Applicant: ITC LIMITED
Application No.:

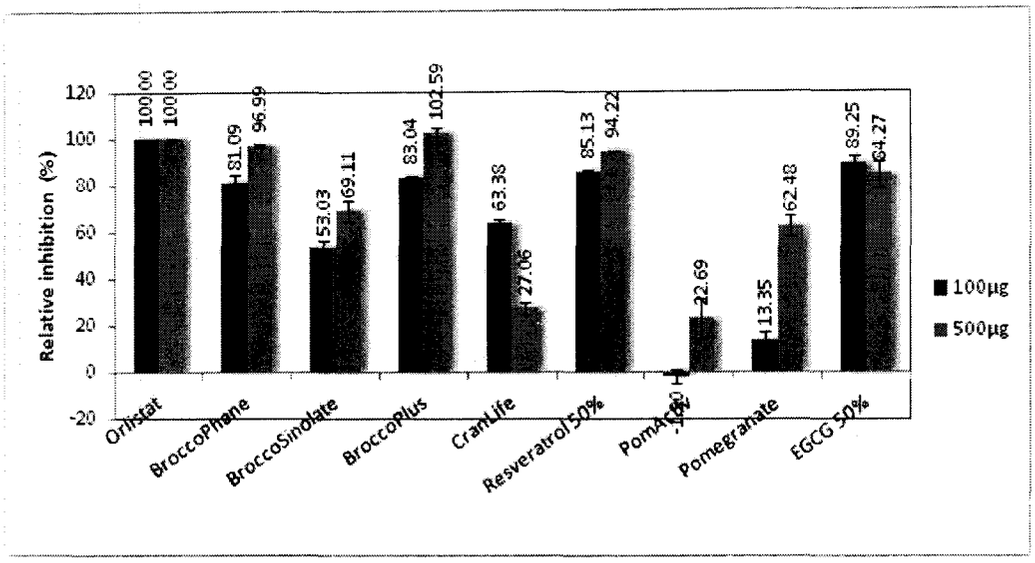

Figure 1

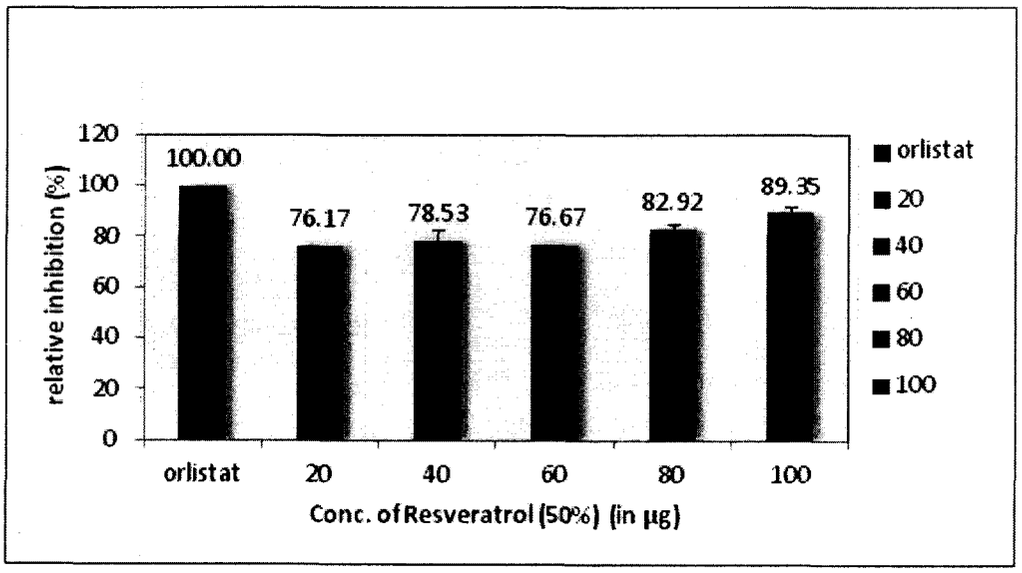

Figure 2

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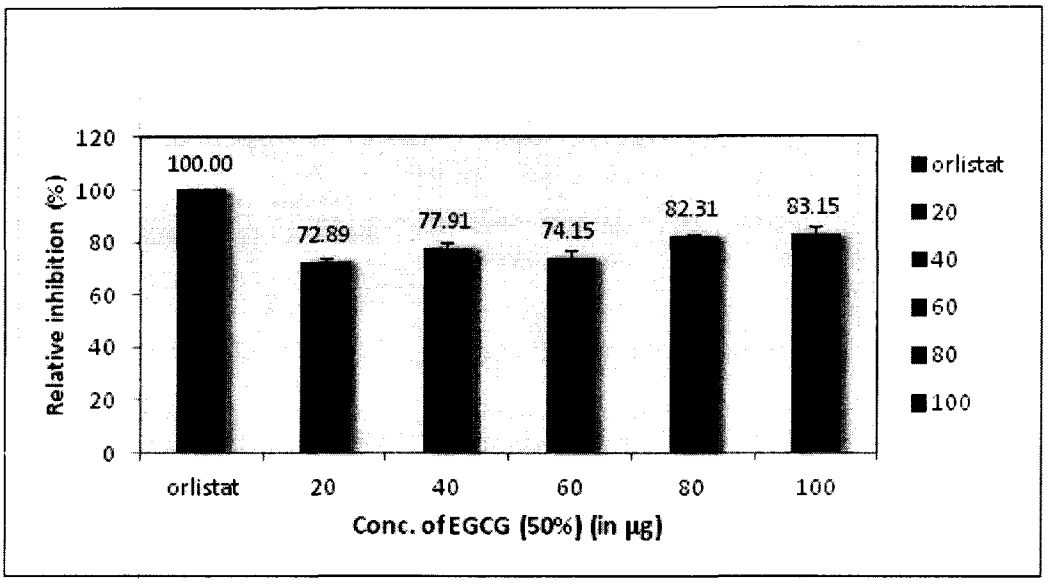

Figure 3

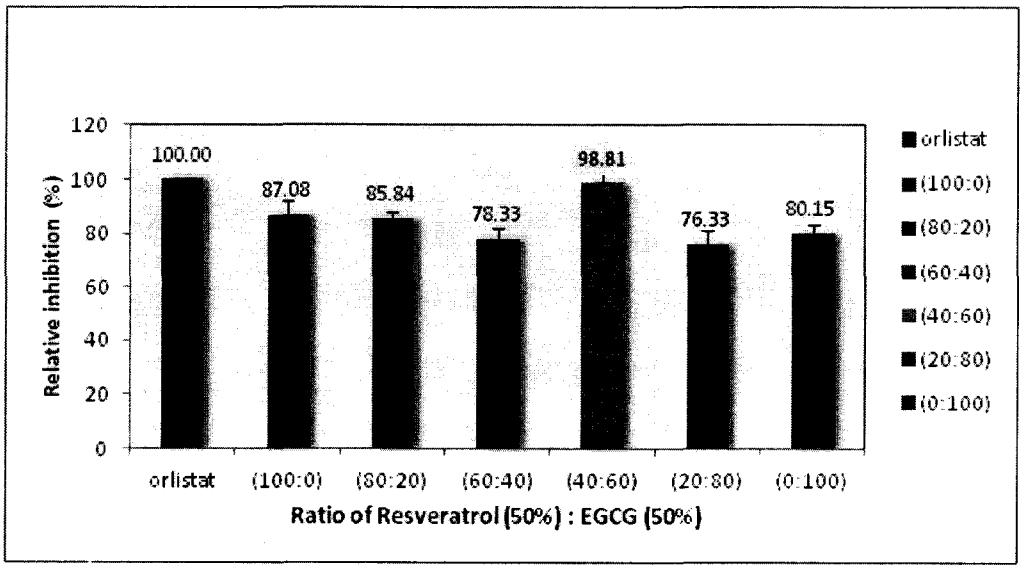

Figure 4

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FIELD OF INVENTION

The present invention relates to a synergistic composition for prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders. The present invention particularly relates to a synergistic composition comprising plant extracts comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof.

BACKGROUND OF THE INVENTION

Obesity is an increasingly common clinical disorder and pathology in both highly developed and developed countries. Over 300 million adults are deemed to be clinically obese worldwide, according to the latest statistics from the World Health Organization and the International Obesity Task Force. In addition to harming physical health, they can wreak havoc on mental health because these diseases affect self-esteem, which ultimately can affect a person's ability to interact socially with others. Unfortunately, obesity and overweight are not well understood, and societal stereotypes and presumptions regarding obesity and overweight only tend to exacerbate the psychological effects of these diseases. Because of the impact of obesity and overweight on individuals and society, much effort has been expended to find ways to treat them, but little success has been achieved in the long-term treatment and/or prevention. There is also substantial clinical and epidemiological evidence accumulated to date that reveals that obesity now represents an additional independent risk factor for the development of both cardiovascular disease and hypertension. Obesity and overweight is generally a chronic disease that requires long-term behavior modification supplemented with drug therapy.

Glucose and lipid metabolism play an important role in development of obesity, overweight, cardiovascular diseases and other metabolic disorders. Excessive lipids or dietary fat uptake has been widely accepted as one of the main causes of obesity and overweight leading to cardiovascular and other metabolic diseases.

The conventional choices to generate a meaningful weight loss for an obese human include medicaments and pharmaceuticals. However, the range of presently available pharmacologically effective compositions is extremely limited; they are frequently quite expensive; and they exhibit many undesirable side effects. Representative examples of FDA-approved orally-prescribed medications, available by prescription, include phentermine, subutramine, and orlistat.

Phentermine is one of the older appetite-suppressing drugs approved by the FDA, with a mechanism of action similar to the stimulant, amphetamine, and therefore is classified as a Class IV controlled drug. Phentermine is a centrally-acting stimulant which increases catecholamine activity, triggering appetite suppression. The pharmacologic effects are generally well-tolerated, but associated with significant potential for physical and psychological dependence [Nelson et al., Endocrine, 2006, 29, 49-60.]

Sibutramine (under the trade names Meridia.RTM. in the U.S.), is a centrally-acting serotonin-norepinephrine reuptake inhibitor structurally similar to amphetamine. Sibutramine increases serotonergic action, which is thought to suppress appetite, but is associated with a significant side effect profile and potential drug interactions; in particular, monoamine oxidase inhibitors (MAOIs), which can cause `serotonin syndrome,` a rare and serious adverse drug reaction. Other side effects include increase in blood pressure, heart rate, and cardiac arrhythmias [Portyansky et al., Drug Topics, 1998, 142, 23.]

Orlistat (marketed in the U.S. under the trade name Xenical.RTM.) inhibits the action pancreatic lipase on triglycerides in the intestine, preventing the absorption of fats in the human diet, and thereby reducing caloric intake. The drug is associated with significant gastrointestinal side effects, such as steatorrhea, fecal incontinence, and excessive flatulence [Zhi et al., J. Clin. Pharmacol., 1995, 35, 1103-1108.] Long-term side effects include a link between orlistat and aberrant crypt foci, lesions found in the colon, believed to be an early precursor of colon cancer [Garcia et al., Cancer Lett., 2006, 240, 221-224.]

Physical exercise is a second conventional technique for achieving weight loss. The benefits of physical exercise and its capability for causing a loss of weight are very publicly advertised. However, despite the many different excise programs and fitness regimens publicly offered today, very few obese persons seem to find sufficient motivation, or adequate determination, or enough physical endurance to continue such exercise programs for any meaningful length of time.

The third general approach is to decrease the amount of caloric consumption per day i.e., a controlled caloric count diet. Although the range and variety of such dieting programs is large, most utilize one of two different techniques: directly decreasing the amount of food and limiting the total calories ingested to a fixed quantity per day; and controlling which kinds of foods or food groupings are eaten in order to trick the metabolic mechanisms and energy producing pathways of the body. Most of the substitute dietary foods contain proteins in large percentage quantities; and typically are powdered admixtures containing soy, whey, or casein (milk) proteins. The commonly encountered feature of this dietary regimen is the relatively high caloric content of these substitute high protein foods. Once eaten, the higher caloric content of proteins will typically increase the glycemic index within the person's bloodstream i.e., generate very high concentrations of glucose in the blood, a medical condition which is nether healthy nor beneficial for any person in general, and one which is particularly hazardous and adverse for those persons having medical problems related to glucose intolerance, insulin resistance, or diabetes.

Another different approach has been to use nutritional intervention compositions which are taken prior to a meal in order to produce a feeling of satiety before the meal, and/or to extend the period of satiety following consumption of a meal in order to lengthen the time interval between one meal and the next.

US Patent 4491578 describes the oral administration of a trypsin inhibitor to enhance satiety. This technique relies upon an *in-vivo* secretion and release of cholecystokinin (or "CCK") as a negative feedback signal, which is caused by a release of trypsin from

the pancreas. The purposeful administration of the trypsin inhibitor blocks the release of trypsin from the pancreas, thereby interfering with the negative feedback mechanism.

US Patent 4833128 discloses the oral administration of phenylalanine in conjunction with protein, carbohydrate and fat to stimulate satiety. When this dietary supplement is consumed fifteen minutes before a meal, a feeling of satiety is generated which causes that person to eat less food during the meal.

US Patent 5932561 reveals that dietary supplements that bind lipids can aid in weight loss and reduce cholesterol levels in the body. It also teaches that dietary supplements that contain saponins from aloe (saponins being any of a group of varied triterpene or steriod glycosides occurring in many plants including aloe) will increase the capacity of chitosan (partially or fully deacetylated chitin) to bind fat.

Another different approach has been to use compositions which inhibit lipase activity. Lipases are ubiquitous enzymes required for all aspects of fat metabolism. Found in the gastrointestinal tract, these enzymes mediate the digestion of triglycerides and their uptake into tissues. Lipases hydrolyze the ester bonds in the triacylglycerides releasing free fatty acids and mono-acylglycerols. Pancreatic lipase is a key enzyme involved in the digestion of dietary triglycerides. The digestion of triglycerides starts in the upper digestive tract by lipases of preduodenal origin (gastric lipase in humans), and is essentially carried out in the intestine, under the action of pancreatic lipase. The latter converts the triglycerides to free fatty acids and to 2-monoglycerides, more polar products of hydrolysis, which are capable of crossing the enterocyte brush border membrane, after incorporation into mixed micelles of bile salts and phospholipids. Pancreatic lipase therefore plays a role in the emergence of diseases linked to the presence of an excess of lipids, such as cardiovascular diseases, hyperlipemias and obesity, by allowing the assimilation of practically all the triglyercides ingested. In addition, it promotes the intestinal absorption of cholesterol, since the solubility of cholesterol is increased in the mixed micelles, which are high in fatty acids.

In the absence of lipases, dietary triacylglycerides are not absorbed and pass into the stool. Consequently, reduced absorption of digested triacylglycerides results in weight loss. In view of the above, current research is increasingly geared towards products which induce inhibition of pancreatic lipases.

Prior art literature reveals that few studies have been conducted on natural compounds from plant extract having pancreatic lipase inhibition activity. Studies in literature reveal various health benefits provided by two such compounds, namely resveratrol and epigallocatechin gallate (EGCG) isolated from various plants. The potential role of resveratrol and epigallocatechin gallate in prevention of cancers, cardiovascular disease and treatment of inflammatory diseases has been documented.

Resveratrol (3,4',5-trihydroxy-trans-stilbene), a phytoalexin found in grape skins, peanuts, and red wine, exhibits a wide range of biological and pharmacological properties. Dietary resveratrol acts as an antioxidant, promotes nitric oxide production, inhibits platelet aggregation, and increases high-density lipoprotein cholesterol and thereby serves as a cardioprotective agent.

EGCG is the major catechin found in green tea. Green tea has been shown to have an effect on increasing fat oxidation and metabolism. By helping to decrease body fat mass, green tea can inadvertently shift the body ratio of fat mass to lean mass in a favourable way. Green tea has been studied for its effects on fat oxidation and thermogenesis in a variety of research models, including *in vitro*, animal and clinical trials.

US Patent No. 7582674 describes compositions comprising resveratrol, its derivative, metabolite or analogue thereof, and at least one additional component selected from epigallocatechin gallate (EGCG), genestein, vitamin E, polyunsaturated fatty acids, gamma-linolenic acid and vitamin K. The patent also discloses method of making such composition. The document further discloses the use of the composition for treatment or prevention of inflammatory diseases such as arthritis, asthma, inflammatory bowel diseases and inflammatory diseases of skin.

US Patent No. 8029830 describes a composition comprising substances that promote DNA repair, reduce body fat levels, increase lean body mass, decreases wrinkle appearance and improves skin surface. The composition comprises forskohlin, astaxanthin, carboxy alkyl ester, beta-alanine, a neuroprotectant, epigallocatechin-gallate (EGCG), rosavins and salidrosides. The patent also describes a method of promoting internal health and external appearance in a subject using the composition.

US patent no. 8129337 describes a composition comprising epigallocatechin gallate and protein hydrolysate for treatment and management of Diabetes. The composition optionally, comprises one or more compounds selected from ligustilide or other phthalides or herbal extracts containing ligustilide or other phthalides, coenzyme Q-10, resveratrol, pantethine or a metabolite thereof, phytanic acid, lipoic acid, and policosanol.

Despite all the foregoing, an effective combination of natural ingredients, which addresses all of the various medical concerns and considerations and is directed specifically to treatment of obesity, overweight and other metabolic disorder has not yet been found to date. Thus, a composition which is effective for use with obese persons with minimal or no side effects and is economically viable would be seen today as an unique and unforeseen development in the technical field.

OBJECTS OF THE PRESENT INVENTION

It is an object of the present invention to provide a composition comprising one or more plant extracts comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof and pharmaceutically acceptable salts.

It is another object of the present invention to provide a composition comprising one or more plant extracts comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof and pharmaceutically acceptable salts, wherein the plant extract is selected from the group consisting of grape juice, pomegranate juice, Japanese knotweed root extract, blueberries fruit extract, bilberries fruit extract, raspberries fruit extract, peanut extract, green tea and carob flour.

It is yet another object of the present invention to provide a composition comprising one or more plant extracts comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof and pharmaceutically acceptable salts, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof are present in a weight ratio between 4:1 and 1:4.

It is still another object of the present invention to provide a composition comprising one or more plant extracts comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof and pharmaceutically acceptable salts, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof are present in a weight ratio of 2:3.

It is another object of the present invention to provide a food product comprising a composition, wherein the composition comprises one or more plant extracts comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof and pharmaceutically acceptable salts.

It is another object of the present invention to provide a beverage comprising a composition, wherein the composition comprises one or more plant extracts comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof and pharmaceutically acceptable salts.

It is yet another object of the present invention to provide a feed supplement comprising a composition, wherein the composition comprises one or more plant extracts comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof and pharmaceutically acceptable salts.

It is still another object of the present invention to provide a feed product comprising a composition, wherein the composition comprises one or more plant extracts comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof and pharmaceutically acceptable salts.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

The following drawings form part of the present specification and are included to further illustrate aspects of the present invention. The invention may be better understood by reference to the drawings in combination with the detailed description of the specific embodiments presented herein.

Figure 1 shows the bar graph illustrating the performance of various plant extracts Orlistat, BroccoPhane, BroccoSinolate, BroccoPlus, CranLife, Resveratrol 50%, PomActiv, Pomegranate and EGCG 50% at concentration of 100 µg and 500 µg on pancreatic lipase enzyme.

Figure 2 shows the bar graph illustrating the inhibitory activity of Resveratrol 50% on Human Pancreatic Lipase at different concentrations (20 µg, 40 µg, 60 µg, 80 µg and 100 µg).

Figure 3 shows the bar graph illustrating the inhibitory activity of EGCG 50% on Human Pancreatic Lipase at different concentrations (20 µg, 40 µg, 60 µg, 80 µg and 100 µg).

Figure 4 shows the bar graph illustrating the synergistic inhibitory effect of Resveratrol 50% and EGCG 50% on human pancreatic lipase at different concentrations.

DETAILED DESCRIPTION OF THE INVENTION

Those skilled in the art will be aware that the invention described herein is subject to variations and modifications other than those specifically described. It is to be understood that the invention described herein includes all such variations and modifications. The invention also includes all such steps, features, compositions and methods referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Definitions

For convenience, before further description of the present invention, certain terms employed in the specification, examples are collected here. These definitions should be read in light of the remainder of the disclosure and understood as by a person of skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. The terms used throughout this specification are defined as follows, unless otherwise limited in specific instances.

The articles "a", "an" and "the" are used to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article.

The term "plurality" means more than one.

The terms "at least two", "more than one" and "plurality" are used interchangeably.

Throughout this specification, unless the context requires otherwise the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated element or step or group of element or steps but not the exclusion of any other element or step or group of element or steps.

The term "including" is used to mean "including but not limited to". "Including" and "including but not limited to" are used interchangeably.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions.

A "therapeutically effective amount" or "an effective amount", which are used interchangeably, is an amount sufficient to decrease or prevent the symptoms associated with the conditions disclosed herein, including diseases associated with diabetes, obesity, overweight, cardiovascular diseases and other metabolic disorders and other related conditions contemplated for therapy with the compositions of the present invention.

The term "lipase inhibitors" refers to a compound that inhibits the metabolic cleavage of dietary triglycerides into free fatty acids and monoglycerides. Under normal physiological conditions, lipolysis occurs via a two-step process that involves acylation of an activated serine moiety of the lipase enzyme. This leads to the production of a fatty acid-lipase hemiacetal intermediate, which is then cleaved to release a diglyceride. Following further deacylation, the lipase-fatty acid intermediate is cleaved, resulting in free lipase, a monoglyceride and a fatty acid. The resultant free fatty acids and monoglycerides are incorporated into bile acid-phospholipid micelles, which are subsequently absorbed at the level of the brush border of the small intestine. The micelles eventually enter the peripheral circulation as chylomicrons. Accordingly, compounds, including lipase inhibitors that selectively limit or inhibit the absorption of ingested fat precursors are useful in the treatment of conditions including obesity, hyperlipidemia, hyperlipoproteinemia, Syndrome X, and the like.

Pancreatic lipase mediates the metabolic cleavage of fatty acids from triglycerides at the 1- and 3-carbon positions. The primary site of the metabolism of ingested fats is in the duodenum and proximal jejunum by pancreatic lipase, which is usually secreted in vast excess of the amounts necessary for the breakdown of fats in the upper small intestine. Because pancreatic lipase is the primary enzyme required for the absorption of dietary triglycerides, inhibitors have utility in the treatment of obesity and the other related conditions.

As used herein the terms "treatment of obesity" and the "management of obesity", are used interchangeably and do not necessarily mean a complete cure. It means that the

symptoms or complications of the underlying disease are reduced, and/or that one or more of the underlying cellular, physiological, or biochemical causes or mechanisms causing the symptoms or complications are reduced. It is understood that "reduced", as used in this context, means relative to the untreated state of the disease, including the molecular state of the disease, not just the physiological state of the disease.

The term "treating" refers to the administration of an effective amount of a composition of the present invention to a subject, who has obesity, overweight and other metabolic disorders, or a symptom or a predisposition of such diseases, with the purpose to cure, alleviate, relieve, remedy, or ameliorate such diseases, the symptoms of them, or the predispositions towards them.

The term "administration" covers oral or parenteral delivery to a subject a composition of this invention in any suitable form, e.g., food product, beverage, tablet, and capsule.

The term "parenteral" refers to subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional, and intracranial injection, as well as various infusion techniques.

The term "oral administration" includes oral, buccal, enteral and intra-gastric administration.

The term "Synergistic effect/Synergize" refers to a combination of two or more treatments, which is more effective to produce advantageous results than the additive effects of these agents.

The term "synergist" refers to an agent which when present results in a greater-than-additive increase, augmentation or enhancement of the biological or functional effect of the agent. In some cases, it may be difficult to determine which compound in a mixture is of primary importance and which only secondary. Thus, in a synergistic mixture of compounds, any of the active compounds within the mixture can be considered a synergist.

A composition comprising "synergistic activity" or a "synergistic composition" is a combination of compounds which exhibits increased biological or functional activity as a non-linear multiple of the biological or functional activity of the individual compounds. In other words, the combined biological or functional activity of two or more compounds being tested is significantly greater than the expected result based on independent effects of the compounds when tested separately. Synergism may be apparent only at some ranges or concentrations.

The term "a metabolic disorder" refers to any pathological condition resulting from an alteration in a patient's metabolism. Such disorders include those resulting from an alteration in glucose homeostasis resulting, for example, in hyperglycemia. According to this invention, an alteration in glucose levels is typically an increase in glucose levels by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% relative to such levels in a healthy individual. Metabolic disorders include obesity and diabetes (e.g., diabetes type I, diabetes type II, MODY, and gestational diabetes).

The term "salts" refers to inorganic and organic salts of compounds. These salts can be prepared in situ during the final isolation and purification of a compound, or by separately reacting a purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, palmitiate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, besylate, esylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts, and the like. These may include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, and the like, as well as non-toxic ammonium, quaternary ammonium, and amine cations including, but not limited to, ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purposes of exemplification only. Functionally-equivalent products, compositions, and methods are clearly within the scope of the invention, as described herein.

The present invention provides a composition comprising plant extract(s) comprising resveratrol and EGCG, wherein these two compounds have synergistic lipase inhibition activity at an appropriate ratio and can be used for prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders.

The composition of the present invention comprises a unique combination of natural plant extracts which facilitate enhanced inhibition of pancreatic lipase. The present invention also provides an optimized combination of these natural plant extracts at a desired ratio to inhibit pancreatic lipase enzyme for prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders.

The present invention provides a composition comprising a mixture of at least two plant extracts, wherein the first plant extract comprises resveratrol or its analogs, and the second plant extract comprises EGCG or its analogs, wherein the ratio of two plant extracts is in the range of 1:99 to 99:1 by weight, preferably 4:1 to 1:4 by weight, more preferably 2:3 by weight.

The present invention provides a composition comprising a mixture of at least two plant extracts, wherein the first plant extract comprises resveratrol or its analogs, and the second plant extract comprises EGCG or its analogs, wherein the ratio of two plant extracts is in the range of 1:99 to 99:1 by weight, preferably 4:1 to 1:4 by weight, more preferably 2:3 by weight.

The present invention provides a composition comprising one or more plant extracts comprising resveratrol and epigallocatechin gallate and pharmaceutically acceptable salts, wherein the concentration of resveratrol in the composition is in the range of 10 μg to 50 μg and the concentration of epigallocatechin gallate in the composition is in the range of 10 μg to 50 μg.

The plant extract of the present invention comprising resveratrol or its analogs is selected from the group consisting of grape juice, pomegranate juice, Japanese knotweed root extract, blueberries fruit extract, bilberries fruit extract, and raspberries fruit extract. The plant extract comprises at least 20% resveratrol by weight, preferably 40%, more preferably 50%. The plant extract comprising EGCG or its analogs is selected from the group consisting of green tea and carob flour. The plant extract comprises at least 20% EGCG by weight, preferably 40%, more preferably 50%.

Surprisingly it was found that the composition of the present invention comprising plant extracts comprising resveratrol and EGCG show an enhanced pancreatic lipase inhibition activity of about 98%. In comparison, the plant extract comprising only resveratrol shows maximum pancreatic lipase inhibition activity of about 89% and the plant extract comprising only EGCG shows maximum pancreatic lipase inhibition activity of about 83%. Thus, it was demonstrated that Japanese knotweed root extract (Resveratrol 50%) and green tea extract (EGCG 50%) in the specific weight ratio of 40:60 (2:3) ratio shows enhanced inhibitory activity of pancreatic lipase as compared to Japanese knotweed root extract (Resveratrol 50%) or green tea extract (EGCG 50%) alone. It was concluded that the synergistic effects of Japanese knotweed root extract (Resveratrol 50%) and green tea extract (EGCG 50%) in inhibiting pancreatic lipase activity can be used for development of pharmaceutical compositions for the treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders, for development of food and feed products for patients suffering from such disease and for development of satiety enhancement foods and nutraceutical composition.

The synergistic composition comprising one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof may be compounded, for example with a pharmaceutically acceptable carrier for solid compositions such as tablets, pellets or capsules, capsules containing liquids, suppositories, solutions, emulsions, suspensions or any other form suitable for use. Suitable carriers include, for example, sterile water, sterile physiological saline, gum

acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents may be used.

Solid dosage forms for oral administration include capsules, tablets, powders, and granules. In such solid dosage forms, the active compound is admixed with at least one inert excipient (or carrier) such as sodium citrate or dicalcium phosphate or (a) fillers or extenders, for example, starches, lactose, sucrose, mannitol, and silicic acid; (b) binders, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia; (c) humectants, for example, glycerol; (d) disintegrating agents, for example, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate; (e) solution retarders, for example, paraffin; (f) absorption accelerators, for example, quaternary ammonium compounds; (g) wetting agents, for example, cetyl alcohol and glycerol monostearate; (h) adsorbents, for example, kaolin and bentonite; and/or (i) lubricants, for example, talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. In the case of capsules and tablets, the dosage forms may also comprise buffering agents.

Solid dosage forms such as tablets, dragees, capsules, and granules can be prepared with coatings and shells, such as enteric coatings and others well known in the art. They may also contain opacifying agents, and can also be of such composition that they release the active compound or compounds in a delayed manner. Examples of embedding compositions that can be used are polymeric substances and waxes. The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Further, compositions in tablet form may be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable orally administered compositions. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the

driving compound, which swells to displace the agent or agent composition through an aperture.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage form may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents and emulsifiers, as for example, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils, in particular, cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame seed oil, glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, or mixtures of these substances, and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

These compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. Prevention of microorganism contamination of the compositions can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of injectable pharmaceutical compositions can be brought about by the use of agents capable of delaying absorption, for example, aluminum monostearate and gelatin.

The compounds identified by the present invention in combination with other pharmaceutically active compounds, if desired, can be administered to a patient either orally, rectally, parenterally, (for example, intravenously, intramuscularly, or subcutaneously) intracisternally, intravaginally, intraperitoneally, intravesically, locally (for example, powders, ointments or drops), or as a buccal or nasal spray. They are advantageously effective when administered orally.

Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions, or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water, ethanol, polyols (propylene glycol, polyethylene glycol, glycerol, and the like), suitable mixtures thereof, triglycerides, including vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The composition of the present invention may comprise additional anti-obesity agents preferably selected from but not limited to the group consisting of a β_3-adrenergic receptor agonist, a cholecystokinin-A agonist, a monoamine reuptake inhibitor, a sympathomimetic agent, a serotoninergic agent, a dopamine agonist, a melanocyte-stimulating hormone receptor agonist or mimetic, a melanocyte-stimulating hormone receptor analog, a cannabinoid receptor antagonist, a melanin concentrating hormone antagonist, leptin, a leptin analog, a leptin receptor agonist, a galanin antagonist, a lipase inhibitor, a bombesin agonist, a neuropeptide-Y antagonist such as NPY-1 or NPY-5, a thyromimetic agent, dehydroepiandrosterone or an analog thereof, a glucocorticoid receptor agonist or antagonist, an orexin receptor antagonist, a urocortin binding protein antagonist, a glucagon-like peptide-1 receptor agonist, and a ciliary neurotrophic factor.

The synergistic composition of the present invention described herein can be used in the form of a food additive, food supplement, dietary supplement for example, in solid, semisolid or liquid form comprising a first plant extract comprising resveratrol or analogs of resveratrol and a second plant extract comprising epigallocatechin gallate or analogs of epigallocatechin gallate.

The synergistic composition of the present invention may be incorporated into food product alone or in combination with another anti-obesity, anti-hyperglycemic (blood

glucose lowering), or anti-lipidemic compound, in admixture with a carrier or an excipient suitable for oral administration.

Any conventional food processing technique may be used to achieve a product comprising the effective amount of the composition described herein. There is much information on the art and technology of the various conventional food processing techniques and their practices in food industries, and it is accordingly assumed that the general principals of these techniques are understood by the person skilled in the art.

In an embodiment of the present invention, there is provided a composition for the prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders comprising one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof are present in a weight ratio in the range of 1:99 to 99:1.

In yet another embodiment of the present invention, there is provided a composition for the prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders comprising one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof are present in a weight ratio in the range of 4:1 and 1:4.

In another embodiment of the present invention, there is provided a composition for the prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders comprising one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof are present in a weight ratio of 2:3.

In another embodiment of the present invention, there is provided a composition for the prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders comprising one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof, wherein the concentration of resveratrol in the composition is in the range of 10 μg to 15 μg and the concentration of epigallocatechin gallate in the composition is in the range of 10 μg to 15 μg.

In still another embodiment of the present invention, there is provided a composition for the prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders comprising one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof, wherein the concentration of resveratrol in the composition is 20 μg and the concentration of epigallocatechin gallate in the composition is 30 μg.

In yet another embodiment of the present invention, there is provided a composition for the prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders comprising one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof are present in a weight ratio in the range of 4:1 and 1:4, wherein the analogs of resveratrol and EGCG are natural analogs or synthetic analogs.

In an embodiment of the present invention, there is provided a composition for the prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders comprising one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof are present in a weight ratio in the range of

4:1 and 1:4, wherein the composition comprises a pharmaceutically acceptable additive or a carrier.

In still another embodiment of the present invention, there is provided a composition for the prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders comprising one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof are present in a weight ratio in the range of 4:1 and 1:4, wherein the composition optionally comprises one or more other therapeutic agents selected from a group consisting of pancreatic lipase inhibitor, calcineurin inhibitor, blood tissue barrier (BTB) transport protein modulator, α-glucosidase inhibitor, insulin, M1 receptor antagonists, prolactin inhibitors, agents acting on an ATP-dependent channel of β-cells, metformin, an intestinal glucose transporter inhibitor, a glycation inhibitor, a nitric oxide production inhibitor, an aldose reductase inhibitor, a PPAR agonist, an adipocytokine activator, a glucose uptake enhancer, a dipeptidyl peptidase-IV inhibitor, and a thermogenesis enhancer.

In still another embodiment of the present invention, there is provided a composition for the prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders comprising one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof are present in a weight ratio in the range of 4:1 and 1:4, wherein the composition is in a form selected from the group consisting of an immediate-release composition, a controlled-release composition, sustained-release orally-administrable compositions, topically-administrable compositions, liquid solutions, liquid sprays, lozenges, throat sprays, ointments, solutions, foams, cough drops, dissolvable strips, a jelly, a mouthwash; a gargle, a lollipop, a gum, aqueous or oily suspensions, dispersible powders or granules, a syrup, an elixir, emulsions, a

cream, a paste, a gel, a lotion, impregnated dressings, occularly-administrable compositions, inhalable particles, inhalable solutions, droplets, and aerosols.

The present invention further provides a process for the preparation of the composition, wherein the process comprising mixing a plant extract comprising resveratrol or analogs thereof and a plant extract comprising EGCG or analogs thereof in Dimethyl sulfoxide (DMSO) in a weight ratio in the range between 4:1 and 1:4; and optionally adding a pharmaceutically acceptable carrier to obtain the composition.

An embodiment of the present invention provides a food, beverage or feed supplement comprising the composition of the present invention, wherein the composition comprises one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof are present in a weight ratio in the range of 4:1 and 1:4.

In still another embodiment of the present invention, there is provided a feed product comprising the composition of the present invention, wherein the composition comprises one or more plant extract comprising resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof are present in a weight ratio in the range of 4:1 and 1:4.

The composition of the present invention may be compounded with foods such as, but not limited to dairy products, grains, breads, meats, fruits, vegetables, rice and the like. The composition of the present invention can be compounded with additional ingredients. For example, the composition can be mixed with carbohydrates lipids, polypeptides, fatty acids, phytochemicals, and combinations thereof.

Industrial Applicability

The present invention provides a useful composition for prevention and/or treatment and management of patients suffering from obesity, overweight, cardiovascular diseases

and other metabolic disorders. The composition of the present invention comprises two or more extracts, wherein the plant extract comprising resveratrol or analogs thereof and the plant extract comprising epigallocatechin gallate or analogs thereof as the main components is highly safe, useful and can be administered over a long period of time. Further, the composition of the present invention can be used for development of anti-obesity food, feed, and nutraceutical composition and for development of satiety enhancement foods.

Although the subject matter has been described in considerable detail with reference to certain preferred embodiments thereof, other embodiments are possible.

Screening of Natural ingredients as pancreatic lipase Inhibitors

The natural ingredients belonging to the group: Broccophane, Broccosinolate, Broccoplus, CranLife, Resveratrol 50%, PomActiv, Pomegranate extract (Pomegranate peel/husk/seed extract with 40% Punicalagins) and Epigallocatechin gallate (EGCG) 50% were selected for the pancreatic lipase inhibition assay. The assay is based on the action of pancreatic lipase enzyme which catalyses hydrolytic cleavage of triglycerides into free fatty acids and monoglyceride. The substrate used in the assay is para-nitrophenol tagged laurate molecule. The enzyme acts on the substrate and p-nitrophenol is released, absorbance of which is measured colorimetrically at 410 nm. The assay was carried out in a total reaction volume of 200µL in 96-well microwell plate comprising assay buffer, emulsifier, substrate, inhibitor and enzyme. 0.1M Tris-HCl buffer at a pH of 7.5 was used as the assay buffer as well as the solvent for enzyme and emulsifier solutions. The substrate was prepared in isopropanol. The assay components are added in their respective volumes and the reaction was started by adding the enzyme. The reaction was carried out at 37°C for one hour with the absorbance readings being measured every one minute. Each assay was performed in duplicates against its blank ingredient comprising assay buffer, emulsifier, substrate and inhibitor or ingredient [at 100 µg and 500 µg concentration].

Enzyme preparation: Human pancreatic lipase procured from Sigma was diluted to 200µL with assay buffer so as to obtain a final concentration of 50U/200µL. The enzyme preparation was stored at 4°C till use.

Orlistat was used at 5µM as a positive control in the pancreatic lipase inhibition assay. Percentage inhibition was calculated as: % Inhibition = ($Absorbance_{Control}$ – $Absorbance_{Test}$)/ Absorbance $_{Control}$ × 100. The results are provided in Figures 1 to 3.

Figure 1 shows the bar graph illustrating the performance of various plant extracts Orlistat, BroccoPhane, BroccoSinolate, BroccoPlus, CranLife, Resveratrol 50%, PomActiv, Pomegranate and EGCG 50% at concentration of 100 µg and 500 µg on pancreatic lipase enzyme.

Based on the results obtained, pancreatic lipase inhibition assay was performed using Resveratrol 50% and EGCG 50% at a concentration in the range from 20 µg to 100 µg (Figure 2 and 3). Figure 2 shows the bar graph illustrating the inhibitory activity of Resveratrol 50% on Human Pancreatic Lipase at different concentrations (20 µg, 40 µg, 60 µg, 80 µg and 100 µg). Figure 3 shows the bar graph illustrating the inhibitory activity of EGCG 50% on Human Pancreatic Lipase at different concentrations (20 µg, 40 µg, 60 µg, 80 µg and 100 µg).

It is inferred from Figure 2 that maximum % inhibition of pancreatic lipase enzyme was obtained with 100 µg concentration of Resveratrol 50% with respect to Orlistat which was taken as positive control. The % inhibition with 100 µg Resveratrol 50% was calculated to be around 89%.

It is inferred from Figure 3 that maximum % inhibition of pancreatic lipase enzyme was obtained with 100 µg concentration of EGCG 50% with respect to Orlistat which was taken as positive control. The % inhibition with 100 µg of EGCG 50% was calculated to be around 83%.

Example 2

Synergy of Resveratrol 50% and EGCG 50% for enhanced pancreatic lipase inhibition

Resveratrol 50% and EGCG 50% were selected for synergy studies and pancreatic lipase inhibition assay was performed. The amount of Resveratrol 50% and EGCG 50% were taken as provided in Table 1.

Table 1: Amount of Resveratrol 50% and EGCG 50% taken for pancreatic lipase inhibition assay

Test Ratio [R:E] (Resveratrol: EGCG)	**Resveratrol 50% [R] (Stock 5mg/mL) (Resveratrol 2.5mg/ml)**	**EGCG 50% [E] (Stock 5mg/mL) (EGCG 2.5mg/ml)**
100:0	50µg (4µL)	0 µg (0µL)
80:20	40 µg (3.2µL)	10 µg (0.8µL)
60:40	30 µg (2.4µL)	20 µg (1.6µL)
40:60	20 µg (1.6µL)	30 µg (2.4µL)
20:80	10 µg (0.8µL)	40 µg (3.2µL)
0:100	0 µg (0µL)	50µg (4µL)

The assay is based on the action of pancreatic lipase enzyme which catalyses hydrolytic cleavage of triglycerides into free fatty acids and monoglyceride. The substrate used in the assay is para-nitrophenol tagged laurate molecule. The enzyme acts on the substrate and p-nitrophenol is released, absorbance of which is measured colorimetrically at 410 nm. The assay was carried out in a total reaction volume of 200µL in 96-well microwell plate. The reaction mixture comprises 180 µL of assay buffer, 4 µL of emulsifier, 4 µL of substrate, 4 µL of inhibitor and 8 µL of enzyme. 0.1M Tris-HCl buffer (pH 7.5) was used as the assay buffer, an emulsifier comprising para-nitrophenol tagged laurate molecule prepared in isopropanol. Human pancreatic lipase and inhibitors (Resveratrol and EGCG) in the ratio of 100:0, 80:20, 60:40, 40:60, 20:80, 0:100 was taken as provided in Table 1.

The assay components are added in their respective volumes and the reaction was started by adding the enzyme. The reaction was carried out at 37°C for one hour with the absorbance readings being measured every one minute. Each assay was performed in duplicates against its blank ingredient comprising assay buffer, emulsifier, substrate and inhibitor or ingredients. The result is provided in Figure 4. Figure 4 shows the bar graph illustrating the synergistic inhibitory effect of Resveratrol 50% and EGCG 50% on human pancreatic lipase at different concentrations. It was inferred from Figure 4 that maximum % inhibition of pancreatic lipase with respect to Orlistat was obtained in composition comprising Resveratrol 50% and EGCG 50% were taken in the weight ratio of 40:60, wherein the composition comprises 1.0 µM Resveratrol and 1.5 µM EGCG. The % inhibition was calculated to be more than 98 %.

Thus, it was demonstrated that Resveratrol 50% and EGCG 50% in a particular combination in the weight ratio of 40:60 (2:3) ratio shows enhanced inhibitory activity of pancreatic lipase as compared to Resveratrol 50% and EGCG 50% alone. These results suggest that the synergistic effects of Resveratrol 50% and EGCG 50% in inhibiting pancreatic lipase activity can be used for development of pharmaceutical compositions for the treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders, for development of food and feed products for patients suffering from such disease and for development of satiety enhancement foods and nutraceutical composition.

Any publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior disclosure.

Dated this 29 October 2012

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AGENT FOR THE APPLICANT

To
The Controller of Patents
The Patents Office at Chennai

ABSTRACT

"SYNERGISTIC COMPOSITION COMPRISING PLANT EXTRACTS COMPRISING RESVERATROL AND EPIGALLOCATECHIN GALLATE AND USES THEREOF"

The present invention relates to a composition comprising one or more plant extracts, wherein the plant extract comprises resveratrol or analogs thereof and epigallocatechin gallate or analogs thereof. The composition as disclosed herein is useful for prevention and/or treatment of obesity, overweight, cardiovascular diseases and other metabolic disorders.