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DEVELOPMENT AND EVALUATION OF ANTIPSORIATIC HERBAL CREAM OF QUERCETIN RICH EXTRACT OF DIOSPYROS EMBRYOPTERIS BARK

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ABSTRACT

In the present study, it was envisaged to standardization of plant bark on the basis of presence of quercetin, preparation of herbal cream from quercetin rich extract and its evaluation was carried out. The oil free uncharacterized methanol extract of plant bark, extracted by Soxhlet apparatus showed presence of flavonoids. The optimum resolution of quercetin in plant sample was obtained employing mobile phase containing toluene: ethyl acetate: formic acid in the ratio (7.5:5.5:1). The content of marker compound in plant bark part was found to be 0.875 ± 0.0001% w/w. The parameters mentioned in ICH guidelines were found to be in prescribed limits as described by ICH guidelines. Finally, it can be suggested that quercetin is responsible for various pharmacological actions of plant on the basis of literature records. Further, the dichloromethane extract for preparation of uniform o/w cream was found to provide 15.25 % w/w yield. The pH of herbal cream was noted as 6.6 ± 0.10. No sign of irritation were observed on any skin part with application of herbal cream. The rheometer apparatus was used to check the viscosity of herbal cream and suggested that herbal cream exhibited good pseudo plastic nature. The increment in shear rate suggested the decline in viscosity of herbal cream and further inferring its pseudo plastic nature. At last, the observations of spreadability testing parameters such as firmness and work of shear suggested that prepared herbal cream could be easily applied on infected areas of skin with no signs of separation. The content of herbal plant extract in prepared herbal cream was quantified with the help of UV/VIS spectrophotometry and recorded as 98.85% w/w.

KEYWORDS: *Diospyros embryopteris*, flavonoid, dichloromethane, herbal cream, psoriasis.

INTRODUCTION

Psoriasis vulgaris is a typical skin problem described by central production of inflamed, raised plaques that continually shed scales got from extreme development of skin epithelial cells (Krueger and Bowcock, 2005). Regardless of accessibility of medicines and treatments for relieving psoriasis, its commonness and rates are expanding step by step. It proposes that there is as yet a great need of more current, more secure and viable prescriptions for the treatment of psoriasis. Examining plants, in light of their conventional cases is by all accounts more feasible methodology (Farber and Mc Clintock, 1968).

The exhaustive survey of literature suggested that various herbal plants such as *Aloe vera*, *Centella asiatica*, *Panax ginseng*, *Rubia cordifolia*, *Saccharum officinarum*, and so on have been accounted for antipsoriatic activity (Syed *et al.*, 1996; Sampson *et al.*, 2001; Shin *et al.*, 2005; Tse *et al.*, 2006; Ledón *et al.*, 2007).

Diospyros embryopteris is commonly known by local names such as Black and White Ebony, Pale Moon Ebony, Gaub tree, Indian persimmon, Kalatendu, Tendu, Deshi gab, Makurkendi in different local names and belongs to family Ebenaceae. The plant is mainly found in the wild regions of Eastern India, Sri Lanka, Burma, Cambodia, Laos, Vietnam, Thailand and Indonesia (Kaushik *et al.*, 2013).

Various traditional uses of plant for treatment of different health problems have been mentioned in old texts such as inflammation, leucorrhoea, anaemia, fever, cold, cough, leprosy, wound, urinary problems (Kaushik *et al.*, 2013; Warrier *et al.*, 1996; Asolkar *et al.*, 1992; Benthall, 1946; Viswanathan *et al.*, 2002), skin related problems, sexual problem and malaria (Anjaria *et al.*, 2002).

The exhaustive survey of literature review has suggested that various phytomolecules have been scientifically separated from different parts of *D. embryopteris* such as aliphatic ketol (Chauhan and Kumari, 1980), flavonoids (Chauhan *et al.*,1979; Sahu *et al.*, 2012), monoterpene hydrocarbons, sesquiterpenes, phenyl propanoids (Viswanathan *et al.*, 2002) and triterpene (Jain and Yadava, 1994).

The plant under investigation named *Diospyros embryopteris* has been known for various scientific reported pharmacological actions such as analgesic (Sarwar *et al.*, 2011; Uddin *et al.*, 2006), antibacterial (Uddin *et al.*, 2008), anticancer (Alex *et al.*, 2012; Venugopal *et al.*, 2011), anthelminitic (Ramaiah *et al.*, 2017), antidiarrheal (Rode *et al.*, 2013); antidiabetic (Dewanjee *et al.*, 2008), antiulcer (Gopalakrishna *et al.*, 2014), neuroprotective (Shilpi *et al.*, 2004), hypolipidemic (Dewanjee *et al.*, 2009a) and antiurolithiatic activities (Purane, 2015).

A survey of literature revealed that no work has been carried out yet to standardize this traditionally used and medicinally promising plant on the basis of marker compounds and preparation of herbal cream from its bioactive compound. Thus,in the present study, standardization of extract from *D. embryopteris* bark on the basis of main bioactive compound such as quercetin and preparation of herbal cream from quercetin rich extract and its evaluation studies were carried out.

MATERIALS AND METHODS

Plant material

The crude plant material of *Diospyros embryopteris* bark under present investigation was purchased from Himalaya Herbs Store, Madhav Nagar, Saharanpur in the month of November 2023. Further, identification certificate was issued by Dr. Preet Kawal Kaur, Professor, Saraswati Institute of Pharmaceutical Education and Research, Saraswati Group of Colleges, Gharuan, Mohali, Punjab, India with vide reference number: Pcog/Auth/06/2024, dated 24/01/2024.

Antipsoriatic activity

Antipsoriatic activity reports on various chemical constituents of plant origin have been studied under this ection such as artesunate (Jin *et al.*, 2007), camptothecin (Huang & Lin, 1996), cannabinoids (Wilkinson & Williamson, 2007), capsaicin (Bernstein *et al.*, 1986; Ellis *et al.*, 1993), colchicine (Wahba & Cohen, 1980; Zachariae *et al.*, 1982), curcumin (Traub & Marshall, 2007; Kurd *et al.*, 2008), embelin (Kumar *et al.*, 2011), fumaric acid esters (Harries *et al.*, 2005), gossypol (-) **(**Dodou *et al.*, 2005), hypericin (Kamuhabwa *et al.*, 1999), iso-camptothecin (Lin *et al.*, 2008), koumine (Zhang *et al.*, 2005), podophyllotoxin (Lassus & Rosen, 1986), psoralen (Briffa & Warin, 1979); isoquinoline (Sphingosine (2-amino-4-octadecene-1,3-diol); tannic acid, isoquinoline and tannic acid (Arnold *et al.*, 1993).

Phytochemical screening

The methanol extract was screened through phytochemical screening based shinoda test for the affirmation of the flavonoid presence in the plant (Farnsworth, 1996).

Shinoda test

To the alcoholic test arrangements, magnesium turnings and concentrated hydrochloric corrosive were added. An appearance of red tone demonstrated presence of flavonoids.

TLC densitometric method development

Test solutions

The methanol extract of plant bark (10 g) for TLC densitometric studies after defatting with n-hexane was prepared using well standardized process named Soxhlet apparatus (Sujata *et al.*, 2017; Madaan *et al.*, 2022). The methanol extract was tested for flavonoids by shinoda test (Farnsworth, 1996). The extract was dried, and volume was then acclimated to 25 ml with methanol in a volumetric flask of glass material.

Preparation of standard plot

A stock solution of quercetin (1 mg/ml) was freshly prepared in analytical grade solvent methanol for the TLC densitometric studies. The stock solution of quercetin was further diluted to prepare various concentrations (10, 20, 30, 40, 50 and 60 µg/ml). A volume of 10 µl from every dilution was applied in three-fold on pre-covered TLC plate. The plate was kept in mobile phase toluene: ethyl acetate: formic acid (7.5:5.5:1) in a chamber immersed for 10 min, to a distance of 8 cm. The created plate was dried in a current of hot air and scanned in Camag TLC scanner at 254 nm. The Area Under the Curve of the each peak of quercetin was noted in each track and a standard plot was prepared against amount of quercetin/ different concentrations.

Estimation of marker compound

Three different amounts of test solution such as 5, 10 or 15 µl of methanol extract were applied on pre-covered TLC plate (5 × 10 cm) along with quercetin as standard marker compound. The plate was created and examined following a similar system as utilized for the preparation of standard plot. The normal Area under the curve of each peak of corresponding to peak of quercetin was noted at 254 nm in the test, and its concentration was determined from the standard plot.

Validation of TLC densitometric method

The created TLC densitometric technique was validated as per the various paramters described in ICH guidelines (Randhawa *et al.*, 2015; Richa *et al.*, 2017).

Preparation of dichloromethane extract of plant bark

Dried coarsely powdered plant material bark (1 kg) was extricated comprehensively with dichloromethane (5 L) in Soxhlet equipment. The extraction was completed at the steady temperature of 70°C. The unrefined concentrate was sifted and brought under diminished pressure utilizing rotational vaccum evaporator. The dried concentrate was kept in a desiccator. The dried extract was further subjected to formulation studies to prepare topical cream.

Formulation and Evaluation of herbal cream

Twenty grams of prepared dichloromethane extract of plant bark was placed in one litre beaker and mixed properly with 5 g of PEG 400 with the help of glass rod. The prepared mixture was further mixed with 375 g suitable cream base with a slow motion and triturated using mechanical stirrer to get uniform cream (o/w) of 5% w/w strength. The inert cream base was used for the formulation studies. The inert plastic tube material was used for packing. The herbal cream was evaluated for various paramanters such as organoleptic characteristics, pH of formulation, viscosity, spreadability and drug content.

Methods for evaluation of topical formulation (Herbal cream)

Physical evaluation

Organoleptic characteristics

The various organoleptic properties of formulation such as physical appearance, colour, texture and phase separation and homogeneity was tested as per standard procedures. The above-mentioned parameters were investigated by naked eyes under sun light or highly beam light. Homogeneity and surface were checked by squeezing a little amount of the prepared cream between the thumb and finger / index pointer. The consistency of the prepared cream and presence of coarse particles were utilized to assess the surface and homogeneity of cream. Prompt skin feel (incorporate firmness, coarseness and oiliness) was additionally assessed.

pH of formulation

One gm of prepared cream was mixed in 50 ml deionized water and still up in the air by utilizing a pH meter. Estimations were made in three-fold. The pH meter was aligned with standard buffer solution (pH 4, 7, and 9.2).

Viscosity

The rheometer was utilized to calculate the viscosity / consistency of topical prepared cream. The axle was rotated at 0, 0.5, 1, 2, 2.5, 4, 5, 10, 20, 50, and 100 rpm. All estimations were made in three-fold.

Spreadability

The spreadability testing mechanical assembly (surface analyser) was utilized to decide the surface of prepared cream. Surface analyser chart was acquired among force and time / power; normal properties (immovability and work of shear) were acquired from the diagram. Immovability addresses the protection from pressure or power (in g) and work of shear communicates the appropriateness of the cream to the skin.

Drug content

1gm of prepared cream was broken down in 2 ml of DCM and afterward sonicated for 30 minutes to completely extract the medication from cream. This solution was centrifuged at 2000 rpm and the supernatant was collected and filtered it after that this solution was evaluated by utilizing UV spectrophotometer and the medication content was determined.

RESULTS AND DISCUSSION

The flavonoid rich methanol extract of plant bark was prepared by Soxhlet apparatus and tested positive for flavonoids by shinoda test. The quantity of quercetin in plant bark was calculated with help of standard plot between amount of quercetin (ng) loaded vs. area under the curve obtained (Figure 1). The content of quercetin in plant bark was found to be 0.875 ± 0.0001% w/w. The thin layer chromatogram overlay of quercetin and methanol extract of plant bark is shown in figure 2. The ultra violet overlay of quercetin and methanol extract of plant bark is shown in figure 3. The results of validation of developed TLC densitometric method have been presented in table 1.

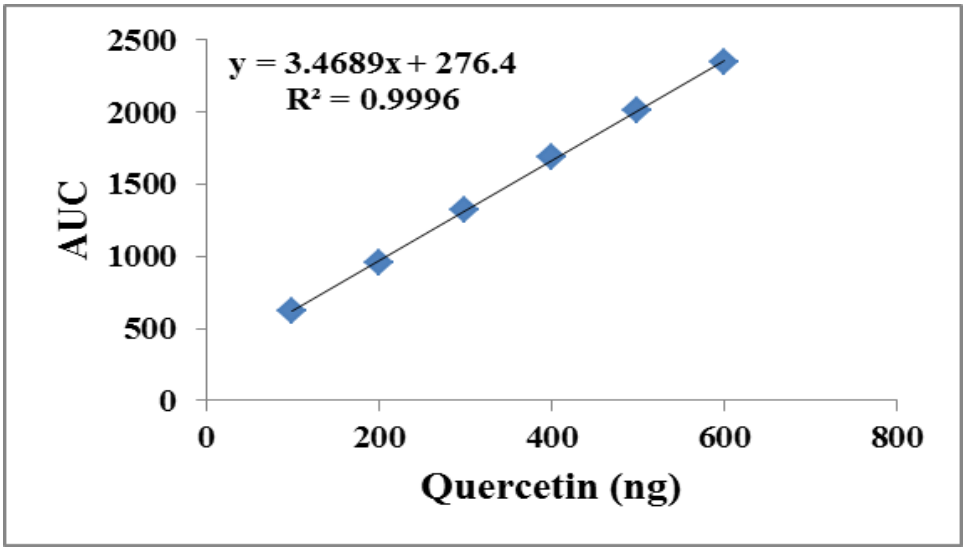

Figure 1: Standard plot of quercetin.

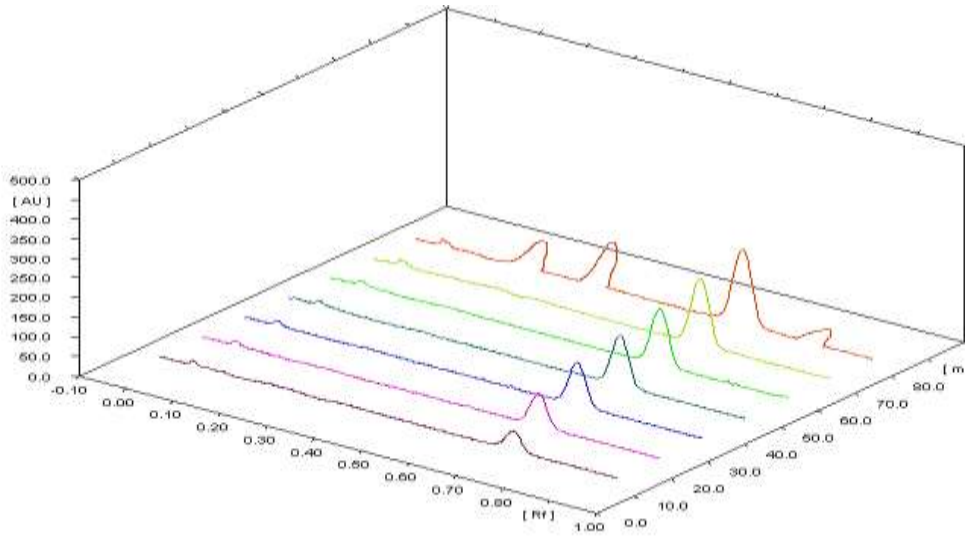

Figure 2: Thin layer chromatogram overlay of quercetin and methanol extract.

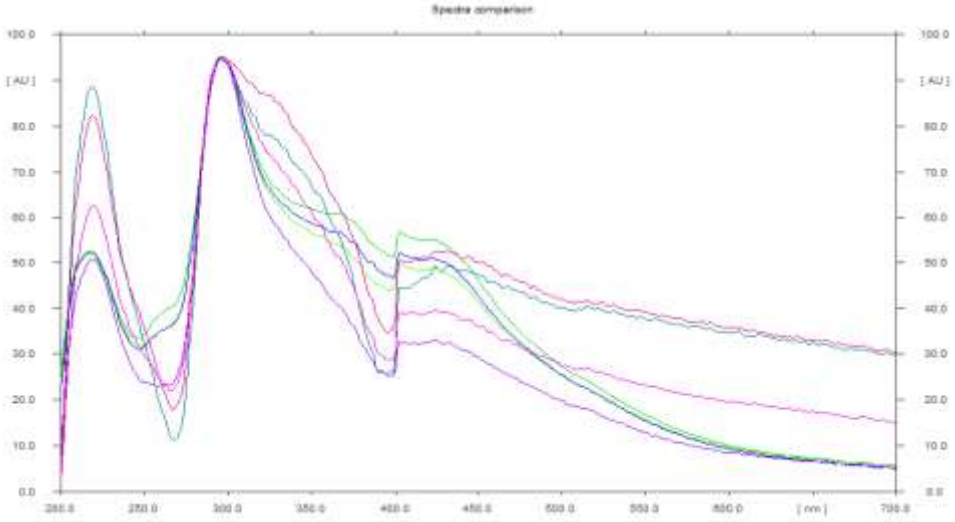

Figure 3: Thin layer spectra overlay of quercetin and methanol extract.

Confirmation of flavonoids presence in methanol extract of plant bark

The methanol extract of plant bark was subjected to phytochemical test named shinoda test to detect the availability of flavonoids. The phytochemical test indicated the presence of flavonoids in methanol extract.

Table 1: Method validation parameters in TLC densitometric analysis.

Parameter	AP-1
Instrumental precision (% CV, n=7)	0.89
Repeatability (% CV, n=5)	0.70
Linearity (Coefficient of correlation r^2)	0.9996
Linearity Range (ng)	100-600 ng
LOD (ng)	10
LOQ(ng)	32
Intra-day precision (%CV, n=9)	1.31
Inter-day precision (%CV, n=9)	1.07
Accuracy (average % recovery)	98.32 ± 1.06
Specificity	Specific
Robustness 252 nm	1.67
Robustness 254 nm	1.69
Ruggedness Analyst 1	1.49
Ruggedness Analyst 2	1.19

In present examination, TLC-densitometric technique was utilised for the assessment of quercetin in plant bark. Further, the created strategy was approved for the boundaries depicted in ICH rules. The content of quercetin in plant bark was discovered to be 0.875 ± 0.0001% w/w. The created strategy for assessment of quercetin in plant bark was approved according to the rules of ICH. Percentage coefficient of variance observed in validation parameters of developed method such as instrumental precision, inter and intra-day precision, robustness, ruggedness and repeatability complied with the prescribed limit. Recovery of quercetin in accuracy studies was more than 98%. Further, no deviation was observed in ultraviolet spectra and thin layer chromatograms of sample and standard. These observations infer that the developed method for estimation of quercetin in plant bark was precise, accurate, reproducible and specific.

The online scientific reports suggested that quercetin show their pharmacological activities through various related mechanisms such as antiviral, antibacterial, anticarcinogenic, anti-inflammatory and antidepressant actions (Naidu et al, 2012). Thus, it is suggested from the observations that quercetin may be one of the bioactive compounds of plant. Therefore, it is selected as a marker to standardize plant bark.

The uniform o/w cream was prepared and assessed for standard boundaries. The standard technique was embraced to get ready dichloromethane extract. The yield of extract was found to be 15.25 % w/w. The extract obtained was semisolid with sticky nature dull greenish dark in shading. The cream of plant leaves dichloromethane extract was arranged utilizing PEG 400 as suspending specialist and reasonable cream / ointment base.

Various organoleptic properties such as physical appearance, colour, texture, phase separation, homogeneity and immediate skin feel have been presented in table 2. The results from above mentioned studies suggested that cream has smooth texture free from coarse particles and homogenous with no sign of phase separation as shown in table 3. The pH of prepared herbal cream was recorded using pH meter and noted as 6.6 ± 0.10. The herbal cream did not produce any irritation on skin after its application on skin. The viscosity of prepared herbal cream was estimated by well-established equipment known as rheometer. It is suggested from observations that the prepared herbal cream exhibited good pseudo plastic nature. A sharp decline in viscosity of prepared herbal cream was noted with increase in shear rate, inferring its pseudo plastic nature as shown in figure 4. A texture analyser graph between force and time for spreadability of herbal cream has been shown in figure 5. Spreadability of cream was calculated in terms of two well-known main parameters i.e., firmness and work of shear. The result of spreadability testing parameters i.e., firmness and work of shear suggested that prepared herbal cream will spread with ease on the applied skin areas. The content of drug in prepared herbal cream was estimated using UV/VIS spectrophotometry and calculated as 98.85% w/w.

Table 2: Various organoleptic characteristics of formulation.

Formulation	Physical appearance	Colour	Texture	Phase separation	Homogeneity	Immediate skin feel
Antipsoriatic cream of plant bark dichloromethane extract	Semi-solid	Light brownish green	Smooth	No	Uniform consistency	No rough and oily feeling on apply, moisturizing

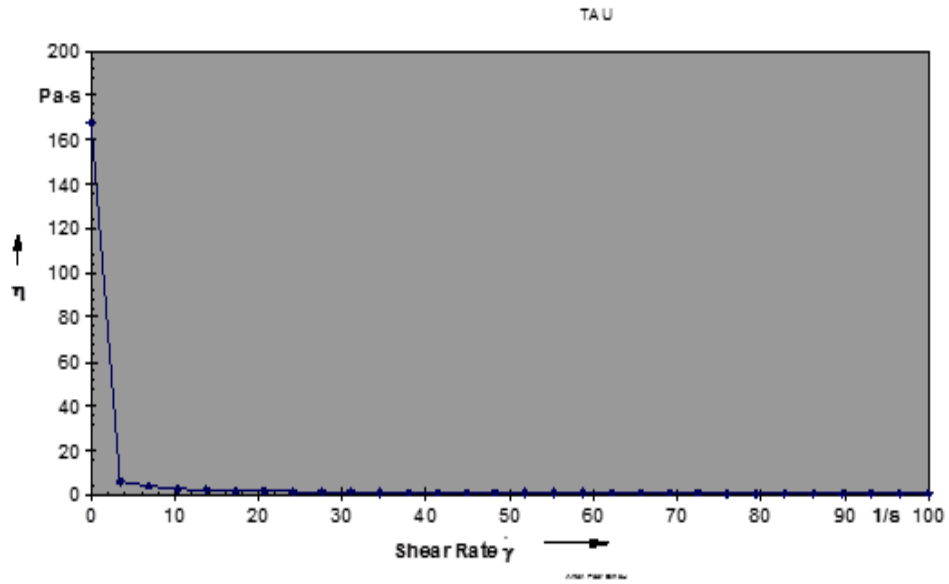

Figure 4: Relationship between shear Rate and Viscosity of formulation.

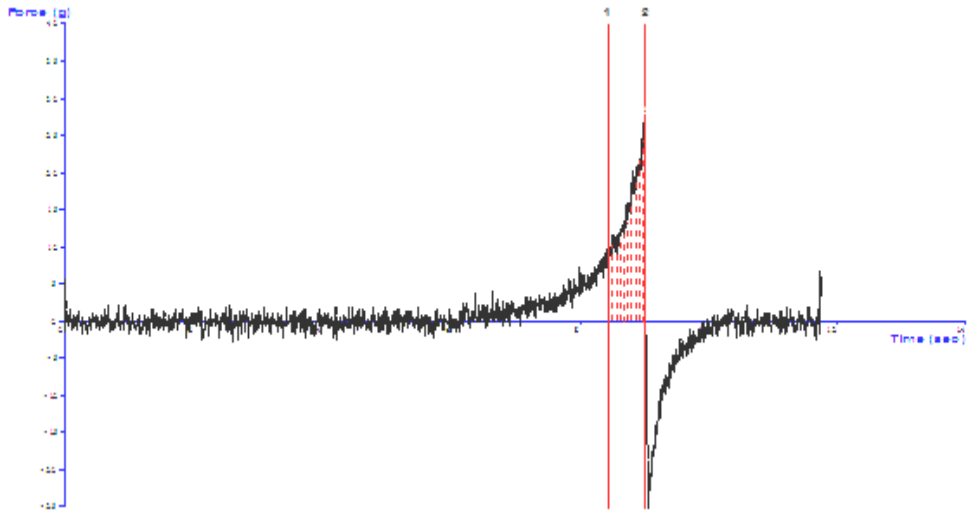

Figure 5: Texture analyzer graph between Force and Time for spreadability of formulation.

Table 3: Spreadability testing parameters of formulation.

Parameter	Result
Firmness	25.542 g
Work of shear	8.256 g per area

CONCLUSION

Finally, it can be suggested that quercetin is responsible for various pharmacological actions of plant. Dichloromethane extract for preparation of uniform o/w cream was prepared using standard protocol and yield of extract was found to be 15.25 % w/w. The semi-solid light brownish green coloured, smooth, uniform, homogenous, free from coarse particles with no sign of phase separation herbal cream was prepared. The pH of herbal cream was noted as 6.6

± 0.10. There was no sign of irritation on any skin part with application of herbal cream. The rheometer apparatus was used to check the viscosity of herbal cream and suggested that herbal cream exhibited good pseudo plastic nature. The increment in shear rate suggested the decline in viscosity of herbal cream and further inferring its pseudo plastic nature. At last, the observations of spreadability testing parameters such as firmness and work of shear suggested that prepared herbal cream could be easily applied on infected areas of skin with no sign of separation. The content of herbal plant extract in prepared herbal cream was quantified with the help of UV/VIS spectrophotometry and recorded as 98.85% w/w.

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