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FORMULATION, CHARACTERIZATION AND PROTEIN TYROSINE PHOSPHATASE-1B (PTP1B) INHIBITORY ACTIVITY OF POLYHERBAL TABLET

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ABSTRACT
The present study involves preparation and characterization of polyherbal tablet followed by evaluation for Protein Tyrosine Phosphatase-1B (PTP1B) inhibitory activity as a preliminary anti-diabetic potential of formulation. The polyherbal tablet prepared from methanolic extracts of *Carissa carandus, Moringa oleifrea, Ocimum sanctum* and *Manilkara zapota*. The extracts of plants were mixed with other excipient as per the prerequisite parameters of wet granulation method and finally compression machine was utilized to prepare polyherbal tablet. Tablets were prepared by varying compositions of ingredients and in this way four different tablets formulations (F1, F2, F3 and F4) were prepared. This herbal formulation was evaluated for its preliminary anti-diabetic potential using PTP1B enzyme inhibitory activity which was performed on *in-vitro* assay kit. The findings of study revealed success of formulation method since all characteristics parameters of tablet formulations were observed within the acceptable range. Moreover formulations **F1 & F3** exhibited appreciable PTP-1B inhibitory activity with percentage inhibition 73.33% and 69.75% respectively. This result suggested probable anti-diabetic potential of polyherbal tablet formulation.

KEYWORDS: Carissa carandus, Moringa oleifrea, Ployherbal, Anti-diabetic, PTP-1B.

INTRODUCTION
Diabetes is considered as chronic disease in which hyperglycemia arises due to the pathological disturbances of metabolic activities. Disturbed insulin secretion and insulin sensitivity leads consequences of diabetes. The disease also associated with other complications including heart problems, vision impairment and renal problems, etc.[1] The cardinal symptoms of disease are polyuria, thirst, blurring of vision, infrequent urination and weight loss, etc.[2]

Diabetes has become common health issue and its incidences increases world widely due to the deteriorated pattern of life style. Therefore currently it is required to find out effective and safe therapeutic option for preventing and treating diabetes mellitus, in this regards natural medicine can work effectively. Many medicinal plants till now have been evaluated successfully for their anti-diabetic potential. The biologically active constituents present in medicinal plant offers beneficial effects in pathological complications of hyperglycemia.[3] The anti-diabetic potential of medicinal plant can be evaluated by various means including *in-vitro* evaluation of PTP1B enzyme inhibitory activity.

Protein Tyrosine Phosphatase 1B is an enzyme identified as a negative regulator of insulin and leptin pathways. PTP1B dephosphorylates activated insulin receptors and alter insulin sensitivity. Therefore inhibition of PTP1B can serve as an anti-diabetic mechanism and in past few years many compounds have been evaluated for their PTP1B inhibitory activity towards the finding of potent anti-diabetic agent.[4,5]

MATERIALS AND METHODS
Collection and identification of plant materials
The plant materials *Carrisa carandus, Ocimum sanctum, Moringa olefera* and *Manilkara zapota* were collected from local area, the plants were dried on shade, converted into coarse powder and stored in air tight container. Plant specimens were identified and authenticated in Department of Pharmacognosy, RKDF College of Pharmacy, Bhopal.

Extraction
The powdered plant materials was defatted using petroleum ether then subjected for methanolic extraction in soxhlet apparatus. The solvent was removed from extract of plant materials then desiccator was used to store dried plant extracts. Phytochemical investigation

was performed after extraction process; phytochemical investigation was performed to identify active constituents present in plant material. It involves various qualitative tests for the identification of constituents present in selected plant extracts.[6]

Preparation of Polyherbal Tablets

The dried plant extracts of selected plant materials were mixed with different ingredients in varying compositions and wet granulation method was used to prepare solid granules. Compressing machine was employed to prepare tablets using previously prepared granules. The compositions of tablet in different formulations were depicted in **Table 1**.

Table 1: Composition of Polyherbal tablet formulations.

Ingredients	Quantity (mg)			
	F1	F2	F3	F4
Carissa Carandus	100	100	100	100
Ocimum Sanctum	100	100	100	100
Moringa Oleifera	100	100	100	100
Manilkara Zapota	100	100	100	100
Ethyl Cellulose	50	40	40	30
Microcrystalline Cellulose	40	40	40	40
Dibasic calcium phosphate	30	40	30	50
PEG 400	20	10	20	20
Methyl paraben	10	20	20	10

Characterization of Polyherbal tablets

Particle size measurement was done using sieving method, size of formulation affects average weight of tablet therefore it was considered as an important parameter.

Tapped Bulk Density

The graduated cylinder containing known amount of powder allowed to fall under its own weight on to a hard surface from the height of 10 cm at two second intervals.[7] The process of tapping was continued until no further change in volume was observed. Tapped Bulk Density was calculated using following formulae:

Tapped Bulk Density = Weight of the powder / Vol. of the tapped packing

Weight Variation Test

Weight variation test is done to ensure proper amount of drug and other ingredients in tablets. Average weight was measured by selecting and weighing 20 tablets randomly. Weight of each tablet was also determined then variation from average weight in each tablet was calculated and reported as percentage weight variation.[7]

Hardness and Friability Test

The strength and shock wearing capacity of tablet was determined on the basis of "hardness and friability test". Monsanto hardness tester was used to test hardness while Roche friabilitor was used to check friability of tablets. The friability of tablets were determined by putting tablets into chamber and subjected for 100 revolutions. The weight difference before and after experiment was utilized to calculate the percentage friability for each formulations.[8]

Disintegration test for tablet formulations was performed using IP disintegration apparatus.[9] The 900 ml of 0.1N hydrochloric acid was used as disintegration medium; six tablets were placed in the glass of plastic tubes, tablets disintegrated and passed through mesh 10 screen.

Thickness and diameter of the tablets formulations were evaluated using Vernier calipers.

***In vitro* drug release** was performed using USP paddle apparatus. It was done with 900 ml 1.2 pH buffer solution maintained at 37±0.5°C and agitation was set 75 rpm. The dissolution medium (5 ml) was withdrawn, filtered and diluted after fixed intervals to measure percentage drug release.[8,9]

PTP1B inhibitory Assay (Preliminary Anti-diabetic activity)

The formulations were evaluated for their PTP1B inhibitory activity using *in vitro* assay. The diluted solution was prepared by triturating tablet formulations and these solutions were used for assay after filtration. Suramin served with assay kit used as positive control, Insulin receptor was used as PTP1B substrate. Assay was performed as per the standard protocol provided with assay kit and supplier instructions. The each wells of 96 well plate titers were filled with assay buffer (85µl), PTP 1B enzyme (5 µl) and PTP1B substrate (50 µl) and incubated at 30 °C. Reaction was terminated after 30 min. and allowed to stand for few min, which after absorbance was measured at 620 nm on ELISA plate reader.[10]

RESULTS AND DISCUSSION

Extraction of different plant materials was done using successive extraction method in which polarity increases from petroleum ether to methanol. The grey-brown colored extracts of all plant materials were further evaluated for their phytochemical compositions using various qualitative tests. Extract of *Carissa carandus* showed presence of flavonoids and tannins, extract of *Ocimum sanctum* showed presence of amino acids, alkaloids and flavonoids. *Moringa oleifrea* showed presence of sterols, resins, alkaloids and tannins. *Manilkara zapota* extract showed presence of phenolic compounds, and tannins.

Polyherbal tablets formulations was prepared using herbal extracts of *Carissa carandus, Ocimum sanctum, Moringa oleifrea* and *Manilkara zapota*. The pre-formulation parameters were observed within range of acceptable limit. Polyherbal tablets were subjected to quality parameters including uniformity of weight, hardness, friability and thickness, and disintegration time. The weight variation test showed percentage

weight variation within the pharmacopoeial limit for all tablet formulations. The hardness was also found within acceptable range which was measured using Monsanto tester. Appreciable hardness of formulations contributed towards fast disintegration of tablets. The results of friability confirmed mechanical stability of formulations.

The disintegration time of formulation was found not more than 25 minutes, the time required for disintegration of tablets was found in the range of 18.40±1.27 to 24.15±1.83 min. This range confirmed optimum disintegration time of formulations.

The **Table 2** depicted results of quality parameters and these findings confirmed good flow properties and quality of formulations.

Table 2: Results of quality parameters of poly-herbal tablet formulations.

S. No.	Parameters	F1	F2	F3	F4
1	Tapped Bulk Density (g/ml)	0.45±0.04	0.53±0.07	0.51±0.10	0.49±0.12
2	Weight Variation Test (%)	1.85± 0.21	2.30±0.18	2.35±0.24	2.65±0.31
3	Hardness (Kg/cm2)	3.1 ± 0.02	3.8 ± 0.05	4.1 ± 0.03	3.6 ± 0.04
4	Friability Test (%)	0.59 ± 0.01	0.89 ± 0.01	0.76 ± 0.03	0.92 ± 0.01
5	Disintegration test (min)	18.40±1.27	24.15±1.83	20. 20±1.75	21.30±1.61
6	Thickness (mm)	4.3 ± 0.01	4.7 ± 0.03	4.6 ± 0.01	3.9 ± 0.02

PTP-1B Inhibitory Assay

The formulations were evaluated for their PTP1B inhibitory activity using enzyme assay kit to confirm probability of anti-diabetic potential of poly-herbal formulations prepared from different plant extracts. The solution of tablet formulations were used for assay, Suramin served as positive control provided with assay kit while insulin receptor was used as PTP1B substrate. The formulations exhibited appreciable PTP1B inhibitory activity as depicted in **Figure 1**. The % inhibition of enzyme was found maximum with formulations F1 and F3. Formulation F1 exhibited 73.33% inhibition of PTP-1B enzyme while formulation F3 showed 69.75% inhibition of enzyme PTP-1B. The results of PTP1B inhibitory activity suggested probable anti-diabetic activity of poly-herbal formulations prepared from various plant extracts. The formulations exhibited remarkable PTP1B inhibitory activity. PTP1B is considered down regulator of insulin signaling; therefore inhibition of PTP1B may offer anti-hyperglycemic effects.

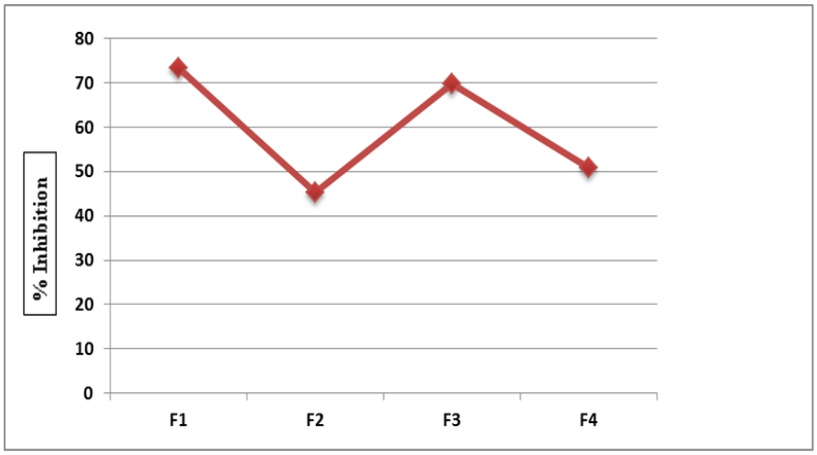

Figure 1: PTP inhibitory activity of poly-herbal formulations prepared from extracts of *Carrisa carandus, Ocimum sanctum, Moringa olefera* and *Manilkara zapota*.

CONCLUSION

The poly-herbal formulations prepared from extracts *Carrisa carandus, Ocimum sanctum, Moringa olefera* and *Manilkara zapota* offers optimum pharmaceutical parameters and possess desired quality characteristics. The values of quality parameters were found within range which indicated success of preparation method utilized to prepare poly-herbal formulations. The formulations further evaluated for their preliminary anti-diabetic potential using *in vitro* PTP-1B inhibitory assay. Formulations exhibited prominent PTP-1B activity this suggested that the plant may be beneficial in prevention and treatment of diabetes mellitus.

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