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Research

Formulation, Evaluvation And Comparison of the Poly Herbal Anti-Diabetic Tablet with Commercial Tablets

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Abstract

Diabetes, which has been known about since ancient times, is one of the oldest diseases in the world. Given what we know now, it is pretty amazing how early this illness was understood, as may be deduced from the available ancient medical texts. To effectively cure hyperglycaemia and maintain normal blood sugar levels, ancient Indian medicine made considerable use of medicinal herbs. Numerous medicinal plants have been studied recently to discover their effects and determine whether they have any ntihyperglycemic potential. An ethanolic extract of the Psidium guajava, Cassia auriculata,Andrographis paniculata was used in the current experiment to make and evaluate a polyherbal tablet. The added anti-diabetic efficacy of the enhanced formulation was investigated in an α- Amylase Inhibition Assay. Results show that the improved batch demonstrated significant activity compared to standard medicine.

Keywords: Diabetes mellitus, Polyherbal, Psidium guajava, Cassia auriculata, Andrographis paniculata, α- Amylase.

INTRODUCTION

Diabetes is a metabolic disorder that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. According to World Health Organization, there are two main types of diabetes mellitus (WHO, 2013), type 1 diabetes mellitus also known as insulin dependent diabetes mellitus (IDDM) and type 2 known as non-insulin dependent diabetes mellitus (NIDDM). Type 1 diabetes mellitus (Insulin-dependent, juvenile or childhood-onset) is characterized by deficiency in insulin production and requires daily administration of insulin. Risk factors of type 1 diabetes mellitus include genetic susceptibility, chemicals, drugs and autoimmune diseases. Symptoms normally include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, loss of weight, changes in vision and fatigue. These symptoms may occur suddenly or after some time. Type 2 diabetes mellitus (Non-insulin dependent or adult-onset) results from the body's ineffective use of insulin. Type 2 diabetes mellitus affects 90% of people with diabetes around the world (WHO, 1999), and is largely the result of excess body

weight, old age, physical inactivity, family history of diabetes, unhealthy diet and impaired glucose tolerance (IGT). Symptoms may be similar to those of Type 1 diabetes mellitus, but are often less marked. As a result, the disease may be diagnosed (Fig 1).

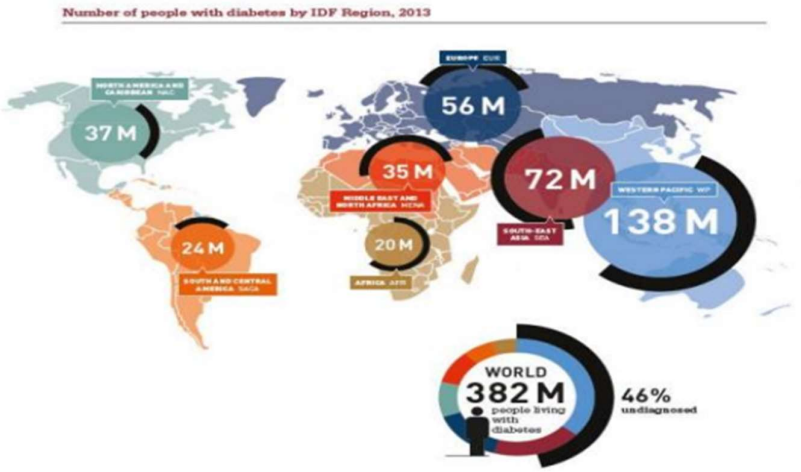


Fig 1: Global distribution of diabetes mellitus (IDF, 2013)

It is estimated that 382 million people live with diabetes in the world which represents prevalence rate of 8.3% (International Diabetes Federation, 2013). More than 80% of diabetes associated deaths occur in low- and middle-income countries (Mathers & Loncar, 2006). In Africa there are 20 million people with diabetes corresponding to a prevalence of 4.8% (IDF, 2013). It is estimated that in the next 20 years the number of people with diabetes in Africa will almost double. In Kenya, the prevalence of diabetes is 3.58% with 20,350 diabetes related deaths of patients between the age of 20-79 in 2013 (International Diabetes Federation, 2013). It is projected that diabetes will be the 7th leading cause of death in the world by 2030 (WHO, 2011).

Management of diabetes involves lowering blood glucose and risk factors associated with it. Healthy diet, maintaining healthy body weight, physical activity as well as tobacco use cessation is important to avoid complications. Feasible interventions include moderate blood glucose control. Those people having type 1 diabetes require insulin. Type 2 diabetic patients can be treated with oral medication, but may also need insulin, blood pressure control and foot care(WHO, 2013). There are two main classes of oral hypoglycaemic drugs, the sulphonylureas such as Tolbutamide and Glibenclamide, and the Biguanides such as Phenformin and Metformin. Other classes are Meglitinides, Thiazolidinedione and Alpha-glycosidase inhibitors.

Aetiology of diabetes mellitus

Diabetes mellitus is a chronic disease that occurs when the body cannot produce enough insulin or do not utilize insulin effectively (Harris et al., 1997). Insulin is a hormone produced by the pancreas that allows glucose from the food to enter the body's cells where it is converted to energy needed by muscles and tissues to function. Depending on the aetiology, diabetes mellitus can be divided into four principal forms. These are Type 1 diabetes mellitus, Type 2 diabetes mellitus, Gestational diabetes mellitus and pre-diabetes (IDF, 2013). Type 1 diabetes mellitus occurs in childhood and is primarily due to autoimmune-mediated destruction of pancreatic beta cells resulting in absolute insulin deficiency. People with type 1 diabetes mellitus must take exogenous insulin for survival (Clement et al., 2004). Type 2 diabetes mellitus is characterized by insulin resistance and/or abnormal insulin secretion. It is caused by a combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors such as obesity, lack of physical activity, stress, as well as aging (Kaku, 2010; Basu et al.,2003). People with type 2 diabetes mellitus are not dependent on exogenous insulin, but may require it for control of blood glucose levels if diet or oral hypoglycemics cannot normalize the blood glucose (Taylor, 2013; Thevenod, 2008) (Fig 2). Gestation diabetes manifest in women who develop a resistance to insulin and subsequent high blood glucose during pregnancy. Pre-diabetes occurs when blood glucose levels are higher than normal but not high enough to be diagnosed as diabetes mellitus (IDF, 2013).

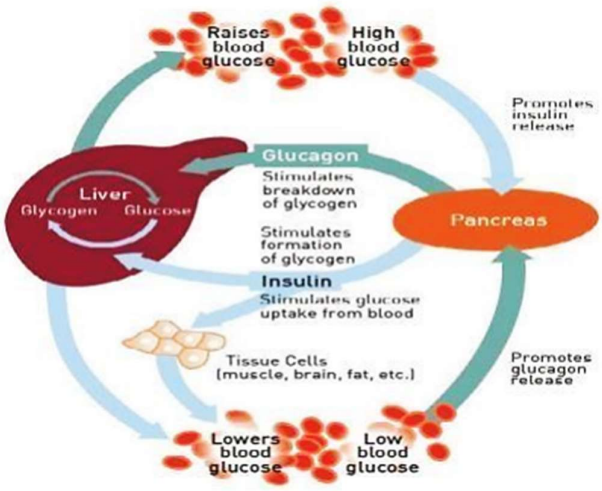


Fig 2: Insulin production and action (IDF, 2013)

Pathogenesis of diabetes mellitus

Type 2 diabetes mellitus involves at least two primary pathogenic mechanisms. These are progressive decline in pancreatic islet cell function resulting in reduced insulin secretion and peripheral insulin resistance resulting in a decrease in the metabolic responses to insulin (Boada & Moreno, 2013; American Diabetes Association, 2010). The transition from normal control of glucose metabolism to type 2 diabetes mellitus occurs through intermediated states of altered metabolism that worsen over time. As the blood glucose levels rises even a small amount above normal, then acquired defects in the glucose homeostasis system occur initially to impair the beta cell's glucose responsiveness to meals by impairing the first phase insulin response and cause increase in blood glucose into the range of impaired glucose tolerance (IGT). This rise in blood glucose and insulin resistance cause deterioration in beta-cell function and the blood glucose levels rise to full-blown diabetes (Thevenod, 2008; Leahy, 2005).

Clinical signs of diabetes mellitus

The clinical signs of untreated diabetes are related to increased blood glucose and loss of glucose in the urine. A high amount of glucose in the urine causes increased urine output and lead to dehydration which causes increased thirst and water consumption. Other symptoms of untreated diabetes include weight loss, fatigue, nausea, vomiting and patients are prone to infections of the bladder, skin and vaginal areas. Fluctuations in blood glucose levels can also lead to blurred vision and extremely elevated glucose levels can lead to lethargy and coma.

MATERIAL AND METHODS

Collection And Identification Of Herbal Plant

Psidium guajava (Guava),Cassia auriculata Linn (Senna) And Andrographis paniculata (Siriyanangai) was purchased Sri Harshini Herbali, Madurai, shade dried for a period of time according to their rate of drying to get powder.

1. *Psidium guajava (guava)*
   Psidium guajava is a common tree widely distributed as a native plant in Africa, Asia and America from Mexico to Brazil. In traditional folk medicine extracts of roots, bark and leaves of Psidium guava (family: Myrtaceae) have been used to treat gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat and inflamed gums. The leaves contain essential oil with major chemical components being alpha-pinene, beta-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, caryophyllene oxide, curcumene, crategolic and guayavolic acids
2. *Avaram (Cassia auriculata Linn)*
   Avaram (Cassia auriculata Linn), (family; Caesalpiniaceae), is also known as Avaram tree,Its flowers are irregular, bisexual, bright yellow and large (nearly 5 cm across), the pedicels glabrous and 2.5 cm long. Various phytoconstituents have been isolated from the various parts of Cassia auriculata Linn, which may be categorized as: Flower terpenoids, tannin, flavonoids, saponin.
3. *Andrographis paniculata (Siriyanangai)*
   Andrographis paniculata (Siriyanangai) commonly known as Kalmegha in Hindi, Kalamegha in Sanskrit

and Kalmegh in Bengali is an erect herb belonging to (family; Acanthaceae) which grows in many South east Asian countries and in India.The herb grows upto 3-4 feet in height, the leaves are lanceolate and 2-3 inches long.

Extract Preparation

The dried powdered plant material Psidium guajava (Guava leaves), Cassia auriculata Linn (Senna flower), Andrographis paniculata (Siriyanangai leaves) was successively extracted 70%v/v alcohol in a Soxhlet apparatus and were also prepared by using Ethanol by maceration process. The liquid extracts obtained with Ethanol solvents were collected and the consistency, color, appearance of the dried extracts and their percentage yield were noted. The extracts obtained from powder by successive solvent extraction were subjected to Physical Evaluation, Loss on Drying Ash Value.

Extraction Standardization

i) Loss On Drying
ii) Ash Value
iii) Calibration Curve

i) Loss On Drying

A sample is weighted on the analytical balance and the wet weighed is thus determined. Then the water is removed through heating the residual weighing give the dry weight and the difference between the initial weight and residual weight tells us the mass of water in sample. Then calculate the moisture content and loss on drying by following formula.

$$\textbf{\% Loss on drying} = \frac{\boldsymbol{Weight\ of\ water\ in\ sample}}{\textbf{Total weight of wet sample}} \times \mathbf{100}$$

ii) Ash Value

The method determines the amount of active constituents in a given amount of crude drugs when extracted with the solvents. The extraction process of crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents provides the preliminary information on the quality of a particular drug sample.

Alcohol Soluble Extractive value

stoppered conical flask was filled with 5 gm of powdered medication and 100 ml of 99.9% percent Ethanol. Using an electric shaker, the flask was shaken constantly for six hours before being allowed to macerate overnight. It was then filtered, and the filter was evaporated to dryness in a subsequent step. The proportion of the extractive was determined by weighing the substance.

Alcoholsoluble extractive =Wt. of extractive / Wt. of drug ×100

Water-Soluble Extractive value

In a stoppered conical flask, 100 ml of chloroform water was added to 5 grams of carefully weighed powdered drug, and the mixture was agitated continually for six hours on an electrical shaker. After maceration, the flask was allowed to sit overnight before being thoroughly filtered and dried using the filter. The proportion of the extractive was determined by weighing the substance.

Water soluble extractive=Wt. of extractive / Wt. of drug×100.

Ether soluble extractive value

The type of ether soluble extractive values determined for evaluation of crude drugs are volatile and non-volatile ether soluble extractives. The volatile ether soluble represents volatile oil content of the drug, while non-volatile ether soluble represent resin, fixed oils or colouring matter present in drugs. The percentage of ether soluble extractive was calculated.

Ether soluble extractive = Wt. of residue /Wt. ofsample x 100.

iii) Calibration Curve

Preparation of standard solutions, buffer, reagent and extract

Psidium guajava solution: Psidium guajava extract was prepared in Acid buffer of different concentration such as 5, 10, 15, 20, 25 µg/ml.

Cassia auriculata Linn (Senna) solution: Cassia auriculata Linn (Senna) extract was prepared in Acid buffer of different concentration such as 5, 10, 15, 20, 25 µg/ml.

Andrographis paniculata (Siriyanangai): Andrographis paniculata (Siriyanangai) extract was prepared in Acid buffer of different concentration such as 5, 10, 15, 20, 25 µg/ml.

The absorbance was measured at 400-200 nm spectrophotometer using UV-visible spectrometer instrument. The blank was performed using Acid buffer. The samples were performed in triplicates. The calibration curve was plotted against concentration and absorbance (Satish Kumar et al., 2008; Patel et al., 2012)

Physical Evaluation

Evaluation of physical properties on polyherbal tablet (Psidium guajava (Guava), Cassia auriculata Linn (Senna) And Andrographis paniculata (Siriyanangai)).

Making A Preparation For Polyherbal Tablets

The extracts, were shade dried for a period of time according to their rate of drying. Excipients like Starch solution, microcrystalline cellulose and magnesium stearate, crospovidone were dried. All active ingredients was weighed according to the formula, mixed with excipients. The mixture was blended thoroughly for 30mins. Then the powders was accurately weighed and filled in the compression of best formulation by using direct compression method. Various batches of tablet Psidium guajava (leaves), Cassia auriculata Linn (flower), Andrographis paniculata (Siriyanangai leaves) were prepared by direct compression technique with each batch containing tablets with 1000mg of drugs. All the ingredients were thoroughly mixed. Then the powder was passed through sieve mesh #20 to get uniform size of particles. The above powder was compressed with the help of tablet punch machine, by keeping average weight 1.15g. After compression the tablet were evaluated for weight variation, hardness, thickness, friability, disintegration and dissolution.

Evaluation Of Polyherbal Preparation (Tablet)

Appearance

The manufactured herbal tablets' appearance and colour were evaluated. In this study, colour, consistency and % yield were noted.

Pre-Compression Formulation Studies

Physical characteristics like bulk density, tap density, angle of repose, Hausner ratio and Carr's index were determined for different formulation.

Bulk Density

The term bulk density refers to method used to indicate a packing of practices or granules. The equation for determining bulk density.

$Db = M/Vb$

Where,

M is the mass of practices and Vb is the total volume of packing.

Tapped Density

The volume of the packing can be determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device that has a specially cut rotating can of weighed formulation powder was taken and carefully added to the cylinder with the aid of a funnel. The initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, giving the value of tapped density.

Angle Of Repose

Angle of repose has been used as an indirect method of quantifying powder flowability, because of its relationship with interparticle cohesion. The fixed funnel and the free standing cone method employs a funnel that is secured with its tip at a given height(H) above the graph paper that is placed on a flat horizontal surface . Powder or granules was carefully poured through the funnel until the apex of conical pile just touched the tip of the funnel. The, width R being the radius of the conical pile.

$Tan\theta = H/R$

Where,

Θ is the angle of repose.

Hausner's Ratio

Hausner's ratio been also used as indirect method of quantifying powder flowability from the bulk density.

Hausner ratio = Dt/Db.
Where,
Db= bulk density and Dt= tapped density.

Carr's Index

Carr's index has been used as an indirect method of quantifying powder flow ability from bulk density; this method was developed by Carr. The percentage compressibility of a powder is direct measure of the potential powder arch or bridge strength and stability and is calculated according to following equation.
Carr's index (% compressibility) = 100 X (1- Db/Dt)
Where,
Db = bulk density, Dt =tapped density.

Post Compression Formulation Studies

Uniformity of Weight Variation

Test of uniformity of weight is carried out to capsules to ensure accurate and consistent dosage form to be administered by patients. The percentage deviation of tablets was calculated and compared with standard specifications. Procedure Weigh individually 20 units selected at random or single dose preparations in individual containers, the contents of 20 units and Calculate the average weight. Not more than two of the individual weights deviate from the average weight by more than the percentage shown in the table and none deviates by more than twice that percentage.

Friability Test

Friability of the best formulation was (F5-P) 0.3247%. The results indicated that the friability for tablets of best formulations was below 1% (I.P. limit 1%) and hence exhibit good mechanical resistance.

Hardness Test

Tablets were selected at random from each formulation and hardness was checked using Monsanto Hardness Tester.

Disintegration test

The disintegration time of the best formulation (F5-P) tablets were determined using Disintegration test apparatus. The prepared tablet formulation showed best disintegration time in 10mins. It was lesser than 15 min, which indicates the formulation was within the acceptable limits as per IP.

Invitro Antidiabetic Activity

Invitro Dissolution Studies

Dissolution studies of formulation was carried out using USP type 2 dissolution apparatus for 30 minuts in 900ml of buffer (pH 1.2) at 50 rpm maintaining the temperature at 37±0.5°C. Sample of 1ml of each were collected over a period of 30minuts at an interval of every 5min. the withdrawn sample was immediately replaced by equal volume of fresh buffer. Collected samples were analyzed spectrophotometrically on a UV-Visivle spectrop hotometer at measured wavelength of cumulative percent drug release was calculated. A plot of cumulative % drug release v/s time in min was plotted.

Invitro Antidiabetic Activity

Determination of alpha – Amylase inhibitory activity

Principle

The α -amylase activity is measured using a colorimetric method with 3,5- dinitrosalicylic acid (DNS) reagent. In this method, starch by α – amylase is converted into maltose. Maltose released from starch is measured by the reduction of 3,5-dinitrosalicylic acid.

Starch + H2O alpha – Amylase Maltose (reducing agent) →

Maltose reduces the pale yellow coloured alkaline 3, 5-Dinitro salicylic acid (DNS) to the orange-red colored.
The intensity of the color is proportional to the concentration of maltose present in the sample. This intensity change in color is measured using a spectrophotometer as the absorbance at 540nm wavelength. Wave length is set to 540 nm because it is the region where orange-red color absorbs. This procedure applies to all products that have a specification for αamylase.

Procedure

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 7ml with water. 1ml of DNS reagent was added. The mixture was incubated for 10mins at 1000C and observed for colour 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 was obtained using standard curve prepared from Maltose.

$$\text{3,5 Dinitrosalicylic acid} \xrightarrow[\text{Incubated for 10mins}]{\text{Maltose}} \text{3 Amino 5 Nitro Salicylic Acid}$$

Estimation of alpha- amylase activity

To achieve different concentrations, the stock solution of plant extracts was prepared and diluted with acid buffer pH 1.2. The -amylase solution in acid pH 1.2 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 dinitrosalicylic 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-nitrosalicylic was detected at 520 nm. The enzyme activity calculated for extract and glucose was used to compute the percentage of enzyme inhibition. The reaction mixture including starch, amylase and DNS served as the control.

$$\text{Enzyme activity} = \frac{\text{µg of product Released}}{\text{M.Wt. of Maltose x Incubation time}} \text{ X 100}$$

Accelerated Stability Studies

The stability properties of a medication dosage form may be affected by several environmental factors during storage, including temperature, light, air, humidity, and the components of the packaging. The various formulations underwent accelerated stability testing for a duration of three months under certain temperature circumstances. These parameters included room temperature (25 ± 2^{oC}) with a relative humidity of 60%, as well as temperatures of 5^{oC} under ambient conditions and $40^{o}C$ with a relative humidity of 75%. The study examined many factors, including colour, odour, and texture of the tablets, as well as average weight, hardness, friability, and disintegration time. These parameters were investigated at accelerated temperature circumstances.

RESULTS AND DISCUSSION

Physical Evaluation

Loss on Drying

Loss on drying for the raw materials was done. The results obtained and the standard values are given Table 1.

Table: 1 Loss on drying

S.no	Plant Name	Lod (%W/W)
1	Psidium guajava	10%
2	Cassia auriculata	5.8%
3	Andrographis paniculata	12%

Ash Value

Ash content of raw materials was determined, the values obtained and their acceptable limits defined are given in the Table 2.

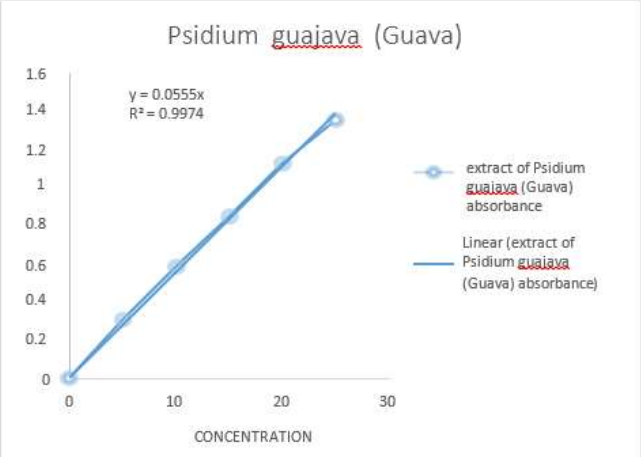
Table 2: Ash Value

Plant Name	Total Ash Value (%W/W)	Water Soluble Ash (%W/W)	Acid Insoluble Ash (%W/W)
Psidium guajava	7.60	2.50	6.66
Cassia auriculata	6.50	0.99	5.33
Andrographis paniculata	11.64	0.8	1.3

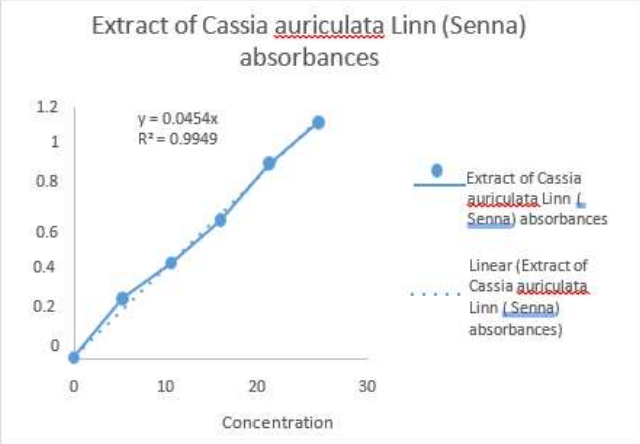
It was observed that the ash values of the individual drugs were within the acceptable range, Total ash: not more than 15.0%; Acid insoluble ash: not more than 3.0%; Water soluble ash: not less than 18 ash. This signifies the ash value determination as an important parameter to standardize the herbal drugs.

Calibration Curve

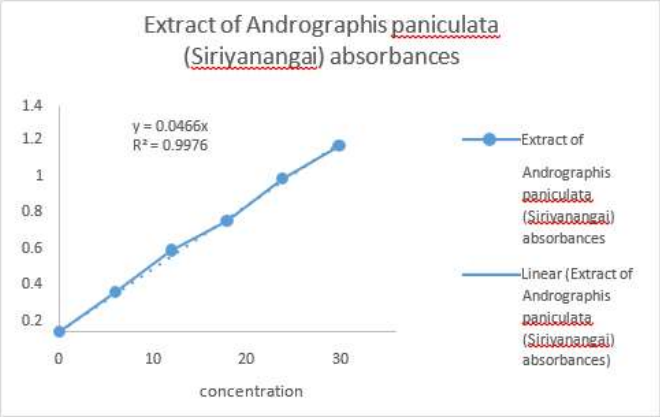
Calibration curve for Extract of Psidium guajava (Guava), Cassia auriculata Linn (Senna) And Andrographis paniculata (Siriyanangai)following as (graph: 1,2&3).



(Graph: 1, Calibration curve for Extract of Psidium guajava (Guava))



(Graph: 2, Calibration curve for Extract of Cassia auriculata Linn (Senna))



(Graph: 3, Calibration curve for Extract of Andrographis paniculata (Siriyanangai))

Extractive Values

Extractive values for the raw materials were determined and the results are tabulated in Table 3.

Table 3: Extractive values

Plant Name	Water Soluble Extractive (%W/W)	Alcohol Soluble Extractive (%W/W)	Ether Soluble Extractive (%W/W)
Psidium guajava	10.2	4.0	9.7
Cassia auriculata	7.6	7.1	6.9
Andrographis paniculata	12.24	18.45	10.89

Extraction

The extracts are observed for colour, consistency and % yield (Table 4, Extraction).

Table 4: Extraction

Parameters	Psidium guajava	Cassia auriculata	Andrographis Paniculata
Colour	Light green	Tan	Light green
Consistency	Solid	Solid	Solid
% Yield	92%	95%	88%

Development Of Formulation

The composition of each formulation is given in Table 5.

Table 5: Development of Formulation.

Formulation Code	Plant Extract (Mg)	Starch	Microcrystalline Cellulose	Mangnisum Strearate	Crospovidone	Strength (In Mg)
Psidium Guajava (F1)	300	60	120	10	10	500
Andrographis Paniculata (F2)	300	60	120	10	10	500
Cassia auriculata (F3)	300	60	120	10	10	500
Psidium Guajava and Andrographis Paniculata (F4)	300	60	120	10	10	500

Pre Formulation Studies

The drug and the powder blends are evaluated for Preformulation parameters. The results are given in the tables Totally three trials of formulation were carried out using different choices of excipients considering different facts of manufacturing problems as well as quality defects in mind. All the resultant formulations were evaluated for their flow property Table 6.

Table 6: Pre-Formulation Studies

Plant Name	Bulk density (g/cm2)	Tapped density (g/cm2)	Compressibility index (%w/w)	Hausner's Ratio	Angle of repose (degrees)
Psidium guajava	0.467	0.588	17.9	1.21	28
Cassia auriculata	0.474	0.569	19.3	1.24	30
Andrographis paniculata	0.474	0.558	17.7	1.17	26

- The Hausner's ratio is within the range of 1.19 – 1.25.
- The Compressibility index is within the range of 16-20.
- From the values of Hausner's ratio and compressibility index, we conclude that the powder has Fair flow property.

Post Comparsion Evaluvation Studis

The formulated tablets were evaluated for general appearance such as color, size and shape and are summarized.

Weight variation

Concerning mass variation, the tablets complied with the standards accepted by the Eighth edition of Indian Pharmacopoeia (IP-2018).

Average wt of 10 tablets =450mg

The lower limit of the weight of tablets is 470mg and the upper limit of tablets is 501mg.

Therefore the weight variation of 10 tablets fall under the limit of ±7.5%.

Friability Test

Friability was found in between 0.62-0.69%. The friability value below 1% was an indication of good mechanical resistance of the tablet.

Hardness Test

Hardness of tablets was between 3.2-3.7 kg/cm2 for all the formulations.

Disintegration Test

The Disintegration Test is given in Table 7.

Table 7: Disintegration Test

S.No	Plants Name	No. of Tablets (500mg)	Disintegration Time (Min) Initial Time
1	Psidium guajava	6	12 sec
2	Cassia auriculata	6	25 sec
3	Andrographis paniculata	6	22 sec

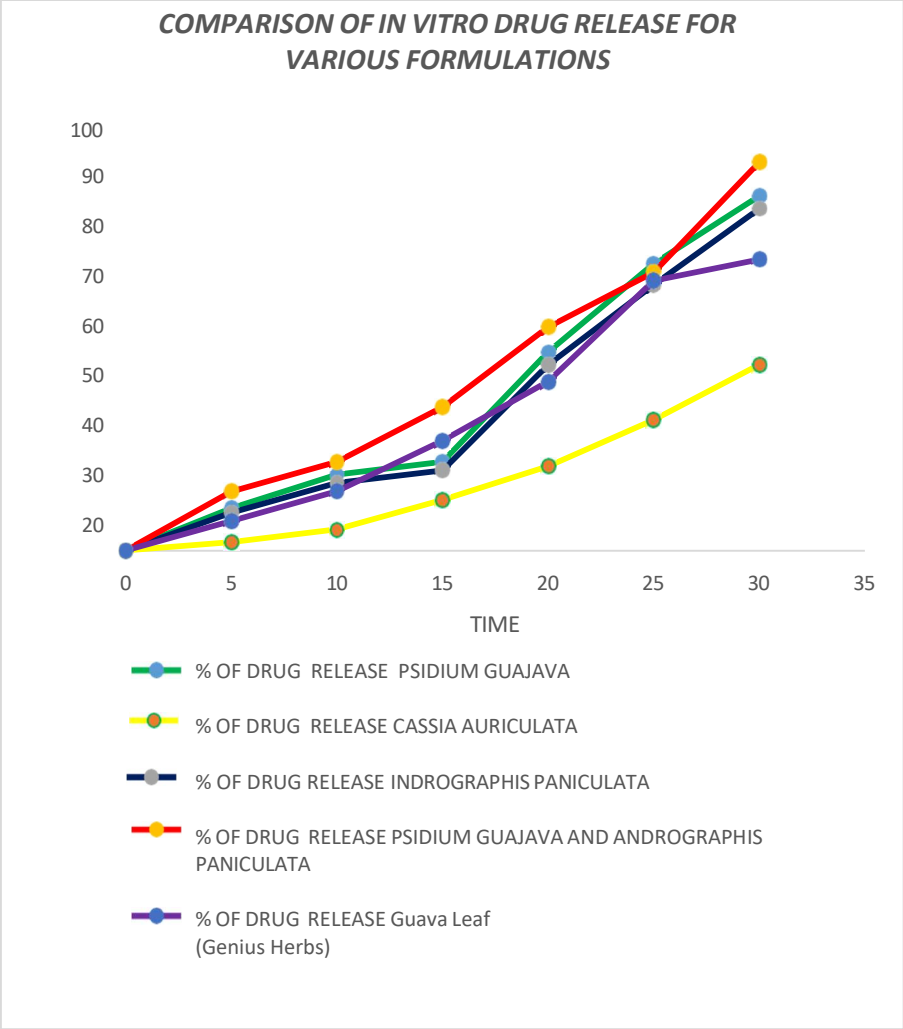
The Disintegration time for polyherbal Tablets 12, 25, 22 sec and the values were found within the standard limits.

In-Vitro Dissolution Studies

Dissolution studies of formulation were carried out and collected sampleswere analyzed spectrophotometrically on aUV-Visible spectrophotometer at measured wavelength of cumulative percent drug release was calculated. A plot of cumulative % drug release v/s time in min was plotted. Comparative study of different formulation and marketed formulation Table 8.

Table 8: *In-Vitro* Dissolution Studies

Time	% Of Drug Release Psidium Guajava	% Of Drug Release Cassia Auriculata	% Of Drug Release Andrographis Paniculata	% Of Drug Release Psidium Guajava And Andrograp His Paniculata	% Of Drug Release Guava Leaf (Genius Herbs)
5	10	2	9	14	7
10	18	5	16	21	14
15	21	12	19	34	26
20	47	20	44	53	40
25	68	31	63	66	64
30	84	44	81	92	69



Graph:4 Comparison Of *In Vitro* Drug Release For Various Formulations

Invitro Anti-Diabetic Activity
α- Amylase Inhibition Assay
The results are given in the Table 9.

Table 9: α-Amylase Inhibition Assay

S. No	Volume of Maltose (ml)	Concentration Of Maltose (mg)	Volume of Distilled Water (ml)	Volume of DNS (ml)		Colorimetry at 520nm
Blank	0.2	200	5.0	1.0	Incubation	0.00
F1	0.2	200	5.0	1.0	for 10 minutes at	0.23
F2	0.2	200	5.0	1.0	100	0.12
F3	0.2	200	5.0	1.0		0.21
F4	0.2	200	5.0	1.0		0.26
F5	0.2	200	5.0	1.0		0.16

Graph 5: Inhibition Range.

Table 10: Enzymatic activity of Samples

Sample	µg of product released	Enzyme Activity (µ mol / ml)
F1	76.24	82.98
F2	28.41	14.56
F3	70.17	79.43
F4	79.42	89.24
F5	72.78	85.45
Glucose	62.49	68.87

Accelerated Stability Studies

The results are given in the Table 11.

Table 11: Accelerated Stability Studies

Sample	Observations																	
	Initial Days						15 Days						30 Days					
	Colour	Texture	Avg.Wt %	Hardness	Friability	Disintegration	Colour	Texture	Avg.Wt %	Hardness	Friability	Disintegration	Colour	Texture	Avg.Wt %	Hardness	Friability	Disintegration
F1	Light Green	Solid	92%	(10 Tablets) 450mg	0.64%	12sec	Nc	Nc	91%	443	0.63%	11sec	Nc	Nc	90%	441	0.63%	10sec
F2	Tan	Solid	95%	450	0.65%	25sec	Nc	Nc	93%	444	0.64%	24sec	Nc	Nc	92%	442	0.63%	23sec
F3	Light Green	Solid	88%	450	0.63%	22sec	Nc	Nc	86%	441	0.62%	21sec	Nc	Nc	85%	440	0.62%	20sec
F4	Light Green	Solid	93%	450	0.65%	20sec	Nc	Nc	92%	440	0.64%	19sec	Nc	Nc	90%	439	0.63%	18sec

CONCLUSION

When the aqueous extract of (Psidium guajava (Guava), Cassia auriculata Linn (Senna) And Andrographis paniculata (Siriyanangai) were formed into the form of polyherbal tablets, it was discovered that the formulation produced effective effects. The tablets that were produced had an appropriate level of hardness and a suitable rate of disintegration. Quality control parameters according to WHO guidelines were examined such as appearance, colour, odour, ash values, extractive value and moisture content. Although the extracts showed up to have the inhibitory activity against the pancreatic enzymes in the in vitro study, but it cannot be considered effective on human beings until the pre-clinical and clinical studies are carried out which are required to prove the efficiency of the extracts. Results of the in vitro study can be considered as the background highlights for the future investigations of the herbal extracts which can be developed to the medicinal value ingredient for treatment and prevention of diabetic.

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