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Physiocochemical Standardization and Formulation Development of Poly-herbal Tablet for Diabetes

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Authors' contributions

This work was carried out in collaboration between both authors. Author MP designed the study and wrote the protocol. Author PM managed the literature searches, physicochemical analyses of the study sample, studies the spectroscopy analysis, experimental process and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Diabetes is one of the leading health problems in the current world population. Very near future India will be the capital of Diabetes mellitus (DM).
Objective: The objective of this present study was to develop and evaluate polyherbal tablet for diabetes. Developing herbal formulations for oral usages is still a challenge in modern pharmaceutical aspects and the tablet formulation presents many technical problems to the industrial pharmacist.
Methods: Potential anti-diabetic herbs were used for developing tablets. Phytochemical parameters for standardization of plants were, according to standard methods and aqueous extract of the whole plant of *Cassia auriculata, Cinnamomum tamala, Ficus benghalensis, Mangifera indica* and *Trichosanthes dioica* were used in the formulations. Pre-formulations studies were performed for powder blends. Drug excipients compatibility and microbiological limits were also evaluated.
Results: All the tablets were prepared by using hand rotating single punch tablet punching machine and were evaluated for various tablet compression parameters, i.e. tab densities, bulk

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densities, an angle of repose, general appearance, weight uniformity, hardness, friability, and disintegration. All formulations are found to be nil or under the standard limit.
Conclusion: The laboratory scale preparation of poly-herbal tablet may lead to a new potent and stable oral dosage formulations for DM and enlighten the area of synergistic action of herbs.

Keywords: Poly-herbal tablet; pre-formulation studies; formulation development; physico chemical standardization.

1. INTRODUCTION

The world's population is turning toward alternative systems of medicine for adverse effects and complexity associated with chemical drugs. Ancient Ayurveda has an admirable means of treating diseases like "Madhumeha" and "Bahumutra". Since the time of Charaka and Sushruta many herbal medicines in different oral formulations have been recommended for the treatment of diabetes mellitus (DM) [1]. The increasing demand for plant medicine in both developing and developed nations elaborated double fold in last two decades. Quality control of medicinal plant based formulations by using modern tools and use of appropriate standards is always emphasized by world health organization (WHO). Standardization of Ayurvedic preparations using advanced techniques for analysis of their constituents is, therefore, extremely important for establishing their authenticity, credibility, and acceptance worldwide [2]. In DM, by restoring healthy blood sugar regulation and normalizing insulin production, herbal formulas have been shown promising outcome. The metabolic and chemical disturbances in the body caused by elevated insulin level and blood glucose levels can be controlled by different herbs once in combining together. Herbal drug synergism plays a major role in the therapeutic efficacy of herbs or herbal formulations where the combination of ingredients is significantly reduced or significantly increased with respect to each individual herbal component. It is very crucial to make separate from additive effects and mostly rely on high margins of differentiation.

The poly-herbal formulation composed of five well-researched plants ie. *Cassia auriculata* L. [3,4], *Mangifera indica* [5-8], *Ficus banghalensis* [9-11], *Cinnamomum tamala* [12-14], *Trichosynthis diocia* [15-16] which all are individually documented for their effect against DM. Though DM is a metabolic disorder, plant synergism may play a potential role in comparison with individual herbs. *Cassia auriculata* L. belongs to the family of Caesalpiniaceae, commonly known as Tanners Senna or cassia, is a shrub and distributed throughout hot deciduous forests of India and also have a prestigious position in Ayurveda and Siddha systems of medicine. The flower has been reported to contain flavanoids, proanthocyanidins, and ß-sitosterol [17,18]. *Cinnamomum tamala* (family: Lauraceae) is also known as Indian Cassia and the leaves are commonly known as Bay leaves, widely used a medicinal plant to treat DM which contributed by Cinnamaldehyde (3-phenyl-2- propenal), a potential antidiabetic agent [19]. *Ficus bengalensis* (family: Moraceae) is commonly known as Banyan tree or Vata or Vada tree in Ayurveda, is a large tree with aerial roots. It grows wild in the lower Himalayas and is found all over India. leucocyanidin and pelargonidin were reported found to have the antihyperglycemic effect of *Ficus banghalensis* Arial root bark [20]. *Mangifera indica* is a large evergreen tree, with a heavy, dome-shaped crown. It belongs to the family Anacardiaceae and found all over the tropical regions of the world where it is used as a horticultural and medicinal plant. C-glucoside xanthone mangiferin [21], homomangifirin [22] responsible for the antihyperglycemic effect in *Mangifera indica*. Trichosanthes, a genus of family Cucurbitaceae is an annual or perennial herb distributed in tropical Asia and Australia. *Trichosanthes dioica* Roxb. is known by a common name of parwal. The main chemicals which found in this plant are 24α- ethylcholest-7-enol & 24β- ethylcholest-7-enol [23]. The present work was undertaken to conceal the standardization of those medicinal plant materials first and later focused on the development of a potential polyherbal formulation and a convenient tablet dosage form for diabetic patients.

2. MATERIALS AND METHODS

2.1 Plant Material

Poly-herbal formulation consists of five herbal ingredients, viz., *Cassia auriculata, Cinnamomum tamala, Ficus benghalensis,*

Mangifera indica, Trichosanthes dioica. All these plant parts were procured from three different places like Kerala, Madhya Pradesh and Himachal Pradesh, India, and were authenticated by Dr. Jomy Augustine, Postgraduate and research Dept. of Botany, St. Thomas College. Pala, Kerala.

Fig. 1. *Cassia auriculata* flower *(CAF)*

Fig. 2. *Cinnamomum tamala* leaves (CTL)

Fig. 3. *Mangifera indica* leaves (MIL)

Fig. 4. *Ficus benghalensis* arial root barks (FBARB)

Fig. 5. *Trichosanthes dioica* Arial parts (TDAP)

Fig. 6. Formulated poly-herbal Tablet

2.2 Extraction and Standardization of Botanicals

All the plant parts (Table 1) were collected, dried and powdered separately, passed through 20 # sieve. All these plants, dried powders were macerated with distilled water at a 1:10 ratio and all the plant extracts were subjected to lyophilization. Lyophilized, and finally brown, green powders obtained, having an acrid taste and typical herbal odor. These powders were used for the preparation of poly-herbal antidiabetic tablet. As per requirement, extracts were prepared and used for the study.

Physico-chemical studies like total ash, water soluble ash, acid insoluble ash, water, alcohol, acetone and petroleum ether soluble extract, loss on drying at 105℃, pH and fluorescence analysis were carried out as per the WHO guidelines [24,25]. Preliminary phytochemical tests and heavy metal analysis were performed according to standard methods [26,27].

2.3 Development of Poly-herbal Formulation

An appropriate amount of dry extracts has been taken for developing the tablet formulations with the requisite quantity of excipients (Table 2). Power blends were compressed to 600 mg tablet

on rotating single punch tablet pressurizing 11 × 8 mm punch set with compression pressure. The dried extracts were mixed with ethyl cellulose, Microcrystalline cellulose, Diabesic calcium phosphate, lactose anhydrous, calcium carbonate, sodium starch glycolate, sodium alginate and starch (Loba chemicals Ltd. Mumbai) and other chemicals of analytical grade were used. Before punching the die and the cavity was adjusted for required weight and the granules were punched to tablets. All the polyherbal extracts and excipients mixture were subjected to per formulation studies according to standard recommended procedure before punching tablets. After tablet preparation, all the tablet batches were kept in the dry self-sealing pouch in a moisture free desiccator.

2.4 Pre-formulation Studies

The following pre-compression parameters were tested [28,29].

2.4.1 Angle of repose

It is the three-dimensional angle formed by a cone-like pile of the material during the determination. The angle of the cone formed was calculated after the product was passed through a funnel with the following dimensions: Funnel height 9.5 cm, an upper diameter of spout 7.2 cm, internal diameter at the bottom, narrow end of spout 1.8 cm. The funnel was placed on a support at 20 cm from the table surface, centered over a millimeter grid sheet on which two intersecting lines were drawn, crossing at the center. The narrow end of the funnel spout was plugged and the funnel was filled with the product under study until it was flush with the top end of the spout when smoothed with a spatula. Thereafter, the plug was removed and the powder was allowed to fall onto the millimeter sheet. The radius of the cone base was measured with a slide caliper and the mean value (r) was calculated. Additionally, the cone height (h) was measured and the angle tangent value (θ) of the cone was calculated employing the following equation:

$$\tan \theta = h/r$$
$$\theta = \tan^{-1} h/r$$

Where θ = angle of repose, h = height of powder cone formed, r = radius of powder cone formed.

Table 1. Composition of Poly-herbal formulation (PHF)

S. no.	Common name	Botanical name of plant	Family	Part used
1.	Tanner's cassia	*Cassia auriculata L.*	Caesalpiniaceae	Flower
2.	Bay leaves	*Cinnamomum tamala (Buch.-Ham.) T. Nees & Eberm.*	Lauraceae	Leaves
3.	Banyan tree	*Ficus benghalensis L.*	Moraceae.	Arial root bark
4.	Mango	*Mangifera indica L.*	Anacardiaceae	Leaves
5.	Pointed gourd	*Trichosanthes dioica Roxb.*	Cucurbitaceae	Arial parts

Table 2. Formulation of tablets

Ingredients	Formulations			
	Quantity per tablet (mg)			
	F1	F2	F3	F4
Weight of powder granules	500	500	500	500
Ethyl cellulose	20	-	-	10
Microcrystalline cellulose	20	40	10	-
Dibasic calcium phosphate	20	20	20	20
Lactose anhydrous	10	10	10	-
Calcium carbonate	-	20	30	20
Sodium starch glycolate	-	10	-	-
Sodium alginate	20	-	30	40
Starch	10	-	-	10
Weight per tablet	600	600	600	600

2.4.2 Loose bulk density (LBD)

LBD was determined by pouring a weighed quantity of powder into a graduated cylinder and measuring the volume and weight.

LBD = Weight of the powder / volume of the packing

2.4.3 Tapped bulk density (TBD)

TBD was determined by placing a graduated cylinder, containing a known mass of powder. The cylinder was allowed to fall under its own weight onto a hard surface from the height of 10 cm at 2-second intervals. The tapping was continued until no further change in volume.

TBD = Weight of the powder / volume of the tapped packing

2.4.4 Hausner ratio

It is the measurement of frictional resistance to the drug. The ideal range should be 1.2 - 1.5. It was determined by using the following formula:

Hausner ratio = TBD / LBD

2.4.5 Compressibility index

The Compressibility index of the blends was determined by the Carr's compressibility index. The Lose bulk densities (LBD) and tapped bulk densities (TBD) were used to calculate the Carr's compressibility index:

Compressibility index (%) = (TBD-LBD) X 100 /TBD

2.5 Drug Excipient Compatibility Study

Compatibility of the drug with excipients was determined by Fourier transform infrared (FT-IR) spectral analysis. This study was carried out to detect any changes in the chemical constitution of the drug after combining it with the excipients. The samples were taken for FT-IR study. IR spectra of the drug in KBr pellets at a moderate scanning speed between 4000-400 cm^{-1} was carried out using FTIR (Jasco FTIR 6100 type A). The peak values (wave number) and the possibility of the functional group were shown in spectra which compare with the standard value. The comparison of these results with chemical structure shows that the sample was pure aqueous extract.

2.6 Standardization/ Evaluation of Poly-herbal Tablets

The following post-compression parameters were employed for evaluation of tablets [30,31].

2.6.1 Uniformity of Weight

Randomly selected 20 tablets of each formulation were individually weighed. The average value was calculated and compared to individual tablet weights.

2.6.2 Hardness test

The tablet requires a certain amount of strength or hardness and resistance friability to withstand mechanical shocks of handling in all processes. The hardness of randomly selected 20 tablets of each formulation was determined by the Monsanto hardness tester.

2.6.3 Percentage friability test

The friability of tablets was determined by Roche friabilator. Percentage of weight loss of 20 tablets randomly selected from each batch tumbled in friability apparatus. After 4 minutes of rotating at 25 rpm, the dust of tablets was removed and the percentage of weight loss was calculated.

2.6.4 Disintegration test

The disintegration time of tablets was determined by using the digital microprocessor based disintegration test apparatus (basket rack assembly, Lab India). One tablet was introduced into each tube and added a disc. The assembly was suspended in a 1000 ml beaker filled in with water. The volume of water was such that the wires mesh at its highest point (at least 25 mm) below the surface of the water, and at its lower point (at least 25 mm) above the bottom of the beaker. The apparatus was operated and maintained at 37±2℃. The time requires to all tablets to disintegrate and pass through wire mesh was noted.

2.7 Microbiological Load Analysis [32,33]

For the safe use of poly-herbal tablet, microbial count was done and checked whether the total count of viable microorganism, growth, and existence of various pathogenic microbes like mold, yeast, *E. coli, Staphylococcus aureus, Salmonella sp., Bacillus subtilis, Candida albicans,* and *Pseudomonas aeruginosa* etc.

were within the prescribed limits and the tests were carried as per standard procedure.

3. RESULTS

Herbal botanicals and developed formulations were subjected to various standardization parameters. Botanical parameters revealed that all the extracts were dark brown and greenish brown in color, with a characteristic odor, characteristic taste, and fine texture. Results of quantitative analysis for various samples for extractive values (petroleum ether, acetone, alcohol, water soluble extractive values), Ash values (acid insoluble ash, water soluble ash), Loss on drying at 105℃, pH (1% and 10% aqueous Solution.), Foreign organic matter, heavy metal analysis were calculated and results were shown in Tables 3, 4, 5, 6, 7 and 8. The heavy metal content was found to be nil in all the used botanicals (Table 8). The results of fluorescent studies of the powdered plant material studied and tabulated in Table 7. The results of preliminary phytochemical analysis of are given in Table 10 which revealed the presence of various phytochemicals like alkaloids, flavonoids, glycosides, steroids, etc. The various compositions of the prepared herbal tablet formulations was shown in Table 2. The micromeritic properties for all the powder formulations were determined and shown in Table 11. The physical property of tablet was determined and the results of the uniformity of weight, hardness, drug content and friability of the tablets are given in Tables 12. The low friability indicates that the herbal tablets were compact and hard. The In-vitro disintegration studies were conducted on tablets of each of the formulations such as PHT1, PHT2, PHT3 and PHT4 which found to be satisfactory and ranges between 29-36 min. The FT-IR spectra of tablet formulations at Figs. 12-15 did not show the presence of any additional peaks for new functional groups. Microbiological examinations like total aerobic count of microorganism and number of *E. Cole* found as per WHO specified limit and *Staphylococcus aureus, Salmonella sp., Bacillus subtilis, Candida albicans,* and *Pseudomonas aeruginosa* etc were found absent in all formulations furnished in Tables 13 and 14.

Table 3. Physicochemical parameter

Extract	Kerala sample	Madhya Pradesh sample	Himachal Pradesh sample	Limit (% NLT)
a) Extractive value of CAF				
Pet. Ether	1.00±0.01	1.11±0.12	1.90±0.11	1.90±0.11
Acetone	8.28±0.10	8.75±0.02	7.92±0.15	8.75±0.02
Methanol	22.40±0.23	22.00±0.15	21.88±0.21	22.40±0.23
Water	11.20±0.13	11.33±0.25	10.81±0.22	11.33±0.25
b) Extractive values of TDAP				
Pet. Ether	2.00±0.02	2.21±0.12	2.11±0.16	2.21±0.12
Acetone	3.88±0.04	4.10±0.02	3.23±0.11	4.10±0.02
Methanol	5.88±0.21	6.72±0.30	6.12±0.22	6.72±0.30
Water	9.88±0.06	9.03±0.41	10.10±0.26	10.10±0.26
c) Extractive values of CTL				
Pet. Ether	1.68±0.08	2.10±0.24	2.01±0.22	2.10±0.24
Acetone	4.88±0.06	5.28±0.11	5.11±0.41	5.28±0.11
Methanol	7.40±0.16	7.10±0.31	8.04±0.34	8.04±0.34
Water	5.28±0.07	6.66±0.19	6.34±0.81	6.66±0.19
d) Extractive values of MIL				
Pet. Ether	3.00±0.13	3.11±0.21	3.09±0.06	3.11±0.21
Acetone	9.40±0.09	10.22±0.24	10.12±0.17	10.22±0.24
Methanol	21.28±0.31	20.91±0.09	21.00±0.15	21.28±0.31
Water	15.68±0.03	16.09±0.21	15.92±0.51	16.09±0.21
e) Extractive values of FBARB				
Pet. Ether	0.90±0.08	1.02±0.35	1.33±0.09	1.33±0.09
Acetone	1.70±0.31	2.23±0.45	2.21±0.13	2.23±0.45
Methanol	6.90±0.51	7.32±0.83	6.00±0.24	7.32±0.83
Water	12.90±0.06	13.02±0.53	13.12±0.41	13.12±0.41

Data represented as mean±SD, n=3

Table 4. Ash values

Sample	Kerala sample	Madhya Pradesh sample	Himachal Pradesh sample	Limit (% NMT)
Total ash				
CAF	4.64±0.20	5.01±0.26	4.96±0.16	5.01±0.26
CTL	4.80±0.31	5.11±0.22	5.00±0.19	5.11±0.22
MIL	4.41±0.22	4.91±0.15	4.89±0.19	4.91±0.15
TDAP	6.51±0.15	6.90±0.09	6.85±0.11	6.90±0.09
FBARB	4.91±0.11	5.22±0.13	5.02±0.21	5.22±0.13
Acid insoluble ash				
CAF	1.06±0.04	1.84±0.06	1.41±0.09	1.84±0.06
CTL	1.00±0.21	1.12±0.11	1.11±0.16	1.12±0.11
MIL	1.10±0.32	1.09±0.31	0.98±0.24	1.10±0.31
TDAP	2.10±0.21	2.01±0.21	2.00±0.15	2.10±0.21
FBARB	2.21±0.34	2.24±0.15	2.13±0.23	2.24±0.15
Water soluble ash				
CAF	3.01±0.11	2.91±0.09	2.97±0.15	3.01±0.11
CTL	3.10±0.15	3.41±0.11	3.01±0.12	3.41±0.11
MIL	3.00±0.21	3.10±0.16	2.98±0.18	3.10±0.16
TDAP	3.10±0.03	3.01±0.12	2.95±0.09	3.10±0.03
FBARB	4.11±0.13	4.01±0.03	4.72±0.08	4.72±0.08

Data represented as mean±SD, n=3

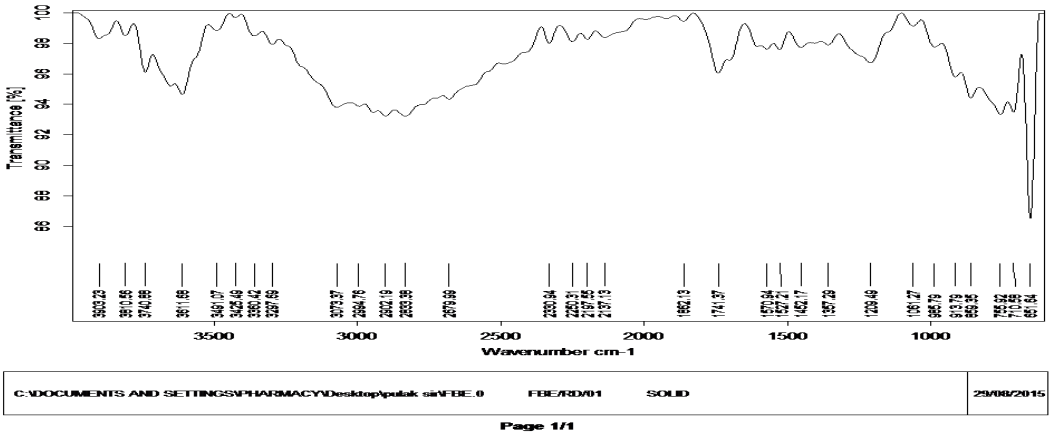

Fig. 7. IR spectra for FBARBE

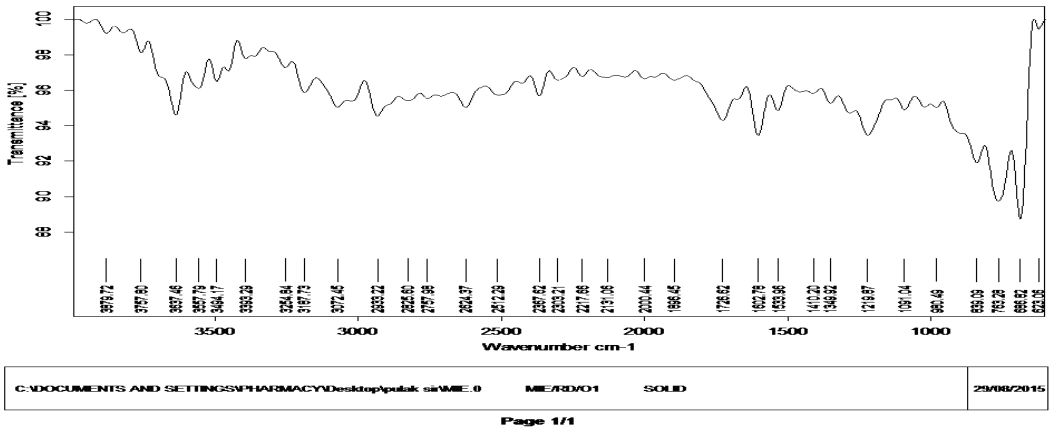

Fig. 8. IR spectra for MILE

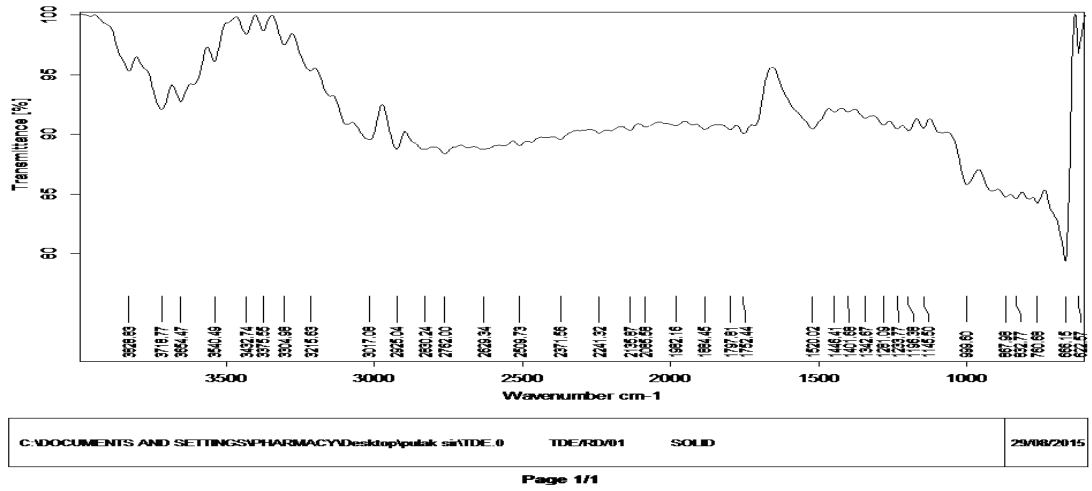

Fig. 9. IR spectra for TDAPE

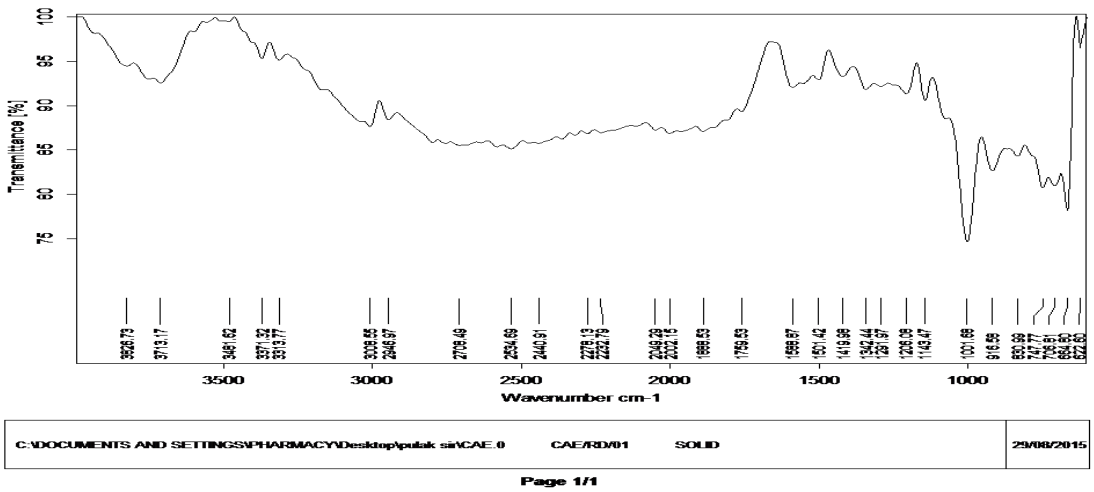

Fig. 10. IR spectra for CAFÉ

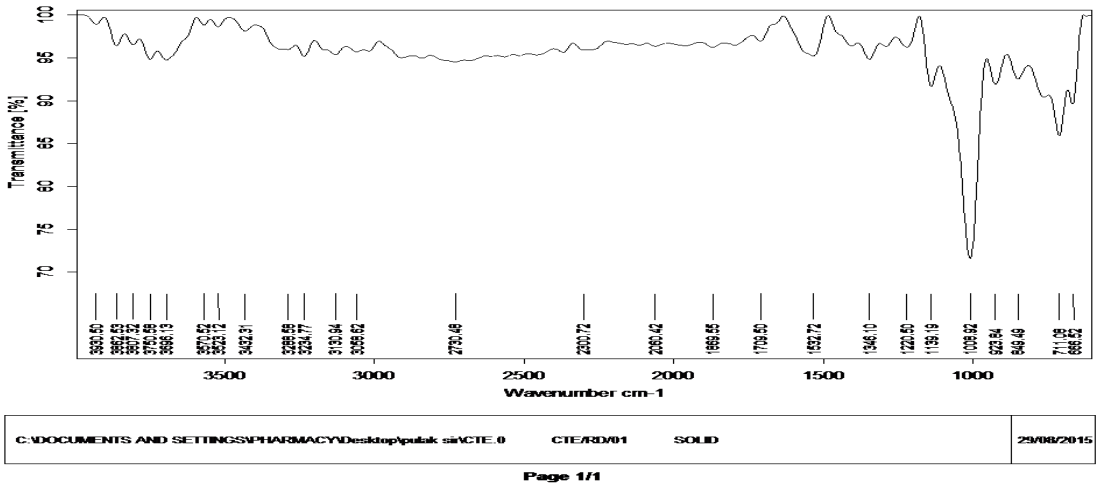

Fig. 11. IR spectra for CTLE

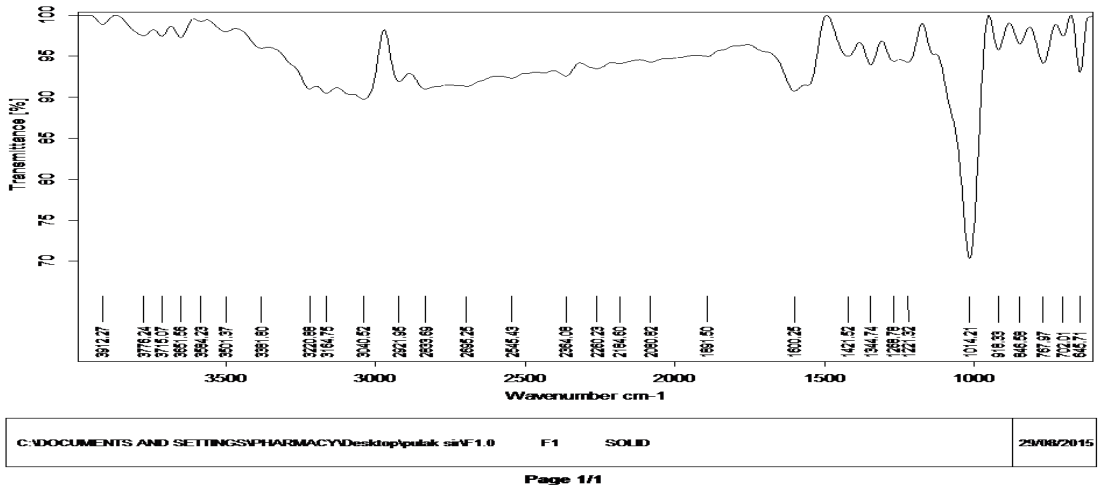

Fig. 12. IR spectra for F1

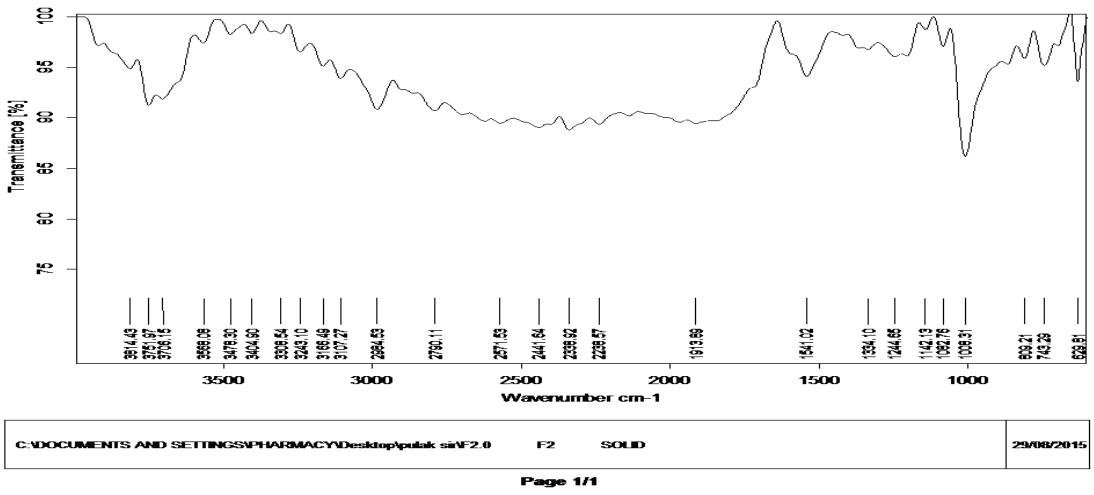

Fig. 13. IR spectra for F2

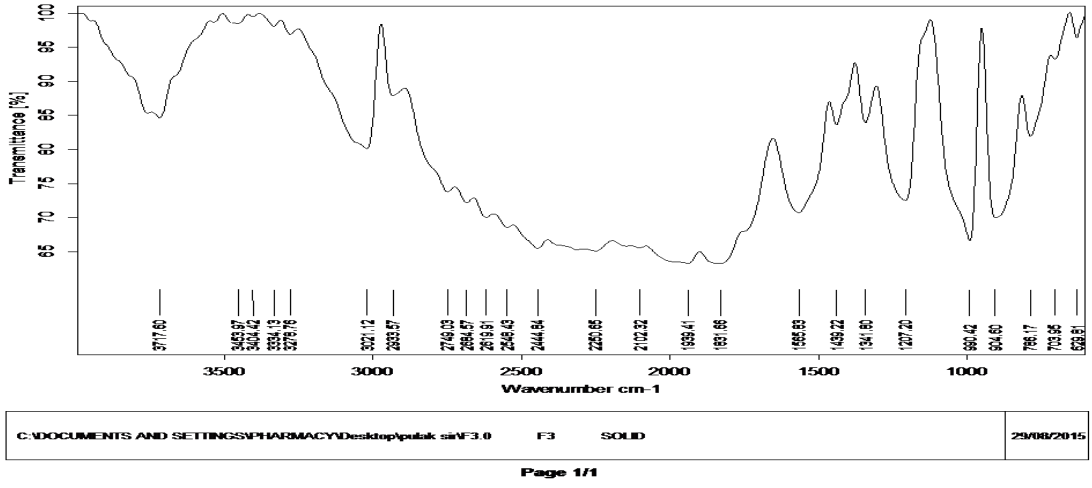

Fig. 14. IR spectra for F3

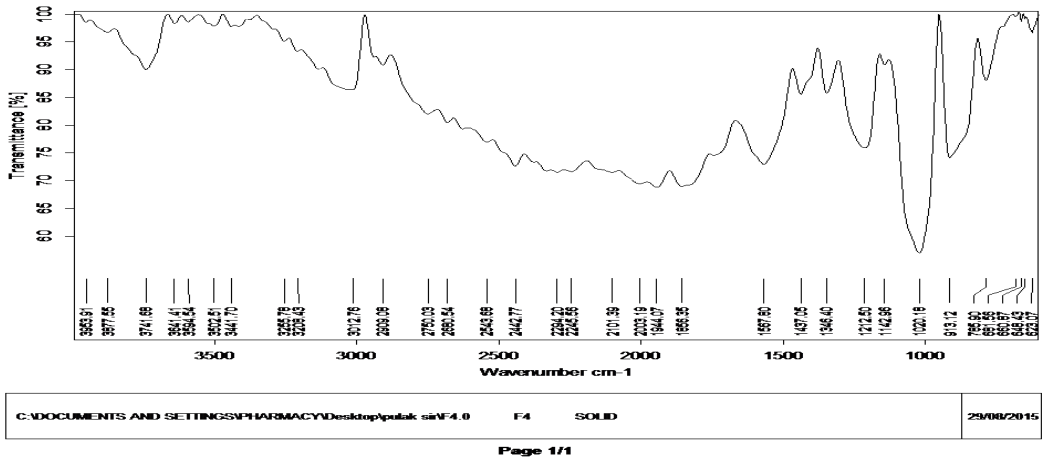

Fig. 15. IR spectra for F4

Table 5. Loss on drying

Sample	Kerala sample	Madhya Pradesh sample	Himachal Pradesh sample	Limit (% NMT)
CAF	12.00±0.01	12.20±0.21	12.20±0.20	12.20±0.20
CTL	13.55±0.11	13.60±0.23	13.50±0.09	13.60±0.23
MIL	09.75±0.22	10.13±0.10	10.09±0.15	10.13±0.10
TDAP	09.80±0.21	09.81±0.25	09.91±0.13	09.91±0.13
FBARB	11.40±0.50	11.42±0.31	12.10±0.20	12.10±0.20

Data represented as mean±SD, n=3

Table 6. pH

Extract	Kerala sample		Madhya Pradesh sample		Himachal Pradesh sample	
	1% solution	10% solution	1% solution	10% solution	1% solution	10% solution
CAF	7.4	7.2	7.1	7.2	7.1	7.0
CTL	6.6	6.9	6.8	6.6	6.9	6.8
MIL	6.1	6.6	6.3	6.1	6.4	6.2
TDAP	6.9	6.8	6.4	6.7	6.5	6.8
FBARB	6.3	5.2	6.0	5.6	6.4	5.4

Data represented as mean±SD, n=3

Table 7. Foreign organic matter

Sample	Kerala sample	Madhya Pradesh sample	Himachal Pradesh sample	Limit (% NMT)
CAF	1.91±0.01	1.98±0.11	1.82±0.15	1.98±0.11
CTL	1.82±0.10	1.77±0.15	1.84±0.31	1.84±0.31
MIL	1.78±0.11	1.82±0.21	1.91±0.20	1.91±0.20
TDAP	1.82±0.14	1.70±0.32	1.80±0.14	1.82±0.14
FBARB	1.66±0.20	1.71±0.11	1.73±0.17	1.73±0.17

Data represented as mean±SD, n=3

Table 8. Heavy metal

Sample	CAF	CTL	MIL	TDAP	FBARB	Observation
Lead	Nil	Nil	Nil	Nil	Nil	Not detected
Cadmium	Nil	Nil	Nil	Nil	Nil	Not detected
Arsenic	Nil	Nil	Nil	Nil	Nil	Not detected

Table 9. Fluorescence analysis

Reagent	CTF			MIL			TDAP			CAF			FBARB		
	Day light	Long UV	Short UV	Day light	Long UV	Short UV	Day light	Long UV	Short UV	Day light	Long UV	Short UV	Day light	Long UV	Short UV
Powder as such	Ash green	Dark brown	Woody brown	Green	Green	Green	Light brown	Brown	Pale brown	Greenish yellow	Dark brown	Greenish brown	Brown	Dark Brown	Pale brown
Powder + conc. HNO_3	Green	Faint green	Yellowish green	Yellow	Dark Green	Pale yellow	Golden yellow	Brown	Reddish yellow	Pale red	Dark brown	Brown	Intense Brown	Brown	Brown
Powder + conc. HCl	Green	Brown	Light green	Pale Green	Dark Green	Deep green	Brown	Brown	Pale Brown	Greenish brown	Dark brown	Greenish brown	Brown	Dark Brown	Pale Brown
Powder + conc. H_2SO_4	Yellowish green	Dark green	Green	Intense yellow	Green	Light yellow	Yellow	Green	Pale yellow	Pale orange	Dark brown	Greenish yellow	Reddish Brown	Dark green	Green
Powder + 5% I_2	Green	Brown	Brown	Woody brown	Green	Pale green	Deep brown	Green	Intense green	Pale yellow	Dark brown	Greenish brown	Brown	Dark Brown	Intense green
Powder + 1% Glacial acetic acid	Pale green	Brown	Green	Light brown	Green	Pale green	Light brown	Faint green	Pale green	Faint yellow	Dark brown	Faint yellow	Brown	Dark Brown	Brown
Powder + Glacial acetic acid + conc. HNO_3	Reddish orange	Dark green	Intense green	Yellow	Green	Pale yellow	Yellow	Green	Pale yellow	Golden yellow	Intense brown	Dark green	Intense reddish brown	Green	Greenish brown
Powder + 10% NaOH	Brown	Dark brown	Green	Brown	Dark brown	Pale brown	Intense brown	Green	Intense green	Dark brown	Blackish brown	Green	Dark Brown	Dark Brown	Brown
Powder +10% NaOH + Conc. HNO_3	Pale brown	Dark brown	Brown	Yellow	Green	Pale yellow	Pale yellow	Dark green	Green	Brown	Black	Intense green	Brown	Dark green	Green
Powder + dil. 1M HNO_3	Faint brown	Intense green	Brown	Pale brown	Dark Green	Green	Fade yellow	Green	Pale green	Pale yellow	Black	Orange yellow	Light Brown	Dark green	Pale green
Powder + dil. 1M H_2SO_4	Fade brown	Dark brown	Green	Light brown	Green	Pale Green	Faint brown	Pale Brown	Light green	Faint yellow	Brown	Light green	Brown	Dark Brown	Intense green
Powder + dil. 1M HCl	Light brown	Fade green	Light green	Brown	Green	Pale Green	Pale brown	Brown	Pale green	Light brown	Dark brown	Pale green	Brown	Pale Brown	Green
Powder + 10% KOH (Methanolic)	Dark brown	Fade green	Green	Reddish brown	Greenish brown	Intense Green	Brown	Dark Brown	Pale green	Brown	Greenish black	Instance green	Chocolate Brown	Dark Brown	Intense green
Powder +	Fade	Brown	Light	Woody	Faint	Faint	Brown	Dark	Faint	Faint	Dark	Pale	Brown	Dark	Pale

Reagent	CTF			MIL			TDAP			CAF			FBARB		
	Day light	**Long UV**	**Short UV**	**Day light**	**Long UV**	**Short UV**	**Day light**	**Long UV**	**Short UV**	**Day light**	**Long UV**	**Short UV**	**Day light**	**Long UV**	**Short UV**
$CuSO_4$	brown		green	brown	brown	green		Brown	green	brown	brown	green		Brown	green
Powder + Picric acid	Brown	Brown	Green	Golden yellow	Green	Light Green	Yellow	Dark Brown	Intense green	Yellow	Dark green	Green	Golden yellow	Intense green	Green
Powder + 5% CH_3COOPb	Faint brown	Brown	Green	Light brown	Faint brown	Pale Green	Woody brown	Dark Brown	Pale green	Brown	Dark brown	Light green	Shade brown	Intense brown	Green
Powder + MeOH	Greenish brown	Brown	Pale green	Brown	Reddish brown	Pale green	Yellowish brown	Brown	Yellowish green	Brown	Black	Fade yellow	Brown	Dark brown	Emrolled green
Powder + dil. NH_3	Brown	Dark brown	Green	Intense brown	Dark Green	Intense Green	Faint brown	Fade Brown	Fade Brown	Brown	Intense green	Green	Intense Brown	Dark brown	Intense green
Powder + dil. NH_3 + conc. HNO_3	Light brown	Pale green	Green	Yellow	Pale green	Green	Intense brown	Shade brown	Pale green	Light Brown	Intense brown	Emrolled green	Intense Brown	Green	Pale green
Powder + 10% $FeCl_3$	Black	Black	Black	Greenish black	Dark Green	Green	Intense brown	Green	Green	Dark brown	Dark green	Green	Blackish brown	Green	Intense green
Powder + acetone+ MeOH	Faint brown	Dark brown	Pale Green	Pale brown	Dark brown	Faint Green	Woody brown	Brown	Pale green	Brown	Black	Faint green	Faint Brown	Intense brown	Green
Powder + Drangdroff reagent	Intense brown	Faint brown	Green	Pale brown	Dark brown	Green	Golden yellow	Reddish Brown	Green	Reddish brown	Pale brown	Intense green	Reddish brown	Intense brown	Intense green
Powder + Wagner's Reagent	Greenish brown	Faint green	Light Green	Chocolate brown	Dark brown	Pale Green	Faint brown	Faint Brown	Pale green	Brown	Pale brown	Pale green	Brown	Faint brown	Green
Powder + Benedicts reagent	Grayish brown	Dark brown	Intense brown	Intense brown	Dark brown	Faint Green	Greenish brown	Faint Brown	Pale green	Yellowish brown	Faint brown	Faint brown	Blackish Brown	Brown	Black
Powder + fehling's Reagent	Greenish brown	Fade brown	Intense Green	Golden brown	Brown	Pale green	Blackish brown	Faint Brown	Pale green	Brown	Brown	Green	Faint Brown	Pale Brown	Pale green
Powder + Ninhydrin Reagent (2% in acetone)	Faint grenish brown	Faint brown	Pale Green	Brown	Brown	Emrolled green	Woody brown	Intense Brown	Fade green	Intense brown	Brown	Faint green	Intense Brown	Dark Brown	Pale green

Table 10. Phyto-chemical analysis

Constituents	CAF				CTL				MIL				TDAP				FBARB			
	PEE	AE	ME	AE	PEE	AE	ME	AE	PEE	AE	ME	AE	PEE	AE	ME	AE	PEE	AE	ME	AE
Alkaloid	-	++	++	++	-	-	+	+	-	-	+	+	-	-	+	+	-	+	-	-
Flavanoids	-	-	++	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	-
Tannins	-	+	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+
Terpenoids	+	++	++	-	+	+	+	-	+	-	+	-	+	-	+	-	+	-	-	-
Saponins	-	-	++	++	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Steroids	+	++	++	-	+	+	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Cardiac glycosides	-	-	++	++	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	-
Proteins	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	+

CAF= Cassia auriculata Flower, CTL= Cinnamomum tamala Leaves, MIL= Mangifera indica Leaves, TDAP= Trichosanthes dioica Arial part, FBARB= Ficus benghalensis Arial Root bark.PEE= Petroleum ether extract, AE= Acetone extract, ME= Methanol extract, AE= Aqueous extract

Table 11. Pre-formulation studies of powder blends

Parameters	Powder blends for			
	TAB1	TAB2	TAB3	TAB4
Angle of repose	24.3±1.13°	26.2±1.41°	24.9±1.12°	30.5±1.22°
Loose bulk density (g/cm^3)	0.325±0.023	0.383±0.015	0.357±0.022	0.356±0.009
Tapped bulk density (g/cm^3)	0.516±0.015	0.505±0.005	0.511±0.021	0.526±0.005
Hausner ratio	1.35±0.003	1.20±0.012	1.31±0.009	1.36±0.011
Compressibility index (%)	24.52±1.27	21.32±1.10	30.56±1.09	27.09±1.02
Loss on drying (%)	0.86±0.004	0.89±0.009	0.91±0.011	0.85±0.033

Data represented as mean±SD, n=3

Table 12. Standardization of formulated ant-diabetic polyherbal tablets

Parameters	Formulations			
	F 1	F2	F3	F4
Colour	Dark brown	Dark brown	Dark brown	Dark brown
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Texture	Smooth	Smooth	Smooth	Smooth
Weight variation (%)	±2.84	±2.56	±2.58	±2.46
Hardness (kg/cm^2)	7.5±0.2	9.5±0.15	9.7±0.17	8.0±0.21
Friability (%)	0.15±0.01	0.16±0.10	0.11±0.01	0.10±0.02
Disintegration time (min)	29±1.03	36±1.08	32±1.41	31±1.02

Data represented as mean±SD, n=3

Table 13. Total microbial count and pathogen

Test parameters	Observation (CFU/gm)	Limit (CFU/gm)
Total aerobic viable count	10^3	NMT 10^5
Yeast and moulds	Nil	NMT 10^2
E. coli	4	10

Table 14. Microbial examination of formulated anti-diabetic polyherbal tablets

Name of the microorganism	Formulations			
	F1	F2	F3	F4
Staphylococcus aureus	Absent	Absent	Absent	Absent
Salmonella sp.	Absent	Absent	Absent	Absent
Bacillus subtilis	Absent	Absent	Absent	Absent
Candida albicans	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent	Absent

CFU= Colony forming unit

4. DISCUSSION

Diabetes is a metabolic disease comprises with various biochemical dysfunctions leads to coronary heart disease, microvascular disease, neuropathy, nephropathy, retinopathy, etc. Various plant isolated constituents are reported to improve the diabetic condition. The therapeutic efficacies of herbals or herbal formulations are mostly based on the synergistic property of each constituent. In some traditional medicine systems, mixtures of plants are used rather than one species ie. a mixture of the two (or more) species gives a better activity than either species on its own. 'Triphala is a well-known polyherbal formulation in Ayurveda since ancient time; used for several therapeutic purposes. This formulation was prepared as powdered preparation, in combination of dried fruits of *Emblica officinalis* (Family: Euphorbiaceae), *Terminalia belerica* (Family: Combretaceae) and *Terminalia chebula* (Family: Combretaceae), in equal proportions as described in Ayurvedic formulary of India (AFI) as the first line treatment for many ailments such as laxative in chronic constipation, detoxifying agent of the colon, food digestive problems and rejuvenator of the body etc. [34]. Although this traditional formulation in

the form of raw powder. The synergy between different herbs in a formulation was also shown pharmacologically by a combination of Nettle (*Urtica Ioica*) and Pygeum bark (*Pygeum africanum*), which was taken for benign prostate hyperplasia, both inhibits 5-reductase and aromatase more significantly than the sum of either alone [35]. In this functional formulation, various constituents of herbs would produce the similar effect as mentioned like *Urtica Ioica* and *Pygeum africanum*. *Cassia auriculata, Ficus bengalensis* leads to suppression of gluconeogenesis and HMG-CoA reductase respectively [36]. *Cinnamomum tamala, Mangifera indica* and *Trichosanthes dioica* also had shown the antidiabetic effect by promoting insulin release from ß-cells [37]. The presence of various phytochemical constituents like flavanoids, alkaloids, steroids, terpenoids, and tannins etc combine activity likely leads to the antidiabetic potential of this formulated tablet (Fig. 6). Similarly the mixture of *Psidium guajava* L. leaf extract, *Lagerstroemia speciosa* L. leaf extract, *Morus indica* L. leaf, *Pinus densiflora* needles and *Acanthopanax senticosus* M. roots extract combination of functions such as intestinal glucose uptake blocking activity, increase activity of glucose transportation of cells, etc. ensures a higher antidiabetic synergy effect compared to that of the individual extracts [38]. The Authenticity and purity of herbals are indicated by Ash values, quantitative standards, and physicochemical parameters. Fluorescence is an important phenomenon in plant materials which exhibited by various chemical constituents present in the plant. A physical mixture of all herbal extract showed a good micromeritics properties like the angle of repose, Carr's Index and Hausner ratio indicated good flow properties and good packing ability. All formulated tablets complied with the official requirements of uniformity of weight. The low friability indicates that the herbal tablets were compact and hard. Drug-excipient binding interaction in the dosage form could affect in vitro drug release The drug and excipients compatibility study first require an aforementioned acquaintance of physicochemical properties for the development of pharmaceutical formulation [39]. Drug–excipients compatibility studies lays a base in the suspicious selection of most suitable excipients which play an important role in the design of optimum and effective dosage form concerning ideal Physico-chemical characteristic and good stability [40,41]. This compatibility study observed the incompatibility between drug and excipients and also affects their nature, bioavailability, stability and their chemical properties distressing their chemical nature in order to give affection their therapeutic efficacy and safety [42]. In IR spectra, the major peaks of O-H (3100-3600 cm^{-1}), C=O (1650-1700 cm^{-1}), C-OH (1350-1400 cm^{-1}), C-H (680-850 cm^{-1}) of the drugs remained unchanged in the mixtures. These results suggest the absence of any chemical interaction between the drug (PHF) and the excipients used in tablet formulations. It was suggested that the poly herbal formulation was compatible with the excipients which all were used in poly-herbal formulations. The FT-IR spectra of tablet formulations did not show the presence of any additional peaks for new functional groups. The major peaks of the drug remained unchanged in the mixtures. These results suggest the absence of any chemical interaction between the drug and the excipients used in tablet formulations. Hence, the drug was found to be compatible with all the excipients used. The *In-vitro* disintegration studies showed the better disintegration time as per standards. Various microbiological test results compile as per standard limits which show its safety.

5. CONCLUSION

Botanical standardization and development of poly-herbal tablet formulation for the treatment of diabetics was done with the combination of various parts of five anti-diabetic plants. The prepared formulation was screened for pre- and post-formulation standardization parameters as per pharmacopoeial standards and found to be appropriate. The research outcomings of the standardization parameters can be used for evaluating the quality, purity, and development of the poly-herbal formulations for the better and futuristic approach in the anti diabetic arena.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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