Plant Extracts and Methods and Uses Therefore

Field of Invention

The invention relates to extracts from *Terminalia* plant species that are capable of being used in methods for managing disease such as diabetes, obesity, cardiovascular diseases, and cancer, and related disease conditions. More particularly, the invention relates to certain extracts from *Terminalia arjuna*, their uses as agents for the treatment of certain diseases such as diabetes, obesity, cardiovascular disease and cancer in mammals, particularly humans, processes for obtaining them and delivery formats therefore.

Background of the invention

Diabetes mellitus

Diabetes mellitus is a common, serious disease characterized by hyperglycemia. The disease can be divided into two major subclasses: insulin-dependent diabetes mellitus (IDDM), also known as type I diabetes, and non-insulin-dependent diabetes mellitus (NIDDM), also known as type II diabetes (World Health Organization Study Group. Diabetes mellitus. WHO Tech. Rep. Ser. 727:1-113, 1985). IDDM results from insulin deficiency caused by cell-mediated autoimmune destruction of pancreatic beta cells, and generally develops in the young (Yoon J W., Insulin-dependent diabetes mellitus. In: Roitt I M and Delves P J. (Eds.) Encyclopedia of Immunology, Second Edition. Academic Press Ltd., London, pp. 1390-1398, 1998; Bach J F., Insulin-dependent diabetes mellitus as a beta cell targeted disease of immunoregulation. J. Autoimm. 8:439-463, 1995). IDDM accounts for approximately 10-15% of the

CONFIRMATION COPY

diabetic population worldwide (World Health Organization Study Group. Diabetes mellitus. WHO Tech. Rep. Ser. 727:1-113, 1985). In contrast, NIDDM results from a variable combination of insulin resistance and insulin deficiency, and generally develops in adults (Jun H S, et al., Pathogenesis of non-insulin-dependent (Type II) diabetes mellitus (NIDDM)--Genetic predisposition and metabolic abnormalities. Advanced Drug Delivery Reviews 35:157-177, 1999; DeFronzo R A., The triumvirate: .beta.-cell, muscle, liver: a collusion responsible for NIDDM. Diabetes 37:667-687, 1988). However, NIDDM can also develop at a younger age, as seen in the maturity-onset diabetes of the young (Pirart J., Diabetes mellitus and its degenerative complications: a prospective study of 4400 patients observed between 1947 and 1973. Diabetes Care 1:168-188, 1978). NIDDM accounts for over 85% of the diabetic population worldwide. Both IDDM and NIDDM can cause microvascular and macrovascular complications, resulting in increases in morbidity and mortality (Fajans S S, et al., Prediabetes, subclinical diabetes, and latent clinical diabetes: interpretation, diagnosis and treatment. In: Leibel D S, Wrenshall G S. (Eds.) On the Nature and Treatment of Diabetes. Excerpta Medica, Amsterdam, pp. 641-656, 1965).

NIDDM is a complex disease that is currently thought to be influenced by more than a single gene or environmental factor (Ghosh S, et al., Genetic analysis of NIDDM. Diabetes 45:1-14, 1995; Kobberling J. Studies on the genetic heterogeneity of diabetes mellitus. Diabetologia 7:46-49, 1971; Rotter J L, et al., Genetics of diabetes mellitus. In: Rifkin H, Porte D (Eds.) Diabetes Mellitus Theory and Practice. Elsevier, N.Y., pp. 378-413, 1990). Familial aggregation and the high concordance rate for the disease (60-100%) in identical twins suggest that genetic factors play an important role in the pathogenesis of NIDDM (O'Rahilly S, et al., Type 2 (noninsulin dependent) diabetes mellitus. New genetics for old nightmares. Diabetologia 31:407-414, 1988; Barnett A H, et al., Diabetes in identical twins. A study of 200 pairs. Diabetologia 20:87-93, 1981). In addition, environmental factors such as obesity,

physical activity and diet also play a strong role in the development of the disease (Knowler W C, et al., Gm and type 2 diabetes mellitus: an association in American Indians with genetic admixture. Am. J. Hum. Genet. 43:520-526, 1988; Bennett P H, et al., Epidemiology and natural history of NIDDM: non-obese and obese. In: Alberti KGMM, DeFronzo R A, Keen H, Zimmett P (Eds.) International Textbook of Diabetes Mellitus. Wiley, N.Y., pp. 147-176, 1992; Helmrich S P, et al., Physical activity and reduced occurrence of NIDDM. N. Engl. J. Med 325:147-152, 1991). Although the relative contribution of genetic and environmental factors to the development of NIDDM differs among individuals, patients generally have two common metabolic abnormalities, insulin resistance and defects in glucose-stimulated insulin secretion, which lead to the disease state (Saad M F, et al., A two step model for development of non-insulin-dependent diabetes. Am. J. Med. 90:229-235, 1991; DeFronzo R A, et al., Pathogenesis of NIDDM: A balanced overview. Diabetes Care 15:318-368, 1992; Lillioja S, et al., Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. N. Engl. J. Med. 329:1988-1992, 1993).

The insensitivity of the target tissue in response to insulin (insulin resistance) appears to develop first in genetically predisposed subjects in the presence of the necessary environmental factors (Jun H S, et al., Pathogenesis of non-insulin-dependent (Type II) diabetes mellitus (NIDDM)--Genetic predisposition and metabolic abnormalities. Advanced Drug Delivery Reviews 35:157-177, 1999). To compensate for this, that is, to lower blood glucose and maintain normoglycemia, the secretion of insulin from the beta cells increases, resulting in hyperinsulinemia. Over time, the insulin resistance worsens, and the compensatory action fails, leading eventually to impaired glucose tolerance. Insulin secretion reaches a plateau, and beta cell function is impaired, resulting in insulin deficiency, and leading finally to hyperglycemic NIDDM. In addition, hyperglycemia itself leads to impaired insulin resistance and insulin secretion, exacerbating the disease.

The regulation of diet and exercise and/or treatment with insulin or hypoglycemia drugs has been used for the control of diabetes. Treatment with these agents is successful in some cases, but the mortality index continues to rise. Insulin treatment provides symptomatic relief rather than a cure for NIDDM. Hypoglycemic agents such as sulfonylureas and biguanides (metformin) also lower blood glucose, but again, simply provide symptomatic relief. Sulfonylureas lower the blood glucose level by stimulating the release of insulin from pancreatic beta cells. These agents directly stimulate insulin release by closing adenosyl triphosphate (ATP)-sensitive potassium channels and depolarizing the cell membrane (Aguilar-Bryan L, et al., Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 268:423-426, 1995; Tan G H, et al., Pharmacologic treatment options for non-insulin-dependent diabetes mellitus. Mayo Clinic Proceedings 71:763-768, 1996; Lubbos H, et al., Oral hypoglycemic agents in type II diabetes mellitus. American Family Physician. 52:2075-2078, 1995) The side effects of sulfonylureas include hypoglycemia, renal and hepatic disease, gastrointestinal disturbances, increased cardiovascular mortality, dermatological reactions, dizziness, drowsiness and headache. Biguanides lower blood glucose levels by reducing intestinal glucose absorption and hepatic glucose, but not by stimulating insulin secretion. The major side effects of biguanidine are lactic acidosis and increased cardiovascular mortality. Alpha glucosidase inhibitors inhibit intestinal alpha glucosidases and consequently delay the digestion of sucrose and complex carbohydrates. The side effects of alpha glucosidase inhibitors include gastrointestinal side effects and hypoglycemia. Thiazolidinediones improve insulin resistance directly, enhancing the effects of circulating insulin, directly stimulate peripheral glucose uptake and inhibit glucose production in the liver. Thiazolidinediones are only effective in the presence of insulin and may cause red blood cell abnormalities, and headache.

Therefore, more effective drugs for the treatment of diabetes are clearly needed.

Obesity

It is generally recognized in the medical profession that obesity among the populations of the United States and some other countries has been steadily increasing in the last several decades.

Obesity is a condition characterized by excessive accumulation of fat on the body. Obesity can be measured by either body weight or by body mass index (BMI). By convention, obesity is said to be present when body weight exceeds by 20 percent the weight listed in typical height-weight index tables. The other measurement of obesity, BMI, is the amount of fat present in the body and is considered a reliable indication of fatness in non-athletic adults. The BMI may be calculated by using the following formula: BMI equals [body weight in kg] divided by [height in meters].sup.2. In general, a normal BMI is between the range of 20 to 25, whereas the BMI of obese individuals is greater than or equal to 30.

Such obesity has caused or contributed to marked increases in the occurrence of heart disease, hypertension, diabetes, osteoarthritis of the knees and hips, and increased morbidity resulting from related medical conditions. It has been reported that 50% of all American adults are overweight.

There are a variety of methods commonly used for treating obesity. These include dietary adjustments such as caloric restriction, exercise regimens, pharmacotherapy, surgery, and psychotherapy. However, there are problems associated with many of these treatments.

The use of human growth (hGH) hormone to treat obesity is described in U.S. Pat. No. 5,597,595. hGH, the most abundant hormone secreted by the pituitary gland, is the primary hormone responsible for growth in mammals, accelerating lipolysis (fat

metabolism), and protein synthesis. hGH secretion is markedly decreased in obese individuals. hGH interacts with specific growth hormone receptors, which are widely distributed throughout the body. For example, fat cells (adipocytes) have hGH receptors, and when hGH binds to these receptors, it stimulates the adipocytes to breakdown triglycerides as well as suppresses their ability to take up and accumulate circulating lipids. Administration of hGH needs to be closely monitored, however, as diabetes mellitus may develop in individuals receiving hGH treatment. Diabetes may result because hGH reduces the amount of glucose uptake by cells, thus causing an increase of blood glucose concentration. Not only does this increase in blood glucose levels stimulate the .beta.-cells of the pancreas to secrete extra insulin, but hGH also directly stimulates the .beta.-cells to produce insulin. The combination of these two stimulatory effects so greatly overstimulates insulin secretion that the .beta.-cells literally "burn out." When this occurs, diabetes mellitus may develop. Guyton and Hall; TEXTBOOK OF MEDICAL PHYSIOLOGY, 938 (1995).

One of the best ways to induce weight loss is through dieting or caloric restriction, i.e., establish a caloric deficit by bringing intake below output. The most common method of reducing caloric intake is by means of a low calorie diet. There are, however, a couple of common pitfalls to dieting. One is that when the dieter stops the diet and returns to the usual fare, the incentives to overeat are multiplied. Second, many obese people choose novel or bizarre diets. One example of this type of diet is a high-fat/high-protein diet. These diets are known as ketogenic diets because they contain a high level of cholesterol, which facilitates ketosis. Ketosis is associated with nausea, hypotension, and lethargy. Kaplan et al., supra, 735.

Pharmacotherapy may include the administration of drugs that function by increasing serontonin levels in the brain and ultimately controlling appetite. Serotonin (5-hydroxytryptamine, 5HT) is related to several general behaviors such as sleep, emotion, sex, and appetite. The drug Redux.RTM. (dexfenfluramine), which is

commonly used as a weight control medication, functions by increasing serotonin levels. Specifically, Redux.RTM. functions by inhibiting re-uptake of serotonin and at the same time stimulating release of serotonin. Several controlled clinical trials have demonstrated that Redux.RTM. can help individuals maintain caloric restriction and a lower body weight for a least a year. It should also be noted that Redux.RTM. appears to reduce appetite in obese more than non-obese individuals. Katzung, BASIC & CLINICAL PHARMACOLOGY 276 (1998). However, there are some adverse side-effects affiliated with Redux.RTM. such as headache, insomnia, and pulmonary hypertension. Id.

Surgical methods that cause malabsorption of food or reduce gastric volume have been widely used in people who are markedly obese. Gastric bypass is a procedure used to make the stomach smaller. In particular, the stomach is reduced by stapling or transecting one of the curvatures of the stomach. The primary objective of reducing the volume of the stomach is to slow the passage of food, thereby limiting the amount of food the individual can intake. Although the results are successful, many adverse side effects result. For example, vomitting, electrolyte imbalance, and obstructions may occur. An alternate surgical procedure is lipectomy, which is the removal of fat. This technique is primarily used for cosmetic purposes, however, and has no real impact on weight loss in the long run. Kaplan et al., supra, 276.

Hence, for all of the reasons detailed above, a better method for facilitating weight loss in obese or overweight individuals that is readily available and cost-efficient is needed.

Cardiovascular diseases

Cardiovascular disease (CVD) is a leading cause of mortality and is responsible for one-third of all global deaths. Nearly 85% of the global mortality and disease burden

from CVD is borne by low- and middle-income countries. In India, for example, approximately 53% of CVD deaths are in people younger than 70 years of age; in China, the corresponding fi gure is 35%. The majority of the estimated 32 million heart attacks and strokes that occur every year are caused by one or more cardiovascular risk factors – hypertension, diabetes, smoking, high levels of blood lipids, and physical inactivity – and most of these CVD events are preventable if meaningful action is taken against these risk factors.

A staggering number of people are destined to experience a cardiovascular-related disorder sometime in their lives. Almost 1 million Americans die each year as a result of cardiovascular disease, whereas 556,000 die each year from cancer. Despite these facts, people are often more afraid of cancer than they are of vascular disease.

Americans have become complacent about the dangers of arterial disease. One reason is that the percentage of young people dying from acute heart attack has plummeted over the past 50 years. Explanations for these reductions include lifestyle changes, greater use of dietary supplements/preventive medications, and improved cardiac medical care.

The question is why are so many Americans continuing to die from heart attack and stroke? The fundamental answer is that people are living longer. What has happened is that much of the human population has succeeded in delaying the development of arterial disease. So instead of suddenly dying from a heart attack at age 50, the vascular symptoms do not manifest until the 60s or 80s are reached. At this point, systemic arteriosclerosis has damaged the major organ systems, and multiple degenerative diseases result in diminished quality and quantity of life.

Many of the underlying causes of arterial disease have been identified in the scientific literature. Regrettably, cardiologists have only addressed a limited number of these factors, such as prescribing cholesterol-lowering drugs, controlling hypertension, etc.

By ignoring the other proven causes for the epidemic of vascular-related diseases, a significant number of Americans are experiencing needless suffering and are dying prematurely.

Terminalia arjuna plant extracts

Terminalia arjuna is a deciduous tree found throughout India growing to a height of around 60-90 feet. *Terminalia arjuna* belongs to the family *Combretaceae* and is called "Arjuna" in vernacular. *Terminalia arjuna* has been used for over 1500 years in India as a cardio tonic and has been indicated for derangement of all three humoursin, vata, pitta and kapha in Ayurveda. The bark of *Terminalia arjuna* has been widely used in Indian system of medicine for a variety of purposes.

US patent 6,162,438 describes an edible herbal compositions, mixture of at least three, preferably at least six, herbs selected from the group consisting of *Terminalia arjuna, Cynara scolymus, Zingibar officinale, Allium sativum, Crataegus oxycantha, Curcuma longa, Boerhaavia diffusa* and *Trigonella foenumgraecum*, for use as agents for the control of hypertension, hyper-cholesterolemia and hyperlipidemia in mammals.

Sharma VN et al. evaluated the antioxidant and hypocholesterolaemic effects of *Terminalia arjuna* tree bark (a popular cardiotonic substance in Indian pharmacopoeia) and compared it with a known antioxidant, vitamin E by a randomised controlled trial. It was concluded from this trial that, *Terminalia arjuna* tree bark powder has significant antioxidant action that is comparable to vitamin E. In addition, it also has a significant hypocholesterolaemic effect. (Antioxidant and hypocholesterolaemic effects of *Terminalia arjuna* tree-bark powder: a randomised placebo-controlled trial, J Assoc Physicians India 2001 Feb; 49:231-235)

Bioassay-guided separation methods was used to determine the cancer cell growth inhibitory constituents residing in the bark, stem and leaves of the Mauritius medicinal plant *Terminalia arjuna* (Combretaceae) by Chapuis et. al. The cancer cell line active components were found to be gallic acid, ethyl gallate, and the flavone luteolin. Only gallic acid was previously known to occur in this plant. Luteolin has a well-established record of inhibiting various cancer cell lines and may account for most of the rationale underlying the use of *T. arjuna* in traditional cancer treatments. Luteolin was also found to exhibit specific activity against the pathogenic bacterium *Neisseria gonorrhoeae*. (Antineoplastic agents 338. The cancer cell growth inhibitory constituents of *Terminalia arjuna* (Combretaceae). J Ethnopharmacol. 1996Aug;53(2):57-63).

The bark of Terminalia arjuna tree has a long history of use as a cardiac tonic as well, and has been indicated in the treatment of coronary artery disease, heart failure, hypercholesterolemia and for relief of anginal pain. (Miller, A. L. Botanical Influences on cardiovascular disease. Alternative Medicine Review. Dec 1998, vol 3. No. 6, pages 421-431.

Water and methanol extracts of stem bark of Terminalia arjuna were found to inhibit the HIV-1 activity by more than 70% at 0.2 mg/ml. (Kandil F. E. et al. A Tannin anti-cancer promoter from Terminalia arjuna. April 1998, Vol 47. No. 8, pages 1567-1568.

Adjuvant therapy with Terminalia arjuna bark powder in post Myocardial Infarction angina patients produced relief in angina alongwith improvement in left ventricular ejection fraction (LVEF) and left ventricular mass (LVM). (Dwivedi, S. et al. Beneficial effects of Terminalia arjuna in coronary Artery Disease. Indian Heart Journal. Spet-Oct 1997, vol. 49. No. 5, pages 507-510.)

Ethanolic extract of Terminalia arjuna tree bark in doses of 100 mg/kg and 500 mg/kg significantly reduced total and LDL cholesterol levels in hypercholesterolaemic rabbits. (Ram et al. Hypocholesterolaemic effects of Terminalia arjuna tree bark. Journal of Ethnopharmacology. Vol 55. No. 3, pages 165-169.)

There is no data or technical information in the prior art, which indicates the use of the Terminalia arjuna extracts in the treatment or prevention or management of Diabetes and Obesity.

It is reported that water and methanol extracts of stem of T. arjuna has shown to have HIV-1 activity. But there are no other references to the use of T. arjuna bark extract with successive extraction solvent system, in the treatment of Cancer.

Terminalia arjuna plant has been known for its cardiotonic effect. Also there are reports indicating the use of T. arjuna in the treatment of coronary artery disease, heart failure, hypercholesterolemia and for relief of anginal pain. But there are no clear cut evaluation of the mechanisms of action responsible for these activities. In the present invention however, we have established that cells treated with *Terminalia arjuna* extracts showed marked decrease in the amount of total lipids. Also the said extracts have shown 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibition activity. HMG-CoA is the first committed step in cholesterol biosynthetic pathway. HMG-CoA reductase the rate-limiting enzyme step in cholesterol biosynthesis has become the most successful drug treatment for lowering total plasma cholesterol, in particular LDL-cholesterol.

There exists a need for the development of new medicines which are effective in treating obesity, cardiovascular disease, certain cancers, and diabetes and related diseases.

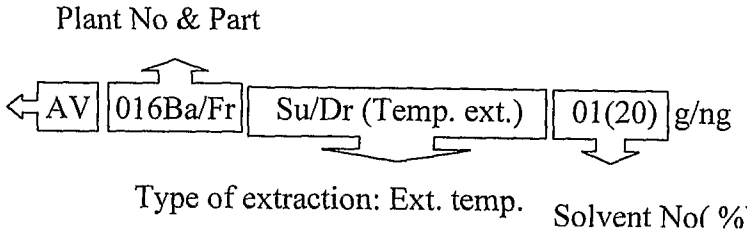
Summary of the invention

Objects of the invention will become apparent from the following description and examples.

The present invention relates to certain extracts of plant parts from plants of the *Terminalia* species, such as *Terminalia arjuna* that are indicated as being potentially useful in the treatment of diseases such as obesity, cardiovascular disease, certain cancers, diabetes and related diseases. Furthermore, the invention relates to prophylactic compositions comprising extracts of *Terminalia* species, such as extracts from *Terminalia arjuna* species.

Brief description of the extract nomenclature:

Nomenclature of Plant extracts.



1. **AV**- first two letters represents **Avesthagen.**
2. Plant Name: The Plants used and in use are assigned with unique 3-digit number, **016** represents ***Terminalia arjuna.***
3. Part of the plant /Tissue: There is a two letter ID for each plant part used. For example **Ba** for **Bark, Fr** for **whole Fruit.**
4. Solvents: The solvents used for extraction are also assigned with two digit numbers **01** for **Acetone**, **02** for **Benzene**, **03** for **Chloroform**, **04** for **Ethanol**, **05** for **Hexane**, **06** for **Methanol**, **07** for **Petroleum ether**, **08** for **water**, **09** for **ethyl acetate. Percentage of solvents** used for extraction is given within **bracket** (20) for 20 % of that solvent. For example if 20% of Ethanol was used for extraction, 04(20).
5. Method of Extraction: **Successive extraction** is referred to as **Su** whereas **direct extraction** is referred to as **Di, temperature for extraction** is written in **bracket**. For example, Su(65) represents successive extraction at 65 °C.
6. Type of extract, g: gluey and ng: non-gluey.

Brief Description of the Tables and Figures:

Table 1: HPLC fingerprint of the extract AV016BaDi(65)04(100).

Table 2: HPLC fingerprint of the extract AV016BaDi(28)04(20).

Table 3: HPLC fingerprint of the extract AV016BaSu(65)09(100).

Table 4: HPLC fingerprint of the extract AV016BaSu(65)01(100).

Table 5: HPLC fingerprint of the extract AV016BaSu(65)01(100)ng.

Table 6: HPLC fingerprint of the extract AV016BaSu(65)01(100)g.

Table 7: HPLC fingerprint of the extract AV016BaSu(65)04(100).

Table 8: HPLC fingerprint of the extract AV016BaSu(65)06(100).

Table 9: HPLC fingerprint of the extract AV016BaSu(105)08(100).

Table 10: HPLC fingerprint of the extract AV016Fr(105)08(100).

Table 11: LC/MS Fingerprint of extract AV016BaDi(28)04(20) (TIC Spectrum (Q1 +ve mode)

Table 12: LC/MS Fingerprint of extract AV016BaDi(28)04(20) (TIC Spectrum (Q1 –ve mode)

Table 13: LC/MS Fingerprint of extract AV016BaDi(65)04(100) (TIC Spectrum (Q1 +ve mode)

Table 14: LC/MS Fingerprint of extract AV016BaDi(65)04(100) (TIC Spectrum (Q1 –ve mode)

Table 15: LC/MS Fingerprint of extract AV016BaSu(65)09(100) (TIC Spectrum (Q1 +ve mode)

Table 16: LC/MS Fingerprint of extract AV016BaSu(65)01(100) (TIC Spectrum (Q1 +ve mode)

Table 17: LC/MS Fingerprint of extract AV016BaSu(65)01(100) (TIC Spectrum (Q1 –ve mode)

Table 18. LC/MS Fingerprint of extract AV016BaSu(65)01(100)ng (TIC Spectrum (Q1 +ve mode)

Table 19.LC/MS Fingerprint of extract AV016BaSu(65)01(100)g (TIC Spectrum (Q1 +ve mode)

Table 20. LC/MS Fingerprint of extract AV016BaSu(65)04(100) (TIC Spectrum (Q1 +ve mode)

Table 21. LC/MS Fingerprint of extract AV016BaSu(65)06(100) (TIC Spectrum (Q1 +ve mode)

Table 22. LC/MS Fingerprint of extract AV016BaSu(105)08(100) (TIC Spectrum (Q1 +ve mode)

Table 23. LC/MS Fingerprint of extract AV016FrDi(65)04(100) (TIC Spectrum (Q1 +ve mode)

Table 24. LC/MS Fingerprint of extract AV016FrSu(105)08(100) (TIC Spectrum (Q1 +ve mode)

Table 25. IC_{50} values of alpha-glucosidase inhibition activity of extracts from different *T. arjuna* plant parts

Table 26: The effect of insulin and *T. arjuna* extracts on glucose up-take in 3T3-L1 adipocytes.

Table 27: Anti-obesity potential of *Terminalia arjuna* extracts.

Table 28: Effect of *Terminalia arjuna* extracts on lipid levels in CHO-K1 cells.

Table 29: Effect of 20% ethanol extract from *T. arjuna* bark AV016BaDi(28)04(20) on HMG-CoA reductase activity.

Table 30: Effect of direct 100% ethanol extract from *T. arjuna* bark AV016BaDi(65)04(100) on HMG-CoA reductase activity.

Table 31: Effect of *Terminalia arjuna* extracts on cell viability of HepG2 cells.

Fig. 1: alpha –Glucosidase inhibition potential of *T. arjuna* direct ethanol extract, AV016BaDi(65)04(100) and AV016BaDi(28)04(20).

Fig. 2: alpha –Glucosidase inhibition potential of *T. arjuna* fruit extracts, AV016FrDi(65)04(100) AV016FrSu(65)08(100).

Fig. 3: alpha –Glucosidase inhibition potential of *T. arjuna* successive extracts, AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100)

Fig. 4: alpha –Glucosidase inhibition potential of *T. arjuna* successive methanol and water, AV016BaSu(65)06(100) and AV016BaSu(105)08(100) extracts

Fig. 5: alpha –Glucosidase inhibition potential of *T. arjuna* successive AV016BaSu(65)09(100) extracts

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect of the invention there is provided a method for treating a disease in a mammal, which comprises administering to the said mammal an effective non-toxic amount of at least an extract from *Terminalia arjuna* as defined herein. Preferably the mammal is a human being. The skilled addressee will appreciate that "treating a disease" in a mammal means treating, that is to say, alleviating symptoms of the disease and may also mean managing a disease in the sense of preventing such a disease state either advancing ie getting worse or becoming more invasive, or slowing down the rate of advance of a disease.

In a second aspect of the invention, there is a provided a prophylactic method for preventing the occurrence of a disease state in a mammal which comprises administering to the said mammal an effective non-toxic amount of an extract from *Terminalia arjuna* as defined herein in the preparation of a comestible (=foodstuff) for prophylaxis against the occurrence of a disease state selected from the group diabetes, obesity, cardiovascular disease, and cancer. Preferably the mammal is human and the said extract comprises a single extract from a plant part of Terminalia arjuna or a combination of extracts therefrom as detailed herein. Thus the present invention further relates to extracts which may be isolated from different parts of the *Terminalia arjuna* plant such as the bark and fruit thereof, the preparation of such extracts, medicaments comprising such extracts, and the use of these extracts and constituents for the preparation of a medicament.

Extracts of the present invention can be isolated from Terminalia tree species, such as *Terminalia arjuna,* using conventional organic solvent extraction and supercritical fluid extraction technology. Generally, extracts of the invention capable of functioning in a prophylactic or therapeutic manner as outlined herein can be

extracted from any *Terminalia arjuna* plant tissue, such as bark or fruit, depending on the end purpose that is required of the extract.

In a third aspect of the present invention there is provided a process for preparing extracts of the invention from plant parts of *Terminalia arjuna* that comprises:

(i) Pulverizing selected plant material to a powder;
(ii) Subjecting the powdered plant material to solvent extraction;
(iii)Lyophilizing the obtained extracts.

The choice of selected plant material may be of any type but is preferably selected from the bark or the fruit of the *Terminalia arjuna* plant.

The solvent extraction process may be selected from direct or successive extraction types such as extraction from plant parts in soxhlet apparatus or in flasks at room temperature or at higher temperature with polar and/or non-polar solvent(s). Typically, the extraction process is as outlined herein.

It will be apparent to the skilled addressee that the selection of solvent, or mixtures of solvents for each step in the isolation of extracts of the invention showing activity can be guided by results of bioassay analysis of separate fractions, for example as indicated herein and/or as shown in the examples.

Also encompassed within the ambit of the invention is a pharmaceutical formulation suitable for use in the treatment of a disease selected from the group diabetes, obesity, cardiovascular disease, and cancer comprising at least one extract as isolated from a *Terminalia* species, such as *Terminalia arjuna*, in admixture with a pharmaceutically acceptable carrier. Preferably, the at least one extract is selected from those listed in Tables 1 – 24 inclusive, depending on design and disease of interest. Preferably the at

least one extract is selected from the group of extracts as defined in Tables 25 – 30 inclusive, again depending on end purpose. Naturally, the skilled addressee will appreciate that such compositions may comprise of two or more plant extracts of the invention in any concentration, which is capable of giving rise to a therapeutic effect. Thus, therapeutic compositions can comprise plant extracts of *Terminalia* substantially devoid of undesirable contaminating compounds. The plant extracts may have, for example, undergone a number of solvent extraction steps substantially to separate out undesirable components from desirable components such as those alluded to in the examples and aforementioned tables.

The invention thus further provides a method for the treatment of a disease selected from the group obesity, cancer, cardiovascular disease, and diabetes in mammals, including humans, which comprises the use of a clinically useful amount of an extract selected from those listed in Tables 1 – 24 inclusive, preferably those listed in Tables 25 – 30 inclusive, in a pharmaceutically useful form, once or several times a day or in any other appropriate schedule for example, orally, or intravenously or by delivery to the lungs in a dry or "wet" spray.

The amount of compound of extract required to be effective in the treatment of the aforementioned diseases will, of course, vary with the disease being treated and is ultimately at the discretion of the medical or veterinary practitioner. The factors to be considered include the condition being treated, the route of administration, and nature of the formulation, the mammal's body weight, surface area, age and general condition and the particular compound to be administered. A suitable effective dose of an extract of the invention generally lies in the range of about 0.01 to about 120 mg/kg bodyweight, e.g. 0.1 to about 120 mg/kg body weight, preferably in the range of about 0.1 to 50 mg/kg, for example 0.5 to 50 mg/kg. The total daily dose may be given as a single dose, multiple doses, e.g. two to six times applications per day. For example, for a 75 kg mammal (e.g. a human) the dose range would be about 8 to

9000 mg per day, and a typical dose could be about 50 mg per day. If discrete multiple doses are indicated treatment might typically be 15 mg of a compound of Formula (I) given up to 4 times per day.

Whilst it is possible for the active extract to be administered alone, it is preferred to present the active extract in a pharmaceutical formulation. Formulations of the present invention, for medical use, comprise an extract of the invention together with one or more pharmaceutically acceptable carriers and optionally other therapeutic ingredients. The carrier(s) should be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and substantially non-deleterious to the recipient thereof.

The present invention, therefore, further provides a pharmaceutical formulation comprising at least one extract selected from those listed in tables 1 – 24 inclusive, preferably from those mentioned in tables 25 – 30 inclusive together with a pharmaceutically acceptable carrier therefore. In a preferment the pharmaceutical formulation comprises at least an extract selected from those listed in tables 25–30, depending on the disease type being treated. Naturally, the skilled addressee will appreciate that when selecting more than one extract from those given in the aforementioned tables for the treatment of any single disease type, that an appropriate selection of extracts fro the disease type will be made. Thus, for example, for the treatment of diabetes, extracts appropriate for doing so will be selected from the said tables.

Naturally, the skilled addressee will appreciate that any pharmaceutical formulation comprising an active extract of the invention can include at least one active extract purified from an extract derived from a *Terminalia* species. Thus a pharmaceutical formulation may contain more than one active extract derived from two or more *Terminalia* species.

There is also provided a method for the preparation of a pharmaceutical formulation comprising bringing into association an extract of the invention, and a pharmaceutically acceptable carrier therefore.

Formulations according to the present invention include those suitable for oral or intravenous administration.

Intravenous formulations including at least one extract of the invention and may also be administered in the form of suitable liposomal or niosomal preparations or other suitable delivery vehicle.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glycerol mono-stearate and sodium laury sulphate.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active extracts(s) into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active extract(s) into association with a liquid carrier or a finely divided solid carrier or both and then, if necessary, shaping the product into desired formulations.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets, tablets, lozenges, comprising the active ingredient in a flavoured based, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier. Each formulation generally contains a predetermined amount of the active extract; as a powder or granules; or a solution or suspension in an

aqueous or non-aqueous liquid such as a syrup, an elixir, an emulsion or draught and the like.

A tablet may be made by compression or moulding optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing an a suitable machine the active extract in a free-flowing form such as a powder or granules, optionally mixed with a binder, (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered extract moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide the desired release profile.

A syrup may be made by adding the active extract to a concentrated, aqueous solution of a sugar, for example sucrose, to which may also be added any necessary ingredients. Such accessory ingredient(s) may include flavourings, an agent to retard crystallisation of the sugar or an agent to increase the solubility of any other ingredients, such as a polyhydric alcohol for example glycerol or sorbitol.

In addition to the aforementioned ingredients, the formulations of this invention may further include on or more accessory ingredients) selected from diluents, buffers, flavouring agents, binders, surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like.

Alternatively, the compositions are dietary supplements, food compositions or beverage compositions suitable for human or animal consumption.

The invention describes the HPLC profiles and Mass spectrums of direct and successive solvent extracts of Terminalia arjuna plant parts thereby giving each extract an identity of itself. The various solvents used for successive extraction are in order from non-polar to polar side i.e hexane, petroleum ether, ethyl acetate, acetone, ethanol, methanol and water. In case of direct extraction alcoholic solvent alone and in combination with water was used as solvent for extraction.

The invention further encompasses novel extracts defined by reference to their HPLC and MS fingerprints as defined in Tables 1 – 24 inclusive, which are isolated from different parts of Terminalia arjuna plant, the preparation of such extracts, the medicaments containing said extracts, and the use of these extracts and constituents for the preparation of a medicament.

In a further aspect of the invention, the pharmaceutical formulations for preventing, treating, or managing diabetes and diabetes related disorders, comprise direct extracts of *T. arjuna* bark with 100% ethanol solvent [AV016BaDi(65)04(100)] and 20% ethanol solvent [AV016BaDi(28)04(20)], successive extracts of *T. arjuna* bark with ethyl acetate solvent [AV016BaSu(65)09(100)], successive extract of *T. arjuna* bark with acetone solvent [AV016BaSu(65)01(100)g], successive extracts of *T. arjuna* bark with acetone solvent [AV016BaSu(65)01(100)ng], successive extracts of *T. arjuna* bark with ethanol solvent [AV016BaSu(65)04(100)], successive extracts of *T. arjuna* bark with methanol solvent [AV016BaSu(65)06(100)] and successive extracts of *T. arjuna* bark with water solvent [AV016BaSu(105)08(100)], direct extract of *T. arjuna* fruit with ethanol solvent [AV016FrDi(65)04(100)] and successive extract of *T. arjuna* fruit with water solvent [AV016FrSu(105)08(100)], alone or in combination thereof. Such pharmaceutical formulations may contain a pharmaceutically acceptable carrier, excipient, or diluent as outlined herein.

In a further aspect of the invention, the pharmaceutical formulations for preventing, treating, or managing obesity and obesity related disorders, comprise direct extracts of *T. arjuna* bark with 100% ethanol solvent [AV016BaDi(65)04(100)] and 20% ethanol solvent [AV016BaDi(28)04(20)], successive extracts of T. arjuna bark with bark ethyl acetate, gluey acetone, non-gluey acetone, ethanol and methanol extracts AV016BaSu(65)09(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100), AV016BaSu(65)06(100) and AV016FrDi(65)04(100), alone or in combination thereof. The pharmaceutical formulations may contain a pharmaceutically acceptable carrier, excipient, or diluent as outlined herein.

In a further aspect of the invention, the pharmaceutical formulations for preventing, treating, or managing cardiovascular diseases (CVD) and CVD related disorders, comprise a direct extract of *T. arjuna* bark with 100% ethanol solvent [AV016BaDi(65)04(100)] and 20% ethanol solvent [AV016BaDi(28)04(20)], successive extract of *T. arjuna* bark with ethyl acetate [AV016BaSu(65)09(100)] and acetone [AV016BaSu(65)01(100)], alone or in combination thereof. The pharmaceutical formulations may contain a pharmaceutically acceptable carrier, excipient, or diluent as outlined herein.

In a further aspect of the invention, the pharmaceutical formulations for preventing, treating, or managing cancer and cancer related disorders, comprise successive extracts of *T. arjuna* bark with ethyl acetate [AV016BaSu(65)09(100)], acetone [AV016BaSu(65)01(100)], ethanol [AV016BaSu(65)04(100)], methanol [AV016BaSu(65)06(100)] and water [AV016BaSu(105)08(100)], alone or in combination thereof. The pharmaceutical formulations may contain a pharmaceutically acceptable carrier, excipient, or diluent as outlined herein.

In a further aspect of the invention there is provided a comestible, that is to say, a foodstuff comprising at least an extract of the invention, typically in dried form, such

as in a lyophilized form selected from those listed in Tables 1 – 24 herein, and in particular, from those extracts selected from those mentioned in Tables 25 – 30. The skilled addressee will appreciate that such comestibles may contain more than one extract of the invention and may be used. Such foodstuffs may be used in a prophylactic manner and may contain further extracts having a similar function to the first added extract or further added extracts may be added that have a different prophylactic function. Thus a foodstuff could either comprise extracts that provide for a comestible having a single functional aspect, for example that of having a prophylactic effect against the occurrence of diabetes, or a comestible may have a multi-functional prophylactic effect against two or more disease types. It is thought that a similar multi-functional role could also be assigned to pharmaceutical formulations comprising two or more extracts possessing dissimilar therapeutic or prophylactic properties designed either for prophylaxis or for the treatment of more than one disease(s) in a mammal, particularly in a human.

The type of foodstuff or comestible to which at least an extract of the invention may be added includes any processed food such as confectionaries, baked products including breads such as loafs, and flat breads such as pitta bread, naan bread and the like, cakes, snack foods such as muesli bars, compressed dried fruit bars, biscuits, dairy products such as yoghurts, milk and milk-based products such as custards, cream, cheese, butter and crème fraiche, simulated dairy food products such as margarine, olive oil-based spreads, and low fat cream substitutes such as Elmlea products, fruit and vegetable juices, aerated drinks, such as carbonated soft drinks and non-aerated drinks such as squashes, soya milk, rice milk and coconut milk and the like, pastas, noodles, vegetable, seed and nut oils, fruited oils such as sunflower oil, rapeseed oil, olive oil, walnut, hazelnut, and sesame seed oil and the like, and frozen confections such as ice creams, iced yoghurts and the like.

A suitable effective dose of an extract of the invention to be included in a comestible generally lies in the range of about 0.01 to about 120 mg/kg bodyweight, e.g. 0.1 to about 120 mg/kg body weight, preferably in the range of about 0.1 to 50 mg/kg, for example 0.5 to 50 mg/kg. The total daily dose may be given as a single dose, multiple doses, e.g. two to six times applications per day.

In a further aspect of the invention there is provided use of an extract selected from those of Tables 1 – 24, and in particular those of Tables 25 – 30 for the preparation of a medicament for the treatment of a disease selected from the group consisting of diabetes, cancer, cardiovascular disease and obesity.

Thus, there is provided use of an extract selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100), and AV016FrSu(105)08(100) for the preparation of a medicament for the treatment or prophylaxis of diabetes and/or related diseases.

Additionally, there is provided use of an extract selected from the group consisting AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100) and AV016BaSu(65)06(100), and AV016FrDi(65)04(100)) for the preparation of a medicament for the treatment or prophylaxis of obesity.

Additionally, there is provided use of an extract selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100), either singly or in one or more combinations thereof for the preparation of a medicament for the treatment or prophylaxis of cardiovascular disease.

Additionally, there is provided use of an extract selected from the group consisting of AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)04(100), AV016BaSu(65)06(100)and AV016BaSu(105)08(100), either singly or in one or more combinations thereof for the preparation of a medicament for the treatment or prophylaxis of cancer.

The invention will now be exemplified with reference to the following Examples section and accompanying tables and Figures. It is to be understood that the examples are not to be construed as limiting the scope of the invention in any way.

Examples Section

Example 1 : Extraction of *Terminalia arjuna*:

Extraction of *Terminalia arjuna* plant parts was carried out by both direct extraction as well as successive extraction method, at room temperature as well as in soxhlet apparatus and related liquid-liquid techniques followed by lyophilization.

I. Successive Extraction:

Successive extraction from bark of *Terminalia arjuna* was carried out using soxhlet extractor. The solvents used, were based on their sequential polarity starting from non-polar to polar, viz; Hexane, petroleum ether, chloroform, acetone, ethanol, methanol and water at 65°C.

The detailed process is given below:

1. Weigh 100 grams of powdered plant material into the extractor (Soxhlet extractor body) with the cotton on the bottom of the soxhlet apparatus. Cover with cotton on the top. Add 1000 ml of solvent (start with Hexane) in to the round-bottomed flask and place it on the mantle and add few ceramic chips in to it. Add 100ml of solvent over the material just wetting it.
2. Place the extractor on the flask, which is in turn connected with the condenser.
3. Let the cold water circulate continuously in the condenser from the tap. The set up fits tightly as it is fabricated as one set.
4. Switch on the mantle and set it at 65 °C. The vapors of the solvent from the flask would pass through the inlet of the extractor and will get condensed. The condensed (distilled) Solvent will get collected in the Extractor (body) thus extracting the compounds from it.
5. When the plant material is completely filled with solvents, it will get drained in the flask. This process is continuous as long as there is stable heat and water circulation.
6. Continue the extraction for 8 hours, 4-5 cycles per hour.
7. The extract collected in the flask is concentrated by vacuum lyophilization.
8. Follow the same procedure as above successively for the following solvents in the same order. Petroleum Ether, Ethyl acetate, Acetone, Ethanol, Methanol and Water.

II. Direct Extraction

a. Soxhlet based extraction procedure with 100% ethanol solvent:

1. Weigh 100 grams of powdered plant material in the cloth bag and transfer it into the extractor (Soxhlet extractor body). Cover with cotton on the top. (Make sure the level of material is below one inch of the vapour inlet tube.)
2. Add 1 liter of solvent (start with Pet. ether) in to the round-bottomed flask and place it on the mantle and add few ceramic chips in to it. Add 100ml of solvent over the material just wetting it.
3. Place the extractor on the flask, which is in turn connected with the condenser.
4. Let the cold water circulate continuously in the condenser from the tap. The set up fits tightly as it is fabricated as one set.
5. Switch on the mantle and set it at 65 °C. The vapours of the solvent from the flask would pass through the inlet of the extractor and will get condensed. The condensed (distilled) Solvent will get collected in the Extractor (body) thus extracting the compounds from it.
6. When the plant material is completely filled with solvents, it will get drained in the flask. This process is continuous as long as there is stable heat and water circulation.
7. Continue the extraction for 8 hours, 4-5 cycles per hour.
8. The extract collected in the flask is concentrated by vacuum lyophilization.

b. Extraction of T. arjuna bark with 20% ethanol solvent at room temperature:

1. Weigh known quantity (100 grams) of powdered plant material into the conical flask and cover the mouth with aluminum foil to avoid solvent evaporation.

2. Add known volume (500 ml) of 20 % ethanol (100 ml ethanol + 400 ml water) solvent in to the flask and place it on to the orbital shaker and set the speed at 210 rpm and room 28 °C temperature for the extraction.
3. Extract the plant material for 4hr and drain the solvent through
4. Centrifuge the filtrate at 1000 rpm for 10 mins. Collect the supernatant and subject it to lyophilization.
5. Re-extract with 250 ml of solvent for (2 x 2hrs).
6. Centrifuge the filtrate at 1000 rpm for 10 mins.
7. Concentrate extract using lyophilizer under vacuum.

Example 2: Metabolic Fingerprinting of the Terminalia arjuna extracts:

Metabolic fingerprinting of all the direct and successive extracts from *Terminalia arjuna* plant parts is done by HPLC and LC/MS technique.

I. HPLC Fingerprinting:

The plant extracts obtained by direct/successive extraction are subjected to HPLC analysis. High Performance Liquid Chromatography (HPLC) is a technique wherein small quantity of the sample is injected into a C-18 column under high pressure and the constituents are allowed to separate based on their interaction with the column and their retention time within the column. The main purpose of HPLC analysis is to find out the total number of constituents in the plant extracts.

The samples are prepared for HPLC analysis by dissolving the appropriate weight of the extract in methanol. These samples are filtered and collected in the total recovery HPLC vials. These samples are subjected to separation by Waters 2695 HPLC instrument and then analyzed.

Run conditions:

1. The software used for HPLC analysis is Waters Millennium^{32}.
2. The HPLC column used for separation is Waters μBondpack C-18, 5μ, 4.6x150mm.
3. Column temperature is maintained at 25^0C.
4. Solvent flow rate is set at 1.0ml per min. HPLC conditions included Gradient chromatography - solvents used are acetonitrile (solvent A), methanol (solvent B) and water (Solvent C and D).

***Terminalia arjuna* extracts and HPLC Run Conditions:**

1. *Terminalia arjuna* extracts:

1. AV016BaDi(65)04(100)
2. AV016BaDi(28)04(20)
3. AV016BaSu(65)04(100)
4. AV016FrDi(65)04(100)
5. AV016BaSu(65)06(100)

I. **Method Set: Ethanol_11**

Pressure Limits:

High Limits 4000 psi Low limits 0 psi

Programmed Flow:

Pump Mode: Gradient

Accelerated to 10 ml/min in: 2.0 min (5ml/min/min)

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	1.00	10.0	0.0	0.0	90.0	6
2	1.00	1.00	10.0	0.0	0.0	90.0	6
3	15.00	1.00	30.0	0.0	0.0	70.0	6
4	30.00	1.00	40.0	0.0	0.0	60.0	6

A: Acetonitrile, B: Methanol, C: Water

2. *Terminalia arjuna* extracts

1. AV016BaSu(65)01(100)
2. AV016BaSu(65)01(100)g
3. AV016BaSu(65)01(100)g

II. Method Set: Ethyl Acetate_10a

Pressure Limits:

High Limits 4000 psi Low limits 0 psi

Programmed Flow:

Pump Mode: Gradient

Accelerated to 10 ml/min in: 2.0 min (5ml/min/min)

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	0.75	5.0	2.5	92.5	0.0	6
2	1.00	0.75	5.0	2.5	92.5	0.0	6
3	25.00	0.75	25.0	2.5	72.5	0.0	6
4	30.00	0.75	5.0	2.5	92.5	0.0	1

A: Acetonitrile, B: Methanol, C: Water

3. *Terminalia arjuna* extract:

1. AV016BaSu(65)09(100)

III. **Method Set: Ethyl Acetate_4a**

Pressure Limits:

High Limits 4000 psi Low limits 0 psi

Programmed Flow:

Pump Mode: Gradient

Accelerated to 10 ml/min in: 2.0 min (5ml/min/min)

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	0.75	0.0	5.0	95.0	0.0	6
2	1.00	0.75	0.0	5.0	95.0	0.0	6
3	15.00	0.75	0.0	20.0	80.0	0.0	6
4	25.00	0.75	0.0	50.0	50.0	0.0	6
5	30.00	0.75	0.0	5.0	95.0	0.0	1

A: Acetonitrile, B: Methanol, C: Water

4. *Terminalia arjuna* extract:

1. AV016BaSu(105)08(100)
2. AV016FrSu(105)08(100)

IV. **Method Set: Gy_Water_12**

Pressure Limits:

High Limits 4000 psi Low limits 0 psi

Programmed Flow:

Pump Mode: Gradient

Accelerated to 10 ml/min in: 2.0 min (5ml/min/min)

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	0.75	5.0	5.0	90.0	0.0	6
2	1.00	0.75	5.0	5.0	90.0	0.0	6
3	20.00	0.75	25.0	15.0	60.0	0.0	4
4	26.00	0.75	70.0	5.0	25.0	0.0	4
5	30.00	0.75	5.0	5.0	90.0	0.0	1

A: Acetonitrile, B: Methanol, C: Water

II. Liquid Chromatography Mass Spectrometry (LC/MS) Fingerprinting:

Mass spectroscopy, is an instrumental approach that allows for the mass measurement of molecules. The five basic components of mass spectrometer are a vacuum system, a sample introduction device, an ionization source, a mass analyzer and an ion detector. Combining these parts a mass spectrometer determines the molecular weight

of chemical compounds by ionizing, separating and measuring molecular ions according to their mass-to-charge ratio (m/z).

Run conditions used for LC/MS fingerprinting of *Terminalia arjuna* is shown down.

1. Q-Trap LC/MS instrument from Applied Biosystems was used. The software used for LC/MS analysis is Analyst.
2. The HPLC column used for separation is COSMOSIL® $5C_{18}$-MS-II Packed Column C-18, 5μm, 4.6mmI.D.x 150mm.
3. Column temperature is maintained at 25^0C.
4. Solvent flow rate is set at 1.0ml per min. HPLC conditions included Gradient chromatography - solvents used are acetonitrile (solvent C), methanol (solvent B) and water (Solvent D).

1. *Terminalia arjuna* extracts:

AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)g, AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(65)06(80), AV016BaDi(65)04(100), and AV016FrDi(65)04(100)

a. LC/MS Sample Run Conditions for all the above-mentioned *Terminalia arjuna* samples:

Mass Spectrometer SPEC	QTrap	0	MASS
Config Table Ver	01		
Firmware Ver	M401400 B4T0301 M3L1400 B3T0300		

Component Name	Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer
Component ID	QTrap
Manufacturer	AB Sciex Instruments
Model	027170 - C
S/N	M1100301

Time from start =0.0500 min

Mass Spectrometer SPEC	QTrap	0	MASS
Start of Run - Detailed Status			
Vacuum Status		At Pressure	
Vacuum Gauge (10e-5 Torr)		0.7	
Backing Pump		Ok	
Dual Turbo Pump		Normal	
Sample Introduction Status		Ready	
Source/Ion Path Electronics		On	
Source Type		Turbo Spray	
Source Temperature (at setpoint)		400.0 C	
Source Exhaust Pump		Ok	
Interface Heater		Ready	

Mass Spectrometer SPEC	QTrap	0	MASS
End of Run - Detailed Status			
Vacuum Status		At Pressure	
Vacuum Gauge (10e-5 Torr)		0.7	
Backing Pump		Ok	

Dual Turbo Pump	Normal
Sample Introduction Status	Ready
Source/Ion Path Electronics	On
Source Type	Turbo Spray
Source Temperature (at setpoint)	400.0 C
Source Exhaust Pump	Ok
Interface Heater	Ready

Time from start =61.4833 min

PE LC-200 Pump Method Properties

PE LC-200 Quaternary Pump

Minimum Pressure (psi):0.0

Maximum Pressure (psi): 6100.0

Shutdown Time (min): 999.9

Step Table:

Step	Total Time (min)	Flow Rate (µl/min)	GradientProfile	A (%)	B (%)	C (%)	D (%)	TE#1	TE#2
0	0.5	1000.00	1.0	0.0	0.0	10.0	90.0	open	open
1	1.0	1000.00	1.0	0.0	0.0	10.0	90.0	open	open
2	15.0	1000.00	1.0	0.0	0.0	15.0	85.0	open	open
3	40.0	1000.00	1.0	0.0	0.0	25.0	75.0	open	open
4	50.0	1000.00	1.0	0.0	0.0	35.0	65.0	open	open
5	60.0	1000.00	1.0	0.0	0.0	50.0	50.0	open	open

Quantitation Information:

Sample Type: Unknown

Dilution Factor: 1.000000

Custom Data:

Quantitation Table:

Period 1:

Scans in Period: 2243

Relative Start Time: 0.00 msec

Experiments in Period: 1

Period 1 Experiment 1:

Scan Type: Q1 MS (Q1)

Polarity: Positive

Scan Mode: Profile

Resolution Q1: UNIT

Intensity Thres.: 0.00 cps

Settling Time: 0.0000 ms

MR Pause: 5.0070 ms

MCA: No

Center/Width: No

Step Size: 0.10 amu

Start (amu)	Stop (amu)	Time (sec)	Param	Start	Stop
50.00	1700.00	1.60	CEP	6.47	66.65

Parameter Table(Period 1 Experiment 1)

CUR: 20.00

TEM: 400.00

GS1: 20.00

GS2: 50.00

ihe: ON

IS:	4500.00
DP	90.00
EP	10.00

2. *Terminalia arjuna* extracts:

AV016BaSu(105)08(100), AV016FrSu(105)08(100) and AV016BaDi(28)04(20).

a. LC/MS Sample Run Conditions for all the above-mentioned *Terminalia arjuna* samples:

Mass Spectrometer SPEC	QTrap	0	MASS
Config Table Ver	01		
Firmware Ver	M401400 B4T0301 M3L1400 B3T0300		
Component Name	Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer		
Component ID	QTrap		
Manufacturer	AB Sciex Instruments		
Model	027170 - C		
S/N	M1100301		

Time from start =2.1000 min

Mass Spectrometer SPEC	QTrap	0	MASS
Start of Run - Detailed Status			
Vacuum Status		At Pressure	
Vacuum Gauge (10e-5 Torr)		0.7	

Backing Pump	Ok
Dual Turbo Pump	Normal
Sample Introduction Status	Ready
Source/Ion Path Electronics	On
Source Type	Turbo Spray
Source Temperature (at setpoint)	400.0 C
Source Exhaust Pump	Ok
Interface Heater	Ready

Time from start =2.1167 min

Mass Spectrometer QTrap 0 MASS SPEC

End of Run - Detailed Status

Vacuum Status	At Pressure
Vacuum Gauge (10e-5 Torr)	0.7
Backing Pump	Ok
Dual Turbo Pump	Normal
Sample Introduction Status	Ready
Source/Ion Path Electronics	On
Source Type	Turbo Spray
Source Temperature (at setpoint)	400.0 C
Source Exhaust Pump	Ok
Interface Heater	Ready

Time from start =42.9333 min

PE LC-200 Pump Method Properties

PE LC-200 Quaternary Pump

Minimum Pressure (psi):0.0

Maximum Pressure (psi): 6100.0

Shutdown Time (min): 999.9

Step Table:

Step	Total Time(min)	Flow Rate(µl/min)	GradientProfile	A (%)	B (%)	C (%)	D (%)	TE#1	TE#2
0	0.5	750.00	1.0	0.0	5.0	5.0	90.0	open	open
1	1.0	750.00	1.0	0.0	5.0	5.0	90.0	open	open
2	20.0	750.00	1.0	0.0	15.0	25.0	60.0	open	open
3	26.0	750.00	1.0	0.0	5.0	70.0	25.0	open	open
4	30.0	750.00	1.0	0.0	5.0	5.0	90.0	open	open
5	40.0	750.00	1.0	0.0	5.0	5.0	90.0	open	open

Analog/Digital Converter Properties

Interval (sec): 0.200

Rate (pts/sec): 5

Polarity: Bipolar

Channel Summary

No. Name: Interpreted Value Full Scale: Interpreted Unit:

Voltage (volts): Status:

1	100.0	%	1.0 Used

Quantitation Information:

Sample Type: Unknown

Dilution Factor: 1.000000

Custom Data:

Quantitation Table:

Period 1:

Scans in Period: 1495

Relative Start Time: 0.00 msec

Experiments in Period: 1

Period 1 Experiment 1:

Scan Type: Q1 MS (Q1)

Polarity: Positive

Scan Mode: Profile

Resolution Q1: UNIT

Intensity Thres.: 0.00 cps

Settling Time: 0.0000 ms

MR Pause: 5.0070 ms

MCA: No

Center/Width: No

Step Size: 0.10 amu

Start (amu)	Stop (amu)	Time (sec)	Param	Start	Stop
50.00	1700.00	1.60	CEP	6.47	66.65

Parameter Table(Period 1 Experiment 1)

CUR:	20.00
TEM:	400.00
GS1:	20.00
GS2:	50.00
ihe:	ON
IS:	4500.00
DP	90.00
EP	10.00

Example 3: Determination of the bio-therapeutic potential of *Terminalia arjuna* extracts:

I. Anti-Diabetic Potential:

Acute glucose elevations after meal ingestion are associated with a variety of glucose-mediated tissue defects oxidative stress, glycation, and advanced glycation end product formation which have far reaching structural and functional consequences for virtually every human organ system. Lowering glycosylated hemoglobin to levels that prevent or delay these complications can be achieved only by reducing both postprandial and fasting plasma glucose levels.

The α –Glucosidase complex in the small intestine is involved in sugar absorption. Thus, inhibitors of α –Glucosidase would limit the absorption of dietary carbohydrates and in turn suppress postprandial hyperglycemia. Therefore, α – Glucosidase inhibitors are promising drug candidates in the treatment and prevention of diabetes. The α –Glucosidase inhibitors (acarbose, voglibose, miglitol) have been effective in delaying the digestion and absorption of carbohy-drates, thus diminishing the postprandial surge in blood glucose levels without loss of calories.

1. Alpha-glucosidase assay

Alpha-Glucosidase hydrolyzes the terminal, non-reducing 1,4-linked alpha-D-glucose residues with release of alpha-D-glucose. In the assay the enzymatic activity is determined by measuring the increase in absorbance at 400nm caused by the hydrolysis of p-nitrophenyl- α -D-glucopyranoside with the release of p-nitrophenol.

a. *Reagents:*

- Potassium phosphate buffer 0.1M, pH 6.8
- PNPG 4 mM
- Sodium carbonate 0.2 M
- BSA 0.2 % in Buffer
- Enzyme 1.2U/ml in 0.2% BSA

b. *Procedure:*

Buffer - 600 µl
Inhibitor - 100 µl
Enzyme - 25 µl

↓

Incubate at 37° C for 15mins

↓

PNPG - 25 µl

↓

Incubate at 37° C for 15mins

↓

Add Na_2CO_3 - 750 µl

↓

Read absorbance at 400nm

c. Results and Discussion:

Successive and direct extract from *Terminalia arjuna* bark and fruit extracts assayed for their alpha-Glucosidase inhibition potential showed that *T. arjuna* extract showed dose dependent decrease in alpha-Glucosidase inhibition. IC_{50} is defined as the amount of extract required for 50% inhibition in the enzyme activity.

It was found that all the extracts of *T. arjuna* showed higher alpha-Glucosidase inhibition potential than commercially available acarbose, as indicated by IC_{50} values. (Table 25).

2 Glucose Uptake assay:

The deoxy-glucose uptake assay by fat cells or adipoctes (3T3-L1) help to identify the extracts/ bioactives that helps to increase the glucose uptake by the fat cells. Differentiating 3T3-L1 cells exhibit a dramatic increase in the rate of insulin-stimulated glucose transport during their conversion from proliferating fibroblasts to nonproliferating adipocytes. Green and Kehinde(1975) first demonstrated that confluent 3T3-L1 cells could be hormonally induced to differentiate into adipocytes over the course of 6–9 d.

The assay will thereby help in screening of phytoextracts that will help the diabetic individuals by quick removal of glucose from blood stream and simultaneously will help the fat cells to build a better storage of energy.

Tritiated deoxyglucose uptake by 3T3-L1 is one of the most accepted assay for the in-vitro screening of active principles with anti-diabetic activity

a. 3T3-L1 cell culture:

3T3-L1 fibroblasts were cultured in DME-F12 media containing 10% FCS, NEAA, glutamine, antibiotics, anti-mycotics, etc., in an atmosphere of 5% CO2 at 37 °C. Fibroblasts were cultured up to confluency. Subcultures were done at three-day intervals with trypsin-EDTA solution treatment.

b. Maturation of the fibroblasts to adipocytes

Differentiation was induced by treating the cells with DMEM hi glucose (4.5 gm/litre) containing 0.5mmol/l 3-isobutyl-1-methylxanthine, 4 mg/ml dexamethasone, 1 mg/ml insulin and 10% FCS for 48 h. The cells were cultured in DMEM media with standard pre-described supplements for the next 6-10 days. Cells were used only when at least 95% of the cells showed an adipocyte phenotype by accumulation of lipid droplets. These were taken up for glucose uptake studies with tritiated glucose.

c. Assay Procedure

Distribute 50,000 of differentiated 3T3-L1 adipocytes in each well of a new 96 well plate. Incubate with KRH buffer at 37°C for 10 min. Add insulin 10 nM, 25 nM and 50 nM to the respective. To the experimental sets treatment was done with or without

insulin 10 nM in presence of test compound (333.0 μg). This was followed by incubation further at 37°C for 15 min. Wash the cells once with KRH buffer. Initiate glucose uptake reaction by adding 0.1 mM 2-deoxy glucose containing 2-deoxy[3H] glucose (final concentration 12.2 kBq/ml). Incubate for 1 hr at 37°C. Terminate assay by adding 40 μM Cytochalasin B. Wash the cells trice with ice-cold KRH buffer Solubilize cells in 20 μl 1% Triton X. Incubate for 10 min at 37°C. Count radioactivity on a micro-titre plate radioactive counter

d. Results and Discussion:

Glucose uptake activity was analyzed by measuring the uptake of 0.1 mM deoxy-glucose containing deoxy-tritiated-glucose containing 2.2 kBq/mL.

As seen from Table 26, direct extract of T. arjuna bark with 100 % ethanol solvent [AV016BaDi(65)04(100)] showed stimulation index of 3.0 in absence of insulin and 2.0 in presence of 10 nM Insulin. The data thus suggest that AV016BaDi(65)04(100) has potent insulin mimetic potential.

2. Anti-Obesity potential:

Studies have demonstrated that central obesity is more strongly related to lipid/lipoprotein abnormalities than is general obesity. Obesity may confer cardiac dysfunction due to lipid accumulation in cardiomyocytes and also induces type II diabetes. These adverse lipid/lipoprotein profiles in obese individuals are important, because they may be responsible for their increased risk for cardiovascular disease (CVD).

The assay is a semi quantitative method used for quantification of Adipose differentiation and hence the lipid and triglycerides contents in the cell after the

stimulation of the cells for differentiation in presence and absence of the plant extracts.

a. Assay Principle

Oil Red staining is a semi quantitative method used for quantification of Adipose differentiation. Staining with oil-soluble dyes is based on the greater solubility of the dye in the lipoid substances than in the usual hydroalcoholic dye solvents. Oil Red O is a lipophilic red dye which stains intracellular lipid drops. The quantitation of lipid can be done following the extraction of intracellular lipid with organinc solvent. Hence it is used to estimate lipid and triglycerides contents in the cell after plant extract stimulation of cells

b. Assay Protocol

Seed 3T3-L1 cells at a density of 10^5 cells/well with the differentiating medium and extracts (0.33mg) and incubated for 24h at 37^0 C and 5% CO_2. The medium was then removed after 24 hr and fresh differentiating medium was added and the cells were kept in the medium for another 24hrs. Post 48hrs from day one the medium was removed and Insulin only medium (DMEM F12 + FBS + Insulin) was added and the cells were kept for24h. Post 24-hr incubation medium was aspirated and Oil O red staining was performed.

c. Oil Red O Staining:

Total volume in each well for reagents is 500µl / well. Media was removed and cells were washed with PBS thrice. For fixing the cells they were incubated for 1h with 4% Formalin. Remove the Formalin and again wash with PBS twice. For staining with Oil Red O, incubate it with the stain for 1h. After 1h remove the stain and wash it

with PBS and dry it for 10 minutes. Add 4% NP40. Read absorbance at 520nm in Spectrophotometer.

d. Results and Discussion:

Anti-obesity assay with 3T3-L1 cells showed that *Terminalia arjuna* extracts showed inhibition in the lipid accumulation. As seen from Table 27 *Terminalia arjuna* bark direct 100% and 20% ethanol extracts, AV016BaDi(65)04(100) and AV016BaDi(28)04(20) extracts showed 76.3 and 64.3 % inhibition as compared to control.

In case of *Terminalia arjuna* successive bark ethyl acetate, gluey acetone, non-gluey acetone, ethanol and methanol extracts AV016BaSu(65)09(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100) and AV016BaSu(65)06(100) gave inhibition of 61.9, 58.5, 86.8, 49.4 and 57.9% inhibition as compared to control. *Terminalia arjuna* fruit direct 100% ethanol extract AV016FrDi(65)04(100) gave inhibition of 41.9 % inhibition as compared to control.

Assay +ve control showed 104% inhibition as compared to control.

3. Cardiovascular Disease:

Elevated cholesterol is associated with a greater-than-normal risk of atherosclerosis and cardiovascular disease. High blood triglyceride levels alone do not cause atherosclerosis. But lipoproteins that are rich in triglycerides also contain cholesterol, which causes atherosclerosis in many people with high triglycerides. So high triglycerides may be a sign of a lipoprotein problem that contributes to CHD.

1. Cell Based Assay for checking levels of lipid accumulation:

a. Assay Principle:

Growing the cells in presence of lipoprotein deficient serum induces the lipid biosynthesis in the cells. Assay is performed on the cells induced by growing in lipoprotein deficient serum followed by total lipid estimation using oil red staining. Oil Red O is a lipophilic red dye used to stain neutral lipids in cells. The amount of staining is directly proportional to the amount of lipid in the cell. The content of lipid was checked across the extract treated and control sets and the amount of dye and hence lipid content was measured spectrophotometrically.

b. Assay Procedure

CHO-K1 cells were seeded at density of 10^5 cells / well in 12 well-plate format. The cells were grown to 80% confluence in DMEM-F12 medium with 10% fetal calf serum, 2mM glutamine, 1.5 gm/L sodium bicarbonate and anti-mycotics and penicillin and streptomycin at 37 C in 5% CO2 environment. After 2 days of incubation the medium was replaced with DMEM-F12 medium with 10% lipoprotein deficient serum, 2mM glutamine, 1.5 gm/L sodium bicarbonate and anti-mycotics and penicillin and streptomycin and grown for additional 24 hrs at 37 C in 5% CO2 environment. At the end of 24 hrs to the extract treated sets *Terminalia arjuna* at concentration of 0.5 mg/ml were added whereas the control sets were treated with vehicle (DMSO). The incubation was continued for additional 4 hrs after which the cells were washed with sterile PBS buffer, fixed and stained with Oil Red O.

c. Oil Red O Staining:

Total volume in each well for reagents is 500µl / well. Media was removed and cells were washed with PBS thrice. For fixing the cells they were incubated for 1h with 4% Formalin. Remove the Formalin and again wash with PBS twice. For staining with Oil Red O, incubate it with the stain for 1h. After 1h remove the stain and wash it with PBS and dry it for 10 minutes. Add 4% NP40. Read absorbance at 520nm in Spectrophotometer.

d. Results and Discussion:

As seen from Table 28, *Terminalia arjuna* extracts showed marked decrease in the amount of total lipids in the extract treated cells. *Terminalia arjuna* bark successive extracts AV016BaSu(65)09(100) and AV016BaSu(65)01(100) showed 22% and 21% inhibition as compared to control set. *Terminalia arjuna* direct ethanol extracts AV016BaDi(65)04(100) and AV016BaDi(28)04(20) showed 31% and 28% inhibition in the levels of total lipids as compared to the control set.

2. HMG-CoA reductase assay:

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is the first committed step in cholesterol biosynthetic pathway. HMG-CoA reductase the rate-limiting enzyme step in cholesterol biosynthesis has become the most successful drug treatment for lowering total plasma cholesterol, in particular LDL-cholesterol.

a. Assay Principle:

HMG-CoA reductase assay is based on the oxidation of NADPH to NADP catalyzed by a fixed concentration of HMG-CoA reductase from liver microsomal fraction.

$$\text{HMG-CoA} + 2\ \text{NADPH} + 2H^{+} \xrightarrow{\text{HMG-CoA reductase}} \text{Mevalonate} + 2\ NADPH^{+}$$

The oxidation of NADPH to $NADP^{+}$ is accompanied by a decrease in absorbance at 340 nm and is directly proportional to the HMG-CoA reductase activity in the sample.

b. Results and Discussion:

The potential of *Terminalia arjuna* bark direct 100% ethanol and 20% ethanol extracts AV016BaDi(28)04(20) and AV016BaDi(65)04(100) were checked for their HMG-CoA reductase inhibition potential. It was found that both AV016BaDi(28)04(20) and AV016BaDi(65)04(100) showed 93% inhibition (Table 29) and 63.1% (Table 30) in HMG-CoA reductase activity as compared to control set.

4. Anti-cancer assay:

The Anti-cancer potential of the *Terminalia arjuna* plant extracts was determined by screening the plant extracts in vitro for cytotoxicity on the human hepatic cell line HepG2.

a. Experimental Details:

The cells were seeded at a density of $2.5x10^{5}$ cell per ml in a 24 well plate. The cells were allowed to attach for 2 hour before adding the extracts at a concentration of 10µg/ml. 48 hour after incubation with vehicle i.e DMSO, ascorbic acid and extracts AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)04(100). The cell viability was determined after 24 hours.

b. Assay Protocol

Seed HepG2 at a cell density of $2.5x10^5$ cells per ml. Add of the Ascorbic acid and *Terminalia arjuna* extracts to final concentration of 10 µg/ml. Incubate in 5% CO_2 incubator at 37 °C for 48 hour. Trypsinize cell. Mix with 1 part trypan blue and 4 parts PBS and count the viable cells and the non-viable cells by counting the cells that has taken typan blue (non-viable cells) and the ones that has excluded it (viable cells).

c. Results and Discussions:

It was found that treatment of HepG2 cells with *T. arjuna* successive ethyl acetate AV016BaSu(65)09(100), acetone AV016BaSu(65)01(100), and ethanol AV016BaSu(65)04(100), extracts there was decrease in the total cell count and in the number of viable cells in case of drug treated set as compared to the untreated set or the cells with DMSO set (Table 31).

Claims:

1. A method for treating a disease selected from the group comprising obesity, cancer, cardiovascular disease and diabetes in a mammal, which comprises administering to the said mammal an effective non-toxic amount of at least an extract from *Terminalia arjuna* selected from those as defined in Tables 1 – 24.

2. A method according to claim 1 wherein the disease is obesity and the extract is selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100) AV016BaSu(65)06(100) and AV016FrDi(65)04(100) or a combination of two or more thereof.

3. A method according to claim 1 wherein the disease is cancer and the extract is selected from the group consisting of AV016BaSu(65)09(100), AV016BaSu(65)01(100) and AV016BaSu(65)04(100) or a combination of two or more thereof.

4. A method according to claim 1 wherein the disease is cardiovascular disease and the extract is selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100) and AV016BaSu(65)01(100) or a combination of two or more thereof.

5. A method according to claim 1 wherein the disease is diabetes and the extract is selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100),

AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100), and AV016FrSu(105)08(100) or a combination of two or more thereof.

6. A method according to claim 1 wherein the disease is diabetes, obesity and cardiovascular disease and the extract is selected from the group consisting of AV016BaDi(65)04(100) and AV016BaDi(28)04(20) or a combination of two.

7. A method according to any one of claims 1 – 6 wherein the said treatment is a prophylactic treatment.

8. A pharmaceutical formulation for use in the treatment of a disease selected from the group diabetes, obesity, cardiovascular disease, and cancer comprising at least one extract isolated from *Terminalia arjuna*, and selected from those listed in Tables 1 – 24 in admixture with a pharmaceutically acceptable carrier.

9. A formulation according to claim 8 for use in the treatment of diabetes comprising at least one extract selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100), and AV016FrSu(105)08(100) or a combination of two or more thereof.

10. A formulation according to claim 8 for use in the treatment of cancer comprising at least one extract selected from the group consisting of AV016BaSu(65)09(100), AV016BaSu(65)01(100) and AV016BaSu(65)04(100) or a combination of two or more thereof.

11. A formulation according to claim 8 for use in the treatment of cardiovascular disease comprising at least one extract selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100) and AV016BaSu(65)01(100) or a combination of two or more thereof.

12. A formulation according to claim 8 for use in the treatment of obesity comprising at least one extract selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100), AV016BaSu(65)06(100) and AV016FrDi(65)04(100) or a combination of two or more thereof.

13. A formulation according to claim 8 for use in the treatment of diabetes, obesity and cardiovascular disease comprising at least one extract selected from the group consisting of AV016BaDi(65)04(100) and AV016BaDi(28)04(20) or a combination of two.

14. A formulation according to any one of claims 8 – 13 for prophylactic use.

15. A method for the preparation of a pharmaceutical formulation comprising bringing into association at least an extract of the invention, and a pharmaceutically acceptable carrier therefore.

16. An extract from *Terminalia arjuna* selected from the group consisting of the extracts having the HPLC and/or MS characteristics shown in Tables 1 – 24.

17. A comestible comprising at least an extract from *Terminalia arjuna* selected from the group consisting of the extracts having the HPLC and/or MS characteristics shown in Tables 1 – 24.

18. A comestible according to claim 17 comprising at least an extract for use in the prophylaxis of cancer.

19. A comestible according to claim 17 comprising at least an extract for use in the prophylaxis of cardiovascular disease.

20. A comestible according to claim 17 comprising at least an extract for use in the prophylaxis of diabetes.

21. A comestible according to claim 17 comprising at least an extract for use in the prophylaxis of obesity.

22. A comestible according to claim 17 comprising at least an extract selected from AV016BaDi(65)04(100) and AV016BaDi(28)04(20) either singly or in one or more combinations thereof, for use in the prophylaxis of diabetes, obesity and cardiovascular diseases.

23. Use of an extract selected from the group consisting of the extracts having the HPLC and/or MS characteristics shown in Tables 1 – 24 for the preparation of a medicament for the treatment of disease selected from the group consisting of diabetes, cancer, cardiovascular disease and obesity.

24. Use according to claim 23 of an extract selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100) and AV016FrSu(105)08(100) for the preparation of a medicament for the treatment or prophylaxis of diabetes and/or related diseases.

25. Use according to claim 23 of an extract selected from the group consisting AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100) AV016BaSu(65)06(100) and AV016FrDi(65)04(100) for the preparation of a medicament for the treatment or prophylaxis of obesity.

26. Use according to claim 23 of an extract selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100) and AV016BaSu(65)01(100), either singly or in one or more combinations thereof for the preparation of a medicament for the treatment or prophylaxis of cardiovascular disease.

27. Use according to claim 23 of an extract selected from the group consisting of AV016BaSu(65)09(100), AV016BaSu(65)01(100) and AV016BaSu(65)04(100) either singly or in one or more combinations thereof for the preparation of a medicament for the treatment or prophylaxis of cancer.

28. Use according to claim 23 of an extract AV016BaDi(65)04(100) and AV016BaDi(28)04(20) either singly or in one or more combinations thereof for the preparation of a medicament for the treatment or prophylaxis of Diabetes, Obesity, and Cardiovascular diseases.

Table 1: HPLC fingerprint of extract AV016BaDi(65)04(100)

	Retention Time	Area	% Area	Height
1	1.746	83501	0.03	18746
2	1.887	21939706	9.17	3508953
3	2.482	424664	0.18	27549
4	3.165	694426	0.29	38196
5	4.499	535430	0.22	20883
6	5.860	1140766	0.48	31447
7	6.488	2287279	0.96	105829
8	7.680	2247351	0.94	73739
9	8.259	1172094	0.49	53148
10	8.702	2209397	0.92	93831
11	9.425	2269048	0.95	89553
12	10.177	3848206	1.61	111334
13	10.485	1536103	0.64	99241
14	10.730	1306318	0.55	102448
15	11.155	3705535	1.55	129030
16	12.263	10146875	4.24	464227
17	12.335	6747979	2.82	548102
18	13.000	30474340	12.74	1778085
19	13.697	3437393	1.44	233361
20	14.232	8223596	3.44	277375
21	14.532	14638851	6.12	819721
22	15.023	12657916	5.29	590365
23	15.452	5471266	2.29	306520
24	15.998	16599596	6.94	1078704
25	16.299	4397452	1.84	318952
26	16.463	2424066	1.01	310668
27	16.863	6414264	2.68	310396
28	17.166	12652492	5.29	355032
29	17.683	4940680	2.07	294832
30	18.118	11457746	4.79	318670
31	18.673	2951869	1.23	232444
32	18.872	6643592	2.78	219376
33	19.475	1933618	0.81	176183
34	19.668	2454443	1.03	182659
35	19.884	4858876	2.03	167366
36	20.329	950214	0.40	139162
37	20.543	6254360	2.61	134329
38	21.427	1804592	0.75	107259
39	21.716	7198552	3.01	109172
40	23.185	2885830	1.21	72715
41	23.905	3042057	1.27	54167
42	24.977	665797	0.28	38055
43	25.258	1486354	0.62	35170

Table 2: HPLC fingerprint of extract AV016BaDi(28)04(20).

	Retention Time	Area	% Area	Height
1	1.724	259750	0.10	58489
2	1.897	27826788	10.53	3799266
3	2.486	571316	0.22	50306
4	3.045	1106233	0.42	51910
5	3.732	674291	0.26	29021
6	4.182	1056425	0.40	32454
7	5.572	2403677	0.91	47788
8	6.082	3557804	1.35	161895
9	6.484	757991	0.29	55308
10	7.264	3549212	1.34	97606
11	7.883	2512425	0.95	92337
12	8.299	3676625	1.39	140954
13	9.060	4300244	1.63	140449
14	9.699	6186918	2.34	155916
15	10.124	2085208	0.79	151077
16	10.796	7036369	2.66	179944
17	11.555	17109248	6.48	350960
18	12.574	31568344	11.95	1286226
19	13.038	2562494	0.97	285400
20	13.307	4871494	1.84	288682
21	14.002	26201944	9.92	838854
22	14.597	14156853	5.36	465017
23	15.150	4497591	1.70	322199
24	15.663	16422598	6.22	962872
25	15.912	3863537	1.46	326789
26	16.103	6416104	2.43	315587
27	16.564	4817209	1.82	305649
28	16.767	8560395	3.24	305526
29	17.221	2824887	1.07	283965
30	17.400	2813123	1.06	282612
31	17.661	16467191	6.23	382391
32	18.625	6447644	2.44	200677
33	19.302	3348258	1.27	168434
34	19.568	11717199	4.44	149595
35	21.405	4945619	1.87	87932
36	22.629	4393424	1.66	69440
37	24.162	2305522	0.87	34152
38	25.963	255400	0.10	9559

Table 3: HPLC fingerprint of extract AV016BaSu(65)09(100).

	Retention Time	Area	% Area	Height
1	2.475	25395907	5.13	3334075
2	3.244	1688929	0.34	136464
3	4.656	9262251	1.87	280029
4	5.254	1188708	0.24	48754
5	6.603	12296585	2.48	364675
6	6.899	5848912	1.18	389909
7	7.233	1367772	0.28	130776
8	7.428	1332175	0.27	138611
9	7.896	7084034	1.43	280115
10	8.260	6705156	1.35	380995
11	8.576	2924953	0.59	246136
12	8.782	2453519	0.50	228061
13	9.184	8669542	1.75	320819
14	9.521	4295969	0.87	304404
15	9.816	4279516	0.86	324956
16	10.243	30792046	6.22	3248747
17	10.516	84937402	17.16	4333685
18	11.442	12774541	2.58	1056539
19	11.603	33723533	6.81	4613519
20	11.705	63394649	12.81	4142892
21	12.316	44870739	9.06	3991785
22	12.575	5106719	1.03	582499
23	12.754	2812210	0.57	307827
24	12.983	7556632	1.53	836964
25	13.270	4609110	0.93	360754
26	13.418	4222758	0.85	346063
27	13.925	16253486	3.28	1431902
28	14.456	7163578	1.45	605726
29	14.797	1631474	0.33	141283
30	15.240	3874910	0.78	285386
31	15.388	3029615	0.61	225745
32	15.588	2719921	0.55	159504
33	16.139	1333368	0.27	123152
34	16.346	3614771	0.73	345601
35	16.570	1244982	0.25	111424
36	16.813	1256674	0.25	123386
37	16.995	1494705	0.30	110575
38	17.367	1278577	0.26	73925
39	17.823	2517030	0.51	117093

40	18.123	2322326	0.47	179787
41	18.419	841112	0.17	86329
42	18.673	2150256	0.43	93219
43	18.954	429553	0.09	73333
44	19.255	1852803	0.37	78786
45	19.627	1385755	0.28	84299
46	19.912	1175927	0.24	106435
47	20.169	2238619	0.45	198261
48	20.362	1903752	0.38	136402
49	20.725	1388092	0.28	86158
50	21.002	2038182	0.41	112055
51	21.221	957990	0.19	84615
52	21.507	1049496	0.21	79603
53	21.799	1772753	0.36	102185
54	22.203	1643957	0.33	76435
55	22.543	831012	0.17	71872
56	22.690	572833	0.12	72239
57	22.941	3535908	0.71	220985
58	23.299	2133293	0.43	142496
59	23.648	2144927	0.43	151460
60	23.960	1493722	0.30	100416
61	24.429	2297448	0.46	123572
62	24.639	908084	0.18	85150
63	24.933	2402551	0.49	104466
64	25.280	583388	0.12	83878
65	25.564	1925541	0.39	92355
66	25.800	1153719	0.23	90624
67	25.974	1727846	0.35	88759
68	26.404	1397501	0.28	91036
69	26.591	795071	0.16	89416
70	26.751	1482526	0.30	89528
71	27.018	763878	0.15	86118
72	27.157	1700407	0.34	86915
73	27.647	2637337	0.53	78919
74	28.193	4360949	0.88	662358

Table 4: HPLC fingerprint of extract AV016BaSu(65)01(100).

	Retention Time	Area	% Area	Height
1	2.391	6928	0.00	852
2	2.556	1739539	0.82	304280
3	3.313	84646	0.04	9680
4	3.686	223139	0.10	9176
5	4.269	63325	0.03	3419
6	5.024	13893	0.01	769
7	6.157	4281	0.00	572
8	6.736	197041	0.09	6916
9	7.391	117641	0.06	5898
10	7.878	616604	0.29	17813
11	8.308	278599	0.13	15829
12	8.860	611104	0.29	22047
13	9.391	187896	0.09	12237
14	9.564	276548	0.13	14167
15	12.058	8846367	4.16	183309
16	12.644	751247	0.35	38688
17	13.199	1719360	0.81	43466
18	13.988	1735451	0.82	56406
19	14.555	2169673	1.02	76948
20	14.875	2254289	1.06	115963
21	15.626	4155110	1.95	123796
22	15.956	1080279	0.51	86186
23	16.580	5912054	2.78	175797
24	17.766	7200465	3.38	141141
25	18.423	2986827	1.40	111890
26	19.067	6971841	3.28	151382
27	19.737	4017460	1.89	145309
28	20.104	1488984	0.70	136621
29	20.459	4173594	1.96	175505
30	20.795	3235535	1.52	167773
31	21.079	1788929	0.84	164753
32	21.272	4796914	2.25	175363
33	21.910	4124406	1.94	168234
34	22.267	6071222	2.85	185861
35	22.997	5930428	2.79	190259
36	23.451	5868309	2.76	231002
37	23.866	16287689	7.65	1076501
38	24.287	4035419	1.90	266032
39	24.627	3569391	1.68	266672
40	24.844	42015183	19.75	2046204

41	26.107	8757964	4.12	239370
42	26.900	25586681	12.02	821474
43	27.926	7429374	3.49	323025
44	28.156	13400123	6.30	654328

Table 5: HPLC fingerprint of extract AV016BaSu(65)01(100)ng.

	Retention Time	Area	% Area	Height
1	2.579	1411822	0.59	207187
2	3.336	80911	0.03	9988
3	3.518	367100	0.15	14428
4	4.289	54629	0.02	3130
5	4.766	46285	0.02	1682
6	6.659	58963	0.02	2489
7	7.316	3635	0.00	454
8	7.852	277194	0.12	10198
9	8.240	150308	0.06	8971
10	8.774	321493	0.14	13773
11	9.207	113505	0.05	7075
12	9.458	151700	0.06	8682
13	10.403	841535	0.35	23389
14	11.877	5609438	2.36	151067
15	12.469	548895	0.23	28503
16	12.997	1315579	0.55	34524
17	13.798	1396765	0.59	46557
18	14.301	1552115	0.65	56533
19	14.658	1693118	0.71	88234
20	15.378	2309177	0.97	76351
21	15.719	1849902	0.78	89567
22	16.341	3751422	1.58	131368
23	16.651	705622	0.30	59259
24	17.537	5824011	2.45	121156
25	18.170	2202916	0.93	92276
26	19.153	16377423	6.89	384476
27	20.514	11154160	4.69	206032
28	21.248	27468304	11.56	1141614
29	22.005	3503203	1.47	178231
30	22.665	5722669	2.41	184191
31	23.185	15337448	6.45	535651
32	23.666	13965998	5.88	926816
33	24.155	2815614	1.18	263179
34	24.360	4975496	2.09	287271
35	24.677	30074237	12.65	1853497
36	25.316	9050757	3.81	411682
37	25.712	6189308	2.60	264816
38	26.607	20990123	8.83	826640
39	26.996	4842260	2.04	310728
40	27.520	16829287	7.08	814850
41	27.942	15732031	6.62	542909

Table 6: HPLC fingerprint of extract AV016BaSu(65)01(100)g.

	Retention Time	Area	% Area	Height
1	2.384	12028	0.01	947
2	2.570	2633549	1.23	435477
3	3.507	735108	0.34	33546
4	5.043	9805	0.00	651
5	6.822	78175	0.04	3779
6	7.045	83478	0.04	5089
7	7.203	66115	0.03	5355
8	7.820	581086	0.27	16595
9	8.749	775713	0.36	25475
10	9.447	325440	0.15	11555
11	10.387	882547	0.41	20400
12	11.017	953840	0.45	28086
13	11.853	2915239	1.36	86837
14	12.467	565867	0.26	27905
15	13.088	924183	0.43	31678
16	13.160	468207	0.22	31979
17	13.766	1224906	0.57	43299
18	14.633	3503010	1.64	84712
19	15.471	1966123	0.92	60085
20	15.728	1016548	0.48	66817
21	16.317	3374916	1.58	110591
22	16.627	663866	0.31	60558
23	17.526	4303710	2.01	88642
24	17.688	741644	0.35	86630
25	18.026	1554964	0.73	90925
26	18.356	1800352	0.84	101584
27	18.852	3360093	1.57	117653
28	19.607	6271811	2.93	186866
29	19.840	4324472	2.02	201700
30	20.208	6132533	2.87	247918
31	20.820	2351992	1.10	151443
32	21.075	3520296	1.65	173642
33	23.221	22630392	10.58	235431
34	23.623	17865849	8.35	1114913
35	24.057	4708441	2.20	290733
36	24.359	4086311	1.91	276895
37	24.661	35429569	16.57	1765464
38	26.639	30653977	14.33	480095
39	26.977	5710437	2.67	393204
40	27.595	12852254	6.01	398867
41	27.911	21792801	10.19	563202

Table 7: HPLC fingerprint of extract AV016BaSu(65)04(100).

	Retention Time	Area	% Area	Height
1	1.241	10089	0.00	743
2	1.737	139563	0.04	29368
3	1.897	10508564	3.02	2120419
4	2.474	168462	0.05	20793
5	3.560	244245	0.07	13079
6	5.156	118208	0.03	5005
7	6.455	515048	0.15	12630
8	7.011	397795	0.11	16720
9	7.792	1203998	0.35	35331
10	8.408	1782066	0.51	81114
11	9.287	3153097	0.90	85209
12	10.010	3363741	0.97	117298
13	10.521	3211695	0.92	129563
14	10.919	3272829	0.94	151739
15	11.201	2910895	0.84	187760
16	11.469	2011099	0.58	187543
17	12.570	22668982	6.51	608697
18	13.072	22315645	6.40	1372355
19	13.943	15908037	4.56	466306
20	14.267	13002097	3.73	755966
21	14.499	14043370	4.03	677681
22	15.168	18500419	5.31	803270
23	15.443	8340109	2.39	561189
24	15.645	7732494	2.22	554586
25	15.999	15671975	4.50	569336
26	16.333	14724201	4.23	556235
27	16.825	11690960	3.35	545534
28	17.178	11285355	3.24	522522
29	18.532	79310513	22.76	612406
30	19.864	57401105	16.47	441406
31	25.389	2531990	0.73	47750
32	29.165	4724	0.00	733
33	29.850	337961	0.10	60806

Table 8: HPLC fingerprint of extract AV016BaSu(65)06(100).

	Retention Time	Area	% Area	Height
1	1.726	325346	0.17	47632
2	1.893	23371224	12.48	3640871
3	2.482	714853	0.38	60900
4	3.177	1875237	1.00	99118
5	3.690	485133	0.26	25416
6	4.103	679406	0.36	27133
7	4.359	352324	0.19	24825
8	4.857	272252	0.15	14769
9	5.596	1486044	0.79	49063
10	6.072	4857288	2.59	165611
11	7.074	715830	0.38	46771
12	7.247	928001	0.50	52209
13	7.925	1065970	0.57	45480
14	8.330	2132695	1.14	79394
15	9.096	2308356	1.23	80800
16	9.743	2396105	1.28	79390
17	11.179	12808928	6.84	205854
18	12.413	25125074	13.42	602504
19	13.130	1899754	1.01	160549
20	14.031	28439970	15.19	935325
21	14.646	9859006	5.27	326112
22	15.208	3351792	1.79	211034
23	15.744	11230538	6.00	540161
24	16.030	4547672	2.43	212887
25	16.651	5194350	2.77	232212
26	16.896	3162049	1.69	216298
27	17.244	5839204	3.12	208158
28	17.782	12488631	6.67	281615
29	18.729	4904610	2.62	146569
30	19.386	2196346	1.17	115299
31	19.673	8273125	4.42	99688
32	21.529	667601	0.36	50722
33	21.763	1946885	1.04	46660
34	22.802	1281629	0.68	26705
35	26.305	16760	0.01	1806
36	27.757	52103	0.03	5397

Table 9: HPLC fingerprint of extract AV016BaSu(105)08(100).

	Retention Time	Area	% Area	Height
1	2.531	251954	2.83	45170
2	3.153	91508	1.03	7618
3	3.590	53827	0.60	2846
4	5.809	95383	1.07	5176
5	6.237	84290	0.95	3408
6	7.377	237161	2.66	7025
7	7.781	208394	2.34	12462
8	7.968	105188	1.18	10471
9	8.523	386758	4.34	15438
10	8.922	255053	2.86	13182
11	9.164	103465	1.16	11779
12	9.356	146409	1.64	11519
13	9.690	263555	2.96	11664
14	10.035	197458	2.22	12951
15	10.220	188840	2.12	14479
16	10.471	188182	2.11	14258
17	10.814	371997	4.18	13832
18	11.142	112966	1.27	12605
19	11.344	318863	3.58	13928
20	11.735	280148	3.15	11849
21	12.451	609274	6.84	21533
22	13.107	585271	6.57	38319
23	13.648	210651	2.37	8967
24	14.205	227248	2.55	13908
25	14.513	2594830	29.14	208042
26	15.951	40742	0.46	3155
27	16.683	52708	0.59	5023
28	17.691	70180	0.79	6181
29	24.062	198328	2.23	4386
30	24.791	13467	0.15	1398
31	24.993	12465	0.14	996
32	25.557	2542	0.03	494
33	26.037	62889	0.71	3648
34	26.578	50279	0.56	5059
35	28.119	5587	0.06	665
36	28.951	42494	0.48	3689
37	29.707	184276	2.07	17288

Table10: HPLC fingerprint of extract AV016Fr(105)08(100).

	Retention Time	Area	% Area	Height
1	2.238	10762330	4.73	1150702
2	2.553	5628824	2.47	655829
3	2.914	2203056	0.97	125091
4	3.208	3512641	1.54	318280
5	3.357	1070868	0.47	212573
6	3.602	1899749	0.84	121669
7	4.052	3407226	1.50	136020
8	4.503	2919013	1.28	94633
9	5.093	2190949	0.96	89619
10	5.889	12371237	5.44	338787
11	6.223	10361831	4.55	315486
12	7.163	4782640	2.10	237340
13	7.700	40604091	17.85	1251589
14	9.163	11851832	5.21	268920
15	9.857	3502688	1.54	228636
16	10.125	4862586	2.14	227948
17	10.448	2710010	1.19	231182
18	10.625	2889073	1.27	233695
19	10.818	1656694	0.73	207765
20	11.218	23908811	10.51	1250520
21	12.067	40804495	17.94	1657258
22	13.556	507546	0.22	127495
23	13.790	6006453	2.64	134107
24	14.940	8534623	3.75	102587
25	17.771	960263	0.42	27876
26	18.313	820905	0.36	25276
27	20.696	13722	0.01	1129
28	23.988	77588	0.03	2021
29	24.355	9612	0.00	1658
30	25.622	204766	0.09	9215
31	25.787	301167	0.13	11470
32	26.472	205806	0.09	9613
33	27.231	486773	0.21	12154
34	28.270	5013669	2.20	94934
35	29.001	2956931	1.30	213028
36	29.400	7494149	3.29	238402

Table 11: LC/MS Fingerprint of extract AV016BaDi(28)04(20) (TIC Spectrum (Q1 +ve mode) Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
2.1775	1.06E+08	12.3131	1.97E+07	17.7412	0.1874	Valley
2.3167	3.07E+08	35.522	3.45E+07	31.1894	0.2408	Valley
2.8588	2.07E+07	2.398	2.52E+06	2.2768	0.2408	Base to Base
3.2879	1.84E+07	2.1314	2.99E+06	2.7	0.1873	Base to Base
3.7661	1.19E+07	1.3822	1.74E+06	1.5668	0.2408	Base to Base
5.0086	9.58E+06	1.1091	1.08E+06	0.9759	0.2941	Base to Base
6.1538	8.09E+06	0.9365	1.03E+06	0.9294	0.2141	Base to Base
6.8962	6.00E+06	0.6949	1.39E+06	1.2572	0.1605	Base to Base
7.7876	8.31E+06	0.9627	1.05E+06	0.9452	0.2676	Valley
7.9719	1.18E+07	1.3624	1.89E+06	1.7057	0.214	Valley
10.735	8.06E+06	0.9334	1.45E+06	1.3135	0.2408	Base to Base
12.0425	2.48E+06	0.2866	6.74E+05	0.6081	0.107	Base to Base
12.3092	1.54E+06	0.1783	8.28E+05	0.7472	0.0803	Base to Base
12.7311	4.83E+06	0.5597	9.68E+05	0.8739	0.1605	Base to Base
14.3893	3.76E+06	0.4351	9.54E+05	0.8611	0.1605	Base to Base
15.0886	3.66E+06	0.4237	9.12E+05	0.8235	0.1338	Base to Base
17.5521	1.82E+07	2.1125	2.34E+06	2.1145	0.3211	Base to Base
17.9476	1.90E+07	2.1971	1.86E+06	1.6753	0.2408	Base to Base
19.5586	1.44E+07	1.6648	1.53E+06	1.3819	0.3211	Base to Base
20.3886	4.78E+06	0.553	1.22E+06	1.1028	0.1605	Base to Base
21.5431	1.49E+07	1.7195	1.31E+06	1.1854	0.3746	Base to Base
23.6233	8.28E+06	0.9586	1.77E+06	1.5935	0.1873	Base to Base
24.5583	8.58E+06	0.9939	1.18E+06	1.0651	0.2676	Base to Base
25.1938	9.77E+06	1.131	1.24E+06	1.117	0.3211	Base to Base
25.6005	5.11E+07	5.9143	5.26E+06	4.7467	0.3478	Base to Base
25.9137	1.34E+07	1.5528	1.76E+06	1.5921	0.2141	Base to Base
26.7469	1.82E+07	2.1106	2.05E+06	1.8533	0.2943	Base to Base
27.3985	1.09E+07	1.2594	1.39E+06	1.2536	0.2675	Base to Base
27.6349	4.60E+06	0.5329	1.06E+06	0.9601	0.1338	Base to Base
28.7272	1.89E+07	2.1893	1.77E+06	1.5968	0.3478	Base to Base
28.8934	1.03E+07	1.1905	2.09E+06	1.8883	0.1338	Base to Base
29.1613	3.01E+06	0.3489	9.23E+05	0.8329	0.1338	Base to Base
29.9922	8.20E+07	9.495	5.03E+06	4.5397	0.6957	Base to Base
34.628	1.16E+07	1.3485	1.01E+06	0.9137	0.3746	Base to Base
36.8649	2.86E+06	0.3315	8.91E+05	0.804	0.107	Base to Base
37.1874	1.77E+06	0.2051	6.81E+05	0.6146	0.0802	Base to Base
37.7494	4.85E+06	0.5616	7.24E+05	0.6535	0.1873	Base to Base

Table 12: LC/MS Fingerprint of extract AV016BaDi(28)04(20) (TIC Spectrum (Q1 –ve mode)

Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
2.1961	2.85E+08	24.4168	3.04E+07	25.3851	0.2676	Valley
2.4834	4.77E+08	40.8241	2.58E+07	21.5134	0.5084	Valley
3.4214	7.47E+06	0.6387	1.44E+06	1.2067	0.1873	Base to Base
4.4188	3.03E+06	0.2592	1.25E+06	1.0442	0.107	Base to Base
7.9809	8.39E+06	0.7181	1.33E+06	1.1079	0.1873	Base to Base
9.6559	5.75E+06	0.4914	1.14E+06	0.9551	0.1873	Base to Base
11.4246	2.60E+06	0.2223	8.53E+05	0.7128	0.1338	Base to Base
12.387	6.72E+06	0.575	1.29E+06	1.0785	0.1873	Base to Base
12.7886	3.28E+07	2.8027	4.15E+06	3.4643	0.2408	Base to Base
14.0996	3.48E+06	0.2974	1.12E+06	0.9338	0.107	Base to Base
14.8736	3.87E+06	0.3307	5.13E+05	0.4282	0.1605	Base to Base
15.1974	2.00E+06	0.1714	1.08E+06	0.9018	0.0803	Base to Base
16.2118	1.20E+07	1.0275	1.28E+06	1.0691	0.2676	Base to Base
17.1494	6.42E+06	0.5495	1.26E+06	1.0499	0.214	Base to Base
17.3337	1.35E+07	1.157	2.57E+06	2.1439	0.1873	Base to Base
17.6906	3.73E+07	3.1891	2.51E+06	2.0939	0.4013	Valley
17.8922	9.86E+06	0.8435	1.99E+06	1.6607	0.1338	Valley
18.3572	4.74E+06	0.4051	2.29E+06	1.9131	0.0803	Base to Base
18.7319	9.90E+06	0.8468	1.37E+06	1.144	0.2408	Base to Base
19.6901	6.66E+06	0.5693	1.68E+06	1.4049	0.1338	Base to Base
20.1151	1.26E+07	1.082	1.77E+06	1.4749	0.2408	Base to Base
21.084	5.17E+06	0.4419	1.45E+06	1.2082	0.1338	Base to Base
21.4301	6.15E+06	0.5263	2.11E+06	1.7665	0.0803	Valley
21.5887	1.05E+07	0.8972	2.53E+06	2.1134	0.1605	Valley
23.7467	8.39E+06	0.7173	1.24E+06	1.0376	0.2675	Base to Base
23.9916	4.44E+06	0.3795	8.19E+05	0.6845	0.1338	Base to Base
24.7774	6.82E+06	0.5835	1.14E+06	0.9516	0.2943	Base to Base
25.5831	2.43E+07	2.0804	2.38E+06	1.9909	0.3211	Base to Base
25.9059	1.37E+07	1.1725	2.04E+06	1.7081	0.2675	Base to Base
26.2502	1.00E+07	0.8555	1.08E+06	0.9016	0.2408	Base to Base
26.7168	1.72E+07	1.471	2.26E+06	1.8859	0.2408	Valley
26.9142	2.14E+07	1.8288	2.20E+06	1.8395	0.2676	Valley
28.3515	3.10E+07	2.6494	3.47E+06	2.8963	0.4281	Valley
28.6747	2.53E+07	2.1615	3.53E+06	2.9518	0.3478	Valley
28.9455	4.47E+06	0.3823	1.21E+06	1.0119	0.1338	Base to Base
29.6212	7.70E+06	0.6586	7.87E+05	0.6574	0.2408	Base to Base
30.0197	1.10E+07	0.939	2.58E+06	2.1565	0.1873	Base to Base
30.4766	9.79E+06	0.8377	1.86E+06	1.552	0.1605	Base to Base

Table13: LC/MS Fingerprint of extract AV016BaDi(65)04(100) (TIC Spectrum (Q1 +ve mode)

Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.6819	5.13E+07	3.3673	1.07E+07	7.702	0.1873	Base to Base
2.1223	4.05E+07	2.6634	4.33E+06	3.1211	0.2408	Base to Base
2.4137	3.19E+08	20.9505	4.49E+07	32.3621	0.3479	Base to Base
35.37	7.19E+07	4.7236	3.26E+06	2.3544	0.6422	Base to Base
43.1584	2.30E+08	15.0758	1.02E+07	7.3293	0.7759	Base to Base
46.4236	1.38E+07	0.9069	1.66E+06	1.1971	0.3746	Base to Base
48.1523	2.43E+07	1.5953	2.55E+06	1.8369	0.3211	Base to Base
48.5731	7.28E+07	4.7831	6.02E+06	4.3446	0.3746	Base to Base
49.1844	4.59E+07	3.0145	4.22E+06	3.0466	0.4014	Base to Base
49.7221	1.53E+08	10.0401	1.05E+07	7.5644	0.6421	Base to Base
50.41	1.98E+08	13.0047	1.31E+07	9.4266	0.6422	Base to Base
53.0591	1.83E+07	1.2049	2.30E+06	1.6573	0.3478	Base to Base
53.7262	1.26E+08	8.2723	8.93E+06	6.4387	0.5619	Base to Base
55.4126	1.19E+08	7.8049	1.12E+07	8.068	0.4013	Base to Base
55.8032	3.95E+07	2.5926	4.92E+06	3.5508	0.3478	Base to Base

Table 14: LC/MS Fingerprint of extract AV016BaDi(65)04(100) (TIC Spectrum (Q1 –ve mode)

Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.5858	4.95E+08	33.2934	5.09E+07	33.5845	0.3211	Valley
1.6837	4.68E+08	31.4658	4.70E+07	30.9815	0.2675	Valley
2.281	2.71E+08	18.2632	2.23E+07	14.6901	0.3479	Valley
2.3736	1.40E+08	9.4462	2.07E+07	13.6345	0.214	Valley
42.7019	3.33E+06	0.2239	1.08E+06	0.7156	0.2141	Base to Base
51.6555	9.78E+06	0.6583	1.30E+06	0.8605	0.2408	Base to Base
54.1648	7.99E+07	5.3782	4.28E+06	2.8245	0.7224	Base to Base
55.1059	3.72E+06	0.2501	1.06E+06	0.697	0.107	Base to Base
57.6108	6.57E+06	0.4422	1.30E+06	0.855	0.214	Base to Base
58.0334	6.71E+06	0.4517	9.03E+05	0.5959	0.2141	Base to Base
58.9956	1.89E+06	0.127	8.50E+05	0.5609	0.0802	Base to Base

Table 15: LC/MS Fingerprint of extract AV016BaSu(65)09(100) (TIC Spectrum (Q1 +ve mode).

Peak List

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
0.6837	2.30E+07	1.9654	2.66E+06	2.8378	0.3211	Base to Base
1.7359	3.23E+08	27.6122	2.10E+07	22.4515	0.6155	Base to Base
2.1817	6.42E+08	54.9509	5.24E+07	55.9679	0.3212	Base to Base
46.2873	2.68E+06	0.2294	1.06E+06	1.1283	0.0802	Base to Base
51.3977	1.34E+07	1.1443	1.86E+06	1.987	0.2408	Base to Base
51.7551	1.14E+07	0.9773	2.30E+06	2.4614	0.1605	Base to Base
53.5882	4.82E+07	4.1246	4.09E+06	4.3665	0.4281	Base to Base
56.8025	3.06E+07	2.6205	2.29E+06	2.4491	0.2943	Base to Base
57.7937	7.45E+07	6.3753	5.94E+06	6.3504	0.4549	Base to Base

Table 16: LC/MS Fingerprint of extract AV016BaSu(65)01(100) (TIC Spectrum (Q1 +ve mode)

Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
0.9306	6.79E+06	0.9084	1.15E+06	1.8547	0.1873	Base to Base
1.6126	3.36E+07	4.493	1.23E+06	1.9756	0.2943	Base to Base
2.2467	5.48E+08	73.275	2.92E+07	46.9161	0.5887	Base to Base
2.6959	5.61E+06	0.7509	2.37E+06	3.8115	0.0803	Base to Base
8.7292	4.57E+06	0.6115	7.87E+05	1.2645	0.1605	Base to Base
22.1814	1.19E+07	1.5974	9.94E+05	1.5977	0.3478	Base to Base
22.538	5.22E+06	0.6979	8.29E+05	1.3331	0.1873	Base to Base
24.7315	5.89E+06	0.7887	1.07E+06	1.7263	0.1605	Base to Base
26.7098	3.29E+06	0.4408	1.01E+06	1.6234	0.107	Base to Base
28.9401	1.24E+07	1.6572	1.40E+06	2.2533	0.2676	Base to Base
29.316	3.87E+06	0.5185	1.47E+06	2.3673	0.0803	Base to Base
33.8775	4.43E+06	0.5927	1.17E+06	1.883	0.1605	Base to Base
35.7436	4.34E+06	0.5805	8.92E+05	1.4329	0.1873	Base to Base
37.7239	6.26E+06	0.8374	1.17E+06	1.8777	0.214	Base to Base
37.8343	1.99E+06	0.2659	7.72E+05	1.2402	0.107	Base to Base
39.1183	5.39E+06	0.7212	9.96E+05	1.6006	0.1606	Base to Base
44.5179	7.33E+06	0.9815	9.63E+05	1.5473	0.2676	Base to Base
49.8635	3.66E+06	0.4897	8.38E+05	1.347	0.1338	Base to Base
51.9325	3.25E+06	0.4342	1.23E+06	1.9711	0.0803	Base to Base
52.2278	3.39E+06	0.4532	1.25E+06	2.0023	0.1071	Base to Base
52.5806	1.33E+07	1.7735	1.73E+06	2.7832	0.2676	Valley
52.735	1.12E+07	1.5033	1.69E+06	2.7234	0.1873	Valley
53.8915	1.27E+07	1.706	2.22E+06	3.5732	0.2141	Base to Base
54.4726	7.86E+06	1.0524	1.51E+06	2.4278	0.1338	Base to Base
57.8304	1.22E+07	1.6307	2.45E+06	3.9445	0.1873	Base to Base
59.5907	5.79E+06	0.7754	1.11E+06	1.7919	0.1338	Base to Base
59.9224	3.46E+06	0.463	7.03E+05	1.1303	0.1338	Base to Base

Table 17: LC/MS Fingerprint of extract AV016BaSu(65)01(100) (TIC Spectrum (Q1 –ve mode)

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
0.9354	4.30E+06	1.2293	1.22E+06	2.4333	0.214	Base to Base
2.2806	1.29E+08	36.7178	1.08E+07	21.6409	0.4549	Base to Base
2.6726	4.11E+07	11.7354	1.62E+07	32.297	0.0803	Base to Base
2.9712	3.79E+07	10.8273	7.74E+06	15.4562	0.1873	Base to Base
51.3175	1.61E+06	0.4586	6.88E+05	1.3736	0.0803	Base to Base
53.8048	2.24E+07	6.3996	1.62E+06	3.2379	0.3211	Base to Base
54.3764	3.18E+07	9.0759	2.29E+06	4.5779	0.5351	Base to Base
56.2363	1.47E+07	4.2035	1.55E+06	3.1017	0.3478	Base to Base
56.9396	2.67E+06	0.7637	1.65E+06	3.2962	0.0535	Base to Base
57.4693	1.54E+07	4.4064	2.12E+06	4.2359	0.2676	Valley
57.8919	4.50E+07	12.8633	3.30E+06	6.595	0.5886	Valley
58.5708	4.62E+06	1.3192	8.79E+05	1.7544	0.2141	Base to Base

Table 18: LC/MS Fingerprint of extract AV016BaSu(65)01(100)ng (TIC Spectrum (Q1 +ve mode)

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
0.2815	2.64E+07	2.56	1.89E+06	2.0165	0.4014	Base to Base
0.6177	2.15E+07	2.0898	4.89E+06	5.2322	0.214	Base to Base
1.4595	6.17E+07	5.9816	5.53E+06	5.911	0.3478	Valley
1.6305	2.63E+07	2.5549	3.33E+06	3.5575	0.1873	Valley
2.2215	5.21E+08	50.5534	3.02E+07	32.3009	0.5352	Base to Base
4.4158	5.86E+06	0.5685	1.43E+06	1.5317	0.1605	Base to Base
10.5664	6.14E+06	0.5957	7.92E+05	0.8469	0.2408	Base to Base
23.1719	7.20E+06	0.6985	1.10E+06	1.1733	0.2141	Base to Base
26.6471	2.14E+06	0.2071	1.07E+06	1.1409	0.0803	Base to Base
28.4723	8.22E+06	0.7973	9.73E+05	1.04	0.2676	Base to Base
29.1292	3.99E+06	0.387	7.08E+05	0.7574	0.1873	Base to Base
31.5736	8.16E+06	0.7917	8.24E+05	0.8813	0.2408	Base to Base
31.6945	2.56E+06	0.2481	7.61E+05	0.8141	0.107	Base to Base
33.9826	1.00E+07	0.9715	1.28E+06	1.3722	0.2943	Base to Base
36.5048	4.65E+06	0.4512	8.70E+05	0.93	0.1338	Base to Base
37.6212	3.23E+06	0.3131	1.19E+06	1.2726	0.107	Base to Base
39.8509	3.18E+06	0.3083	7.69E+05	0.822	0.1338	Base to Base
42.0646	4.16E+06	0.4038	9.70E+05	1.0375	0.1605	Base to Base
42.4115	3.05E+06	0.2954	6.68E+05	0.7142	0.1338	Base to Base
42.5886	5.36E+06	0.5203	1.14E+06	1.2137	0.1338	Base to Base
43.7214	1.66E+06	0.1606	8.64E+05	0.9238	0.0803	Base to Base
49.6266	3.44E+06	0.3332	6.23E+05	0.6663	0.1605	Base to Base
49.8477	1.80E+06	0.175	9.30E+05	0.9949	0.0803	Base to Base
50.2725	2.67E+06	0.2585	1.22E+06	1.3044	0.0803	Base to Base
50.4625	2.57E+06	0.2489	1.25E+06	1.3321	0.0803	Base to Base
51.6971	9.21E+06	0.8936	1.33E+06	1.4175	0.3211	Base to Base
52.2644	3.77E+06	0.3661	1.08E+06	1.1526	0.1338	Base to Base
52.6743	1.05E+07	1.0181	1.27E+06	1.3617	0.3478	Base to Base
53.3229	1.80E+06	0.175	7.11E+05	0.7607	0.0803	Base to Base
54.0466	5.16E+07	5.0087	5.02E+06	5.3685	0.4281	Base to Base
54.5611	7.42E+06	0.7194	1.35E+06	1.439	0.1873	Base to Base
55.786	3.29E+06	0.3187	1.22E+06	1.304	0.107	Base to Base
56.3205	9.29E+06	0.9008	1.81E+06	1.9397	0.1605	Base to Base
56.982	1.01E+07	0.9748	1.91E+06	2.0474	0.1873	Base to Base
57.5849	6.16E+07	5.9742	5.18E+06	5.5408	0.4549	Valley
57.9722	1.07E+08	10.4	6.43E+06	6.8743	0.6421	Valley
59.2956	8.01E+06	0.7772	9.41E+05	1.0067	0.2676	Base to Base

Table 19: LC/MS Fingerprint of extract AV016BaSu(65)01(100)g (TIC Spectrum (Q1 +ve mode)

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
0.2462	4.54E+06	0.4516	1.28E+06	1.5367	0.1605	Base to Base
0.7783	2.91E+07	2.896	2.30E+06	2.7508	0.4281	Base to Base
1.4229	1.44E+08	14.3325	1.54E+07	18.4396	0.2676	Valley
1.6808	2.48E+08	24.6093	1.67E+07	20.0141	0.4281	Valley
2.2103	5.05E+08	50.1865	3.12E+07	37.4091	0.5084	Base to Base
2.6741	2.06E+07	2.0484	6.02E+06	7.2093	0.1338	Base to Base
3.5511	4.01E+06	0.3982	1.49E+06	1.7817	0.107	Base to Base
4.8158	1.41E+07	1.4004	2.22E+06	2.6599	0.2676	Base to Base
27.5026	4.41E+06	0.4385	1.10E+06	1.3192	0.2141	Base to Base
53.9115	1.52E+07	1.5124	1.73E+06	2.0716	0.3479	Base to Base
55.1432	1.04E+07	1.0317	1.45E+06	1.735	0.3478	Base to Base
56.3186	2.19E+06	0.2172	8.58E+05	1.0289	0.0803	Base to Base
57.4751	1.54E+06	0.153	7.41E+05	0.8876	0.0803	Base to Base
57.9589	3.26E+06	0.3241	9.65E+05	1.1566	0.107	Base to Base

Table 20: LC/MS Fingerprint of extract AV016BaSu(65)04(100) (TIC Spectrum (Q1 +ve mode)

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.3402	1.14E+08	17.1348	1.35E+07	13.7133	0.2676	Valley
1.6073	1.74E+07	2.6038	1.93E+06	1.9612	0.1606	Valley
2.2967	1.87E+08	28.0404	1.01E+07	10.3069	0.4817	Base to Base
4.0358	4.53E+06	0.6786	1.01E+06	1.0332	0.1338	Valley
4.1691	3.10E+06	0.4647	1.31E+06	1.3305	0.0803	Valley
4.385	5.02E+06	0.7515	1.42E+06	1.4467	0.1338	Base to Base
4.9989	5.94E+06	0.8892	1.16E+06	1.1771	0.1605	Base to Base
5.3019	3.46E+06	0.5183	1.06E+06	1.0776	0.1338	Base to Base
5.9113	2.78E+06	0.4167	1.30E+06	1.3198	0.0803	Base to Base
6.1778	4.49E+06	0.6721	7.58E+05	0.7719	0.1873	Base to Base
6.3704	2.71E+06	0.4056	7.84E+05	0.7985	0.107	Base to Base
6.8924	6.19E+06	0.9275	7.75E+05	0.7891	0.214	Base to Base
7.2702	3.77E+06	0.5654	1.17E+06	1.1885	0.107	Base to Base
7.4357	2.04E+06	0.3051	9.28E+05	0.945	0.0803	Base to Base
7.9721	4.93E+06	0.7391	9.22E+05	0.939	0.1873	Base to Base
8.8605	6.09E+06	0.9128	7.53E+05	0.7667	0.2141	Base to Base
9.4252	2.87E+06	0.4292	7.85E+05	0.7996	0.1071	Base to Base
9.789	4.10E+06	0.6138	6.12E+05	0.6234	0.2676	Base to Base
10.7492	1.94E+06	0.2902	7.58E+05	0.7718	0.0803	Base to Base
13.0858	2.60E+06	0.3897	6.98E+05	0.7105	0.1094	Valley
13.1888	3.36E+06	0.5029	7.11E+05	0.7238	0.1582	Valley
15.4704	4.27E+06	0.64	1.29E+06	1.3121	0.1338	Base to Base
16.5549	2.37E+06	0.3545	8.66E+05	0.8822	0.0802	Base to Base
17.1515	4.19E+06	0.6275	1.24E+06	1.2608	0.107	Valley
17.2577	5.17E+06	0.7752	9.38E+05	0.9557	0.1606	Valley
17.4256	5.27E+06	0.7899	8.05E+05	0.8196	0.1605	Base to Base
17.7689	5.45E+06	0.816	1.09E+06	1.1149	0.1338	Base to Base
19.3648	2.95E+06	0.4416	8.37E+05	0.8528	0.1606	Base to Base
19.5311	1.19E+06	0.1786	5.57E+05	0.5667	0.0803	Base to Base
20.302	5.74E+06	0.8605	9.81E+05	0.9987	0.1605	Base to Base
23.1422	4.84E+06	0.7254	8.61E+05	0.8767	0.1605	Base to Base
23.4055	3.51E+06	0.5262	7.76E+05	0.7901	0.1345	Base to Base
25.5012	2.20E+06	0.3299	1.04E+06	1.0621	0.0803	Base to Base
25.9266	2.59E+06	0.3882	9.14E+05	0.931	0.107	Base to Base
26.7339	3.31E+06	0.496	1.09E+06	1.1142	0.107	Base to Base
27.8535	3.91E+06	0.5853	1.02E+06	1.0382	0.1873	Base to Base
28.7851	2.65E+06	0.3968	9.95E+05	1.0129	0.0803	Valley
28.9276	5.31E+06	0.7949	8.69E+05	0.8849	0.1873	Valley
29.0503	4.46E+06	0.6684	1.13E+06	1.1496	0.1605	Valley
29.6159	2.76E+06	0.413	1.10E+06	1.1195	0.107	Base to Base
31.7858	2.66E+06	0.3987	7.40E+05	0.7538	0.1338	Valley
31.9243	2.84E+06	0.4248	5.33E+05	0.543	0.1605	Valley
32.5063	3.51E+06	0.5259	1.20E+06	1.2204	0.107	Base to Base

33.128	3.73E+06	0.558	7.30E+05	0.7436	0.2141	Base to Base
33.3651	1.37E+06	0.2059	5.85E+05	0.596	0.0803	Base to Base
34.7807	3.81E+06	0.5705	9.24E+05	0.9412	0.1338	Base to Base
35.2633	2.05E+06	0.3075	7.05E+05	0.7181	0.107	Base to Base
36.9489	5.65E+06	0.8458	9.78E+05	0.9955	0.1605	Base to Base
38.1394	2.87E+06	0.4303	8.43E+05	0.8586	0.107	Base to Base
38.6896	6.18E+06	0.9252	1.03E+06	1.0486	0.2408	Base to Base
40.5898	2.15E+06	0.3224	5.81E+05	0.5917	0.1338	Base to Base
41.5733	7.00E+06	1.0491	9.43E+05	0.96	0.2408	Base to Base
42.0674	3.46E+06	0.5184	8.98E+05	0.9143	0.1338	Base to Base
42.2718	8.14E+06	1.2191	1.05E+06	1.0705	0.2676	Base to Base
42.4327	2.55E+06	0.3818	6.87E+05	0.6998	0.107	Base to Base
44.1144	7.48E+06	1.1211	1.08E+06	1.1033	0.2943	Base to Base
46.1964	6.08E+06	0.9103	8.94E+05	0.9104	0.1873	Base to Base
46.8725	3.21E+06	0.4811	7.64E+05	0.7785	0.1338	Base to Base
47.3852	1.47E+06	0.2207	7.06E+05	0.7194	0.0803	Base to Base
50.6192	1.54E+06	0.2307	6.74E+05	0.6865	0.0803	Base to Base
50.8303	3.42E+06	0.5122	1.03E+06	1.0527	0.107	Base to Base
51.2141	6.26E+06	0.9376	1.25E+06	1.2757	0.1873	Base to Base
51.531	8.74E+06	1.3088	1.14E+06	1.1594	0.2408	Valley
51.6971	7.62E+06	1.1419	1.49E+06	1.5193	0.1606	Valley
51.9366	3.58E+06	0.5358	1.13E+06	1.146	0.1338	Base to Base
52.5223	4.27E+06	0.6399	1.01E+06	1.0277	0.1338	Base to Base
53.2728	4.39E+06	0.6573	7.78E+05	0.7923	0.1606	Base to Base
53.8949	1.32E+07	1.9795	1.63E+06	1.6568	0.3211	Base to Base
54.4009	4.66E+06	0.6975	1.34E+06	1.3633	0.107	Valley
54.5073	1.82E+07	2.7254	1.63E+06	1.6624	0.2676	Valley
55.9786	4.07E+06	0.6101	9.34E+05	0.9515	0.1338	Base to Base
56.3475	1.33E+07	1.9928	1.13E+06	1.1551	0.3211	Valley
56.5847	4.61E+06	0.6907	8.48E+05	0.864	0.1605	Valley
56.8275	9.26E+06	1.3875	1.22E+06	1.243	0.1873	Valley
57.0145	4.88E+06	0.7309	9.98E+05	1.016	0.1606	Valley
57.1188	2.45E+06	0.3669	7.75E+05	0.7891	0.107	Base to Base
57.7155	6.35E+06	0.952	1.11E+06	1.1327	0.1606	Valley
57.8152	9.52E+06	1.4268	1.38E+06	1.4031	0.2141	Valley

Table 21: LC/MS Fingerprint of extract AV016BaSu(65)06(100) (TIC Spectrum (Q1 +ve mode)

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.3425	1.34E+08	15.6624	2.35E+07	34.2569	0.2141	Base to Base
2.3068	6.22E+08	72.7416	3.24E+07	47.2835	0.5886	Base to Base
4.4357	5.31E+06	0.6201	1.63E+06	2.3849	0.1605	Base to Base
5.2161	2.96E+06	0.3463	1.29E+06	1.8752	0.107	Base to Base
5.6491	9.55E+06	1.1164	1.58E+06	2.3015	0.1873	Base to Base
6.6417	9.07E+06	1.0594	2.04E+06	2.9753	0.1605	Base to Base
17.6827	1.27E+07	1.4888	1.34E+06	1.954	0.4281	Base to Base
54.1984	5.00E+07	5.8447	3.32E+06	4.8386	0.4548	Base to Base
57.3586	9.59E+06	1.1203	1.46E+06	2.1301	0.1873	Base to Base

Table 22: LC/MS Fingerprint of extract AV016BaSu(105)08(100) (TIC Spectrum (Q1 +ve mode)

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
2.0569	2.14E+08	27.1727	2.15E+07	36.3993	0.2677	Base to Base
3.0165	5.13E+08	65.1587	2.56E+07	43.3466	0.7224	Base to Base
4.4937	1.06E+07	1.3463	2.07E+06	3.4978	0.1873	Base to Base
4.8116	9.88E+06	1.255	1.36E+06	2.304	0.2141	Base to Base
7.1128	9.55E+06	1.2133	1.79E+06	3.0334	0.1606	Base to Base
20.2582	6.97E+06	0.8856	1.05E+06	1.7785	0.1873	Base to Base
21.0285	2.37E+06	0.3008	8.07E+05	1.3647	0.107	Base to Base
21.9949	5.91E+06	0.751	1.41E+06	2.3921	0.1605	Base to Base
26.1397	4.67E+06	0.5933	1.60E+06	2.7139	0.1338	Base to Base
28.479	1.04E+07	1.3233	1.87E+06	3.1697	0.1873	Base to Base

Table 23: LC/MS Fingerprint of extract AV016FrDi(65)04(100) (TIC Spectrum (Q1 +ve mode)

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.6028	1.52E+09	29.4877	6.12E+07	29.3519	0.6423	Valley
2.2961	3.50E+09	68.0186	1.30E+08	62.3426	0.99	Valley
4.0708	3.33E+06	0.0647	1.35E+06	0.6474	0.107	Base to Base
4.8702	1.71E+07	0.3327	2.48E+06	1.1882	0.2675	Base to Base
5.4024	9.12E+06	0.1773	2.48E+06	1.1914	0.1338	Base to Base
6.2587	4.84E+06	0.0941	1.53E+06	0.7348	0.107	Base to Base
47.0653	5.85E+07	1.1379	2.31E+06	1.1097	0.6689	Base to Base
48.8324	4.52E+06	0.0879	1.54E+06	0.7369	0.1338	Base to Base
49.5832	5.13E+06	0.0998	1.18E+06	0.5668	0.1338	Base to Base
51.2074	3.34E+06	0.065	1.38E+06	0.6601	0.0802	Base to Base
51.9303	6.03E+06	0.1171	1.52E+06	0.7281	0.1338	Base to Base
57.0683	1.63E+07	0.3173	1.55E+06	0.7421	0.3211	Base to Base

Table 24: LC/MS Fingerprint of extract AV016FrSu(105)08(100) (TIC Spectrum (Q1 +ve mode)

Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.9597	2.64E+08	29.489	2.96E+07	45.0514	0.2677	Base to Base
3.1068	6.21E+08	69.4326	3.28E+07	49.9315	0.6689	Base to Base
5.6942	4.34E+06	0.485	1.88E+06	2.8549	0.0802	Base to Base
22.1023	5.31E+06	0.5934	1.42E+06	2.1622	0.1605	Base to Base

Table 25. IC_{50} values of alpha-glucosidase inhibition activity of extracts from different *T. arjuna* plant parts

Plant Part	Extract-ID	IC_{50} (µg/ml)
1. Bark	AV016BaDi(65)04(100)	13.2
2. Bark	AV016BaDi(28)04(20)	14.2
3. Bark	AV016BaSu(65)01(100)g	14.3
4. Bark	AV016BaSu(65)01(100)ng	15.0
5. Bark	AV016BaSu(65)04(100)	16.5
6. Bark	AV016BaSu(65)06(100)	24.0
7. Bark	AV016BaSu(105)08(100)	31.5
8. Bark	AV016BaSu(65)09(100)	42.7
9. Fruits	AV016FrDi(65)04(100)	16.3
10. Fruits	AV016FrSu(105)08(100)	31.5
11. Acarbose (Standard)		80.7

Table 26: The effect of insulin and *T. arjuna* extracts on glucose up-take in 3T3-L1 adipocytes.

Set	Treatment	cpm			Average	SD value	Stimulation Index
		Set I	Set II	Set III			
1	cells alone	314.6	390	1200.8	635.13	491.33	
2	cells + Insulin (10 nM)	2185.8	1705.4	3010.7	2300.63	660.18	2.6
3	cells + Insulin (25 nM)	4806.8	1399.7	996.7	2401.07	2093.15	2.8
4	cells + Insulin (100 nM)	2906.5	3776.6	2215.6	2966.23	782.21	3.7
5	cells + AV016BaDi(65)04(100) (**Ter1**)	1946.5	2570.2	3106.7	2541.13	580.65	3.0
6	cells + Insulin (10 nM) + AV016BaDi(65)04(100) (**Ter1**)	2178.5	1873	1667.3	1906.27	257.22	2.0

Stimulation index ={mean of sample – mean of control (cells alone) / mean of control (cells alone)}

Table 27: Anti-obesity potential of *Terminalia arjuna* extracts.

Treatment	Reading OD520 nm	Average	Final Reading (Reading - Well Blank)	% Inhibition
Well Blank (- cells)	0.887		0	-
Cells Alone	2.883	2.883	1.996	0.0
	2.883			
Cells + DMSO	2.786	2.786	1.899	0.0
	2.786			
Cells + AV016BaDi(65)04(100)	1.428	1.344	0.457	76.3
	1.26			
Cells + AV016BaSu(65)09(100)	2.105	1.616	0.729	61.9
	1.127			
Cells + AV016BaSu(65)01(100)ng	1.161	1.146	0.259	86.8
	1.131			
Cells + AV016BaSu(65)01(100)g	2.23	1.6805	0.7935	58.5
	1.131			
Cells + AV016BaSu(65)04(100)	1.813	1.8535	0.9665	49.4
	1.894			
Cells + AV016BaSu(65)06(100)	1.905	1.693	0.806	57.9
	1.481			
Cells + AV016BaDi(28)04(20)	1.832	1.6	0.713	64.3
	1.368			
Cells + AV016FrDi(65)04(100)	1.941	1.9895	1.1025	41.9
	2.038			
Cells + +ve Control	0.774	0.807	-0.08	104.0
	0.84			

% Inhibition = {(Mean of Control –Mean of Sample)/Mean of Control}*100

Table 28. Effect of *Terminalia arjuna* extracts on lipid levels in CHO-K1 cells.

Sr. No.	Treatment	OD_{520nm}	% Inhibition w.r.t control
1.	Cells alone + DMSO	1.206	0
2.	Cells + AV016BaSu(65)09(100)	0.944	22
3.	Cells + AV016BaSu(65)01(100)	0.964	21
4.	Cells + AV016BaDi(65)04(100)	0.844	31
5.	Cells + AV016BaDi(28)04(20)	0.873	28

% Inhibition ={(Control – Extract treated / Control) * 100 }

Table 29. Effect of 20% ethanol extract from *T. arjuna* bark AV016BaDi(28)04(20) on HMG-CoA reductase activity.

Time (mins)	Without Inhibitor Reading	With Inhibitor Reading
0	1.3465	1.7372
1	1.3103	1.726
2	1.2891	1.726
3	1.2768	1.726
4	1.2689	1.7042
5	1.2534	1.7042
Substrate Blank	0.0277	0.0307
	0	
Final Reading	0.0654	0.0046
% Inhibition	0	93.0

% Inhibition ={(Control – Extract treated / Control) * 100 }

Table 30. Effect of direct 100% ethanol extract from *T. arjuna* bark AV016BaDi(65)04(100) on HMG-CoA reductase activity.

Time (mins)	Without Inhibitor Reading	With Inhibitor Reading
0	1.162	1.7815
1	1.1125	1.7637
2	1.1048	1.7637
3	1.0828	1.7637
4	1.0756	1.746
5	1.0652	1.7467
Substrate Blank	0.023	0.0212
	0	
Final Reading	0.0738	0.0272
% Inhibition	0	63.1

% Inhibition ={(Control – Extract treated / Control) * 100 }

Table 31: Effect of *Terminalia arjuna* extracts on cell viability of HepG2 cells.

Treatment	No. of live cells	No. of dead cells
Cells	85	20
Cells + DMSO	82	11
Cells + Ascorbic acid	66.5	18.5
Cells + AV016BaSu(65)09(100)	27	7
Cells + AV016BaSu(65)01(100)	55	2
Cells + AV016BaSu(65)04(100)	11	1