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Quality Assesment And Designing Of Nano Topical Gels Of Psoralea Corylifolia (Bakuchi Seed), Pongamia Pinnata (Karanj Seed Oil) And Holarrhena Pubescens (Kutaja Leaves) Extract.

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Abtract:
Pongamia pinnata Linn and Psoralea corylifolia Linn, both from the Fabaceae family, were chosen to study their combined effects on antib acterial and anti-psoriatic activities. The goal was to create a nanogel using Psoralea Corylifolia (Bakuchi seed), Pongamia Pinnata (Karanj seed oil), and Holarrhena Pubescens (Kutaja Leaves) extracts, assess its quality and effectiveness, and formulate a topical gel for skincare. Karanja Oil is used to treat various skin conditions, while Pongamia Seed oil has insecticidal and antibacterial properties. Wrightia tinctoria (Kutaja) offers anti-psoriatic, anti-fungal, and anti-bacterial benefits, promoting collagen production to alleviate psoriasis symptoms. Bakuchi Rasayana was selected as an oral drug for the study due to its efficacy in treating skin disorders. A polyherbal nanogel was developed for transdermal drug delivery, characterized, optimized, and evaluated. Quality control tests included phytochemical analysis, physical and chemical evaluations, and antimicrobial activity testing. The nanogel showed promising results, with positive phytochemical analyses and no significant changes in the pure drug. The study suggests that the combination of Bakuchi, Kutaj, and Karanj can be effectively prepared and evaluated.

Keywords: Bakuchi, Kutaj, Karanj, Polyherbal nanogel, Quality Assesment

1. Introduction

Psoriasis is the commonest chronic autoimmune disorder. It is a skin disorder that causes skin cells to multiply up to 10 times faster than normal. This makes the skin build up into bumpy red patches covered with white scales. They can grow anywhere, but most appear on the scalp, elbows, knees, and lower back. Psoriasis is a common, long-term (chronic) disease with no cure. It tends to go through cycles, flaring for a few weeks or months, then subsiding for a while or going into remission [1-2] .It shows strong genetic predisposition and autoimmune pathogenic traits. psoriasis is a plaques of red skin, often covered with silver-coloured scales. These plaques may be itchy and painful, and they sometimes crack and bleed. In severe cases, the plaques will grow and merge, covering large areas. Disorders of the fingernails and toenails, including discoloration and pitting of the nails. The nails may also crumble or detach from the nail bed,many types of psoriasis are reported such as Plaque psoriasis,Nail psoriasis, Guttate psoriasis, Inverse psoriasis, Pustular psoriasis, Erythrodermic psoriasis, Psoriatic arthritis, many alternative natural treatments for psoriasis are Aloe vera, Curcuma longa/ Curcuma domestica, Bakuchi, Karanj [Pongamia pinnata] and Kutaj[2-3] .

Formulation of nanogel have many benefits like it Improves skin penetration and better skin localization was found to be beneficial for effective topical delivery of drugs, for which stratum corneum permeability is a limiting factor, Nanogel systems proved their potential to deliver drugs in controlled, sustained and targetable manner, By selecting appropriate polymers, it is possible to modify the surface charge of the nanogel system and thus control the interaction with skin and hence the transdermal flux, As compared with other nano size drug carriers, nanogel, show a properties like capacity to decrease off target impacts, stretch out medication flow time because of high stability compared to micelles, control drug discharge, to target particular tissues by means of conjugation of the nanogel surface with affinity ligands, to give security to the medication cargo from quick degradation, and to encourage crossing tissue barrier[3-5].Nanogel are more effective because of their small size and magnificent properties, they may represent a suitable drug carrier for skin penetration and retention. Novel formulations based on nanocarriers are a promising prospect to overcome the limitation of conventional formulations by offering a reduction in dose, dosing frequency, dose-dependent, side effects with enhanced efficacy. Presently nano-formulations have gained widespread application for effective and safe treatment of psoriasis.In the Present study Bakuchi powder, Kutaj leaves powder and Karanj seed powder was evaluated as per the specifications and test procedure mentioned in the WHO guideline and the pharmacopoeia.All Raw material specification was under the limits of pharmacopoeia and Phytochemical analysis of raw material was observed positive. All the 3 formulations were homogenous and free of grittiness. formulation showed good physical appearances. No phase separation after centrifugation was found in formulations A1, A2, A3. The pH of all formulation from A1 To A3 was found to be in the range of 6.0 to 6.9. A2 formulation showed better spreadability than the other formulations. The antimicrobial activity was performed by well diffusion method by measuring the zone of inhibition (in mm) the activity results of the polyherbal gel showed antimicrobial activity in dose dependent manner. The methanolic extract of Psoralea corylifolia (Bakuchi), Pongamia Pinnata (Karanj) and Holarrhena pubescens (Kutaj) shows the best result in the solvent system of Ethyl acetate: methanol (3:7) and in the solvent system of Toluene: ethyl acetate (3:7) and gave the spot. Their Rf values were 0.7, 0.5, 0.9. FTIR spectra of polyherbal nanogel are shown in graph. it was observed that principal peak. of the drug was found in FTIR spectra of drug. It was suggested that there were no physical and chemical changes of pure drug.The major diffraction pic for polyherbal formulation was observed at 14.82˚, 24.26˚ and 38.144˚ corresponding to interplanar [5-7] .

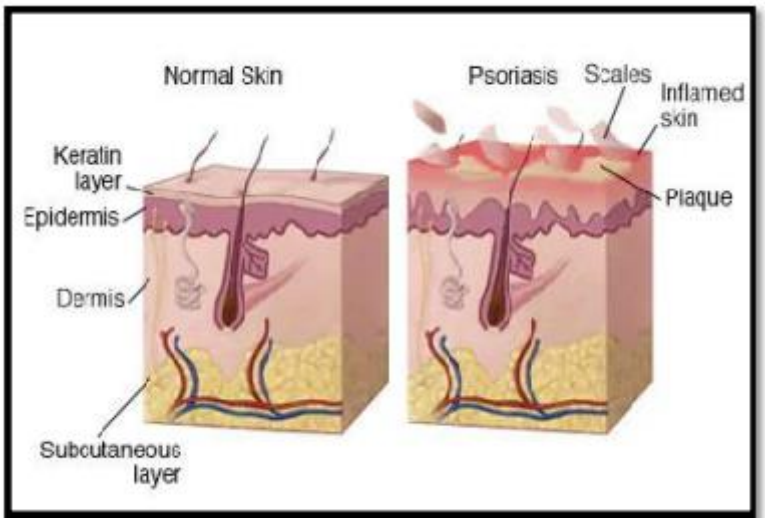

Fig 1.1 Development of Psoriasis

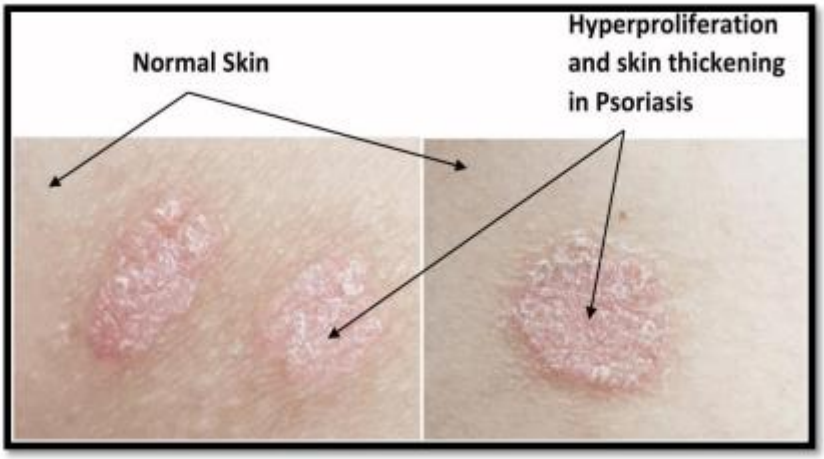

Fig 1.2 Psoriatic plaques covered with silvery scales compared to normal skin parts.

2.Materials and Methods

Materials:

Bakuchi (Psoralea corylifolia) was obtained from herbal plant from forest of Dhaga, Karanj seed (Pongamia glabra) was obtained from Reema oil Industries, Mumbai, Kutaj (Holarrhena Antidysentrica) was obtained from herbal plant obtains from forest of Dhaga, Carbopol- 940 and di-methyl sulphoxide was obtained from Research-Lab Fine Chem Industries, Mumbai, Methyl paraben, Propylene glycol and Triethanolamine was obtained from S d fine- chem limited, Mumbai, Ethanol, Methanol was obtained from Thermo Fisher Scientific India Pvt. Ltd, Mumbai

Method:

Method of Extraction of Bakuchi & Kutaj

Preparation of Bakuchi (Psoralea corylifolia)

Seed Extract:

Bakuchi seed extract prepared by maceration method.Fine powder of each of the seed (25gm) with methanol (250ml) and diethyl ether respectively were taken in round bottom flask. For successive extraction with these solvents, seed powder was allowed incubation for 48 hrs with intermittent shaking at room temperature. After that the liquid extract obtained were filtered with Whatman filter paper and they were filter sterilized. All the extract stored at -20˚C in air tight bottle[3,8].

Fig 1.3 Bakuchi extract carried out by maceration

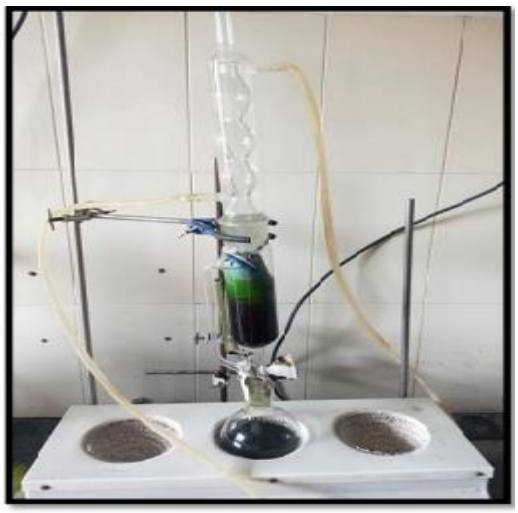

Fig 1.4 Kutaj extract carried out by Soxhlet method

3. Method of preparation of polyherbal nanogel

The nanogel is formulated and prepared from modified Emulsion Solvent Diffusion method. It is having 4 steps. In Step I -in the first step Accurately weighed quantity of bakuchi extract. kutaj extract and karanj oil is dissolved in ethanol and propylene glycol with stirring (organic phase). Step II -In the second step aqueous phase is prepared by using Carbopol -940 (1 gm) dissolved in water (100ml) with continuous stirring and heat for a 20min in a magnetic stirring. And the drug phase is sonicated under ultrasonic bath Sonicator for 10min [8,10].Step III -In this step drug phase is added drop by drop into aqueous phase during high-speed homogenization for 30 min at 6000rpm to from emulsion. The emulsion is converted into nanodroplet by homogenizer results in o/w emulsion formed. Step IV- In this step o/w emulsion added triethanolamine with continues stirring to from nanogel.

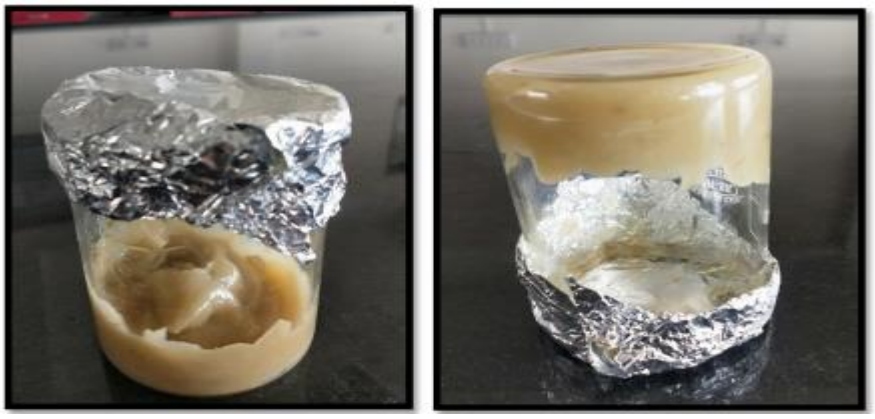

Fig1.5 Formulated polyherbal nanogel

Table. 1 Composition of batches A1 to A3

Sr.no	Ingredients (%)	A1	A2	A3
1	Bakuchi extract	0.5	1	1
2	Kutaj extract	0.5	0.5	1
3	Karanj oi	0.5	0.5	1
4	Carbopol-940	1	1	1
5	Propylene glycol	5ml	5ml	5ml
6	Methyl paraben	0.5 g	0.5 g	0.5 g
7	Triethanolamine	1 ml	1 ml	1 ml
8	Distilled water	Up to 100 ml	100 ml	100 ml

4.Evalution and characterization of prepared polyherbal nanogel

Physical Evaluation

Appearance

The polyherbal nanogel formulated were observed or their visual appearance, color, texture, and feel upon application such as grittiness, grassiness, smoothness, stuffiness and Tackiness.

Centrifugation Centrifugation test for base and formulation kept at different storage conditions were performed for 15 days. No phase separation after centrifugation was found in formulations A, B, C and base at 8 and 40°C during one-month study

pH

The pH of the develop gels base was determined by standardized digital pH meter at room temperature. One gram of Herbal nanogel gel was dissolved in 100ml of distilled water and stored for two hours. The measurement of pH of each formulation was in triplicate and the average values are presented. [10].

Viscosity

The viscosity of formulated polyherbal nanogel was determined using Brook-field viscometer (spindle number 7) Mounted the guard leg Attached the spindle (left hand thread) to the viscometer lower shift by lifting the coupling screw slightly. It washed firmly with one hand while screwing the spindle on with the other (note left hand thread) Avoid spindle, do the following before attaching the spindle. Begin by immersing the spindle in a diagonal path, slowly drag the spindle across the fluid surface, and bring the spindle to an upright position and thread on to screw Lower and center spindle in the test material until the "meniscus" of the fluid is at the center of the immersion groove on the spindle shaft. To make a viscosity measurement, turn the motor switch "ON" This energizes the viscometer drive motor. Allow time for the indicated reading to stabilize. The required for stabilization will depends on the speed at which the viscometer was running and the characteristics of the sample fluid. When making a viscosity measurement, the reading should be noted. [11,12].

Spreadability

Spreadability is expressed in terms of time in seconds taken by A modified apparatus consisting of two glass slides containing gel in between with the lower slide fixed to a wooden plate and the upper one attached to a balance by a hook was used to determine spread ability. It is calculated by Using the formula: - S=ML/T Were, M-weight tied to upper slide L-length of glass slides T-time taken to separate the slides The results are shown in table no. From the study of physical evalution of all three batches we considered batch A2 as it gives better result. [11,12].

Antibacterial Activity

Preparation of inoculum Uniform suspension of microorganism is obtained by Suspending 24 h fresh culture of bacteria (S. aureus and S. Epidermis) in an amount of 15mL of the sterile water [12,13].

Determination of zone of inhibition

Agar well diffusion method was used to determine the Antibacterial activity of the polyherbal nanogel. Transferred 20 mL of liquefied agar medium previously inoculated with 0.1 mL Bacteria into the sterile petri

dish having an internal diameter of 8.5 cm and allowed to form uniform thickness of the medium in the petri dish. After complete solidification of liquefied Inoculated medium, the wells were made aseptically with corn Borer having 6mm diameter[14-23].
Specific quantity of the polyherbal nanogel was Carefully added into the well and the plates were kept for 30 min for prediffusion of the extracts. After pre-diffusion, the petri Plates were incubated at 37 ºC for 24 h in the incubator and measured the zone of inhibition for its antibacterial activity[14-16].

Chemical Evalution

Thin layer Chromatography (TLC)

The extracts were subjected to the separation using different Mobile phase based on the phytoconstituents present in them. TLC analysis was conducted on pre-coated silica gel 60F 254 TLC plates[22-23].The plates were visualizing in day light, in short UV and long UV. The Rf value is the "retardation factor" or "ratio-to-front" value expressed as a decimal fraction. The Rf Value was calculated using following formula:

Rf = Distance travelled by solute/ Distance travelled by solute

Procedure

Standard solution: 10mg of standards dissolved in 10ml of Methanol and filter it to remove insoluble matter. The filtrates Were used for spotting on silica gel plate[23-25].

Test solution: 0.5g of extract dissolved in 100ml of methanol and filters it to remove insoluble matter. The filtrates were Used for spotting on silica gel plate.

FTIR

FTIR of pure drug was carried out and the significant absorption peaks were recorded were match with standard FTIR. The FTIR spectrum of the drug was recorded on the infrared spectrophotometer (Shimadzu Affinity-1) IR spectrum of drug was recorded in the frequency range 400 to 4000 cm-1. The significant peaks were recorded with standard FTIR[24-26].

X-ray crystallography

Experimental science determines the atomic and molecule structure of crystal in which the crystalline structure causes a beam of incident X- rays to diffract into many specific direction.it is also useful for the determining average particle size. Firstly, prepare the crystal powder of polyherbal gel after that the powder crystal is placed in an intense beam of X- rays usually of single wavelength producing the regular pattern of reflections. The angle and intensities of diffracted X-rays are measured [24-26].

Development of method by High Performance Thin Layer Chromatography (HPTLC)

Selection of solvents Practically, it was found that polyherbal nanogel is freely soluble in, methanol, DMSO and water. On the basis of several trials, toluene: ethyl acetate (14:6) was selected as ideal solvent system for qualitative analysis of polyherbal nanogel.

Standard preparation of polyherbal nanogel

The standard solution was prepared containing known concentration of 10 mg/mL by dissolving 10 mg standard of psoralen in 10 mL of methanol. take 1 ml of this solution and diluted to 10 ml with methanol.

Sample preparation of polyherbal nanogel

A total of 10 mg of polyherbal gel was weighed accurately in 10 mL conical flask; 10 mL of methanol was added and mixed thoroughly. The solution kept into sonicator for 15 min. After that the solution was centrifuged for 10 min.

TLC development and scanning

The plate was developed by immersing sample HPTLC plate in a CAMAG glass chamber (20 cm × 10 cm) containing the solvent system toluene: ethyl acetate (7:3) (v/v). After complete development, the plate was allowed to dry by keeping in fume cupboard for 10 min and then kept in hot air oven for 5 min at 105˚C. The

plate was scanned in the densitometer by linear scanning at 254 nm for gallic acid by using a TLC Scanner III CAMAG with a D2 source, and integrated the area of the spots corresponding to psoralen standard.
With the studied of all three batches I found batch A2 as a final appropriate batch as it gives better result than A1 and A3.

5.VALIDATION OF METHOD BY UV SPECTROPHOTOMETRIC METHOD

Selection of solvent

Practically, it was found that polyherbal nanogel is freely soluble in, methanol, dimethyl sulphoxide. So, methanol and DMSO was selected as ideal solvent for spectrophotometric analysis of prepared polyherbal nanogel.

Spectrophotometric method for the estimation of polyherbal nanogel

The spectrophotometric method for the estimation of polyherbal nanogel prepared in methanol by using UV spectrophotometer Shimadzu 1800.

Standard calibration curve of psoralen in DMSO

Optimization of scanning and determination of maximum wavelength (λ max) Weight accurately about 0.1 g dissolved in sufficient DMSO to produced 100ml with DMSO and measured the absorbance of the resulting solution at the maximum at about 254 nm. In order to the wavelength of maximum absorption (λ max) of the drug, solution of the drug (20 ug/ml) in methanol was scanned using spectrophotometer within the wavelength region of 400-200 nm against methanol as blank. The absorption curve showed characteristic absorption maxima at λ max 254 nm. Same procedure carried out for the methanol.

Preparation of standard solution

0.1 g polyherbal gel was accurately weighed.it was dissolve in sufficient DMSO to produced 50 ml. the volume was made up to the mark with DMSO to get the concentration of 100µg/ml this was treated was working standard.

Preparation on working standard solution

From solution having concentration 100µg/ml aliquots of 2, 4,6,8 ml was pipette out into 100 ml volumetric flask the volume was made up to the mark with DMSO to get the final concentration of 20, 40, 60 and 80 µg/ml respectively. The absorbance of each concentration was measured at 254 nm. Result is showed in table no.8.15 A graph of absorbance versus concentration was plotted and it shown if fig.8.11 it shows the line meaning the calibration curve obeys Beer's laws.

Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity show in Fig. No. 8.16, page no.74

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy polyherbal gel were weighed and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples show in Table No. 8.17

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation (CV) show in Table No.8.18

Intra and Inter day precision

Variation of results within the same day (intraday), variation of results between days (inter day) were analyzed. Intraday precision was determined by analyzing polyherbal nanogel individually for three times in the same day at 254nm. Inter day precision was determined by analyzing drug daily at 254 nm show in Table No.8.18, page no.75

Repeatability

Standard solutions of polyherbal nanogel (50 ug/ml.) were prepared and spectrums were recorded. Absorbance was measured at 254 nm taking the DMSO as the blank. The absorbance of the same concentration solution was measured six times and RSD was calculated. show in Table No.8.19

5.RESULT AND DISCUSSION

Different parameter for raw material evalution

Raw material specification of Bakuchi seed powder

Bakuchi powder was evaluated as per the specifications and test procedure mentioned in the WHO guideline and the pharmacopoeia. Table no show the limit and result of bakuchi powder evaluated.

Table 1 Raw material specification and test observation of Bakuchi seed powder

Sr. no	Specification	Limit	Observation
1	**Organoleptic properties**		Bakuchi
	State	Seed powder	Seed powder
	Colour	Dark brown black	Dark brown black
	Odour	Characteristic aromatic	Characteristic aromatic
2.	**Physicochemical characterization**		
	Loss on drying	-	7.4 %w/w
	Total ash	NMT 10%w/w	5.4% w/w
	Acid insoluble ash value	NMT 2.5%w/w	0.2 %w/w
	Water soluble ash value	_	1.55% w/w
	Water soluble extractive	NLT 20%w/w	21.6 %w/w
	Alcohol soluble extractive	NLT 10%w/w	44 %w/w
	Bulk density	-	0.50 g/m
	Tapped density	-	0.92 g/ml
	Angle of repose	NMT 40	30.16
	Hausner's ration	-	1.84
	Carr index	-	46 %
	Compressibility index	-	0.84 %

Raw material specification of kutaj leaves powder

Kutaj leaves powder powder was evaluated as per the specifications and test procedure mentioned in the WHO guideline and the pharmacopeia. Table 2. show the limit and result of Kutaj powder evaluated.

Table 2 Raw material specification and test observation of Kutaj seed powder

Sr. no	Specification	Limit	Observation
1	Organoleptic properties Kutaj		
	State	Powder	Powder
	Colour	Brown	Brown
	Odour	Characteristic	Characteristic
2			
	Loss on drying	-	8.41%w/w
	Total ash	NMT 10%w/w	4.65%w/w
	Acid insoluble ash Value	NMT 2.5%w/w	2 %w/w

	Water soluble ash value	-	4.2 %w/w
	Water soluble extractive	NLT 20%w/w	22% w/w
	Alcohol soluble extractive	NLT 10%w/w	29% w/w
	Bulk density	-	0.30 g/ml
	Tapped density	-	0.33 g/ml
	Angle of repose	NMT 40	33.30
	Hausner's ration	-	1.031
	Carr index	-	2.61%
	Compressibility index	-	0.03 %

Raw material specification of Karanja seed powder

Karanj seed powder was evaluated as per the specifications and test procedure mentioned in the WHO guideline and the pharmacopeia. Table no 3 show the limit and result of Karanj seed powder evaluated.

Table 3 Raw material specification and test observation of karanj seed powder

Sr. no	Specification	Limit	Observation
1	Organoleptic properties		Karanj
	State	Karanj seed powder	Karanj seed powder
	Colour	Brown	Brown
	Odour	Characteristic	Characteristic
2			
	Loss on drying	-	7.35 %w/w
	Total ash	NMT 10%w/w	3.15%w/w
	Acid insoluble ash Value	NMT 2.5%w/w	0.2 %w/w
	Water soluble ash value	-	2.1 %w/w
	Water soluble extractive	NLT 20%w/w	34 %w/w
	Alcohol soluble extractive	NLT 10%w/w	28 %w/w
	Bulk density	-	0.49 g/ml
	Tapped density	-	0.66 g/ml
	Angle of repose	NMT 40	35.53
	Hausner's ration	-	1.34
	Carr index	-	22.27%
	Compressibility index	-	0.25 %

Fig.1.6 Alcohol soluble extract of Bakuchi, Kutaj and Karanj

6. Phytochemical analysis of raw material

Table 6.1 indicates phytochemical analysis of raw material,all the test like for Carbohydrate, Alkaloids, Glycoside, steroid(except Karanj), Flavonoid, Protein was found to be +ve.

Sr. no	Specification	Limit	Observation		
	Phytochemical analysis of raw material		Bakuchi	Kutaj	Karanj
1.	Test for Carbohydrate				
	Molish test	+ve	+ve	+ve	+ve
2.	Test Alkaloids				
	Dragendoff's test	+ve	+ve	+ve	+ve
3.	Test for Glycoside				
	Killer killani test	+ve	+ve	+ve	+ve
4.	Test for Tannin				
	Gelatine test	+ve	+ve	+ve	+ve
5.	Test for steroid	+ve in bakuchi and-ve in karanj			
	Salkowski test	+ve	+ve	+ve	+ve
6.	Test for Flavonoid				
	Shinoda test	+ve	+ve	+ve	+ve
7.	Test for Protein				
	Burette test	+ve	+ve	+ve	+ve

Table 4 Phytochemical analysis of raw material

7.Evalution of prepared polyherbal nanogel

Table5 indicate Physical Appearance A1, A2, A3 formulations show clear yellowish appearance, all the 3 formulations were homogenous and free of grittiness.

Table 5 Physical appearance of prepared polyherbal nanogel

Formulation	Appearance	Homogeneity	Grittiness
A1	Yellow	Homogenous	No
A2	Yellow	Homogenous	No
A3	Yellow	Homogenous	No

7.2.Centrifugation test

Table 6 shows centrifugation test of prepared polyherbal nanogel. one month study was performed at 10 and 40°C and No phase separation after centrifugation was found in formulations A1, A2, A3.Appearance and colour was found to be semisolid and pale yellow.

Table 6 centrifugation test of prepared polyherbal nanogel

Duration			**Storage condition**	
	7 days		**15** days	
Parameter	10˚C	40˚C	10˚C	40˚C
Appearances				
Formulation A1	Semisolid	Semisolid	Semisolid	Semisolid
Formulation A2	Semisolid	Semisolid	Semisolid	Semisolid
Formulation A3	Semisolid	Semisolid	Semisolid	Semisolid
Color				
Formulation A1	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Formulation A2	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Formulation A3	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Centrifugation test				
Formulation A1	NSL	NSL	NSL	NSL
Formulation A2	NSL	NSL	NSL	NSL
Formulation A3	NSL	NSL	NSL	NSL

*NSL- No separation of layer

7.2.Viscosity

The measurement of viscosity of the prepared polyherbal nanogel was done using the Brookfield viscometer, formulation showed viscosity was in given (Table7)

Table7 Viscosity of prepared polyherbal nanogel

RPM	VISCOCITY OF FORMULATED GEL (cps)		
	A1	A2	A3
0.5	4053	6795	5415
1	2703	2962	3295
1.5	1880	1920	2701
2.0	1312	1420	1780
2.5	1021	1250	1556

7.3.pH

The pH of all formulation from A1 To A3 was found to be in the range of 6.0 to 6.9. All measurements were carried out triplicate. pH of topical formulation should less than 7 as pH of skin is 5.5 so the formulation pH should compatible with skin and topical formulation.

Table 8 pH of prepared polyherbal nanogel

Sr. no.	Formulation code	Observed pH
1	A1	6.3
2	A2	6.7
3	A3	6.9

7.4.Spreadability of nanogel formulations

The spreadability of polyherbal nanogel was carried out, of which A2 showed better spreadability than the other formulations.

Table 9. Spreadability of prepared polyherbal nanogel

Sr. no	Formulation code	Load Applied	Length Of Glass Slide	Time Taken	Spreadability (Cm/Sec)
1	A1	5gm	7.4	1.20	30.83
2	A2	5gm	7.4	1.29	28.68
3	A3	5gm	7.4	1.17	31.62

7.5.Antimicrobial test

The antimicrobial activity was performed by well diffusion method by measuring the zone of inhibition(in mm) the activity results of the polyherbal gel showed antimicrobial activity in dose dependent manner.

Table10. Antimicrobial test of prepared polyherbal nanogel

Sr. no	Formulation	Diameter of zone of inhibition (in mm)
1	Fluconazole	5
2	A1	4
3	A2	3.5
4	A3	3

7.6.Thin layer chromatography

The methanolic extract of Psoralea corylifolia (Bakuchi), Pongamia Pinnata (Karanj) and Holarrhena pubescens (Kutaj) shows the best result in the solvent system of Ethylacetate: methanol (3:7) and in the solvent system of Toluene: ethyl acetate (3:7) and gave the spot. Their Rf values were 0.7, 0.5, 0.9.

Table11.TLC of prepared polyherbal nanogel

Drug	Psoralea corylifolia (Bakuchi)	Pongamia Pinnata (Karanj)	Holarrhena pubescens (Kutaj)
Mobile phase	Ethyl acetate: methanol (3:7)	Toluene: ethyl acetate (3:7)	Ethyl acetate: methanol (3:7)
Rf value	0.7	0.6	0.9

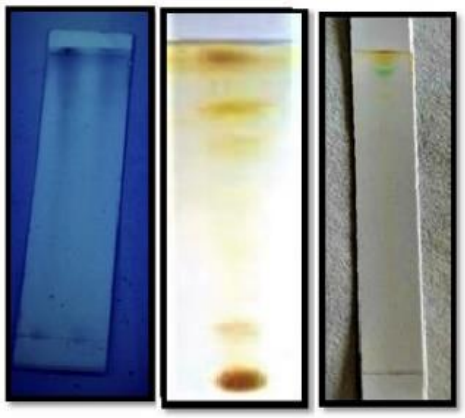

Fig 1.7 TLC plate of Psoralea corylifolia (Bakuchi), Holarrhena pubescens (Kutaj) and Pongamia Pinnata (Karanj)

7.7.FTIR

The spectrum of polyherbal nanogel shows the following functional group in their frequencies mentioned in table12. Drug characterization study by FTIR was carried out as per standard procedure FTIR spectra of polyherbal nanogel are shown in graph. it was observed that principal peak of the drug was found in FTIR spectra of drug. It was suggested that there were no physical and chemical changes of pure drug. The result are shown in Fig.4

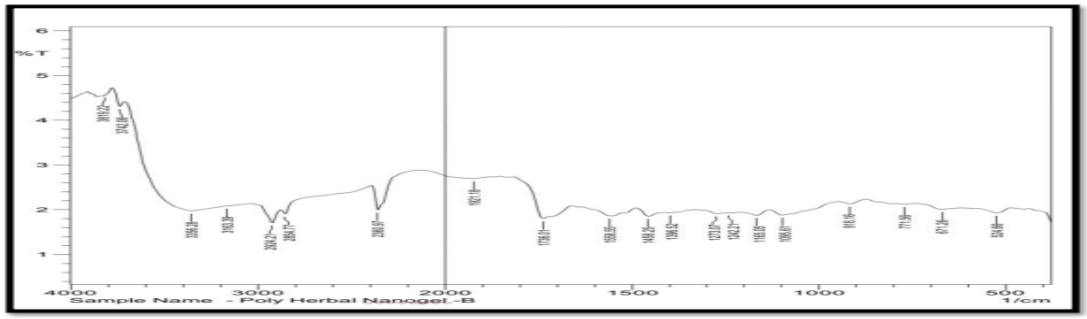

Fig.(4). FTIR spectrum of polyherbal nanogel

Table 12. Functional group and their frequencies of FTIR spectra of prepared polyherbal nanogel

Group	Principle peak (cm-1)	Peak observed (cm-1)
C-Br stretching	690-515	671.26
C=C bending	1210-1163	1165.05
C-O stretching	1250-1020	1242.21
S=O stretching	1410-1380	1396.52
C=O stretching	1740-1720	1736.01
C-H bending	2000-1650	1921.18
C-H stretching	3000-2840	2924.21
N-H stretching	3400-3300	3356.28

7.8. XRD of prepared polyherbal nanogel

XRD was performed using a Rigaku MiniFlex X-ray diffractometer with CuK alpha radiation source (λ= 1.54178A); ˚ a scanning rate 0f 0.02˚/s was used to record the pattern in the 2ɵ range of 10˚ to 80˚. The major diffraction pic for polyherbal formulation was observed at 14.82˚, 24.26˚ and 38.144˚ corresponding to interplanar distance of 5.971, 3.665 and 2.3574 A˚ respectively. The XRD pattern showed in fig.5 and the identified components particle size are listed in Table13.

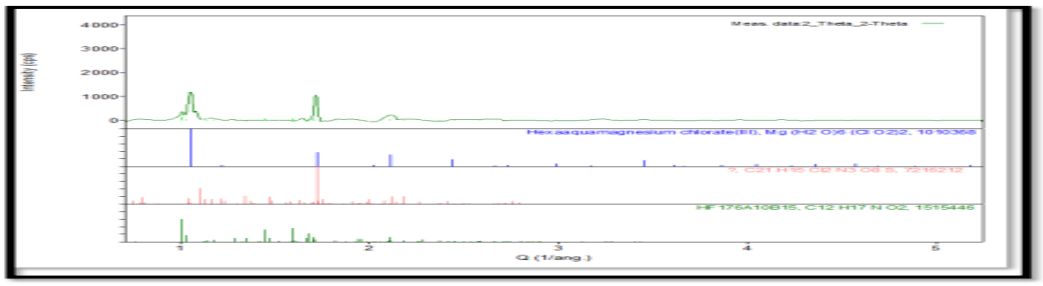

Fig 1.8 XRD of prepared polyherbal nanogel

Table13. XRD of prepared polyherbal nanogel

Sr.No.	2- theta(deg)	d(ang.)	Height(cps)	FWHM (deg)	Int. I(cps deg)	Int. W(deg) Int. W(deg)	Size(ang.)
1	14.82(3)	5.971(10)	802(82)	0.44(4)	632(22)	0.79(11)	189(17)
2	24.26(2)	3.665(3)	724(78)	0.296(19)	232(18)	0.32(6)	287(18)
3	38.144(19)	2.3574(11)	199(41)	0.33(5)	69(14)	0.35(14)	270(45)

7.9 Development of method by High Performance Thin Layer Chromatography

Psoralen is a common phytoconstituent; therefore, in the qualitative estimation of psoralen, it is well represented in chromatogram. For optimization of method, different mobile phase compositions were employed to achieve good separation. solvent system containing toluene: ethyl acetate 7:3 (v/v) resulted in good resolution of psoralen in the presence of other compounds in formulation. TLC plate was observed under UV light for the presence of psoralen, detected by prominent green color spot. The Rf value (0.68) for psoralen in both sample and reference standard was found comparable under UV light at 366 nm.

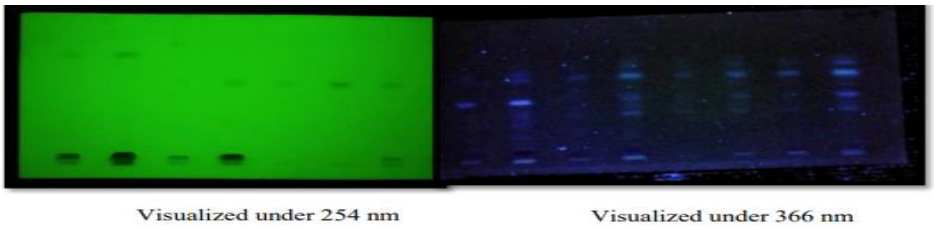

Fig 1.8 TLC plate of prepared polyherbal nanogel

Table 14. TLC data of prepared polyherbal nanogel

Drug	Mobile phase	Rf value	Start Rf	End Rf
Psoralen std	Ethyl acetate: methanol (3:7)	0.63	0.57	0.69
A1	Ethyl acetate: methanol (3:7)	0.65	0.64	0.69
A2	Ethyl acetate: methanol (3:7)	0.64	0.59	0.70
A3	Ethyl acetate: methanol (3:7)	0.64	0.65	0.70

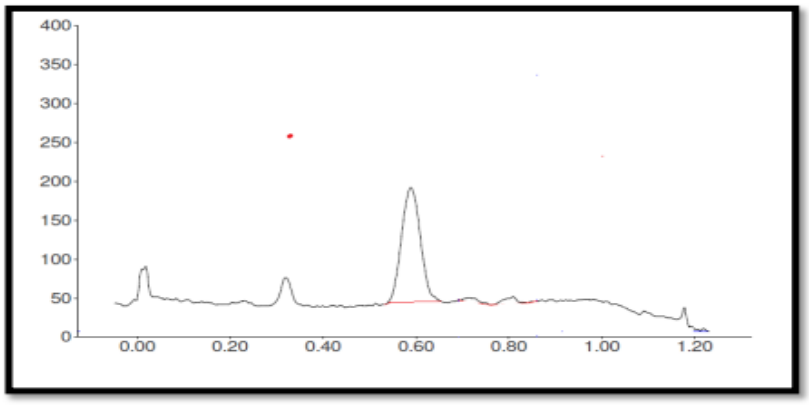

Fig 1.9 Peak response of psoralen std

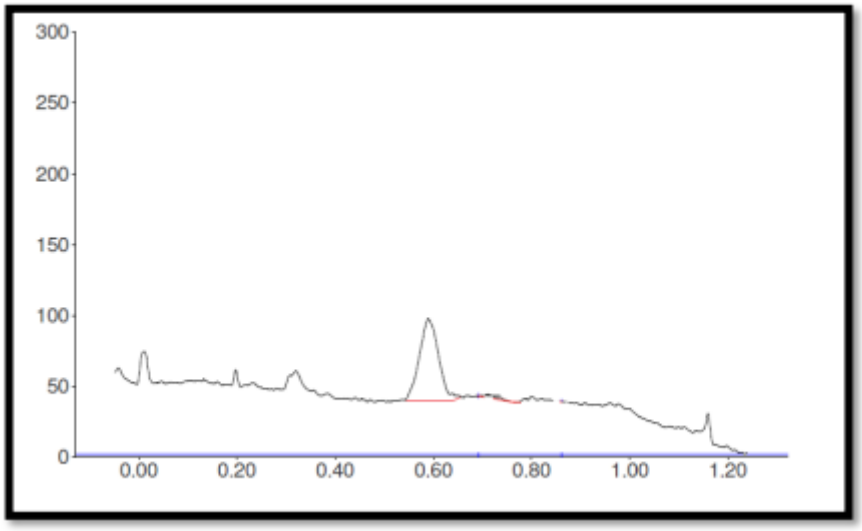

Fig 1.10 Peak response of psoralen in polyherbal nanogel

8.Validation of method by UVspectrscopy.

8. **1.Selection of solvent**

Practically, it was found that polyherbal nanogel is freely soluble in, methanol, dimethyl sulphoxide. So, methanol and DMSO was selected as ideal solvent for spectrophotometric analysis of prepared polyherbal nanogel.

8.2. Preparation of standard stock solution

Standard stock solution of polyherbal nanogel, as prepared by dissolving 10 mg of polyherbal nanogel in 10 mL of methanol and to give 1 mg/mL. solution, from above stock solution 1 mL of aliquot was pipette out in a 10 mL volumetric flask and volume was made up to the mark with methanol to obtain the final concentration of 100 µg/mL.

8.3. **Study of Spectra and Selection of Analytical wavelength**

The maximum absorption value of pure drug, prepared polyherbal nanogel was found to be 200-400 nm Wavelength. Therefore 254 nm were recorded as λ max of the prepared polyherbal nanogel. The Observed λmax value of drug was found to be similar as given in literature. Hence the prepared formulation was considered to be pure. The UV spectrum of prepared polyherbal nanogel was showed in Fig.9.

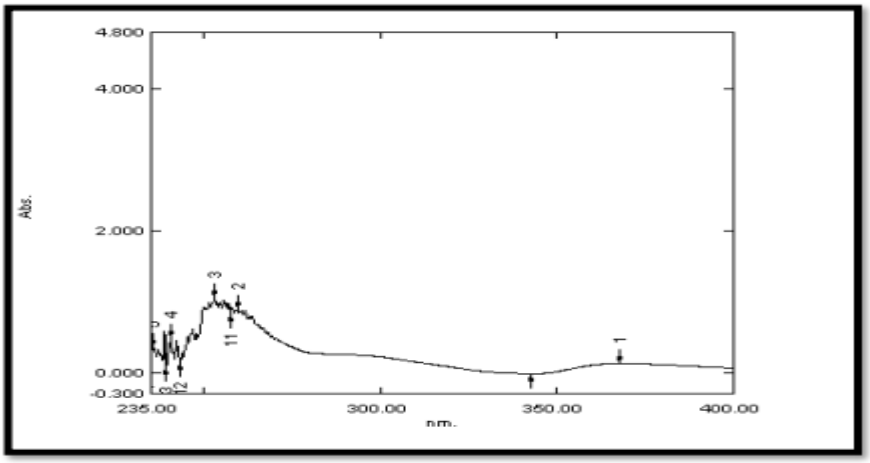

Fig 1.11 Spectra of standard of polyherbal nanogel

8.4. Preparation of standard calibration curve

Appropriate aliquots were pipette out from the standard stock solution in to a series of 10 mL volumetric flasks. The volume was made up to the mark with methanol for each volumetric flask to get series of

concentration range of 20 to 80 µg/mL. Absorbance of the above solutions was assured at 254 nm and a calibration curve of absorbance against concentration was plotted. The drug obeys Beers Law in the concentration range of 20 to 80 µg/mL. The regression equation and coefficient were determined

Table15.Standard Calibration curve of prepared polyherbal nanogel at 254 nm

Sr.no	Concentration (µg/ml)	Absorbance at 254 nm
1	20	1.320
2	40	1.525
3	60	1.812
4	80	1.985

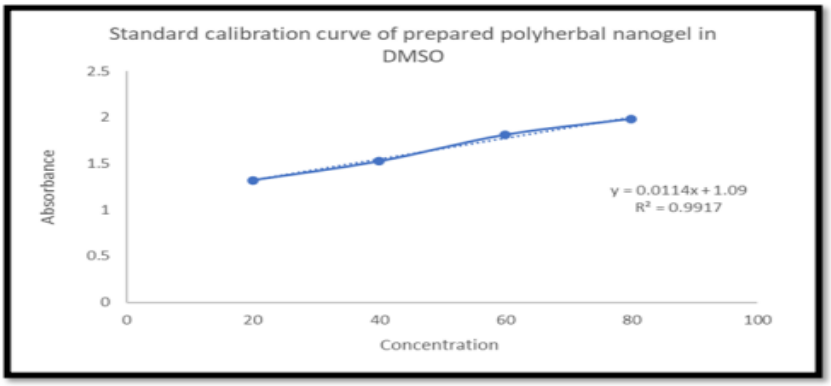

Fig 1.12 Calibration curve of prepared polyherbal nanogel in DMSO

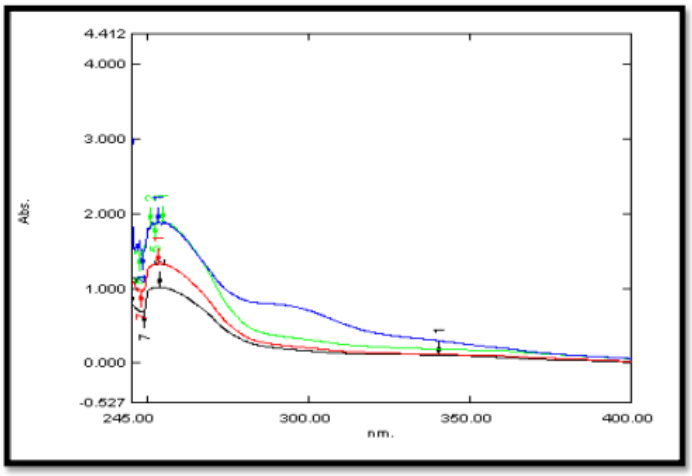

Fig 1.13 Overlain spectrum of prepared polyherbal nanogel

9.Validation parameter of polyherbal nanogel by UV spectrophotometry

9.1) Linearity

linearity curve at 254 nm

Table16. Data of linearity for polyherbal nanogel at 254 nm

Sr.no	**Concentration (µg/ml)**	**Absorbance**
1	20	1.102
2	40	1.335
3	60	1.591
4	80	1.913
5	100	1.978
		Mean = 1.583
		R2 = 0.9732

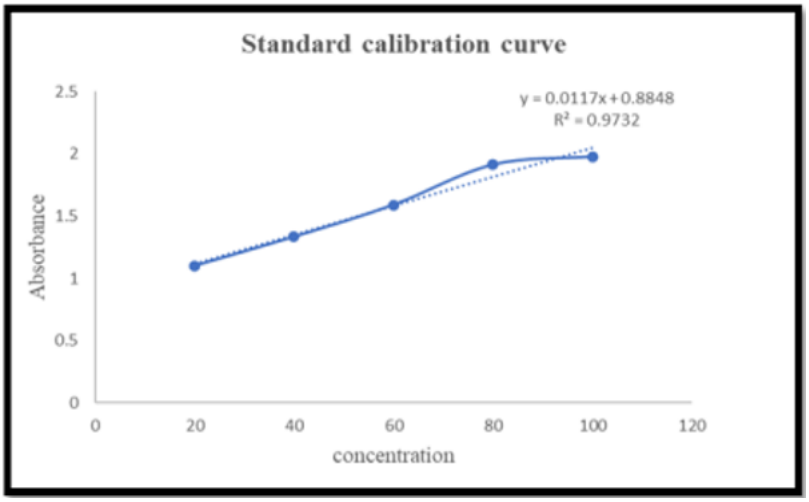

Fig 1.14 Standard calibration curve of linearity

9.2) Accuracy

Table17. Determination of accuracy (% recovery)

Sr no.	Formulation	Amount of drug taken (µg/ml)	Amount of drug added (µg/ml)	Amount of drug found (µg/ml)	% Recovery ± SD
1	Polyherbal nanogel	20 µg/ml	5	26.8	104.32
2		20 µg/ml	10	30.96	103.2
3		20 µg/ml	15	33.81	96.57

9.3) Precision

The precision of polyherbal nanogel with interday and intraday % RSD were found to be 38.36% & 34.59%

Table 15. Inter day precision data of polyherbal nanogel at 254 nm

Sr no	Concentration (µg/ml)	Absorbance	Mean	SD	% RSD
1	10	0.432	0.808	0.31010	38.36%
2	20	0.546			
3	30	0.873			
4	40	1.045			
5	50	1.146			
Intraday precision data of polyherbal nanogel at 254 nm					
Sr no	Concentration (µg/ml)	Absorbance	Mean	SD	% RSD
1	10	0.561	0.908	0.31433	34.59%
2	20	0.642			
3	30	0.894			
4	40	1.171			
5	50	1.275			

9.4) Repeatability

Repeatability data of polyherbal nanogel at 254 nm

Table 16 Repeatability data of polyherbal nanogel at 254 nm

Sr no	**Concentration (µg/ml)**	**Absorbance**	**Mean**
1	50	0.694	0.739
2	50	0.714	
3	50	0.729	
4	50	0.751	
5	50	0.762	
6	50	0.789	

6.Conclusion

On the basis of the study, the data showed that the polyherbal gels prepared from the dried methanolic extracts of Bakuchi and Kutaj. Polyherbal nanogel formulation was developed and Evaluated for its physicochemical properties and preformulation evaluation of raw material that contain bulk density, Tapped density, car's index. Hausner ratio, angle of repose and also formulation was validated and optimized by UV-spectroscopy methods.

As phytochemical tests showed the presence of glycosides, carbohydrates, flavonoids, steroids and protein. the nanogel of Bakuchi, Kutaj and Karanj in combination can be successfully prepared and evaluated. It was prepared by modified solvent diffusion emulsification method by using Carbopol- 940 and varying the speed of homogenization. The formulation was found stable as per ICH guidelines. Appearance of the gel was found to be homogenous with no grittiness and yellowish in color. The pH was found to be 6.3 to 6.9, Viscosity 6795, Spreadability 28.68.

HPTLC was performed to confirm the quantitative presence of Psoralen (Rf value 0.68) employing toluene: ethyl acetate 7:3 (v/v) as a solvent system at a wavelength of 254 nm. Sample preparation and development of appropriate mobile phase are two imperative stages in analytical procedures, which becomes more considerable for plant-based medicines owing to their complexity of the chemical compounds and their affinity towards different solvent systems. Also, formulation was validated by UV-spectroscopy methods by using DMSO as solvent.

The developed UV-spectroscopic method was found to be simple, accurate, sensitive, precise, specific, economical and rapid. The polyherbal nanogel was well validated and optimized by this method. This method was found to be highly specific. The UV Spectroscopic method was found to be linear over wider concentration range. Therefore, the developed method can be applied for routine quantitative and qualitative analysis of polyherbal nanogel. This method was validated as per the ICH guidelines. The developed UV spectroscopic method can be employed for pharmaceutical preparations within pharmaceutical industry.

Abbreviations

RP-HPLC: Reversed-phase high-performance liquid chromatography; ODS: Octa-decyl silane; v/v: Volume by volume; Cmax: Concentration maxima; Tmax: Therapeutic maxima; CLD: Cilnidipine; VAL: Valsartan; CV: Coefficient of variance; LOD: Limit of detection; LOQ: Limit of quantitation; DAD: Diode array detector

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Authors' contributions

RNK was design and optimized method. The manuscript was drafted by RNK. The method was performed and validated by SSY. AGM have contributing in grammatically molding and writing of manuscript and gives their scientific suggestion. All authors have read and approved the manuscript.

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