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Research Article

FORMULATION AND PHARMACEUTICAL EVALUATION OF POLYHERBAL CAPSULE (FEMITEX-SP$_4$) FOR TREATING MENORRHAGIA

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ABSTRACT

The aim of the present study was to formulate and evaluate the pharmaceutical quality of a polyherbal capsule. Polyherbal formulation was prepared using extracts of *Saraca indica L.,* bark extract*, Vitex agnus castus L.,* fruit extract*, Embelica officinalis L.,* fruit extract*,* and *Symplocos racemosa Roxb.,* bark extract*,* in 1:1:1:1 ratio to obtain the best formulation; in order to increase the acceptability and adoptability of herbal medicine in the treatment of menorrhagial condition. To attain the purpose; identification of herbs used, pre-formulation and post formulation studies on polyherbal capsules were done. The quality of individual herbs was evaluated by observing physical behavior of different solvent with the herbal extracts and also by TLC assay that indicated the presence of basic active constituent in the herbal extracts. For appropriate formulation the plant materials underwent cleaning, drying, grinding, extraction, percolation, and lyophilization where as for the pharmaceutical quality analysis screening of particle size, flow ability of the blended mixture of polyherbal herbal extracts were analyzed. The flow capability of blended powder was calculated by angle of repose, porosity, bulk and tap density, compressibility and hausner ratio, these properties helped to estimate the right size of capsule for the desired strength (250mg). The quality of polyherbal formulation was evaluated through weight variation, length, diameter, disintegration time (Mean ± SD; 9.83± 2.64), and dissolution (Mean ± SD; 95.1± 3.725) and stability studies that indicated light, temperature and humidity had no significant effects on the physiochemical properties of the formulated capsules. The conclusion of the study is that the herbal medicine can be used more conveniently and safely in various diseased conditions, if used in proper portion and combination.

Keywords: Pharmaceutical quality, *Saraca indica L.*, *Vitex agnus castus L.*, *Embelica officinalis L.*, *Symplocos racemosa Roxb*, and Femitex-SP$_4$.

INTRODUCTION

Herbal medicine, a form of complementary and alternative medicine, have always been the principal form of medicine in Pakistan and India, and now they are becoming popular throughout the developed world, as people make an effort to stay healthy in the face of chronic stress and pollution[1-2].

The World Health Organization estimates that in some Asian and African countries 80% of the population depend on traditional medicine for primary health care; in many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine[3].

To enhance the acceptability of the Herbal Medicine by consumers, many of the herbs have been converted into conventional dosage forms such as tablets, capsules, suspensions, solutions and powders. These have found a great sense of acceptability within the Pakistani population. So it is the need of time to evaluate the pharmaceutical qualities of these Herbal Products, irrespective of the medicinal content and therapeutic claims. Thus in the present study, the pharmaceutical quality of capsule dosage form was evaluated that was formulated by combining the powdered extract of four different herbs as a single dose i.e. were *Saraca indica L.* (Family *Caesalpinaceae)*, *Vitex agnus castus L.* (Family *Verbenaceae)*, *Embelica officinalis L.*(Family *Euphorbiaceae)*, and *Symplocos racemosa Roxb* (Family *Symplocaceae).*

Saraca indica has a drastic effect when used for treating feminine disorders. It was also used for the treatment of various skin disorders, obesity, and has shown a powerful immunostimulant effect too[4-6]. *Vitex agnus castus* has revealed marvelous activity in various gynecological problems along with analgesic, anti-inflammatory, antiviral, antifungal, and antibacterial activity[7-11]. *Embelica officinalis* is a rich source of Vitamin C, a good antioxidant, can be used as cardio protective, anticancer and antibacterial. Along with improving vigor and bone strength, it is also used for treating menstrual disorders[12-16]. *Symplocos racemosa* has recognized for value in diarrhea, dysentery, menorrhagia, and bleeding gums, along with anti-inflammatory effect and as an emollient[17-20].

Although these plants were extensively been used to treat various illness and in feminine disorders too, there was no exclusive finding available in previous 100 years literature regarding the use of these four plants in combination. The study was attempted for the first time and a new formulation (250 mg capsule) was prepared from combined powder extracts of these plants for treating menorrhagia.

MATERIAL AND METHODS

Instrumentation

Soxhlet apparatus, Rotary evaporator (Eyela, Japan), Lyophilizer (freeze dryer model FDI, Eyela, Tokoio Rikakekai Co. Ltd. Japan), Electronic Microscope (Laboval-4, Germany), Stereomicroscope (Wolfe, Carolina, 59.18.18, Burlington, North Carolina 2715), Ultraviolet Lamp (λ max 254 and 366), Water Bath with Thermostat (Model No. HH. S21.8, (Jiangsu Medical Instrument Factory China), Physical Balance: i. Libro AEG – 120 Shimadzu (Japan), ii.Libro EB – 3200D, Shimadzu (Japan), Grinder WestPoint automatic series TSK-333 France, blender, Disintegrator apparatus (Erweka alpha/numeric), Dissolution apparatus (Erweka Dt 600), Stabilizer.

Materials and reagents

Sieves (Mesh# 40 to 60). Hard gelatin capsules (size # 1) were of highest quality commercially available. Silica Gel for TLC Plate: 20 × 20 cm silica gel 60 florescence at 254 nm (Merck, Germany). 5 × 10 cm gel 60T (0.2 mm thick) pre coated TLC plates, florescence at 254 nm (Merck, Germany).Pre coated Silica gel F_{254} TLC plate (Merck, Germany).

Ethyl Acetate, Methanol, Hexane, Chloroform, Iodine solution, Acetone, Ferric chloride, Lead acetate, Butanol, Ethanol (Merck, Germany), and distilled Water (prepared freshly).

Plant material

Dry herbs of all four plants (i.e. plant-A: bark of *S. indica*, plant-B: fruit of *V. castus*, plant-C: fruit of *E. officinalis*, and plant-D: bark of *S. racemosa*; approximately 2,000gm each) were procured from the standard shop of herbal market (Hakeem & Company, Herb Medicine Supplier, Allah Bakaya Street, Jodia bazaar, Karachi-74000,

Pakistan) in May 2007 and authenticated at source by Prof. Dr. Ghazala H. Rizwani, Dept of Pharmacognosy, Faculty of Pharmacy, University of Karachi. The plant material was further identified by Department of Botany, Faculty of Science, University of Karachi. A voucher specimen has been deposited at the Herbarium of Faculty of Pharmacy, University of Karachi under the numbers 0040, 0041, 0042, 0043 for plants-A, B, C and D respectively.

Preformulation Studies

Extraction of Herbs

In phase I; the above mentioned plant parts were oven dried at 35°-45° and coarsely powdered in grinder separately. About 500 g coarse powder of each plant drugs was kept for getting the methanolic extract for phytochemical and chromogenic testing; whereas remaining mixed into 1:1:1:1 ratio and finely powdered. Powder of each plant material at the amount of 50 g was extracted in 250 ml of 80% methanol for 18 hr in a soxhlet apparatus. Further methanol extract was recovered and evaporated to dryness by distillation under reduced pressure in rotary evaporator. The concentrate methanol fraction obtained was stored at 4°C and used for phytochemical and chromogenic testing for the next 7 days of experiment. In phase II; the fine powder (gained after mixing fine powdered extracts of all herbs in 1:1:1:1 ratio and thoroughly blended) extracted in 80% methanol in soxhlet apparatus, methanol extract then obtained was evaporated under reduced pressure in rotary evaporator. A reddish brown gummy residue obtained, which was then lyophilized and finally shiny brown powder obtained, having acrid taste and typical herbal odor. This powder was used for the preparation of Femitex-SP_4 capsules. As per demand extracts were prepared further throughout the experimental period and used for the study.

Phytochemical Analysis

Preformulation studies also involves the characterization of a drug's chemical properties in order to choose what other ingredients should be used in the preparation; but in case of plant material, it is one of the important parameter in the plant's standardization.

For this, studies were performed by two methods:

(i) Physical behavior of different solvent with the individual drug extracts

Behavior of solvents with the methanolic extract of drugs also exhibit chemical nature of the drugs. For checking the nature of plant extracts in different reagents by colour reaction, lead acetate, ferric chloride, n-hexane and iodine solution were used.

(ii) Preliminary screening methods

For knowing the existence of the naturally occurring compounds in methanolic extract of all four plants extracts (A-D), chromogenic testing was performed. The observation was made on the basis of thin layer chromatographic procedures. For the identification of different chemical classes of compounds, different tests were performed; such as Lieberman test was performed for triterpenes, Dragendorff's test for Alkaloids, Lead acetate and Phenazone test for Tannins, Froth and ether test for Saponins, Salwaski's test for α, β unsaturated Sterols, Millon's test for proteins, and test for detection of steroids was also performed. A positive response against the specific reagent of each chemical type of compound was observed.

Preparation of Femitex-Sp_4 Powder for Encapsulation

Screening of particle size

It is done in order to obtain equivalent diameter with which to interpret the particle size of a powder. For the uniformity of particle size, powder material passed through different sieves mesh # 40 and 60, according to B.P[21]. Uniformity of particle size is one of the important parameter for getting the optimum efficacy of the therapeutic moiety.

Flow Ability of Powder

Powder flow is the key condition for the pharmaceutical manufacturing process. Flow ability of the powder is very useful for uniform volume filling of powder in the capsule formulation as well as in clinical effectiveness of drug. Flow is also crucial during mixing of different ingredients and packaging. Flow ability of powder was checked by different methods[22].

Angle of Repose of Powder

For the determination of cohesiveness and non cohesiveness of the powdered material, the angle of repose of powder of Femitex-SP_4 was measured by fixed cone method. For this purpose, 3 gm of powdered material was used and then the calculation of angle of repose was measured by following formula:

$$\text{Tan } \theta = h/r$$

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

Where h is height or heap of powder and r is diameter of Petri-dish.

Porosity of Powder

To check the porosity of powder, a cylinder was freely filled up to 100 ml (bulk volume of powder). Then the cylinder was tapped for 100 times on smooth surface in order to obtain the pack volume of powder.

Porosity was calculated by formula:

$$\epsilon = \text{porosity of powder} = Vb - Vp / Vp$$

Where Vb is bulk volume and Vp is packed volume.

Bulk Density and Tapped Density

Bulk and tapped densities were determined.

$$\rho_{bulk} = m / Vb$$

$$\rho_{tapped} = m / Vp$$

Where m is weight of powdered drug.

Compressibility Index and Hausner Ratio

The bulk and tapped densities were used to calculate the Carr's compressibility index and the Hausner ratio to provide a measure of the flow properties and compressibility of powders.

$$C.I = \frac{\rho_{tap} - \rho_{bulk}}{\rho_{tap}}$$

$$HR = \frac{\rho_{tap}}{\rho_{bulk}}$$

Where ρtap is the tap density and ρbulk is the bulk density.

Formulation Of Femitex-Sp_4 Capsule

The dosage should have a uniform appearance, homogeneity, with an acceptable taste, tablet hardness, or capsule disintegration.

Preparation of capsules

For ease of swallowing and greater patient acceptance, a pharmacist generally chooses the smallest capsule that will contain the prescribed dose[23]. Size # 1 capsule was selected for encapsulating the desired strength (250 mg) of the drug (blended extract).

Physiochemical parameters

For the analysis and evaluation of the quality of single dosage form, a number of different pharmacopeial and non- pharmacopeial physicochemical tests were performed.

Uniformity of weight

For capsules, granules (uncoated, single dose); if average mass is less than 300 mg, percent deviation is 10. Not more than 2 of the individual masses can deviate from the average mass out of 20 units taken or weighed at random[24].

For the estimation of weight uniformity; weight variation was checked along with diameter and length of the capsule.

Disintegration

Complete disintegration is defined as the state in which any residue of the unit, except fragments of the insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the disc; if used is a soft mass having no palpably firm core. At least six capsules should be used to determine the disintegration time of capsules[25].

For the test, a 1000 ml beaker was filled with distilled water (approx. 900ml), equilibrated to 37±0.5ºC. Six capsules were subjected to the test. Time required for the last capsule to disintegrate was recorded.

Dissolution

The rate of absorption and bioavailability are dependent upon how fast the drug dissolves in GI fluid. This means that drugs administered orally in solid dosage forms (tablets, capsule, etc) than dissolve in the GI fluid before absorption. So that dissolution is used as the predictor of bioavailability.

For Dissolution six capsules were taken to determine the rate at which the active drug substance dissolved in the fluid of gastrointestinal tract[26].

Stability

The stability of a pharmaceutical product is the capability of a particular formulation in a specific container / closure system to remain within its physical, chemical, microbiological, therapeutic, and toxicological specification. For the studies, capsules were exposed to environmental factors; light, temperature, and humidity.

Light

Capsules were kept in different intensities of light i.e. sun light, florescent (tube) light, UV light, and Infra red light. Then, for detection of degradation of powdered material; the powder of capsules was subjected to TLC chromatography on silica gel fluorescence (245 nm) plate in solvent system {EtOAC – MeOH – H_2O (7:10:0.1)}.

Temperature

The effect of temperature was checked at four different temperatures i.e. ambient, 35° C, 55° C, 65° C and observed the effect with ½, 1, 3, and 6 hours intervals.

Humidity

In humidity studies powdered sample was evaluated at 30%, 50%, 70%, and 90%.

RESULTS

Many herbs are used by people throughout the world in the form of complementary and alternative medicine after their indigenous study of remedies and their bio-scientific investigation. There is a need to convert these herbal extracts in to unit dosage form to provide uniform amount of drug for clinical efficiency. In the present study the blended mixture (1: 1: 1: 1 ratio) of four plants were converted into unit dosage form (capsule 250 mg named as Femitex-SP_4) in order to enhance the acceptability of the herbal medicines by consumers, at the same time the herbs were also standardized by phytochemical investigation.

Phytochemical investigation of all four plant drugs

Proper and complete identification is one of the most important parameter incase of herbal medicine because the formulation cannot produce desired effects, if the herbs are not properly identified. (Table: 1)

Chemical composition of drugs

Before the blends filled in to the capsule dosage form it was necessary to evaluate the presences of different chemical composition of the herbs. In case of herbal medicine the pharmacological efficiency can only be estimated by evaluating the chemical composition of herbs. (Table: 2)

Flow property of powdered extract

The powdered material of Femitex-SP_4 was filled in size # 1 capsules. Prior to filling of powder in capsules, its flow property was checked. (Table: 3)

Table 1: Solubility and color reaction of Herbal Drugs (A- D) in Different Chemical Reagents

Chemical Reagent	Plant Drugs (Color Reaction with Chemical reagent)			
	Plant-A	Plant-B	Plant-C	Plant-D
Iodine Solution				
Spontaneous	Reddish-Brown	Brown	Dark Brown	Brown
After 5 minutes	Reddish	Brown turbid	No change	No change
After 10 minutes	No change	Light brown	No change	No change
After 30 minutes	No change	No change	No change	No change
Chloroform				
Spontaneous	Brown	Yellow	Light brown	Light Brown
After 5 minutes	No change	Clear yellow	Very light Brown	No change
After 10 minutes	No change	No change	No change	No change
After 30 minutes	No change	No change	No change	No change
Hexane				
Spontaneous	Dark Brown	Turbid Solution	Very light Brown	Light Brown
After 5 minutes	Light Brown	No change	No change	No change
After 10 minutes	No change	No change	No change	No change
After 30 minutes	No change	No change	No change	No change
Methanol				
Spontaneous	Dark Brown	Turbid solution	Light Brown	Light Brown
After 5 minutes	No change	No change	No change	No change
After 10 minutes	No change	No change	No change	No change
After 30 minutes	No change	No change	No change	No change
Acetone				
Spontaneous	Slight Reddish Brown	Clear yellow	Light Brown	Brown
After 5 minutes	Brown	No change	Very light Brown	No change
After 10 minutes	No change	No change	No change	No change
After 30 minutes	No change	No change	No change	No change
Ferric Chloride				
Spontaneous	Black	Greenish brown	Bluish Black	Greenish Brown
After 5 minutes	Bluish Black	No change	No change	No change
After 10 minutes	No change	No change	No change	No change

After 30 minutes	No change	No change	No change	No change
Lead Acetate				
Spontaneous	*ppt with clear yellow solution	Brown ppt with pale yellow solution	White ppt with clear solution	Peach color ppt
After 5 minutes	light brown ppt with clear yellow solution	No change	Thick white ppt with clear solution	No change
After 10 minutes	No change	No change	No change	No change
After 30 minutes	No change	No change	No change	No change

*ppt = Precipitates

Table 2: Chemical Composition of Plants (A-D) used in the preparation of Femitex-SP$_4$

Chemical composition	Plant Drugs			
	Plant-A	Plant-B	Plant-C	Plant-D
Proteins	+	+	+	+
Lipids	+	+	+	+
Alkaloids	_	+	_	+++
Glycosides	++	++	_	+
Sterols	+	_	_	+
Triterpenes	_	+++	_	+
Tannins	+++	_	+++	+
Saponins/ Flavonoids	+	+++	_	_
Steroids	++	++	_	_

Table 3: Flow characteristic of Femitex-SP$_4$ granules

S. No	AOR*	P*	BD*	TD*	CI*	HR*
1	28.61	26.01	0.458	0.619	0.260	1.351
2	29.01	26.28	0.460	0.624	0.2628	1.356
3	28.82	25.89	0.458	0.618	0.2589	1.349
Mean	28.81	26.06	0.4587	0.620	0.2605	1.352
%CV	0.694	0.766	0.250	0.516	0.768	0.266

AOR* = Angle of repose

P*= Porosity

BD*= Bulk Density

TD*= Tapped Density

CI*= Compressibility Index

HR*= Hausner Ratio

Formulation Analysis

In the present study, quality of the Femitex-SP$_4$ capsules was evaluated through weight variation, length, diameter, disintegration time, dissolution and stability studies. It helps to recognize the quality control test / parameters and the effect of these differences on the release of drug from the formulation.

Weight variation

In physiochemical features of capsules that containing powdered extract, weight variation along with diameter and length were checked. It was observed that all capsules come under the upper and lower limit of weight variation[24] and was found the average weight of 250 mg ± 7.5% (Figure: 1).

Disintegration and Dissolution

Disintegration test was conducted on six capsules of Femitex-SP$_4$ (250mg). The minimum time of disintegration was 6 minutes and the maximum time observed was 13 minutes[25].All the six capsules fulfill the criteria of dissolution according to the BP[26]. (Table: 4)

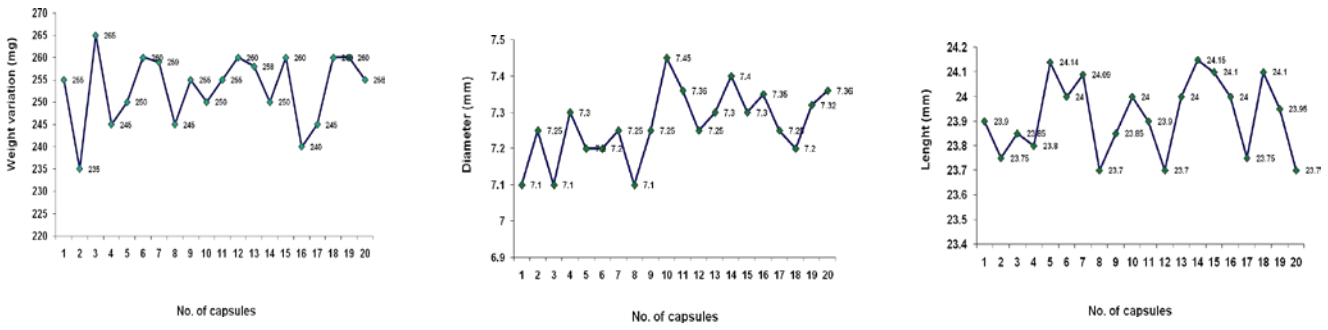

Fig. 1: Weight Variation of 20 Capsules (250 mg) of Femitex-SP$_4$ with length and diameter

Table 4: Disintegration and Dissolution Pattern of six capsules of Femitex-SP$_4$ (250mg)

No of Capsules	Disintegration Time (min)	Extent of Dissolution %
1st capsule	6 min	99
2nd capsule	8 min	93.85
3rd capsule	9 min	90.02
4th capsule	11 min	91.85
5th capsule	12 min	98.0
6th capsule	13 min	97.9
Mean X	9.83 min	95.103
±S.D	2.64	3.725
%CV	26.856	3.917

Stability studies

Stability studies of formulated oral dosage form were also carried out for determining the physical, chemical, and therapeutic changes occurred in the drug material by extrinsic factors. (Table.5a, b, and c)

Table 5a: Effect of Different Intensities of Light on Powdered Capsules

Light source	Sun light	Fluorescence tube light	UV light	Infra red light
Hours	½ 1 3 6	½ 1 3 6	½ 1 3 6	½ 1 3 6
Effect	- - - -	- - - -	- - - -	- - - +

Table 5b: Effect of Different Temperatures on Powdered Capsules

Temperatures	Ambient	35° C	55° C	65° C
Hours	½ 1 3 6	½ 1 3 6	½ 1 3 6	½ 1 3 6
Effect	- - - -	- - - -	- - - +	- - + +

Table 5c: Effect of Humidity on the Stability of drug

Temperatures	Humidity			
	30%	50%	70%	90%
Ambient	-	-	-	-
35° C	-	-	-	-
55° C	-	-	+	++
65° C	-	-	++	+++

(-) No Change, (+) Degradation

DISCUSSION

The rate and extent of absorption of a drug into the bloodstream is an important quality characteristic of a dosage form. In vivo bioavailability and in vitro dissolution studies are important in the development and ultimately in the quality control of a dosage form. Formulation studies involve developing a preparation of the drug which is both stable and acceptable to the patient. For orally taken drugs, this usually involves incorporating the drug into a tablet or a capsule.

The medicine derived from plants can be used more conveniently and safely in various diseased conditions, if used in proper portions and combination. To enhance the acceptability of the herbal medicine by consumers, many of the products have been formulated into conventional dosage forms such as tablets, capsules, suspensions, and powders.

Methanolic extract of four different plant drugs were used in this research to formulate a unit solid dosage form (capsule 250mg) to increase the compliances, acceptability and adaptation of the consumers. As it is also very important to estimate the pharmaceutical quality of the Herbal products irrespective of their medicinal content and therapeutic states; so in the present study, the pre-formulation and formulation studies of the formulated capsules (Femitex-SP$_4$) were evaluated. Before converting the blended powder extract into dosage form it was passed through different procedures to estimate the flow ability of the powder extract that was necessary for getting pharmaceutically equivalent dosage. The flow property of powdered extract was evaluated by performing angle of repose, porosity, compressibility and density. These are very preliminary test for concluding the flow of powder but it provides a basic foundation for formulation scheme (Table: 3).

Absorption of drug in the blood is controlled by the availability of drug from solid dosage into the GI fluid. Hence the rate of absorption and availability may be improved by improving the disintegration and the rate of dissolution of drug. In present study, the maximum time for disintegration was 13 min and the minimum time was 6 minutes where as the mean time for disintegration of six capsules with Mean ± S.D was 9.88 ± 2.64minutes. (Table: 4) In case of dissolution it was found that all the six capsules containing powdered extracts were dissolved in 30 minutes up to 85% (Tablet: 4). That fulfill the criteria of BCS rapid dissolution. Rapid dissolution is causing rapid absorption.

Phytochemical tests were done to assess the class of compounds in the formulation (Table: 2). This analysis was helpful to recognize the base line occurrence of chemical components found in these plants along with their identification.

For any new formulation, stability is one of the vital parameter for its evaluation. In the present study, results signified the safety and stability of extracted powdered samples. Effect of light was observed in sunrays, fluorescent (tube) light, UV, and Infra red light. The results had shown that no degradation observed in sun rays, fluorescent, and UV light after ½, 1, 3, and 6 hours, where as in the exposure to IR lamp, the powder underwent degradation after 5 hours (Table: 5a). Similarly, after keeping the capsules at four different temperatures i.e. ambient, 35°C, 55°C, and 65°C for ½, 1, 3, and 6 hours, it was observed that the best stability of sample was at 50°C after 6 hours (Table: 5b). In humidity studies after keeping capsules at different humidity 30%, 50%, 70% and 90%, slight degradation started from 70% and 90% (Table: 5c).

CONCLUSION

The formulation and phytopharmaceutical evaluation of oral dosage form (250 mg capsule named Femitex-SP$_4$) according to the British Pharmacopeia was successfully done. It was prepared in allopathic way to get the equivalent dose for obtaining maximum clinical efficacy. The assessment of developed formulation also indicated that the purposed extract was stable under worst condition (i.e. temperature, light and humidity).

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