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FORMULATION AND EVALUATION OF ANTIDIABETIC TABLET FROM CNIDOSCOLUS ACONITIFOLIUS LEAF EXTRACT

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ABSTRACT Diabetes is a growing health concern worldwide, leading researchers to explore natural alternatives for managing blood sugar levels. Cnidoscolus aconitifolius, commonly known as Chaya Mansa, is a plant known for its potential antidiabetic properties. The purpose of this study was to create, develop and assess antidiabetic tablets from the leaf extract of Chaya Mansa. The study utilized the direct compression method to form the tablets and focused on optimizing their properties, particularly their physical characteristics and their effectiveness in managing Diabetes compared to the standard drug Acarbose. Various physicochemical properties were assessed. Preformulation studies were done to evaluate pre-compression parameters of powder blends. Compatibility of the drug with excipients was deterimined by FT-IR spectral analysis. Three different tablet formulations, labeled F1, F2, F3 were created using the direct compression method and then evaluated based on several physical properties & organoleptic characteristics. Additionally, the tablet formulations were tested for their in vitro anti diabetic activity using Alpha amylase enzyme inhibition method. However, among the three formulations, F3 emerged as the most promising candidate, demonstrating effective physical properties and superior in vitro anti diabetic activity compared to other formulations.

KEYWORDS : Cnidoscolus aconitifolius, Chaya Mansa, Diabetes Mellitus, FT-IR Spectroscopy, Anti diabetic tablets, In vitro Antidiabetic activity, Alpha amylase inhibition assay

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder, affects millions worldwide, has emerged as a major global health concern[1]. India's diabetes prevalence is one of the highest in the world, earning it the dubious distinction of being the "Diabetes capital of the world"[2].The southern states of Kerala, Karnataka & Tamil Nadu have the highest rates of Diabetes[3] .The search for effective and safe anti diabetic treatments has led to increased interest in natural products. Traditional medicinal plants, rich in bioactive compounds, offer promising solutions. Cnidoscolus aconitifolius, a plant commonly used in folk medicine, also known as Chaya or Tree Spinach, is a perennial shrub that belongs to the family Euphorbiaceae. It is native to the Yucatan Peninsula in Mexico and is widely cultivated in various tropical and subtropical regions around the world. Chaya is a medium sized shrub that can grow up to 3-6 meters in height. Chaya leaves are the primary edible part of the plant. They are rich in nutrients and have been used as a traditional food source for centuries. Chaya possess antidiabetic, antioxidant, anti-inflammatory properties. This plant's leaf extract has shown promising in vitro anti diabetic activity, warranting further investigation.[4,5,6]

This study aims to formulate and evaluate anti diabetic tablets from Cnidoscolus aconitifolius leaf extract, exploring its potential as a complementary therapy for diabetes management. The specific objectives are:

- To extract and characterize the bioactive compounds from Cnidoscolus aconitifolius leaves.
- To evaluate the antidiabetic activity of leaf extract and to formulate anti diabetic tablets using the extract.
- To evaluate the physicochemical properties, organoleptic characteristics, pre-compression and post-compression parameters of the formulated tablets.

MATERIALS & METHODS

Collection of Plant Material

The fresh leaves of Cnidoscolus aconitifolius was collected from Kasaragod district, Kerala, India. The plant material was identified and authenticated by Dr. Harikrishnan. E, Assistant Professor, Department of Botany, Payyanur College, Payyanur, Kannur, Kerala.

Chemicals/Solvents Used for Formulation

Ethanol was procured from Excise department, Kasargod. Microcrystalline cellulose & Magnesium stearate from Burgoyne Burbidges & Co, Mumbai; Lactose from Medilise chemicals, Kannur and Methyl paraben was procured from Yarrow chem. Products, Mumbai.

Determination of Physicochemical Parameters[7]:

The physicochemical parameters like total ash value, water soluble ash, acid insoluble ash, loss on drying, water soluble extractive value, alcohol soluble extractive value were determined and calculated. The standard procedures were adopted to perform the study.

Preliminary Phytochemical Screening[11-13]:

The coarsely powdered leaves of Cnidoscolus aconitifolius were macerated with solvents like petroleum ether, ethanol and water separately. The extract obtained after maceration process was then used for phytochemical screening to detect chemical constituents like Alkaloids, Carbohydrates, Proteins, Amino acids, Glycosides,Tannins, Phenols, Phytosterols and Flavonoids. The standard procedures were adopted to perform the study. Preliminary phytochemical screening of extract in different solvents was carried out to detect various biologically active constituents. From the result, the water gave more active constituents. Hence for maceration water was taken as solvent.

Drug Excipient Compatibility Studies[14,15]

The drug and excipients should be compatible with one another to produce stable, efficacious, attractive and easy to administer and safe dosage form. Hence FT-IR spectra of pure drugs were taken and compared with the different excipients. The drugs in the formulation never should react with the excipients under experimental condition and it should not affect the shelf life of product. This is confirmed by Fourier Transform Infrared Spectroscopy (FT-IR). It is a powerful technique for functional group identification of the molecules hence the chemical interactions of drugs and drugs with other excipients.

Method

The sample was taken for FT-IR study. IR spectra of drug in KBr pellets at moderate scanning speed between 4000-400 cm^{-1} was carried out using FT-IR. The peak values (wave numbers) and the possibility of functional group are shown in spectra which compare with the standard value.

Preformulation Studies Of Herbal Tablet Blends[17-19]

Angle Of Repose:

Angle of repose is determined by using the funnel method. Angle of repose was calculated from the following formula.

$tan\theta = {}^{h}/_{r}$

$\theta = tan^{-1}(h/r)$

where,

θ = Angle of repose

h = Height of powder cone formed

r = Radius of cone formed

Carr's Compressibility Index

It helps in measuring the force required to break the friction between the particles and the hopper. It is expressed in %.

Carr's compressibility index I(%) = Dt-Db/Dt x 100

Where, Dt = Tapped density

Db = Bulk density

I = Carr's index

Hausner's Ratio:

It is the measurement of frictional resistance to the drug. Hausner's ratio is the rate of tapped density to the bulk density. It is determined by using the following formula;

Hausner's ratio = Dt/Db

Where, Dt = Tapped Density; Db = Bulk density

Bulk Density:

Bulk density is determined by pouring a weighed quantity of granules in to a graduated cylinder and measuring the volume and weight.

Bulk density (Db) = weight of the powder/volume of the packing.

Tapped Density:

Tapped density is determined by placing a graduated cylinder, containing a known mass of granules..

Tapped density (Dt) = weight of the powder/Volume of the tapped packing.

Porosity:

The porosity is expressed as;

Porosity = $V_0 - V_1/V_0$

Where: V_0 = Bulk volume

V_1 = True volume

Formulation Of Cnidoscolus Aconitifolius Tablet[16-18]:

Antidiabetic tablets containing Chayamansa leaf extract were prepared by Direct compression method. Other ingredients such as lactose used as diluents, Magnesium stearate as lubricant and Microcrystalline cellulose act as diluents and disintegrants. Methyl Paraben act as preservatives. All the excipients along with API weighed as shown in table and passed through sieve no.22. Then, all ingredients were mixed following geometric mixing excluding glidant and lubricant thoroughly for 15 minutes. Glidant and lubricant were later added and compressed into a 400mg tablet using Tablet Punching machine.

Tableno1:- Composition Of Chaya Mansa Tablet

SI.NO	INGREDIENTS	F1(mg)	F2(mg)	F3(mg)
1	Drug	350	340	330
2	MCC	20	25	30
3	Lactose	15	20	25
4	Magnesium stearate	10	10	10
5	Methyl paraben	5	5	5

Evaluation Of Herbal Tablet[20-27]

All the formulated tablet were subjected to the following evaluation parameters.

- **General Appearance:**

The general appearance involves the measurement of tablet size, shape, colour, presence or absence of odour, taste, surface texture and consistency of identification marks.

- **Tablet Size And Thickness:**

The tablet's diameter size and punch size depends on the die punches selected for making the tablet. The thickness of tablet is measured by Vernier Callipers scale. Tablet thickness should be controlled within ±5%.

- **Hardness Test:**

Hardness is determined by using Monsanto hardness tester. It has a graduated scale which gives the reading in Kg/Sq cm. Hardness of 5Kg is considered as suitable for handling the tablet.

- **Friability Test:**

Friability test is done to evaluate the ability of tablet to withstand the abrasion in packing, handling and transporting. Friability is determined by Roche friabilator. Compress tablet that lose less than 0.5 to 1.0% of the tablet weight are considered acceptable.

- **Average Weight Of Tablets:**

Take randomly 3 tablets, weigh accurately and calculate the average weight by the equation:

Average weight = Total weight of 20 tablets/20

Weight Variation Test:

It is desirable that all the tablets of a particular batch should be uniform in weight. If any variation is there, that should fall within the prescribed limits:

Table no:2:- Percentage Deviation In Weight Variation

Average weight of the tablet	Percentage deviation
130 mg or less	±10%
More than 130 mg and less than 324 mg	±7.5%
More than 324 mg	±5%

- **Disintegration Test:**

This is one of the important quality control test for disintegrating tablets. Place one tablet in each of the 6 tubes of the basket, and if the tablet has a soluble external coating, immerse the basket in water at room temperature for 5 minutes. Then add a disk to each tube, suspend the assembly in water maintained at 37±2°C and operate the apparatus for 60 minutes or as indicated in monograph. The tablets pass the test if all 6 tablets are disintegrated. In case any of the tablet has not disintegrated, repeat the test on further 6 tablets replacing water with 0.IN HCl. The tablets then pass the test if all the 6 tablets have disintegrated in the acid medium. The time in seconds taken for complete disintegration of the tablet with no palpable mass remaining in the apparatus was measured and recorded.

In-vitro Anti-diabetic Activity[29-31]

Inhibition Of Alpha Amylase Enzyme:

A starch solution (0.1% w/v) was obtained by stirring 0.1g of

potato starch in 100 ml of 16mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg if alpha-amylase in 100 mol of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartrate solution and 3, 5- dinitro salicylic acid solution 96mM. Both control and plant extracts were added with starch solution and left to react with alpha-amylase solution under alkaline conditions at 25°C. The reaction was measured over 3 times. The generation of maltose was quantified by the reduction of 3, 5- dinitro salicylic acid. This reaction is detectable at 540 nm.

The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (1%) was calculated by, I%= (Ac-As)/Ac × 100, (Shai et al., 2010).

Where Ac is the absorbance of the control and As is the absorbance of sample. Statistical Evaluation: The data were statistically analyzed by one way ANNOVA followed by dunnet's t-test and values were considered significant. And value were expressed + SEM. And $p<0.001$.

RESULTS AND DISCUSSION

Cnidoscolus aconitifolius leaves are used in traditional medicine and is believed to have anti diabetic property. From the present study it was obvious that the extract has got significant antidiabetic potential and thus formulated the herbal tablets and evaluated them.

Pharmacognostic Studies:

Morphological Studies

The leaves of Cnidoscolus aconitifolius are large, deeply lobed,and palmately compound. The leaves are alternate, with long petioles (leaf stalks). Each leaf is composed of 3 to 7 lobes with serrated or toothed edges. The leaf blades are generally ovate to triangular in shape and can measure around 6 to 8 inches (15 to 20 centimeters) in diameter.

Table No3:-physicochemical Parameters Of Cnidoscolus Aconitifolius

Test	Value (%)
Total ash (% w/w)	13.50
Acid insoluble ash (%w/w)	02.00
Water soluble ash (% w/w)	08.00
Water soluble extractive value (%w/w)	10.15
Alcohol soluble extractive value (%w/w)	04.25
Moisture Content (%w/w)	10.56

Table No.4: Phytochemicals Present In Petroleum Ether, Ethanol And Water Extracts Of Leaves Of Cnidoscolus Aconitifolius

Sl.NO	Chemical constituents	Petroleum ether extract	Ethanol extract	Aqueous extract
1	Alkaloids	+	++	+
2	Carbohydrate	_	+	++
3	Proteins & amino acid	_	++	+
4	Glycosides	_	+	++
5	Tannins and phenols	_	+	++
6	Phytosterols	+	+	++
7	Flavonoids	_	+	++

Preliminary phytochemical screening of extract in different solvent was carried out to detect various biologically active constituents. Tannins and flavonoid components present in the plant reported to have antidiabetic activity. Upon basis of preliminary phytochemical studies, most of the chemical constituents are present in the aqueous macerated product. Hence the extraction of plant materials was carried out by water as solvent. The Cnidoscolus aconitifolius extract was green in colour and nature of the extract was semisolid.

Drug Excipient Compatibility Study

Compatibility of the drug with excipients was determined by FT-IR spectral analysis.The FTIR spectra of plant extract is given as follows:

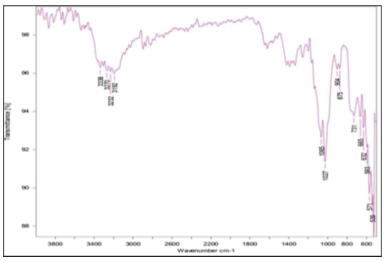

Figure No 1: IR Spectrum Of Lactose + Cnidoscolus Aconitifolius Leaf Extract

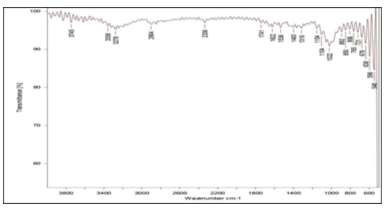

Figure No 2: IR Spectrum Of Methyl Cellulose + Cnidoscolus Aconitifolius Leaf Extract

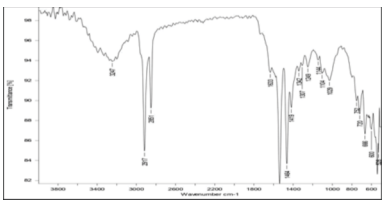

Figure No 3: IR Spectrum Of Magnesium Stearate + Cnidoscolus Aconitifolius Leaf Extract

FTIR spectra of the mixture were compared with FTIR spectra of pure samples, FTIR spectra of the mixture showed all the relevant peaks of individual components. All the characteristics peaks of herbal extracts were present in the spectra thus indicating compatibility between the drugs and polymers. The results of FT-IR suggest the absence of any chemical interaction between the drug and the excipients which confirmed that there were no significant changes in the chemical integrity of the drug.

Preformulation Studies Of Herbal Tablet Blend:

The powder indicated passable flowability with 32.56±0.142 to 35.92±0.278 angle of repose. Carr's index value was found in the range of 10±0.341 to 22.01±0.298 indicated excellent and passable flowability. Hausner's ratio was found in the range of 1.11 ±0.013 to 1.32±0.016 , which is within the limits as per Indian Pharmacopoeia(2010). These studies showed better compressibility of the blend which is suitable for tableting.

Table No.5: Evaluation Of Flow Properties Of Tablet Blends (Angle Of Repose, Carr's Index, Hausner's Ratio)

Sl.NO	Formulation code	Angle of repose	Carr's index	Hausner's ratio
1	F1	35.92±0.278	22.01±0.298	1.32±0.016
2	F2	34.12±0.282	15.21±0.482	1.17±0.018
3	F3	32.56±0.142	10±0.341	1.11±0.013

Evaluation Of Physical Properties Of Tablet Blend

The powder was evaluated for bulk density, tapped density and porosity. They were found in the prescribed range. Bulk density value was found to be in the range of 0.3615±0.013 to 0.4312 ±0.015. Tapped density value was found to be in the range of 0.4032±0.002 to 0.6451±0.007. Porosity value was found to be in the range of 0.1111±0.006 to 0.3333±0.004 (Table no.6). After evaluation of precompression parameters,

it showed that there is no presence of moisture in the powder blend and showed the uniformity of powder blend. After the study of flow rate, it concludes that powder blend exhibit optimum proportion that leads to maximum flow rate. So the result showed that the powder has the good flowing property which does not affect the process of tablet punching.

Table No.6: Evaluation Of Physical Properties Of Tablet Blend (bulk Density, Tapped Density, Porosity)

Sl. NO	Formulation code	Bulk density (g/cm3)	Tapped density (g/cm3)	Porosity
1	F1	0.4312±0.015	0.6451±0.007	0.3333±0.004
2	F2	0.3922±0.009	0.4635±0.006	0.1428±0.003
3	F3	0.3615±0.013	0.4032±0.002	0.1111±0.006

Formulation of Cnidoscolus Aconitifolius Tablet

The antidiabetic tablets of Chayamansa leaf extract were prepared by direct compression method. The plant extract was mixed with excipients and compressed in to tablet by using 14 station tablet punching machine. All the excipients along with API were thoroughly mixed and passed through sieve no.22 to get uniform size of particles. Glidant and lubricant were later added and compressed into a 400 mg tablet using tablet punching machine.

Figure No:4 Antidiabetic Herbal Tablet

Evaluation Of Herbal Tablet

Chaya Mansa tablets were evaluated based on their general appearance, thickness, hardness , weight variation, friability and disintegration.

General appearance of all the formulations were greenish in colour, have characteristic odour and taste, and a smooth surface.

Table No.7: Physical Evaluation Of Tablet Formulation

Sl. No	Formulation code	Weight variation (%)	Hardness (kg/cm2)	Friability (%)	Thickness (mm)	Disintegration time (min)
1	F1	1.238±0.02	4.21±0.03	0.370±0.0	4.67±0.02	13.15±0.01
2	F2	1.219±0.01	4.50±0.02	0.412±0.0	4.73±0.01	13.26±0.02
3	F3	1.233±0.02	4.71±0.04	0.530±0.0	4.96±0.02	14.56±0.01

The tablets were evaluated for % weight variation, hardness, friability, thickness and disintegration time. They were found in the prescribed range. Weight variation value was found to be in the range of 1.219±0.01 to 1.238±0.02%. Hence tablet passes the test for weight variation. Hardness value was found to be in the range of 4.21±0.03 to 4.71±0.04 Kg/cm^2. Hardness is within the limit. Friability value was found to be in the range of 0.370±0.0 to 0.530±0.0 %. The tablet passes the test of friability, because % friability is less than 1% of initial tablet weight. Thickness (4.67±0.02 to 4.96±0.02mm) and Disintegration time(13.15±0.01 to 14.56±0.01 min)showed that the tablets have enough strength to withstand physical abrasion. All the formulations prepared were evaluated for various post-compression parameters and showed satisfactory results. F3 has been found to be the most acceptable one in terms of pre-compression parameters and postcompression parameters (Table no.7).

In Vitro Antidiabetic Test:

Alpha Amylase Inhibition Assay:-

Alpha amylase is responsible for hydrolyzing dietary starch which breaks down into glucose prior to absorption. Inhibition of alpha amylase can lead to reduction in post prandial hyperglycemia in diabetic condition. In the present study the active components in the extract compete with the substrate for binding to the active site of the enzyme thereby preventing the breakdown of oligosaccharides to disaccharides. In this study, the plant extract showed potent inhibition of alpha amylase compared to that of standard drug Acarbose. There was a dose dependent increase in the percentage inhibitory activity against alpha amylase enzyme by both standard drug Acarbose and Cnidoscolus aconitifolius test samples. IC_{50} vaue of standard drug Acarbose was found to be 19.15 µg/ml and that of Cnidoscolus acontifolius aqueous extract is 365.5µg/ml.

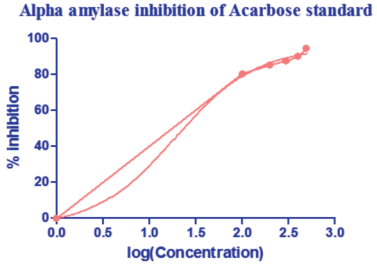

Figure No. 5: Standard Graph Of Acarbose

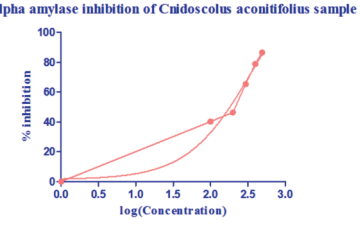

Figure No.6: Percentage Inhibition Of Aqueous Extract Of Cnidoscolus Aconitifolius Leaves By Alpha Amylase Activity.

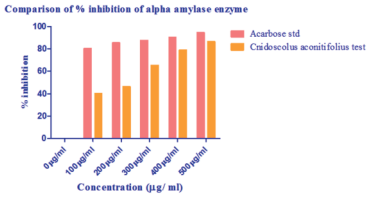

Figure No.7:comparison Of Alpha Amylase Inhibition Assay

CONCLUSION

This study has demonstrated the potential of Cnidoscolus aconitifolius leaf extract as a natural anti diabetic agent. The formulation and evaluation of this tablet have shown promising results. However, further studies are necessary to fully elucidate the antidiabetic potential, including its long term efficacy, safety and potential interactions with other medications. Additionally, studies on the extract's bioactive compounds and their mechanisms of action may provide valuable insights into its antidiabetic effects.

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