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Formulation and Evaluation Of In Vitro Antidiabetic Activity Of Polyherbal Capsules

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ABSTRACT
For the treatment and maintenance of postprandial blood glucose increase (i.e., diabetesmellitus), alpha (α)-amylase is a well-known therapeutic target. The present study evaluated the antidiabetic potential of polyherbal capsules formulated from ethanolic fractions of Mangifera indica, Psidium guajava, Syzygium cumini, Cassia auriculata, and Cinnamomum Verum. The extract possesses numerous classes of chemicals such as alkaloids, glycosides, tannins, polyphenols, and terpenoids, which can contribute to antidiabetic activity through alpha-amylase inhibition. The α-amylase inhibitory potential of polyherbal capsules was compared with acarbose tablets and the inhibition range of polyherbal (0.053) had favorable results as acarbose (0.056). The enzymatic activity with reducing sugar maltose was found to be 51.46 µmol/ ml. The obtained extracts were also subjected to preliminary phytochemical screening. From the present investigation, it is evident that this extract can be used for the treatment of diabetes.

Keywords: Polyherbal formulation, Antidiabetic activity, Mangifera indica, Psidium guajava, Syzygium cumini, Cassia auriculata, Cinnamomum Verum, in vitro.

I. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease, involving inappropriately elevated blood glucose levels. DM has several categories, including type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes due to endocrinopathies, steroid use, etc. The main subtypes of DM are Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM), which classically result from defective insulin secretion (T1DM) and/or action (T2DM).T1DM is characterized by the destruction of beta cells in the pancreas. T2DM involves a more insidious onset where an imbalance between insulin levels and insulin sensitivity causes a functional deficit of insulin. Insulin resistance is multifactorial but commonly develops from obesity and aging [1].

Hyperglycemia is a concern for DM patients. The pancreatic beta-cell's potential to secrete insulin can be impaired by hyperglycemia. In this setting, blood sugar levels over 180 mg/dL are commonly considered hyperglycemic. According to the International Diabetes Federation (IDF), the total adult population in the age group of 20–79 years stands at 463 million with diabetes which is set to increase to 578 million by 2030and 373.9 million Adults aged 20-79 years worldwide are estimated to have impaired glucose tolerance. There is one patient who dies of diabetes mellitus every 6s. Herbal treatments may be used to help with hyperglycemia and muscle versus fat production.[2]

Medicinal plants, minerals, and organic materials constitute the basis for many of the ancient medicines still in use today. This article focuses on Indian herbal medicines and plants that are utilized, particularly in India, to treat diabetes. A list of medicinal plants with proven antidiabetic and related beneficial effects and herbal drugs used in the treatment of diabetes iscompiled. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Approximately 800 plants have been identified in the treatment or prevention of T2DM. Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. 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.[3]

A polyherbal treatment for diabetes is preferable to a single plant because of its synergism and low risk of side effects in most conventional systems. We developed a workshop to formulate a polyherbal

International Journal of Pharmaceutical Research and Applications
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capsule for the treatment of diabetes mellitus after reading the aforementioned literature. Mangifera indica (leaves), Psidium guajava (leaves), Syzygium cumin (seeds), Cassia auriculata (flowers), and Cinnamomum verum (bark) were chosen for the formulation The potential antidiabetic activity of each of the chosen plants has been documented. In this article, we present the results of a preliminary investigation into the project, namely the in vitro alpha-amylase activity of the hydroalcoholic polyherbal extract of the chosen plants**.**

Collection of Materials

The different parts of the plants selected for the study showed antidiabetic activity. The plants used Mangifera indica(leaves), and Pisdium gujava(leaves), were collected on (Nov-Dec)around Thevur, Salem district, Tamilnadu, Indian region. Syzygium cumini(seeds), Cassia auriculata (flower), and Cinnamomum verum(bark) were collected on (Nov-Dec) from the Shree ramajayam store in Bhavani, Erode district, Tamilnadu.

Methods

Physical evaluation

According to the WHO quantitative guideline examination of ash value, the extractive value was determined.

Preparation of plant extract

The collected plant materials are washed thoroughly to remove any foreign materials or dust and shade dried for 10 days to prevent the loss of active phytochemicals from the plant. The dried plant materials are then coarsely powdered and 50gm of each plant Mangifera indica, Psidium gujava, Syzygium cumini, Cassia auriculata, and Cinnamomum verum was taken to carry out the extraction by cold maceration.

The powder of 50g of each plant was drenched in 150 ml of 99.9% ethanol in a 500 ml round-bottomed flask and kept for 72hrs for allowing total extraction at room temperature. After that, the soaked it was filtered by Whatman's filter paper no. 41. The filtrate was collectedin a porcelain dish and evaporated through a rotary evaporator. The semisolid extract was preserved and dried to remove moisture in a hot air oven.

Preliminary phytochemical screening

All the extract was subjected to preliminary phytochemical screening for the detection of various plant constituents. Different tests such as tests for alkaloids, carbohydrates, steroids, triterpenoids, cardiac glycosides, saponins, tannins, and flavonoids were performed.

Determination of Flow property

The purpose of this study was to develop an effective laboratory method for characterizing powder flow properties and correlating such properties to weight variability in filled capsules. The methods used for powder flow characterization were bulk and tapped densities, and angle of repose.

Formulation of Herbal capsules

All active ingredients were weighed according to the formula and mixed with excipients. The mixture was blended thoroughly for 30mins. Then the powder was accurately weighed and filled in the hard gelatin capsule of size "00" and then capped tightly.

Table 1: Ingredients for the Capsule formulation

SNO	ACTIVE INGREDIENTS	STRENGTH (in mg)
1.	Mangifera indica	100mg

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2520-2530 www.ijprajournal.com ISSN: 2249-7781

2.	Cassia auriculata	100mg
3.	Syzygium cumini	30mg
4.	Psidium guajava	70mg
5.	Cinnamomum verum	50mg
6.	Lactose	30mg
7.	Sodium Starch glycolate	15mg
8.	Magnesium stearate	5mg

Pharmacological Studies
Invitro Antidiabetic activity
α-amylase inhibitory assay
Reagents
Reducing Sugar (Maltose), Dinitosalicylic acid (DNS), Sodium Phosphate (Buffer), Amylase enzyme, Herbal extract (sample), and Acarbose (standard).

Estimation of Reducing Sugar
To the samples (S1, S2, S3, S4, S5), add 0.2, 0.4, 0.6, 0.8, and 1 ml of stock solution of Maltose and make up to 7 ml with water. 1 ml of DNS reagent was added. The mixture was incubated for 10mins at 100^0C and observed for color change. The tubes were cooled under running tap water. Absorbance was recorded against blank at 520nm. Blank was prepared by adding all reagents used in sample preparation except plant material. The inhibition range wasobtained using a standard curve prepared from Maltose.

3,5 *Dinitrosalicylic acid*
Maltose
incubated for 10mins > 3 *Amino* 5 ~~*Nitro Salicylic Acid*~~

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2520-2530 www.ijprajournal.com ISSN: 2249-7781

Estimation of alpha-amylase activity

To achieve different concentrations, the stock solution of plant extracts was prepared and diluted with 0.06 M sodium phosphate buffer pH 6.9. The -amylase solution in

M sodium phosphate buffer pH 6.9 was mixed with the plant extract solution. The best pH for maximal enzyme activity is the one above. After 20 minutes at 37 °C, the solution mixture was incubated at 100 °C for 10 minutes before adding the 1% starch solution with dinitro salicylic acid (DNS) solution. Maltose, a by-product of the enzymatic activity, turned the orange-red 3-amino-5 nitrosalicylic acid from the alkaline DNS's pale-yellow hue. Using a UV-microplate reader, this 3-amino-5-nitro salicylic was detected at 520 nm. The enzyme activity calculated for extract and acarbose was used to compute the percentage of enzyme inhibition. The reaction mixture including starch, -amylase, and DNS served as the control.

$$Enzyme\ activity = \frac{\mu g\ of\ product\ released}{\text{M. wt of Maltose x Incubation time}}\ X\ 100$$

Results and discussion

Determination of ash value

Table 2: Ash Values of Mangifera indica, Cassia auriculata , Syzygium cumini,Psidium guajava, Cinnamomum verum

PLANT NAME	TOTAL ASH	ACID INSOLUBLEASH	WATER SOLUBLEASH
Mangifera indica	4.80	1.80	1.04
Cassia auriculata	6.50	5.33	0.99
Syzygium cumini	11.50	2.50	5.25
Psidium guajava	7.60	6.66	2.50
Cinnamomum verum	11.70	3.76	3.43

Determination of Extractive Value

Table 3: Extractive Values of Mangifera indica, Cassia auriculata , Syzygium cumini,Psidium guajava, Cinnamomum verum

PLANT NAME	WATER SOLUBLE EXTRACTIVE	ALCOHOL SOLUBLE EXTRACTIVE	ETHER SOLUBLE EXTRACTIVE
Mangifera indica	3.4	3.2	2.8
Cassia auriculata	7.6	7.1	6.9
Syzygium cumini	3.2	4.4	3.0

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2520-2530 www.ijprajournal.com ISSN: 2249-7781

Psidium guajava	10.2	4.0	9.7
Cinnamomum verum	4.1	3.8	3.6

EXTRACTION

Table 4: Extraction Parameters.

PARAMETERS	Mangiferaindica	Psidiumguajava	Syzygiumcumini	Cassia auriculata	Cinnamomum verum
COLOUR	Green	Light green	Light brown	Tan	Reddishbrown
CONSISTENCY	Solid	solid	solid	solid	solid
% YIELD	7.03	5.4	5.96	9.5	4.12

Preliminary phytochemical screening of the extract

From qualitative screening, it was observed that the majority of the phytochemicals were present in the extract.

Table 5: Preliminary phytochemical screening of the extract

Phytoconstituents	Mangifera indica	Syzygium cumini	Psidium guajava	Cinnamomumverum	Cassia auriculata
Flavanoids	+	+	+	+	+
Tannins	+	_	+	_	+
Alkaloids	+	+	+	_	+

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2520-2530 www.ijprajournal.com ISSN: 2249-7781

Steroids	_	_	+	+	_
Glycosides	+	_	+	_	+
Saponins	+	+	+	_	+
Proteins	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Terpenoids	+	+	+	+	_
Phenolic Compounds	+	+	+	_	+

Determination of flow property

The powder extract was examined for the flow properties by determining Bulk density, Tapped density, Carr's index, Hausner's ratio, and Angle of repose.

Table 6: Parameters for Flow Property

Parameters	**Trial 1**	**Trial 2**	**Trial 3**
Bulk density($g/cm^{2)}$	0.467	0.474	0.474
Tapped density($g/cm^{2)}$	0.569	0.588	0.558

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2520-2530 www.ijprajournal.com ISSN: 2249-7781

Compressibility index(%w/w)	17.9	19.3	17.7
Hausner'sRatio	1.21	1.24	1.17
Angle of repose (degrees)	21	23	25

Pharmacological Studies
Invitro anti-diabetic activity
α-Amylase Inhibition Assay Table 7: DNS assay for Reducing sugar

S. No	**Vol. Of maltose(ml)**	**Conc of Maltose(mg)**	**The volume of Distilled Water (ml)**	**Volume of DNS(ml)**		**OD at 520nm**
BLANK	0.0	0	7.0	1.0	**Incubation for 10 minutes at 100 ^{0}C**	0.00
S1	0.2	200	6.8	1.0		0.05
S2	0.4	400	6.6	1.0		0.12
S3	0.6	600	6.4	1.0		0.18
S4	0.8	800	6.2	1.0		0.24

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2520-2530 www.ijprajournal.com ISSN: 2249-7781

S5	1.0	1000	6.0	1.0		0.30

Figure 1: Graphical representation of standard

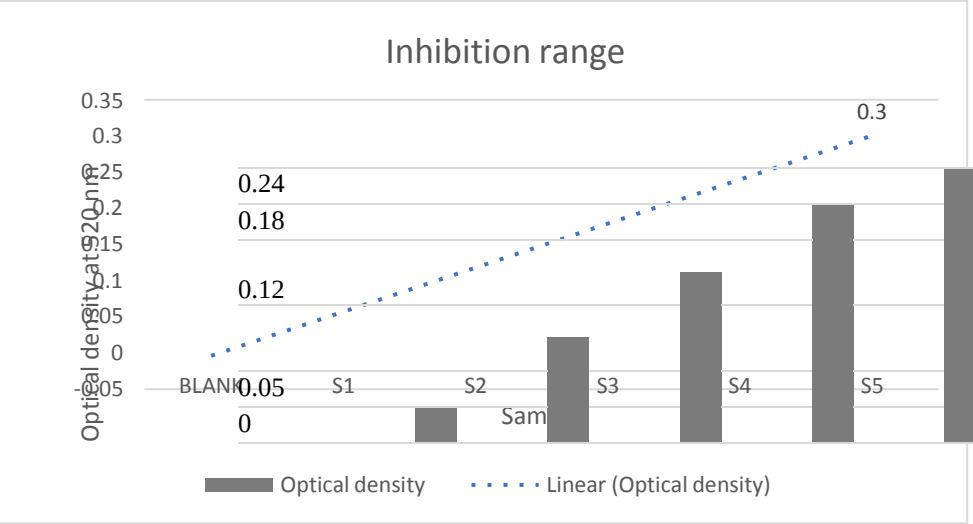

Table 8: DNS Assay of salivary amylase enzyme

Tube	Vol. of Starch (ml)	Vol Of buffer (ml)		Vol. of Sample (ml)	Vol.of Enzyme (ml)		Vol.of DN S (ml)	Vol.of Enzyme (ml)		OD at 520nm	T –C	SD
HC	0.5	5.0		1.0	-		1.0	0.5		0.00	-	
HT1	0.5	5.0		1.0	0.5		1.0	-		0.03	0.03	0.053
HT 2	0.5	5.0		1.0	0.5		1.0	-		0.08	0.08	
HT3	0.5	5.0	Pre Incubation for 5 Minutes at 37 °C	1.0	0.5	Incubation for 20 Minutes at 37 °C	1.0	-	Incubation for 10 minutes at 100 °C	0.05	0.05	
TC	0.5	5.0		1.0	-		1.0	0.5		0.00	-	
TT 1	0.5	5.0		1.0	0.5		1.0	-		0.04	0.04	0.056
TT 2	0.5	5.0		1.0	0.5		1.0	-		0.08	0.08	
TT 3	0.5	5.0		1.0	0.5		1.0	-		0.05	0.05	
NC	0.5	5.0		1.0	-		1.0	0.5		0.00	-	
NT	0.5	5.0		1.0	0.5		1.0	-		0.11	0.11	

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2520-2530 www.ijprajournal.com ISSN: 2249-7781

Figure 2: Graphical representation of Inhibition range of Sample and Acarbose.

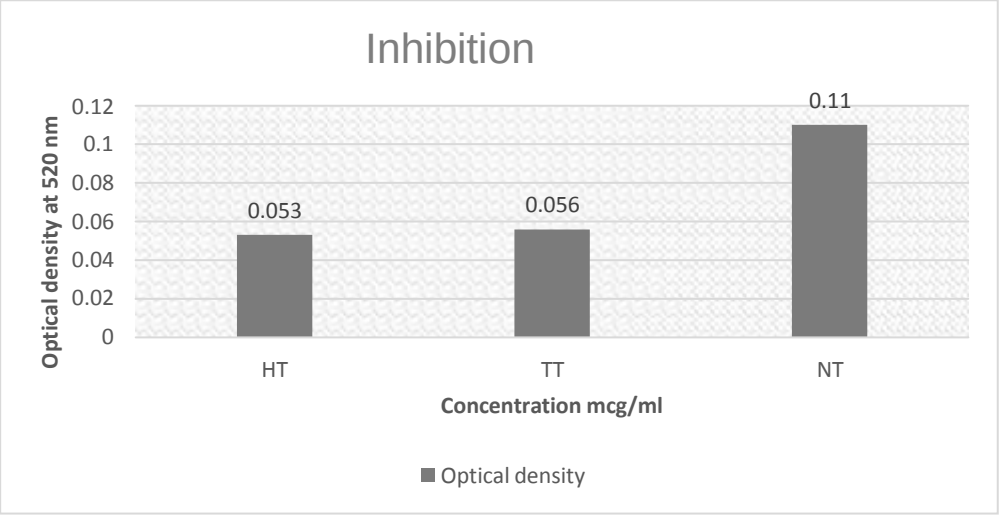

Table 9: Enzymatic Activity Acarbose vs Herbal extract

SAMPLE	µg of product released	ENZYME ACTIVITY(µmol / ml)
Herbal extract	176.6	51.46
Acarbose	186.6	54.56
Blank	366.7	107.19

II. DISCUSSION

The raw materials were first gone through physical evaluation for ash value and extractive value. It was observed that the ash values of the individual drugs were within the acceptable range, Total ash: not more than 15.0%; Acid insoluble ash: not more than 3.0% Water soluble ash: not more than 5.0 ash. This signifies the ash value determination as an important parameter to standardize herbal drugs. Extractive values for the raw materials were determined and the yield was calculated for water-soluble, alcohol-soluble, and ether-soluble extracts.

The extraction was carried out by the process of cold maceration. The extracts are observed for color, consistency, and % yield for ethanolic fraction were determined. The phytochemical screening tests also indicate the presence of alkaloids, flavonoids, Tannins, and saponins which is essential for antidiabetic activity.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2520-2530 www.ijprajournal.com ISSN: 2249-7781

The extracts are assorted and evaluated for flow property by determining parameters such as bulk density, tapped density, Hausner's ratio, and Carr's index. From the values of Hausner's ratio and compressibility index, we conclude that the powder has fair flow properties.

The extracts were finally formulated as polyherbal capsules (400mg). The polyherbal capsules are assessed for the weight variation and disintegration test. The weight variation of 20 Capsules falls under the limit of ±7.5%. Thus, none of the analyzed capsules containing powder mixtures exceeded the acceptable range.

The developed polyherbal capsules were standardized for the Disintegration test and the values were found within the standard limits of not more than 15 mins

The pharmacological activity was studied by in vitro DNS assay for reducing sugar (Maltose). The formation of 3- amino 5-nitrosalicylic acid results in a change in the amount of light absorbed, at wavelength 520nm.

Evaluating the plot of % α-amylase inhibition as a function of extract concentrations and measuring the absorbance at different concentrations. From the linear plot (fig.2), it was found that the sample extracts showed significant inhibition of α-amylase, on increasing the concentration of the extract.

The herbal extract and acarbose tablet were compared for the enzyme activity of α- amylase and it was found that the herbal extract has equivalent enzymatic activity

III. CONCLUSION

In this conclusion, the polyherbal formulation is made with 5 plants that are Mangifera indica, Psidium guajava, Syzygium cumini, Cassia auriculata, and Cinnamomum Verum containing secondary metabolites and bioactive components that have therapeutic potential. In comparison with the individual herb, the PHF has the best antidiabetic capability. The result obtained suggests that the PHF of the ethanolic extract is stated to have greater bioactive components.

The result shows that PHF has the best inhibitory impacts on alpha-amylase indicatingthat PHF has favorable activity with acarbose because of the presence of phytochemicals that act as potential alpha-amylase inhibitors including glycosides, steroids, phenol, triterpenoids, and others. The interest of alpha-amylase enzyme therapy can be utilized as an oral hypoglycemic agent to control postprandial hyperglycemia (PPHG).

Future Prospects

Toxicology tests on the aforementioned polyherbal formulations are necessary to create a formulation's safety profile. The effectiveness of polyherbal formulations as antidiabetic agents should be studied at the molecular level. It guarantees high levels of pharmacological activity in polyherbal formulations. For the disclosed polyherbal formulation, pharmacokinetic and pharmacodynamic tests may also be conducted. It is possible to undertake clinical trials for polyherbal formulations that have greater medicinal and nontoxic effects.

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