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Original Article

Formulation, Evaluation, and Stability testing of Polyherbal Antidiabetic Capsules

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ABSTRACT

Background: The phytoconstituents of single plants are inadequate to produce the desired efficacy and results. The combination of various herbal drugs in a specific proportion will improve the therapeutic efficacy by simultaneously acting on multiple targets, leading to enhanced patient compliance and therapeutic outcome. The objective of the present study was to formulate a polyherbal dosage form and to evaluate its efficacy and stability. **Materials and Methods:** The polyherbal extract was prepared by mixing different alcoholic extracts of all four drugs in a ratio of 1:1:1:1. A total of six polyherbal formulations (PHF) prepared were and evaluated for their preformulation studies. The phytoconstituents present were determined through Fourier transformer Infra-Red spectroscopy evaluation. The drug was further filled into capsules of 000 size and checked for all the evaluation parameters of capsules. The drug was checked for stability studies under various conditions. **Results:** The PHF1 showed the best flow properties. The capsules showed a drug release of 94% in 30 min. The spectroscopic results suggested the presence of terpenes, tannins, phenols, and flavonoids in granules. The capsules were found to be stable in various types of lights but get degraded at 70% humidity and temperatures above 55°C. **Conclusion:** This releasing pattern of medication from their capsule shell *in vitro* can be used to anticipate the releasing sequence *in vivo*. The Polyherbal capsule was shown to be fairly stable in light, temperature, and humidity.

Keywords: *Azadirachta indica* leaves, *Pterocarpus marsupium* heartwood, *Picrorhiza kurroa* rhizomes, *Withania coagulans* berries and fruit coat, Stability study, Dissolution, FTIR.

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INTRODUCTION

Diabetes is a serious and chronic ailment that leads to various other complications in the body like cardiovascular diseases, obesity, liver disease, and kidney problems.[1] Diabetes affects 463 million people globally, with a 9.3% occurrence rate, which is expected to reach 10.2% by 2030 and 10.9% by 2045. Asia has played a significant role in the fast-spreading Type 2 Diabetes Mellitus (T2DM) epidemic. China and India are the epicenters of the worldwide T2DM epidemic.[1,2]

Recently, a rapid expansion of different classes of modern oral antidiabetic drug therapy with diverse toxicological profiles has taken place but the need of the day is an effective but safe treatment. A safe alternative for the treatment of diabetes could be herbal drug therapy.[3]

Compared to single herbal products, all these advantages resulted in success of polyherbal formulations in the market. They show high performance, a broad therapeutic window, cost efficiency, easy availability, and minimal side effects.[4,5] As a result, determining the hypoglycaemic potential of therapeutic herbs has become critical.

Herbal drugs play a vital role in the treatment of diabetes. There is various mechanism of action acting behind the antidiabetic activity of the herbal drug. These include stimulus to the secretion of insulin from β-cells of the pancreas, enhanced binding of insulin to receptors, decreases resistance to insulin, improvement in tolerance to glucose, improvement in the metabolism of glucose, enhancing the number and activity of β-cells and increase in insulin levels in the plasma leading to decrease in levels of glucose in blood.[6] The first category is plant drugs acting like insulin and promoting regranulation of pancreatic cells and controlling the blood glucose levels: *Pterocarpus marsupium*[7,8] and herbal drugs act on the beta cells, recover them to increase the production of insulin, enhancing uptake of glucose in adipocytes e.g. *Picrorhiza kurroa*[9-11] is the second category. The third and fourth categories may be defined as herbal drugs stimulating glucose utilization

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and oral tolerance in diabetic patients: *Azadirachta indica*[8,12] and plants with hypoglycaemic potency through various mechanisms like reducing haemostasis of glucose and resistance to insulin. E.g.: W*ithania coagulans.*[13,14]

The present study involves the preparation of polyherbal extract consisting of an equal ratio of *Azadirachta indica* leaves, *Pterocarpus marsupium* heartwood, *Picrorhiza kurroa* rhizomes, and *Withania coagulans* berries and fruit coat and the formulation and evaluation of polyherbal capsules along with their stability studies. In the evaluation part, the polyherbal formulation will be evaluated for the functional groups present in the polyherbal granules using Fourier transformer infra-red (FTIR) spectroscopic method so that the constituents responsible for the antidiabetic activity of the drug can be predicted. The polyherbal granules will be evaluated for their flow properties and the best composition of the granules will be selected further for filling into the capsules. The polyherbal capsules so formulated will be further evaluated for the capsule evaluation parameters and stability studies.

MATERIALS AND METHODS

Plant collection and authentication

Herbal drugs were collected from Ropar and validated at NIPER's Natural Product Field Laboratory and Nursery, SAS Nagar, Punjab. The authenticated plants' voucher specimens were subsequently sent to the A.S.B.A.S.J.S. college of pharmacy in Bela, Ropar, and Punjab for future reference.

Extract preparation

The authenticated drugs *Azadirachta indica* (Neem) leaf, *Withania coagulans* (paneer dodi) fruit, *Picrorrhiza kurroa* (Kutki) rhizomes, *Pterocarpus marsupium* (Vijay Sar) wood were dried, and powdered properly in a grinder and were extracted using ethanol by using cold maceration method for 18h individually. The extracts so obtained were concentrated to dryness on a water bath below 60°. The dried extracts were then stored in a cool place for further usage. A polyherbal extract was prepared[15] using the above-obtained extracts by mixing them in the ratio of 1:1:1:1.

Polyherbal granules formulation

The polyherbal granules were prepared using the wet granulation method. The composition of the different polyherbal granular formulations is mentioned below in Table 1.

Pre-formulation study

The preformulation study includes evaluation of different parameters like tap and bulk density, Carr's index, Hausner's ratio, angle of repose for determining flow properties, and size of polyherbal granules.[16]

Fourier Transformer Infra-Red Spectroscopy

The IR spectrum of individual extracts and polyherbal granules was obtained using the Bruker A-E IR. The potassium bromide (KBr) was used in preparing the sample disks. The sample was observed in the range of 700-4000 cm^{-1}.

Capsule evaluation parameters

The polyherbal capsules were assessed for organoleptic characteristics, weight variation, average weight, disintegration time, moisture content, and dissolution rate.[17-19]

Disintegration time

To determine the disintegration time of capsules six random capsule samples were taken and placed in every tube of the Basket rack and placed in the beaker containing 1l medium. The medium used is Simulated Gastric fluid (SGF). The temperature of the assembly was upheld at 37°C± 2°C. To move the basket 5-6cm in height, at a frequency of 28-32 cycles per minute, a motor was utilized. If they pass through a 10 mesh screen in a certain period, the capsules have disintegrated.[18]

Preparation of Calibration Curve

For making a standard curve, a stock solution of 1000 µg/ml concentration was prepared and it was then serially diluted with water to get 0, 30, 50, 100, 200, 300, 400 up to 500 µg/ml. The absorbance of the solutions of various concentrations was taken using distilled water as blank in UV spectrophotometer at wavelength of 260 nm. The absorbance values so obtained were plotted against concentration (µg/ml).[18]

In vitro Dissolution studies

This test is used as a measure to determine the absorption rate, bioavailability, and bioequivalence of the drug dosage form. In this method, Paddle Type Dissolution Apparatus (Lab India Disso 2000- 6 basket dissolution apparatus), was used containing 900 ml of simulated gastric fluid (SGF) solution (2g sodium chloride + 3.2g Purified Pepsin (with activity 800 to 2500 units/mg of protein) +7ml hydrochloric acid (stock solution) + adequate water to make 1000 ml) in 1000ml capacity cylindrical vessel and the temperature was maintained at 36.5°C to 37.5°C. The paddle speed was maintained at 75 rpm for 30 min. At each period 5ml of the sample was taken from the vessel and the replacement of medium was done with fresh medium. The samples so taken were analysed using UV spectrophotometer at 260nm.[18]

Stability study

The stability study experiment is carried out to determine the effect of accelerated conditions on the quality of pharmaceutical formulation. In the proposed work durability of the polyherbal capsules is examined by exposing to increased temperature, humidity, and light intensity conditions. The effect of above

factors was seen on the polyherbal capsules[20] and is well depicted from the Table 2.

RESULTS

Preformulation study

The polyherbal granules prepared were tested for various parameters like particle size analysis, bulk density, tapped density, compressibility index, Carr's index, Hausner's ratio, and angle of repose. The granules cleared all the above-mentioned tests and showed good flow properties. The exact results are mentioned below in Table 3.

Fourier Transformer Infra-Red Spectroscopy

The FTIR study of alcoholic extracts of *Azadirachta indica* leaves, *Pterocarpus marsupium* heartwood, *Picrorhiza kurroa* rhizomes, and *Withania coagulans* berries and fruit coat was done using

Table 1: Composition of six formulations of polyherbal granules.

Ingredients	PHF 1 (mg)	PHF 2 (mg)	PHF 3 (mg)	PHF 4 (mg)	PHF 5 (mg)	PHF 6 (mg)
Azadirachta indica	125	125	125	125	125	125
Withania coagulans	125	125	125	125	125	125
Picrorhiza kurroa	125	125	125	125	125	125
Pterocarpus marsupium	125	125	125	125	125	125
Lactose / Mannitol	250	300	350	400	400	300
Microcrystalline cellulose	150	125	100	75	50	100
Pregelatinized Starch	75	50	25	0	25	75
Talc	20	20	20	20	20	20
Sodium Benzoate	5	5	5	5	5	5
Total	1000	1000	1000	1000	1000	1000

PHF: Polyherbal formulation

Table 2: Effect of different types of lights, temperature and humidity on polyherbal capsules.

Effect of light	Light Source	Sunlight				Fluorescence				Tube light				UV light				Infra-Red Light				Lamp Light			
	Exposure time (h)	½	1	3	6	½	1	3	6	½	1	3	6	½	1	3	6	½	1	3	6	½	1	3	6
	Degradation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Effect of temperatur	Storage condition	Time duration (h)	Time duration (h)				Results
			½	1	3	6	
	Ambient	30	-	-	-	-	No degradation in 6
	Warm (30-40 oC)	35	-	-	-	-	No degradation in 6 hrr
	Accelerated	50	-	-	-	-	No degradation in 6 hr
	Accelerated	55	-	-	-	+	Degraded after 4 hr
	Accelerated	60	-	-	+	+	Degraded after 2 hr

	Temperature (°C)	Humidity			
		30%	50%	70%	90%
Effect of humidity	30	-	-	-	-
	35	-	-	+	+
	55	-	-	+	++
	65	-	-	++	+++

(+) Degradation (-) No change

the KBr pellet method and the polyherbal granules prepared were also evaluated for FTIR study. The results so obtained are mentioned here in Figure 1.

Capsule evaluation parameters

The capsules formulated from the polyherbal granules were tested for various evaluation parameters to ensure proper quality, drug content, and drug release. Capsules are of sky blue coloured cap and transparent body filled with brown coloured powder with 000 sizes, bitter taste, characteristic odour, with an average weight of 1017 mg, weight variation of 1079-1105mg, disintegration time of 10.35 (min) and moisture content of 3.25%. The aliquots prepared were scanned at λ_{max} value 260 nm. The absorbance range was found to be 0.095-1.267. These solutions obeyed Beer-Lambert's law in the above concentration range with a regression of 0.9998.

Dissolution study

The dissolution study gives the estimate of *in vitro* drug release in SGF or SIF. It gives the pattern in which the drug gets released from its dosage form to ensure proper efficacy and quality. The dissolution profile of polyherbal antidiabetic capsules is given below in Figure 2.

Stability Study

The stability parameters analysed for 30 min, 1, 3 and 6 hr of storage at accelerated conditions of temperature, light, and humidity have been tabulated in Table 3.

DISCUSSION

Polyherbal capsules were formulated by preparing the polyherbal granules. The granules are porous and secondary particles, with different properties from the original particles in dimensions as well as in microstructure.[21] The polyherbal granules were prepared into six formulations while altering the concentrations of excipients and checked for their flow properties as well as other parameters. The angle of repose <30° designates 'excellent' flow, and>56° shows 'very poor' flow. Therefore, PHF1, PHF2, and PHF3 showed excellent flow. The Carr's index and Hausner's ratio were calculated as 10.52 and 1.10 in the case of PHF1. The lesser the value of Carr's index and Hausner's ratio, the better the flow properties. A value of Carr's index <10 or Hausner's ratio of <1.11 represents 'excellent' flow, but Carr's Index >38 or Hausner's ratio >1.60 represents 'very very poor' flow properties. Therefore, the results showed that PHF1 has 'excellent' flow properties. This represents that the selection of excipients in PHF1 was excellent as its flow properties.[20]

The excipients play a major role in the flow properties of the granules. The microcrystalline starch acted as a diluent, pre-gelatinized starch acted as a disintegrant, talc acted as a binder, and sodium benzoate was used as a preservative. The preservatives are required there in herbal formulations as they are more prone to microbial contaminations.[22]

It was found that PHF1 showed all the desired characteristics of the granules. The flow properties of PHF2 and PHF3 were also

Table 3: Evaluation parameters for polyherbal granules.

Parameters	PHF 1	PHF 2	PHF 3	PHF 4	PHF 5	PHF 6
Bulk density (g/ml)	0.47	0.52	0.5	0.45	0.55	0.43
Tapped Density (g/ml)	0.52	0.66	0.62	0.58	0.71	0.55
Carr's index (%)	10.52	21.21	19.35	22.41	22.54	21.82
Hausner's ratio	1.10	1.27	1.24	1.29	1.29	1.28
Angle of repose (^{O})	24.81	27	28.49	32	35.53	34.04

PHF: Polyherbal Formulation

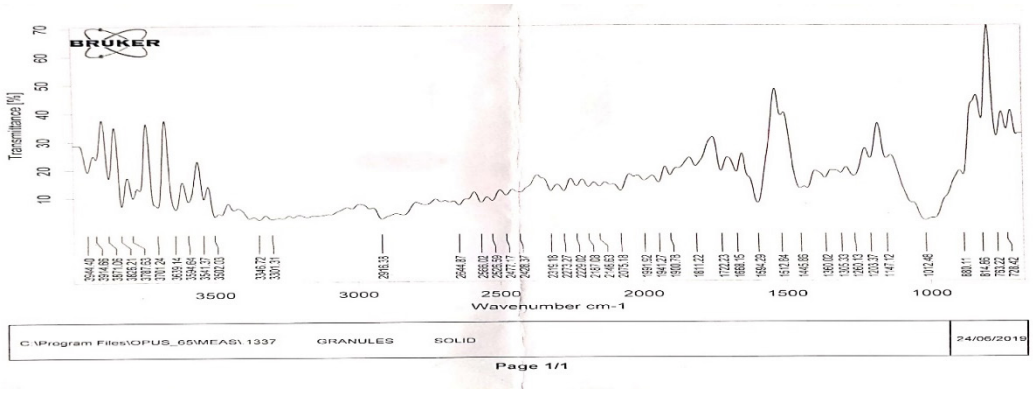

Figure 1: FTIR study of polyherbal granules.

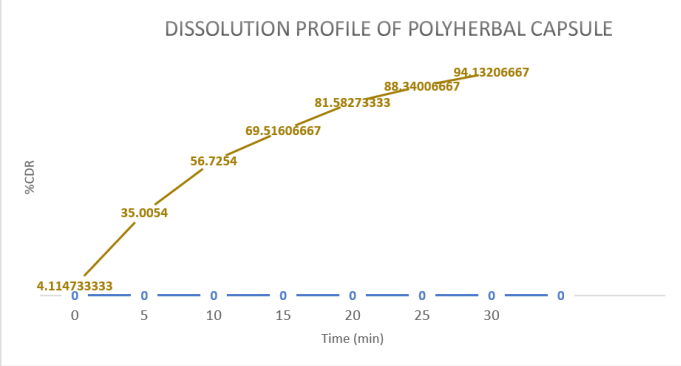

Figure 2: The dissolution profile of Polyherbal capsules. %CDR: Percent Cumulative Drug Release

good but the PHF1 acts ideally for the condition. So the PHF1 was selected and was filled into the 000-sized capsules. The polyherbal capsules were further tested for the capsule evaluation parameters. The capsules of sky-blue coloured cap and transparent body had a characteristic odour and with an average weight of 517 mg. The disintegration time was found to be 10.35 min. with a moisture content of 3.25%.

The dissolution study of capsules was done in which the drug reached a % CDR of 94.13 in 30 min as depicted in Figure 2. This releasing sequence of drug from their capsule shell *in-vitro* help in expecting the *in vivo* releasing order and bioavailability studies of the polyherbal capsule.[23] The rate and amount of drug absorption in the bloodstream is a significant feature of a dosage form. *In vivo* bioavailability and *in vitro* dissolution studies play a major role in it. These are required to prepare a dosage form that is stable and adequate for the diseased person. The herbal drugs are usually more convenient and safe with more patient compliance, acceptance, and adaptation.[24,25]

The FTIR spectra of polyherbal granules is shown in Figure 1 in which the presence of specific functional groups is marked by the bands. Each sample has a peak in the spectral range between 3400–3200 cm^{-1} and 2800–3000 cm^{-1}, which shows the presence of OH groups (such as water, alcohols, and phenols) and methylene CH symmetric/asymmetric, which indicates the existence of alkane vibration.[26] When measured between 1600 and 1800 cm^{-1}, alkenes are detected. The FTIR showed the peaks at 3502.03 cm^{-1} representing O-H stretching, leading to an alcohol group, similarly, 3346.72 (C-H stretching, alkyne), 2916.38 (medium C-H stretching, alkane), 2075.18 (N=C=S stretching, isothiocyanate), 1722.23 (C=O stretching, aliphatic ketone or cyclohexanone or cyclopentanone) 1668.15 (C=C stretching, alkene disubstituted), 1594.29 (C=C stretching, cyclic alkene), 1203.37 (C-O stretching, vinyl ether), 814.86 (C-H bending, 1,4-disubstituted), 763.22 (C-H bending, 1,2,3-trisubstituted), 728.42 (C=C bending, alkene disubstituted).[26] They indicated the existence of water and alcohol, alkane and cyclohexanone, and vinyl ether in their composition. This confirms the existence of relative active chemicals (flavonoids, tannins, terpenes, and phenols) in polyherbal granules. The formulation was stabilized by adding excipients to the granule preparation. Wavenumber changes are minimal, which is consistent with the inert nature of the excipients introduced.[27-29]

In the evaluation of any novel formulation, stability is one of the most important factors to consider. As a consequence of this investigation, the capsules were shown to be safe and stable.[30] Where the drug was found to be stable in different spectrums of lights like Sunlight, Fluorescence, tube light, UV light, IR, and lamp light during the whole 6hr. In case of stability studies at different temperatures the drug showed no signs of degradation even after 6hr of exposure to 50°C but started to degrade after 4hr and 2hr at a temperature of 55°C and 60°C respectively. The drug responded to degradation after exposure to 70% and 90% humidity above 35°. It was found that in phyto-pharmaceutical stability studies, the polyherbal capsules were found almost stable.

CONCLUSION

Following the pharmacopoeial criteria, all four medicines were deemed to be of high quality. The 500 mg polyherbal capsules decomposed in between 10.35 min and *in vitro* condition. All six capsules dissolved to 90% in 30 min. This releasing pattern of medication from their capsule shell *in vitro* can be used to anticipate the releasing sequence *in vivo*. The Polyherbal capsule was shown to be fairly stable in light of phyto-pharmaceutical investigations. To determine the mechanism of action, elements responsible for the antidiabetic activity and shelf life of polyherbal capsules, more investigations utilizing more specific approaches are necessary.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FTIR: Fourier Transformer Infra-Red; **PHF:** Polyherbal Formulation; **CDR:** Cumulative drug release; **T2DM:** Type 2 Diabetes Mellitus.

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