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Research Article

EXPLORING THE ANTIDIABETIC MECHANISM OF *EMBLICA OFFICINALIS* AND *MANGIFERA INDICA* EMPLOYING CELL BASEDASSAY

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ABSTRACT

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine. The term of medicinal plants includes some numerous types of herbal plants used in herbalism and some of these plants have a medicinal activity. Use part of the plant like fruit, seed, stem, bark, flower, leaves, bark or a root, as well as a non-woody plant. The herbal plant used in treatment as cancer, Hyperglycaemic, heart desisses, gastric, and they mostly plant is also used in diabetes. Diabetes is a group of metabolic disorder they can be affected increase in blood glucose level and decrease an insulin production and secretion. Traditional plant treatments have been used throughout the world for the therapy of diabetes. This article presents a resurch on some reported antidiabetic medicinal plants and plant based marketed and herbal formulations and exploring the antidiabetic mechanism of *emblica officinalis* and *mangifera indica* employing cell based assay.

INTRODUCTION

Medicinal Plant

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. The term of medicinal plants includes some various types of plants used in herbalism and some of these plants have a medicinal activity. The word "herb" has been derived from the Latin word, *"herba"* and an old French word *"herbe"*. Now days, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaves, bark or a root, as well as a non-woody plant. These medicinal plants consider as some rich resources of ingredients which can be used in drug development and synthesis. Besides that, these plants play a critical role in the development of human cultures around the whole world. Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies. Chevallier A *et.,al* 1996.

Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. Sofowora A *et.,al* 1982. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes. Medicinal plants are considered as some rich resources of ingredients which can be used in drug development pharmacopoeia, non-pharmacopoeia or synthetic drugs. In the treatment of various diseases of humans and animals, the medicinal plants were used from the ancient times and it is an efficient source to treat the diseases. Many plant extracts have different activities, such as antidiabetic antimicrobial, antioxidant, anticancer, etc. The medicinal plants also have Antidiabetic activity. Many herbal drug preparations and plants used in the treatment of diabetes mellitus. Kirtikar KR *et.,al* 1918.

Diabetes

Diabetes is a group of metabolic disorder they can be affected increase in blood glucose level and decrease an insulin production and secretion. Insulin is hormone that can convert food in to the energy and the insulin is released from B cell of pancreas. This results in elevated blood glucose levels, a

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condition known as hyperglycemia. If blood glucose levels remain high over a long period of time, this can result in long-term damage of organs such as the kidneys, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to Death. Rahmatullah M *et.,al* 2011 Jul 1.

For Normal Person without Diabetes

Before meal of blood glucose- 70-99mg/dl
After meal less then- 130mg/dl

For The Person with Diabetes

Before meal of blood glucose- 80-130mg/dl
After meal less then- 180mg/dl

Two Major Types of Diabetes Mellitus

Type 1

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the pancreatic islets, leading to insulin deficiency. Destruction of beta cell by auto immune attack by T cell is the primary cause of type1 diabetes it is also called insulin dependent diabetes mellitusand they not produce body does any Insulin. It regularly occurs in children and young adults' persons. Eisenbarth GS. *et.,al*1986 May 22.

Type 2

Type 2 diabetes is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion and production. When insulin produced by beta cell is resist by cell, it is also called non-insulin dependent diabetes mellitus in type 2 diabetes cells not responded to the insulin produced by beta cell. In the body does not produce enough or use of secretion and production of insulin is the most common form of the diabetes mellitus disorder, accounting for 85–95% of diabetes mellitus. Pradhan AD *et.,al* 2001 Jul 18.

Basis of Diabetes Mellitus Treatment

- Patient education concerning the disease.
- Physical exercise.
- Diet and proper sleeping.
- Hypoglycaemic agents.

It is a very common chronic metabolic disorder; diabetes is fetching the health of mankind along with cardiovascular, cancer, diabetes and cerebrovascular diseases because of its high prevalence, mortality and morbidity. Therefore, once diagnosed, it is well regulated by means of various therapeutically effective drugs. Besides, the therapy based on chemotherapeutic agents, the present century has progressed towards brain. Thus, herbal medical plants have an ever-emerging role to play in treatment or management of lifelong prolonging diseases like diabetes mellitus, cancer and especially in developing countries where resources are meagre. Herbal medicine the treating the related disorders such as polydipsia, polyuria, diabetes, glycosuria, sulfonylureas, cancer and glinides etc. along with curing the chronic metabolic disorders such as diabetes mellitus. Alberti KG, *et.,al* 1998 Jul;15.

Ethology

Type 1 Diabetes

- Antibodies to islet cells and insulin are present at diagnosis.
- Caused by the immune destruction of the beta cells of the pancreas.
- Insulin secretion gradually diminishes.
- Insulin by injection is necessary for survival.
- May present at any age, but most common in childhood and adolescence.
- Contributing factors:
  - Environmental triggers
  - Genetic predisposition

Type 2 Diabetes

- Insulin secretion decreases with gradual beta cell failure.
- Caused by insulin resistance in the liver and skeletal muscle, increased glucoseproduction in the liver, over production of free fatty acids by fat cells and relativeinsulin deficiency.
- Reductions in blood glucose levels often can be achieved with changes in foodintake andphysical activity patterns. Oral medication and/or insulin injections areeventually required.
- Contributing factors:
- Age (onset of puberty is associated with increased insulin resistance) Lack of physical activity.
- Racial/ethnic background (African American, Native American, Hispanic and Asian/Pacific Islander).
- Genetic predisposition.
- Conditions associated with insulin resistance, (e.g.,polycystic ovary syndrome).
- Obesity.

Diabetes Complications

Acute complications

- Diabetic Ketoacidosis (DKA)
- Hyperglycaemic Hyperosmolar Non-Ketotic Syndrome (HHNS)

Chronic complications

- Kidney Damage: Nephropathy
- Eye: Diabetic retinopathy, cataracts, glaucoma
- Nerve Damage: Neuropathy
- Cardiovascular: Heart disease, peripheral vascular disease, stroke

Chronic complications are responsible for most illness and death associated with diabetes. Chronic complications usually appear after several years of elevated blood sugars (hyperglycaemia). Since patients with type 2 diabetes may have elevated blood sugars for several years before being diagnosed, these patients may have signs of complications at the time of diagnosis.

Drug Used As Antidiabetic

Antidiabetic drugs are medicines developed to control blood glucose levels and stabilise between people with diabetes mellites. Antidiabetic drugs are generally used to manage diabetes. There are different types of antidiabetic drug including: Insulin.

Marketed drugs	Mechanism	Side effect
Glimepiride	Stimulates insulin release from pancreas	hypoglycaemia and weight gain
Rosiglitazone	Lowers insulin resistance in peripheral tissue	Weight gain, fluid retention andheart failure
Nate glinide	Increases insulin secretion in the pancreas	Mild gastrointestinal complaints and weight gain
Metformin	Reduce hepatic glucose production, increases Peripheral glucose utilisation and insulin sensitivity	Gastrointestinal complaints and lactic acidosis
Bromocriptine	Resets abnormally elevated	Fatigue, nausea, vomiting
glipizide	Blocking K channel and causes membrane, depolarization which result Ca channel opening and release insulin	Weight gain, nausea, vomiting, lactic acidosis or fatigue.
Vildagliptin	DPP-4 inhibitors	Few gastrointestinal disturbance

Medicinal Plants Used As Antidiabetic Drug

Botanical name	Common name	Part used
Zingiber officinale	Ginger	Bulb
Mangifera indica	Mango tree	Leaves, Stem, Bark, Fruit
Emblica officinalis	Amla	Fruit
Aloe vera	Barbados aloe	Leaves
Piper betle	Pan	Leaves
Musa paradisiaca	Banana	Fruit
Aegle marmelos	Golden apple	Leaves, Seed, Fruit
Artocarpus heterophyllus	Jackfruit	Fruit, Leaves

Medicinal Plants Scientifically Documented For Management of Diabetes

Zingiber officinale

Roscoe belonging to the Family Zingiberaceae is a perennial herb with thick tuberous rhizomes. Zingiberene as the main component. Other components include β-sesquiphellandrene bisabolene, gallic acid, polyphenol, carbohydrate, flavonoids and farnesene which are also sesquiterpenes, lipids, and ascorbic acid also produced from gingerols during this process, and it is less pungent and has a sweet aroma. Ginger is a minor chemical irritant, and has a sialagogue action, stimulating the production of saliva. Mature ginger roots are fibrous and nearly dry. Medically ginger is used as a stimulant, purgative, carminative, and is used frequently for dyspepsia and colic. It is also used to disguise the taste of medicines. ginger has been used for stomach upset, gastric disorder, motion sickness, nausea, and vomiting. The FDA has not reviewed this product for safety or effectiveness. Take this product by mouth as directed; burning feeling in mouth and throat, diarrhea, abdominal pain, or heartburn may occur. Ali BH, Blunden G, *et., al* 2008 Feb 1.

Zingiber Officinale

Emblica Officinalis

Emblica officinalis which is most common food from ayurvedic medicine. Ayurveda is an ancient system of holistic health care. Its name is derivative from two words first is ayur means life and second are Veda means science. Emblica officinalis which is contains vitamin C, ascorbic acid, gallic acid, ellagic acid, carbohydrate and polyphenol contents. It helps in absorption of food, protect the liver function, balances stomach acid, nourishes the brain and mental functioning, strengthens the lungs, support the heart, regulates elimination, helps the urinary problem, enhance fertility, supports healthier hair, and also it is good for skin acts as a body flushes out toxins, increases vitality, coolant, strengthens the eyes, improves muscle tone and it acts as an antioxidant, polyphenol, lipid, protein, carbohydrate. Ankamwar B*, et., al* 2005 Oct 1.

Emblica Officinalis

Aloe Vera

It grows in arid climates and is widely distributed in Africa, India and other arid areas. *Aloe vera* gel at 200 mg/kg 1 possesses significant antidiabetic, cardio protective activity, reduces the increased TBARS, maintains the Superoxide dismutase and Catalase activity up to the normal level and increases reduced glutathione by four times in diabetic rats. The leaves pulp extract showed hypoglycemic activity on IDDM and NIDDM rats, the effectiveness being enhanced for type II diabetes in comparison with glibenclamide. Chandran SP, *et.,al* 2006 Jan 1.

Aloe Vera

Aegle Marmelos

A species of tree native to India, it is present throughout Southeast Asia as a naturalized species. A significant decrease in liver glycogen of diabetic rats is reversed to almost the normal level by the leaves extract and it also decreases the blood urea and serum cholesterol. A similar effect is seen with insulin treatment and the results indicate that the active principle in *Aegle marmelos* leaves extract has similar hypoglycemic activity to insulin treatment. Rana BK, *et.,al* 1997 jan 1.

Aegle marmelos

Mangifera Indica

The aqueous extract produces reduction of blood glucose level in normoglycemic and glucose-induced hyperglycaemia but does not have any effect on streptozotocin-induced diabetic mice under the same conditions when compared with that of an oral dose of chlorpropamide. The result indicates that the aqueous extract of the leaves of *Mangiferaindica* possess hypoglycaemic activity. Aderibigbe AO, *et.,al* 1999 sep 1.

Mangifera Indica

Some Benefits of Ayurvedic Formulation Medicine

Herbal drugs, as defined by regulatory measures constitute only those traditional herbal medicines, which primarily use herbal medicinal plant preparations for therapy. WHO has recently defined traditional herbal medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, almost for several hundreds of years, Mostly herbal drugs are well tolerated by the patient, having fewer unintended consequences and fewer side effects than traditional medicine, and may be safer to use. Cost of herbal drugs is much less than prescription medications. Research, testing, and marketing add considerably to the cost of prescription medicines. Herbs tend to be inexpensive compared to drugs. Herbs are available without a prescription. Simple herbs, such as peppermint and chamomile, can be cultivated at home. Herbal drugs are more effective for long-standing health complaints that don't respond well to traditional medicine. The use of herbal medicinal plants is the most common form of traditional medication worldwide. Regulation of herbal medicines is a key means of ensuring safety, efficacy and quality of herbal medicinal products. Martínez G, *et.,al* 2000 sep 14.

Mechanism of Action of Herbal Antidiabetics

- Inhibition in renal glucose reabsorption.
- Reduction in insulin resistance.
- Regenerating and repairing pancreatic beta cells.
- Increasing the size and number of cells in the islets of Langerhans.
- Stimulation of glycogenesis and hepatic glycolysis.
- Stimulation of insulin secretion and production.
- Improvement in digestion along with reduction in blood sugar, urea and cardiac.
- Protective effect on the destruction of the beta cells.
- Prevention of pathological conversion of starch to glucose.
- Cortisol lowering activities.
- Inhibition of β -galactosidase and α–glucosidase.
- Inhibition of alpha-amylase.
- Adrenomimeticism, pancreatic beta cell potassium channel blocking, and calcium channel opining, cAMP (2nd messenger) stimulation.
- Stimulation of insulin secretion and production from beta cells of islets or inhibition of insulin degradative processes.

Life Style for Patient

Some of the home and herbal remedies prescribed by Ayurveda are described below.

- Include turmeric, coriander, ginger, Beal, aloe Vera, fenugreek and cinnamon diets.
- Avoid white bread, rice, potatoes, sweet and sugary foods.
- Regular exercise. Walk for at least 40 minutes a day.
- Increase intake of vegetables like spinach, cucumber, tomatoes, onion, sprouts, beans, garlic, jackfruit, bitter melon etc.
- Avoid fried, oily and starchy foodstuffs.

- Avoid coffee, sugar, refined flour and alcohol Eat smaller meals (low fat diet) five to six times a day instead of having three large meals.
- Refrain from taking stress and tensions.
- Avoid red meat and excessive salt in your meals. Fish and soy can be taken due to their good protein value, vitamins, carbohydrate.

Plant Profile

Emblica Officinalis

Embilica officinalis which is most common food from ayurvedic medicine. It can be used in herbal medicinal medicine. Embilica officinalis which is contain ascorbic acid, gallic acid, ellagic acid, polyphenol (free polyphenol and bound polyphenol), vitamin C, lipid, protein, and carbohydrate etc. it can be help protect the liver function, balances stomach acid, absorption of food, strengthens the lungs, support the heart, helps the urinary problem, enhance fertility, and it can also good for skin acts as a body flushes out toxins, increases vitality, coolant, strengthens the eyes, improves muscle tone and it acts as an antioxidant.

Taxonomical Classification

- Latin name- *Emblica officinalis.*
- Other names- Amla, Indian goosebery, Aamalaki, Nellaka, Anwla, Ambla, Nelli, kayi, Emblica.
- Family- Euphorbiaceae.
- Species- phyllanthus emblica L.
- Division- Angiosperms.
- Class- Dicots.
- Kingdom- Plantae.
- Parts used- Fruit.
- Active constituent- Polyphenols, Flavonoids, Gallic acid, Vitamin C, Tanin, Pectin, Ascorbic acid, Ellagic acid, etc.

Pharmacological Investigation

Pharmacologyical Activity

Various crude extracts of this plant have shown activities including antiulcer, antidiabetic, ntihyperlipidaemic, antioxidant, anticancer, antimicrobial, radio protective, antiinflammatory, antipyretic, analgesic and antispermatogenic effects on various animal models.

Hypoglycemic Activity

The hypoglycaemic effect of the water extract of the fruits of *Emblica officinalis* was examined in streptozotocin-induced diabetic Wistar rats. Oral administration of the water extract (125 and 250 mg kg−1) twice a day for 4 weeks resulted in significant reductions in blood gluose.

Traditional Uses

Paste - it is helpful in resolving pains and inflammation.

Powder –it helps in curbing the digestive related problems like indigestion and diarrhea. It also promotes appetite. It nourishes heart and helps in clotting of blood in case of injuries and hemorrhages. It helps in relieving from inflammation of the internal organs especially of uterus. It is a good nerving tonic.

Juice - It is very effective in diabetes and urinary problems. It is also helpful in respiratory disorders like cough, cold and asthma. It is effective in gonorrhea, leucorrhea and menstrual disturbances. It also helps in increasing gensral body strength. It is also effective in heart related problems.

Mangifera Indica

Mangifera indica, commonly known as mango, is a species of flowering plant in the family Anacardiaceous. The epidermis was covered with a single layer of cuticle. The upper and lower epidermis is single layered and in between single layer of palisade tissue and spongy parenchyma. Epidermis cell thick and irregular in shape.*Mangifera indicia* is a largeevergreen tree, and it can be used is commonly in folk medicine for a wide variety of remedies. The root, bark, leaves, flowers, unripe and ripe fruit are acrid, cooling and astringent to the bowels and have been employed to cure “vata”, “pitta”, and “kapha”.

Taxonomical Classification

- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order: Sapindales
- Family: Anacardiaceae
- Genus: Mangifera
- Species: *Mangifera indica*
- Part used: fruit, leave, bark etc.
- Active constituents: polyphenols, flavonoids, triterpenoids. Mangiferin a xanthine glycoside major bio-active constituent, isomangiferin, tannins & Gallic acid derivatives.

Pharmacological Investigation

Antidiabetic

Find out the Single oral administration of a dose of 250 mg/ kg body weight produces a potent and strong hypoglycemic effect in Type-2 diabetes on rats.

Anti-Hemorrhagic

Evaluated the Anti-hemorrhagic and antidermonecrotic activities of mango extract against snake venoms.

Anti-Ulcer

The antiulcer potential of the petroleum ether and ethanol extracts of leaves of mango was evaluate against in vivo aspirin-induced gastric ulcer.

Anticancer

Compared the anticancer properties of polyphenolic extracts from several mango varieties in cancer lines, including Molt-4 leukemia, A-549 lung, MDA-MB-231 breast, LnCap prostate, SW-480 colon cancer cells and non-cancer colon cell line CCD-18Co. Determined that ethanol extract had significant cytotoxicity to HeLa cells and the bioactive fraction from the crude extract had antiproliferative effects with an IC50 value of<10µg/ml.

Analgesic and Antipyretic

The stem bark extract of MI was evaluated for antipyretic activity in mice. A reduction in yeast-induced hyperpyrexia was also produced by the extract.

Anti-Diarrheal

The potential anti-diarrheal activity of methanolic and aqueous extracts of seeds of *Mangifera indica* was studied.

Antibacterial

The aqueous and ethanol extract of leaves and stems of mango at 50 and 25 mg/mL has been
found sufficient activity against bacteria; *Staphylococcus aureus*, *Streptococcus pyogenes, Streptococcus pneumoniae, Pseudomonas aeruginosa, Candida albicans, Enterococcus Faecalis.*

Laxative

Mangiferin significantly accelerated gastro intestinal tract (GIT) movement at oral doses of 30 mg/kg and 100 mg/kg by 89% and 93%, respectively.

MATERIAL AND METHOD

List of Chemicals

Chemicals	Manufacturer/ Supplier
Streptomycin	Ambistryn-S from AHPL
Ethanol	SD Fine chemicals
DMEM	SD Fine chemicals
EDTA	SD Fine chemicals
NaCl	LoBA Chemie
Kcl	LoBA Chemie
KH2Po4	LoBA Chemie
Na2HPo4	LoBA Chemie
MTT Dye	Himedia , Mumbai

List of Instruments

Instruments	Manufacturer/ supplier
Elisa multiple plate reader	Jupitar UV
Phase contrast microscope	Leica
Sonicator	PCI Mumbai
Magnetic Stirrer	Suntek
Incubator	BINDER/Germany
Water Bath	Labtech
Hemocycomiter	Labtech

Method

Collection of Plant Material

The *emblica officinalis* pulp and *mangifera indica*leaves were collected from the Dhamtari and Raigarh. Districts in 11 Nov. 2016

Drying

Drying was done in shade in closed room for 15 days at room temperature For the Spread out wet herbs until the water evaporates before using extraction method.

Size Reduction

The size reduction of *Magifera indica* and *emblica officinalis* pulp was done by Hand mill Grinder in Laboratory.

Preparation of Extraction

- The preparation of the aqueous fruit extract, 100 g of the dried amla fruits were ground in an electrical grinder and soaked in 500 ml distilled water.
- The mixture was left for 24 h with a magnetic stirrer at room temperature.
- 24 hours later, the mixture was strained out using a fine sieve and the crude extract air evaporated for 3 days.

Extraction of Leaves

The powdered plant material (100 g) was packed into a soxhlet apparatus (1 l) and extracted up to 4h with petroleum ether (60–80 °C) for defatting. It was then extracted with methanol for further 4 h. The extract was filtered, and the solvent was evaporated under reduced pressure using a rotary vacuum evaporator, which was kept in a desiccator for further use.

Extraction of Leaves Done By The Soxhlet Extractor

1. Firstly defatting leaves is done by petroleum ether for removal of fatty material in leaves. Further it will be extracted with ethanol.
2. The weight of a blank thimble filter (22 90 mm) (Advantec, Japan) and a blank round bottom extraction flask were weighed before and after placing 3 grams of sample into thimble.
3. The cotton is then placed into the thimble and its weight along with sample was recorded again. The purpose of using cotton was to ensure the presence of samples inside the thimble during the experiment.
4. The Soxhlet extraction processes using ethanol (99.8% assay) as extraction solvents were carried out to compare the percentage of extraction and their quality of extraction between the two solvents.
5. 350 mL of solvent is poured into the round bottom extraction flask, weighed and placed on the heating mantle.
6. After this, the thimble containing the sample was placed into the extraction chamber. Lastly, the condenser was placed on top of the extraction flask and all these parts were fixed vertically.
7. The extraction was carried out for four different pre-set intervals of times, which is 3hours, 6 hours, 9 hours and 12 hours respectively.

Yield of Extract

The yield of extract will be determined. The extraction yield is a measure of the solvent efficiency to extract specific components from the original material. Evaporate the extract to dryness by the rotary evaporator under vacuum at 40°C. Lyophilize the dried product in the freeze-dryer to obtain a freeze-dried extract. Weigh the freeze-dried extract. Calculate the extraction yield as the percentage of the weight of the crude extract to the raw material. Extraction yield (%) = (weight of the freeze-dried extract x 100) / (weight of the original sample).Mezzomo N, *et.,al* 2010 jul 1.

Phytochemical Screening of Emblica Officinalis and Magifera Indica

Detection of carbohydrates

Extracts were dissolved separately and were tested with Molisch reagent, Fehling's reagent, test for detection of carbohydrates

Benedict's Test

The presence of carbohydrates is confirmed by Benedict's Test. 0.5ml of Benedict's reagent was added to 0.5 ml of extract and the mixture was heated on a boiling water bath for 2 mins.

Iodine Test

The plant extract was treated with few drops of iodine solution (0.1M potassium iodide) to confirm the presence of carbohydrate (starch) in it. The formation of blue-black colour confirms the presence of starch.

Detection of Glycosides

Glycosides were confirmed by subjecting the acid hydrolysed extract to Legal's test, Borntrager test and Libermann-Burchard's test

Keller-Kilani Test

Keller –Kilani test was used to identify the presence of glycosides in the plant extract. Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2% solution of FeCl3.The mixture was then poured into another test tube containing 2 ml of concentrated H2SO4. The presence of cardiac glycosides is indicated by the formation of a brown ring at the interphase.

Borntrager's Test

A few milliliters of hydrolysate treated with chloroform, decanted off chloroform layer, added equal quantity of dilute ammonium solution. A pink colour is produced in ammonical layer in presence of glycosides.

Libermann-burchard's Test

Hydrolysate treated with chloroform, to this added Libermann-burchard reagent; a colour change result in presence of glycosides.

Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a yellow cream precipitate indicates the presence of Alkaloids.

Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in potassium iodide) Formation of brown/reddish brown precipitate indicates the presence of alkaloids

Detection of Flavonoids

Alkaline Reagent Test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Lead Acetate Test

Extracts were treated with few drops of lead acetate solution. Formation of a yellow colour precipitate indicates the presence of flavonoids.

Detection of Steroids and Terpenoids

Salkowski Test

The crude extract (about 100 mg) was separately shaken with chloroform (2 mL) followed by the addition of concentrated H2SO4 (2 mL) along the side of the test tube, a reddish brown coloration of the interface indicates the presence of terpenoid.

Detection of Phenols

Ferric Chloride Test

Extracts were treated with few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Detection of Resins

Acetone-Water Test

Extracts were treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins.

Detection of Phenolic Compounds & Tannins

All the dry extracts were dissolved in minimum amount of water, filtered and subject to Ferric chloride test, Gelatin test. Filtrate on addition of few drips of ferric chloride produce a violet colour precipitate in presence of tannins. A white precipitate is resulted in presence of tannins on addition of 1ml 1% solution of gelatin to the filtrate. Nascimento GG, *et.,al* 2000 oct 31.

Preparation of Buffer Solution

There are many different ways to prepare PBS solutions (one of them is DPBS, or Dulbecco's phosphate-buffered saline, which has a lower phosphate concentration than standard PBS). Some formulations do not contain potassium and magnesium, while other ones contain calcium and/or magnesium. Composition of PBS are listed in table

Common Composition of PBS

SALT	Concentration (MMOL/L)	Concentration (G/L)
NaCl	135	8.5
KCl	3.7	1.2
Na2HPO4	12	1.44
KH2PO4	1.9	1.24

Start with 800 mL of distilled water to dissolve all salts. Adjust the pH to 7.4 with HCl. Adddistilled water to a total volume of 1 liter. The resultant 1x PBS should have a final concentration of 10 mM PO43−, 137 mMNaCl, and 2.7 mMKCl.

Cell Culture

A rat insulinoma cell line (RINm5F) and 3T3-L1 pre-adipocyte was provided by National Centre for Cell Science (NCCS), Pune and was grown in Dulbecco's modified eagle Medium(DMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C,100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week. Kohn AD, *et.,al* 1996 dec 6.

Cell Lines Used 3T3-L1, RINm5f.

L1 Cell Line Description

- Organism: Mouse
- Tissue: Embryo
- Cell type: Fibroblast
- Morphology: Fibroblast
- Culture properties: Adherent
- Storage condition: Liquid nitrogen vapor phase

RINM5F Cell Line Description

- Organism: Rat
- Tissue: Pancreas
- Cell type: Beta cell
- Morphology: Epithelial
- Culture properties: Adherent
- Storage condition: Liquid nitrogen vapor phase

Sub-Culture of Cells

Volumes used in this protocol are for a 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for sub culturing this product. Fang XK, *et.,al* 2008 mar 12.

1. Removed and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Added 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer was dispersed (within 5 to 15 minutes).
4. Added 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Added appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C.

Determination of Cytotoxicity by Micro Culture Tetrazolium (MTT) Assay

Method

1. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0x106 cells/ml using DMEM medium containing 10% fetal bovine serum.
2. To each well 100 µl of the diluted cell suspension was added. After 24 hours, the Different concentration of test extract & Standard was added the concentration rang is 6.25 to 400 ug\ml.
3. The final volume of the well was made up to 200µl with medium (Serum Free) to the cells.
4. The plates was then incubated at 370C for optimum time, and microscopic examination was carried out and observations recorded every 24 hours.
5. After 72 hours, the drug solutions in the wells were discarded and 20µl of MTT was added to each well. The plates shall be gently shaken and incubated for 3 hours at 370C.
6. The supernatant has to be removed and 200µl of solubilization solution was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured at a wavelength of 570nm. The percentage growth inhibition was calculated.

Glucose Uptake Assay

1. Glucose uptake activity of test drugs were determined in differentiated, 3T3-L1 cell Line.
2. 2.The 3T3L1 (mouse fibroblast) cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% Fetal Calf Serum (FCS), 4mm Glutamine and 1% antibiotic/antimitotic, in a 5% CO2 incubator at 370CIn brief, the 24 hr cell cultures with 70 80% confluence in 40mm petri plates and allowed to differentiate 4-6 day.
3. The extent of differentiation was established by observing multinucleation of cells. The differentiated cells were serum starved overnight and at the time of experiment cells were
4. Washed with HEPES buffered Krebs Ringer Phosphate solution (KRP buffer) once.
5. After then incubated with KRP buffer with 0.1% BSA for 30min at 37 °C.
6. Cells were treated with different non-toxic concentrations of test and standard drugs for 30 min along with negative controls at 37 °C.
7. Concentrations ranging from 6.25 µg/ml to 400 µg/ml of extract.
8. D-glucose solution was added simultaneously to each well and incubated at 370C for 30 min.
9. After incubation, the uptake of the glucose was terminated by aspiration of solutions from wells and washing thrice with ice-cold KRP buffer solution.
10. Cells were lysed with 0.1M NaOH solution and an aliquot of cell lysates were used to measure the cell-associated glucose.
11. The glucose levels in cell lysates were measured using glucose assay kit. Three independent experimental values in duplicates were taken to determine the percentage enhancement of glucose uptake over controls. Ashcroft SJ, *et.,al* 1973 feb 1.

Insulin Release Assay

1. The assay is carried out in RIN m5F cells.
2. Cells are washed with KRB (Krebs-Ringer Bicarbonate) buffer (100 µL) to remove serum and then the cells (1x105cells/well) are seeded into the 96 well microtitre plate and incubated for 24 hours at 37°C.
3. Various concentrations of test samples are prepared using 100 µL KRB buffer and incubated for an hour at 37°C.
4. After incubation, the insulinis measured by sandwich ELISA methods. Sandwich ELISA Procedure (Mercodia

Rat Insulin ELISA) Twenty five µL of the cellly sates and 50 µL of theenzyme conjugate are added to anti-insulin coated 96 well micro titre plate.

5. The whole mixture is incubated for 2 hours at room temperature; the incubated solution isthen washed with KRB buffer. Two hundred µL of the substrate (tetramethylbenzidine) isadded to each well and incubated for 15 minutes.
6. Afterthe incubation, 50 µL of the stop solution (0.5%H2SO4) is added and is placed on ashaker for 5sec to ensure proper mixing. The absorbance ismeasured at 450 nm. Horwitz DL, *et.,al* 1975.

RESULTS AND DISCUSSION

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites.The phytochemical screening and qualitative estimation of *Emblica officinalis*and *Magifera indica*are important for further study. Results of preliminary phytochemical screening of the leaves and *Mangifera indica* are presented in table Results showed the presence of alkaloids, glycoside, tannins, flavonoids, saponin, polyphenol and glycoside while steroid and triterpenoides were absentin the extract.

Cell Viability and Glucose Uptake Activity

In the present study, the ethanolic extract of the plants of *Magifera indica* and *Emblica officinalis*was screened using MTT for its cytotoxicity against three cell lines namely 3T3-L1 and RINm5f at different concentrations. The cytotoxicity of the ethanolic extract fruits of *Magifera indica* and *Emblica officinalis* was found to be dose dependent. Result of the percentage cell viability of ethanolic *Magifera indica* (MI) leaves extract in 3T3-L1 cell line with concentration rang 6.25 to 400 µg/ml are presented in table.

Effects of Magifera Indica on 3T3-L1

Concentration (MG/ML)	Ethanol Leaves Extract of MI
50	103.29 ±0.42
100	83.37±1.24
150	62.49± 1.09
200	17.89± 1.07
250	11.09± 1.07

% Cell Viability of Cell Line- RINM5F

Concentration (MG/ML)	Ethanol Leaves Extract of MI
50	96.32 ±0.36
100	85.73 ±1.20
150	66.63± 0.56
200	41.23± 0.88
250	25.63± 1.19

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CONCLUSION

Emblica officinalis and *Magifera indica* is widely used in traditional medicine to combat and cure various ailments and found to be rich in secondary metabolites. The presence of alkaloids, polyphenol, carbohydrates, phenol, lipid, resins, proteins, gallic acid, amino acids, flavonoids, tannins steroids, ascorbic acid, and terpenoids in medicinal herbal plant may be attributed to their curative properties. *Emblica officinalis* and*Magifera indica* probably exerts its anti-diabetic properties by stimulating glucose uptake in adipocytes with significant inhibition of adipogenesis. Leaves extract of *Emblica officinalis* and *Magifera indica* were also observed to enhance basal and insulin-stimulated glucose uptake. This research paper has presented various anti-diabetic plants that have been pharmacologically tested and shown to be of some value in treatment of Diabetes Mellitus.

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