

FORMULATION AND EVALUATION OF HERBAL FOOD SUPPLEMENT CONTAINING LETTUCE LEAVES (PISONIA GRANDIS R.BR) EXTRACT

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Abstract

Medicinal plants have curative properties due to the presence of various complex chemical substance of different composition. PisoniagrandsR.Br belongs to family Nyctaginaceae is widely distributed throughout India, commonly known as 'Leechaikottaikeerai' and extensively used in different disease conditions. The present paper deals with formulation and evaluation (colour, moisture, weight variation, hardness, thickness, diameter and disintegration time) of herbal food supplement in the form of tablets prepared from herbal leaves extract of the selected plant. A solid pharmaceutical dosage formulation with leaves extract using various excipients by direct compression and optimization and standardization of control was performed. The present study design the concentration/activity of D-pinitol, an anti-diabetic agent present in the food supplement and physical parameter was determined at different intervals for 1 month. The physical parameters results that it has hardness of $>5 \text{ kg/cm}^2$, water absorption ratio of 65% to 80%, weight variation, thickness and diameter are within the IP limits (± 5). Then the moisture and microbial analysis shows the stability of the tablet prepared using extract remains good at room temperature for 1month. The antidiabetic activity was analysed using HPTLC method which shows that the drug rate was high in PGPET than PGEET.

Keywords: *Diabetes, PisoniagrandsR.Br, tablets, Anti-diabetic activity, CMC sodium salt, MCC, HPMC k4 and Vanilla essence, Herbal formulation.*

Introduction

In the last few years there has been an exponential growth in the field of herbal medicine both in developing and developed countries because of their natural origin and less side effects. Diabetes mellitus is caused due to deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. It is a global problems and number of those affected is increasing day by day. The plants provide a potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes(Mukund S.C., 2007). Application of medicinal plants in the control of diabetes has renewed and the WHO expert committees on diabetes recommended such as alternative treatment.

Herbal drugs are prescribed widely even when their biologically active compounds are unknown, because of their effectiveness, less side effects and relatively low costs. The objective of the present study was to develop the herbal formulation of the plant *Pisoniagrands* which has anti-diabetic activity. The herbal formulation was more reliable with the less risk of side effects than compared to allopathic system of medicine on

continued therapy. The reason for selecting *Pisoniagrandsis* for this work was that anti-diabetic activity has been reported individually in this plant. So based on the literatures collected this plant was selected for the formulation of conventional dosage of herbal tablet used for the treatment of Diabetes.

Materials and Methods

A study on formulation and evaluation of herbal supplement in the form of tablet containing *Pisoniagrandsis* leaves, the experiments trails were conducted with herbal leaves extract, Finally these tablet was granulated and compressed using cookie sheet, dried and evaluated.

Design of Experiment

Preliminary Trial

Optimization of herbal tablet ingredients and standardization of control *Pisoniagrandsis R.Br* supplement in the form of tablet.

Herbal (*Pisoniagrandsis*) tablet was formulated at 5 different combinations to optimize the control herbal tablet (Chandira et al., 2012). In the five different combinations sample B was selected as good combination by viewing the colour, odour and phytochemical present of the tablet and these samples was used as a control sample in this study.

Raw Materials

Excipients added for this study are aerosil, sodium carboxy methyl cellulose, micro crystalline cellulose, hydroxy propyl methyl cellulose K4M (Zulfeequar M.A., 2008). The leaves of the plant *PisoniagrandsisR.Br* for the formulation of herbal tablets was collected. Cookie sheet is used in this study for the compression and drying of tablets in solar drier.

Formulation of tablets containing herbal extracts using direct compression method

Weighed amount of excipients was added separately to weighed amount of herbal extracts accurately. All the materials were mixed well. Then the remaining excipient aerosil is added and mixed well until a coherent mass was obtained.

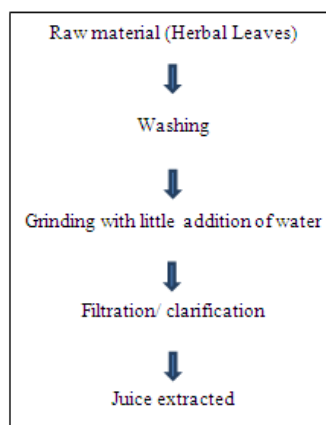


Figure 1: Flow chart on Preparation of *Pisoniagrands* Powder and Extract

Preparation of Herbal (*Pisoniagrands R.Br*) Powder and Extract

The collected plant leaves are subjected to grinding with desired water content and filtering results in juicy extract of herbal leaves. Dark greenish juicy extract is obtained. The preparation of extract for the study was carried out as detailed in the Fig.1. The formulation of herbal supplement in the form of tablets for the study was carried out as detailed in the Fig.2.

Evaluation of Formulated Tablets (Analytical Procedure)

The formulations were stored at room temperature for 1 month and analyzed the following parameters at 0th day, 10th day, 20th day and 30th day respectively.

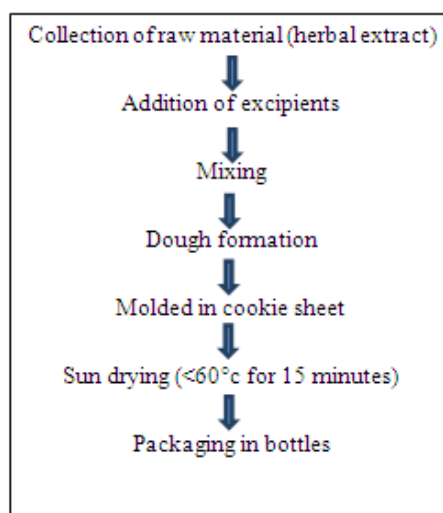


Fig.2: Flow chart on formulation of herbal tablets from its leaf powder and extract

Physical Parameters

The major physical parameters analyzed for herbal tablets prepared for the study are hardness, water absorption ratio, weight variation, thickness, diameter, disintegration time.

Hardness

Hardness of tablets was determined individually with the Monsanto hardness tester. Hardness for compressed tablet is to 3 to 5 kg/cm².

Disintegration Time

Disintegration time was measured using a modified disintegration method. For this purpose, a petri dish was filled with 10 ml of water at 37⁰C±0.5⁰C. The tablet was carefully put in the centre of the petridish and the time for the tablet to completely disintegrate into fine particles was noted. Time required for complete disintegration of six tablets was recorded. The procedure was done for tablets at different intervals and the mean disintegration time of capsules calculated.

Disintegration time: Uncoated tablet: 5-30 minutes, Coated tablet: 1-2 hours

Water Absorption Ratio

A piece of tissue paper folded twice was placed in a small Petri dish containing 6 ml of water. A tablet was put on the paper and the time required for complete wetting was measured. The wetted tablet was then weighed.

Water absorption ratio R, was determined using following equation

$$R = \frac{W_a - W_b}{W_a * W_b} \times 100$$

Where, W_a-Weight of tablet after absorption

W_b -Weight of tablet before absorption

Weight Variation Test

Twenty tablets were selected at random and their average weight was determined using an electronic balance. The tablets were weighed individually and compared with average weight.

Diameter and Thickness

Tablet diameter and thickness were also an important test, screw gauge and calipers for measurement. It can be done through using micrometer or by other device. Ten tablets from each batch were examined using micrometer screw gauge. Tablet diameter and thickness should be controlled within ± 5% variation of standard value. The thickness of tablet is measured by screw gauge. The average value and standard deviation were reported.

Proximate Analysis

Determination of Moisture (AOAC, 2000)

About 2-3g of the sample was weighed in to a pre-weighed clean petridish and was placed in a hot air oven maintained at 130°C for 1 hour. After drying petridish was cooled in desiccator and weighed. This was repeated until a constant weight was obtained moisture was expressed as percentage.

$$\text{Moisture content (\%)} = \frac{\text{Moisture loss (g)}}{\text{Weight of the sample (g)}} \times 100$$

Microbial analysis (APHA., 1984)

Sample Preparation for Microbial Analysis

Weigh 10g of the sample into a sterile blender jar or into a stomacher bag. Add 90ml of diluents i.e. physiological saline. Blend for 2 minutes at low speed (approximately 800rpm) or mix in the stomacher for 30-60 seconds (Shake vigorously). From this 1ml of 10⁻¹ dilution is mixed with 9ml of the diluents (10⁻² dilution) and so on to get appropriate dilutions for plating. This type of dilution was prepared and called as serial dilution.

Microbial Analysis of Total Plate Count and Yeast & Mould

The selective medium used was Tryptone glucose extract (TGE) agar for TPC and Potato Dextrose agar (PDA) for yeast & mould to detect the presence of TPC in the sample. The serial dilution was carried out from 10⁻¹ to 10⁻⁶. Among these serial dilutions, 10⁻¹ and 10⁻² dilutions were chosen and pipette out 1ml from these dilutions, and add respectively to marked petriplates. Then pour about 15-18ml medium of XLD and PDA agar to each of the Petri-dishes, and mix well with inoculums and allowed to set till the medium gets solidify. Then incubate the plates at 37°C for 48hrs for TPC and 5-7 days for yeast & mould .

$$\text{TPC} = \text{Average count} \times \text{Dilution factor}$$

Phyto-Chemical Parameter

The standard and sample solutions were applied to the silica gel 60 F254 TLC aluminium sheet as 6mm band using Hamilton syringe with CamagLinomat automated TLC applicator. The spots were dried in a current of air. After the sample application, the plate was developed in a camag twin trough glass tank pre saturated with the mobile phase up to 90 mm. After the development, the plate was dried in hot air and the images captured in the photo-documentation chamber under white light and in UV (254 nm and 366 nm). The developed plate was sprayed with ammonical silver nitrate and dried at 100°C for 10 minutes and documented again in day light and in UV. The retention factor (Rf) and % area was calculated using Wincats software.

Colour and Odour

Colour and odour are the important parameter for tablets. Colour was tested through naked eye and odour through untrained panelist. After storing it at room temperature for 1month, the tablets prepared from *Pisoniagrands* leaves extract was tested at different intervals.

Results and Discussion

Anti-diabetic plants play an important role in inhibiting the glucose level and inflammation thus providing protection to human against hyperglycemia. Realizing the fact this research was an attempt to formulate, develop and evaluate the anti-diabetic activity of herbal leaves extract tablets of *Pisoniagrands* R.Br and to provide as a diet supplement.

Optimization of herbal tablet ingredients and standardization of control *Pisoniagrands* supplement in the form of tablet.

Optimization technique is widely used developing optimal dosage forms and a better process of manufacture. In this study direct compression method is applied using cookie sheet as given in Table 1

Table 1: Different formulations of prepared tablets

S.No	Ingredients	Proportion of Composite Mixes				
		Sample				
		A	B	C	D	E
1.	Herbal extract	100	150	200	240	260
2.	HPMC K4M	75	50	25	15	10
3.	CMC Sodium	45	35	25	18	14
4.	Thulasi juice	3	1.15	0.25	0.5	0.75
5.	Micro-crystalline cellulose	260	250.35	240.25	220.5	210.25
6.	Aerosil	7	3.5	2	1	2
7.	Vanilla	10	10	7.5	5	3
8.	Net weight	500mg	500mg	500mg	500mg	500mg

Physical parameters

Evaluation of physical parameters of prepared herbal tablets

The results obtained on various parameters of post formulations studies of tablets were found satisfactory and shown in table 2 and 3. Hardness for tablets of two formulations was in the range of 4.1 to 4.5 kg/cm², which falls above the limit of not less than 3.0 kg/cm². The water absorption ratio of the tablets of both the formulations was found in the range of 77.1 ±3.005 and 71.76±7.24 indicating fairly acceptable tablets (Bandariet *al.*, 2008). Disintegration time is an important parameter of tablet. As a coated tablet should disintegrate within 60 min, the tablets of all the batches disintegrated within the range of 54 to 60 minutes (Athawaleet *al.*, 2011).

Sample C- Herbal extract

Sample	Hardness kg/cm ²	Disintegration time (min)	R (%)	Thickness	Weight	Diameter
PGEET	4.5	58	71.76±7.24	4.8515±0.25	0.1406± 0.02	10.2015±0.50

Table 2: Effect of Physical Parameters on Storage of *Pisoniagrandis* R.Br Extractenriched Tablet

Sample	Moisture	TPC	Yeast and Mould
PGEET	9.04±0.015	8.96±0.015	9.13±0.020

The maximum weight variation of the tablets was $\pm 3.62\%$ and $\pm 2.0\%$ which falls within the acceptable weight variation range of $\pm 5\%$, hence the tablets of all batch passed the weight variation test. The thickness of the tablets formulated was found in the range between $4.8495\pm 0.25\text{mm}$ and $4.8515\pm 0.25\text{mm}$ and diameter between $10.2115\pm 0.54\text{mm}$ and $10.2015\pm 0.50\text{mm}$ indicating fairly acceptable tablets (Lieberman *et al.*, 1990).

Moisture analysis is an important parameter of tablets to find the stability. It shows that the herbal tablets prepared using extract remains good after 1month storage at room temperature are within the limit prescribed. Similarly, microbial analysis was carried out in which TPC and yeast and mould test shows that TPC and fungi were in acceptable limits in the tablets prepared powder and extract tablets, remains good (Ramesh *et al.*, 2010). The colour and odour of the tablets are found to be characteristic (Gahlot *et al.*, 2012).

D-pinitol analysis

HPTLC Fingerprint

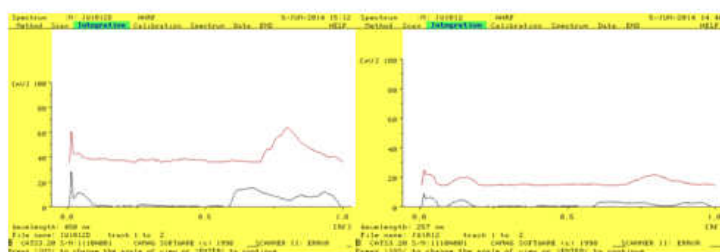


C

HPTLC Fingerprint: Derivatized with 10% sulphuric acid



C



Comparative chromatogram for sample C before and after derivatization

Phytochemical test was carried out to determine the antidiabetic activity of the supplement prepared which was performed using HPTLC method. It was found that antidiabetic activity of herbal tablet containing *Pisoniagrandis* R.Br leaves powder has reported to have more activity than herbal tablet prepared using extract(Sripathi et al., 2011).

Conclusion

Herbal products may contain a single herb or combinations of several different herbs believed to have complementary and/ or synergistic effects. Perceiving the potential of herbal plants with higher levels of therapeutic activity, the present study was undertaken with an aim to formulate and evaluation of herbal food supplement in the form of tablet of the *Pisoniagrandis* R.Br for the treatment of diabetes mellitus. From the ten combinations sample C (PGEET) were found to be the best formulations in terms of the physical and proximate parameters. And therefore, these two formulations were selected for antidiabetic activity. Finally the anti-diabetic activity was reported which was found to be significant.

Hence this study gave a support on the selected medicinal plants which ascertain its folklore uses and interplay with phytochemical compound (antidiabetic activity), lead to rapid development in herbal food supplement for diabetes treatment. Thus, this holds great promise for future research for the formulation of potent antidiabetic drug from these plants.

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