

Development and Standardisation of Polyherbal Antidiabetic Formulation

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ABSTRACT

Ayurvedic formulations are used to treat a wide variety of diseases including Diabetes Mellitus. Standardization of Herbal Formulation is Essential in order to assess the Quality of drugs. The Present Study Reports for Standardization of six herbal Antidiabetic drugs terminalia chebula (fruits) emblica officinallis gaertn (fruits) momordica charantia (seed) annona squamosa (seed) Nigella sativa (seed) Fenugreek (seed) individually and in the form of polyherbal marketed sample baidyanath madhuhari churna Shivayu Madhuhari Churna and were compared to the in-house preparation for Physicochemical properties as per WHO Guidelines. The Limits obtained from the different Physicochemical Parameters of the individual Eight Herbal drugs. And the Marketed Formulations could be used as Reference Standard for Standardization of the Anti-diabetic drugs in a Quality Control laboratory.

Herbal medicines are the oldest form of health care known to mankind. A number of traditional herbal medicinal practices have been adopted for the diagnostic prevention and treatment of various diseases. Based on the extensive review of literature, five raw materials were selected for the formulated as polyherbal capsules and the antidiabetic potency was evaluated in animal model. The herbal raw materials were analyzed for identity, quality and purity as per the standards prescribed by WHO and Ayurvedic Pharmacopoeia of India. The Physicochemical parameters like Loss on drying, ash values and extractive values were determined, which will help in preventing variation in quality of the drugs. Preliminary phytochemical investigation revealed the presence of various phytoconstituents such as alkaloid, steroids, glycosides, Flavonoids, Phenols, Tannins, and terpenoid in the raw materials. The safety of the raw materials was analysed by heavy metals and microbial screening and the results found within the standard limits given by WHO. The coarse powders of the selected plants were extracted by using ethanol as solvent. The ethanolic extracts

were dried by freeze drying and used for the formulation. TLC for the each individual plant extracts and polyherbal extract was performed. Further HPTLC fingerprinting of the polyherbal formulation was also performed and the resultant chromatogram showed the presence of peaks indicating different constituents. The chromatogram can be used as an index for the qualitative analysis of the formulation. The dried polyherbal extract was optimized for its quality measures and its batch consistency by making four different trial batches (Trial I, II, III, IV). The trials were subjected to preformulation parameters to confirm the uniformity and quality. The result concludes that the

Trial IV was excellent in all parameters and the values were found within the standard limits and it was used for formulate Polyherbal Capsule. The developed polyherbal capsules were standardized for its Description, uniformity of weight, disintegration time, moisture content, pH, Physicochemical parameters, and phytochemical studies.

Multifactorial metabolic diseases, for instance diabetes develop several complications like hyperlipidemia, hepatic toxicity, immunodeficiency etc., Hence, instead of mono-drug therapy the management of the disease requires the combination of herbs. Marketed herbal drugs comprise of irrational combinations, which makes their quality control more difficult. Phytoconstituents, despite having excellent bioactivity in vitro demonstrate less or no in vivo actions due to their poor lipid solubility, resulting in high therapeutic dose regimen; phospholipids encapsulation can overcome this problem.

I. INTRODUCTION

The last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from

medicinal plants, minerals and organic matter .A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparations of Indian traditional health care systems in Indian systems of medicine most practitioners formulate and dispense their own recipes.The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world . The current review focuses on herbal drug preparations and plants used in the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses

Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. This review focuses on Indian Herbal drugs and plants used in the treatment of diabetes, especially in India. Diabetes is an important human ailment afflicting many from various walks of life in different countries. In India it is proving to be a major health problem, especially in the urban areas. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects and low cost. A list of medicinal plants with proven antidiabetic and related beneficial effects and of herbal drugs used in treatment of diabetes is compiled. These include, *Allium sativum*, *Eugenia jambolana*, *Momordica charantia* *Ocimum sanctum*, *Phyllanthus amarus*, *Pterocarpus marsupium*, *Tinospora cordifolia*, *Trigonella foenum graecum* and *Withania somnifera*. One of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals and hence an antidiabetic compound with antioxidant properties would be more beneficial. Therefore information on antioxidant effects of these medicinal plants is also included

Ayurvedic and herbal medicinal products contain a combination of botanicals; each of these contains a number of chemical compounds that may give the anticipated activity in combination. Therefore, it is very important to analyze and evaluate the compatibility of various active constituents and markers from different medicinal plants for their possible chemical interactions with various excipients at different storage conditions during the development of a stable polyherbal

formulation

POLYHERBAL CONCEPT :-

Polyherbal formulation (PHF) is the use of more than one herb in a medicinal preparation. The concept is found in Ayurvedic and other traditional medicinal systems where multiple herbs in a particular ratio may be used in the treatment of illness.

ANTIDIABETIC:-

Drugs used in diabetes treat diabetes mellitus by altering the glucose level in the blood. With the exception of insulin, most GLP receptor agonists, and pramlintide, all are administered orally and are thus also called oral hypoglycemic agents or oral antihyperglycemic agents.

The Indian traditional Ayurvedic system of medicine has advocated the use of natural products to promote healthy living by preventing unnecessary ailments. This traditional medical system is well-known using a variety of herbal medications to treat various causes of imbalance in human health. The fundamental principle behind the use of herbal formulations in Ayurveda is not only the restoration of a disease-free body, but also the prevention of its recurrence .Herbal formulations are also common in other ancient medicinal systems, such as those of China, Egypt and Greece According to World Health Organization (WHO), around 80% of people across the world still resort to these natural herbal products for the maintenance of a good lifestyle . These natural products are used as a single herb or in combination with other herbs. The latter, referred to as "Polyherbal Formulations," has recently gained significant attention. The concept of "Polyherbalism" was espoused in Ayurvedic literature such as the Sarangdhar Samhita' because sometimes a single herb cannot achieve the desired health effects . The literature also implies that combining different herbs in an optimum ratio reduces the **toxicity** of each herb and thus improves therapeutic effects. Thus, the term "Polyherbal Formulations" refers to those pharmaceutical preparation that uses more than one herb as a component for increased therapeutic effectiveness and decreased toxicity of individual herbs.

The ancient herbal medicine system from many parts of the world have made use of polyherbal formulations due to their multifaceted pharmacological actions [4].

Polyherbal utilizes the concept of synergies which indicates a positive herb-herb

interaction. This principle suggests that the phytochemical constituents from one plant get activated in the presence of constituents from another plant. Such interactions have been demonstrated using two mechanisms namely pharmacokinetics and pharmacodynamics. The former mechanism focuses on the facilitation of distribution, metabolism, absorption, and elimination of one herbal constituent by another. However, the latter mechanism focuses on the synergistic effect of one herb with other, when the phytochemical constituents have similar therapeutic activities and thus when targeted to a similar receptor enhances the overall pharmacological effect. It also advocates the fact that the use of multiple herbs in a single formulation may help in targeting many potential targets in the physiological system at the same time imparting faster relief. Therefore both these mechanism supports the use of multiple herbs rather than single herbal formulation. Medical practitioners also believe that polyherbal formulations help in lowering the dose size for each herbal constituent, reducing the risk of any side effect due to highdose of herbs [5]. Other than this, polyherbal formulations make it easy for patients to consume these herbs by nullifying the need to consumemany medicines separately. This improves compliance along with improved pharmacological effects.

Contemporary science has proven the effectiveness of these polyherbal formulations in the treatment of various ailments. The therapeutic potential of these Polyherbal formulations have been demonstrated against various acute and chronic diseases such as diabetes, wound care, hypertension, cardiovascular disorders, anxiety, neurological imbalances, disorders related to the gastrointestinal tract, respiratory tract, and endocrine system. Out of many applications of polyherbal formulations, the wound healing potential is the most recognized one. Traditional healing agents have been significant in wound care since ancient times. The combination of these traditional therapies with clinical therapies has helped in the development of various wound healing products with greater efficiencies.

Therefore the current review is a systematic compilation of various Polyherbal formulations tested on different models for investigating their wound healing potentials during the last decade.

● MATERIAL AND METHOD :-

- Animals
- Prepration of drugs
- Chemicals
- Drugs administration
- Experimental design
- Biochemical analysis

Diabetes mellitus (DM) is defined as a heterogeneous metabolic syndrome characterized by chronic hyperglycemia, glycosuria. Negative balance.

Hyerlipidaemia and ketonaemia. It is the most commonly occurring chronic endocrinal disorder which effects. Almost each and every cell of human body.

Diabetes is a chronic metabolic disorder characterized by either the insufficient production or the lack of response to a key regulatory hormone of the body's metabolism, insulin.

It can be categorized as Type-1 diabetes [insulin dependent diabetes mellitus (IDDM)] and Type-2 diabetes [non- insulin dependent diabetes mellitus (NIDDM)].

The overall prevalence of diabetes is approximately 10% of the population, of which 90% is Type-2,

Collection of the plant

Taxonomically identified stem bark of *Glycosmis pentaphylla* (Rutaceae), whole plant of *Tridax procumbens* (Asteraceae), and leaves of *Mangifera indica* (Anacardiaceae) were collected from the Alagar kovil region, Madurai district. The collected plants were authenticated at the Department of Botany, American college, Madurai, Tamil Nadu. The voucher specimen of the plant was deposited in the Department of Pharmacology, Ultra College of Pharmacy, Madurai, India for further reference

HARBAL DRUGS AND IT'S INFORMATION :

Momordica charantia:-

-Bitter gourd is a green-skinned vegetable with white to translucent flesh and a taste that fits its name. Unless you grew up with bitter gourd as part of your regular diet, it might take you a while to warm up to the bitter flavor.

Bitter gourd is also a vegetable of many names. It is equally known as bitter melon, bitter cucumber, balsam-pear, bitter apple, or bitter

squash. This vegetable is also called karela in India, nigauri in Japan, goya in Okinawa, ampalaya in the Philippines, and ku-gua throughout China.

It has been shown to possess anticancer, antidepressant, antidiabetic, anti-inflammatory, antimicrobial, antiobesity, antioxidant, and antiulcer properties. Its common phytochemical components include alkaloids, charantin, flavonoids, glycosides, phenolics, tannins, and terpenoids.

Biological name : momordica charantia
linn, bitter gourd
Synonyms: karela, Balsam pear,
momordica muricata
Family: cucurbitaceae,
pumpkin family
Biological source: karela consists of fresh green
fruits of the plant known as Charanta Linn
Chemical constituents: charantin, momordicin



Kingdom: plantae Division: Magnoliophyta. Class: Magnoliopsida

Order: Viales

Family: Cucurbitaceae. Genus: Momordica Species: charantia Uses:-

- Contains at least three active substances with anti-diabetic properties, including charantin, which has been confirmed to have a blood glucose-lowering effect, vicine and an insulin-like compound known as polypeptide-p.
- These substances either work individually or together to help reduce blood sugar levels.
- Contains a lectin that reduces blood glucose concentrations by acting on peripheral tissues and suppressing appetite - similar to the effects of insulin in the brain.

- The said lectin is thought to be a major factor behind the hypoglycemic effect that develops after eating bitter melon.

Bitter melon powder marketed by Garry and Sun. It lowers blood & urine sugar levels. It increases body's resistance against infections and purifies blood. Bitter melon has excellent medicinal virtues. It is antidotal, antipyretic, tonic, appetizing, stomachic, antibilious and laxative. The bitter melon is also used in native medicines of Asia and Africa. The bitter melon is specifically used as a folk medicine for diabetes. It contains compounds like bitter glycosides, saponins, alkaloids, reducing sugars, phenolics, oils, free acids, polypeptides, sterols, 17-amino acids including methionine and a crystalline product named p-insulin. It is reported to have hypoglycemic activity in addition to being antihaemorrhoidal, astringent, stomachic, emmenagogue, hepatic stimulant, anthelmintic and blood purifier.

Bitter melon and its anti-diabetic properties

Bitter melon (*Momordica charantia*) is an important market vegetable in Southern and Eastern Asia and is widely spread throughout most of tropical Africa. It is also referred to as bitter melon, balsam pear, bitter apple, and bitter African or wild cucumber. Fruits and leaves of most wild *Momordica* species are consumed as vegetables and have a similar bitter taste and almost identical medical uses. It has been used as a traditional anti-diabetic remedy in eastern countries for many years.

It is now commercially available as tea (from fruits or leaves), juice, extracts, and tablets. Although these products promise health benefits, most of the manufacturers do not provide scientific evidence on the effectiveness of bitter melon or their products. However, in recent years researchers have focused on the anti-diabetic effects of bitter melon. The goals of these studies are to provide safe and clear preparation and dosage recommendations. This article provides information about the nutritional importance of bitter melon as a vegetable and scientific evidence about its anti-diabetic properties.

Annona squamosa/ sugar apple

Botanical name :- *Annona squamosa* Family:- Annonaceae

Origin:- Tropical America.

Synonyms:- Sitaphal, sugar apple, sweetsop, naina, a



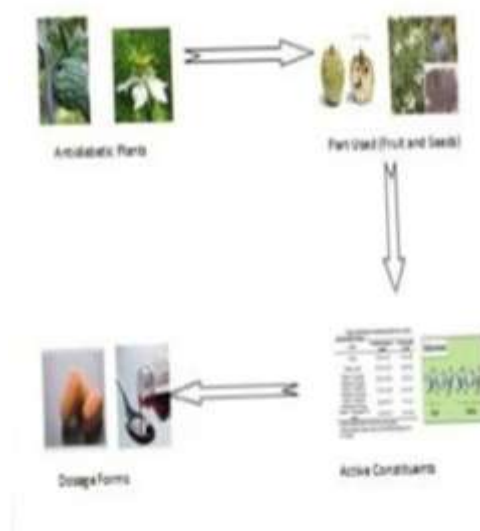
Plant formulation and combination extract of plants are used a drug of choice rather the individua Various herbal formulations such as diamed, coagent db, Diasulin, and hyponidd, are well known for their antidiabetic effects Polyherbal formulation of Annona squamosa and Nigella sativa is composed of medicinal plants (Table 1), which are traditionally used for antidiabetic and antihyperlipidemic activity. The present investigation was undertaken to study the effect of the polyherbal formulation of Annona sqamosa and Nigella sativa on lipidperoxidation and tissue lipid profile in streptozotocin induced diabetic rats.

Polyherbal formulation of Annona squamosa and Nigella sativa on blood glucose, plasma insulin, tissue lipid profile, and lipidperoxidation in streptozotocin induced diabetic rats. Aqueous extract of Polyherbal formulation of Annona squamosa and Nigella sativa was administered orally (200 mg/kg body weight) for

30 days

The different doses of Polyherbal formulation on blood glucose and plasma insulin in diabetic rats were studied and the levels of lipid peroxides and tissue lipids were also estimated in streptozotocin induced diabetic rats. The effects were compared with tolbutamide.

Preparation of polyherbal Antidiabetic drugs



Uses of Annona squamosa:-

The leaf extract of custard apple combined with black pepper could help reduce the severity of diabetes in a lab study. Consumption of leaves extract of custard apple could also improve the activities of plasma insulin and reduces the levels of blood glucose during the study. Custard apple may increase the effect of diabetes medicines, resulting in fewer requirements for insulin therapy and also fewer side effects.^{1,2} However, before you start consuming custard apple for its anti-diabetic benefits, or making any diet changes, reach out to your healthcare provider and get all the necessary information.

The leaf extract of custard apple showed positive effects against arthritis. This anti-arthritic activity causes a reduction in inflammation and may help people with arthritis. Custard apple helps to manage malaria. The leaf extract of custard apple contains bioactive compounds, which act against malaria-causing Aedes mosquitos.¹

The leaf extract of custard apple has strong anti-microbial properties that may help in reducing the growth of bacteria, fungi and viruses.⁴ Though there are studies that show the benefits of

custard apple in various conditions, but these are insufficient and there is a need of further studies to establish the true extent of benefits of herb on human health.

Nigella sativa:- (kalonji)

Common Name : Black cumin or Black seed

Botanical Name : Nigella sativa

Family. : Ranunculaceae

Nigella sativa uses:-

Nigella sativa is an erect annual herb of the Mediterranean region and Asia.

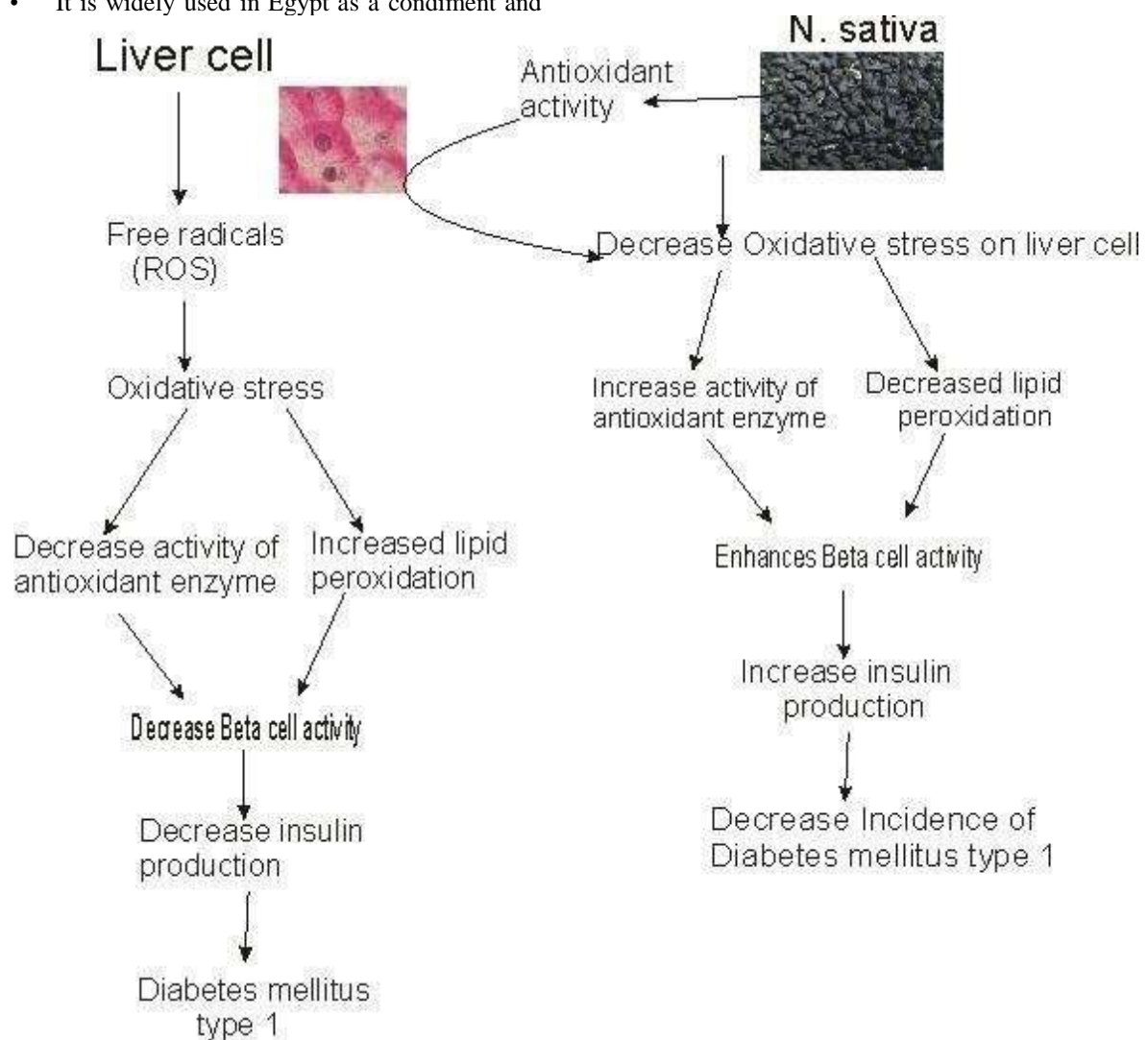
- The seeds are known as al habbah al sawdaa, Habbet el baraka, black seed, black cumin, Shung & others.
- It is widely used in Egypt as a condiment and

as a natural remedy formany ailments.

The expressed oil from the seeds is available in the market in the form of gelatin capsules used in folk medicine for treatment of many diseases, among which asthma & other inflammatory disorders.

Insulin Secretion and Insulin Sensitivity Glucose uptake by muscle cellsand adipose tissue
 Glucose absorption from intestine and glucose production fromhepatocyte

Fasting blood glucose and Post- prandial blood glucose level HbA1c glycosylated plasma protein concentration



FENUGREEK:- (methi)

Synonyms: Methi, Methika, Chandrika

Biological source: Methi consists of dried ripe seeds of *Trigonella foenum-graecum* Family: Leguminaceae

Geographical source: The plant grows wild in Northern India and is cultivated as a crop throughout India. It is also cultivated in Southern and Eastern Europe, Pakistan, France, Morocco and Egypt.

Shape: Rhomboidal, smooth

Size: 5-8mm long and 1-2mm thick

• Colour: Light to dark yellow, brown, green

Odour: Characteristics

Taste: Mucilaginous and slightly bitter

On one side run a diagonal depression, dividing the radial and two yellow accumbent cotyledons

Uses:-

In Indian cuisine it is used as a spice. Gastric protector: powerful anti-ulceric. It helps control diabetes.

It has many properties for women. To treat high cholesterol.

Useful to prevent hair loss. Medicinal Actions

The phytoestrogens and steroidal saponins in fenugreek have been shown to reduce both menopausal symptoms and PMS, as well as increasing testosterone levels and enhancing sexual function in men. On the other hand, galactomannan has been shown to help regulate digestive enzymes, also inhibiting the absorption of glucose, thus helping reduce blood sugar.



The health benefits of fenugreek include relief from anemia, loss of taste, fever, dandruff, stomach disorders, biliousness, respiratory disorders, mouth ulcers, sore throat, diabetes, inflammations, wounds, and insomnia. It is beneficial in lactation and helps in improving

digestion and hair health. It is also shown to reduce cholesterol levels and protect heart health, while simultaneously boosting the immune system and protecting against flu and various infections.

FENUGREEK:-

In North Africa, Asia, and southern Europe, fenugreek was traditionally used for diabetes and to increase milk supply in women who were breastfeeding. Today, fenugreek is promoted as a dietary supplement for diabetes, menstrual cramps, and other conditions and to stimulate milk production during breastfeeding.

Synonym: Fenugreek, Methi

Biological source: Consist of seeds and leaves of *Trigonella foenum-gracum* Family: Fabaceae

Dose :- 2- 3 gm of drugs in powder form.

TERMINALIA CHEBULA:-



Plants Taxonomy. Kingdom. Planta Order - Myrtales Family Combretaceae
Species.-Terminalia chebula. Description:

Deciduous trees, Leaves ovate or elliptic. Flowers yellow or creamy white, in spikes. Fruits 5-ridged.

PROPERTIES

Phytoconstituents:

Fruits contain-Tannic acid, gallic acid, Chebulinic acid, mucilage, glycoside (Anthroquinone), Carbohydrates, aminoacids, phosphoric acid

Ethno-Medicinal uses:

Fruits Diabetes, dysentery, diarrhoea, gout, malaria, sore throat, pneumonia, Anaemia, typhoid

Bark. Ezema

Dose: 3-6g of the drug in powder form

EMBLICA OFFICINALIS GAERTN



Synonyms phae lanthus emblica L Common name- Amla

PLANT TAXANOMY

Kingdom – plantae Order- malpighiales Family – Euphorbiacea

Genus-phyllanthus. Species-Phyllanthus emblica AMLA

Description

Large, deciduous trees, with distichous, linear-oblong leaves. Flowers greenish- yellow, in fascicles on leafless branches. Fruits globose, fleshy; Seeds bony.

Phytoconstituents:

Fruits contain protein, fats, fibres, carbohydrates, vitamin c, Nicotinic acid, Tannins

After Drying fruits contain Gallic acid, Ellagic acid, Flavin & glucose. Seed contains Linoleic acid, Linolenic acid, Oleic acid.

Dose: 3-6g of the drug in powder form

AIM:-

The aim of the present work is to develop a polyherbal anti-diabetic powder from the selected plant material and evaluate the same.

OBJECTIVE

- To perform the raw material analysis
- To extract the plant material by continuous Hot Percolation method using ethanol as solvent.
- To formulate and evaluate polyherbal powder from drugs
- To evaluate the antidiabetic activity by In vitro and in vivo models

PLAN OF WORK:-

Collection and Authentication Processing of raw materials

RAW MATERIALS STANDARDIZATION

- Organoleptic Evaluation.
- Microscopical evaluation.

- Phytochemical study.
- Development of formulation
- Physico-chemical property.
- Standardization
- Pharmacological study
- In vivo studies
- Blood glucose levels
- Lipid levels

PHYTOCHEMICAL STUDIES

- Preliminary phytochemical screening of powder and extracts
- Preparation of Extract
- Fluorescence analysis of raw materials
- Thin Layer Chromatography
- HPTLC-Finger print analysis

DEVELOPMENT OF FORMULATION

- Pre formulation studies
- Selection of excipient
- Flow property measurement
- Bulk density
- Tapped density
- Compressibility index
- Hausner's ratio
- Angle of repose
- Trial batches (I, II, III, IV) (Selection of optimized batches)
- Formulation of capsules
- Description
- pH
- Uniformity of weight
- Disintegration time
- Ash value
- Extractive value
- Quantitative estimation of Phytoconstituents
- Quantitative Estimation of Heavy metals and Inorganic elements
- Microbial load

PHARMACOLOGICAL STUDIES

- In vitro studies:
- a-amylase inhibitory assay.
- In vivo Streptozotocin induced diabetes in rats
- Blood glucose level
- Lipid profile

COLLECTION AND AUTHENTICATION

Herbs used for formulation were procured from the authentic suppliers and further authenticated by Dr. K.N. Sunil Kumar R.O. and

HOD Pharmacognosy, Central Siddha Research Institute, Government of India, Arumbakkam, Chennai-106.

PROCESSING OF RAW MATERIALS

The procured plant materials were cleaned thoroughly. They were then dried under shade for a week or so. Once they were completely dried, they were ground into coarse powder and stored in air tight containers and preserved for the further processing.

STANDARDISATION OF RAW MATERIALS

Shade dried powdered plant materials of the plants, *Berberis aristata* (dried Stem), *Terminalia chebula* (pericarp of matured fruit), *Emblca officinalis* (pericarp of dried matured fruit), *Terminalia bellerica* (pericarp of dried ripe fruit) and *Cyperus rotundus* (dried rhizome) used for the standardization of raw materials.

ORGANOLEPTIC EVALUATION

Organoleptic evaluation defines the majority of information on the identity, purity of the material which are of primary importance for the establishment of degree of quality done by sensory organs for the evaluation of drugs colour, odour, taste and specific characters.

In this study the following organoleptic characters like physical appearance, taste and odour of plant materials were evaluated and confirmed with reference samples.

MICROSCOPICAL EVALUATION

POWDER MICROSCOPY

Powder characters show the detailed examination of a drug which is mainly used to identify the organised drugs by their known structural characters. The

PHYTOCHEMICAL STUDIES

Herb is a biosynthetic laboratory, which contains chemical compounds such as carbohydrates, proteins and lipids that are utilized as food. It also contains secondary products like glycosides, alkaloids, flavonoids, tannins etc. The detection of these active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude drugs and extracts and is very important in regard to their potential pharmacological effects. These tests facilitate the quantitative estimation and qualitative separation of pharmacologically active chemical compounds and subsequently may lead to the drug discovery and

development.

All the plant raw materials were subjected to preliminary phytochemical screening for the detection of various plant constituents

PRELIMINARY PHYTOCHEMICAL SCREENING

Triterpenoids

Salkowski test Powdered crude drug was treated with few drops of concentrated sulphuric acid, formation of yellow colour indicates the presence of triterpenoids.

Flavones

Shinoda test – To the powdered crude drug, few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes formation of red coloration indicates the presence of flavones.

Department of Pharmacognosy, MMC, Chennai-0

Alkaloids

Materials and methods

Dragendorff's reagent. To the powdered crude drug, few drops of potassium bismuthiodide solution was added, reddish brown colour indicates the presence of alkaloids.

Carbohydrates

Molisch's test In a test tube containing powdered drug, 2 ml of distilled water and 2 drops of freshly prepared 20% alcoholic solution of a naphthol were added. Mixed well and added 2ml of concentrated sulphuric acid along the sides of the test tube.

Formation of red violet ring, which disappears on addition of excess alkali solution, indicates the presence of carbohydrate

Glycosides

Extracted 200 mg of drug with 5 ml dilute sulphuric acid by warming on a water bath, filtered and neutralized the acid extract with 5% solution of sodium Hydroxide 1ml of Fehling's solution A and B were added until it became alkaline (test with pH paper) and heated on a water bath for 2 minutes. Formation of red precipitate was observed, which indicates the presence of glycosides.

Phenols

Ferric chloride test Dissolved a small quantity of the drug with 2ml of distilled water, added a few drops 10% aqueous ferric chloride solution. A blue or green colour was produced,

which indicates the presence of phenols.

Resin

Dissolved a small quantity of the ethanolic extract of the drug with 5-10 ml of Acetic anhydride by gently heating the solution. Cooled and added 0.05 ml of Concentrated sulphuric acid. A bright purplish red colour was seen, which rapidly Changed to violet indicates the presence of resin.

CHROMATOGRAPHY:-

Chromatographic fingerprinting has been in use for a long time for single chemical entity drug substances. Recently it has become one of the most powerful tools for quality control of herbal medicines. The use of chromatographic fingerprinting for herbal drugs tends to focus on identification and assessment of the stability of the chemical constituents observed by various chromatography techniques such as HPLC, TLC, HPTLC, GC, capillary electrophoresis

THIN LAYER CHROMATOGRAPHY

Principle

It consists of a thin layer of adsorbent coated on a chromatographic plate, the mobile phase (developing solvent) flows against gravitational force by means of capillary action. The separation is mainly on the differential migration that occurs when the solvent flows along the thin layer of stationary phase. The principle involved thin layer chromatography is adsorption.

Thin layer chromatographic study Materials and methods

The ethanolic extracts of *Berberis aristata*, *Terminalia chebula*, *Emblica officinalis*, *Terminalia bellerica* and *Cyperus rotundus* were subjected to thin layer chromatography (TLC) as per conventional method using silica gel 60F254, 5x3 cm. (Merck). Plate markings were made with soft pencil. Glass capillary tubes were used to spot the extract in TLC plates. Different solvent systems ranging from lower to higher polarities were tested for the separation of bioactive components.

In the TLC chamber the solvent system viz Butanol: Acetic acid: Aqueous (40:10: 20) were used. After pre-saturation with mobile phase for 30 min the plates were kept inside the chamber and the elution was performed using above mentioned solvent systems. After completion of the elution the plates were dried and subjected to visualized under UV chamber and sprayed using different spray reagents.

R, values determined by using following formula $R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$

HPTLC FINGERPRINT PROFILE

HPTLC is one of the advanced and versatile chromatographic technique which Helps in the identification of compound and thereby authentication of purity of herbal Drugs. It is very quick process. In addition to qualitative detection, HPTLC also Provides semi-quantitative information on major active constituents of a drug thus Enabling an assessment of drug quality.

HPTLC serves as a convenient tool for finding the distribution pattern of the Phytoconstituents which is unique to each plant. The finger print obtained is suitable For monitoring the identity and purity of drugs and for detecting adulteration and Substitution. HPTC technique is helpful in order to check the identity, purity and Standardize the quantity of active principles present in the herbal extracts.

Sample Preparation: 1mg of Polyherbal extract was dissolved in 1 ml of methanol. PROCESS AND MATERIALS

The curation of ant materials were cleaned thoroughly. They were then dried under shade for a week or so. Once they were completely dried, they were ground into powder and stored in air tight containers and preserved for the further processing.

- Standardization
- Organoleptic evaluation.
- Microscopic evaluation.
- Physicochemical evaluation.
- Loss On drying.
- Determination extraction value
- Water soluble extractive
- Ether soluble extractive
- Determination of Ash value PROCEDURES PHYTOCHEMICAL STUDIES

Herb is a biosynthetic laboratory, which contains chemical compounds such as carbohydrates, proteins and lipids that are utilized as food. It also contains secondary products like glycosides, alkaloids, flavonoids, tannins etc. The detection of these active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude drugs and extracts and is very important in regard to their potential pharmacological effects. These tests facilitate the quantitative estimation and qualitative separation of

pharmacologically active chemical compounds and subsequently may lead to the drug discovery and development.

PREPARATION OF EXTRACTS

Extraction is the preliminary step involved in the phytochemical studies. Ethanol proves to be the universal solvent. As the present study utilizes a combination of herbs, ethanol was opted for the extraction of the active constituents from the individual plants separately. The method of extraction is Hot Percolation method.

- Hot percolation method.
- Chromatography
- TLC
- HPTLC
- Fluorescence analysis.
- Preformulation studies

II. RESULT & CONCLUSION

- Raw material standardization
- Oranoleptic evaluation
- Physicochemical evaluation
- Determination of Ash value
- Standardization of finished product
- Activity
- In vivo activity calculation

Herbal medicines are the oldest form of health care known to mankind. A number of traditional herbal medicinal practices have been adopted for the diagnostic prevention and treatment of various diseases.

Based on the extensive review of literature, five raw materials were selected for the formulation as polyherbal capsules and the antidiabetic potency was evaluated in animal model. The Physicochemical parameters like Loss on drying, ash values and extractive values were determined, which will help in preventing variation in quality of the drugs. Preliminary phytochemical investigation revealed the presence of various phytoconstituents such as alkaloid, steroids, glycosides, Flavonoids, Phenols, Tannins, and terpenoid in the raw

Fasting blood glucose parameters and lipid profile were analyzed. The formulation showed significant effect compared to the normal range before induction of diabetes. The polyherbal at the dose level of 400 mg/kg showed significant decrease in blood glucose level on 7th day. The

elevated levels of total cholesterol, L.DI. And triglycerides were also reduced and increases HDL level. The phytochemical study showed the presence of flavonoids. This may be responsible for the potent anti diabetic activity. Further studies are recommended for stability studies in the formulated polyherbal powder from drugs and also clinical trials have to perform in future in Human Volunteers. Since Ancient times medicinal plants as single drug and in combination with other herbal drugs are using in the treatment of various chronic and non-chronic disorders. Ayurveda is one of the most traditional systems of medicine which describes the methodology to use the medicinal plants as healing power in treating the disease. Polyherbalism is also the best concept of Ayurveda, which consists of magical power of healing the disease. Ayurveda is one of the reliable and trustworthy medicine systems. In developing countries mostly 75-95% of populations rely on herbal drugs. Deep research and investigation still needed on this magical system of medicines. Research Studies pertaining to safety, toxicological studies, Standardization, clinical trial studies are still required to grow Ayurveda and increasing its wide acceptability. Numbers of commercialized standardized herbal drugs are quiet less in market since we are lacking in developing the regulatory standards implemented protocols. Diabetes mellitus has appearing as dreadful disorder for society. It directly impacts our metabolic system by making it sluggish in catabolic activities. It is mainly characterized by hyperglycaemia resulted from decrease insulin secretion. This dreadful disease can lead to many more complications like blindness, kidney failure and organ dysfunction. Several synthetic drugs are available in market but with long use of these drugs could lead to serious side effect including the kidney failure there is greater risk of using these synthetic drugs for long term.

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