The effect of formulation variables on the physicochemical and antimicrobial properties of cream formulations of *Phyllanthus amarus* whole plant extract

Tolulope O. Ajala[1], Mbang N. Femi-Oyewo [2], Oluwatoyin A. Odeku[1]
[1]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy
University of Ibadan, Ibadan, Nigeria
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy
Olabisi Onabanjo University, Ago-Iwoye, Nigeria

Corresponding author: Tolulope Ajala
E-mail: tolulola1721@gmail.com, Phone: +2348022171674

ABSTRACT

Background: *Phyllanthus amarus* Schum and Thonn (Euphorbiaceae) is a plant of ethnomedicinal importance whose cream formulations have shown antimicrobial properties in a previous study.

Objective: To determine the quantitative effects of formulation additives on the physicochemical and antimicrobial properties of *Phyllanthus amarus* cream.

Methods: The ethanol extract of *Phyllanthus amarus* was prepared by maceration for 72 hours and the cream formulated and assessed using established procedures. The individual and interaction effects of nature (N) of humectant, concentration (C) of humectant and extract concentration (E) on the physicochemical and *in vitro* antimicrobial properties of the cream formulation were determined using a 2^3 factorial experimental design.

Results: The independent coefficient values ranked N>C >E on viscosity, E>N>C on globule size and C >E > N on spreadability. The ranking on antibacterial property was E > N > C, while on fungi it changed to E > C > N. The physicochemical properties of the formulations were differently influenced by N, C and E. The individual and interaction effects on the physicochemical and antimicrobial properties were found to be significant ($p<0.05$).

Conclusion: In the design of *P. amarus* cream, careful consideration of formulation variables is required to obtain optimum physicochemical and antimicrobial properties.

Keywords: *Phyllanthus amarus* cream, formulation variables, factorial experimental design

West African Journal of Pharmacy (2017) 28 (1) 52-60

L'effet des variables de formulation sur les propriétés physico-chimiques et antimicrobiennes des formulations de crème de l'extrait végétal entier de *Phyllanthus amarus*

Auteur de correspondance: Tolulope Ajala
E-mail: tolulola1721@gmail.com Téléphone: +2348022171674

RESUME

Contexte: Le *Phyllanthus amarus Schum* et Thonn (Euphorbiaceae) est une plante d'importance ethnomédicale dont les formulations de crème ont montré des propriétés antimicrobiennes dans une étude antérieure.

Objectif: Déterminer les effets quantitatifs des additifs de formulation sur les propriétés physico-chimiques et antimicrobiennes de la crème de *Phyllanthus amarus*.

Méthodes: L'extrait d'éthanol de *Phyllanthus amarus* a été préparé par macération pendant 72 heures et la crème a été formulée et évaluée selon des procédures établies. Les effets individuels et d'interaction de la nature (N) de l'humectant, de la concentration (C) de l'humectant et de la concentration de l' extrait (E) sur les propriétés physico-chimiques et antimicrobiennes *in vitro* de la formulation de la crème ont été déterminés en utilisant un modèle expérimental factoriel (de niveau 2).

Résultats: Les valeurs de coefficients indépendants classés N>C>E sur la viscosité, E>N>C sur la taille des globules et C>E>N sur l'étalement. Les propriétés physico-chimiques des formulations ont été influencées différemment par N, C et E. Les effets individuels et d'interaction sur les propriétés physico-chimiques et antimicrobiennes ont été jugées significatives ($p<0,05$).

Conclusion: Dans la conception de la crème de *P. amarus*, une étude minutieuse des variables de formulation est nécessaire pour obtenir des propriétés physico-chimiques et antimicrobiennes optimales.

Mots-clés: Crème de *Phyllanthus amarus*, variables de formulation, conception expérimentale factorielle

INTRODUCTION

Topical drug delivery systems are designed to deliver a drug formulation to the skin for the treatment of cutaneous disorder or the cutaneous manifestations of a general disease such as psoriasis with the aim of restricting the pharmacological effect of the drug to the surface of the skin or within the skin.[1] The advantages of topical dosage forms over oral forms include the ease of application and withdrawal of medication, avoidance of First Pass effect, maintenance of activity for a prolonged period and reduction of potential side effects.[2] Pharmaceutical creams are typical examples of topical drug delivery systems and the most common are oil-inwater emulsion systems, whose structure or semisolid character is dependent upon emulsified liquid droplets or particles that make up the internal phase.[3] The physicochemical properties of pharmaceutical creams have been reported to depend on the drug and formulation additives.[4] For example, high oil content (25-35 %) has been shown to alter viscosity and particle sizes of cream systems leading to reduced homogeneity.[5] Glycerin and propylene glycol are excellent examples of commonly used cream additives that exhibit a wide range of variability in its effect on the consistency and quality of the finished cream[6]. They are useful as co-solvents and humectants and are known to alter rheology and drug delivery characteristics of topical gels. This has been attributed to their different properties due to the change in solvent-polymer and solvent-solvent interactions.[5]

The assessment of formulation variables on pharmaceutical preparations has been done previously using diverse statistical experiments.[7,8,9] Generally, this type of study enables a clear understanding of the effects of various formulation variables on whatever pharmaceutical formulations it is applied to. A factorial experiment is a statistical test whose design comprises of two or more factors having separate possible values or 'levels'.[10] The experimental entities take on all possible combinations of these levels across all such factors. A factorial experiment allows a formulator to study the effect of each factor on the response variable and the effects of interactions between factors on the response variable. The design allows the effect of several factors and even interactions between them to be determined with the same number of trials as are necessary to determine any one of the effects by itself with the same degree of accuracy. When a design is denoted as 2^3 factorial, it implies the number of factors i.e 3; how many levels each factor has (i.e 2); and how many experimental conditions there are in the design (2^3=8). [11] The analysis of a factorial experiment may be done using parametric and non-parametric tests.

The topical treatment of dermatologic conditions with plant-derived medicines predates the cultures of ancient Egypt and still exists today in the industrialized countries.[12] Many herbal preparations are used for

various ailments including those of the skin.[13] Previous studies [14, 15, 16] have shown that the formulation of herbs as topical dosage forms have potential for the development of new therapies depending on the specific activity of the herb.[17] *Phyllanthus amarus* (*P. amarus*) Schum and Thonn (family-Euphorbiaceae) is commonly called *small leaves* in English language, *Eyin Olube in Yoruba* (a Nigerian language) and *Ngwu in Igbo* (a Nigerian Language).[18] The plant is widely used in folklore medicine for the treatment of diabetes, kidney stones and malaria.[18] The extracts of *P. amarus* has been shown to possess antibacterial [19, 20] and antiplasmodial [21] properties.

Ajala *et al*., also studied the physicochemical, safety and antimicrobial properties of *P. amarus* cream and ointment formulations.[22] The results showed that the cream demonstrated superior properties when compared to the ointment. Previous studies have reported that the performance of pharmaceutical creams is affected by formulation additives.[4,5] However; no study had been carried out to verify the effect of formulation additives on the performance of the cream formulations of *P. amarus*. Thus, the objective of the present study was to investigate the effect of formulation variables on the physicochemical and *in vitro* antimicrobial effect of *P. amarus* cream using the 2^3 factorial experimental designs.

MATERIALS AND METHODS Materials

The materials used were dimethyl sulphoxide (DMSO) from Gaylord Chemical Corporation, (USA), propylene glycol, glycerin, chlorocresol, emulsifying wax, white soft paraffin, liquid paraffin, woolfat, hard paraffin and cetostearyl alcohol all products of BDH Chemicals Limited (Poole, England), nutrient broth was obtained from Oxoid Ltd., (Basingstoke, United Kingdom), nutrient agar (Difco Laboratories, USA) and Saboraud Dextrose agar (Biolife Laboratories, V. Le Monza, Italy). The fresh plants of *P. amarus* were collected from the natural habitat in Sagamu, Ogun state, Nigeria. The plant was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan and given a voucher number (FHI-108345). The bacteria isolates (*Pseudomonas aeruginosa and Staphylococcus aureus)*

were obtained from the Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria. The fungal isolates (*Candida albicans and Trichophyton rubrum) were* obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, University of Ibadan.

Plant preparation and extraction

The whole plant of *P. amarus* was cleaned, cut and dried in the shade for two weeks and then in the oven (Model BS 250, GallenKamp Co., UK) at 60 C for four hours. The ^{0} dried plant was powdered using a laboratory mill (Kenwood Ltd, Hertfordshire, UK) and one kilogram was macerated for 72 hours with 4 Liters of absolute ethanol (96 %), and then filtered using a Whatman filter paper (Whatman Plc., Kent, UK). The extract was concentrated in vacuo at 40 C using a rotary evaporator ^{0} (Buchi Model R 210, Switzerland). The remaining solvent in the extract was evaporated in a vacuum dessicator.

Cream formulation

Aqueous cream (BP) used as the base was prepared using established methods.[23] Briefly, emulsifying ointment (30 %$^{w}/_{w}$) was melted on a water bath (I) and chlorocresol (0.1 %$^{w}/_{w}$) was dissolved in the purified water (69.9 %$^{w}/_{w}$) with the aid of gentle heat (II). The solution (II) was added to the melted wax (I) while still hot with continuous stirring until cool (35 ^{o}C). The extract of *P. amarus* was incorporated into the base at concentrations of 5 and 10 %$^{w}/_{w}$ by first mixing the required quantity of extract with the humectants (glycerin or propylene glycol). Stirring continued until the product was formed. The details of the ingredients used for the various *P. amarus* cream formulations are presented in Table 1.

Physicochemical properties of cream formulations

The pH values of the cream formulations were measured using Jenway model 3520 pH meter (Barloworld Scientific Ltd.,UK), while the viscosities were determined at 25±2°C using a rotational viscometer (Brookfield Viscometer® VT 181, Haake, Karlsruhe, Germany) using size 7 spindle at 100 revolutions per minute. The globule size of the internal phase was also measured using an optical microscope (Olympus Light Microscope) fitted with a camera and computer software-Motic MC 1000 (Motic China Group Co.Ltd.,) for image analysis. The samples were prepared by spreading a very thin layer of the cream evenly on a specimen slide, staining with methylene blue and covered with a cover slide. Spreading time was done by placing 0.5 g of each formulation on a previously cleaned slide and covered with another slide. Weight (1 kg) was placed on the covered slides for 5 minutes for even spread and the length of time taken to separate the two slides were recorded.[24] All the measurements were done in triplicate.

***In vitro* antimicrobial assessment of cream formulations**

The formulations were evaluated for antibacterial activity on *Staphylococcus aureus* and *Pseudomonas aeruginosa.* One (1 g) of the cream was weighed and mixed with 1 ml of sterile water using a vortex mixer (Griffin and George Ltd., RAM 2411 London, Britain) until it became homogeneous. Molten agar which has cooled to about 40 ^{0}C was poured into petri dish and about 0.2 ml of 10^{-3} diluted 24-hour old culture of the micro-organisms were added to it, swirled and allowed to set. Wells (6 mm) were bored into the set agar using a sterile cork borer. Sterile spatula was used to fill the holes with the cream formulations and diffusion was allowed to occur for 30 min before incubating at 37 ^{0}C for 24 hours.

The antifungal activity of the cream (1.0 g) against *Candida albicans* and *Trichophyton rubrum* was done using sterilized Saboraud Dextrose Agar (SDA) as the growth media. The SDA (20 ml) was transferred into sterile petri-dish, allowed to set and about 0.2 ml of a 24-hour old culture of the fungal isolates in nutrient broth were streaked unto the agar plates and then the diluted cream were transferred into the wells as previously described. The dishes were incubated at 27 ^{0}C for a minimum of 48 hours and control experiments were set up using- cream base, humectants, sterile water, neomycin-bacitracin cream (ACME Laboratories, Dhaka, Bangladesh) for the bacteria and ketoconazole cream (Drugfield pharmaceuticals, Ota, Nigeria) for the fungi. Inhibition zones for both bacteria and fungi were then measured using first a divider and the reading placed on a ruler to obtain values in millimetres.

Factorial experiments

The quantitative effect of formulation variables on the physicochemical and antimicrobial properties of *P. amarus* cream formulations was studied using a 2^{3} factorial experimental design (18). The relative

quantitative effects of the nature of humectant (N), concentration of humectant (C), and extract concentration (E) on the physicochemical and *in vitro* antimicrobia properties were studied. The basis of the experimental design was that each of the three variables was utilized at a 'high' level (denoted by the subscript H) and a 'low' level (denoted by the subscript L). The number of experiments in the design was 2^3=8. Using the nomenclature above, the various combinations between the variables used in the design were:

$N_LC_LE_L$ $N_LC_HE_H$ $N_LC_LE_H$ $N_LC_HE_L$

$N_HC_HE_H$ $N_HC_LE_L$ $N_HC_LE_H$ $N_HC_HE_L$

Where:

N_L=Nature of humectant (propylene glycol)

N_H=Nature of humectant (glycerin)

C_L= Low Concentration of humectant (1 %$^w/_w$)

C_H=High Concentration of humectant (4 %$^w/_w$)

E_L=Low Extract concentration (5 %$^w/_w$)

E_H= High Extract concentration (10 %$^w/_w$)

In addition, by grouping the results into a number of sets, the effects of the three variables on the physicochemical and antimicrobial properties of the cream formulations were possible. It was also used to determine whether the variables were interacting or acting independent of each other.

The effects of increasing N, from its 'low' level to its 'high' level on the various parameters were found by adding all the results (pH or viscosity or globule size or spreadability) of samples with 'high' level of N and subtracting the sum of the results with 'low' level of N. This is exemplified below:

¼{($N_HC_HE_H$+ $N_HC_LE_L$+ $N_HC_HE_L$ + $N_HC_LE_H$) – ($N_LC_LE_L$ + $N_LC_HE_H$ + $N_LC_LE_H$ + $N_LC_HE_L$)}. The quantitative measure of the effect of N on the values of the relevant parameter is the amount by which the result of this treatment departs from zero. Similar expressions were used for finding the effects of C and E. Furthermore, to determine if there was any interaction between two variables, the results of the physicochemical parameters and antimicrobial activity in which two variables appear together at either 'high' or 'low' levels were summed and the sum of other combinations subtracted from this to obtain the interaction coefficient. For example for N and C we have:

¼{($N_HC_HE_H$+ $N_HC_HE_L$ + $N_LC_LE_H$ + $N_LC_LE_L$) – ($N_HC_LE_H$ + $N_HC_LE_L$ + $N_LC_HE_H$ + $N_LC_HE_L$)}

A result of zero for the interaction coefficient will indicate that there was no interaction between the variables. Likewise, if the result was significantly removed from zero, it is concluded that the two variables were interacting with each other. The extent of removal from zero is a measure of the magnitude of interaction.

Statistical analysis

Statistical analysis to compare the individual and interaction effects of the formulation variables on the physicochemical and antimicrobial properties of the cream was done with the Kruskal-Wallis non-parametric test for multiple comparisons using Graphpad Prism 4 (Graphpad Software Inc., San Diego, CA, USA). P values less than or equal to 0.05 were considered significant.

RESULTS

The results of the physicochemical and antimicrobial properties of *P. amarus* cream are presented in Tables 2 and 3. The values were used to calculate the individual and interaction coefficients and the results are shown in Tables 4 and 5. The values of these coefficients provide a clear indication of the quantitative effect of the formulation variables on the physicochemical and antimicrobial properties of *P. amarus* cream. The ranking of the independent coefficient values for the formulations was N>C>E on viscosity, E>N>C on globule size and C >E >N on spreadability.

The ranking for the antibacterial effect was E >N >C while on the fungal isolates, it ranked E >C >N showing that extract concentration (E) had a greater effect on all organisms than other variables. This effect was also positive presenting an improved antimicrobial property, while the individual effects of N and C on viscosity and globule size were negative showing that the parameters reduced. The changes observed in the physicochemical and antimicrobial properties of the cream due to the individual effect of the variables were significant ($p<0.05$).

The result of the interaction coefficient showed the effect of the variables in combination. N, C and E interacted with one another to alter physicochemical and antimicrobial properties of *P. amarus* cream. The ranking of the interaction effects was N-E>N-C=C-E on viscosity, N-E>N-C>C-E on globule size and N-C>N-E>C-E on spreadability. Furthermore, the interaction

between N and C was positive for the fungal strains (*C. albicans* and *T. rubrum*) and negative for the bacteria (*S. aureus and P. aeruginosa*). Generally, the interaction between N-C and N-E yielded greater results than for CE. The changes produced from the interaction effects of the formulation variables on both physicochemical and antimicrobial properties were significant ($p<0.05$).

Table 1 Details of the ingredients used for *Phyllanthus amarus* cream formulations

Formulation code	Factorial description of formulation	ients Used (‰w/w)				
		Aqueous cream BP	*P. amarus* extract	Glycerin	Propylene glycol	Total
F1	$N_HC_LE_L$	94	5	**1**	**-**	100
F2	$N_HC_LE_H$	89	10	1	-	100
F3	$N_HC_HE_L$	91	5	4	-	100
F4	$N_HC_HE_H$	86	10	4	-	100
F5	$N_LC_LE_L$	94	5	-	1	100
F6	$N_LC_LE_H$	89	10	-	1	100
F7	$N_LC_HE_L$	91	5	-	4	100
F8	$N_LC_HE_H$	86	10	-	4	100

Table 2: The physicochemical properties of *P. amarus* cream formulations for factorial experimental design.

Variables and combination codes	Viscosity (cP)	Globule size (µm)	Spreadability (secs)
$N_LC_LE_L$	770	16.5	12.1
$N_HC_HE_H$	767	10.2	8.9
$N_LC_HE_H$	880	11.7	9.5
$N_LC_LE_H$	1057	7.7	12.0
$N_HC_HE_L$	570	12.5	11.4
$N_HC_LE_H$	600	16.3	10.4
$N_LC_HE_L$	936	68.3	8.6
$N_HC_LE_L$	913	12.6	11.5

Table 3: The antimicrobial properties of *P. amarus* cream formulations for factorial experimental design.

Variables and combination codes	*S. aureus*	*P. aeruginosa*	*C. albicans*	*T. rubrum*
$N_LC_LE_L$	15	15	15	15
$N_HC_HE_H$	23	18	16	16
$N_LC_HE_H$	23	21.5	15.5	14
$N_LC_LE_H$	17	20.5	15	18
$N_HC_HE_L$	22	17	15	13
$N_HC_LE_H$	21	21	15.5	17.5
$N_LC_HE_L$	15.5	19	15	12
$N_HC_LE_L$	19	17	14.5	13.5

Table 4: The individual and interaction effects of nature of humectant (N), concentration of humectant © and extract concentration (E) on the physicochemical parameters of *P. amarus* creams for factorial experimental design

Variables	Viscosity	Globule size	Spreadability
Independent coefficient			
N	-198.34	-13.17	0.02
C	-46.67	-12.42	1.90
E	28.34	-16	-0.69
Interaction coefficient			
N-C	-41.67	-15.53	1.13
C-E	41.67	-13.47	-0.12
N-E	-86.42	16.72	-1.10

p<0.05 for independent and interaction coefficients

Table 5: The individual and interaction effects of nature of humectant (N), concentration of humectant (C) and extract concentration (E) on the antimicrobial property of *P. amarus* creams for factorial experimental design.

Variables	*S. aureus*	*P. aeruginosa*	*C. albicans*	*T. rubrum*
Independent coefficient				
N	12.5	-3	0.5	1.0
C	11.5	2	1.5	-11
E	14.5	13	2.5	12
Interaction coefficient				
N-C	-1.5	-8	0.5	3
C-E	4.5	-6	0.5	-1
N-E	-16.5	-3	1.5	2

The effect of N, C and E and their interaction on the antimicrobial property was found to be significant (p<0.05).

DISCUSSION

The physicochemical and antimicrobial properties of *P. amarus* cream were altered by the formulation variables. The effect of the nature of humectant (N), concentration of humectant (C) and the extract concentration (E) varied on the formulations depending on the parameter being assessed. The nature and concentration of humectant reduced the viscosity and globule size of the cream but increased the susceptibility of *S. aureus, C. albicans and T. rubrum* to the cream. This implies that a shift from propylene glycol to glycerin caused a decrease in these physicochemical parameters but resulted in a boost in the antimicrobial properties. Creams with smaller globule sizes (3-10µm) will penetrate into deep skin layers due to improved surface area compared to larger ones (>10 µm) which remains on the skin surface.[25] The reduced globule size could have been the outcome of improved solubility when the concentrations of humectants were increased hence a greater mixing

efficiency was achieved. Generally, the globule sizes of *P. amarus* cream maintained an acceptable range (≤100µm). In addition, the viscosity of a topical formulation has been reported to affect the delivery of an active agent across the skin with variation in therapeutic performance.[24]

The presence of glycerin enhanced the activity of the cream against *S. aureus, C. albicans and T. rubrum* while it produced a decrease in the susceptibility of *P. aeruginosa*. A possible explanation of this is that glycerin potentiates the effect of the metabolites in the extract to weaken the defences of *S. aureus*, *C. albicans* and *T. rubrum.* For infections caused by *S. aureus, C. albicans and T. rubrum,* the use of glycerin as a humectant for *P. amarus* cream will enhance the antimicrobial activity. High concentrations (>30 %) of glycerin and propylene glycol have been documented to have antimicrobial properties [26, 27] but the studies used much lower values of these humectants. Likewise, a reduced viscosity will assist in the spreadability of the cream to cover larger areas when the cream is applied on the skin hence the increased effect on that parameter.

Furthermore, the effect of extract concentration (E) was positive on viscosity and antimicrobial property implying that increasing the concentration from 5 to 10 $\%^{w}/_{w}$ increased the activity of the formulations on all the organisms. The positive effect on viscosity indicates that as the extract concentration increases, the viscosity of the formulations increased producing a more rigid shape. This could lead to higher adhesion of the cream to the skin membrane during use due to the plastic rheologic behaviour of semi-solid dosage forms thus prolonging the activity of the formulation. The effect of extract concentration increased antimicrobial properties of the cream on all organisms than any other variable because as extract quantity increases, the molecules of the active metabolites in the cream also increased leading to an improved inhibition zone for the microorganisms. It also illustrates that the cream has a concentration-dependent antimicrobial activity and this effect is higher than that of the humectant and its concentrations.

The result of the interaction coefficient specifies the effect of the variables in combination. N, C and E interacted with one another to alter the viscosity, globule size and spreadability of *P. amarus* cream. The interaction between N and C were higher on spreadability, while N and E had higher effects on viscosity and globule size. In all, the formulation variables (N, C and E) were not only acting independently but also interacting to influence the physicochemical parameters. The significant difference ($p < 0.05$), for both individual and interaction variables suggests that changes in the physicochemical parameters and antimicrobial activity produced by the formulation variables cannot be ignored. Generally, $N_LC_HE_H$ will be preferred because it yielded higher activity on *S. aureus*, *P. aeruginosa* and *C. albicans* without compromising the physicochemical parameters. The study limitation was the lack of Franz Diffusion Cell which could have been used to study the permeation properties of the formulations.

CONCLUSION

In the design of *P. amarus* cream, careful consideration of extract concentration, type and concentration of humectant is required to obtain optimum physicochemical and antimicrobial properties.

ACKNOWLEDGMENT

The authors did not receive financial support from any agency or individual for this study. In addition, there were no conflicts of interest attached to the study.

REFERENCES

1. Kumar KK, Sasikanth K, Sabareesh M, Dorababu N (2011). Formulation and evaluation of diacerein cream. *Asian Journal of Pharmaceutical and Clinical Research* 4 (2): 93-98.
2. Chien YW (1982). Transdermal Drug Delivery, in Novel Drug Delivery Systems, Chien Y.W. Ed. (Marcel Dekker, New York); pp. 200–213.
3. Reuter J, Merfort I, Schempp CM (2001). Botanicals: In Dermatology: An Evidence-Based Review. *American Journal of Clindermatology* 11 (4): 247-267.
4. O'Laughlin R, Sachs C, Brittain H, Cohen E, Timmins P and Varia S (1989). Effects of variations in physicochemical properties of glyceryl monostearate on the stability of an oil-in-water cream. *Society for Cosmetic Chemistry* 40: 215 229.
5. Juntawong S, Charoenteeraboon J, Chansiri G, Tuntarawongsa T, Katewongsa P and Phaechamud T (2010). Emulsifying Polymer: Effect of Oil Content, Stirring Intensity & Mixing Temperature.

Research Journal of Pharmaceutical, Biological and Chemical Sciences 1 (2): 360-365.

6. Islam MT, Rodríguez-Hornedo N, Ciotti S, and Ackermann C (2004). Rheological characterization of Topical Carbomer Gels Neutralized to Different pH. *Pharmaceutical Research* 21 (7): 24-29. 7. El-Kattan AF, Asbill CS, Kim N, Michniak BB (2000). Effect of Formulation Variables on the Percutaneous Permeation of Ketoprofen from Gel Formulations. *Journal of Drug Delivery* 7 (3): 147-153.
8. Mani N, Park MO, Jun HW (2005). Effects of formulation variables and characterization of guaifenesin wax microspheres for controlled release. Pharmaceutical Development and Technology 10 (1):71-83.
9. Gupta B, Poudel BK, Pathak S, Tak JW, Lee HH, Jeong JH, Yong CS, Kim JO (2016). Effects of Formulation

Variables on the Particle Size and Drug Encapsulation of Imatinib-Loaded Solid Lipid Nanoparticle**s.** AAPS PharmSciTech 17 (3): 652-62.

10. Box GE, Hunter W. G, Hunter J. S. (2005). Statistics for Experimenters: Design, Innovation, and Discovery (2nd ed.). Wiley. ISBN 0-471-71813-0.
11. Woolfall RC (1964). An approach to product formulation. *Soap, Perfume and Cosmetics* 37: 965-
970.
12. Brown D.J and Dattner A.M (1998). Phytotherapeutic Approaches to Common

Dermatologic Conditions. *Archives of Dermatology* 134: 1401 – 1404.

13. Bedi MK and Shenefelt PD (2002). Herbal therapy in dermatology. *Archives of Dermatology* 138: 232 242.
14. Aremu OI and Femi-Oyewo MN (2008). Antifungal effect of topically administered Neem (*Azadirachta indica*) seed oil cream against *Pytiriasis versicolour*. *Journal of Pharmaceutical and Allied Science* 5 (2): 591-597.
15. Bhaskar G, Arshia S and Priyadarshini S (2009). Formulation and evaluation of topical polyherbal antiacne gels containing *Garcinia mangostana* and *Aloe vera*. *Pharmacognosy Magazine* 5: 93-99.
16. Akhtar N, Waqas MK, Ahmed M, Saeed T, Murtaza G, Rasool A, Aamir MN, Khan SA, Bhatti NS and Ali A (2010). Effect of Cream formulation of *Fenugreek* Seed Extract on some Medicinal Parameters of Human Skin. *Tropical Journal of Pharmaceutical Research* 9 (4):329-337.
17. Kamkaen N, Phuntuwe W, Samee W, Boonrod A and Treesak C (2007). The Investigation of the Rabbit and Human Skin Irritation of Herbal Anti-wrinkle cream. *Thai Pharmaceutical and Health Science* 2 (1):20-25.
18. Iranloye B, Oyeusi K and Alada A (2010). Effect of aqueous extract of *Phyllanthus amarus* leaves on Implantation and Pregnancy in Rats. *Nigerian Journal of Physiological* Science 25: 63 – 66.
19. Oluwafemi F and Debiri F (2008). Antimicrobial Effect of *Phyllanthus amarus* and *Parquetina nigrescens* on *Salmonella typhi*. *African Journal of Biomedical Research* 11: 215-219.
20. Adegoke AA, Iberi PA, Akinpelu DA, Aiyegoro OA and Mboto CI (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products* 3 (3): 6-12.
21. Ajala TO, Igwilo CI, Oreagba IA and Odeku OA (2011). The antiplasmodial effect of the extracts and formulated capsules of Phyllanthus amarus on Plasmodium yoelii infection in mice. Asian Pacific Journal of Tropical Medicine 4 (4): 283-287.
22. Ajala TO, Femi-Oyewo MN, Odeku OA, Aina OO,

Saba AB and Oridupa OO (2016). The Physicochemical, safety and antimicrobial properties of Phyllanthus amarus herbal cream and ointment. Journal of Pharmaceutical Investigation 46 (2): 169-178.

23. BPC (1979). British Pharmaceutical Codex 11th Edition. Wade. L. (ed), The Pharmaceutical Press, London, UK. pp. 55.
24. Kryscio DR, Sathe PM, Lionberger R, Yu L, Bell MA, Jay M, Hilt JZ (2008). Spreadability Measurements to Assess Structural Equivalence (Q3) of Topical Formulations-A Technical Note. *AAPS PharmSciTech*, 9 (1): 84-86.
25. Rolland A (1993). Site-Specific Drug Delivery to

Pilosebaceous Structures using Polymeric Microspheres. *Pharmaceutical Research* 10 (12): 1738–1744.

26. Kinnunen T, Koskela M (1991). Antibacterial and antifungal properties of propylene glycol, hexylene

glycol, and 1, 3-butylene glycol *in vitro*. *Acta Dermatology Venereology* 71(2):148-50.

27. Saegeman VSM, Ectors NL, Lismont D, Verduyckt Bert, Verhaegen J (2008). Short- and long-term bacterial inhibiting effect of high concentrations of glycerol used in the preservation of skin allografts. Burns. 34: 205–211.