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Development of multicomponent formulation of herbal drugs for evaluation of Antidiabetic activity

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ABSTRACT

The study was designed to evaluate the antidiabetic potential of formulation of hydro-alcoholic extract of Luffa acutangula and Madhuca longifolia. The formulation was evaluated in the alloxan monohydrate induced diabetic model. Graded doses of the "hydroalcholic extract" were administered to "normal" and "experimental" diabetic rats for 10 days. Significant ($p < 0.05$) reduction in fasting blood glucose levels were observed in the normal as well as in the treated diabetic animal. The formulation treated diabetic rats were compared with diabetic control and normal animals. Positive results were obtained in the observed parameters, thereby favouring the use of the plant in the "indigenous system" of medicine. The extract were designed in solid dosage form (Tablet) and standardized as per the pharmacopoeial standards. Antidiabetic potential of formulation of hydro-alcoholic extract (Luffa acutangula and Madhuca longifolia), evaluated. Appreciable hardness characteristics (3.24±0.58), shown by formulation "which facilitates its fast disintegration". The friability of formulated tablets were (0.28±0.02) indicated that the tablets were mechanically stable. The average weight of tablets were [340 mg], and the acceptable weight variation range was (±7%). Hence the entire formulated tablet passed the weight variation test. The disintegration time of formulations was more than (3.01±1.3) minute. Phytomedicines offer greater advantage over synthetic drugs as they are cheaper and show no side effects.

Keywords - Antidiabetic potential, *Luffa acutangula*, *Madhuca longifolia*, hydroalcholic extract

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INTRODUCTION

In plants chemical compounds mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs, thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects.From the time immemorial, plants were used as medicine. To discover future medicines, ethnobotany (the study of traditional human uses of plants) is recognized as an effective tool. From "ethnomedical" plant sources 122 compounds were identified in 2001, that were used as modern medicine, about (80%) of the plants had an ethnomedical use identical or related to the current use of the active elements of the plant [1].

Luffa acutangula (Family: Cucurbitaceae) is commonly known as Ridge gourd. It is a widely growing vegetative climber. The fruits are base ball club shaped. Various pharmacological activities include hepatoprotective activity, antidiabetic activity, antioxidant activity, fungistatic poperty, CNS depressant activity.

In northern, central and southern part of peninsular India, Sri Lanka and Burma distribution of *Madhuca longifolia* belonging to family Sapotaceae occurs. There are two varieties of *Madhuca longifolia*. First variety is "longifolia" is distributed in Sri Lanka, Southern India extending northwards to Maharashtra and Gujarat and the second variety "latifolia" is found in Central and North India and Burma. Commonly it occurs in dry mixed deciduous,dry sal,dry teak forest [2]. Best growth of tree occurs on sandy soil. On shallow, bouldery, clayey and calcareous soils, growth

of *Madhuca longifolia* trees occurs. Following are the requirements for the effective growth of trees altitude (1200 m), mean annual maximum temperature (28°C-50°C), minimum (2°C-12°C), annual rainfall (550-1500 cm). In the present study,the antidiabetic potential of the formulation of hydro-alcoholic extract of drug (*Luffa acutangula* (fruit) and *Madhuca longifolia* (stem bark) was evaluated. It was investigated that the hydro-alcoholic extracts of fruit of *Luffa acutangula* and stem bark of *Madhuca longifolia* showed significant Antidiabetic Activity.

MATERIALS AND METHODS

Collection, authentication and extraction

The fruit of *Luffa acutangula* and stem bark of *Madhuca longifolia*, collected from a village (Gajraula). All plant parts were authenticated by botanist and air-dried before pounding into the powder. A voucher specimen was also deposited in IFTM Museum (Specimen No. 97872).

A solid material containing *Luffa acutangula* and *Madhuca longifolia* was placed inside a thimble made from thick filter paper, which was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor [3] was placed onto a flask containing the extraction solvent. The Soxhlet was then equipped with a condenser. The solvent contains half of the alcohol and half of the water which was heated to reflux. The hydro-alcoholic extract run through the siphon tube reaches its top and fall in the round bottom flask again. The cycle was repeated few times until the siphon tube become transparent.

Then with the help of rotary evaporator, the solvent was evaporated yielding the extracted compound. In the thimble, non-soluble portion of the extracted compound remains and discarded.

Ant diabetic activity

In polypropylene cages 4–6 weeks old (175–200 g body weight (bw)) male wistor rats were housed at ambient temperature (22±3°C) and humidity (55±5%) in light and dark cycle (12/12). Intraperitonially, alloxon monohydrate solution (10 mg/ml) was prepared in ice cold [pH 4.5] kept in ice and was administered in 5 min to the rats at a dose of 50 mg/kg of body weight. Rats with moderate diabetes having glycosuria and hypoglycemic were taken for the experiment after [48 hr] of alloxon monohydrate administration. Rats [150-160 g] fasted overnight were used for induction of diabetes. Rats were divided into two sets - diabetic and non-diabetic. Normal control [Group I] received normal diet. Alloxan-induced rats [Group II] receiving normal diet serve as diabetic control. Alloxan induced rats[Group III] receiving Glibenclamide (synthetic antidiabetic drug) [0.5 mg/kg body] weight once a day orally for 10 days. Alloxan-induced rats [Group IV] receiving *Luffa acutangula* and *Madhuca longifolia* (1 ml) once a day orally for 10 days. After drug administration blood samples were collected through the tail vein just prior to and on day 10. The Institutional Animals Ethics Committee (IACE) approved the study protocol and Reg. No is 837/ac/04/CPCSEA [4]. The blood glucose, were determined for all the samples, as depicted in **Table 1**.

Table 1 - Comparison of blood glucose

Group	Mean±sem	Response
Control	5.31 ± 2.17	
Diabetic control	2.14 ± 0.87	
Diabetic+glibenclamide	.51 ± 0.61	***
Diabetic + extract	1.17 ± 0.48	***

ANNOVA followed by Dunnett test P< 0.001 when compared with Diabetic control

Formulation of Antidiabetic tablet

In the present study dried powder of ethanolic extract of *Luffa actangula* and *Madhuca longifolia* was formulated into tablet dosage form by wet granulation method. Formulation has the following composition as depicted in the following **Table 2**.

Table 2 - Composition on formulation ingredients

Formulation Ingredients	Composition
Luffa acutangula	100 mg
Madhuca longifolia	100 mg
Lactose	166 mg
Starch	250 mg
Dicalcium phosphate	416 mg
Gelatin	5%
Magnesium stearate	5 mg/kg

Preparation of granules by wet granulation method:

1. Starch was weighed and made into granulating liquid with water and the preservative dicalcium phosphate was added.
2. The starch emulsion along with preservative was cooked well on a water bath until translucent semisolid mass was formed.
3. The gelatin paste was prepared by using required quantity of water separately.
4. The weighed quantities of excipients were mixed thoroughly with extract, the cooked starch and gelatin paste were added slowly till the powder became a damp mass.
5. This damp mass was passed through sieve number 20 and dried in an oven at a temperature of 600°C for 3 hr, until granules were properly dried.
6. Then the dried granules were passed through sieve number 20 and were subjected to lubrication.

Lubrication

All the ingredients mentioned as lubricating agents were mixed thoroughly and sieved through Sieve No.100 and mixed with the dried granules. Finally the tablets were compressed by using single punch machine.

Evaluation of parameters

Preformulation studies [5]

Angle of repose

By using funnel method, angle of repose was determined. In a funnel,the accurately weighed blend was taken. The funnel height was arranged in a manner that the funnel tip just touches the "apex of the heap" or "head of blend". Through the funnel "the drug excipient blend" was allowed to flow freely on to the surface. **Table 3** shows the relationship between Angle of Repose and Powder Flow. The diameter of the powder cone and angle of repose were calculated by using the following equation -

Tan θ = h/r Where h = height of powder cone formed r = radius of the powder cone formed

Table 3 - Relationship between angle of repose (θ) and powder flow

Angle of Repose (θ)	Type of flow
25	Excellent
25-30	Good
30-40	Passable
>40	Very poor

Loose bulk density

By pouring a weighed quantity of blend into graduated cylinder and measuring the volume and weight .

Loose Bulk Density = Weight of the powder / volume of the packing

Tapped bulk density

A known mass of drug excipient blend was placed in a graduated cylinder. The cylinder was tapped on to a hard surface from the height of 10 cm at two second interval.Tapping was continued, "until no further change in volume was noted".

Tapped Bulk Density = Weight of the powder / volume of the tapped packing

Compressibility index

The Compressibility index of the blends was determined by Carr's compressibility index. **Table 4** shows grading of powders for their flow properties

Compressibility index (%) = (Tapped Bulk Density-Loose Bulk Density) x 100 / Tapped Bulk Density

Table 4 - Grading of powders for their flow properties

Consolidation Flow index (Carr's index)	Flow
5-15	Excellent
12-16	Good
18-21	Fair to Passable
23-35	Poor
33-38	Very poor
<40	Very Very poor

Preformulation parameters were determined for the Pharmaceutical dosage form was depicted in **Table 5**

Table - 5 Preformulation parameters

S No.	Parameters	Result
1	Angle of repose	26.30
2	Loose bulk density	0.27 g/cm^3
3	Tapped bulk density	0.36 g/cm^3
4	Compressibility index	29.62 %

Physical evaluation of Tablets
All the formulated tablets were subjected to following evaluation parameters:

Colour and appearance
For the colour and appearance – "the compressed tablets were examined".

Weight variation test
By randomly selecting and weighing 20 tablets, "the average weight was determined". Individually, each tablet was also weighed. In each case "deviation from the average weight was calculated and expressed as percentage. Not more than two of the tablets from the "sample size" deviate from the average weight by a "greater percentage" and none of the tablets deviate by more than "double that percentage".

Hardness and Friability test
By using calibrated hardness tester (Monsanto) and Roche friabilitor (4 min at 25 rpm) tests respectively, hardness and friability were tested for the tablets.

Disintegration test for tablets
Glass of plastic tube [80-100 mm] long with an internal diameter [28 mm] and external diameter [30-31 mm] fitted at the lower end with a disc of rust proof wire gauge. Six tablets were placed in the tube, the tube was raised and lowered in such a manner that the complete up and down movement was repeated [28 to 32] per min. The tablets were disintegrated when no particle remains above the gauge, which readily pass through mesh (10 mesh screen).

Thickness
The thicknesses of the tablets were evaluated by Vernier calipers.
Physical parameters observed were determined below in **Table 6**

Table 6 - Physical parameters

S.No	Parameters	Result
1	Colour	Grayish White
2	Weight variation Test	±4.24
3	Hardness	3.24±0.58
4	Friability	0.28±0.02
5	Disintegration Time	3.01±1.3
6	Thickness	0.38±0.03

RESULTS AND DISCUSSION

Antidiabetic potential of formulation of hydro-alcoholic extract of drugs (*Luffa acutangula and Madhuca longifolia),* evaluated. Appreciable hardness characteristics (3.24±0.58), shown by formulation "which facilitates its fast disintegration". The friability of formulated tablets were (0.28±0.02) indicated that the tablets were mechanically stable. The average weight of tablets were [340 mg], and the acceptable weight variation range was (±7%). Hence the entire formulated tablet passed the weight variation test. The disintegration time of formulations was more than (3.01±1.3) minute.

CONCLUSION

Phytomedicines are better than allopathic drugs in some of the respective, one of them is that they are less expensive than allopathic drugs and they also doesn,t show any side effects. The powder of fruits of *Luffa acutangula* and stem bark of *Madhuca longifolia* were extracted from hydroalcoholic solvent. The semisolid extract of fruits of *Luffa acutangula* and stem bark of *Madhuca longifolia* formulated and the antidiabetic activity was performed. The formulation of hydroalcoholic extract 100 mg/kg fruits of *Luffa acutangula* and 100 mg/kg stem bark of *Madhuca longifolia* decrease dose dependent diabetes index. Antidiabetic Potential offered by 200 mg/kg body weight of test

formulation was found to be comparable to that of standard i.e. Glibenclamide. The hydro-alcoholic extract suppressed the glucose level, hence the formulation of fruits of *Luffa acutangula* and stem bark *of Madhuca longifolia* attributed to the antidiabetic principle that are present in plants.

The formulation of fruits of *Luffa acutangula* and stem bark of *Madhuca longifolia* were design in solid dosage form i.e. Tablet and evaluated for physical parameters and standardize as per pharmacopoeial standards. Preformulation study and Physical Parameter revealed that all the values were within acceptable limit. The polyherbal formulation showed significant antidiabetic activity and the solid dosage form i.e. tablet standardize as per Pharmacopoeial standards. Moreover, further study is required to isolation, purification, and characterization of active component(s) from most active extracts which might pave a good independent and/or complementary regimen for the treatment of diabetes mellitus.

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