFRULLANOLIDE AND ITS ANALOGS FOR PREVENTION, CONTROL AND TREATMENT OF METABOLIC DISORDERS

Abstract: The invention discloses biologically active ingredient(s) selected from 7-hydroxyfrullanolide, its analogs, the extracts and fraction(s) standardized to 7-hydroxyfrullanolide or its analogs or both or mixtures thereof or their compositions for the prevention, control and treatment of one or more obesity, overweight, metabolic syndrome, diabetes and other metabolic disorders or for producing lean body mass in a warm blooded animal in need thereof.
7-HYDROXYFRULLANOLIDE AND ITS ANALOGS FOR PREVENTION, CONTROL AND TREATMENT OF METABOLIC DISORDERS

Field of Invention:
The present invention discloses biologically active ingredient(s) comprising at least one component selected from 7-hydroxyfrullanolide, its analog(s) or the herbal extract(s) and fraction(s) standardized to 7-hydroxyfrullanolide or its analog(s) or mixtures thereof as a biologically active ingredient or their compositions, optionally containing one or more of pharmaceutically or dietetically acceptable diluents, vehicles, carriers and actives or mixtures thereof for the prevention, control and/or treatment of one or more of the metabolic disorders selected from metabolic syndrome, obesity, diabetes, endothelial dysfunction and other disease indications related thereto.

The invention also relates to the amelioration of one or more of the biological marker proteins or metabolic processes related to metabolic syndrome, obesity and other metabolic disorders by 7-hydroxyfrullanolide or its analog(s) or the extract(s)/fraction(s) standardized to 7-hydroxyfrullanolide or its analog(s) or mixtures thereof or their compositions.

Background of the invention:
Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems. Body mass index (BMI), a measurement which compares weight and height, defines people as overweight (pre-obese) when their BMI is between 25 kg/m² and 30 kg/m², and obese when it is greater than 30 kg/m². Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, breathing difficulties during sleep, certain types of cancer, and osteoarthritis. It is a leading preventable cause of death worldwide, with increasing prevalence in adults and children, and authorities view it as one of the most serious public health problems of the 21st century.

Metabolic Syndrome also known as Syndrome X, insulin resistance syndrome and DysMetabolic Syndrome is a condition, wherein a group of diseased states, which
increase atherosclerosis, stroke and diabetes. It was first described by Reaven in 1988 as a cluster of interrelated common clinical disorders, including obesity, insulin resistance, glucose intolerance, hypertension and dyslipidemia.

A criteria for diagnosing Metabolic Syndrome was established by The Adult Treatment Panel-III (ATP-III) of the National Cholesterol Education Program in 2001. Five Criteria were selected by this Panel to identify individuals with Metabolic Syndrome including abdominal obesity, impaired fasting glucose, high triglyceride (TG), low HDL cholesterol (HDL-C) concentrations and increased blood pressure. Metabolic Syndrome is diagnosed, if any three of the components are present in an individual.

A lot of research is being carried out over a decade to develop agents to control Metabolic Syndrome. The application of metabolic markers for the control of this syndrome has also been attempted.

Based on the information cited above and several other documents, the inventors of the present invention have felt the need for the development of an effective compound or composition which can efficiently be used for the prevention, control and treatment of obesity, metabolic syndrome and several other associated and related diseases.

3a-hydroxy-5a,9-dimethyl-3-methylene-3a, 4, 5, 5a, 6, 7, 8, 9β-octahydro-3H-naphthol [1,2-b] furan-2-one (7-a-Hydroxy-4,11(13)-eudesmadien-12,6-olide or 7-hydroxyfrullanolide) is a natural compound isolated from the flower heads of Sphaeranthus indicus. 7-hydroxyfrullanolide (7-HF) is a sesquiterpene compound.

None of the published literature describes anti-obese or anti-metabolic syndrome potential of 7-HF. The amelioration of biomarkers or biological processes related to metabolic disorders such as obesity, metabolic syndrome and other disease conditions associated with metabolic syndrome by 7-hydroxyfrullanolide also not known.

Some of the Patent literature of Sphaeranthus is quoted below:
US patent US7635494 relates to a novel herbal composition comprising an extract of flowering and fruiting heads of the plant, *Sphaeranthus indicus*. The said extract of *Sphaeranthus indicus* contains a compound, 3a-hydroxy-5a,9-dimethyl-3-methylene-3a,4,5,5a,6,7,8,9b-octahydro-3H-naphtho[1,2-b]furan-2-one (7-Hydroxy-4,11(13)-eudesmadien-12,6-olide/7-hydroxyfrullanolide) (compound 1), as a bioactive marker. The application also relates to a composition comprising 3a-hydroxy-5a,9-dimethyl-3-methylene-3a,4,5,5a,6,7,8,9b-octahydro-3H-naphtho[1,2-b]furan-2-one (compound 1) as an active ingredient, methods of manufacture of the said compositions, methods of administration of the said compositions to a subject in need of treatment for an inflammatory disorder. The publication also disclosed tumor necrosis factor-a (TNF-a) and interleukin (IL-1, IL-6, IL-8) inhibitory activity of the said compositions.

PCT Publication WO07036900A2 relates to a novel herbal composition comprising an extract of flowering and fruiting heads of the plant, *Sphaeranthus indicus* containing 3a-hydroxy-5a,9-dimethyl-3-methylene-3a,4,5,5a,6,7,8,9b-octahydro-3H-naphtho[1,2-b]furan-2-one (7-Hydroxy-4,11(13)-eudesmadien-12,6-olide), as a bioactive marker and relates to methods of manufacture of the said compositions.

PCT Publication WO06016228A2 relates to a compound or group of compounds present in an active principle derived from plants of the species *Sphaeranthus*, for the preparation of pharmaceutical formulations or food supplements for the prophylaxis and/or treatment of tumor diseases. The said invention furthermore relates to a novel method for the isolation of an active principle from *Sphaeranthus* plant parts which are effective in prophylaxis and/or treatment of cancers.

According to our knowledge, there is no prior art relating to the usage of 7-hydroxyfrullanolide or its analogs and their compositions for the amelioration of metabolic markers or for the control, prevention and treatment of diseased conditions associated with or related to obesity, metabolic Syndrome and other metabolic disorders.

**Summary of the invention:**

In the primary embodiment, the invention provides biologically active ingredient(s) comprising at least one component selected from 7-hydroxyfrullanolide, its analog(s);
extract(s) and fraction(s) containing 7-hydroxyfrullanolide or its analog(s) or both; or mixtures thereof for the prevention, control and/or treatment of one or more metabolic disorders.

In another embodiment the invention provides biologically active composition comprising at least one component selected from the list comprising 7-hydroxyfrullanolide, its analog(s); the extract(s) or fraction(s) containing 7-hydroxyfrullanolide/its analog(s) or both; or mixture(s) thereof as an active in combination with one or more ingredients selected from other biologically active components derived from plants, animals and microorganisms; pharmaceutically or dietetically acceptable active ingredients, vitamins, amino acids, minerals, vehicles, carriers and diluents or mixtures thereof for the prevention, control and/or treatment of one or more metabolic disorders.

In yet another embodiment, the invention provides biologically active ingredient(s) or their composition(s) for the amelioration of the expression/production of one or more biological marker proteins related to metabolic disorders.

In yet another embodiment, the invention provides compositions comprising at least one component selected from 7-hydroxyfrullanolide, its analogs, the extract(s) and fraction(s) standardized to 7-hydroxyfrullanolide or its analogs or mixtures thereof as an active ingredient and at least one component selected from pharmaceutically or dietetically acceptable phytochemical actives, plant extracts, diluents, vehicles, carriers and actives or mixtures thereof for the control, prevention and treatment of metabolic disorders, which include but not limited to metabolic syndrome or obesity, and/or one or more disease indications related to or associated with metabolic syndrome.

In still another embodiment, the invention provides pharmaceutical or dietary supplement or food ingredient selected from 7-hydroxyfrullanolide, its analog(s) and the extract(s) and fraction(s) standardized to 7-hydroxyfrullanolide alone or its analogs or mixtures thereof or their composition(s) for the amelioration of the expression or production of one or more biological marker proteins related to or associated with metabolic syndrome, obesity and other disease conditions associated with metabolic syndrome including but
not limited to Peroxisome proliferator-activated receptor gamma (PPARγ), Adipose Differentiation Related Protein (ADRP), adipocyte CD36, Macrophage CD36, Monocyte Chemotactic protein (MCP-1), Oxidized LDL (Ox-LDL), adipocyte fatty-acid-binding protein (aP2/FABP4/A-FABP), beta-3 Adrenergic Receptor (P3AR), Perilipin, Adiponectin, Protein tyrosine phosphatase-IB (PTP-1B), Metalloproteinase-1 (MMP-1), Matrix Metalloproteinase-3 (MMP-3) and Matrix Metalloproteinase-13 (MMP-13).

In the other embodiment, the invention provides method(s) for the prevention, control and/or treatment of metabolic disorders, which include but not limited to obesity, overweight, diabetes, arteriosclerosis, cardiovascular diseases, hypertension, hypercholesteremia, hyperlipidemia, triglyceridemia, metabolic syndrome, endothelial dysfunction and other metabolic disorders in warm blooded animals, wherein the method comprises of administering to a warm blooded animal in need thereof an effective amount of a component selected from 7-hydroxyfrullanolide, its analog(s), the extract(s) and fraction(s) containing 7-hydroxyfrullanolide alone or its analogs or mixtures thereof or their composition(s).

**Brief Description of Figures:**

**Figure I:** Illustration of anti-adipogenic activity of 7-hydroxyfrullanolide (7-HF). 7-HF decreases the lipid content in mouse adipocytes by inhibiting adipogenesis process. Photomicrographs show lipid accumulation in Oil Red O stained 3T3-L1 adipocytes treated with either 0.1% DMSO as the vehicle control, or 1 µg/ml 7-HF. Arrows indicate the lipid vesicles in the cytoplasmic compartment of adipocytes.

**Figure II:** Illustration of prolipolytic activity of 7-hydroxyfrullanolide (7-HF). Bar diagram represents 7-HF increases glycerol release into the cell culture supernatant in a dose dependent manner. Equal number of 3T3-L1 mouse pre-adipocytes was differentiated and maintained in post differentiation medium to obtain mature adipocytes. The mature adipocytes were treated with 2.5 and 5.0 µg/ml of 7-HF for 2 h. Lypolytic potential of 7-HF was evaluated by measuring the released glycerol in the culture supernatant by Glycerol assay reagent. Each bar represents the mean ± SD of released glycerol (n= 6). P value of <0.05 was considered as statistically significant (Students t test).
**Figure III:** Amelioration of marker proteins of Adipogenesis and lipolysis by 7-hydroxyfrullanolide (7-HF). Figure illustrates modulation of marker proteins of adipogenesis and lipolysis processes by 7-HF in 3T3-L1 adipocytes. Representative immuno blots indicate down-regulation of various marker proteins such as PPARy, ADRP, CD36, aP2, and perilipin. The 3T3-L1 mouse pre-adipocytes were allowed to differentiate in absence or presence of 1 µg/ml of 7-HF. Vehicle control cultures received only similar concentrations of DMSO. Expression of actin protein was evaluated in each blot as the internal control. Expression of each protein was measured densitometrically and normalized with actin expression. The comparative levels protein expressions are represented as bar diagrams (side panels).

**Figure IV:** Illustrates Representative photomicrographs show 7-HF inhibits lipid accumulation in high glucose induced macrophage cells of an in vitro model of atherosclerosis. The J774 mouse macrophage cells were exposed to high glucose (600 mg/dL) for 5 days in presence or absence of 1 µg/ml of 7-HF. Control cultures (A) received low glucose (100 mg/dL). B and C represent the macrophage cells supplemented with 600mg/dL of glucose alone or in combination with 1 µg/ml of 7-HF, respectively.

**Figure V:** Illustration of the down-regulation of high glucose induced CD36 expression in macrophage cells 7-HF. The J774 mouse macrophage cells were exposed to high glucose (600 mg/dL) for 5 days in presence or absence of 1 µg/ml of 7-HF. Control culture received low glucose (100 mg/dL). Representative immuno-blot assay demonstrates down regulation of CD36 protein and expression of actin protein is considered as the internal control. Bar diagram shows the CD36 expression normalized with actin protein (lower panel).

**Figure VI:** Bar diagram represents increased nitric oxide production in human endothelial cells induced by 7-HF in a dose dependent manner. Equal number of human endothelial cells was treated with various concentrations of 7-HF (0.1, 0.25, 0.50, 1.00 ng/ml respectively) as indicated for 24h. The control cultures received 0.01% (v/v) DMSO as the vehicle. Culture supernatants were collected and the nitrite concentrations
were estimated quantitatively by Griess reagent. Each bar represents mean ± SD of nitrite concentration (µM) (n=5). * indicates significance, p < 0.01 (vs. control).

Figure VII: Bar diagram represents inhibition of PTP-1B activity in 3T3-L1 cells. 7-HF inhibits PTP-1B activity in 3T3-L1 cells in a dose dependent manner. Equal number of 3T3-L1 mouse pre-adipocytes was treated with either 1.0 or 2.5 Dg/ml of 7-HF for 48 h. Cells were treated with 50 µM Na2V0₃ as a positive control. PTP-1B activity in cell lysates was analyzed based on the hydrolysis of p-nitrophosphyl phosphate (pNPP) substrate. Each bar represents the mean ± SD of enzyme activity in pmol/min/Dg of cell lysate protein (n=5). * indicates significance at P < 0.05 (vs. control), and # indicates P < 0.001 (vs. control).

Figure VIII: Representative immunoblot showing over expression of adiponectin protein in 3T3-L1 adipocytes treated with 1 µg/ml of 7-hydroxyfrullanolide (7-HF). Protein expressions were densitometrically analyzed and normalized with the actin expression. Bar diagram in each panel shows normalized protein expressions in arbitrary units. In bar diagrams, the bars represent protein expressions in cells treated with vehicle control (a) and 7-HF (b).

Figure IX: Figure represents the natural analogs of 7-hydroxyfrullanolide isolated from Sphaeranthus indicus.

Figure X: Figure represents the semi-synthetic analogs of 7-hydroxyfrullanolide.

Figure XIA: Bar diagrammatic representation of body mean weight gain in HFD induced metabolic syndrome model of SD rats supplemented without (1) or with (2) ethyl acetate extract of Sphaeranthus indicus (SIE) from week-1 to week-8 of treatment. Each bar represents mean ± SD, *p < 0.05.

Figure XIB: Line diagrammatic representations of body weight gain in diet induced metabolic syndrome model of SD rats supplemented with (2) or without (1) 7-hydroxyfrullanolide. Each line indicates change in mean body weight gain during eight-week treatment period.
Figure XII: Bar diagrammatic representation of increase in serum adiponectin concentration in diet induced metabolic syndrome model of Sprague Dawley rats. Each bar indicates mean ± SD of serum adiponectin concentration at 0 day and after 56 days of treatment with either vehicle (1) or ethyl acetate extract of Sphaeranthus indicus (SIE) (2) as indicated in the diagram. N=6, * indicates statistical significance (t-test, 8 weeks vs. 0 week).

Figure XIII: Bar diagrammatic representation of reduction of HOMA Index in ethyl acetate extract of Sphaeranthus indicus (SIE) supplemented metabolic syndrome model of Sprague Dawley rats. Each bar indicates mean ± SD of HOMA Index (arbitrary units) at 0 week and at 8 weeks of supplement with either vehicle (1) or 250 mg/kg of SIE (2). N=6; * indicates statistical significance (t-test, SIE group vs. control at 8 weeks).

Detailed description of the invention:
Obesity is excess body weight for a particular age, sex and height as a consequence of imbalance between energy intake and energy expenditure. The primary causes of obesity are either due to overeating, inadequate exercise or eating disorder, some genetic disorders, underlying illness (e.g. hypothyroidism), certain medications or sedentary lifestyle. Obesity increases the risk of many diseases and health conditions such as hypertension, dyslipidemia (for example, high total cholesterol or high levels of triglycerides), type 2 diabetes, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep disorders, respiratory problems, tumors (endometrial, breast, and colon), arteriosclerosis and heart failure.

Metabolic syndrome is a condition involving a set of disorders that enhances the risk of heart disease. The major components of metabolic syndrome are excess weight, the cardiovascular parameters (high blood pressure, dyslipidemia, high levels of triglycerides and low levels of HDL in the blood), atherosclerosis, diabetes and insulin resistance. A subject suffering with several of these components, i.e. metabolic syndrome is highly prone to heart disease, though each component is a risk factor. Even though several classes of drugs are available in the market for the treatment of different components of Metabolic Syndrome and many of them are associated with a number of side effects, very few medicines are available to treat Metabolic Syndrome and none of them are
comprehensive in addressing all the associated diseases. Hence there exists a great medicinal need for developing agents for the prevention, control and treatment against metabolic syndrome, obesity, diabetes, hypertension and atherosclerosis especially using safe and beneficial natural compounds.

Keeping this in mind the inventors have conducted extensive research investigation involving several in vitro and in vivo experiments on several plant extracts, fractions and pure compounds and found surprisingly that administration of 7-hydroxyfrullanolide (7-HF) in a therapeutically effective amount in cell based studies potently ameliorated metabolic processes which include inhibition of adipogenesis and promotion of adipolysis (lipolysis). In addition, 7-HF was also found to potently modulate the expression and production of many bioactive protein molecules related to different components of metabolic disorders such as obesity and metabolic syndrome. These unexpected aspects of the present invention are summarized below.

![7-Hydroxyfrullanolide](image)

Modulation of metabolic processes by 7-Hydroxyfrullanolide (7-HF)

**Adipogenesis**: Adipogenesis is the differentiation and proliferation of pre-adipocytes into major adipocytes or fat cells and it has been one of the most intensely studied models of cellular differentiation. In the adipogenesis process, proliferation of preadipocytes or precursor fat cells is followed by the differentiation of these cells into mature adipocyte phenotype. The nuclear receptor PPAR-γ is expressed predominantly in adipose tissue, where it is known to play a critical role in adipocyte differentiation and fat deposition. Adipocytes secrete proteins exhibiting either beneficial (leptin, adiponectin) or deleterious effects (angiotensinogen). A disturbance in the balance between these various secreted factors, in association with the effect of secretory products from macrophages (cytokines), leads to the development of metabolic syndrome.
Lipolysis: Lipolysis is the breakdown of stored lipid in adipocytes. p3-Adrenoreceptor agonists can stimulate lipolysis in the white adipose tissue and thermogenesis in the brown adipose tissue. Adipose tissue lipolysis is the catabolic process leading to the breakdown of triglycerides stored in fat cells and release of fatty acids and glycerol. The proteins involved in the lipolytic process constitute drug targets for the treatment of obesity and the metabolic syndrome.

Thus the phytochemical agents having the adipogenesis and lipolysis activities could be useful in the treatment of obesity, metabolic syndrome and other metabolic disorders.

We have disclosed the compositions and synergistic compositions of Sphaeranthus indicus in our earlier Indian provisional application no. 224/CHE/2009 filed on February 2nd, 2009 and PCT application no. PCT/TN2010/000053 filed on February 1st, 2010. The ameliorative effect of biomarkers expression such as PPAR-γ, ADRP, CD 36, aP2, β3AR and Perilipin by Sphaeranthus indicus ethanol extract (SIE) along with 7-hydroxyfrullanolide (LI054A01) was also disclosed.

In a cell based assay, 7-HF potently inhibited lipid accumulation in 3T3-L1 human adipocyte cells as depicted in Figure I. 7-Hydroxyfrullanolide (1) exhibited 52.5% inhibition of lipid accumulation at 1 μg/ml concentration in 3T3-L1 Human pre-adipocyte cells in a cell based in vitro assay. In addition, 7-HF also inhibited lipid accumulation in high glucose induced J774 mouse macrophage cells of an in vitro model of atherosclerosis as depicted in Figure IV. Further, 7-Hydroxyfrullanolide potently enhanced lipolysis in 3T3-L1 Human pre-adipocyte cells. 7-HF showed 47.8% increase in lipolysis at 5 μg/ml concentration in an in vitro cell based assay.

Amelioration of biologically active metabolic biomarker protein by 7-HF:
Adipocytes and macrophages play important role in the pathogenesis of metabolic syndrome and disease components associated with it. They both share a number of common features, including the ability to phagocytize and kill microorganisms and to secrete cytokines such as tumor necrosis factor (TNF) and interleukin-1(IL-1). Critical transcription factors in adipocytes involved in regulating the expression of cytokines,
inflammatory molecules, and fatty acid transporters are also expressed and have similar biologic roles in macrophages. The adipocytes, in addition to accumulating fat during the obesity development, produce and circulate several low molecular weight bioactive protein molecules having powerful effects throughout the body. These protein markers are related to different components of metabolic disorders such as obesity and metabolic syndrome. The expression and production of several of these metabolic markers, which include but not limited to PPAR-γ, Adipose Differentiation Related Protein (ADRP), CD36, Adipocyte Fatty-Acid-Binding Protein (aP2/FABP4/A-FABP), Beta-3 adrenergic receptor (P3-AR), adiponectin and Perilipin, become abnormal during obesity and metabolic syndrome and other disease conditions associated with metabolic syndrome.

A brief description of some of the metabolic biomarker molecules that are involved in the pathogenesis and control of metabolic syndrome and the disease conditions associated is outlined below:

**Peroxisome proliferator-activated receptor-γ (PPAR-γ):** Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a nuclear receptor that plays a pivotal role in obesity and diabetes. An increase in adipose tissue mass can be the result of the production of new fat cells through the process of adipogenesis and the deposition of increased amounts of cytoplasmic triglyceride or lipid droplets per cell. In the adipogenesis process, proliferation of preadipocytes or precursor fat cells is followed by the differentiation of these cells to the mature adipocyte phenotype. PPAR-γ is expressed predominantly in adipose tissue, wherein it is known to play a critical role in adipocyte differentiation and fat deposition.

**Adipose differentiation related protein (ADRP):** ADRP is a 50 kD protein and its mRNA (ADRP mRNA), which is 1.7 Kb in size, is expressed at high level in adipose tissue. The expression of ADRP is very low in undifferentiated adipocytes, but ADRP mRNA reaches 50 to 100-fold in few hours after the onset of adipose differentiation process. The above thus indicate the possible role of ADRP in the formation or stabilization of lipid droplets in adipocytes and other cells. ADRP specifically enhances uptake of long chain fatty acids by adipose tissue. Hence ADRP is an important target to identify the
compounds that can potentially control obesity and diabetes through regulation of the expression of ADRP.

Adipocyte CD36: CD36 is a common protein marker expressed by both adipocytes and macrophages. The CD36 expressed in adipocytes is known to function as a fatty acid transporter (FAT). It is a scavenger receptor that binds and internalizes oxidized LDL (Ox LDL) in macrophages. CD36 also functions as a long-chain fatty acid (LCFA) transporter to facilitate the uptake of LCFAs in adipocytes. The CD36 expression is up-regulated by PPAR during the differentiation of both types of cells. It is also shown that the adipocytes can endocytose and lysosomally degrade Ox LDL, a process mainly mediated by CD36. The CD36 null animals thus found to show significant decrease in binding and uptake of oxidized low density lipoprotein and showed significant increase in fasting levels of cholesterol, nonesterified free fatty acids, and triacylglycerol.

Fatty-Acid-Binding Protein (aP2/FABP4): FABPs are molecular chaperones linked to metabolic and inflammatory pathways. Different members of the FABP family exhibit unique patterns of tissue expression/distribution and are expressed most abundantly in tissues involved in active lipid metabolism. FABPs play numerous functions. As lipid chaperones, for example, FABPs may actively facilitate the transport of lipids to specific compartments in the cell, such as to the lipid droplet for storage; to the endoplasmic reticulum for signaling, trafficking and membrane synthesis; to the mitochondria or peroxisome for oxidation. A-FABP is abundantly present in human serum and it may play a central role in the development of major components of the metabolic syndrome such as obesity, type 2 diabetes and cardiovascular diseases, through its distinct actions in adipocytes and macrophages. Hence aP2 is an important marker for metabolic disorders.

Perilipin: Perilipin is a protein that forms a coating around the lipid droplets in adipocytes. It is a protective coating against body’s natural lipases, such as hormone-sensitive lipase, that breaks triglycerides into glycerol and free fatty acids by a process called lipolysis. Perilipin [PLIN] may play key role in obesity. Following β-adrenergic receptor activation, protein kinase A (PKA) hyperphosphorylates perilipin localized at the surface of the lipid droplet. Phosphorylated perilipin changes conformation and translocate away from the lipid droplet, exposing the stored lipids to hormone-sensitive
lipase-mediated hydrolysis of triglycerides (lipolysis) to release non-esterified fatty acids (NEFA). Perilipin is thus an important regulator of lipid storage, lipolysis and energy balance and is an important target for developing anti-obesity drugs.

The inventors have thus evaluated the efficacy of 7-hydroxyfrullanolide in the modulation of the above metabolic biomarkers that are primarily responsible for the adipogenesis processes, insulin resistance in type 2 diabetes, obesity, metabolic syndrome and other metabolic disorders using an immunoblot assay in 3T3-L1 adipocytes. It was found surprisingly that 7-hydroxyfrullanolide potently ameliorated the levels of several adipocyte differentiation markers such as Peroxisome proliferator-activated receptor gamma (PPARγ), ADRP, CD36, Fatty Acid Binding Protein 4 (aP2/FABP4) and intracellular lipid droplet surface associated protein (perilipin) (Figure III) in a dose dependent manner. This unexpected result confirms the potential of 7-HF to control, prevent and treat metabolic disorders through modulating multiple disease targets.

**Efficacy of 7-hydroxyfrullanolide in the improvement of cardiovascular health**

**Macrophage CD36:** CD36 is a prototypic member of the class B scavenger receptor family. It is widely expressed on the surface of monocytes and macrophages, and mediate uptake of oxidized low-density lipoprotein (Ox-LDL) as well as play a role in diverse cellular processes including foam cell formation, fatty acid transport, engulfment and removal of senescent cells, suppression of angiogenesis, and cell-matrix interactions. As such it can be an important risk factor of cardiovascular disease and a potential molecular maker of atherosclerosis. Hyperglycemia-induced synthesis of CD36 in macrophages has been associated with increased uptake of Ox-LDL by macrophages and foam cell formation in atherosclerotic lesions in people with diabetes.

Inhibition of CD36 protein expression in high glucose induced J774 macrophage cells in presence or absence of 7-HF was evaluated using immunoblot assay and the results are summarized in **Figure V**. The CD36 levels were significantly enhanced in the cells treated with glucose. However, these levels were reduced back towards their base values in the cells treated with 7-HF when compared to the untreated control cells. This unexpected observation provides support in favor of the potential use of 7-HF for the prevention, control and treatment of cardiovascular diseases.
Nitric oxide (NO): Nitric oxide (NO) is a key biological molecule that, either directly or through intracellular signaling, stimulates host defenses in the immune system, maintains blood pressure in the cardiovascular system and modulates neural transmission in the brain. NO is an activator of soluble guanylyl cyclase, which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) and leads to vasodilatation and inhibition of leukocyte and platelet activation. As the biologically active component of endothelium-derived relaxing factor, NO plays critical roles in the maintenance of vascular homeostasis. Hence, compounds that enhance the NO levels can have potential health benefits in humans. However, the volatile nature of NO makes it unsuitable for most detection methods. In the cell, NO undergoes a series of reactions with several molecules present in biological fluids and is eventually metabolized to nitrite (NO$_2^-$) and nitrate (NO$_3^-$). Hence measuring the nitrite level gives an indication of nitric oxide. The ability of 7-HF to modulate the NO level was evaluated in a human endothelial (HE) cellular model and found surprisingly significant and dose dependent increase in NO concentration in cell culture supernant following administration of the HE cells with 7-HF. The data is summarized in Figure VI.

Inhibition of Protein tyrosine phosphatase 1B (PTP-IB) by 7-HF:
Resistance to the hormone insulin is the hallmark of type 2 diabetes and obesity. Protein tyrosine phosphatase 1B (PTP-IB) is regarded as a physiological negative regulator of insulin signal transduction in insulin sensitive cells such as adipocytes, muscle cells and hepatocytes. In insulin resistant diabetes and obesity, the PTB-1B is over expressed and its enzyme activity is increased. Over expression of PTP-IB decreases insulin receptor and IPs-1 Phosphorylation and hence produces insulin resistance. Silencing of PTP-IB gene in an animal study astonishingly provided resistance from developing type 2 diabetes. Therefore, inhibition of PTP-IB has recently been emerged as a potential target to treat type 2 diabetes. The ability of 7-HF in modulating PTP-IB was evaluated in 3T3-L1 mouse preadipocytes. Surprisingly, the adipocytes treated with 7-HF in the present invention exhibited significant inhibition of PTP-IB activity as summarized in Figure VI. This observation thus indicates that 7-HF can also be used as a potential therapeutic intervention to prevent, control and treat type 2 diabetes and insulin resistance.
Modulation of adiponectin by 7-HF:

Adiponectin is an important adipokine hormone exclusively secreted from the adipose tissue and it modulates a number of metabolic processes including glucose homeostasis and lipid metabolism. It is known that low levels of adiponectin are associated with disease states such as obesity, diabetes and cardiovascular disease. Administration of adiponectin was proven to be beneficial in animal models of diabetes, obesity and atherosclerosis. High plasma concentrations of adiponectin were also found to associate with lower risk of Myocardial Infarction in men. Therefore, adiponectin has been established as a promising marker of obesity, metabolic syndrome and other metabolic disorders. The modulation of adiponectin protein by 7-HF in 3T3-L1 adipocytes was evaluated in Western immunoblot assay. The cell culture, treatment protocol and immunoblot assay methodology were as per the standard protocol. 7-HF showed potent upregulation of adiponectin protein expression in 3T3-L1 mature adipocytes as depicted in Figure VIII, manifesting its potential use in the prevention, treatment and control of metabolic disorders, such as obesity, insulin resistance or Type 2 diabetes and endothelial dysfunction.

It was quite unexpected and surprising to see that a single ingredient, 7-hydroxyfrullanolide could be able to modulate the marker proteins related to many disease conditions associated with metabolic disorders. This suggests that 7-hydroxyfrullanolide could be a potential therapeutic agent to prevent, treat and control metabolic syndrome, obesity, diabetes, atherosclerosis, endothelial dysfunction, chronic kidney disease (CKD) and other metabolic disorders in animals and humans.

Several natural analogs, namely compound-1 to compound-9 and their structures are depicted as those with numbers 3 to 11 respectively in Figure IX, have been isolated as congeners of 7-hydroxyfrullanolide from Sphaeranthus indicus alcohol extract and their structures characterized using IR, H NMR, 13C NMR and Mass Spectral data. Several of these natural analogs have shown potent anti-adipogenesis activity in the cell based assay. Out of these compound-2(4), compound-3(5), compound-5(7), compound-7(9) and compound-8(10) are found to be new to the best of our knowledge. The anti-adipogenic potential of some of these analogs are summarized in Table-1.
Similarly, several synthetic analogs of 7-hydroxyfrullanolide, named sequentially as compound-10 to compound-23 in the present invention, have been prepared using a semi-synthetic process and their structures are depicted as those with numbers 12 to 25 respectively as in Figure X. Their biological activity was evaluated in a cell based assay. These compounds also exhibited antiadipogenic potential and inhibited lipid accumulation in human 3T3-L1 adipocytes as summarized in Table-I.

The extracts containing 7-hydroxyfrullanolide and/or one or more of the analogs of 7-hydroxyfrullanolide also showed potent anti-adipogenic activity in 3T3-L1 Human pre-adipocyte cells. The ethyl acetate extract of Sphaeranthus indicus (SIE) containing 11% of 7-hydroxyfrullanolide, for example, showed 65.9% inhibition of lipid accumulation at 10 µg/mL concentration in human 3T3-L1 pre-adipocyte cells. Further, SIE accelerated the lipolysis by 26.7% at 25 µg/mL as indicated by the percentage increase in glycerol release in the lipolysis assay.

The efficacy shown by SIE in vitro models was further evaluated in an in vivo rat model of metabolic syndrome (MS). Metabolic syndrome condition was experimentally induced in male Sprague Dawley rats by feeding the rats with high fat, high cholesterol, high salt and high sucrose diet for eight weeks. After eight weeks of induction period, the rats were randomly divided into two groups with six animals in each group and the treatment group animals were supplemented orally with 250 mg/kg body weight of SIE in 10 mL of 0.5% CMC in water for further 8 weeks. The control group of animals received only the vehicle (10 mL/kg of 0.5% CMC in water) during this period. Body weight of individual animal was recorded weekly for the entire duration of the study. Mean body weights for the treatment group and control group were determined. The body weight gain was calculated at the end of 1st week, 4th week and 8th week after initiation of treatment in comparison to initial body weight. SIE at a dose of 250 mg/kg exhibited highly potent and statistically significant (p<0.01) reduction in body weight gain (66.04%) in comparison to control group as summarized in figures XIA & XIB.

Assessment of serum adiponectin in MS rats: The serum adiponectin concentration in the treatment and control groups of animals was assessed using double antibody based sandwich rat adiponectin ELISA kit. The data revealed that daily supplementation of SIE at 250 mg/kg body weight for 8 weeks resulted in significant (p=0.00618) improvement
in serum adiponectin concentration, when compared to the baseline as summarized in Figure XII. The control group, however, did not show such improvement in serum adiponectin concentration.

Supplementation of SIE at 250 mg/kg also resulted in improvement in fat profile with 15.3, 12.7 and 22.9 percentage reductions respectively in serum cholesterol, LDL and triglycerides. This is well corroborated with its efficacy observed in improvement of adiponectin levels.

Homeostasis Model Assessment (HOMA):

- The HOMA index was calculated based on serum insulin and glucose levels using the formula: Fasting insulin concentration (µIU/mL) × Fasting glucose concentration (mmol/L)/22.5. The data presented in Figure XIII manifested that compared to the control group, supplementation of a daily dose of 250 mg/kg of ethyl acetate extract of Sphaeranthus indicus (SIE) for 8-weeks resulted in significant reduction of HOMA index. The Homeostatic Model Assessment (HOMA) is widely considered as a measure of insulin resistance and beta cell function in clinical research. The data indicates that (SIE) can be a therapeutic agent to improve insulin sensitivity and beta-cell function.

Based on the above animal study, it is obvious that ethyl acetate extract of Sphaeranthus indicus (SIE) not only reduces obesity but also ameliorates various symptoms of metabolic syndrome including body weight gain, visceral and organ fat deposition and improves lipid profile, glucose homeostasis, insulin resistant type-II diabetes, beta-cell function etc.

The foregoing thus suggest that 7-hydroxyfrullanolide, its analogs, the extracts/fractions containing 7-hydroxyfrullanolide or its analogs or both or mixtures thereof or their compositions can be potential pharmaceutical/dietary supplement/food ingredient for the control, prevention and treatment of one or more metabolic disorder(s) and/or for the amelioration of the expression/production of one or more of the biological markers related to metabolic disorders.

The present invention comprises different aspects cited below:
For the purpose of this invention, the word "component" or "biologically active ingredient" widely used in the specification and claims of the present invention refer to 7-hydroxyfrullanolide alone, or one or more of its analog(s); or the extracts or fraction standardized to 7-hydroxyfrullanolide or analog(s) or both; or mixtures thereof. The word "component" or "biologically active ingredient" are used interchangeably through out the specification and the same may be appreciated as such by the person skilled in the art.

The word "composition" used in the specification and claims of the present invention refers to combination of one or more of 7-hydroxyfrullanolide or one or more of its analog(s); or the extracts or fraction standardized to 7-hydroxyfrullanolide or analog(s) or both; or mixtures thereof with one or more of other biologically active components, vehicles, carriers and diluents etc.

The phrase "other biologically active components" refers to extract(s) or fraction(s) or compound(s) derived from plants, animals and microorganisms.

In an important embodiment, the invention provides biologically active ingredient(s) selected from one or more of 7-hydroxyfrullanolide alone, its analog(s) and the extract(s) or fraction(s) containing 7-hydroxyfrullanolide alone/its analog(s) or both or mixture(s) thereof as an active for the control, prevention and treatment of one or more metabolic disorder(s) and/or for the amelioration of the expression/production of one or more of the biological markers related to metabolic disorders.

In another important embodiment, the invention provides biologically active ingredient(s) compositions comprising at least one component selected from 7-hydroxyfrullanolide, its analog(s), the extract(s) or fraction(s) containing 7-hydroxyfrullanolide/its analog(s) or both or mixture(s) thereof as an active in combination with one or more selected from biologically actives derived from plants, animals and microorganisms, pharmaceutically or dietetically acceptable active ingredients, vitamins, aminoacids, minerals, vehicles, carriers and diluents or mixtures thereof for the prevention, control and treatment of one or more metabolic disorder(s) and/or for the amelioration of the expression/production of one or more of the biological markers related to metabolic disorders.
In the other important embodiment, the metabolic disorders to be controlled/prevented/treated by the biologically active ingredient(s) or compositions described comprise obesity, over weight, diabetes, arteriosclerosis, cardiovascular diseases, hypertension, hypercholesteremia (LDL, HDL, VLDL), hyperlipidemia, triglyceridemia, metabolic syndrome, endothelial dysfunction and other metabolic disorders.

In the other embodiment, the invention provides biologically active ingredient(s) selected from one or more of the components 7-hydroxyfrullanolide, its analog(s) and the extract(s) or fraction(s) containing 7-hydroxyfrullanolide alone or its analog(s) or mixture(s) thereof or their composition(s) for the amelioration of the expression or production of one or more biological marker proteins related to or associated with metabolic syndrome, obesity and other disease conditions associated with metabolic syndrome including but not limited to Peroxisome proliferator-activated receptor gamma (PPARy), Adipose Differentiation Related Protein (ADRP), adipocyte CD36, Macrophage CD36, Monocyte Chemotactic protein (MCP-1), Oxidized LDL (Ox-LDL), adipocyte fatty-acid-binding protein (aP2/FABP4/A-FABP), beta-3 Adrenergic Receptor (P3AR), Perilipin, Adiponectin Protein tyrosine phosphatase-IB (PTP-1B), Metalloproteinase-1 (MMP-1), Matrix Metalloproteinase-3 (MMP-3) and Matrix Metalloproteinase-13 (MMP-13).

In another embodiment of the invention the 7-hydroxyfrullanolide and its analog(s) mentioned in the previous embodiments are of synthetic or semi-synthetic origin or natural origin, wherein the natural origin can be any plant species that produces 7-hydroxyfrullanolide or its analog(s) or mixtures thereof, more selectively Sphaeranthus indicus.

In another embodiment the invention provides the extract(s) and fraction(s) comprising 7-hydroxyfrullanolide or its analogs or mixtures thereof, wherein these extracts or fraction can be derived from any plant species that produces 7-hydroxyfrullanolide or its analog or mixtures thereof, more selectively Sphaeranthus indicus.

In another embodiment the invention provides biologically active ingredient(s) comprises of the extracts and fractions containing 7-hydroxyfrullanolide or its analogs or mixtures.
thereof wherein the said extracts and fractions contain 7-hydroxyfrullanolide or its analog(s) or mixtures thereof in the range of 0.001% to 100%, preferably 0.01 to 99%.

In another embodiment, the invention provides biologically active ingredient(s) compositions wherein the percentage of the extract or fraction standardized to 7-hydroxyfrullanolide or its analog(s) or both varies in the range from 0.01% to 99%, preferably 1% to 90% by weight in the composition.

In the other embodiment, the invention provides extracts, fractions and compositions comprising 7-hydroxyfrullanolide or its analog(s) for the control, prevention and treatment of one or more metabolic disorder(s), wherein the concentration of 7-hydroxyfrullanolide or its analog(s) or mixtures thereof varies in the range from 0.01% to 99.9%.

In the other embodiment, the invention provides biologically active ingredient(s) compositions comprising at least one component selected from 7-hydroxyfrullanolide, its analog(s), the extract(s) or fraction(s) containing 7-hydroxyfrullanolide or its analog(s) or mixture(s) thereof as an active for the control, prevention and treatment of one or more metabolic disorder(s), wherein the concentration of active in the composition varies in the range from 0.001% to 99.9%, preferably 0.01 to 95% by weight.

In a further embodiment the invention provides analogs of 7-hydroxyfrullanolide as described above for the control, prevention and treatment of metabolic disorder(s) and/or for the amelioration of the expression/production of one or more of the biological marker(s) related to metabolic disorder(s), where in the analogs comprises of the compounds represented by the general formula I given below:

![Formula I](image-url)
Wherein Ri, R2, R3, R4 and R5 are each independently selected from H, hydrogen, hydroxy, halogen, -OORn, alkoxy, -OC(0)Ri 2 and C(0)Ri 2; optionally Ri and R2 are taken together to form a ketone (=0).

The tricyclic ring system consisting of one or two or three double bonds.

Optionally R2 and R3 together form a double bond;
Optionally R3 and R4 together form a double bond;
Optionally R5 and R6 together form a double bond;
Optionally R8 and R9 together form a double bond;
Further optionally R3 and R4 together form an epoxide ring

R7 is selected from hydrogen, hydroxy, halogen, alkoxy and -OC(0)Ri 2; R8 is selected from hydrogen, hydroxy, halogen, alkoxy, -OC(0)Ri 2, -C(0)Ri 2 and NRi 3Ri 4; R9 is selected from hydroxy, alkyl, cycloalkyl, alkoxy, aryl, heterocyclyl, halogen, -OC(0)Ri 2, -C(0)Ri 2, azido and -NRi 3Ri 4, -S(0)mRi 5, -OS(O)mRi 5j wherein m is 0, 1 or 2;
R6 and R11 are each independently selected from hydrogen, alkyl, halogen, ORi 6, -NH R12 and SRi 2; wherein R16 is selected from hydrogen, alkyl and -C(0)Ri 2 or R6 and R11 together form one of ketone (=0), thioketone (=S), imine (=NH) and selenoketone (=Se);
R12 is selected from hydrogen and alkyl;
Ri 3 and Ri 4 are each independently selected from hydrogen, alkyl, cycloalkyl, aralkyl, aryl, heterocyclyl, -C(0)Ri 2 and -C(S)NH Ri 2; or Ri 3 and Ri 4 together with the N atom to which they are bonded, form a 5-, 6-, or 7-membered heterocyclic ring, optionally having one or more additional heteroatoms selected from O, N, S and Se;
R16 is selected from hydrogen, alkyl, cycloalkyl, aryl, heterocyclyl
X is selected from O, NH, S and Se.

The 7-hydroxyfrullanolide or its analogs used for the prevention, control or treatment of obesity, metabolic syndrome and other metabolic disorders or for making the composition
of the present invention can be naturally derived from plant species or can be produced through synthesis or semisynthesis.

In other embodiment, the natural analogs of 7-hydroxyfrullanolide described above comprises of frullanolide/eudesmanoid sesquiterpene compounds selected from but not limited to frullanolides, 7-hydroxyfrullanolide (1); 1la,13-dihydro-3a,7a-dihydroxy-4,5-epoxy-6p,7-eudesmanolide ; 1la,13-dihydro-7a-acetoxy-3β-hydroxy-6p,7-eudesm-4-enolide;3-keto-β-eudesmol; 1la,13-dihydro-3a,7a-dihydroxyeudesm-4-en-6a,12-olide; lla,13-dihydro-3a,7a-dihydroxyfrullanolide; 1la,13-dihydro-7a,13-dihydroxyfrullanolide; lla,13-dihydro-7a-hydroxy-13-methoxyfrullanolide (8); 2a,7a-dihydroxy-4-en-1,13-dihydroeudesm-6,12-olide; 2a-hydroxyxystic acid; 3-keto-7a-hydroxyeudesm-4-en-6,12-olide (cryptomeridiol); 4-epicryptomeridiol; sphaerantholide (11); 2a-hydroxyxysphaerantholide; 2a-acetoxysphaerantholide; 2a,7a-dihydroxysphaerantholide; 2a-acetoxy-7a-hydroxyxysphaerantholide; 2a-acetoxy-5a-hydroxisosphaerantholide, eudesmanolide dimer [compound-2, (4)], (3aR,5aR,9aR,9bR)-decahydro-9a-hydroperoxy-3a-hydroxy-5a-methyl-3,9-dimethylenenaphtho[1,2-b]furan-2(9bH)-one [compound-3, (5)], (3aS,5aR,9bR)-3,3a,4,5,5a,6,7,8-octahydro-3,5a,9-trimethynaphtho[1,2-b]furan-2(9bH)-one [compound-4, (6)], (R)-5,5a,6,7-tetrahydro-3,5a,9-trimethynaphtho[1,2-b]furan-2(4H)-one [compound-5, (7)], (3R,3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-3-( methoxymethyl)-5a,9-dimethynaphtho[1,2-b]furan-2(9bH)-one [compound-6, (8)], 2-(3R,8aR)-1,2,3,7,8,8a-hexahydro-5,8a-dimethynaphthalen-3-yl)acrylic acid [compound-7, (9)], (3aR,5aR,9bS)-3-((6-amino-9H-purin-9-yl)methyl)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethynaphtho[1,2-b]furan-2(9bH)-one [compound-8, (10)], (3R,3aR,5aR,8R,9bS)-8-((2R,3S,4R,5R)-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yloxy)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-3,5a,9-trimethynaphtho[1,2-b]furan-2(9bH)-one [compound-9, (11)].

In another embodiment, the invention provides biologically active ingredient(s) selected from 7-hydroxyfrullanolide or their analog(s) or their compositions as described above, wherein the synthetic and semi-synthetic analogs of 7-hydroxyfrullanolide comprises (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a,8-dihydroxy-5a,9-dimethyl-3-methylenenaphtho[1,2-b]furan-2(9bH)-one [compound-
10. (12), (3aR,5aS,9bS)-3a,4,5,5a,6,7-hexahydro-3a-hydroxy-5a,9-dimethyl-3-methylenenaphtho[1,2-b]furan-2,8(3H,9bH)-dione [compound-1 1, (13), (R)-2,4,5,5a,6,7-hexahydro-5a,9-dimethyl-2-oxonaphtho[1,2-b]furan-3-carbaldehyde [compound-12, (14)], 4,5-epoxy -7-hydroxyfrullanolide [compound-1 3, (15), (R)-3-(bromomethyl)-5,5a,6,7-tetrahydro-5a,9-dimethynaphtho[1,2-b]furan-2(4H)-one [compound- 14, (16), (3aR,5aR,9bS)-3,3a,4,5,5a,6,7-octahydro-3a-hydroxy-3,5a,9-trimethylnaphtho[1,2-b]furan-2(9bH)-one [compound-15, (17)], (2E)- (3aR,5aR,9bS)-2,3,3a,4,5,5a,6,7,8,9b-decahydro-3a-hydroxy-3,5a,9-trimethyl-2-oxonaphtho[1,2-b]furan-8-yl 3-(2,5-dimethoxyphenyl)acrylate [compound-16, (18)], (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethyl-3-(morpholinomethyl)naphtho[1,2-b]furan-2(9bH)-one [compound- 17, (19)], (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethyl-3-(morpholinomethyl)naphtho[1,2-b]furan-2(9bH)-one [compound-18, (20)], (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethyl-3-((piperidin-1-yl)methyl)naphtho[1,2-b]furan-2(9bH)-one [compound-19, (21)], (5aR)-5,5a,6,7,8,9-hexahydro-9-hydroxy-5a,9-dimethyl-3-((piperidin-1-yl)methyl)naphtho[1,2-b]furan-2(4H)-one [compound-20, (22)], (3aR,5aR,9bS)-3-((4H-1,2,4-triazol-4-yl)methyl)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethynaphtho[1,2-b]furan-2(9bH)-one [compound-21, (23)], (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethyl-3-((piperazin-1-yl)methyl)naphtho[1,2-b]furan-2(9bH)-one [compound-22, (24)] and piperazine bis-7-hydroxyfrullanolide [compound-23, (25)] represented by the structure numbers 12 through 25 respectively as depicted in Figure X.

In another embodiment, the invention provides the extracts and fractions derived from Sphaeranthus indicus containing 7-hydroxyfrullanolide/other frullanolide(s)/eudesmanoid sesquiterpene(s)/other phytochemicals for the prevention, control and treatment of obesity, metabolic syndrome and other metabolic disorders or for making the compositions described above comprises, 7-hydroxyfrullanolide/other frullanolide(s)/eudesmanoid sesquiterpene(s)/other phytochemicals or mixture thereof varies in concentration range of 0.001% to 100%, preferably 0.01 to 99%.
In another embodiment, the concentration of the active compound 7-
hydroxyfrullanolide/other frullanolide(s)/eudesmanoid sesquiterpene(s)/
other phytochemicals in the compositions comprising *Sphaeranthus indicus* derived component
as described in the previous embodiments varies in the range from 0.001% to 99%,
preferably 0.01 to 95% by weight.

In another embodiment of the invention, the other biologically active components used
for making the compositions comprise components having any health benefit selected
from but not limited to anti-diabetic activity, anti-hyperlipidemic activity, anti-obesity
activity, anti-hypertensive activity, anti-platelet aggregation activity, anti-infective
activity, anti-atherosclerotic activity and anti-inflammatory activity, anti-oxidant activity
and bio-enhancing activity.

In another embodiment, the invention provides biologically active ingredient(s) selected
from 7-hydroxyfrullanolide, its analogs, the extracts and fractions containing 7-
hydroxyfrullanolide or its analogs or mixtures thereof, derived from *Sphaeranthus
indicus*, or their composition, wherein said extract(s) or active fraction(s) or active
compound(s) or phytochemicals or mixtures thereof are derived from at least one of the
plant parts selected from but not limited to leaves, flower heads, fruits, stem, bark, root,
whole plant or mixtures thereof, preferably flower heads.

In another embodiment of the invention, biologically active ingredient(s) and their
compositions as described in previous embodiments, wherein said 7-hydroxyfrullanolide,
it natural analogs, the extract(s) or active fraction(s) containing 7-hydroxyfrullanolide or
it natural analog(s) or mixtures thereof or phytochemicals or mixtures thereof derived
from *Sphaeranthus indicus* are obtained through extraction using solvents selected from
one or more of organic solvents, alcohols, hydroalcohols, water or mixtures thereof or
those followed by partitions and/or chromatography.

The examples of the biologically or pharmaceutically acceptable excipients, vehicles and
carriers employed in the present invention include, but are not limited to, surfactants,
binders, diluents, disintegrators, lubricants, preservatives, stabilizers, buffers, suspensions
and drug delivery systems.
The examples of the biologically or pharmaceutically acceptable excipients, carriers and diluents comprise glucose, fructose, sucrose, maltose, yellow dextrin, white dextrin, aerosol, microcrystalline cellulose, calcium stearate, magnesium stearate, sorbitol, stevioside, corn syrup, lactose, citric acid, tartaric acid, malic acid, succinic acid, lactic acid, L-ascorbic acid, dl-alpha-tocopherol, glycerin, propylene glycol, glycerin fatty ester, poly glycerin fatty ester, sucrose fatty ester, sorbitan fatty ester, propylene glycol fatty ester, acacia, carrageenan, casein, gelatin, pectin, agar, vitamin B group, nicotinamide, calcium pantothenate, amino acids, calcium salts, pigments, flavors, preservatives, distilled water, saline, aqueous glucose solution, alcohol, propylene glycol and polyethylene glycol, various animal and vegetable oils, white soft paraffin, paraffin and wax.

In another embodiment, the invention provides biologically active ingredient(s) or their composition(s) as claimed in preceding embodiments, wherein said component or composition is administered orally, topically or parenterally or by inhalation to a subject or mammal or warm blooded animal in need thereof.

In another embodiment, the invention provides biologically active ingredient(s) or their composition(s) as claimed in preceding embodiments, wherein said components or compositions can be formulated into any suitable formulation like oral agents such as tablets, soft capsule, hard capsule, soft gel capsules, pills, granules, powders, emulsions, suspensions, syrups, pellets, food, beverages, concentrated shots, drops and the like; and parenteral agents such as injections, intravenous drip and the like; suppositories; and transdermal agents such as patches, topical creams and gel; ophthalmic agents; nasal agents; and food or beverages.

In another embodiment, the invention provides a method for the control/prevention/treating of a metabolic disorder selected from but not limited to obesity, over weight, diabetes, arteriosclerosis, cardiovascular diseases, hypertension, hypercholesteremia, hyperlipidemia, triglyceridemia, metabolic syndrome, endothelial dysfunction and other metabolic disorders in a mammal or warm blooded animal in need thereof, wherein the method comprises administering a therapeutically effective amount
of atleast one biologically active ingredient(s) selected from 7-hydroxyfrullanolide, its analog(s), the extract(s) or fraction(s) comprising 7-hydroxyfrullanolide/its analogs or both as an active or mixtures thereof or their compositions as described in preceding embodiments.

In another embodiment, the invention provides a method of promoting lipolysis and/or inhibiting adipogenesis comprising administering to a subject or mammal or warm blooded animal in need thereof a therapeutically effective quantity of atleast one biologically active ingredient(s) selected from 7-hydroxyfrullanolide, its analog(s), the extract(s) or fraction(s) comprising 7-hydroxyfrullanolide/its analog(s) or both or mixtures thereof as an active or their compositions as described in the preceding embodiments.

In another embodiment, the invention provides a method of using biologically active ingredient(s) selected from 7-hydroxyfrullanolide or its analogs; the extract(s) or fraction(s) comprising 7-hydroxyfrullanolide or its analog(s) or both or their compositions for the amelioration of the expression or production of biological markers selected from but not limited to PPAR-γ, C-reactive protein (CRP), Adipose Differentiation Related Protein (ADRP), adipocyte CD36, macrophage CD36, Monocyte Chemotactic protein (MCP-1), Oxidized LDL, Adipocyte Fatty-acid-Binding Protein (aP2/FABP4/A-FABP), Beta-3 adrenergic receptor (P3-AR), adiponectin, Perilipin, Protein tyrosine phosphatase IB (PTP IB), Metalloproteinase-1 (MMP-1), Matrix Metalloproteinase-3 (MMP-3) and Matrix Metalloproteinase-13 (MMP-13).

In a further embodiment of the present invention, the components selected from 7-hydroxyfrullanolide, its analogs; the extract(s) or fraction(s) or mixtures thereof derived from Sphaeranthus indicus comprising 7-hydroxyfrullanolide as an active ingredient or their compositions as described above can be optionally combined with bio-availability enhancing agents selected from but not limited to extract(s), fraction(s), pure compound(s) derived from Piper nigrum or Piper longum, and Stevia rebaudiana..

In alternative embodiment of the invention, the components selected from 7-hydroxyfrullanolide or its analogs or compositions comprising the extract(s), fraction(s),
active compound(s) or phytochemical(s) or mixtures thereof derived from *Sphaeranthus indicus* comprising 7-hydroxyfrullanolide as an active ingredient or their compositions claimed in the present invention are delivered in the form of controlled release tablets, using controlled release polymer-based coatings by the techniques including nanotechnology, microencapsulation, colloidal carrier systems and other drug delivery systems known in the art. The said formulation can be designed for once a daily administration or multiple administrations per day.

In other embodiment of the invention, the components selected from 7-hydroxyfrullanolide or its analogs or the extracts or fractions containing 7-hydroxyfrullanolide or their compositions described/claimed in the present invention can also be formulated into or added to existing or new food and beverage form(s) and animal feeds as a healthy food or beverage or feed for prevention, control or treatment of one or more of the diseases including but not limited to obesity, diabetes, hypertension, cardiovascular diseases, neurological disorders, Alzheimer's, cognitive disorders, oxidative stress, skin disorders, aging of the skin, UV irradiated damage, hypercholesterolemia, variations of LDL, HDL & VLDL, hyperlipidemia, triglyceridemia, immune deficiency, cancer, metabolic syndrome, for bringing about weight loss effectively, for producing lean body mass, for using during weight loss program as well as for other metabolic disorders.

In other embodiment, the invention provides the use of ingredient(s) or composition(s) for prevention, control and treatment of one or more diseases several diseases or disease conditions including but not limited to obesity, diabetes, hypertension, atherosclerosis, cardiovascular diseases, neurological disorders, Alzheimer's, cognitive disorders, oxidative stress, skin disorders, aging of the skin, UV irradiated damage, hypercholesterolemia, variations of LDL, HDL & VLDL, hyperlipidemia, triglyceridemia, immune deficiency, cancer, metabolic syndrome, for bringing about weight loss effectively, for producing lean body mass, for using during weight loss program as well as for other metabolic disorders.

In other embodiment, the invention provides a method of prevention/ control /treatment of one or more metabolic disorders selected from obesity, over weight, diabetes,
atherosclerosis, arteriosclerosis, cardiovascular diseases, hypertension, hypercholesterolemia, variations of LDL, HDL, VLDL, hyperlipidemia, triglyceridemia, metabolic syndrome, endothelial dysfunction and other metabolic disorders in a mammal or warm blooded animal in need thereof, wherein the method comprises administering to mammal or warm blooded animal a therapeutically effective amount of at least one biologically active ingredient(s) from 7-hydroxyfrullanolide, its analog(s), the extract(s) or fraction(s) comprising 7-hydroxyfrullanolide or its analog(s) or both as an active or mixtures thereof or their compositions.

In other embodiment, the invention provides a method of promoting lipolysis and/or inhibiting adipogenesis in a subject or mammal or warm blooded animal in need thereof comprising administering to said subject or mammal or warm blooded animal a therapeutically effective quantity of at least one biologically active ingredient(s) selected from 7-hydroxyfrullanolide, its analog(s), the extract(s) or fraction(s) comprising 7-hydroxyfrullanolide or its analog(s) or both or mixtures thereof as an active or their compositions.

In other embodiment the invention provides a method of amelioration of the expression or production of at least one biological marker selected from PPAR-γ, C-reactive protein (CRP), Adipose Differentiation Related Protein (ADRP), adipocyte CD36, macrophage CD36, Monocyte Chemotactic protein (MCP-1), Oxidized LDL, Adipocyte Fatty-acid-Binding Protein (aP2/FABP4/A-FABP), Beta-3 adrenergic receptor (P3-AR), adiponectin, Perilipin, Protein tyrosine phosphatase IB (PTP IB), Metalloproteinase-1 (MMP-1), Matrix Metalloproteinase-3 (MMP-3) and Matrix Metalloproteinase-13 (MMP-13) in a subject or mammal or warm blooded animal in need thereof, wherein the method comprises administering to the subject or mammal or warm blooded animal a biologically active ingredient(s) selected from 7-hydroxyfrullanolide or its analogs; the extract(s) or fraction(s) containing 7-hydroxyfrullanolide or its analog(s) or both or mixtures thereof in their composition(s).

The unexpected and superior ameliorating effects of 7-hydroxyfrullanolide or its analogs or the extracts/fraction containing 7-hydroxyfrullanolide or mixtures thereof
compositions claimed in the present invention are illustrated by the following non-limiting examples:

Example 1

*Sphaeranthus indicus* ethyl acetate extract (SIE): Sphaeranthus indicus flower heads (2.2 kg) were charged into a pilot extractor and extracted with ethyl acetate (22 L) at reflux temperature for 2 h. The extract was filtered and the spent raw material was re-extracted twice with ethyl acetate (2 x 13 L) under similar conditions. The combined extract was fine filtered and concentrated over a climbing film evaporator to obtain residue (174 g). The ethyl acetate extract showed 11% of 7-hydroxy-4, 11 (13)-eudesmadien-12, 6-olide (7-hydroxyfrullanolide) by HPLC method of analysis.

Example 2

Preparation of methanol extract of *Sphaeranthus indicus* and isolation of 7-hydroxyfrullanolide and its natural analogs: Sphaeranthus indicus flower heads (7 kg) were taken into a pilot extractor and extracted with methanol (56 L) at 80°C temperature for 2 h. The extract was filtered and the spent raw material was re-extracted twice with methanol (2 x 40L) under similar conditions. The combined extract was fine filtered and concentrated under vacuum to obtain a residue (1 kg). The methanol extract was suspended in water (1 L) and extracted with ethyl acetate (3 x 1.5 L). The combined organic layer was evaporated under vacuum and the residue (300 g) was subjected to column chromatography over silica column using eluants of increasing polarity from hexane to acetone. The fractions eluted with 20% acetone/hexane were combined and evaporated under vacuum to give a residue (fraction-I; 49 g). The fractions eluted with 40% acetone/hexane were combined and evaporated under vacuum to give a residue (fraction-II; 35 g). The fraction eluted with 60% acetone/hexane was evaporated under vacuum to give a residue (fraction-III).

The fraction-I was subjected to re-chromatography over silica column using solvents of increasing polarity from chloroform to ethyl acetate. The fraction (3 g) eluted with chloroform was evaporated and the residue subjected to repeated chromatography over silica gel using ethyl acetate/hexane mixture to obtain turmerone (2); 60 mg. The fractions eluted of the fraction-I column with 2-5% ethyl acetate/chloroform were
combined and evaporated, and the residue was subjected to repeated chromatography over silica gel using acetone/hexane and chloroform/hexane mixtures to yield compound-1 (3); 40 mg and compound-2 (4); 50 mg. The fractions eluted with 5-10% ethyl acetate/chloroform mixtures were evaporated under vacuum to obtain 7-hydroxyfrullanolide (1); 13 g. The fractions obtained on elution with 15% ethyl acetate/chloroform mixture were evaporated and the residue was subjected to further chromatography over silica gel using same solvent system to obtain compound-3 (5); 20 mg. The fraction eluted with 20% ethyl acetate/chloroform was subjected to repeated chromatography on silica column using ethyl acetate/chloroform mixtures, followed by final purification on HPLC to obtain compound-4 (6); 24 mg.

The fraction-II obtained of the main column was subjected to re-chromatography over silica column using chloroform and ethyl acetate/chloroform mixtures as eluants. The fractions eluted with chloroform and 5% ethyl acetate/chloroform mixture were combined and evaporated. The residue (5 g) was re-purified on silica column again using ethyl acetate/chloroform mixtures and the fraction eluted with 2% ethyl acetate/chloroform was evaporated under vacuum to provide compound-5 (7); 15 mg. The fraction eluted with 10% ethyl acetate/chloroform mixture was evaporated to yield a further quantity (3 g) of 7-hydroxyfrullanolide. The fraction (12 g) eluted with 20% ethyl acetate/chloroform mixture was subjected to further purification on silica column using acetone/hexane mixtures and the fraction so obtained using 10% acetone/hexane was re-purified on silica column using ethyl acetate/chloroform mixtures to obtain compound-6 (8); 100 mg. The other fraction obtained on elution with 20% acetone/hexane mixture furnished compound-7 (9); 20 mg upon evaporation of the solvent. The fraction (5g) eluted with 60% ethyl acetate/chloroform mixture was subjected to further purification on silica column using ethyl acetate to obtain compound-8 (10); 30 mg.

The fraction-III obtained of the main column was purified on silica column using methanol/ethyl acetate mixtures and the fraction eluted with 5% methanol/ethyl acetate upon evaporation of the solvent yielded compound-9 (11); (1500 mg). 7-hydroxyfrullanolide (1): NMR δH (400 MHz, CDCl3): 6.24 (s,1H), 5.80 (s 1H), 5.02 (s,1H), 2.12 (m,2H), 1.68 (m,1H), 1.95 (td J=1.4,3.2Hz,1H), 1.80 (m,1H), 1.68 (m,1H) 1.65 (m,1H) , 1.52 (td; J=14.3.1Hz,1H), 1.42 (m,2H), 1.35 (m,1H), 1.77 (s,3H ), 1.07
ar-Turmerone (2): $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.10 (4H, s), 6.02 (IH, t, $J = 1.2$ Hz), 3.28 (IH, sextet, $J = 7.6$ Hz), 2.70 (IH, dd, $J = 6.0$, 15.6 Hz), 2.60 (IH, $J = 8.4$, 15.6 Hz), 2.31 (3H, s), 2.10 (3H, d, $J = 0.8$ Hz), 1.85 (3H, d, $J = 0.8$ Hz), 1.24 (3H, d, $J = 7.2$ Hz);

$^{13}$C NMR (CDCl$_3$, 400 MHz): $\delta$ 199.77, 154.84, 143.71, 135.51, 129.08, 126.65, 124.15, 52.70, 35.32, 29.67, 27.53, 21.95, 20.91, 20.64.

3,3a,4,5,5a,6,7,8-octahydro-5a,9-dimethyl-3-methylenenaphtho[1,2-b]furan-2(9bH)-one [Compound-1 (3)]: $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 6.16 (IH, s), 5.57 (IH, s), 5.26 (IH, d, $J = 5.6$Hz), 2.95 (IH, m), 2.09 (2H, m), 1.83 (IH, m), 1.76 (3H, s), 1.61 - 1.71 (4H, m), 1.51 - 1.23 (9H, m), 1.08 (3H, s); LCMS: 232 (M+H)$^+$ +ve ion mode.

![Compound-1 (structure: 3)](attachment:image)

Eudesmanolide dimer [Compound-2, (4)]: $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 6.20 (IH, s), 3.28 (IH, dd, $J = 6.0$, 12.8Hz), 2.74 (IH, m), 2.52 (2H, m), 2.23 (2H, m), 2.14 (IH, m), 2.10 (IH, m), 2.04 (IH, m), 2.02 (IH, m), 1.99 (IH, m), 1.92 (IH, m), 1.88 (3H, s), 1.84 (IH, m), 1.78 (IH, m), 1.74 (IH, m), 1.68 (IH, s), 1.68 (3H, s), 1.61 (IH, m), 1.60 (IH, m), 1.54 (IH, m), 1.48 (IH, m), 1.41 (IH, m), 1.38 (IH, m), 1.19 (2H, m), 1.06 (3H, s), 0.98 (3H, s); $^{13}$C NMR (CDCl$_3$, 100MHz): $\delta$ 206.32, 177.13, 175.07, 140.21, 139.85, 138.13, 127.88, 88.69, 82.78, 52.61, 52.46, 51.26, 39.99, 38.39, 38.14, 37.74, 33.82, 33.05, 32.45, 32.40, 27.74, 27.13, 26.37, 25.38, 24.13, 20.84, 20.63, 19.73, 19.61, 18.51, 18.36; LCMS: 478 (M+Na)$^+$ +ve ion mode.
(3aR,5aR,9aR,9bR)-decahydro-9a-hydroperoxy-3a-hydroxy-5a-methyl-3,9-dimethylenenaphtho[1,2-b]furan-2(9bH)-one [Compound-3, (5)]: \( ^1H \) NMR (CDCl\(_3\), 400 MHz): \( \delta \) 7.82 (IH, br s), 6.19 (IH, s), 5.80 (IH, s), 5.39 (IH, s), 5.29 (IH, s), 4.75 (IH, s), 3.32 (IH, br s), 2.42-2.26 (4H, m), 1.88 -1.98 (4H, m), 1.63 - 1.78 (12H, m), 1.37 - 1.42 (2H, m), 1.16-1.09 - 1.16 (4H, m), 1.04 (3H, s), 0.84 - 0.94 (4H, m); \(^{13}C\) NMR (CDCl\(_3\), 400MHz): \( \delta \) 167.87, 143.34, 142.57, 119.70, 115.98, 86.99, 78.38, 75.69, 38.13, 35.97, 32.36, 30.35, 29.67, 21.92, 20.29; LCMS: 279(M-H) \(^-\) negative ion mode.

(3aS,5aR,9bR)-3,3a,4,5,5a,6,7,8-octahydro-3,5a,9-trimethylnaphtho[1,2-b]furan-2(9bH)-one [Compound-4, (6)]: \( ^1H \) NMR (CDCl\(_3\), 400 MHz): \( \delta \) 5.30 (IH, s), 4.90 (IH,s), 4.80 (IH, d, J=5.2Hz), 3.77 (IH, t, J=5.2Hz), 2.79 (IH, q, J=7.2), 1.87 (IH td, J=4.4Hz), 1.815 (3H, s), 1.61 (IH, m), 1.59 (IH, m), 1.52 (2H, m), 1.48 (IH, m), 1.45 (IH, m), 1.27 (IH, m), 1.18 (IH, m), 1.04 (3H, d, J=7.2Hz), 0.92 (3H, s); \(^{13}C\) NMR (CDCl\(_3\), 400 MHz): \( \delta \) 176.66, 139.38, 130.01, 79.39, 75.92, 67.30, 47.30, 34.46, 33.87, 33.14, 27.38, 24.40, 23.60, 17.04, 6.798.
(R)-5,5a,6,7-tetrahydro-3,5a,9-trimethylnaphtho[1,2-b]furan-2(4H)-one [Compound-5, (7)]: $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 5.83 (1H, m), 2.65 (2H, m), 2.30 - 2.35 (1H, m), 2.16 - 2.23 (2H, m), 2.13 (3H, s), 1.89 (3H, s), 1.841 - 1.60 - 1.84 (7H, m), 1.48 - 1.54 (2H, m), 1.28 - 1.46 (3H, m), 1.08 (3H, s); LCMS: m/z 233 (M+H)$^+$, +ve ion mode.

![Compound-5 (structure: 7)]

(3R,3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-3-(methoxymethyl)-5a,9-dimethylnaphtho[1,2-b]furan-2(9bH)-one [Compound-6, (8)]: $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 5.01 (1H, s), 3.88 (1H, dd, J = 5.2, 9.6 Hz), 3.74 (1H, t, J = 11.2 Hz), 3.40 (3H, s), 3.18 (1H, dd, J = 4.8, 10.8Hz), 1.92 - 2.15 (4H, m), 1.79 (3H, s), 1.60 - 1.68 (3H, m), 1.40 - 1.50 (4H, m), 1.05 (3H, s); LCMS: m/z 303 (M+Na)$^+$, +ve ion mode.

![Compound-6 (structure: 8)]

2-((3R,8aR)-1,2,3,7,8,8a-hexahydro-5,8a-dimethylnaphthalen-3-yl)acrylic acid [Compound-7, (9)]: $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 6.32 (1H, s), 5.71 (1H, s), 5.55 (1H, bs), 5.38 (1H, bs), 3.43 (1H, m), 2.25 (3H, t J = 7.8, 11.2Hz), 2.03 - 2.09 (3H, m), 1.78 (3H, s), 1.63 (5H, m), 1.47 (2H, d, J = 5.2Hz), 1.00 (3H, s); LCMS: m/z 231 (M-H)$^-$, -ve ion mode.

![Compound-7 (structure: 9)]
Example 3

Oxidation of 7-hydroxyfrullanolide to produce (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a,8-dihydroxy-5a,9-dimethyl-naphtho[1,2-b]furan-2(9bH)-one [compound-10, (12)] and (3aR,5aS,9bSV3a,4,5,5a,6,7-hexahydro-3a-hydroyx-5a,9-dimethyl-3-methylenenaphtho[1,2-b]furan-2,8(3H,9bH)-dione [compound-11, (13)]: A mixture of 7-hydroxyfrullanolide (1g, 0.00403mol) and 10mL of ethanol was taken in a RB flask and treated slowly with SeO₂ (1.4g, 0.0126mol) at room temperature. The reaction mixture (RM) was then stirred at 70°C for 12h and then poured into ice-cold water. The mixture was extracted with EtOAc and the organic layer washed with brine, dried over Na₂SO₄ and concentrated. The residue (1.2g) was subjected to chromatography.
eluting with chloroform followed by 2% and 5% methanol/chloroform mixtures as eluants. The fractions eluted with 5% methanol/chloroform were monitored, the fractions containing the compounds were combined and evaporated to obtain 700 mg of compound-10 [(12), 70%] and 50 mg of compound-11 [(13), 4.7%].

Compound-10: \(^1\)H NMR(CDC\(_3\), 400 MHz); \(\delta = 6.29\) (IH, s), 5.84 (IH, s), 4.99 (IH, s), 4.08 (IH, d, \(J = 3.2\) Hz), 3.10 (IH, brs), 2.22 (IH, brs), 2.06 (IH, tt, \(J = 3.2, 14.4\) Hz), 1.94 (3H, s), 1.90 (IH, m), 1.66 - 1.79 (3H, m), 1.55 (IH, m), 1.28 - 1.44 (2H, m), 1.08 (3H, s). \(^13\)C NMR (CDC\(_3\), 100 MHz); \(\delta = 168.0, 142.9, 138.2, 130.9, 120.7, 80.3, 68.4, 33.6, 32.5, 32.1, 31.1, 26.6, 24.2, 16.8\); LC/MS: 287 (M+Na)+, 303 (M+K) + positive ion mode.

Compound-11: \(^1\)H NMR(CDC\(_3\), 400 MHz); \(\delta = 6.398\) (1H, s), 5.952 (1H, s), 5.138 (1H, s), 2.711 (2H, m), 2.583-2.482 (2H, m), 2.09-1.94 (2H, m), 1.913 (3H, s), 1.773-1.617 (2H, m), 1.538-1.431 (1H, m), 1.284 (3H, s); LC/MS: 261 (M-HV) negative ion mode.

Example 4

Oxidation of 7-a-hydroxyfrullaholide to produce (R)-2.4.5.5a.6.7-hexahydro-5a,9-diethyl-2-oxonaphtho[1,2-b]furan-3-carbaldehyde [compound-12, (14)]

A mixture of 2.0 g of 7-hydroxyfrullaholide (0.00806 mol) and 40 ml of dichloromethane was taken in a Round Bottom (RB) flask and treated with 39 g of silica gel adsorbed Jones reagent (13 ml of Jones reagent was adsorbed on 26 gm of silica gel). The reaction mixture was stirred at room temperature. After 3 h, the mixture was filtered and the solid was washed with chloroform. The combined filtrate was concentrated and the residue (2.0 g) was subjected to silica column chromatography using EtOAc/hexane mixtures as eluant. The fractions eluted with 10% EtOAc/hexane were monitored and the fractions containing the oxidized product were evaporated to obtain 50 mg of Compound-12 [(14), 2.3%] as a colorless solid.

Compound-12: \(^1\)H NMR(CDC\(_3\), 400 MHz); \(\delta = 9.98\) (1H, s), 6.15 (IH, m), 3.45 (IH, dt, \(J = 4.0, 20.0\) Hz), 2.96 (IH, p, \(J = 8.8\) Hz), 2.19 (3H, t, \(J = 1.2\) Hz), 1.73 (2H, dd, \(J = 3.2, 9.6\) Hz), 1.61 (2H, dd, \(J = 3.6, 8.4\) Hz), 1.14 (3H, s); LC/MS: 245 (M+H)\(^+\), 267 (M+Na)\(^+\) positive ion mode.

Example 5

Epoxidation of 7-HF to obtain 4,5-epoxy-7-hydroxyfrullanolide [compound-13, (15)].
A mixture of 7-HF (300 mg, 0.0012 mol) and 5mL of methylene chloride in a round bottomed flask was treated slowly with m-chloroperbenzoic acid (417 mg, 0.0024 mol) and the contents stirred at rt. After 1.5h, the reaction mixture was poured into ice-water and the mixture was extracted with EtOAc. The EtOAc layer was washed with NaHCO₃ solution followed by dry over Na₂SO₄ and concentrated under vacuum. The residue (300mg) was purified on a silica column using acetone/hexane mixtures. The fractions eluted with 30% acetone/hexane were monitored on TLC and the fractions containing Compound- 13 (15) were combined and evaporated under vacuum to form a solid (71.5 mg).

**Example 6**

Treatment of 7-HF with N-bromosuccinimde (NBS) to obtain (R)-3-(bromomethyl)-5,5a,6,7-tetrahydro-5a,9-dimethylnaphtho[1,2-b]furan-2(4H)-one [compound-14, (16)]

To a mixture 7-Hydroxyfrullanolide (500 mg, 0.002 mol) in carbon tetrachloride (5 mL) was slowly added 720 mg (0.004 mol) of NBS at Room Temperature (RT). After 1h, the reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na₂SO₄ and evaporated under vacuum. The residue (500 mg) was subjected to column chromatography over silica gel using ethyl acetate/hexane mixtures. The fraction eluted with ethyl acetate/hexane was evaporated to obtain compound- 14 [(16); 200 mg, 32.2%]

**Compound-14**: ¹H NMR (CDCl₃,400MHz): δ 5.95 (1H, m), 4.21 (2H, s), 2.83 - 2.88 (1H, m), 2.67 - 2.76 (1H, m), 2.32 - 2.40 (1H, m), 2.22 - 2.27 (1H, m), 2.14.(3H, m), 1.71 (2H, m), 1.57 (2H, m), 1.11 (3H, m).

**Example 7**

Reduction of 7-a-HF with Pd/CaCOVethanol to obtain (3aR,5aR,9bS)-3.3a.4.5a.5a.6.7.8-octahydro-3a-hydroxy-3,5a,9-trimethylnaphtho[1,2-b]furan-2(9bH)-one [compound- 15].
To a mixture of 7-α-HF (500mg) in 10 ml of ethanol in a RB flask under stirring was added 10mg of Pd on CaCO₃. The RB flask is flushed with H₂ gas and the stirring continued under H₂ atmosphere. After 1h, the reaction mixture was filtered through super cell. The filtrate was concentrated under vacuum and the residue (500 mg) was subjected to column chromatography on silica gel using ethyl acetate/chloroform mixtures to yield 200 mg (40%) of Compound-15 (17).

Compound-15: ¹H NMR(CDC1₃, 400MHz): δ 4.96 (1H, d, J = 0.8 Hz), 2.78 (1H, q, J = 7.2Hz), 2.08 - 2.14 (3H, m), 1.81 (2H, m), 1.79 (3H, s), 1.64 (1H, m), 1.37 - 1.56 (5H, m), 1.20 (3H, d, J = 7.2 Hz), 1.05 (3H, s); LC/MS: 479.3 (M+Na) + positive ion mode, 455 (M-H), 491 (M+2H₂O-H) - negative ion mode.

Example 8

Synthesis of (2E)-(3aR,5aR,9bSV2,3,3a,4,5,5a,6,7,8-octahydro-3a,8-dihydroxy-5a,9-trimethyl-2-oxonaphtho[1,2-b]furan-8-yl 3-(2,5-dimethoxyphenyldacrylate (compound-16). (18): A solution of (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a,8-dihydroxy-5a,9-dimethyl-3-methylenenaphtho[1,2-b]furan-2(9bH)-one (compound-10; 300 mg, 0.0013 mol), 2, 5-dimethoxy cinnamic acid (280 mg, 0.013 mol), 6mL of methylene dichloride (MDC), 50 mg of dimethylaminopyridine (DMAP) was taken in a RB flask and treated slowly with a solution of 351 mg of dicyclohexylcarbodiimide (DCC) (0.0017 mol) dissolved in 4mL of MDC under stirring at 0°C. The RM was then allowed to RT and continued the stirring for 2h. The RM was then poured into ice-cold water and the mixture extracted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under vacuum. The residue (600 mg) was subjected to column chromatography on silica column using acetone/hexane mixtures to obtain 120 mg of compound-16 (18, 24%) in the fraction eluted with 25% of acetone/hexane.

Compound-16: ¹H NMR (CDCl₃, 400MHz): δ 7.91 (1H, d, J = 16.0Hz), 7.44 (1H, d, J = 8.4Hz), 6.50 (1H, dd, J = 8.4, 2.4 Hz), 6.46 (1H, d, J = 16.0 Hz), 6.48 (1H, d, J = 2.0Hz), 5.34 (1H, d, J = 4.0Hz), 3.88 (3H, s), 3.84 (3H, s), 2.52 (1H, q, J = 7.6Hz), 2.05 (2H, m), 1.848(3H, s), 1.84 (2H, m), 1.68 (2H, m), 1.47 (1H, m), 1.138(1H, m), 1.34 (3H, d, J = 7.6Hz), 1.23 (1H, m), 1.10 (3H, s); LC/MS: 479.3 (M+Na) + positive ion mode, 455 (M-H)⁺, 491 (M+2H₂O-H) - negative ion mode.
Example 9
Reaction of 7-HF with morpholine to obtain \((3aR,5aR,9bS)-3.3a,4.5.5a,6.7.8\text{-octahydro}-3a\text{-hydroxy}-5a,9\text{-dimethyl}-3-(\text{morpholinomethyl})\text{naphtho}[1,2-b]\text{furan}-2(9bH)\text{-one [compound-17, (19Y]:}}

To a solution of 7-hydroxyfrullanolide (300 mg, 0.0012 mol) in 5 mL of THF in a RB flask was added slowly morpholine (87 µL, 0.0014 mol) under stirring at RT. After 1.5 h, the RM was poured into ice-cold water and the mixture extracted with EtOAc. The organic layer was washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated under vacuum. The residue (300 mg) was purified on silica column using acetone/chloroform mixtures to obtain 150 mg (yield 38%) of compound 17 (19) in the fractions eluted with 15% acetone/chloroform.

**Compound-17:** \(^1\text{H} \text{NMR (CDC}1\text{3, 400MHz):} \delta 5.02 (1H, s), 3.74 (2H, m), 3.67 (2H, m), 3.08 (1H, dd, \(J = 7.6, 8.8 \text{ Hz}), 2.84 (1H, d, \(J = 7.6 \text{ Hz}), 2.84 (1H, d, \(J = 8.8 \text{ Hz}), 2.73 (2H, m), 2.44 (2H, m), 2.09 (2H, m), 1.91 (1H, dt, \(J = 4.0, 14.0 \text{ Hz}), 1.79 (3H, m), 1.70 - 1.70 (4H, m), 1.40 - 1.47 (3H, m), 1.06 (3H, s); \text{LC/MS:} 336.4(M+H)\text{+}, 358.4(M+Na)\text{+ positive ion mode.}

Example 10
Reaction of (R)-2A5.5a,6 J-hexahydro-5a,9-dimethyl-2-oxonaphtho[1,2-b]furan-3-carbaldehyde [compound-10, (12)] with morpholine to produce \((3aR,5aR,9bS)-3.3a,4.5.5a,6.7.8\text{-octahydro}-3a,8\text{-dihydroxy}-5a,9\text{-dimethyl}-3-(\text{morpholinomethyl})\text{naphtho}[1,2-b]\text{furan}-2(9bH)\text{-one [compound-18, (20):}}

A mixture of 300 mg (0.0011 mol) of compound-10 (12) and morpholine (0.169 mL, 0.00135 mol) in 5 mL of THF in a RB flask was stirred at RT for one and a half hours and poured into ice cold water. The mixture was extracted with EtOAc and the organic layer washed with brine, dried over sodium sulfate and concentrated under vacuum. The residue was subjected to chromatography on silica column using acetone/chloroform mixtures. The fraction eluted with 40% acetone/chloroform yielded 100 mg (yield: 25%) of compound-18 (20).

**Compound-18:** \(^1\text{H} \text{NMR(CDC}1\text{3, 400MHz):} \delta 4.95 (1H, s), 3.95 (1H, d, \(J = 3.2 \text{ Hz}) 3.74 (2H, m), 3.67 (2H, m), 3.08 (1H, dd, \(J = 6.0, 10.0 \text{ Hz}), 2.84 (2H, m), 2.72 (2H, m), 2.44 (2H, m), 2.04 (1H, dt, \(J = 3.2, 13.6 \text{ Hz}), 1.98 (1H, m), 1.96 (3H, s), 1.60 - 1.89 (6H, m), 1.35 - 1.48 (2H, m), 1.04 (3H, s).
Example 11
Reaction of 7-HF with piperidine to produce (3aR,5aR,9bS)-3.3a.4.5.5a.6.7.8-octahydrop-3a-hydroxy-5a,9-dimethyl-3-((piperidin-1-yl)methyl)naphtho[1,2-b]furan-2(9bH)-one [compound-19, (21)].

To a mixture of 7-HF (300 mg, 0.0012 mol) in 5 mL of THF in a RB flask was slowly added piperidine (0.143 mL, 0.0014 mol) at rt and the mixture stirred for 1.5 h. The reaction mixture was then poured into ice cold water and the mixture extracted with EtOAc. The organic layer was washed with brine, dried over Na2SO4 and evaporated under vacuum. The residue (300 mg) was subjected chromatography over silica column using methanol/chloroform mixtures. The fraction eluted with 40% of acetone/hexane yielded compound-19 [(21), yield 44%].

Compound-19: 1H NMR (CDCl3, 400 MHz): δ 5.02 (1H, s), 3.10 (1H, dd, J = 5.6, 10.8 Hz), 2.85 (1H, d, J = 11.2 Hz), 2.79 (2H, dd, J = 5.6, 11.2 Hz), 2.70 (2H, brs), 2.41 (2H, brs), 2.17 (3H, s), 2.151 - 2.06 (3H, m), 1.81 - 1.85 (2H, m), 1.79 (3H, s), 1.56 - 1.71 (4H, m), 1.505 - 1.38 - 1.50 (3H, m), 1.05 (3H, s).

Example 12
Reaction of 4,5-epoxy-7-hydroxy frullanolide [compound-13, (15)] with piperidine to produce (5aR)-5,5a,6,7,8,9-hexahydro-9-hydroxy-5a,9-dimethyl-3-((piperidin-1-yl)methyl)naphtho[2,3-b]furan-2(4H)-one [compound-20, (22)]. To a mixture of epoxide [compound-13, (15); 300 mg, 0.0013 mol] in 5 mL of THF in a RB flask was slowly added piperidine (144 µL, 0.0013 mol) at Room Temperature (RT) and the mixture stirred for 24 h. Then the Reaction Mixture (RM) was poured in to ice-cold water and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na2SO4 and concentrated under vacuum. The residue (400 mg) was purified on a silica column using acetone/chloroform mixtures. The fraction eluted with 30% acetone/chloroform yielded compound-20 [(22); 150 mg] with a percentage yield 40%.

Compound-20: 1H NMR (CDCl3, 400 MHz): δ 3.27 (2H, s), 2.93 (1H, m), 2.27 (1H, m), 2.41 (4H, m), 1.90 (1H, m), 1.63 - 1.85 (6H, m), 1.55 - 1.59 (6H, m), 1.53 (3H, s), 1.46 (4H, m), 1.21 (3H, s). LC/MS: 332 (M+H)+ positive ion mode.
Example 13

Reaction of 7-HF with 1, 2, 4-triazole to produce (3aR,5aR,9bS)-3-((4H-1.2.4-triazol-4-yl)methyl)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethyl-3-((piperazin-1-yl)methyl)naphtho[1,2-b]furan-2(9bH)-one compound-21 (23): To a mixture of 7-HF (300 mg, 0.0012 mol) in 5 mL of THF in a RB flask was slowly added 1, 2, 4-triazole (0.1 g, 0.0014 mol) at RT and the mixture stirred for 1.5 h. The reaction mixture was then poured into ice-cold water and the mixture extracted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and evaporated under vacuum. The residue (300 mg) was subjected chromatography over silica column using methanol/chloroform mixtures. The fraction eluted with 40% of acetone/hexane yielded compound-21 [(23), yield 79%].

Compound-21: ¹H NMR (CDCl₃, 400 MHz): δ 8.21 (1H, s), 8.02 (1H, s), 5.10 (1H, s), 5.06 (1H, s), 4.82 (1H, dd, J = 2.4, 14.4 Hz), 4.36 (1H, dd, J = 11.2, 14.8 Hz), 3.19 (1H, dd, J = 2.8, 11.2 Hz), 2.16 (1H, m), 2.08 (1H, dd, J = 6.0, 17.6 Hz), 1.90 (2H, m), 1.80 (3H, s), 1.77 (2H, m), 1.47 (3H, m), 1.07 (3H, s). LC/MS: 318(M+H)+, 340(M+Na)+, 356(M+K)+ positive ion mode.

Example 14

Reaction of 7-HF with piperazine to produce (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethyl-3-((piperazin-1-yl)methyl)naphtho[1,2-b]furan-2(9bH)-one compound-22. (24): To a mixture of 7-HF (300 mg, 0.0012 mol) in 5 mL of THF in a RB flask was slowly added piperazine (624 mg, 0.0072 mol) at RT and the mixture stirred for 1.5 h. The reaction mixture was poured into ice cold water and the mixture extracted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and evaporated under vacuum. The residue (300 mg) was subjected chromatography over silica column using acetone/hexane mixtures. The fraction eluted with 30% acetone/hexane yielded 170 mg of compound-22 [(24), yield 51%].

Compound-22: ¹H NMR (CDCl₃, 400 MHz): δ 5.02 (1H, s), 3.06 (1H, m), 2.94 (5H, m), 2.71 (2H, brs), 2.73 (2H, m), 2.40 (2H, brs), 2.154-2.03 (2H, m), 1.87 (1H, td, J = 3.6, 14.0 Hz), 1.79 (3H, m), 1.56 - 1.71 (4H, m), 1.38 - 1.49 (4H, m), 1.06 (3H, s); LC/MS: 335(M+H)+ positive ion mode.
Example 15
Reaction of 7-HF with piperazine to produce piperazine bis-7-hydroxyfrullanolide compound-23. (25): To a mixture of 7-HF (300 mg, 0.0012mol) in 5mL of THF in a RB flask was slowly added piperazine (124 mg, 0.0014 mol) at RT and the mixture stirred for 1.5 h. The reaction mixture was then poured into ice cold water and the mixture extracted with EtOAc. The organic layer was washed with brine, dry over Na2SO4 and evaporated under vacuum. The residue (300 mg) was subjected chromatography over silica column using acetone/hexane mixtures. The fraction eluted with 30% of acetone/hexane yielded 30 mg of compound-23 (25).

Compound-23: 1H NMR (CDCl3, 400MHz): δ 5.01 (2H, s), 3.06 (2H, dd, J = 5.2, 11.2 Hz), 2.87 (2H, d, J = 13.2 Hz), 2.80 (4H, dd, J = 5.6, 13.2 Hz), 2.11 (4H, m), 1.91 (2H, td, J = 2.8, 13.2 Hz), 1.80 (2H, m), 1.79 (6H, s), 1.39 - 1.70 (18H, m), 1.05 (6H, s); 13C NMR (CDCl3, 100MHz): δ 174.2, 140.3, 126.7, 79.7, 77.5, 77.2, 53.2, 49.3, 39.5, 35.0, 33.5, 33.2, 19.7, 25.9, 25.4, 19.4, 18.2; LC/MS: 583(M+H)⁺, 605(M+Na)⁺ positive ion mode.

Example 16
Assessment of inhibition of lipid accumulation in differentiated adipocytes by 7-hydroxyfrullanolide (7-HF): One hundred thousand 3T3-L1 Human pre-adipocyte cells in Dulbecco’s Modified Eagles Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) were taken into each well of a 24-well plate and incubated for 48h at 37°C and 5% CO₂. The differentiation of pre-adipocyte cells was initiated in a differentiation medium containing 10 µg/ml insulin, 1.0 µM dexamethasone, and 0.5 mM isobutylmethylxanthine (IBMX) for 48h. After this the medium was replaced by DMEM containing 10ug/ml insulin and incubated for 3 days. Then the differentiating cells were treated with 1.0 or 2 or 2.5 µg/ml of 7-hydroxyfrullanolide (I) or different natural analogs (structure numbers 3 to 11) or semi-synthetic analogs (structure numbers 12 to 25) of 7-HF. The cells were maintained in the medium for another 3-5 days. The cells incubated with 0.1% DMSO were considered as the vehicle control. After the incubation period, cells were washed with phosphate buffered saline (PBS) and fixed with 10% buffered formalin for 1h at room temperature. One small aliquot of cell suspension was separated for cell counting in hemocytometer chamber. Fixed cells were stained with Oil Red O solution to measure the
cellular neutral lipid accumulation. Briefly, cells were washed with PBS, fixed with 10% buffered formalin and stained with Oil Red O solution (0.5 g in 100 ml isopropanol) for 10 min. After removing the staining solution, the dye retained in the cells will be eluted into isopropanol and OD measured at 550 nm. The inhibition of fat accumulation in the treated cells was compared with the mock treated differentiated adipocytes. The treated and control cells were also analyzed and compared for inhibition of lipid accumulation visually under microscope and recorded digitally in suitable image capture system. The anti-adipogenic activity by 7-HF is depicted in Figure 1 and the percentage inhibition of lipid accumulation shown by 7-HF and its natural and semi-synthetic analogs is summarized in Table-1.

Table-1

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compound</th>
<th>Concentration (µg/mL)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7-HF (1)</td>
<td>1.0</td>
<td>52.5</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>2.5</td>
<td>63.5</td>
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<tr>
<td>3</td>
<td>Compound-1</td>
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<td>19.4</td>
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<td>Compound-17</td>
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<tr>
<td>16</td>
<td>Compound-23</td>
<td>1.0</td>
<td>15.2</td>
</tr>
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</table>
Example 17  
Assessment of pro-lipolytic activity of 7-hydroxyfrullanolide (1) and its analogs in differentiated adipocytes: The lipolytic activity was assessed in mature adipocytes as per the procedure of Chemicon International, USA, by measuring free glycerol secreted into the culture medium. One hundred thousand 3T3-L1 Human pre-adipocyte cells in Dulbecco’s Modified Eagles Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) were taken into each well of a 24-well plate and incubated for 48h at 37°C and 5% CO₂. The differentiation of pre-adipocyte cells was initiated in a differentiation medium containing 10 µg/ml insulin, 1.0 µM dexamethasone, and 0.5 mM isobutylmethylxanthine (IBMX). The cells were differentiated for 5 days and then the culture medium was removed. The monolayer was washed twice with wash solution (Hank's balanced salt solution), and then 250 µL of incubation solution (Hank's balanced salt solution plus 2% bovine serum albumin) was added to the wells in triplicate in presence or absence of 7-hydroxyfrullanolide or its analogs or the extracts containing 7-HF, and the cells were further incubated for 16 h. To measure lipolysis, 200 µL of free glycerol assay reagent was added to 25 µL of culture supernatants and controls containing glycerol standard. The samples and the controls were incubated for 15 min, and the absorbance was read at 540 nm. A standard curve constructed from the glycerol was used to calculate the concentration of free glycerol in the culture supernatants. The percentage increase in glycerol concentration in the sample solutions compared to the control containing the known concentrations of glycerol corresponds to the percentage acceleration of lipolysis by test compound. The percentage increase in lipolysis accelerated by 7-HF was found to be 47.8% at 5 µg/ml concentration. The data is summarized in Figure II. The data for other compounds is summarized in Table-2.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compound</th>
<th>Concentration (µg/mL)</th>
<th>% acceleration of lipolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7-HF (1)</td>
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<td>47.8</td>
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<td>2</td>
<td>Compound-1</td>
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<td>44.6</td>
</tr>
<tr>
<td>3</td>
<td>Compound-3</td>
<td>5</td>
<td>39.6</td>
</tr>
</tbody>
</table>
Example 18

Inhibition of Peroxisome proliferator-activated receptor gamma (PPARγ), Adipose Differentiation Related Protein (ADRP), CD36, adipocyte fatty acid binding protein (aP2) and Perilipin in 3T3-L1 adipocytes by 7-hydroxyfrullanolide (7-HFV).

Experimental protocol: Mouse pre-adipocyte 3T3-L1 cells are maintained in Dulbecco’s Modified Eagles Medium (DMEM) supplemented with 2 mM glutamine, 4.5g/L glucose and 10% fetal bovine serum. Equal number of cells was plated in each well of 24-well culture plates. Cells were pre-treated separately with 1 µg/mL 7-hydroxyfrullanolide for 2h and followed by addition of differentiation medium containing 500 nM insulin, 1.0 µM dexamethasone, and 0.5 mM isobutylmethylxanthine (IBMX) for 48h. Thereafter, cells were further incubated with post differentiation medium (DMEM containing 100 nM insulin) in presence or absence of 7-HF. Finally, the cells were harvested, washed with chilled phosphate buffered saline and lysed with the lysis buffer. The protein extracts were clarified at 14,000g for 20 min. Protein content was measured in Bradford method by using Coomassie blue dye and cell lysates were stored in aliquots at -80°C until further use. The modulation of adipocyte differentiation markers such as Peroxisome proliferator-activated receptor gamma (PPARγ), CD36, adipocyte fatty acid binding protein (aP2); and intracellular lipid droplet surface associated protein, perilipin expression were evaluated by immunoblot assay.

Inhibition of protein expression of biomarker molecules adipocytes in presence or absence of 7-hydroxyfrullanolide (7-HF) was evaluated in immunoblot assay. Briefly, equal amount of cell lysates proteins were resolved in 7.5% SDS-PAGE; thereafter, the proteins were transferred to nitrocellulose membrane. After blocking the non-specific sites, the membrane was incubated with either anti-PPARγ, or anti-CD36, or anti-aP2, or anti-ADRP, or anti-perilipin antibody. Finally, the specific immuno-reactive bands were developed with West-pico chemiluminescent substrate (Pierce Biotechnology, IL, USA), and the immunoblot images were recorded in a Kodak Image Station (Kodak, USA).
Band intensities were calculated densitometrically and normalized with expression of actin in respective samples. The data is summarized in Figure III.

Example 19

Inhibition of CD36 production by 7-hydroxylfrullanolide (7-HF) in macrophage cells:
Experimental protocol: This was evaluated in glucose induced J774, mouse macrophage cells. Briefly, the cells were cultured in DMEM with 2 mM Glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin and 10% fetal bovine serum (Hyclone, Logan, UT). Equal number of cells was seeded into 35 mm petri dishes (Corning, USA) one day before the experiment. The culture media was replaced with fresh, glucose free DMEM supplemented with 10% fetal bovine serum. 7-HF was diluted at 1 µg and all cultures were pre-incubated for 2 hours at 5% CO₂ at 37°C, and then incubated with 600 mg/dL of glucose for 5 days. Representative photomicrographs showing inhibition of lipid accumulation by 7-HF in high glucose induced macrophage cells of an in vitro model of atherosclerosis are shown in Figure IV. The control culture was supplemented with 100 mg/dL glucose. The cells were harvested and lysed with lysis buffer. Cell lysates were clarified at 14,000g. Protein concentration was measured by Bradford method.

Inhibition of CD36 protein expression in high glucose induced J774 macrophage cells in presence or absence of 7-HF was evaluated in immunoblot assay. Briefly, equal amount of cell lysates proteins were resolved in 7.5% SDS-PAGE; thereafter, the proteins were transferred to nitrocellulose membrane. After blocking the non-specific sites, the membrane was incubated with CD36 antibody (R&D Systems, Minneapolis, MN). Finally, the specific immuno-reactive bands were developed with West-pico chemiluminescent substrate (Pierce Biotechnology, IL, USA), and the immunoblot images were recorded in a Kodak Image Station (Kodak, USA). Band intensities were calculated densitometrically and normalized with expression of actin in respective samples. The results are summarized in Figure V.

Example 20

Nitrite assay protocol

Equal number (5000 cells) of human endothelial cells was plated in each well of a 96-well cell culture plate. The cells were treated with various concentrations (0.1, 0.25, 0.5
and 1.0 ng/ml) of 7-HF for 24h. The control cultures received 0.01% (v/v) DMSO as the vehicle. After 24h, the culture supernatants were collected and mixed with equal volume of Griess reagent [1:1 mixture of NED solution (0.1% N-1-naphthylethulenediamine dihydrochloride in water) and Sulfanylamide solution (1% sulfanilamide in 5% phosphoric acid)]. The reaction was allowed for 10 min at room temperature. Finally, the color reaction was read at 550 nm in a micro-plate reader (BioRad, USA). Known concentrations of sodium nitrite were reacted to obtain a standard curve. Modulation of nitrite production in 7-HF treated cultures was quantitatively assessed by extrapolating the absorbance readings obtained from the test samples into the standard plot. The data is summarized in Figure VI.

Example 2.1
Inhibition of PTP-1B activity by 7-HF: Equal number of 3T3-L1 mouse preadipocytes was seeded into cell culture dishes. After 24h, the cells were treated either with different concentrations of 7-HF or 50 DM Sodium vanadate (Na<sub>2</sub>V<sub>0</sub>) for further 48h. Thereafter, the washed cells were lysed with cell lysis buffer and the clarified at 14,000 g for 10 min at 4°C. The protein concentrations were calculated by Bradford method and the cell lysates were reacted with equal volume of substrate reagent containing 10 mM p-nitrophenyl phosphate (pNPP). After 1h incubation at 37°C, the reaction was stopped with 1N NaOH and the developed color was read at 405 nm. The specific enzyme activity was calculated by using an extinction coefficient of 1.78 x 10<sup>4</sup> M<sup>-1</sup>cm<sup>-1</sup> for pNPP at A<sub>405</sub>. The inhibition shown by 7-HF is depicted in Figure VII.

Example 22
Modulation of adiponectin by 7-hydroxyfrullanolide (7-HF): Modulation of adiponectin protein by 7-hydroxyfrullanolide (7-HF) in 3T3-L1 adipocytes was evaluated in Western immunoblot assay. The cell culture, treatment protocol and immunoblot assay methodology were the same as described above in Example 18. Figure VIII summarizes the enhancement of adiponectin protein expression in 3T3-L1 mature adipocytes by 7-HF.

Example 23
In vivo efficacy of Sphaeranthus indicus ethyl acetate extract (SIE) against metabolic disorders:
Efficacy of the *Sphaeranthus indicus* ethyl acetate extract (SIE) was tested in high fat, high cholesterol, high salt and high sucrose diet induced model of metabolic syndrome. Induction: A batch of 12 Sprague Dawley Rats was randomly divided into 2 groups, each comprised of 6 animals. Animals were acclimatized for 7 days prior to study initiation. Metabolic syndrome was induced by feeding the rats with the metabolic syndrome diet containing 32 g of roasted bengal gram, 27 g of sucrose, 17 g of milk powder, 5 g of mineral salt mixture, 1 g of yeast, 2 g of butter, 11 g of groundnut oil and 5 g of cholesterol per 100 g of the diet for 8 weeks.

Treatment: Following 8 weeks induction phase, the animals were treated orally (using oral feeding gavage) with allocated test substance or vehicle daily for 8 weeks. The treatment group animals were supplemented orally with 250 mg/kg body weight of SIE in 10 mL of 0.5% CMC in water for further 8 weeks. The control group of animals received only the vehicle (10 mL of 0.5% CMC in water) during this period. During the treatment phase, all animals were provided with the standard rodent diet till the end of the study.

Body weights: Body weight of individual animal was recorded weekly for the entire duration of the study. Mean body weights for the treatment group and control group were determined. The body weight gain was calculated at the end of 1st week, 4th week and 8th week after initiation of treatment in comparison to initial body weight. In comparison to the control group, SIE at 250 mg/kg dose exhibited highly potent and statistically significant (p<0.01) reduction in body weight gain (66.04%) in comparison to control group. The results of body weight gain for the treatment groups and control group are summarized in figures XIA & XIB.

**Fat tissue weight:** Abdominal and epididymal fat were isolated and weighed at the termination of the study and the results were represented in Table-3. Abdominal and epididymal fat weights in the treatment group are lower, when compared to those in the control group. The total fat was significantly reduced (p<0.05) in the treatment group supplemented with ethyl acetate extract of *Sphaeranthus indicus* (SIE).
Weight of fat tissues isolated from abdomen and epididymal area of rats.

Table-3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abdominal fat (g)</th>
<th>Epididymal fat (g)</th>
<th>Total fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10 mL/kg)</td>
<td>4.52 ± 1.16</td>
<td>4.18 ± 1.56</td>
<td>8.70 ± 2.52</td>
</tr>
<tr>
<td>SIE (250 mg/kg)</td>
<td>2.28 ± 0.78</td>
<td>3.07 ± 0.74</td>
<td>5.36 ± 0.89</td>
</tr>
</tbody>
</table>

Values expressed as mean weight ± SD

Serum Biochemistry: Blood sampling was done via sinus orbital plexus under mild anesthesia, before induction, before initiation of treatment and after completion of treatment. Various biochemical parameters including lipid profile were evaluated using biochemistry reagents supplied by Human, Germany, in an automated clinical chemistry analyzer HumaStar300, Make: Human, Germany. Mean values of the biochemical parameters especially serum cholesterol levels and triglycerides levels were estimated before induction, after induction/before treatment and after treatment. Supplementation of ethyl acetate extract of Sphaeranthus indicus (SIE) at 250 mg/kg resulted in improvement in fat profile with 15.3, 12.7 and 22.9 percentage reductions respectively in serum cholesterol, LDL and triglycerides.

Estimation of Biomarker Adiponectin: The serum adiponectin concentration for the control and treatment groups of animals were assessed using double antibody based sandwich rat adiponectin ELISA kit. The assay was performed following the instructions provided by the manufacturer (Linco Research, USA). The sensitivity of the assay is 0.155 ng/ml. Adiponectin assay revealed that supplementation of SIE at a dose of 250 mg/day/kg body weight for 8-weeks resulted in significant (p=0.00618) improvement in serum adiponectin concentration, in comparison with the baseline. The control group, however, did not show improvement in serum adiponectin concentration. The results are summarized in Figure XII.
The Homeostasis Model Assessment (HOMA): The HOMA index was calculated based on serum insulin and glucose levels, using the following formula:

\[
\text{Fasting insulin concentration (µu/mL) } \times \text{ Fasting glucose concentration (mmol/L)/22.5}
\]

The supplementation of treatment group of rats with a daily dose of 250 mg/kg body weight for 8-week treatment period resulted in significant reduction of HOMA index compared to control group. The data is presented in Figure XIII.

Those of ordinary skill in the art will appreciate that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments or examples disclosed, but is intended to cover modifications within the objectives and scope of the present invention as defined in the specification.
We claim:

1. Biologically active ingredient(s) comprising at least one component selected from 7-hydroxyfrullanolide, its analog(s); extract(s) and fraction(s) containing 7-hydroxyfrullanolide or its analog(s) or both; or mixtures thereof for the prevention, control and/or treatment of one or more metabolic disorders.

2. A biologically active composition comprising at least one component selected from the list comprising 7-hydroxyfrullanolide, its analog(s); the extract(s) or fraction(s) containing 7-hydroxyfrullanolide/its analog(s) or both; or mixture(s) thereof as an active in combination with one or more ingredients selected from other biologically active components derived from plants, animals and microorganisms; pharmaceutically or dietetically acceptable active ingredients, vitamins, aminoacids, minerals, vehicles, carriers and diluents or mixtures thereof for the prevention, control and/or treatment of one or more metabolic disorders.

3. The biologically active ingredient or their composition according to claims 1 and 2, wherein said metabolic disorders comprise obesity, over weight, diabetes, arteriosclerosis, cardiovascular diseases, hypertension, hypercholesteremia, hyperlipidemia, triglyceridemia, metabolic syndrome, endothelial dysfunction and other metabolic disorders.

4. The biologically active ingredient(s) or their composition according to claims 1 and 2, for the amelioration of the expression/production of one or more biological marker proteins related to metabolic disorders.

5. The biologically active ingredient(s) or their composition according to claim 4, wherein said biological marker proteins comprise Peroxisome proliferator-activated receptor gamma (PPARy), Adipose Differentiation Related Protein (ADRP), adipocyte CD36, Macrophage CD36, Monocyte Chemotactic protein (MCP-1), Oxidized LDL (Ox-LDL), adipocyte fatty-acid-binding protein (aP2/FABP4/A-FABP), beta-3 Adrenergic Receptor (P3AR), Perilipin, Adiponectin, Protein tyrosine phosphatase-1B (PTP-1B),
Matrix Metalloproteinase-1 (MMP-1), Matrix Metalloproteinase-3 (MMP-3), and Matrix Metalloproteinase-13 (MMP-13).

6. The biologically active ingredient(s) or their composition according to claims 1, 2 and 4, wherein the analog(s) of 7-hydroxyfrullanolide comprises of the compound(s) represented by the general formula I given below:

![Formula I](image)

Wherein R₁, R₂, R₃, R₄, and R₅ are each independently selected from hydrogen, hydroxy, halogen, -OOR₂, alkoxy, -OC(0)R₁₂ and C(0)R₁₂; optionally R₁ and R₂ are taken together to form a ketone (=0).

The tricyclic ring system consists of one or two or three double bonds.

- Optionally R₂ and R₃ together form a double bond;
- Optionally R₃ and R₄ together form a double bond;
- Optionally R₃ and R₅ together form a double bond;
- Optionally R₅ and R₆ together form a double bond;
- Optionally R₈ and R₉ together form a double bond;
- Further optionally R₃ and R₅ together form an epoxide ring

R₇ is selected from hydrogen, hydroxy, halogen, alkoxy and -OC(0)R₂; R₈ is selected from hydrogen, hydroxy, halogen, alkoxy, -OC(0)R₂, -C(0)R₂ and NRᵢ₃Rᵣ₄; R₉ is selected from hydroxy, alkyl, cycloalkyl, alkoxy, aryl, heterocyclyl, halogen, -OC(0)R₂, -C(0)R₁₂, azido and -NRᵢ₃Rᵣ₄, -S(0)ᵢ₅R₁₅, -OS(O)ᵢ₅Rᵣ₅; wherein m is 0, 1 or 2;
Rio and R\textsubscript{11} are each independently selected from hydrogen, alkyl, halogen, OR(6, -NH Ri\textsubscript{2} and SRi\textsubscript{2}; wherein R\textsubscript{i6} is selected from hydrogen, alkyl and -C(0)Ri\textsubscript{2} or R\textsubscript{i6} and R\textsubscript{11} together form one of ketone (=0), thioke tone (S), imine (NH) and selenoketone (Se);

R\textsubscript{12} is selected from hydrogen and alkyl;

R\textsubscript{i3} and R\textsubscript{i4} are each independently selected from hydrogen, alkyl, cycloalkyl, aralkyl, aryl, heterocycll, -C(0)R\textsubscript{12} or -C(0)NHRi\textsubscript{2}; or R\textsubscript{13} and R\textsubscript{i4} together with the N atom to which they are bonded, to form a 5-, 6-, or 7-membered heterocyclic ring, optionally having one or more additional heteroatoms selected from O, N, S and Se;

R\textsubscript{15} is selected from hydrogen, alkyl, cycloalkyl, aryl, heterocycll

X is selected from O, NH, S and Se

7. The biologically active ingredient(s) or their composition according to claim 6 wherein said 7-hydroxyfrullanolide or its analog(s) or mixtures thereof can be of natural, synthetic or semi-synthetic origin.

8. The biologically active ingredient(s) or their composition according to claim 7, wherein the natural origin is of any plant species that produces 7-hydroxyfrullanolide or its analog(s) or mixtures thereof.

9. The biologically active ingredient(s) or their composition according to claim 7, wherein the natural analogs of 7-hydroxyfrullanolide comprises of frullanolides; 11a, 13-dihydro-3a,7a-dihydroxy-4,5-epoxy-6p,7-eudesmanolide; 11a, 13-dihydro-7a-acetoxy-3β-hydroxy-6p,7-eudesm-4-enolide; 3-keto-β-eudesmol; 11la,13-dihydro-3a,7ct-dihydroxyeudesmesm-4-en-6a,12-olide; lla,13-dihydro-3a,7a-dihydroxyfrullanolide; 11a,13-dihydro-7a,13-dihydroxyfrullanolide; 11a,13-dihydro-7a-hydroxy-13-methaoxyfrullanolide; 2a,7a-dihydroxy-4-en-1 1,13-dihydroeudesm-6,12-olide; 2a-hydroxycostic acid; 3-keto-7a-hydroxyeudesmesm-4-en-6,12-olide (cryptomeridiol); 4-epicryptomeridiol; sphaeranthanolide; 2a-hydroxyxyaenarthanolide; 2a-acetoxyxyaenarthanolide; 2a,7a-dihydroxyxyaenarthanolide; 2a-acetoxy-7a-hydroxyxyaenarthanolide; 2a-acetoxy-5a-hydroxyisxyaenarthanolide; eudesmanolide dimer (compound-2); (3aR,5aR,9aR,9bR)-decahydro-9a-hydroperoxy-3a-hydroxy-5a-
methyl-3,9-dimethylenenaphtho[1,2-b]furan-2(9bH)-one (compound-3); (3aS,5aR,9bR)-3,3a,4,5,5a,6,7,8-octahydro-3,5a,9-trimethylnaphtho[1,2-b]furan-2(9bH)-one (compound-4); (R)-5,5a,6,7-tetrahydro-3,5a,9-trimethylnaphtho[1,2-b]furan-2(4H)-one (compound-5), (3R,3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-3-(methoxymethyl)-5a,9-dimethylnaphtho[1,2-b]furan-2(9bH)-one (compound-6); 2-((3R,8aR)-1,2,3,7,8,8a-hexahydro-5,8a-dimethylnaphthalen-3-yl)acrylic acid (compound-7); (3aR,5aR,9bS)-3-((6-amino-9H-purin-9-yl)ethyl)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethylnaphtho[1,2-b]furan-2(9bH)-one (compound-8); (3R,3aR,5aR,8R,9bS)-8-((2R,3S,4R,5R)-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yloxy)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-3,5a,9-trimethylnaphtho[1,2-b]furan-2(9bH)-one (compound-9) or mixtures thereof, preferably 7-hydroxyfrullanolide.

10. The biologically active ingredient(s) or their composition according to claim 7, wherein the synthetic/semi-synthetic analogs of 7-hydroxyfrullanolide comprises (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a,8-dihydroxy-5a,9-dimethyl-3-methylenenaphtho[1,2-b]furan-2(9bH)-one (compound-10); (3aR,5aS,9bS)-3a,4,5,5a,6,7-hexahydro-3a-hydroxy-5a,9-dimethyl-3-methylenenaphtho[1,2-b]furan-2,8(3H,9bH)-dione (compound-11); (R)-2,4,5,5a,6,7-hexahydro-5,6,7,9-trimethyl-2-oxonaphtho[1,2-b]furan-3-carbaldehyde (compound-12); 4,5-epoxy-7-hydroxyfrullanolide (compound-13); (R)-3-(bromomethyl)-5,5a,6,7-tetrahydro-5a,9-dimethylnaphtho[1,2-b]furan-2(4H)-one (compound-14); (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-3,5a,9-trimethylnaphtho[1,2-b]furan-2(9bH)-one (compound-15); (2E)-(3aR,5aR,9bS)-2,3,3a,4,5,5a,6,7,8,9b-decahydro-3a-hydroxy-3,5a,9-trimethyl-2-oxonaphtho[1,2-b]furan-8-yl 3-(2,5-dimethoxyphenyl)acrylate (compound-16); (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethyl-3-(morpholinomethyl)naphtho[1,2-b]furan-2(9bH)-one (compound-17); (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a,8-dihydroxy-5a,9-dimethyl-3-(morpholinomethyl)naphtho[1,2-b]furan-2(9bH)-one (compound-18); (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethyl-3-((piperidin-1-yl)methyl)naphtho[1,2-b]furan-2(9bH)-one (compound-19); (5aR)-5,5a,6,7,8,9-hexahydro-9-hydroxy-5a,9-dimethyl-3-((piperidin-1-yl)methyl)naphtho[1,2-b]furan-2(4H)-one (compound-20); (3aR,5aR,9bS)-3-((4H-1,2,4-triazol-4-yl)methyl)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethylnaphtho[1,2-b]furan-2(9bH)-one (compound-21), (3aR,5aR,9bS)-3,3a,4,5,5a,6,7,8-octahydro-3a-hydroxy-5a,9-dimethyl-3-
((piperazin-1-yl)methyl)naphtho[1,2-b]furan-2(9bH)-one (compound-22) and piperazine bis-7-hydroxyfrullanolide (compound-23) as substantially depicted in figure X by structures 12 through 25.

11. The biologically active ingredient(s) or their composition according to claims 1 and 2, wherein said 7-hydroxyfrullanolide or its analog(s) or fractions or mixtures thereof are derived from any plant species that produces 7-hydroxyfrullanolide or its analog(s) or mixtures thereof, preferably Sphaeranthus indicus.

12. The biologically active ingredient(s) or their composition according to claims 1 and 2, wherein said extract(s) and fraction(s) standardized to 7-hydroxyfrullanolide or its analog(s) or mixtures thereof are derived from any plant species that produces 7-hydroxyfrullanolide or its analog(s) or mixtures thereof, preferably Sphaeranthus indicus.

13. The biologically active ingredient(s) comprising at least one component selected from 7-hydroxyfrullanolide, its analog(s); extract(s) and fraction(s) containing 7-hydroxyfrullanolide or its analog(s) or both; or mixtures thereof according to claims 1 and 4, wherein the said extracts and fractions contain 7-hydroxyfrullanolide or its analog(s) or mixtures thereof in the range of 0.001% to 100%, preferably 0.01 to 99%.

14. The biologically active composition according to claims 2 and 4, wherein the concentration of 7-hydroxyfrullanolide or its analog(s) or mixtures thereof in the compositions varies in the range from 0.001% to 99%, preferably 0.01 to 95% by weight.

15. The biologically active composition according to claims 2 and 4, wherein the percentage of the extract or fraction standardized to 7-hydroxyfrullanolide or its analog(s) or both varies in the range from 0.01% to 99%, preferably 1% to 90% by weight in the composition.

16. The biologically active composition according to claim 2, wherein the other biologically active components used for making the compositions comprise extract(s), fraction(s), active compound(s), phytochemical(s) or powder(s) derived from plant(s), animal(s) or microorganisms having one or more health benefits selected from but not

17. The biologically active ingredient(s) or their compositions according to claims 1 and 2, can be optionally combined with bio-availability enhancing agents selected from but not limited to extract(s), fraction(s), pure compound(s) derived from Piper nigrum or Piper longum or Stevia rebaudiana.

18. The biologically active ingredient(s) or their compositions according to claims 1 and 2, wherein the 7-hydroxyfrullanolide, its analogs; extracts and fractions containing 7-hydroxyfrullanolide or its analogs or mixtures thereof are derived from Sphaeranthus indicus, wherein said extract(s) or active fraction(s) or active compound(s) or phytochemicals or mixtures thereof are derived from at least one of the plant parts selected from leaves, flower heads, fruits, stem, bark, root, whole plant or mixtures thereof, preferably flower heads.

19. The biologically active ingredient(s) or their compositions according to claim 2, wherein, the pharmaceutically or dietetically acceptable excipients, vehicles and carriers comprise surfactants, binders, diluents, disintegrators, lubricants, preservatives, stabilizers, buffers, suspensions and drug delivery systems.

20. The biologically active ingredient(s) or their compositions according to claim 19, wherein the pharmaceutically or dietetically acceptable excipients, carriers and diluents comprise glucose, fructose, sucrose, maltose, yellow dextrin, white dextrin, aerosil, microcrystalline cellulose, calcium stearate, magnesium stearate, sorbitol, stevioside, corn syrup, lactose, citric acid, tartaric acid, malic acid, succinic acid, lactic acid, L-ascorbic acid, dl-alpha-tocopherol, glycerin, propylene glycol, glycerin fatty ester, poly glycerin fatty ester, sucrose fatty ester, sorbitan fatty ester, propylene glycol fatty ester, acacia, carrageenan, casein, gelatin, pectin, agar, vitamin B group, nicotinamide, calcium pantothenate, amino acids, calcium salts, pigments, flavors, preservatives, distilled water,
saline, aqueous glucose solution, alcohol, propylene glycol and polyethylene glycol, various animal and vegetable oils, white soft paraffin, paraffin and wax.

21. The biologically active ingredient(s) or its composition(s) according to claims 1, 2 and 4, wherein said ingredient(s) or composition(s) is administered orally, topically, parenterally or by inhalation to a subject or mammal or warm blooded animal in need thereof.

22. The biologically active ingredient(s) or its composition(s) according to claim 21, wherein said ingredient(s) or composition(s) is administered once daily or multiple administrations per day.

23. The biologically active ingredient(s) or its composition(s) according to claims 1, 2 and 4, wherein said ingredient(s) or composition(s) can be formulated as oral agents such as tablets, soft capsule, hard capsule, soft gel capsules, pills, granules, powders, emulsions, suspensions, syrups, pellets, food, beverages, concentrated shots, drops and the like; and parenteral agents such as injections, intravenous drip and the like; suppositories; and transdermal agents such as patches, topical creams and gel; ophthalmic agents; nasal agents; and food or beverages.

24. The biologically active ingredient(s) or its composition(s) according to claims 1, 2 and 4, wherein the said ingredient(s) or their composition(s) are delivered in the form of controlled release tablets, using controlled release polymer-based coatings by the techniques including nanotechnology, microencapsulation, colloidal carrier systems and other drug delivery systems.

25. The biologically active ingredient(s) or its composition(s) according to claims 1 and 2, wherein the said ingredient or their composition(s) can be formulated into or added to existing or new food and beverage form(s) as a healthy food for warm blooded animals.

26. Use of biologically active ingredient or its composition(s) according to claims 1 and 2 for prevention, control and/or treatment of one or more diseases or conditions including but not limited to obesity, diabetes, hypertension, arteriosclerosis, cardiovascular
diseases, neurological disorders, Alzheimer's, cognitive disorders, oxidative stress, skin disorders, aging of the skin, UV irradiated damage, hypercholesterolemia, hyperlipidemia, triglyceridemia, immune deficiency, metabolic syndrome, for bringing about weight loss effectively, for producing lean body mass, for using during weight loss program as well as for other metabolic disorders.

27. A method of preventing/controlling/treating one or more metabolic disorders selected from obesity, over weight, diabetes, arteriosclerosis, cardiovascular diseases, hypertension, hypercholesterolemia, hyperlipidemia, triglyceridemia, metabolic syndrome, endothelial dysfunction and other metabolic disorders in a mammal or warm blooded animal in need thereof, wherein the method comprises administering to mammal or warm blooded animal a therapeutically effective amount of at least one biologically active ingredient or its composition(s) according to claims 1 and 2.

28. A method of promoting lipolysis and/or inhibiting adipogenesis in a subject or mammal or warm blooded animal in need thereof comprising administering to said subject or mammal or warm blooded animal a therapeutically effective quantity of at least one biologically active ingredient or its composition(s) according to claims 1 and 2.

29. A method of amelioration of the expression or production of at least one biological marker selected from PPAR-γ, C-reactive protein (CRP), Adipose Differentiation Related Protein (ADRP), adipocyte CD36, macrophage CD36, Monocyte Chemotactic protein (MCP-1), Oxidized LDL, Adipocyte Fatty-acid-Binding Protein (aP2/FABP4/A-FABP), Beta-3 adrenergic receptor (P3-AR), adiponectin, Perilipin, Protein tyrosine phosphatase 1B (PTP IB), Matrix Metalloproteinase-1 (MMP-1), Matrix Metalloproteinase-3 (MMP-3) and Matrix Metalloproteinase-13 (MMP-13) in a subject or mammal or warm blooded animal in need thereof, wherein the method comprises administering to the subject or mammal or warm blooded animal at least one biologically active ingredient or its composition(s) according to claims 1 and 2.
Figure III

Vehicle control vs. 7-HF (1μg/ml)

PPARγ
Actin

ADRP
Actin

CD36
Actin

aP2
Actin

Perilipin
Actin

Bar charts showing expression levels for PPARγ, ADRP, CD36, aP2, and Perilipin with groups a and b.
Figure IV
Figure V

| Glucose 100 mg/dL | +  | -  | -  |
| Glucose 600 mg/dl | -  | +  | +  |
| 7-HF (μg/ml)      | -  | -  | 1.0|

CD36
Actin

Figure VI

Nitrite conc. (μM)

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<th>0.25</th>
<th>0.50</th>
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<tr>
<td>7-HF (ng/ml)</td>
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</table>
Figure IX

1. 

2. 

3. 

4. 

5. 

6. 

7. 

8. 

9. 

10. 

11.
Figure X

Chemical structures labeled as 12 to 25.
Figure XIA

![Bar chart showing body weight gain (g) for weeks 1 and 2 with a star indicating a significant difference.]

Figure XIB

![Line graph showing body weight gain in grams over weeks 1, 4, and 8, with two lines representing different groups.]

*Note: The specific details of the data represented in the charts are not provided in the image.*
Figure XII

Adiponectin conc. (μg/ml)

0 5 10 15 20
1 2

0 week
8 weeks

p = 0.00687

Figure XIII

HOMA Index

0 5 10 15 20
1 2

0 week
8 weeks

p = 0.0013
### INTERNATIONAL SEARCH REPORT

**Category**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>X</td>
<td>US 2008/0254157 A1 (CHAUHAN et al.) 16 October 2008 (16.10.2008) claims 1, 7, 14; para 1000I, [0024]-[0025], [0060]-[0063], [0081], [0090], [0156]</td>
<td>1-2, 16 and 19-20</td>
</tr>
</tbody>
</table>

**Date of the actual completion of the international search**

14 April 2011 (14.04.2011)

**Date of mailing of the international search report**

26 APR 2011

**Name and mailing address of the ISA/US**

Mail Stop: PCT, Attn: ISA/US, Commissioner for Patents
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Form PCT/ISA/210 (second sheet) (July 2009)
# INTERNATIONAL SEARCH REPORT

**International application No.**

PCT/IN 10/00494

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

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

1. ☐ Claims Nos.: (Continuation of item 2 of first sheet)
   - because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.: (Continuation of item 2 of first sheet)
   - because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 3, 15, 17, 18 and 21-29
   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

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### Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

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Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)