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Research

Standardization Quality Control and Development of Poly Herbal Formulation for the Management of Type-2 Diabetes Mellitus

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Abstract

In the Ayurvedic system of medicine, Herbal Drugs For Diabetes Mellitus as mentioned in ancient Indian books like Charak Samhita, Mahdhav Nidan and Astang Sanghra, there are about 600 plants, which are stated to have antidiabetic property Polyherbal antidiabetic formulation consists of six herbs viz., *Nigella sativa* (seed), *Moringa oleifera* (seed), *Linum usitatissimum* (seed), *Trogonella foenum* (seed), *Cinnamum zeylanicum* (bark) and *Macrotylom auniflorum* (seed). Crospovidone, Microcrystalline cellulose, Colloidal Silicon dioxide, PVP Magnesium stearate, (Polyvinyl pyrrolidone). Polyherbal formulation of the extracts of all selected plants was subjected to freeze drying process. The extracts were dried for a period of time according to their rate of drying .Diluents like, Microcrystalline cellulose, Magnesium stearate, Lactose, starch were dried. All active ingredients were weighed according to the formula, mixed with MCC followed by diluents and Glidant like aerosil and magnesium stearate as lubricant as specified in formula were mixed well. The mixture was blended thoroughly for 30minutes. Then the powder was transferred to the polythene bags and labeled for further studies.

Keywords: Polyherbal formulation, Standardization, Nigella, Moringa, Linum, Trogonella, Cinnamum, Macrotylom, Type 2 Diabetes.

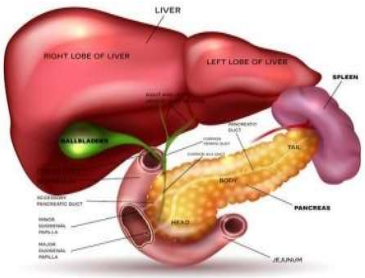
INTRODUCTION

Herbal medicines have become the remedy for most of the diseases. Herbal medicine is still the mainstay of about 75 – 80% of the world population for primary healthcare mainly in the developing countries. However among the estimated 250,000 – 400,000 of plant species, only about 6% have been studied for biological activity, and about15% have been investigated based on its phytochemical. Therefore it seems necessary to evaluate the herbs properly. The reason for the use of herbals is that it is part of the culture and belief of some people for maintenance of health or to treat certain ailments, increased use of herbals is the relatively cheaper cost of herbal products and hence affordability to the lower income group and public has the impression of herbals being natural and that anything natural is safe. There is also this notion that herbal products do not contain chemicals and only those chemicals found in modern medicines, are linked to toxicity, and hence are more harmful.

Standardization is an important aspect for maintaining and assessing the quality and safety of the poly herbal formulation as these are combinations of more than one herb to attain the desire therapeutic effect. Standardization minimizes batch to batch variation, assures safety, efficacy, quality and acceptability of the Polyherbal formulations. Standardization involves: Quality control of crude drugs material, plant preparations and finished products, Stability assessment and shelf life. Safety assessment, documentation of safety based on experience or toxicological studies. Assessment of efficacy by ethnomedical in formations and biological activity evaluations.

Diabetes mellitus[10-13] As per WHO, Diabetes Mellitus is defined as heterogeneous metabolic disorder characterized by common feature of chronic hyperglycemia with disturbance of carbohydrate, protein and fat metabolism. **Type-I diabetes** (insulin dependent diabetes mellitus) **Type-II diabetes** (formerly, non-insulin dependent diabetes mellitus) **Gestational diabetes** (first recognition during pregnancy) **Diabetes due to other causes** (genetic defects or medication)

Type 2 diabetes: Formerly named non-insulin-dependent) which results from the body's inability to respond properly to the action of insulin produced by the pancreas.Type2 diabetes is much more common and accounts for around 90% of all diabetes cases worldwide.



The hormones play an important role in regulating the metabolic activities of the body, particularly the homeostasis of blood glucose. The pancreases both an endocrine and exocrine gland, in which endocrine produces the peptide hormone insulin, glucagon and somatostatin and exocrine gland produces digestive enzymes The peptide hormones are secreted from cells located in the islet of Langerhans (β cells produce insulin, alpha cells produces glucagon and δ cells produce somatostatin).

HERBAL DRUGS FOR DIABETES MELLITUS

In the Ayurvedic system of medicine, as mentioned in ancient Indian books like Charak Samhita, Mahdhav Nidan and Astang Sanghra, there are about 600 plants, which are stated to have antidiabetic property.

Polyherbal formulations may enhance the pharmacological activity and reduce the concentrations of single herbs, thereby reducing adverse effects. Plant formulation and combined extracts of plants have been used as a drug rather than individual. Exploring an effective drug either single or in combination against diabetesis challenging till. Hence we planned to develop antidiabetic Polyherbal formulation in the form of tablet containing extracts of *Nigella sativa* (dried Seed), *Moringa oleifera* (seed), *Linum usitatissimum* (seed), *Trigonella foenum* (seed) and *Cinnamum zeylanicum (bark*), *Macrotylom auniflorum* (seed). Development of this preparation into a suitable drug delivery system in the form of tablet was sought to be of appropriate pharma copoeial quality and would have similar release of the activesas that of the traditional dosage

Preliminary phytochemical screening for selected plants: Triterpenoids, Flavonoids, Alkaloids. Carbohydrate, Glycosides, Phenols, proteins, Resins, Saponins, Tannins, Steroids. HPTL finger print is one of the advanced and versatile chromatographic technique which helps in the identification of compound and thereby authentication of purity of herbal drugs. The finger print obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extracts.

Sample preparation: 1mg of Polyherbal extract was dissolved in1ml of methanol.

Development system: A number of solvent systems were tried, for extract. The satisfactory resolution obtained for the phytochemical constituent alkaloid was in the solvent butanol-acetic acid-water (7:2.5:0.5). Chromatography was performed on silica gel 60 F254 TLC pre- coated plates.

MATERIALS AND METHODS

Polyherbal antidiabetic formulation consists of six herbs viz., *Nigella sativa* (seed), *Moringa oleifera* (seed), *Linum usitatissimum* (seed), *Trogonella foenum* (seed), *Cinnamum zeylanicum* (bark) and *Macrotylom auniflorum* (seed). Crospovidone, Microcrystalline cellulose, Colloidal Silicon dioxide, PVP Magnesium stearate, (Polyvinyl pyrrolidone)

DEVELOPMENT FORMULATION

The extracts of all selected plants were subjected to freeze drying process. The extracts were dried for a period of time according to their rate of drying .Diluents like, Microcrystalline cellulose, Magnesium stearate, Lactose, starch were dried. All active ingredients were weighed according to the formula, mixed with MCC followed by diluents and Glidant like Aerosil and magnesium stearate as lubricant as specified in formula were mixed well. The mixture was blended thoroughly for 30minutes.Then the powder was transferred to the polythene bags and labeled for further studies.

Table 1: Proposed strength of formulation

S.NO.	ACTIVE INGREDIENTS	STRENGTH (in mg)
1	*Nigella sativa*	62.5
2	*Moringa oleifera*	62.5
3	*Linum usitatissimum*	125
4	*Trogonella foenum*	62.5
5	*Cinnamum zeylanicum*	125
6	*Macrotyloma uniflorum*	62.5

PREFORMULATIONSTUDIES[36]: Prior to formulation, it is essential that fundamental physical and chemical properties of the drug molecule and other derived properties of the drug powder are determined. This information decides many of the subsequent events and approaches in formulation development. This first learning is known as Pre-formulation. It aims to optimize the process of turning a drug into a drug product. During pre-formulation the physiochemical properties of the drug candidate are determined.

Selection of the Excipients: The majority of materials filled in the capsules are formulated as powders that are typically mixtures of the active ingredients together with a combination of different types of Excipients. Normally, there are three types of Excipients used in tablet formulation i.e. diluents, glidants and lubricants.
Diluents/Fillers: Lactose, Di calcium phosphate, microcrystalline cellulose, etc. Lubricants: Magnesium Stearate, Stearic acid, Hydrogenised vegetable oils and talc are commonly used lubricants. Glidant: Colloidal silicon dioxide, Talc and Starch.

The flow property of the blended powder is an important parameter to be measured since it affects the uniformity of dose. It was assessed by the following parameters. Bulk density, Tapped density, Compressibility index, Hausner's ratio Angle of repose.

RESULT AND DISCUSSIONS

PHARMACOLOGICAL STUDIES: *IN-VITRO* ANTIDIABETIC ACTIVITY
ALPHA GLUCOSIDASE[47]

Sample extraction: 25 gram of powdered sample were taken and extracted with ethanol using soxhlet apparatus**.** The extract was collected condensed under reduced pressure in rotary vacuum evaporator and storedat4^{o}C.

Determination of alpha–glucosidase inhibitory activity:

Material required: Phosphate Buffer: **50mM,** *pH:* **6.8,** *Sodium carbonate:* **0.1M,** *PNPG:* 1Mm extract with range of concentrations:20-100µg/ml, *Sample Alpha-glucosidase*: 1u/ml-SRL Alpha-glucosidase inhibitory activity of extracts was carried out according to method of Bachhawat *et al 2011* with slight modification. Reaction mixture containing50µl phosphate buffer, 10µl alpha-glucosidase and 20µl of varying concentrations of extracts was pre-incubated at 37^{o}C for15min.Then 20µlp-nitrophenyl-α-D-Glucopyranoside (PNPG) was added as a substrate and incubated further at 37^{o}C for 30min. The reaction was stopped by adding 50µl sodium carbonate .The yellow color produced was read at 405nm. Each experiment was performed along with appropriate blanks. Acarbose at various concentrations (20-100 µg/ml) was included as a standard. Negative control without extracts was set up in parallel. The result is expressed as percentage inhibition.

Inhibition (%) =Abs.Control –Abs.Sample/Abs.controlX100,

DNS ASSAY[48]

Cellline and culture: 3T3 *cell* line was obtained from NCCS, Pune. The cells were maintained in DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100µg/ml) in a humidified atmosphere of 50µg/ml CO_2at 37 °C. Take T-25 flask from incubator and check under microscope. Check for Confluence of cells. Remove the medium using a pipette. Gently rinse the cells with DMEM without FB S2-3times. Add 4–5ml of TPVG (Trypsin, PBS, Versin and Glucose) over the cells. Allow TPVG to act for 3-5minutes discard the TPVG and add 5ml of

10% FBSDMEM Break off the cell clusters by gently pipetting back and forth with pipette. Count the cells in haemocytometer keep sterile Tissue culture flask properly labeled and corked ready. Add 5ml of growth medium (DMEM with serum) to each of the Tissue Culture flask. Add cell suspension to each of the Tissue Culture flask based on the cell count (1lakh cell per ml of medium). Shake the bottle gently so as to allow uniform dispersion of cells. Label on flask should indicate cellline, date of seeding, passage number. Stopper the newly seeded flask tightly and incubate at 37^0C in 5% CO2 atmosphere.Observethecell growth every day. If attains 60% confluence, follow procedure of MTT assay. Collect the cells with 60% confluence from the TC flask by the means of adding TPVG and passing into a 15ml centrifuge tube Centrifuge and remove the supernatant. To the cell pellet add 15ml of fresh10%FBS, DMEM and re-suspend the cells (Solution A: Cell +Medium).

CELL GROWTH WITH SAMPLE AND GLUCOSE: Take 6 well plates and name it. Take 5ml of Solution A (Cell + Medium) and add in 6 well plate, Add1ml of sample (Sample at selected dosage) to 5ml of solution A in the 6 well plates. Incubate for a day (24hrs). Sub samples were collected from the culture medium at0^{th}hr, 5^{th}hr, 10^{th} hr, 15^{th} and 20^{th}hr. DNS assay was carried out.

GLUCOSESAMPLEPREPARATION: After 24hrs, take the 6-well plate from the incubator and suck the entire medium from the plate. Take 2ml of 10XPBS and add to the 6-well plate for washing and suck out PBS and close the plate. Take 150µl of 10% SDS, add to the plates and shake well. Take a cell scraper and scrap the cells from the bottom of the plate and shift the cells into a corner of the plate.Take150µl, take the total content from the plates and pour in eppendorf tube and named it separately. Allow to settle for 5min.The 6 eppendorf tubes were centrifuged at14000rpm for 15min at 25^0C.Then take pipette and take supernatant and add to labeled eppendorf. Store in refrigerator 4^0C for further use.

DNS ASSAY–STANDARDCURVE

DNS REAGENT: Take 1g of DNS, add 50ml of distilled water and dissolve it. Then, add 30g of sodium potassium tartarate tetrahydrate solution turns milky yellow color. Then add 20ml of 2N NaOH (1.6g of NaOH in 20ml of water) – turns to transparent orange yellow color. Make the final volume 100ml by adding distilled water. Take the solution in are agent bottle and cover it with aluminum foil.

GLUCOSESTOCK: 450mg of glucose is weighed and made up to 100ml with distilled water (0.025m).

PROCDURE: For standard Graph: Take the required no. of test tubes and label as follows, Take 200,400, 600,800,1000µl of glucoses tock in each test tube. Makeup the volume of 2ml with distilled water. Take 100µl of each sample supernatant in each test tube and make up a volume of 2ml with distilled water. Take 2ml of distilled water as a blank. Add 1ml of prepared DNS reagent to all the tubes. Observe all the test tubes are of equal volume (3ml). Cover the entire test tube top with aluminum foil. Keep the test tubes with rack at 100^0C in water bath for 5min. Observe for the color change. Take 1ml of the solution and observe OD at 540 nm. Draw a graph with amount of glucose in mg (mg/ml) as X-axis and OD at 540 nm as Y-axis Measure absorbance of the samples and detect the unknown concentration from the standard.

PHYTOCHEMICALANALYSIS: The chemical tests for various Phytoconstituents in the raw materials were carried out and the results were recorded and detailed in table.

Table 2: 15 Phytochemicalanalysis

Phyto-constituents	*Nigellasativa*	*L.usitatis simum*	*M.oleifera*	*T.foenum*	*C.zeyla nicum*	*M.uni florum*
Phenoliccompounds	-	+	+	+	+	+
Flavanoids	-	+	+	+	-	-
Tannins	-	-	+	+	+	+
Alkaloids	+	+	+	+	+	-
Steroids	-	-	+	-	+	+
Glycosides	+	+	+	+	-	-
Saponins	+	+	+	-	+	-
Proteins	+	+	+	+	-	+
Carbohydrates	+	+	+	+	-	+
Terpenoids	-	-	+	+	+	+

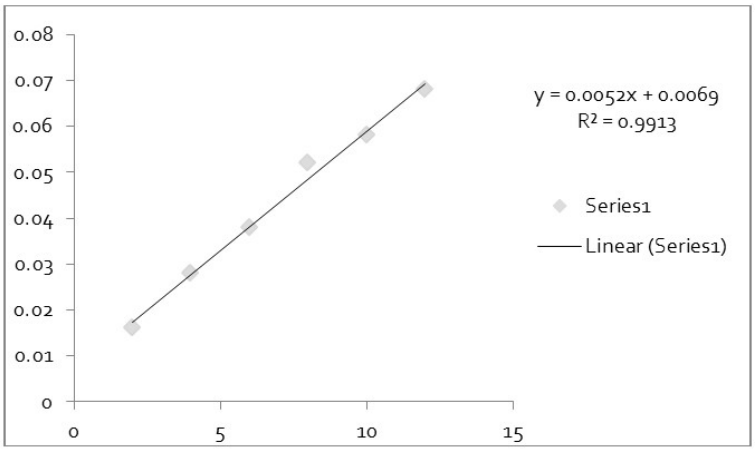


Fig 1: Calibration curve of Phenolic content

Comparison of a standard (Gallic acid) absorbance at various concentration (2µg to10µg) and Polyherbal formulation. Sample absorbance corresponds at standard absorbance at a concentration of 9µg. Hence the amount of flavonoids present in formulation found to be 9µg. The estimated amounts of phenolic, Flavonoids, and Tannins were enumerated in the Table 3.

Table 3: Quantitative estimation of phytoconstituents

S.NO	PARAMETER	OBSERVATION (%w/w)
1	Total tannin content	0.54±0.15
2	Total flavonoids content	3.25±0.37
3	Total phenolic content	1.75±0.21

Result (n=3) are reported as Mean ± Standard

PRE-COMPRESSION STUDIES [47] The drug and the powder blends are evaluated for Pre-compression parameters. These results are given in the tables totally nine 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, uniformity of filling, uniformity of weight, moisture content and disintegration time.

Table 4: Evaluation of trial batches

Parameters	Trial1	Trial2	Trial3	Trial4	Trial5	Trial6	Trial7	Trial8	Trial9
Bulk density(g/cm$^{2)}$	0.36	0.39	0.42	0.43	0.45	0.47	0.47	0.50	0.51
Tapped density(g/cm$^{2)}$	0.50	0.53	0.55	0.55	0.56	0.56	0.55	0.57	0.58
Compressibility index (%w/w)	26.75	26.40	23.63	21.81	19.64	16.07	14.54	12.28	12.06
Hausner's Ratio	1.35	1.36	1.32	1.29	1.26	1.20	1.19	1.15	1.13
Angle of repose (degrees)	46.05	45.66	43.03	42.03	41	38	37	34	32

STANDARDISATION OF THE FINISHED PRODUCT: The final formulation was analyzed for its quality control parameters in three trials. The mean value was obtained and Standard deviation was calculated. Wherever there were no official standard, limits for each parameter was established based on trial and error analysis of Trial 9 batch tablets.

EVALUATION OF TABLETS: Light brown" colored tablets. The Polyherbal tablets were evaluated for organoleptic characters which include color, odour, taste and nature.

Table 5: Organoleptic Characters

S.NO	PARAMETER	OBSERVATION
1.	Description	Light brown color tablet, round shape
2.	Color	Light brown
3.	Odor	Characteristic odor
4.	Taste	Bitter taste

Table 6: Physical Parameters

S.NO	PARAMETER	OBSERVATION
1.	pH(1%aqueoussolution)	7.33 ± 0.21
2.	Moisture content	3.98± 0.5%w/w
3.	Uniformity of weight	625.3 ±3.4mg

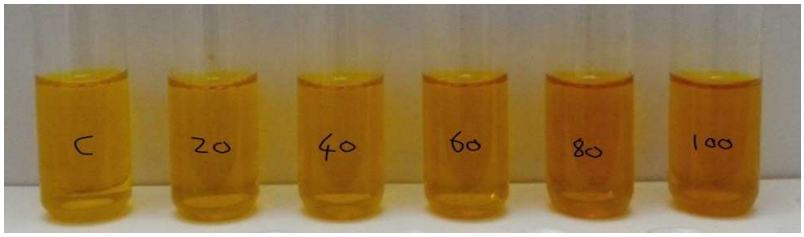
Results are reported as Mean ±Standard deviation. 1% aqueous solution of Polyherbal formulation showed neutral pH. The average weight of the tablet was calculated as per I.P and the obtained value was within the limit (±7.5%).

Table 7: Formulation

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Average weight	625.1	623.3	626.4	619.3	622.2	623.2	621.4	624.2	621.5
Hardness	4.5	4.51	4.53	4.37	4.31	4.54	4.57	4.70	4.83
Thickness	4.08	4.10	4.09	4.08	4.08	4.09	4.10	4.08	4.10
Friability	0.61	0.64	0.63	0.59	0.62	0.87	0.88	0.87	0.88
Disintegration time	18.55	17.35	17.10	16.25	16.20	15.20	14.35	14.20	14.00

POLYHERBALTABLETS: (Polyherbal tablet)

Pharmacological studies *in vitro* anti diabetic activity
α-Amylase Inhibition Assay
Standard carbose



POLYHERBAL FORMULATION (TRIAL9)

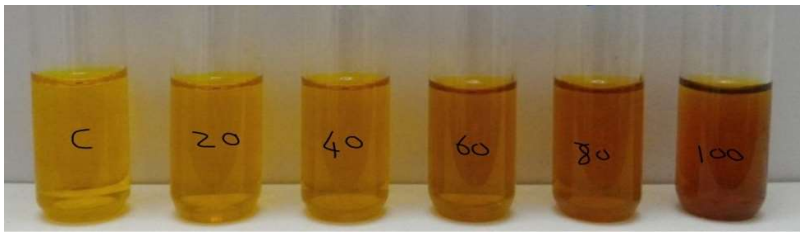


Fig 2: α-Glucosidase assay

Alpha-Glucosidase assay

CONCENTRATION OF SAMPLE(µg/ml)	% INHIBITION OF SAMPLE	%INHIBITION OF ACARBOSE
20	39	45
40	50	54
60	65	68
80	76	78
100	92	89

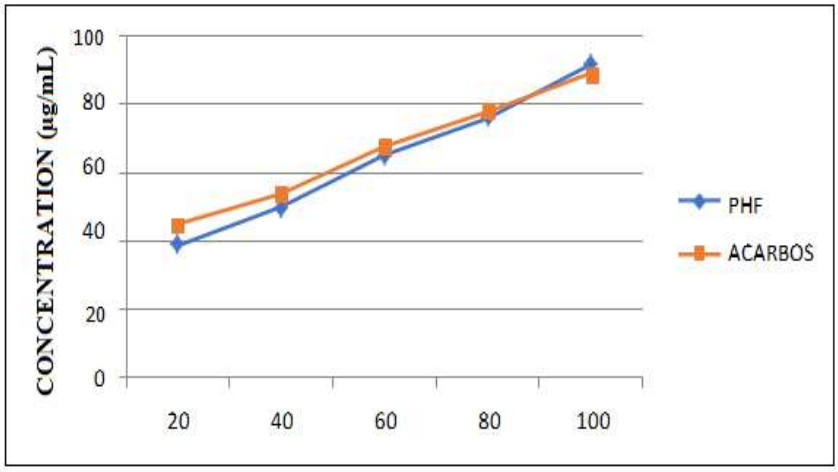


Fig 2: Graphical representation of the α-glucosidase inhibition assay

DNS ASSAY GRAPH

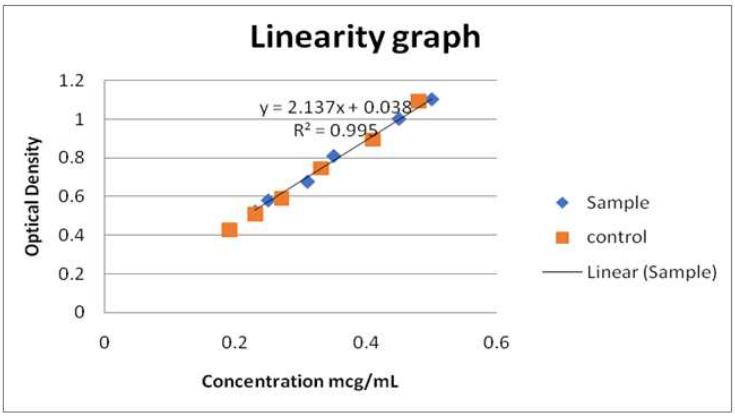


Table 8: DNS ASSAY

S. No.	Samples	0thhr		5thhr		10thhr		15thhr		20thhr		24thhr	
		OD	Conc µg)	OD	Conc µg)	OD	Conc µg)	OD	Conc µg)	OD	Conc µg)	OD	Conc µg)
1	Control	1.102	0.500	1.001	0.450	0.810	0.350	0.621	0.310	0.582	0.250	0.524	0.230
2	Sample	1.094	0.480	0.895	0.410	0.746	0.330	0.592	0.270	0.511	0.230	0.428	0.190

SUMMARY AND CONCLUSION

Herbal medicines are the oldest form of health care known to mankind. A number of traditional herbal medicinal practices have been adopted for the diagnostic prevention and treatment of various diseases. Based on the extensive review of literature, six raw materials were selected for the formulated as Polyherbal tablet and the antidiabetic potency was evaluated in cell lines. The herbal raw materials were analyzed for identity, quality and purity as per the standards prescribed by WHO and Ayurvedic Pharmacopeia of India. The Physiochemical parameters like Loss on drying, ash values and extractive values were determined, which will help in preventing variation in quality of the drugs. Preliminary phytochemical investigation revealed the presence of various phytoconstituents such as alkaloid, steroids, glycosides, Flavonoids, Phenols, Tannins, and terpenoids in the raw materials. The safety of the raw materials was analyzed by heavy metals the results found within the standard limits given by the WHO. The extracts were dried by tray drying and used for the formulation. HPTLC finger printing of the Polyherbal formulation was performed and the resultant chromatogram showed the presence of peaks as same in HPTLC fingerprint of extracts. The chromatogram can be used as an index for the qualitative analysis of the formulation. The dried Polyherbal extract was optimized for its quality measures and its batch consistency by making nine different trial batches (Trial 1,2,3,4,5,6,7,8,9). The trials were subjected to pre-formulation parameters to confirm the uniformity and quality. The result concludes that the trial 9 was excellent in all parameters and the values were found within the standard limits and it was used for formulate Polyherbal Tablet. The developed Polyherbal Tablets were standardized for its Description, uniformity of weight, disintegration time, moisture content, pH, Physiochemical parameters, and phytochemical studies. Quantitative estimation of phytoconstituents was done for flavonoids, phenols, and tannins. The heavy metal analysis was carried out in Polyherbal formulation as per the WHO Guidelines and found within the limits. *In-vitro* anti-diabetic activity was done by using α-glucosidase inhibition assay method. It possesses significant antidiabetic activity as compared to standard A carbose.3T3-L1 cell line was performed. The formulation showed significant effect compared to the standard. The phytochemical study showed the presence of flavonoids. This may be responsible for the potent antidiabetic activity. Further studies are recommended for stability studies in the formulated Polyherbal tablet and also clinical trials have to perform in future in Human Volunteers.

REFERENCES

1. Factsheetno.134.World Health Organization. 2008-12-01.[retrieved2009-5-2].
2. Verma S, Singh SP. Current and future status of herbal medicines. Vet World. 2013;1(11):347-50.
3. Hasan Z. S, Vedant Misra, Swati Singh, Garvita Arora, Sunita Sharma and Sarika Sharma, Current status of herbal drugs and the future perspectives, Biological Forum– An International Journal 2009;1(1),12-17.
4. Balandrin MF, Klocke JA, Wrtele ES, Boilinger WH. Content and purity of extract solasodine in some available species of solanum. Sci Cult. 1985;56(5):214-6.
5. Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. J Nat Prod. 1997;60(1):52-60. doi: 10.1021/np9604893, PMID 9014353.
6. Jone BE. Pharmaceutical capsules. 2nd ed podczeck F, John BE, editors. Vol. 242,449. Pharmaceutical press; 2004. p. 91.
7. Choudhary N, Singh Sekhon B. An overview of advances in the standardization of herbal drugs. J Pharm Educ Res. 2011;2(2):55-70.
8. GMP in pharmaceutical industry – global cGMP and regulatory expectations. TruptiPatil Dongare.
9. ali khan I, Khanum A. Herbal therapy for Diabetes, 1st edi. Vol. 87. Ukaaz Publications; 2007. p. 119-20.
10. Dean L, McEntyre J. The genetic landscape of diabetes. National Center for Biotechnology Information: Introduction to Diabetes; 2004. p. Chapter1.
11. Harshmohan. Text book of pathology. 5th ed. New Delhi: Medical Publisher's Ltd; 2005. p. 818-28.
12. Kumar CH. Dr. Kumar JNS, Ishaq BM, Rani GU, Prkash KV. Antidiabetic Act Polyherbal Preparation Pharmacol Online. 2010;2:780-7.
13. Kaul S. Dewivedi Indigenous Ayurvedic knowledge of some species in the treatment of human disease and disorders. Int J Pharm Life Sci. 2010;1:44-9.

14. Kumar T, Chandrashekhar KS. Bauhinia purpurea Linn: are views of its Ethanobotany, phytochemical and pharmacological profile. Res J Med Plants;5:420-31.
15. Stohs SJ, Hartman MJ. Phytother Res. Epub 2015 Mar 24. Review of the safety and efficacy of Moringa oleifera. Stohs SJ[1],Hartman MJ. 2015 Jun;29(6):796-804. doi: 10.1002/ptr.5325, PMID 25808883.
16. Gaddam A, Galla C, Thummisetti S, Marikanty RK, Palanisamy UD, Paturi V. Rao Role of Fenugreek in the prevention of type 2 diabetes mellitus in prediabetes. J Diabetes Metab Disord 2015Oct2;14:74. 2015. doi: 10.1186/s40200-015-0208-4.eCollection.
17. Ranasinghe P, Perera S, Gunatilake M, Abeywardene E, Gunapala Nuwan, Premakumara S et al. Effects of *Cinnamomum zeylanicum* (Ceylon cinnamon) on blood glucose and lipids in a diabetic and healthy rat model. Pharmacogn Res 2012Apr. 2012;Jun(4 (2)):73-9. doi: 10.4103/0974-8490.94719, PMID 22518078.
18. Mani UV, Mani I, Biswas M, Kumar SN. An open-label study on the effect of flax seed powder (Linum usitatissimum) supplementation in the management of diabetes mellitus. J Diet Suppl. 2011 Sep;8(3):257-65. doi: 10.3109/19390211.2011.593615. PMID 22432725.
19. Gupta LH, Badole SL, Bodhankar SL, Sabharwal SG. Epub2010 Nov 3. Antidiabetic potential of α-amylase inhibitor from the seeds of Macrotyloma uniflorum in streptozotocin-nicotinamide-induced diabeticmice. Pharm Biol 2011 Feb. 2010;49(2): 182-9:507633. doi: 10.3109/13880 209.
20. Bamosa AO, Kaatabi H, Lebdaa FM, Elq AM, Al-Sultanb A. Effect of Nigella sativa Seeds On the glycemic control of patients with type 2 diabetes mellitus. Amarjeet Singh. 2010;54(4):344-54. PMID 21675032[1], Dharmendra Kumar, Jatin Sharma, Dr. H. S. Lamba A Review Article On Linum usitatissimum A Life Saving Drug Now A Days Indian J Physiol Pharmacol.2010Oct- Dec;54(4):344-54.
21. Anonymous. The Ayurvedic pharmacopoeia of India. 1st Ed, Ministry of health and family welfare, Govt. of India. The controllers of publications, New Delhi, volume 1, 74, page no 854.
22. Anonymous. The Ayurvedic pharmacopoeia of India. 1st Ed, Ministry of health and family welfare, Govt. of India. The controller of publications, New Delhi, volume1, 12 page19.
23. Anonymous. The Ayurvedic Pharmacopoeia Of India, First edition, Ministry of health and family welfare, Govt. of India. The controller of publications, New Delhi. Vol. 1(71). p. 113.
24. Anonymous. The Ayurvedic pharmacopoeia of India. 1st ed, Ministry of health and family welfare, Govt. of India. The controller of publications, New Delhi, volume1, page811.
25. Anonymous. The Siddha pharmacopoeia of India. 1st ed, Ministry of health and family welfare, Govt. of India. The controller of publications, New Delhi, volume1, part 1 Kulthi.
26. Lira S, Brush P, Senak L, Wu C, Malawer E. The use of inductively coupled plasma-optical emission spectroscopy in the determination of heavy metals in Crospovidone and povidone as a Replacement for the concomitant visual comparison test. Vol. 3(4). Pharmacopoeial Forum; 2008. p. 6-10.
27. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. London: Chapman & hall; 1973; 434. p. 2nded.
28. Kokate CK, Purohit AP, Gokahle SB. Pharmacognosy. 24th ed. Vallabh Prakashan, Pune.2003. p. 108-9.
30. Rowe RC, Shesky PJ, Quinn ME. Hand book of Pharmaceutical *Excipients.*6thed.London: pharmaceutical press and American pharmacists association; 2009.
31. Jone BE. Pharmaceutical capsules. 2nd ed Podczeck F, editor Pharmaceuticalpress.2004. Vol. 91(242). p. 449.
32. Van Hostetler Hard capsules In: Lachman L, Herbert AL, Joseph LK, editors. The theory and practice of industrial pharmacy. 3rd ed. Bombay: Varghese Publishing House; 1991. p. 374.
33. Shah VK, Zalavadiya V, Sheth NR, Raval NK, Golwala DK. Standardization and assessment of preformulation parameters of rasayana Tablet. Int J Pharm Sci Drug Res. 2010;2(1):58-62.
34. Remingtons pharmaceutical sciences. 17th ed. Easton, PA: Mack Publishing Co.; 1985. p. 1633-43.
35. Indian pharmacopoeia. Govt. India Ministry Health Fam Welf Controller Publ, Ghaziabad. 2007; I: A53-54.
36. The Ayurvedic pharmacopoeia of India, Vol. 4. Ministry of Health and Family Welfare, Govt. of India. The controller of publications, NewDelhi.2002; 158-165.