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ANTI-DIABETIC ACTIVITY OF TRADITIONAL POLYHERBAL FORMULATION OF "SYZYGIUM CUMINI, MOMORDICA CHARANTIA, OCIMUM SANCTUM, MYRISTICA FRAGRANS"

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ABSTRACT

Diabetes has been alarmingly increasing globally as a result of changes in lifestyle. Its increasing prevalence has made it a worldwide problem that needs attention. A common ailment in children and young adults is diabetes mellitus. Diabetes comes in two primary forms. Type II diabetes is a highly prevalent illness that affects about 90% of individuals globally. Type 1 diabetes is an autoimmune disorder with a hereditary propensity where T lymphocytes kill pancreatic B-cells. Many diabetes treatment strategies that aim to control blood sugar levels are available in modern medicine. However, because Ayurveda is readily available and less expensive, it can offer better treatment choices for the disease's worldwide burden. The data was sourced from a variety of published journals and research publications as well as traditional literature.

KEYWORDS: Diabetes Mellitus, Poly-herbal formulation *Syzygium Cumini, Momordica charantia, Ocimum Sanctum, Myristica Fragrans.*

INTRODUCTION

An estimated 537 million persons worldwide were predicted to have diabetes mellitus in 2021, making it one of the top five causes of mortality worldwide. This figure is predicted to increase to 783 million globally by 2045. Type 1 diabetes (T1 DM), the first of the two main forms of the disease, is characterised by hyperglycemia brought on by the immune system's death of the pancreatic beta cells, which lowers insulin production overall.[1]

The more prevalent form Type 2 diabetes mellitus is one of these metabolic diseases (T2 DM), which is characterised by a gradual loss in islet secretary function inside the pancreas, leading to overall insulin insufficiency, and an release of more insulin in order to make up for insulin resistance.[2]

Both genetic and environmental variables have been found to have a part in this autoimmunity, even though the precise pathophysiology is yet unclear. Most of the time, the pancreas and cell-specific autoimmunity and the disease itself either develop slowly, as in adults (late start), or rapidly, as in infants and adolescents (juvenile onset).[3]

In order to maintain normoglycemia in the early stages of the illness, B-cells hyperfunction in response to increased insulin production as a result of decreased insulin sensitivity.

Therefore, hyperinsulinemia, or high levels of circulating insulin, prevents hyperglycemia. However, as time passes, the rise in insulin production by the B-cells cannot sufficiently counteract the reduction in insulin sensitivity. Furthermore, B-cell function begins to decline, ultimately leading to insulin insufficiency. The failure to maintain normoglycemia leads to hyperglycemia.[4]

Basic therapeutic One method of treating diabetes might be to prevent the absorphon of glucose by delaying the activity of digestive enzymes such amilase and glucosidase. Because the complication of disease is mainly due to the higher glucose level in blood which dysfunction the other Organs of Body. Therefore, we may conclude that effective glucosidase inhibitors may be used as chemotherapeutic drugs in paediatric settings to treat obesity and diabetes.[5]

MATERIAL AND METHODS

Momordica Charantia (karela)

The plant *Momordica Charantia*, which belongs to the Cucurbitaceae family, produces fresh green fruit, which is known as karela.

M. charantia is one of the hypoglycemic medicinal plants that is the subject of the most investigation. This climber has been widely cultivated for food in South America, Africa, and Asia. In India, it is also cultivated up to 1500 meters above sea level.

Momordica's name comes derived from the verb mordeo (to bite) in Latin and the Greek term for the species, which means lovely flower. African cucumber, balsam pear, bitter melon, and bitter gourd are some names for the fruit of this plant.

Among the most promising therapeutic herbs is M. charantia treating AD and T2DM as well as for promoting longevity and general human health.

While several herbal medicines claim to be useful in treating AD and T2DM, M.charantia is one that has drawn a lot of interest.

Bitter melon's strong anti-diabetic and cholesterol-lowering properties have long been known.[6]

Triterpenes, such as momordicin and momordicinin, as well as a number of cucurbitanes, momordicosides, and goyaglycosides, are the fruits' active ingredients Polypeptide P, commonly referred to as vegetable or plant insulin (v- or p-insulin); and kuguacins; proteins such as α, β, and γ-momorcharins; and momordins a and b.

The list of every kind of chemical component found in M.charantia is provided below. Glycosides: Charantin, Momordin.
Alkaloids: Momordicin
Additional: Polypeptide-P
Stearic, linoleic, and oleic acids are oils (found only in seeds).
Alpha-momorcharin, beta-momorcharin, and lectins are examples of glycoproteins.
Amino acids include things like glutamic acid, aspartic acid, serine, thscinne, alanine, g-amino butyric acid, and pipecolic acid.
Other: protein, vicine (pyrimidine nucleoside). There is no free pectic acid in the fruit pulp, but there is soluble pectin.[7]

***Ocimum Sanctum* (Tulsi)**
Ocimum sanctum is an upright, robust, fragrant plant with many branches that grows to a height of around 75 cm.
This little plant is cultivated around Hindu temples and residences all across India. Ayurvedic medicine has made use of this plant's leaves, seeds, and root.
An essential part of the Hindu sacred heritage is tulsi. Its other name, Vishnupriya, signifies the one who pleases Lord Vishnu, even though the term Tulsi connotes the matchless one. Its tale has permeated Indian culture throughout the millennia and is found in the majority of Indian homes, where it is venerated.

O. Sanctum may have an important part of the treatment of diabetic and metabolic diseases due to its antimicrobial, antioxidant, and anti-inflammatory qualities. Physicians should be aware of the therapeutic benefits of the plant and encourage people to include it frequently in their diet, especially those with metabolic diseases and diabetes.[8]

***Myristica fragrans* (Nutmeg)**
The Latin term nux muscatus, which means "musky nut," is where the name "nutmeg" originates. The majority of taxonomists are familiar with nutmeg (*Myristica fragrans*), an evergreen tree that is a member of the Myristicaceae family of flowering plants that are native to Asia, Africa, the Pacific islands, and America.

A highly widespread custom that dates back to ancient times is the utilisation of plants for various health advantages. Herbal medications and herbal pharmaceuticals are common names for medicinal items made from plants.

In practically every kitchen, it is used as a spice to add taste to a variety of foods, particularly in North India where it is a component of garam masala. It is utilised in desserts, meats, sausages, sauces and baked goods.[9]

There are around 150 species in the genus Myristica, which are distributed throughout the western Pacific and Asia. Because a single tree does not have enough flowers of both sexes, nutmeg cross-pollinates. There are several names for *Myristica fragrans* across the world.[10]

***Syzygium cumini* (Jamun)**
One of India's significant minor indigenous fruit crops, jamun (*Syzygium cumini*) belongs to the family Myrtaceae. The tree produces noticeable, long seeds and dark purple, date-like fruits that are 2-4 cm long. The fruit's flavour ranges from acidic to somewhat sweet, and it is often astringent and occasionally disagreeable.

A significant traditional medicinal herb, jamun is indigenous to India and its neighbouring nations, including Nepal and Pakistan.

There are several applications for every component of the jamun or jambul tree. In addition to the ripe jamun fruits, other portions such as the bark, leaves, and—most importantly—the seeds are also utilised to treat a variety of illnesses.

People often don't eat jamun seeds uncooked. Every jamun fruit contains one weed.

Because the seeds reduce blood glucose levels, they are quite beneficial. In addition, it includes calcium, phosphorus, riboflavin, choline, folic acid, nicotinic acid, vitamin C, and vitamin A.[11]

The jamun is a large, 30-meter-tall evergreen tree. Bark, which is light brown in colour and rough in texture, is typically seen on old stems.

The leaves are 6 to 12 centimetres long, leathery, and obovate-elliptic. They have numerous nerves that converge at the periphery, are smooth and shiny, and can have a variety of shapes.

The leaf's tip is less acuminate and broader. Branchlets under the leaves produce the majority of the panicles, which are 4 to 6 cm in length.

At the tops of the stems are branching clusters of fragrant, greenish white flowers that range in size from 7.5 to 13 mm.

Each of the four petals is joined to form a cap, and the calyx has a cup-like form. The calyx is 4 mm long, funnel-shaped, and toothed.[12]

Collection, Identification, and Authentication of the plant

The selected plant material were purchased from local market/Nursery and were Authenticated by Identification an authentication of plant specimen from Department of Botany Environment, Forests, and Climate Change Ministry of India Botanical Survey, Allahabad.[13]

Raw Material

The raw material, *Syzygium cumini, Momordica Charantia, Ocimum Sanctum, Myristica Fragrans* is often sourced from agricultural cooperatives or local markets. Specific parts of the plant may be used, such as the fruits, leaves, or pericarp (the outer layer of the fruits). The source location and harvesting time might be mentioned for clarity and reproducibility.[14]

Morphological Studies

Morphological investigations involve the study of organoleptic properties, which refer to characteristics that can be perceived by the senses like smell, appearance, taste, touch, and odor. Various methods exist to evaluate the organoleptic properties of dried samples including chemical analysis, microscopic examination and direct sensory perception. By utilizing the organ of the sense, organoleptic assessment aims to identify specific features of a material serving as an initial step in determining its identity and purity level. The evaluation of organoleptic attributes such as condition, color, odor, taste, texture, and nature was conducted and recorded for further analysis.[15]

Ash Value:- Total and acid-insoluble ash are both included in the Ash value is calculated to evaluate the overall quantity of inorganic salts in the medication. The overall ash content. The objective of the process is to quantify the remaining material following ignition, which comprises "non-Physiological ash" from outside materials adhered to the plant's surface (such as sand and Mud) as well as "physiologicalash" from the plant's tissue.

Procedure:- Precisely transfer 2 grams of finely powered Material that has been dried in the air in to a crucible that has been heated and weighed before hand. Ensure the material is evenly distributed, then fire it slowly, making sure the temperature does not exceed 4500C, till it turns white, indicating that there is no longer any Carbon-weigh the residual material after letting the sample cool in the desicators. Make Carbon-Free ash by doing the following : Cool the crucible first. Then wet the residue with water and dry it. Next, fire the residue until it reaches a consistent weight. After that, chill it in desiccators for 30 Minutes. Finally instantly weigh the sample. Determine the overall amount of ash present in the material that has been dried by exposure to air.

$$\text{Moisture Percentage} = \frac{Pw - Fw}{w} \times 100$$

Where,
Pw = Pre-weight of Crucible
Fw = Final weight of Crucible
W = Total weight of Powdered Plant Material.

Calculation of consecutive Soxhlet extractive values: Using hexane, chloroform, acetone, alcohol, and water in a certain order, 5g of the dried and crushed material must be fully extracted in order to calculate sequential Soxhlet extractive values. The water-soluble extracts, hexane, chloroform, acetone, and alcohol should all be separated, concentrated, and dried separately. Finally, ascertain the percentage of each extraction relative to the drug that has been air-dried.

Sugar estimate entails preparing a 10% combination of plant tissue in 80% ethanol to ascertain the medication's total sugar content. To separate the supernatant, centrifugal force should be applied to the mixture at a rate of 2000 revolutions per minute for 50 minutes. After that, the supernatant is adjusted to a preset volume, usually up to 10 ml, or based on the expected sugar content. Take a 0.1 ml sample and mix it with 5 ml of concentrated H2SO4 and 0.1 ml of 80% phenol. Utilizing a spectrophotometer, find the absorbance at 490 nm after the sample has cooled. Determine the necessary percentage by computing this absorbance value.[16]

Preparation of Extract

Extraction of Ethanol: The extraction process for *Syzygium cumini, Momordica Charantia, Ocimum Sanctum, Myristica Fragrans* using ethanol and glycerol begins with drying and crushing the seed to obtain a fine powder. This powder is then powder combined with a mixture of ethanol and glycerol in a 3:1 ratio, with 100 grams of the powder mixed with 300ml. of the solvent mixture in a glass container. The blend is well mixed to guarantee even distribution of the ingredients, and it is let to stand at room temperature for a full day to promote

the release of bioactive substances, Following incubation, any solid particles are strained out of the mixture using cheesecloth or a coffee filter and disposed away. After the liquid has been filtered, the solvent is extracted and a concentrated extract is obtained by evaporating the liquid under low pressure using a vacuum pump or a rotary evaporator the main bioactive component with anti-diabetic effects, will be present in this ethanolic and glycerol extract of *Syzygium cumini, Momordica Charantia, Ocimum Sanctum, Myristica Fragrans.*[17]

Extraction of Ethyl Acetate: The extraction process for *Syzygium cumini* using ethyl acetate starts with drying and crushing the seed into a fine powder. This powder is then combined with 500 ml of ethyl acetate forevery100 grams of powder in a glass container. After giving the mixture a through stir, it is incubated for a full day at room temperature. To get rid of the solid particles, the mixture is strained through cheesecloth or a coffee filter after the incubation period. To get a concentrated extract, the filtered solution is subsequently evaporated at lower pressure. This extract is further fractionated using a basic anion exchange resin (Dark purple), which is eluted with 10% acetic acid. The resultant ethyl acetate extract has a high concentration of substances called. *Syzygium cumini, Momordica Charantia, Ocimum Sanctum, Myristica Fragrans*. These extraction techniques demonstrate how versatile it is to extract many bioactive compounds – each with unique benefits and uses – from *Syzygium cumini, Momordica Charantia, Ocimum Sanctum, Myristica Fragrans*.[18]

Phytochemical Screening: The examination of plant extracts was conducted to identify various phytoconstituents for their detection.

Detection of Alkaloids

Firstly, Each extract was separately dissolved indiluted HCl before being filtered.

Mayer's Test: In this test, the Mayer's reagent was applied to the filtrates; the presence of alkaloids was shown by the production of a yellow-colored precipitate.

Wagner's Test: In this test, the filtrates were treated with Wagner's reagent, resulting in a brown/reddish precipitate if alkaloids were present.

Hager's Test: Hager's Test revealed the presence of alkaloids when a yellow precipitate was generated by the filtrates.

Table 1: Experimental Design for Anti-diabetic Activity Assessment.

Group	Treatment Type	Dose	Number of Rats
Diabetic control (D.C)	No Treatment (Diabetic)	-	2
Standard Treatment (S.T)	Glibenclamide (Standard Anti-Diabetic Drug)	12 mg/ Day	3
F1	SC : J : OC : MC 10 : 20 : 30 : 40	125mg/Kg	6
F2	OC : J : MC : SC 20 : 20 : 30 : 30	500mg/Kg	6
F3	J : SC : MC : OC 30 : 20 : 20 : 30	75mg/Kg	6

RESULT AND DISCUSSION

Morphological Studies: The organoleptic evaluation of extracts of *Syzygium cumini, Momordica Charantia, Ocimum Sanctum, Myristica fragrans* revealed the following key findings:

These organoleptic properties provide valuable information about the physical and sensorycharacterstics of the *Syzygium cumini, Momordica, Charantia, Ocimum Sanctum, Myristica fragrans* extracts, which can be used in further phytochemical and pharmacological studies to explore the plant's potential medicinal applications.

Table No. 2: Organoleptic Evaluation of *Syzygium cumini, Momordica Charantia, Ocimum Sanctum, Myristica fragrans* Extracts.

Parameter	Syzygium Cumini	Momordica Charantia	Ocimum Sanctum	Myristica fragrans
Condition	Viscous liquid	Harvested Fresh	Warm, Humid Environments	Smooth Shiny
Colour	Reddish Purple	dark Green	Green	Dark Green
Odor	Characterized by a distinet, earthy, Sweet Aroma	Bitter	Clove, Mint and basil	Strong aromatic
Taste	Slightly sweet, slightly sour	Bitter	Spicy, slightly sweet and earthy	Sweet and slightly bitter

Texture	Solid or semi solid	Rough, Bumpy and warty	Smooth	Smooth
Nature	Solid or semi solid	Several Health benefits	Fragreant medicinal Herb	Meditional Herb
Extract Weight (g)	25	90-100 grams	114 Grams	25 Kilograms
PH	3.63-7.0	4.24 to 4.25	6 to 7.5	5.75-14
Solvent used	Ethanol	Flavonoids, Phenolics	Ethanol	Ethanol
Extraction Time	30 to 60 minute	95 minutes	4-5 hours	24 hours
Drying conditions	60^0C to 80^0C	50^0C to 60^0C	35^0C to 45^0C	40^0C to 50^0C

Determination of Extract Yield

Table No. 3: Table for yield value.

S.No.	Name of Drug	Drug taken	Yield	Yield%
1	*Syzygium cumini*	100	20	20%
2	*Momordica charantia*	100	22	22%
3	*Ocimum sanctum*	100	15	15%
4	*Myristica fragrans*	100	25	25%

Assessment of Antidiabetic effect: Results of Antidiabetic Activity Assessment of *syzygium cumini*, *Momordica Charantia*, *Ocimum Sanctum*, *Myristica fragrans* Extracts.

Table No. 4: Blood Glucose Levels.

Group	Day1	Day7	Day14	Day 21
Normal Control (NC)	92.3±4.2	94.1±3.8	93.5±4.1	92.8±3.9
Diabetic Control (DC)	301.4± 16.2	315.6± 18.4	328.2± 20.1	342.5±22.3
Standard Treatment (ST)	298.7± 15.9	212.4± 12.6	158.3±9.7	124.5±7.8
Low Dose Ethanol- Glycerol (LD-EG)	295.2± 14.8	248.3± 13.2	202.4± 11.5	178.6± 10.2
High Dose Ethanol- Glycerol (HD-EG)	292.5±15.3	198.6± 10.9	152.4±8.6	118.3±6.9
Low Dose Ethyl Acetate (LD-EA)	299.1± 16.1	232.4± 12.8	188.2± 10.7	162.5±9.3
High Dose Ethyl Acetate (HD-EA)	296.3± 15.5	205.3± 11.4	144.2±8.1	112.6±6.4

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