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FORMULATION AND CHARACTERIZATION OF POLYHERBAL TOPICAL CREAM

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ABSTRACT

Herbal plants and their combination report therapeutic as well synergistic effect that has been recognized in medicine. So, taking into account this factor, polyherbal topical cream formulation was prepared by using plant extracts, to improve patient compliance, enhance antimicrobial spectrum and enhance aesthetic properties. The objective of this study was to formulate and evaluate topical polyherbal cream for the delivery of the active constituents present in plants to improve skin diseases. The plant extracts of *Ocimum sanctum* (OS), *Rubia cordifolia* (RC) and *Glycyrrhiza glabra* (GG) were utilized for the preparation of cream. The formulated cream was subjected to different evaluation parameters and the results depicted that the spreadability of the formulation was low (17.80±1.10g. cm/sec) and this low value of spreadability coefficient was sufficient suggesting easy spreading and no signs of grittiness. In rheological studies, all the cream formulations also exhibited the same non-Newtonian behavior. Polyherbal topical cream showed potential antimicrobial activity against all selected microorganisms. Polyherbal topical cream (PHC5) was ideal in terms of viscosity than other formulations and showed good drug release. Thus, the formulated polyherbal cream was found to be stable in terms of all physicochemical properties.

Keywords: *Ocimum sanctum, Rubia cordifolia, Glycyrrhiza glabra,* topical cream, Polyherbal cream.

1. INTRODUCTION

In the present era, the use of herbal cosmeceuticals is rapidly increasing. As these possess varied properties in terms of availability of the natural resources, development of successful products and preparation of good quality, these are the potentials in the market [1]. Cosmetics are those products that are applied on the body for the purpose of cleansing, beautifying or altering appearance and enhancing the beauty. For most of the skin conditions, creams are used, for their various benefits they possess [2]. Human skin is the major organ of the body that is acts as a defense mechanism against most of the disorders. The basic three layers of skin include epidermis, dermis and the hypodermis. These layers of skin have specific properties and role that make them to act as a barrier against foreign material to enter the body, through skin [3]. The function of skin is to protect the underlying muscles, ligaments, internal organs etc. [4]. It also interfaces with environment, to protect against pathogens, with loss of excessive water [5, 6]. The other functions of skin include regulation of temperature, insulation, sensation, synthesis and storage of Vitamin D against UV, water resistance etc. [7]

So, the present study is aimed to prepare a polyherbal topical cream useful in the management of various skin diseases, by use of extracts of *Ocimum sanctum (OS), Rubia cordifolia (RC), Glycyrrhiza glabra (GG).*

2. MATERIAL AND METHODS

2.1. Material

Ocimum sanctum, Rubia cordifolia, Glycyrrhiza glabra, were procured from local market and authenticated.

2.2. Methods

The extraction of collected plant materials was carried out using established methods. The part of individual plant was selected, cleaned and powdered to get crude drug. To obtain non polar extracts, the air-dried coarse powders of *Ocimum sanctum, Rubia cordifolia* and *Glycyrrhiza glabra* were extracted separately by Soxhlet extraction process using petroleum ether and chloroform. These extracts were further successively extracted with respective polar extracts hydroalcoholic (60:40) solution. The extracts were then concentrated to dryness under reduced pressure and controlled temperature, respectively and they were preserved in a refrigerator for further study. The extracts obtained were filtered, evaporated to dryness to yield semi solid paste and preserved in refrigerator for further study [8]

2.3. Experimental design

Design expert® software, version 12.0 was used to find correlation between independent and dependent variables. At 5% level of significance, analysis of variance was implemented. In design expert the model was screened out by analyzing adjusted R2 value, which has to be<1. The topical formulations of polyherbal cream were optimized by 32 factorial design. The factors were calculated by low, medium and high, at 3 levels indicating (-1, 0, +1) respectively, as given in Table 2. Two independent formulation variables were evaluated: a) concentration of glycerin b) concentration of methylcellulose. 3^2 Factorial design for formulation of polyherbal topical cream on basis of preliminary studies, using optimization studies. The dependent factors were drug release and viscosity.

2.4. Preparation of polyherbal cream

Creams were formulated by first preparation of the two phases, aqueous and oil separately. In aqueous phase, 1gm of methyl cellulose polymer was dispersed in hot water (at around 75°C) and then cooled to around 5°C with continuous stirring in 100ml of water then 1ml each of glycerin and propylene glycol was added with constant stirring. This prepared aqueous phase was added to the three preselected extracts in different concentrations. The oil phase was prepared, by melting the 0.9gm of bees wax at 70°C, with intermittent stirring and to it 1 ml of almond oil was mixed. After preparation of both the phases, these were mixed together, to get a mixture. To this, prepared above mixture, 0.1 gm of sodium benzoate as preservative and 0.8gm of zinc oxide as skin whitener was added, with continuous stirring.

Table 1: Independent variables and their corresponding levels for optimization studies

Independent variables (%w/w)		Levels		
		-1	0	+1
Concentration of glycerin	A	1.0	1.5	2.0
Concentration of methylcellulose	B	1.0	1.5	2.0

Table 2: Factorial design for formulation of topical polyherbal cream

Formulation number	Factor 1 **(A)**	Factor 2 **(B)**
1	-1	-1
2	-1	0
3	-1	+1
4	0	-1
5	0	0
6	0	+1
7	+1	-1
8	+1	0
9	+1	+1

Table 3: Various compositions of polyherbal topical creams, by use of selected three extracts

Ingredients (%w/v)	PHC1	PHC2	PHC3	PHC4	PHC5	PHC6	PHC7	PHC8	PHC9
Ocimum sanctum extract	1	1	1	1	1	1	1	1	1
Rubia cordifolia extract	1	1	1	1	1	1	1	1	1
Glycyrrhiza glabra extract	1	1	1	1	1	1	1	1	1
Glycerin	1	1	1	1.5	1.5	1.5	2	2	2
Propylene glycol	1	1	1	1	1	1	1	1	1
Zinc oxide	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Methyl cellulose	1	1.5	2	1	1.5	2	1	1.5	2
Bees wax	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Almond oil	1	1	1	1	1	1	1	1	1
Sodium benzoate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Purified water	qs	qs	qs	qs	qs	qs	qs	qs	qs

2.5. Phytochemical analysis of extracts [9]

Phytochemical analysis of different extracts was carried out to record the presence of prominent chemical constituents by the following tests: Test for steroids, Test for triterpenoids, Test for glycosides, Tests for saponins, Tests for carbohydrates, Tests for alkaloids, Tests for Flavonoids, Tests for tannins, Tests for proteins

2.6. Evaluation of polyherbal cream [10]

2.6.1. Physical parameter

The physical parameters of individual topical cream

were studied at room temperature and at accelerated temperature.

2.6.2. Homogeneity

Topical cream was individually tested for the homogeneity by visual appearance and by touch.

2.6.3. Appearance

The appearance of the respective topical cream individually was judged by its color, pearlescent and roughness and graded accordingly.

2.6.4. pH measurement

To measure the pH, 1 gm of respective topical cream was diluted individually with 9 ml of distilled water and then pH was checked using pH meter.

2.6.5. Spreadabilty

One gram of respective topical cream individually was placed on the lower plate and the upper plate was place on the top of the sample. A known weight was applied to generate constant force. The observations were done thrice.

2.6.6. Viscosity measurement

The viscosity of respective topical cream was measured and compared individually before and after accelerated test by Brookfield Viscometer at 100 rpm, using spindle no 763.

2.6.7. Rheological studies

The formulated respective topical cream individually was studied for its rheological property. 10 gms of respective topical cream was taken in a 10 ml beaker and kept for 1 hr. To see whether the cream was in liquid form or not the beaker was leaned to one side. Beaker was shaken to and fro for 5 min using mechanical shaker to check change in consistency. The beaker was again tilted and checked for pourability of the cream.

2.6.8. In vitro diffusion study

Franz diffusion cell (25 ml cell volume) was used for the drug release studies. 1 gm of formulation was applied onto the surface of cellophane membrane evenly over a fixed area. The receptor chamber was filled with freshly prepared Phosphate Buffer (pH 7.4) solution. The receptor chamber was stirred by a magnetic stirrer. The samples (1.0 ml aliquots) were collected at a suitable time interval replaced with fresh buffer solution. Samples were analyzed for drug content by UV visible spectrophotometer at 270 nm after appropriate dilutions. The cumulative amount of drug released across the membrane was determined as a function of time.

2.6.9. Stability study

Stability studies as per ICH guidelines: The stability studies were performed individually for respective topical cream by keeping it at refrigerator temperature (4°C), 25°C± 2°C/60% relative humidity (RH) ±5% RH and 40°C ± 2°C/75% RH ±5% RH for the period of three month. The various parameters such as homogeneity, appearance, spreadabilty, after feel, type of smear removal, pH, viscosity and phase separation were recorded.

3. RESULTS AND DISCUSSION

3.1. Phytochemical evaluation:

The selected plants are known to have Steriods, Trierpenoids, Saponin, alkaloids, flavonoids and tannins as presented in table 1.

3.2. Evaluation of polyherbal formulations

Physical Evaluation included homogeneity, appearance, Spreadability, after feel, Type of smear, Removal and Rheological studies for various topical cream formulation. It was found that the cream was homogeneous and smooth and consistent in nature. All formulations of base produced uniform distribution in cream. Visual appearance and touch, confirmed this test. The prepared creams of individual extracts were light yellow to brown in color having appropriate appearance. Also, it had pleasant aroma. The pH of the cream was found to be in range of 6.4 to 7.2. The spreadability of the formulation was low (17.80±1.10 g.cm/sec) and this low value of spreadability coefficient of the cream was sufficient suggesting easy spreading and no signs of grittiness.

3.3. Experimental Design and Statistical Analysis

A 3^2 factorial design was selected, for the study, as it help to study the effect of factors on the response, with least number of experimental runs. The viscosity of the formulations was found to be in the range 1546 to 1554 cps. Multiple Regression Analysis

To make possible the response parameters, by the effect of the independent variables, it is necessary to fit a mathematical model, that predicts the value of response and that generates the polynomial equations, that is useful for evaluation.

$Y = k + b_1A + b_2B + b_{12}AB + b_{11}A^2 + b_{22}B^2$

Where Y is the response evaluated, k is the intercept; b1 to b22 is the five coefficients of independent variables.

The Model F-value of 6.88 implies the model is significant. There is only a 2.80% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case B is a significant model term. Values greater than 0.1000 indicate the model terms are not significant.

Table 4: Phytochemical Screening of *Ocimum sanctum, Rubia cordifolia, Glycyrrhiza glabra*

Plant Constituents	Test performed	*Ocimum sanctum*	*Rubia cordifolia*	*Glychyrrhiza glabra*
Test for Steroids	Salkowaski Test	+	+	+
	Liebermann-Buchard Test	+	+	+
Test for Triterpenoids	Salkowaski Test	-	+	+
	Liebermann-Buchard Test	+	+	+
Test for Glycosides	Balget's test	-	+	+
	Keller-Killiani test	-	+	+
	Legals test	-	-	+
	Borntrager's test	-	-	+
Tests for Saponin	Foam Test	++	+	+
Tests for Carbohydrates	Molisch's test	-	+	-
	Barfoed's test	-	-	-
	Fehling's test	-	+	-
	Benedict's test	-	+	-
Test for Alkaloids	Mayer's Reagent	+	-	+
	Hager's Reagent	+	+	+
	Dragendorff's Reagent	+	+	+
Tests for Flavonoids	Ferric-chloride test	+	+	+
	Shinoda test	+	+	+
Test for Tannins	$FeCl_3$ Solution	+	+	+
	Gelatin test	+	+	+
Test for Proteins	Millon's test	-	-	-
	Xanthoproteic test	-	-	-
	Biuret test	-	+	-
	Ninhydrin test	-	-	-

Present (+) Absent (-)

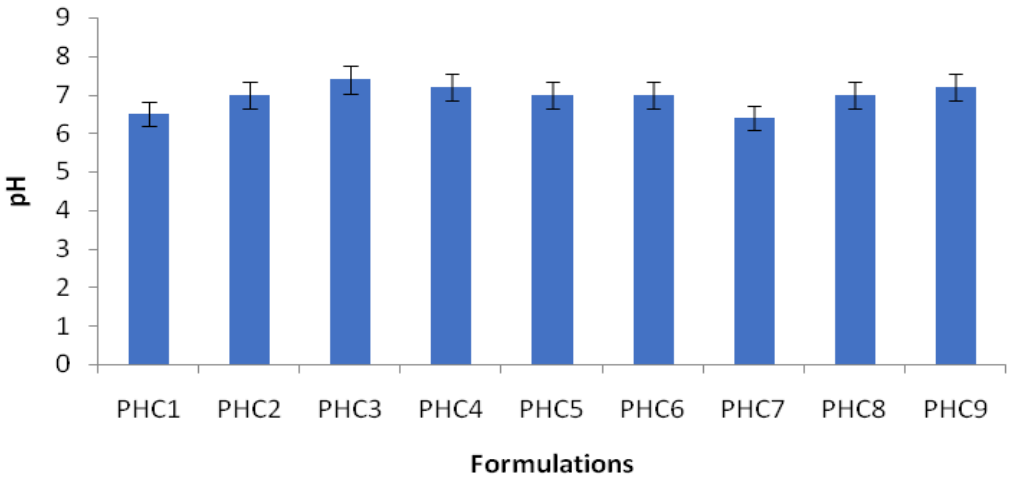

Fig. 1: pH data for polyherbal topical cream

Table 5: Viscosity and Drug release for the prepared polyherbal cream

Formulationnumber	Factor 1 (A)	Factor 2 (B)	Viscosity (cps)	Drug release (%)
PHC1	-1	-1	1546±0.57	87.4±0.57
PHC 2	-1	0	1550±2.30	91.2±0.60
PHC 3	-1	+1	1550±1.52	96.5±1.05
PHC 4	0	-1	1554±1.73	93.3±0.64
PHC 5	0	0	1554±0.57	93.1±0.45
PHC 6	0	+1	1548±1.15	90.1±0.45
PHC 7	+1	-1	1552±1.52	86.4±0.62
PHC 8	+1	0	1551±0.57	89.2±0.47
PHC 9	+1	+1	1544±1.52	90.3±0.45

**Data are mean values (n=3)±SD*

Table 6: ANOVA analysis on Viscosity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	72.28	5	16.86	9.88	0.0479	significant
A-Con of glycerin	2.49	1	2.49	0.8670	0.4205	
B-Conc of methyl cellulose	44.46	1	44.46	15.50	0.0292	
AB	11.18	1	11.18	3.90	0.1428	
A^2	0.6033	1	0.6033	0.2103	0.6777	
B^2	2.07	1	2.07	0.7215	0.4581	
Residual	8.60	3	2.87			
Cor Total	92.89	8				

Table 7: Value of R^2 for Viscosity

Std. Dev.	1.69	R^2	0.9074
Mean	1549.89	Adjusted R^2	0.7530
C.V. %	0.1093	Predicted R^2	0.0470
		Adeq Precision	7.6046

The Predicted R^2 of 0.0470 is not as close to the Adjusted R^2 of 0.7530 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 7.605 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors

Viscosity = +1550.05 +0.5987 A +2.70 B + 2.13+ $0.4557A^2$ -$0.8063B^2$

Final Equation in Terms of Actual Factors

Viscosity= +1556.1810-17.0637 CON OF GLYCERIN +2.28520 CONC OF METHYL CELLULOSE + 8.52891 CON OF GLYCERIN * CONC OF METHYL CELLULOSE +1.82261 CON OF $GLYCERIN^2$- 3.22509 CONC OF $METHYL\ CELLULOSE^2$

The equations I terms of actual factors can be used to make predictions about the response for given levels of each factor.

3.4. Rheological studies

The formulated cream was found to be non-Newtonian. Most topical formulations, when applied on the surface of the skin, show non-Newtonian behavior. Thus, all the cream formulations also exhibited the same Non-Newtonian behavior.

The Model F-value of 10.97 implies the model is significant. There is only a 3.83% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A^2 is a significant model term. Values greater than 0.1000 indicate the model terms are not significant.

The Predicted R^2 of 0.4916 is not as close to the Adjusted R^2 of 0.8617 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 9.580 indicates an adequate signal. This model can be used to navigate the design space

Final Equation in Terms of Coded Factors

Drug release = +95.94 -0.4214 A +1.14 B +1.64 AB -4.03 A^2- 1.89 B^2

Final Equation in Terms of Actual Factors

Viscosity = +55.28451+37.672 CON OF GLYCERIN +15.09623 CONC OF METHYL CELLULOSE+ 6.54099 CON OF GLYCERIN * CONC OF METHYL CELLULOSE -16.10884 CON OF GLYCERIN^2-7.54410 CONC OF METHYL CELLULOSE^2

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor

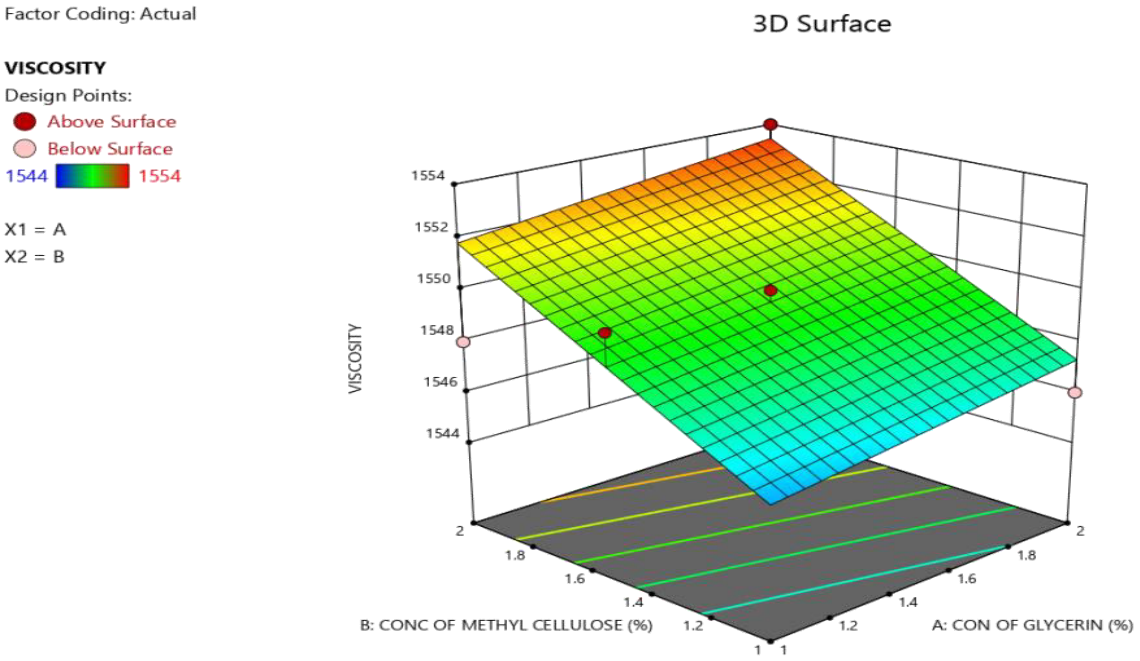

Fig. 2: Three- dimensional response surface plot for viscosity

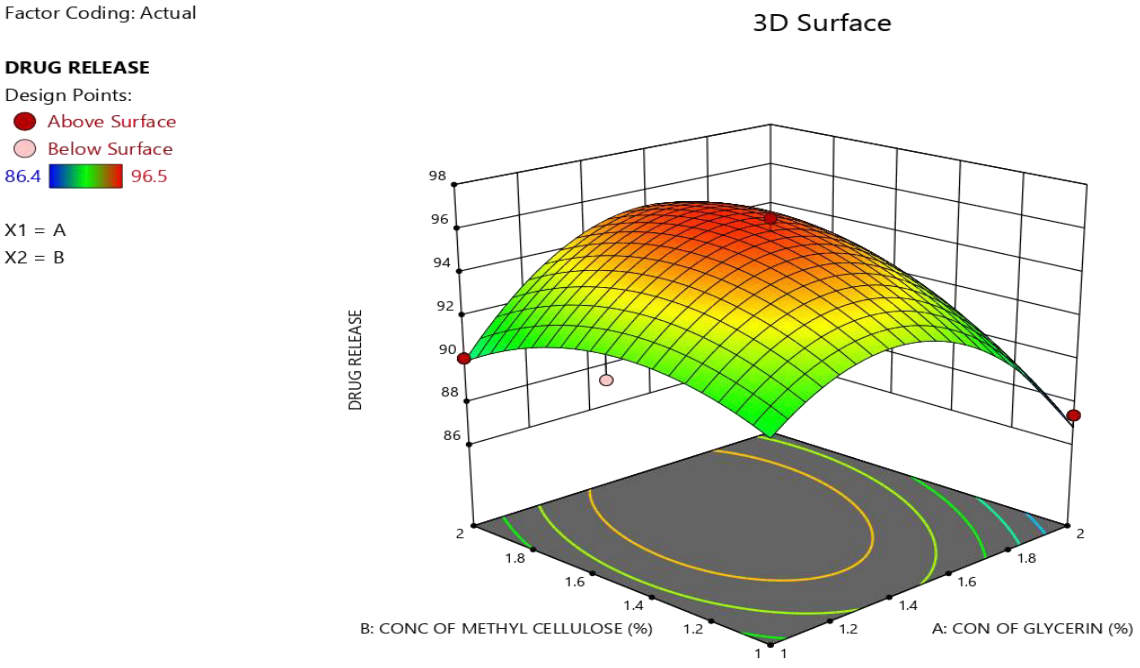

Fig. 3: Three-Dimensional Response Surface Plots For Drug Release

Table 8: ANOVA analysis on Drug release

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	74.33	5	14.87	10.97	0.0383	significant
A-Con of glycerin	1.23	1	1.23	0.9086	0.4108	
B-Conc of methyl cellulose	7.88	1	7.88	5.82	0.0949	
AB	6.58	1	6.58	4.85	0.1149	
A^2	47.12	1	47.12	34.76	0.0097	
B^2	11.32	1	11.32	8.35	0.0630	
Residual	4.07	3	1.36			
Cor Total	78.40	8				

Table 9: Value of R^2 for Drug Release

Std. Dev.	1.16	R^2	0.9481
Mean	90.83	Adjusted R^2	0.8617
C.V. %	1.28	Predicted R^2	0.4916
		Adeq Precision	9.5799

3.5. Selection of Optimized Formula

After generating the reduced model polynomial equations to relate the dependent and independent variables, the process was optimized for all three responses. Optimum formulation was selected based on the constraints set on independent variables. The final optimal experimental parameters were calculated using the extensive grid search and feasibility search provided in the Design Expert software. After generation of the polynomial equations to correlate the dependent and independent variables, the process was optimized for responses. Optimum formulation was selected on the basis of the results of the evaluation tests. Thus, the optimized formulation was PHC5 which had pH of 7.0, viscosity of 1554 cps and drug release of 93.1 %

3.6. Stability studies

In this study, the formulations were subjected at different storage conditions and were examined. There was no change in the color of formulation at the end of observation periods that suggest the physical stability and no chemical reaction between the ingredients. Hence, polyherbal creams were found to be stable in terms of color, pH and viscosity.

4. CONCLUSION

The polyherbal cream was formulated and evaluated by use of excipients. Thus, this cream proved to be of great potential for topical application, due to the properties it possessed. The pH range was observed to be between 6.4 to 7.2. The polyherbal cream also possessed good spreadability, with good emollient property and viscosity. Thus, it can be concluded that the use of combination of plant extracts for formulation of polyherbal cream could provide synergistic effect of all these individual plants.

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***Conflicts of Interest*:** none declared

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