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Formulation, Characterization and Stability Evaluation of Antidiabetic Poly Herbal Suspensions

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ABSTRACT

From the time immortal, herbal combination formulations are also used as herbal therapies for different illnesses, including diabetes. The aim of the current study were to formulateand evaluate polyherbal suspensions using dried Hydroalcoholic extracts of dried seeds of Syzygiumcumini, dried fruit ofMomordicacharantia Linn, dried leaves of Gymnemasylvestre and heartwood of Pterocarpus marsupium. All the plants are regarded as active anti-diabetic behaviour in ancient Indian literature / folklore.Polyherbal suspensions were formulated using a suspending agent and other excipients.All formulations were further characterized for colour, pH, viscosity, sedimentation ratio, redispersibility, particle size, crystal formation, zeta potential, TLC profile and stability tests. The findings showed that the suspension was free flow and quickly dispersible.The organoleptic and physicochemical properties were satisfactory. From these findings it can be suggested that the herbal suspensions PHFA-6 in the sample were physically and chemically stable out of all the prepared formulations.Thus, the prepared oral herbal suspension is safe for use with promising hypoglycemic activity.

1. Introduction

Diabetes mellitus (DM) is a metabolic condition and a chronic disease which is complex. It has become a leading cause of loss of lower limbs through amputation, renal failure and blindness worldwide (Lester, 2019). Diabetes is mostly treated with modern medications, and diabetes treatment remains one of the 21st century's most challenging untreated health problems(Olokoba, Obateru and Olokoba, 2012). To address the shortcomings of modern medicine, the advancement of polyherbal medicine has provided new perspectives for DM treatment, as DM is a complicated disease needing numerous therapeutic approaches(Sridharan *et al.*, 2011). There is increasing interest in using medicinal plants to cure and control diabetes, oxidative stress and associated diseases, as plants are endowed with rich therapeutic bioactive compounds. Herbal treatment is therefore much effective, less adverse effects and more comparable than prescription medicines(Ríos, Francini and Schinella, 2015).

In traditional medicine, plant synergy is well established. Many herbal remedies include various plants or plant components, which may increase potency synergistically or decrease toxicity relative to individual compounds(Yuan *et al.*, 2017). In India, most contemporary health practitioners prepare their medicines as two or more herbal combinations, possibly because of their belief in phytomedicinal synergy.Based on the broad applicability of herbal plants to treat and manage diabetes, we centered on dried seeds of *Syzygiumcumini*, dried fruit of *Momordicacharantia* Linn, dried leaves of *Gymnemasylvestre* and heartwood of *Pterocarpus marsupium* plants.

Momordicacharantia(*M. charantia*)is generally referred to as bitter melon. Antiviral, anti-bacterial , anti-cancer and immune modulation properties have been identified.(Sathish Kumar *et al.*, 2010). It has been commonly used as an additional or supplemental medicine for the management of mellitus because of its decreased glucose effects (Ooi, Yassin and Hamid, 2012). *Syzygiumcumini*(*S.cumini*)commonly knownasblackpluminenglish,Jamunin Hindi(Aeri, Anantha Narayana and Singh, 2020).The seeds of *S. cumini* had already been added to the standard literature in the category of therapeutic herbals. Numerousstudies havebeenperformedtoshow thebeneficialeffects of *S.cuminie*xtractinnormalizing theelevatedlipidprofilesofdiabeticrats('Anti-diabetic activity of Syzygium cumini and its isolated compound against streptozotocin-induced diabetic rats', 2008)'(Peixoto and Freitas, 2013)and increasing superoxidedismutaseandglutathiomeperoxidaseactivities (Peixoto and Freitas, 2013). Jamun seedspossessprotectiveeffect againstdiabetesrelatedcomplications like neuropathy,gastropathy,nephropathy(Grover*etal*.,2002),diabeti ccataract(Rathi*et al*.,2002),dyslipidemia(Sidana*etal*.,2017)and alsoreducedpepticulceration (Chaturvedi*etal*., 2009).*Pterocarpusmarsupium* (Roxb.) (*P. marsupium*) one of the most well-known members of genus *Pterocarpus* (Dhanabal *et al.*, 2006). *P. marsupium* bark is very effective in preventing cataract formation and reducing hyperglycemia in alloxanized diabetic rats(Therrell *et al.*, 2007).*Gymnemasylvestre* (*G. sylvestre*) belong to family Asclepiadaceae(Kanetkar, Singhal and Kamat, 2007). It is a popular medicinal herb with a long history of use in traditional ayurvedic medicine and has been thoroughly investigated for the efficacy of DM treatment (Pothuraju *et al.*, 2014).

The studied herbal mixtures in different combinations are widely used in traditional system of medicine for treatment of diabetes for almost a decade.Therefore, in the present study, for the first time we formulated and evaluated polyherbal suspensions using hydroalcoholic extracts of dried seeds of *Syzygiumcumini*, dried fruit of *Momordicacharantia* Linn, dried leaves of *Gymnemasylvestre* and heartwood of *Pterocarpus marsupium*.

2. Material and methods

2.1. Chemicals

Petroleum ether, ethanol, sodium carboxy methyl cellulose, methyl paraben, propyl paraben, tween 80, chloroform, benzene was used in the study. All the chemicals were procured from Sigma-Aldrich, India. Throughout the study, double distilled water was used to carry out the study.

2.2 Collection and authentication of plant materials

The dried seeds of *Syzygiumcumini*, *dried fruit of Momordicacharantia* Linn, dried leaves of *Gymnemasylvestre*and heartwood of*Pterocarpusmarsupium*were procured from the Khari baoli market, New Delhi. These plant materials were authenticated by Prof. M. P. Sharma, Taxonomist & Botanist, Department of Botany, Faculty of Sciences, JamiaHamdard (Hamdard University), Hamdard Nagar, New Delhi – 110062.All thevoucher specimens are preserved in the herbarium section of Department of Pharmacognosy, Monad University, KastalaKasmabad,Uttar Pradesh, India.

2.3Hydroalcoholic extractionofplant materials (by reflux condensation method)

The dried seeds of ***Syzygiumcumini***(2.5 kg), dried fruits of ***Momordicacharantia* Linn**(4.0 kg), dried leaves of ***Gymnemasylvestre*** (3.5 kg)and heartwood of***Pterocarpus marsupium***(4.0 kg) were taken and standardized.After standardization, it was crushed to smaller pieces, re-dried, coarsely powdered and passed through sieve no 44 to obtain dried powder. The dried powder of the plant components was collected individually using a cold hydroalcoholic percolation process.10 g of the dried powder was taken in 100 mL of petroleum ether in a rounded bottom container, filled with cotton wool, and placed on a rotary shaker for 24 hours at 120 rpm. After 24 hours, eight layers of muslin cloth was washed and centrifuged for 15 minutes at 5000 rpm.The supernatant was obtained and dried air under reduced pressure for dried residue.Petroleum ether has been evaporated from the powder.This dry powder was then taken separately in 100 mL of each solvent i.e. 70 % ethanol and 30 % water and stored for 24 hours on a rotary shaker at 120 rpm.Then the protocol followed was the same as before, and residues were measured to achieve the extractive yield of all extracts and deposited at 4 °C in airtight bottles.

Table 1. Hydro alcoholic extraction of plant materials.

Plant name	Part used	Qty of plant material (kg)	Wt. of dried hydroalcoholic extract ((gm)	% yield of dried hydroalcoholic extract (%)
Momordicacharantia	Dried Fruit	4.0	270	6.84
Pterocarpus marsupium	Heartwood	4.0	290	7.25
Syzygiumcumini	Dried seed	2.5	145	5.83
Gymnemasylvestre	Dried leaves	3.5	220	6.34

2.4. Method for preparation of antidiabetic herbal Suspension

The polyherbal antidiabetic suspensions were formulated by utilizing the individual dried hydroalcoholic extract of the plant materials by trituration method.The calculation for the required amount of the individual extract was based on their therapeutically effective dose (TED) with reference to available literatures. The dose at which the individual dried hydroalcoholic extracts of the plants exhibited the maximum anti-diabetic activity was found to be 150 mg/kg body weightfor dried fruit for *M. charantia*(Ahmed *et al.*, 2004), 100mg/kg b.w. for heartwood of *P. marsupium*(Dhanabal *et al.*, 2006), 100 mg/kg body weight for fried seeds of*S. cumini*(Ravi, Ramachandran and Subramanian, 2004)and 200 mg/kg b.w. for dried leaves of *Gymnemasylvestre*(Pothuraju *et al.*, 2014).

Six polyherbal antidiabetic suspensions namelyPHFA-1, PHFA-2, PHFA-3, PHFA-4, PHFA-5 and PHFA-6 were prepared and their composition is givenin **Table 2**.The quantity of individual dried hydroalcoholic extract required for preparing the suspensions was calculated using the ratio-proportion method.

Procedure

For preparing the suspension of extract, sodium carboxy methyl cellulose, methyl and propyl paraben were weighed accurately. Firstly, hydroalcoholic extractsand parabens were transferred into a clean mortar. Then, tween 80 and a little amount of sodium carboxy methyl cellulose were added and uniformly triturated to form a smooth paste. After that remaining amount of sodium carboxy methyl cellulose was added in portions with proper mixing to form slurry which was then transferred to a 100 mL beaker. The mortar was rinsed with distilled water and rinsed suspension was added to the beaker. The homogeneous mixing of the dispersion was achieved by mechanical stirrer (500 rpm). After the uniform mixing, the suspension was transferred to a 100 mL measuring cylinder and the volume was made up to the desired level (100 mL) with distilled water.

Table No2. Composition of formulation preparation.

Ingredients	Part used	Qty of dried hydroalcoholic extracts in gm in 100 mL formulation					
		PHFA-1	PHFA 2	PHFA 3	PHFA 4	PHFA 5	PHFA 6
Momordicacharantia	Dried Fruit	3.0 g	-	2.0 g	1.0 g	1.0g	1.0 g
Pterocarpus marsupium	Heartwood	-	2.5 g	1.5 g	1.0 g	1.0 g	1.0 g
Syzygiumcumini	Dried seed	-	-	-	1.0 g	-	0.75 g
Gymnemasylvestre	Dried leaves	-	-	-	-	2.0 g	1.25 g
CMC Sodium	Thickening, stabilizing agent	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v
Tween-80	Suspending agent	0.1% w/v	0.1% w/v	0.1% w/v	0.1% w/v	0.1% w/v	0.1% w/v
Propyl paraben	Preservative	0.02% w/v	0.02% w/v	0.02% w/v	0.02% w/v	0.02% w/v	0.02% w/v
Methyl paraben		0.2%	0.2%	0.2%	0.2%	0.2%	0.2%

	Preservative	w/v	w/v	w/v	w/v	w/v	w/v
Distilled water q.s. to	Vehicle	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL

Total volume prepared for stability studies

The dried hydroalcoholic extract of six ployherbal formulation were prepared and volumewasmake up to 3 litre for accelerated stability studies.It is given in **Table 3.**

Table No 3. Composition of formulationfor stability studies.

Ingredients	**Part used**	**Qty of dried hydroalcoholic extracts in gm in 100 mL formulation**					
		PHFA-1	**PHFA 2**	**PHFA 3**	**PHFA 4**	**PHFA 6**	**PHFA 6**
Momordicacharantia	Dried Fruit	90.0 g	-	60.0 g	30.0 g	30.0g	30.0 g
Pterocarpus marsupium	Heartwood	-	75.0 g	45.0 g	30.0 g	30.0 g	30.0 g
Syzygiumcumini	Dried seed	-	-	-	30.0 g	-	22.50 g
Gymnemasylvestre	Dried leaves	-	-	-	-	60.0 g	37.5 g
CMC Sodium	Thickening, stabilizing agent	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v
Tween-80	Suspending agent	0.1% w/v	0.1% w/v	0.1% w/v	0.1% w/v	0.1% w/v	0.1% w/v
Propyl paraben	Preservative	0.02% w/v	0.02% w/v	0.02% w/v	0.02% w/v	0.02% w/v	0.02% w/v
Methyl paraben	Preservative	0.2% w/v	0.2% w/v	0.2% w/v	0.2% w/v	0.2% w/v	0.2% w/v
Distilled water q.s. to	Vehicle	3.0 Lt	3.0 Lt	3.0 Lt	3.0 Lt	3.0 Lt	3.0 Lt

2.5. Characterization of polyherbal anti-diabetic herbalsuspensions

2.5.1. Determination of colour and odour

The colourand odour of poly herbal suspensions are visually examined after1 hour of preparation of formulation at room temperature.

2.5.2. Determination of pH

The pH of all the polyherbal suspensions wereexaminedafter 1 hour of preparation at room temperature by using ELICO India pH analyzer (Model LI612) in standard buffers solution of pH 4.2 and pH 9.0.

2.5.3. Determination of sedimentation rate

Sedimentation rate (F) is defined as a ratio of the final volume of sediment (V_u) to the original volume of sediment (V_o) before settling.The sedimentation volume of all the polyherbal suspensions was measured after 1 hour of preparation at room temperature. 50 mLof each formulation was taken in a 50 mLof graduated measuring cylinder.The vessel was stoppered and inverted thrice to obtain a uniform dispersion and then it was allowed to settle for 3mins, the volume of sediment formed was noted(Schuck, 2000).

2.5.4 Determination of viscosity

The viscosity of the polyherbalsuspensions was determined after 1 hour of preparation at room temperature by Brookfield viscometer (Model-LVDVE). To carried out this, the sample was added in the sampling vessel. And a small adapter was appropriately assembled. The spindle no. S-00 was inserted and placed centrally in the test material kept in small sample adapter. It was maintained until the fluid level was at proper immersion depth. Attached the spindle to the lower shaft of the viscometer very carefully; lift the shaft slightly holding it firmly with one hand, while screwing the spindle on with other. Avoid putting side thrust on the shaft. Proper spindle immersion depth and viscometer level were verified. The temperature of the sample was adjusted and maintained at about 25± 0.1℃ throughout the measurement. The presence of the air bubble trapped around the spindle should be avoided. Again, it was allowed to stabilize for 15 mins, and reading was recorded (in cps).

2.5.5 Determination of redispersibility

The polyherbal suspensionswas placed in a 100 mLgraduated measuring cylinder and was allowed to settle. There dispersibility of the suspension was measured after 1 hour of preparation at room temperature. Then the cylinder was rotated through 180° and the number of inversions necessary to rest homogeneous suspension was determined. If the suspension became homogeneous in one inversion, then there dispersibility of suspension was considered to be 100%. The percentage of ease of redispersibilityis decreased by every 5% with each additional inversion.

2.5.6 Determination of particle size

The eyepiece micrometer was calibrated usingthestagemicrometer. The mountwaspreparedby placingonedropofuniformly dispersedsuspensionontheslide andby placingthecoverslipoverit.Then,thedimensionsof50particleswer emeasured afterfocusingtheslideonacompoundmicroscope.Theaveragepart iclesizeandrange of theparticlesizewascalculated (SundarRajan*etal*., 2012).

2.5.7 Determination of crystal growth (Polarized light microscopy)

Crystals from each composition were analyzed under cross-polarized light to determine morphology (shape and size) of crystal. It gave information regarding crystal habit. The same crystal polymorph exhibited different crystalline habits, which lead to differences in crystal shape and rate of dissolution.

Photomicrographs were obtained using a Leica DM2500P polarizing microscope equipped with a PAXcam3 digital camera controlled by PAX-it! 2 versions one software. Each sample was prepared by dispersing the solid particles onto a glass microscope slide. To look for birefringence in the samples, images were collected using a 40x magnification with

crossed polarizers and the first-order red compensator in place.

2.5.8 Determination of zeta potential

The zeta-potential is used in colloid chemistry for observing the behaviour of dispersive systems in liquids. Besides, the zeta-potential characterizes the electrical double layer on the solid-liquid interface, a fact significant in flotation and flocculation processes (Ocepek, 1989).

Suspensions products are stabilized through Brownian motion of suspended particles. It should be protected from sticking of the particle when it presents in the colloidal stage. This can be achieved by increasing the charge associated with the particle, i.e. zeta potential. Zeta potential of all the formulations was determined by Dynamic Light Scattering (DLS) method using a computerized inspection system Malvern Zetasizer Nano-ZS series at 25±0.5.

2.5.9 Thin-layer chromatography (TLC)

Thin-layer chromatography (TLC) is a traditional method, frequently used for the analysis of botanical raw materials. This method is official in almost all pharmacopoeias for identification of herbal medicines. The visualization of the entire pattern of compounds which is present in the herbal drug and its formulation is essential in the quality and stability testing.

TLC provided for the entire drug in the monographs includes the identification of the drug based on its major chemical constituents as markers. Silica gel GF_{254} plates of uniform thickness were prepared and activated at 105ºC for 30min. and the plates were spotted with the drug extract using capillary tube. The spotted plates were dried at R.T. The solvents used were of analytical grade. Solvent system was prepared and the chromatographic chamber was saturated for 30 min. The plates were placed in the chamber and left for the development. Using different reagents did visualization of the spots and R_f values were calculated.

The TLC of the six polyherbal formulations was performed to check the stability of the isolated Rubiadin present in suspension. The TLC carried out in the solvent system Ethyl acetate: Methanol in 1:1ratio on a glass plate prepared with silica gel.

2.6. Accelerated stability studies for polyherbal formulations

Herbal formulations, singularly and in combinations, contains complex compounds in which no single active constituent. The main objective is to perform a stability study of the developed formulation. It is mainly helpful in determining the shelf-life of the product and compositions. It is primarily the period of storage at a specified condition within which the drug product still meets its established specifications.

The polyherbal formulated suspensionsPHFA-1, PHFA-2,PHFA-3, PHFA-4, PHFA-5 and PHFA-6 were stored at (40°C ± 2°C /75% RH ± 5% RH)in a stability chamber for a period of six months .The suspensions were withdrawn after a period of 30, 60, 90, 120, 150, and 180 days and analyzed for physical characterization like colour, odour, pH, sedimentation rate, viscosity, redispersibility, crystal growth, particle size, zeta potentialand TLC.

Observations

Table 4: TLC Profile of Hydro alcoholic extract of anti-diabetic Poly herbal formulations.

Formulation	Solvent System	No of spots observed under U.V. at 0 day	Rf value observed at 0 day	No of spots observed under U.V. at 30 day	Rf value observed at 30 day	No of spots observed under U.V. at 60 day	Rf value observed at 60 day	No of spots observed under U.V. at 90 day	Rf value observed at 90 day	No of spots observed under U.V. at 120 day	Rf value observed at 120 day	No of spots observed under U.V. at 150 day	Rf value observed at 150 day	No of spots observed under U.V. at 180 day	Rf value observed at 180 day
PHFA-1	PE : CHCl3 : MeOH 3 : 7 : 0	3	0.42 0.54 0.60	3	0.55 0.59 0.71	3	0.48 0.63 0.76	3	0.46 0.56 0.64	3	0.52 0.67 0.64	3	0.61 0.65 0.74	3	0.45 0.53 0.64
PHFA-2	PE : CHCl3 : MeOH 2.5 : 7.5 : 0	3	0.45 0.50 0.62	3	0.45 0.50 0.62	3	0.45 0.50 0.62	3	0.64 0.82 0.96	3	0.68 0.79 0.90	3	0.70 0.78 0.89	3	0.75 0.76 0.91
PHFA-3	PE : CHCl3 : MeOH 2 : 8 : 0	5	0.40 0.45 0.52 0.56 0.64	5	0.40 0.45 0.52 0.56 0.64	5	0.40 0.45 0.52 0.56 0.64	5	0.40 0.45 0.52 0.56 0.64	5	0.40 0.45 0.52 0.56 0.64	5	0.40 0.45 0.52 0.56 0.64	5	0.40 0.45 0.52 0.56 0.64
PHFA-4	PE : CHCl3 :	7	0.42 0.50 0.54 0.60	6	0.42 0.50 0.54 0.62	7	0.40 0.53 0.56 0.60	5	0.40 0.50 0.55 0.60	6	0.40 0.50 0.55 0.60	4	0.40 0.54 0.68 0.72	6	0.40 0.50 0.58 0.62

	MeOH 2.8:7 : 0.2		0.65 0.68 0.74		0.65 0.74		0.65 0.69 0.76		0.75		0.65 0.75				0.65 0.78
PHFA-5	PE : CHCl3 : MeOH 2.7 : 7 : 0.3	8	0.40 0.45 0.52 0.58 0.60 0.64 0.70 0.78	8	0.40 0.44 0.50 0.58 0.60 0.64 0.74 0.78	7	0.40 0.45 0.52 0.58 0.60 0.70 0.78	7	0.40 0.45 0.52 0.58 0.60 0.70 0.78	6	0.40 0.45 0.52 0.60 0.70 0.78	6	0.40 0.45 0.52 0.60 0.70 0.78	5	0.40 0.45 0.52 0.60 0.78
PHFA-6	PE : CHCl3 : MeOH 2.5 : 07 : 0.5	9	0.43 0.46 0.57 0.60 0.68 0.70 0.74 0.77 0.79	9	0.43 0.46 0.57 0.60 0.68 0.70 0.74 0.77 0.79	9	0.43 0.46 0.57 0.60 0.68 0.70 0.74 0.77 0.79	9	0.43 0.46 0.57 0.60 0.68 0.70 0.74 0.77 0.79	9	0.43 0.46 0.57 0.60 0.68 0.70 0.74 0.77 0.79	9	0.43 0.46 0.57 0.60 0.68 0.70 0.74 0.77 0.79	9	0.43 0.46 0.57 0.60 0.68 0.70 0.74 0.77 0.79

PE : Petroleum Ether , CHCI3: Choloroform , MeOH : Methanol

Table 5: Characterization and Stability studies of anti-diabetic herbal suspension at 0thday.

Standard deviation, n=3

Formulation code	Parameters							
	Colour&Odour	**pH (mean±S.D)**	**Viscosity in Cps (mean±S.D)**	**Sedimentation rate**	**Redi(%)**	**Particle size**	**Crystal Growth**	**No. of spots**
PHFA-1	Faded brown Characteristic	6.02±0.08	79.9±0.35	1.30	90 %	32-35 µ	None	3
PHFA-2	Reddish brown Characteristic	6.22±0.06	92.6±0.5	1.42	85 %	52-55 µ	None	3
PHFA-3	Brownish orange Characteristic	6.84±0.12	116.73±0.4	1.27	95 %	35-42 µ	None	5
PHFA-4	Light brown Characteristic	7.77±0.12	96.1±0.70	1.10	85 %	45-52 µ	None	7
PHFA-5	Dark brown Characteristic	6.94±0.13	109.5±0.95	1.15	90 %	34-38 µ	None	8
PHFA-6	Brownish Characteristic	6.26±0.12	124.6±0.1	1.25	95 %	52-56 µ	None	9

Table 6: Characterization and Stability studies of anti-diabetic herbal suspension at 30thday.

Formulation code	Parameters							
	Colour&Odour	**pH (mean±S.D)**	**Viscosity in Cps (mean±S.D)**	**Sedimentation Rate**	**Redi(%)**	**Particle size**	**Crystal Growth**	**No. of spots**
PHFA-1	Faded brown Characteristic	6.12±0.06	105.8±0.5	1.50	90 %	32-35 µ	None	3
PHFA-2	Reddish brown Characteristic	5.94±0.07	94.8±0.8	1.84	85 %	52-55 µ	None	3
PHFA-3	Brownish orange Characteristic	6.86±0.10	120.45±0.6	1.28	95 %	42-44 µ	None	5
PHFA-4	Light brown Characteristic	7.79±0.8	95.08±0.87	1.15	85 %	53-55 µ	None	6
PHFA-5	Dark brown Characteristic	6.72±0.11	79.4±0.75	1.42	90 %	40-43 µ	None	8

PHFA-6	Brownish Characteristic	6.24±0.10	126.7±0.4	1.28	95 %	52-57 µ	None	9

Standard deviation, n=3

Table 7: Characterization and Stability studies of anti-diabetic herbal suspension at 60thday.

Formulation code	Parameters							
	Colour&Odour	pH (mean±S.D)	Viscosity in Cps (mean±S.D)	Sedimentation rate	Redi(%)	Particle size	Crystal growth	No. of spots
PHFA-1	Faded brown Characteristic	6.12±0.08	108.7±0.5	1.45	90 %	32-35 µ	None	3
PHFA-2	Reddish brown Characteristic	6.20±0.06	91.8±0.4	1.42	85 %	52-55 µ	None	3
PHFA-3	Brownish orange Characteristic	6.84±0.10	120.3±0.7	1.23	95 %	35-42 µ	None	5
PHFA-4	Light brown Characteristic	7.73±0.07	109±0.90	1.18	85 %	45-52 µ	None	7
PHFA-5	Dark brown Characteristic	6.72±0.11	89.5±0.95	1.30	90 %	34-38 µ	None	7
PHFA-6	Brownish Characteristic	6.25±0.12	125.6±0.1	1.26	95 %	52-56 µ	None	9

Standard deviation, n=3

Table8: Characterization and Stability studies of anti-diabetic herbal suspension at 90thday

Formulation code	Parameters							
	Colour&Odour	pH (mean±S.D)	Viscosity in Cps (mean±S.D)	Sedimentation Rate	Redi(%)	Particle size	Crystal Growth	No. of spots
PHFA-1	Faded brown Characteristic	6.02±0.08	109.9±0.35	1.40	90 %	32-35 µ	None	3
PHFA-2	Reddish brown Characteristic	6.22±0.06	98.6±0.5	1.50	85 %	52-55 µ	None	3
PHFA-3	Brownish orange Characteristic	6.85±0.12	120.53±0.4	1.28	95 %	35-42 µ	None	5
PHFA-4	Light brown Characteristic	7.77±0.12	112.1±0.70	1.20	85 %	45-52 µ	None	5
PHFA-5	Dark brown Characteristic	6.94±0.13	92.5±0.95	1.45	90 %	34-38 µ	None	7
PHFA-6	Brownish Characteristic	6.26±0.12	125.6±0.1	1.25	95 %	52-56 µ	None	9

Standard deviation, n=3

Table 9: Characterization and Stability studies of anti-diabetic herbal suspension at 120thday

Formulation code	Parameters							
	Colour&Odour	pH (mean±S.D)	Viscosity in Cps (mean±S.D)	Sedimentation rate	Redi(%)	Particle size	Crystal Growth	No. of spots
PHFA-1	Faded brown Characteristic	6.34±0.08	112.9±0.35	1.48	90 %	32-35 µ	None	3
PHFA-2	Reddish brown Characteristic	6.54±0.06	99.6±0.5	1.52	85 %	52-55 µ	None	3
PHFA-3	Brownish orange Characteristic	6.84±0.12	120.54±0.4	1.28	95 %	35-42 µ	None	5
PHFA-4	Light brown Characteristic	7.89±0.12	115±0.60	1.25	85 %	45-52 µ	None	6

PHFA-5	Dark brown Characteristic	7.04±0.13	99.5±0.95	1.35	90 %	34-38 µ	None	6
PHFA-6	Brownish Characteristic	6.26±0.12	125.8±0.1	1.25	95 %	52-56 µ	None	9

Standard deviation, n=3

Table 10: Characterization and Stability studies of anti-diabetic herbal suspension at 150thday**.**

Formulation code	Parameter							
	Colour&Odour	pH (mean±S.D)	Viscosity in Cps (mean±S.D)	Sedimentation Rate	Redi(%)	Particle size	Crystal Growth	No. of spots
PHFA-1	Faded brown Characteristic	6.38±0.08	109.9±0.35	1.52	90 %	32-35 µ	None	3
PHFA-2	Reddish brown Characteristic	6.55±0.06	96.6±0.5	1.58	85 %	52-55 µ	None	3
PHFA-3	Brownish orange Characteristic	6.84±0.12	120.13±0.4	1.27	95 %	35-42 µ	None	5
PHFA-4	Light brown Characteristic	7.90±0.12	102.54±0.80	1.30	85 %	45-52 µ	None	4
PHFA-5	Dark brown Characteristic	7.04±0.13	98.5±0.65	1.40	90 %	34-38 µ	None	6
PHFA-6	Brownish Characteristic	6.26±0.12	125.8±0.1	1.25	95 %	52-56 µ	None	9

Standard deviation, n=3

Table 11: Characterization and Stability studies of anti-diabetic herbal suspension at 180thday**.**

Formulation code	Parameter							
	Colour&Odour	pH (mean±S.D)	Viscosity in Cps (mean±S.D)	Sedimentation rate	Redi(%)	Particle size	Crystal growth	No. of spots
PHFA-1	Faded brown Characteristic	6.40±0.08	110.6±0.35	1.55	90 %	32-35 µ	None	3
PHFA-2	Reddish brown Characteristic	6.58±0.06	93.5±0.5	1.50	85 %	52-55 µ	None	3
PHFA-3	Brownish orange Characteristic	6.84±0.12	120.23±0.4	1.27	95 %	35-42 µ	None	5
PHFA-4	Light brown Characteristic	7.90±0.12	109.76±0.70	1.30	85 %	45-52 µ	None	6
PHFA-5	Dark brown Characteristic	7.08±0.13	105.62±0.54	1.45	90 %	34-38 µ	None	5
PHFA-6	Brownish Characteristic	6.26±0.12	125.8±0.2	1.25	95 %	52-56 µ	None	9

Standard deviation, n=3

3. Results and discussion

3.1 Results and discussions of formulation and stability

The Pharmaceutical evaluation and stability study data of the formulated suspensions PHFA-1, PHFA-2, PHFA-3, PHFA-4, PHFA-5 and PHFA-6 at 0th, 30th, 60th, 90th, 120th, 150th, and 180thday for the physicochemical characteristics likecolour, odour, pH, sedimentation volume (sed. vol.), viscosity, particle size (part.size), redispersibility (redis(%)), crystal growth (crys.gro.) and number of prominent spots observed in TLC pattern.

3.1.1 Organoleptic characteristics

In the study of organoleptic features of the freshly prepared suspensions, PHFA-1 was faded brown, PHFA-2 was reddish brown, PHFA-3 was brownish orange, PHFA-4 was light brown, PHFA-5 was dark brown andPHFA-6 was brownish. All these formulations possessed a characteristic odour. These observations remained same after 1 hour of preparation and did not show any variations, i.e. they retained their pleasant appearance and characteristic odoureven on storing at (40°C ± 2°C /75% RH ± 5% RH) for a period of three months. Thus,all the formulations were stable in terms of their organoleptic properties.

3.1.2 pH

The pH after 1 hour of preparation of formulation was observed to be 6.02, 6.22, 6.84, 7.77, 6.94and 6.26respectively for PHFA-1, PHFA-2, PHFA-3, PHFA-4, PHFA-5 and PHFA-6. The pH after 180 dayaccelerated stability studies was 6.40, 6.58, 7.90and 7.08 for PHFA-1, PHFA-2, PHFA-4 and PHFA-5 respectively. While for PHFA-3

and PHFA-6, the values of pH were almost same on ageing also and there were no chemical changes.

3.1.3 Viscosity in Cps

The viscosities of the freshly prepared suspensions PHFA-1, PHFA-2, PHFA-3, PHFA-4, PHFA-5 and PHFA-6 determined after1 hour of preparation were 79.9cps, 92.6cps, 116.73cps, 96.1cps, 109.5cps and 124.6cps respectively. After storing at accelerated stability study conditions (40°C ± 2°C /75% RH ± 5% RH) for a period of three months, their final viscosities were 110.6cps, 93.5cps, 120.23cps, 109.76cps, 105.62cps and 125.8cps, respectively.

3.1.4 Sedimentation volume

The sedimentation volume of suspensions 1 hour after preparation, were 1.30, 1.42, 1.27, 1.10, 1.15and 1.25 respectively for PHFA-1, PHFA-2, PHFA-3, PHFA-4, PHFA-5 and PHFA-6. These dimentationvolume increased to 1.55, 1.50, 1.30and 1.45 respectively for A-1, A-2, A-4 and A-5 while it remained almost constant throughout the duration of accelerated stability studies for suspensions A-3 and A-6. The caking was not observed in any of the formulations.

3.1.5 Redispersibility

All the six freshly prepared suspensions PHFA-1,PHFA-2, PHFA-3, PHFA-4, PHFA-5 and PHFA-6. Exhibitedexcellent redispersibilityafter 1 hour of preparation. All yielded a homogenous suspension in single inversion, i.e. 100% redispersibility. On storing at accelerated stability study conditions (40°C ± 2°C /75% RH ± 5% RH) for a period of three months, the redispersibilitywas 90% for PHFA-1and PHFA-5, 85% for PHFA-2and PHFA-4and 95% for PHFA-3 and PHFA-6

3.1.6 Crystal growth

There was no indication of crystal growth in any of the freshly prepared formulations PHFA-1, PHFA-2, PHFA-3, PHFA-4, PHFA-5 and PHFA-6 when observed by naked eyes as well as under microscope. There was no crystal growth even after storage at (40°C ± 2°C /75% RH ± 5% RH) for a period of three months, indicating good stability of the formulations.

3.1.7 Particle size

The particle size observations for the freshly prepared formulations PHFA-1, PHFA-2, PHFA-3, PHFA-4, PHFA-5 and PHFA-6 were 32-35µ, 52-55µ, 35-42µ, 45-52µ, 34-38µ and 52-56µ respectively. There were no major variations in the particle size of the formulations throughout the study.

As the observations for pH, sedimentation volume, viscosity and redispersibiltywere almost constant on0^{th}, 30^{th}, 60^{th}, 90^{th}, 120^{th}, 150^{th}, and 180^{th}day for suspensions PHFA-3 and PHFA-6, it lead us to infer that PHFA-3 and PHFA-6 might be the most stable formulations.

3.1.8 Zeta size

The zeta-potential of final formulation PHFA-6 in water was found to be −19.3 ± mV at 0^{th} day and -17.7 ± mV at 180^{th} day as shown in **Figure 1.** The negative charge indicates proper surface charge for longer half-lives and high biodistribution and ensured the stability of the nanocomposite in biological environments, allowing target sites.

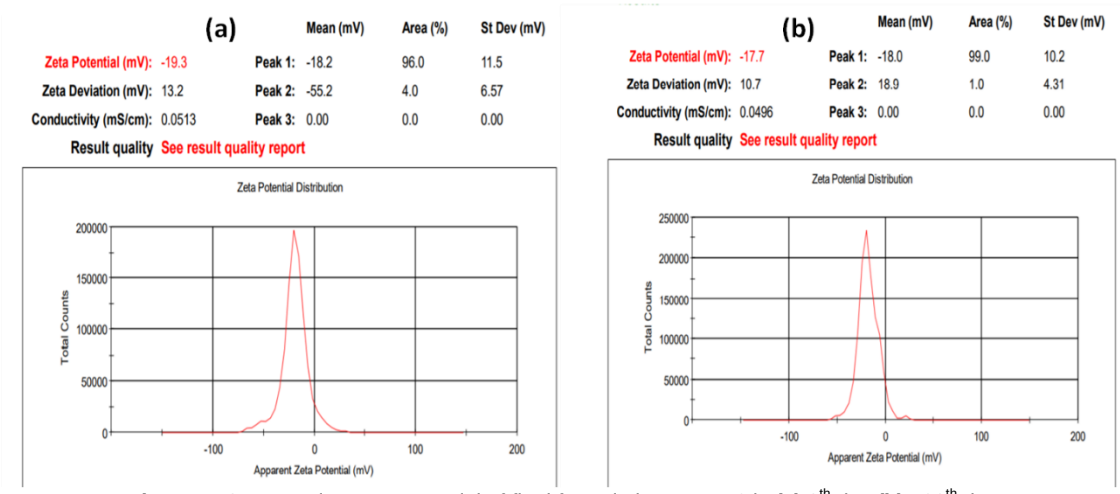

Figure 1. Comparative zeta potential of final formulation PHFA-6 in **(a)** 0^{th} day **(b)** 180^{th} days

3.1.9 TLC

TLC for each formulation: PHFA-1, PHFA-2, PHFA-3, PHFA-4, PHFA-5 and PHFA-6 were carried outin order to assess their stability. The 0^{th} day samples and the 30^{th}, 60^{th}, 90^{th}, 120^{th}, 150^{th}, and 180^{th}day samples obtained after storing the formulations at (40°C ± 2°C /75% RH ± 5% RH) were subjected to TLCanalysis. The TLC profile for 0^{th} day for each of the formulation was used as the standard. Then 30^{th}, 60^{th}, 90^{th}, 120^{th}, 150^{th}, and 180^{th}day sample's TLC profiles were compared with this standard to check whether the formulations were stable or not.

The TLC profile of the Poly herbal suspension

PHFA-1 exhibited 3spots respectively for 0^{th}, 30^{th}, 60^{th}, $90^{th}120^{th}$, 150^{th}, and 180^{th}day samples with changing $R_f$values.

PHFA-2 showed 3spots in 0^{th}, 30^{th}, 60^{th},90^{th}, 120^{th}, 150^{th}, and 180^{th}day TLC profile. Same $R_f$values were observed for 30^{th} and 60^{th} day samples while there was a great variation in the 90^{th} day observations.

TheTLC pattern for 0^{th}, 30^{th}, 60^{th}, $90^{th}120^{th}$, 150^{th} and 180^{th}day samples of formulation PHFA-3 was constant throughout the study and Exhibited 5most prominent spots with their respective R_f values of 0.40, 0.45, 0.52, 0.56, 0.64 . The brightness of the spots did not diminish.

The number of spots in PHFA-4 samples kept on changing from 7 in 0th day, 6 in 30th 7 in 60th day, 5 in 90th day, 6 in 120th day, 4 in 150th day and finally 6in 180th day samples.

The pattern for PHFA-5 showed 8spots in 0th day,30th day which decreasedto 7in 60th day and 90th day thenfinally decreasedto 6and 5respectively for 120th,150th and 180th day samples.

The TLC profile for herbal formulation PHFA-6 exhibited presence of 9 highly prominent spots which maintained their brightness in all the profiles for different day samples. 0.43 0.46, 0.57, 0.60, 0.68, 0.70, 0.74, 0.77and 0.79 were respectively the R_f values for the spots1, 2, 3, 4, 5, 6, 7 , 8and 9.

Thus, the TLC pattern did not change at all throughout the accelerated stability studies for formulation PHFA-3 and PHFA-6. After studying the TLC profile we concluded that the formulations PHFA-3 and PHFA-6 were most stable. So, these two were selected for studying their anti-diabetic effects in streptozotocin induced diabetic rats.

The results led us to conclude that the anti-diabetic herbal suspensions PHFA-6 were physically and chemically stable throughout the study, i.e. they did not undergo any phytochemical variations.

4. Conclusion

Six anti-diabetic herbal formulations: PHFA-1, PHFA -2, PHFA -3,PHFA -4,PHFA -5 and PHFA -6 were prepared using the dried alcoholic extracts of dried seeds of *Syzygiumcumini*, *dried fruit of Momordicacharantia* Linn, dried leaves of *Gymnemasylvestre*and heartwood of*Pterocarpusmarsupium*. Of these PHFA -3 andPHFA -6 were established to be most stable after studying the various physicochemical characteristics like colour, odour, pH, sedimentation volume (sed. vol.), viscosity, particle size (part. size), redispersibility (redis(%)), crystal growth (crys.gro.),zeta potential , number of prominent spots observed in TLC pattern along with their R_f values. The results led us to conclude that the anti-diabetic herbal suspensions PHFA -6 were physically and chemically stable throughout the study, i.e. they did not undergo any phytochemical variations.

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Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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