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EVALUATION AND OPTIMIZATION OF PHARMACEUTICAL TOPICAL FORMULATIONS FROM THE LEAVE EXTRACTS OF *SENNA ALATA* AND *PSIDIUM GUAJAVA*

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ABSTRACTS

This research work is aimed at the formulation and evaluation of cosmetic/pharmaceutical cream from *senna alata* and *psidum guajava* leave extracts in comparison with ketoconazole and gentamicin. Cream formulations was carried out in batches and designated as: batch A (*S.alata* extract), batch B (*P. guajava* extract), batch C (*S. alata* and *P. guajava* extract (1:1) batch D (*S. alata* and *P. guajava extracts* (3:1), batch E (*S. alata* and *P. guajava* extracts (1:3), then evaluated for some physicochemical parameters such as: pH, viscosity, conductivity, type of emulsion, emolliency and thermal stability. Anti-microbial screening of the formulated creams was investigated on pathogenic micro-organisms to determine the medicinal value and the organisms included: *Escherichia coli, proteus spp, salmonella thyphi, Staphylococcus aureus* and the fungi *Candida albicans and Aspergillous albicas*. The results obtained depicts the extracts to have pharmaceutical and antimicrobial properties within acceptable range of activities with formulated batch E dissipating better activity. The cream formulations were observed to be o/w type and with pH values ranging between 7.13 to 8.34. The viscosity of the various creams formulated also ranged between 523±0.03 to 613±0.02cp while upon their subjection to thermal stability, liquefaction at 60^{O}C occurred within the time line of 46 to 65 seconds while batch E was observed to be the most stable as it took the longer time to liquefy.

KEYWORDS: *Senna alata*, *Psidium guajava*, *Leave extracts*, topical formulation, Evaluation, optimization.

INTRODUCTION

Pharmaceutical formulation, involves the process whereby different chemical substances, including the active drug components, are combined and processed to produce a final medicinal product. Formulation factors that could influence such process, includes: the chemical nature and constituent of the drug (ester, salt, complexation etc.), drug solubility in various medium (aqueous and non-aqueous), particle size and surface area, type of dosage form (solution, suspension, capsule, tablet), the excipients used and processes adopted in the formulation of the drug delivery systems. The design of optimal drug delivery systems is thus an important part of providing an effective therapeutic dosage form.[1]

TYPES OF PHARMACEUTICAL FORMUATIONS

These includes such routes as enteral, parenteral and topical formulations:

Topical formulations: These includes, ointment - combines oil (80%) and water (20%), effective barrier against moisture loss, creams and gel - liquefies upon contact with the skin, paste - combines three agents - oil, water, and powder suspended in an ointment and powder - a finely subdivided solid substance.[2]

Pharmaceutical creams: These are emulsions of oil and water in approximately equal proportions with other constituent such as: emulsifier and thickening agent.

Creams are preparations usually for application to the skin and penetrates the stratum corneum (outer layers of skin) easily although those for application to mucous membranes such as the rectum or vagina are also used. Creams are often considered as pharmaceutical products or as cosmetic creams based on techniques developed in the course of the formulations though medicated creams are highly used in a variety of skin conditions (dermatoses).

Creams are semisolid dosage forms which may contain more than 20% of water or volatile components and less than 50% hydrocarbons, waxes, or polyols as vehicles.[3]

They are divided into two types: oil-in-water (o/w) and water-in-oil (w/o) forms. Oil-in-water creams are more comfortable and cosmetically acceptable as they are less greasy and more easily washed off upon application of aqueous solvent. Water-in-oil creams are more difficult to handle but many drugs which are incorporated into creams are hydrophobic and will be released more readily from a water-in-oil cream than an oil-in-water types. Water-in-oil creams are also more moisturizing as they provide hydrophobic barrier which reduces water loss from the stratum corneum.[4]

The ideal properties of cream include: high affectivity, rapid onset of action, biocompatible and bio-miscible, free from grittiness, smooth appearance and texture, readily washable, non-allergic, non-toxic, physically and chemically stable. Others include: provision of barrier for skin protection, aid in the retention of moisture (especially water-in-oil creams)**,** cleansing to remove dead skin cells, oil, dirt from the skin**,** helpful in sun burns, have emollient effects**,** act as vehicle for drug substances such as local anesthetics, anti-inflammatories (NSAIDs or corticosteroids), antibiotics, antifungals or as counter-irritants.[5]

CREAM FORMULATION

In the formulation of creams, there are two phases which include the oil and the aqueous phase. The ingredients which are miscible or soluble in one phase are mixed together and heated with continuous stirring. While maintaining same temperature, both phases are then gradually mixed together to form a viscous entity which on gradual cooling solidifies to give the cream. Important factor to be maintained are: the two phases should be at same temperatures and addition should be continuous and controlled without splashing or vortexing, in order to avoid entrapment of air. Subsequent cooling should be slow with stirring which should be adequate to ensure homogeneity, yet minimize aeration because sudden cooling or excessive aeration can lead to granular product and after cooling to 30˚C -40˚C, the cream can usually be homogenized.[6]

RHEOLOGY AND STABILITY OF CREAM

As emulsified creams are usually non-Newtonian systems, their rheological properties vary with the shear forces applied. Thus, viscosity and flow characteristics may change in accordance to the degree to which the system are homogenized or with the amount of shear applied during processing either in mechanical devices or by a spatula. Oil-in-water creams may have a visco-elastic gel network, the rigidity of which can be increased by the inclusion of higher concentrations of mixed emulsifying agents such as cetrimide and cetostearyl alcohol. In most emulsified systems, the overall viscosity may be increased by: increasing the viscosity of the continuous phase, increasing the content of a single emulsifying agent, or reducing the globular size by homogenization.

Major factors which affects the stability of emulsified creams are temperature, cohesive and gravitational forces, physical and surface properties of the oily constituents, and the relative densities, viscosities and concentration of the two phases. In formulating creams, the chemical, physical and surface properties of the active substance must be carefully considered, for instance anionic substances are incompatible with cationic emulsifying gents, hydrolysis of an active substance may be enhanced if an aqueous base is used instead of an anhydrous base while electrolytes can react with emulsifying agents or may induce gel-formation.[7]

CREAM INSTABILITY: Instability of creams occurs when dispersed phase droplets of the emulsion collide and coalesce, thus producing corresponding larger emulsion droplets, the presence of which begins to destabilize the system. These larger droplets may in turn collide and coalesce and eventually compete causing cream destabilization, accompanied by phase separation. The instability of pharmaceutical creams may be classified as: Creaming and sedimentation (creaming could be more relevant to oil in water system, whilst sedimentation is a property confined to water in oil emulsion), coalescence and breaking, miscellaneous physical and chemical changes then phase inversion.[8] But whereas creaming is the reversible process, breaking is irreversible.

Certain other factors that could lead to breaking includes: If the spheres are arranged in closest packing, theoretically they cannot exceed 74% of the total volume regardless of the size. Ostwald and others showed that an attempt to incorporate more than 74% of the oil in oil in water cream, the oil globules often coalesce and the cream breaks.

The phase volume ratio of an emulsion has a secondary influence on the stability of the product and this term refers to the relative volume of water and oil in the cream.

Phase inversion: This gets out of hand during manufacturing or is brought about by other factors after the cream is formed, it can cause considerable troubles. An emulsion stabilized with sodium salts can be inverted to water in oil cream type by adding calcium chloride to form calcium salt. Inversion can be produced by alterations in phase volume ratio or presence of electrolytes.[9]

SKIN CARE

Skin care is the range of practices that support skin integrity, enhance its appearance and relieve skin conditions. They can include nutrition, avoidance of excessive sun exposure and appropriate use of emollients. Practices that enhance appearance include the use of cosmetics, botulinum, exfoliation, fillers, laser resurfacing, microdermabrasion, peels, retinol therapy and ultrasonic skin treatment. Skin care is a routine daily

procedure in many settings, such as skin that is either too dry or too moist, and prevention of dermatitis and skin injuries.

SKIN INFECTIONS

Skin is the body's largest organ which has many different functions, including covering and protecting the body. It helps keep germs away because sometimes the germs could cause skin infection especially when there is a break, cut, or wound on the skin. This may occur especially when one's immune system is weakened, due to opportunistic infection or as a result of medical treatment. Some skin infections could cover a small area of the skin but some others may go deep into the skin or spread to larger areas. Some symptoms common to many skin infections include rashes, swelling, redness, pain, pus and itching.[10]

ANTIBACTERIAL AGENT

Antibacterial agents are used to treat bacterial infections. Antibiotics are classified generally as beta-lactams, macrolides, quinolones, tetracycline or aminoglycosides. Their classification within these categories depends on their antimicrobial spectra, pharmacodynamics, and chemical composition. Prolonged use of certain antibacterial agents can decrease the number of enteric bacteria, which may have a negative impact on the health of the individual.

Antibiotics are among the most commonly used drugs and among the drugs commonly misused by physicians and patients. As a consequence of widespread and non-judicious use of antibiotics, there has been an increase emergence of antibiotic-resistant pathogens, resulting in a serious threat to global public health. This problem demands that a renewed effort be made to check antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Possible strategies towards this objective include increased sampling from diverse environments, application of metagenomics to identify bioactive compounds and development of natural compounds to combat the menace of micro-organism proliferation in the environment.[11]

ANTIFUNGAL AGENTS

Antifungal agents are also termed as anti-bacterial agents which are used to kill or prevent further growth of fungi and in medicine, are used as treatment for infections such as athlete's foot, ringworm and thrush and they work by exploiting the differences between mammalian and fungal cells. Unlike bacteria, both fungi and humans are eukaryotes thus, fungal and human cells are similar at the molecular level and this makes it more difficult to find a target for an antifungal drug to attack that which does not exist in the host organism.[12]

Senna alata

Figure 1: *Senna alata* leaves.

Medicinal uses: *Senna alata* (also known as *Cassia alata*) is often called the ringworm bush tree because of its very effective fungicidal properties, for treating ringworm and other fungal infections of the skin.

Figure 2: *P. guajava* leaves.

Traditionally, preparations of the leaves of *P. guajava* have been used in folk medicine in several countries, mainly as anti-diarrheal remedy. The decoction is used as gargle for mouth ulcers and as anti-bactericidal in Nigeria. For skin and wound applications, the poultice is externally used in Mexico, Brazil, Philippines, and Nigeria.[13]

This study is aimed at, the assay of the leaf extracts of *S.alata* and *P.guajava* as antibacterial agents against certain fungal and bacterial isolates, pharmaceutical formulation of cream using the extracts singly and in combination and evaluation of the creams formulated.

Materials

Senna alata and *Psidum Guajava leave extracts* (University of Port Harcourt), glycerine (E, Merck, Darustadt), bacterial isolates of *E.coli*, *P. mirabilis*, *S. typhi* and, *S. aureus* and the fungal isolates of *Aspergillus niger* and *Candida albicans* ((Divic Medical laboratories, Port Harcourt). light microscope, pH meter (Helmreasinn, PHS-25), Homogenizer (Binatone), Brookfield viscometer (DV2T), autoclave, incubator, petri dishes (disposable), universal bottles, MacCartney bottles.

Formulation of Cream

Preparation of Emulsifying Ointment

The emulsifying ointment was prepared with reference to official standards from United States Pharmacopoeia.

Oil in water creams of 100g target weight were formulated with the given formula:

Table 1: Formula for cream formulation.

Oil phase **Ingredient**	**Percentage composition (%w/w)**
Glycerol	0.12
Emulsifying ointment	0.26
Stearic acid	0.1
Aqueous phase	
Potassium Hydroxide	0.1
Plant extract	0.2
Water	0.2
Fragrance	0.02
Colour	0.01
Viscosity enhancer	0.03

The oil phase was prepared by mixing the ingredients: Glycerol (0.12%), emulsifying ointment (0.26%) and stearic acid (0.10%) in a vessel. This mixture was melted at a temperature of 70˚C.

The aqueous phase was prepared by incorporating the ingredients in a vessel with aqueous medium. These included water (0.2%), viscosity enhancer (0.03%), color (0.006%). The content in the vessel was heated in a water bath and stirred continuously and at a temperature maintained at 70^{O}C and same as the oil phase.

While stirring on the water bath, the oil phase was gradually added to the vessel containing the aqueous phase until a homogenous (uniform) formulation was obtained. The formed cream was removed from the heat source and submerged in a bowl of cool water while being rotated then left to cool to about 30^{O}C before addition of the plant extract (0.20% w/w) and fragrance (0.015% w/w). The mixture was then homogenized and allowed to cool.

Cream Evaluation

Organoleptic

The appearance of the cream was observed for its color, pearlscence, texture and graded.

Determination of pH of the Cream

2.0%w/w of the sample was made into solution with 10ml of water in a beaker and the pH electrode inserted into the beaker. The tests were conducted in triplicates and the readings recorded when it is stable.

Determination of Viscosity of the Cream

10g of the sample (cream) to be evaluated was transferred into a clean 250ml beaker containing 50ml of water. A temperature probe was attached to the spindle guard, lowered and inserted into the beaker until the spindle is fully immersed in the sample. The procedure was repeated at different temperatures for 30 seconds. The displayed viscosity was recorded in Centipoise (Cp), and displayed on the viscosity data sheet.

Determination of Homogeneity of the Cream

5.0grams each of the formulations were tested for homogeneity by visual appearance and by touch to know how smooth and non-gritty they were. This was done at random and in triplicates.

Determination of Wetness of the Cream

This was determined by applying 10gram of the cream on the skin surface of 14 human volunteers and making observations on whether it moisturizes the skin or not.

Determination of Type of Smear

This was determined by applying 10 gram of the cream on the skin surface of 14 human volunteers, after which, the type of smear formed on the skin was checked by using water to wash off the cream.[14]

Determination of Emolliency of the Cream

Emolliency, slipperiness and amount of residue left was determined after the application of 5.0grams of the cream on the dry skin of 14 human volunteers and the extent to which it lubricated or softened the skin was observed and recorded.[15]

Determination of Type of Emulsion

Dilution test: In this test, 4.0%w/w of the emulsion (cream) was dispersed in 10ml of water and equal volume of oil in a separate beaker. The emulsion type was determined by observing for uniform dispersion and stability or breaking in the different media.[16]

Conductivity Test of the Cream

The electrode of the probe-conductometer was inserted into a beaker containing a solution of 2.0%w/w of the sample. The test was conducted in triplicates at room temperature and the readings recorded when it is stable.

Centrifugation Test of the Cream

Centrifugation test was carried out for the different batches of creams after preparation. About 5.0g of the samples were separately placed in the 10ml centrifugal tube and spanned at 3000rpm for 10 minutes and at room temperature, then the extent of separation observed and recorded.

Determination of Refractive Index

The refractive index was determined using refractometer having refractive prism with water temperature adjusted to 1.0^{O}C.

Thermal Stability Test of the Cream

The cream samples were subjected to room temperature (27^{o}C) and 60˚C so as to determine the appropriate temperature for storage and the form which the cream samples will take or appear when exposed to varying temperature and environmental conditions.

Spreadability Test

Spreadability of the formulations was determined by measuring the spreading diameter of 1.0 g of sample

placed between two horizontal glass slides (10 x 20cm) after 10 seconds. The standard weight applied to the upper plate was 50g. The distance in which the upper glass slide moves over the plate in 10secs was noted. Each formulation was tested and in triplicates.[17]

The Spreadability (S) was calculated using the formula

$$S = M \times \frac{L}{T}$$

S = Spreadability, L = Length moved on the lower glass slide, T = Time (mins), M = weight applied to upper slide.

Determination of Antimicrobial properties

Antimicrobial activity of the resultant creams was evaluated by the agar disk diffusion method.

The creams of the various batches, including ketoconazole and gentamicin were screened for their antibacterial and antifungal activities against the bacteria isolates such as: *Escherichia coli, proteus spp, salmonella typhi, Staphylococcus aureus* and the fungi as: *Candida albicans and Aspergillous albicas*. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains and allowed to stay at 37°C for 3 hours. The zones of growth inhibition around the disks were measured after 18 to 24 hours of incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivities of the microorganism species to the creams were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, values <8 mm were considered as not active against microorganisms.

RESULTS

THE FORMULATED CREAMS

Plate 1: Cream of Senna alata extract

Plate 2: Cream of Psidum guajava extract

Plate 3: Cream of Senna alata extract and Psidum guajava extract (1:1)

Plate 4: Cream of Senna alata extract and Psidum guajava extract (3:1)

Plate 5: Cream of *Senna alata* and *Psidum guajava* extracts (1:3).

Table 2: Physical Characterization of the Formulated Cream.

Physical Parameter		Observation			
	Cream A	**Cream B**	**Cream C**	**Cream D**	**Cream E**
Appearance	Brown	Light brown	brown	Dark brown	Brown

pH	7.86±0.08	7.13±0.04	8.34±0.11	7.32±0.05	7.87±0.12
Homogeneity (By touch and Visual)	Homogenous, smooth and consistent	Homogenous Smooth and Consistent	Homogenous Smooth and consistent	Homogenous Smooth and consistent	Homogenous Smooth and consistent
Viscosity	615±0.02 at 29.0˚C	568±0.04 at 30.6˚C	523±0.03 at 28.3˚C	575±0.03 at 30.3˚C	536±0.03 at 29.3˚C
Dilution Test	O/W type of emulsion	O/W type of emulsion	O/W type of emulsion	O/W type of emulsion	O/W type of emulsion
Robustness (spreadability and wetness)	Easily spreadable and Moisturizes skin surface	Easily spreadable and Moisturizes skin surface	Easily spreadable and Moisturizes skin surface	Easily spreadable and Moisturizes skin surface	Easily spreadable and Moisturizes skin surface
Type of Smear	Non-greasy	Non-greasy	Non-greasy	Non-greasy	Non-greasy
Emolliency	No residue left	No residue left	No residue left	No residue left	No residue left
Conductivity m/s	643.67±4.04 at 29.2˚C	520.47±0.48 at 28.5˚C	754±4.00 at 29.9˚C	796±3.00 at 29.1˚C	764±1.00 at 28.9˚C

Table 3: Centrifugation Test for the Creams.

Sample	**Cream A**	**Cream B**	**Cream C**	**Cream D**	**Cream E**
Observation	No phase separation	No phase separation	No phase separation	No phase separation	No phase separation

Table 4: Thermal Test for Creams.

Temperature	**Cream A**	**Cream B**	**Cream C**	**Cream D**	**Cream E**
Room Temperature ($29.38^{O}C$)	stable	Partially stable	stable	stable	stable
$60^{O}C$	Liquefied within 46 sec	Liquefied within 38 sec	Liquefied within 62 sec	Liquefied within 57 sec	Liquefied within 65 sec

Cream A = Formulated Cream of *S.alata*, Cream B = Formulated Cream of *P. guajava*, Cream C = Formulated Cream of *S. alata* and *P. guajava* (1:1) Cream D = Formulated Cream of *S. alata* and *P. guajava* (3:1), Cream E = Formulated Cream of *S. alata* and *P. guajava* (1:3).

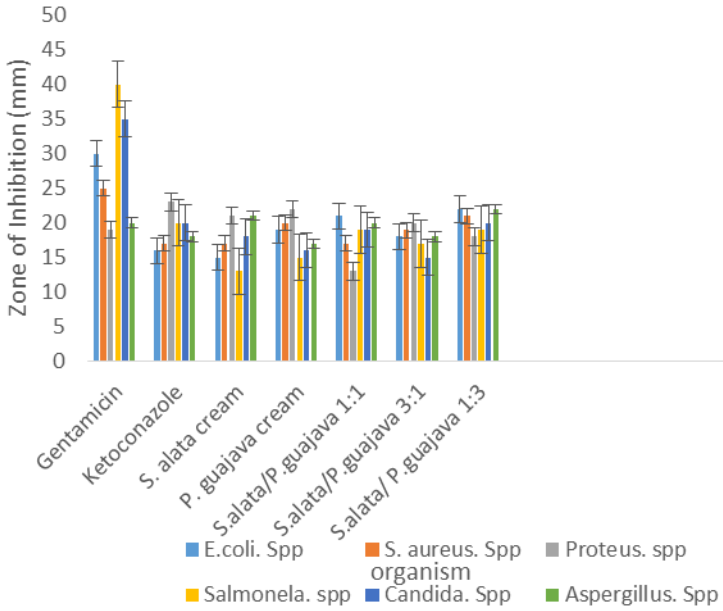

Figure 6: Microbial analysis.

DISCUSSION

Observation of results from the various formulations showed that the creams had characteristic odor relative to the plant source when subjected to olfactory examination and they all had smooth texture when felt between the thumb and index finger end while the homogeneity evaluated by visual inspection were ranked to be excellent. There were some similarities and differences

amongst the batches in relation to the physical characteristics of formulated creams. Such similarities aside from homogeneity, includes, the emulsion type as identified using the dilution test, robustness, type of smear and emolliency as seen in the table 2 and by appearance, all the creams were cosmetically appealing.

pH: The cream formulations had pH values that range between 7.13 to 8.34 which though close to neutrality appears to be slightly alkaline. This indicates that the creams can be applied on the skin without having any harmful or irritating effect more so, since from previous study as observed in results of the proximate analysis of the extracts, there was no presence of deleterious materials such as lead or any related substance.[18] Still in relation to the pH, though the values obtained from the creams are slightly higher than the pH of the of the skin surface assumed to be within the range of 4.5 to 6.0, the creams could still assist to ward off the advances of harmful bacteria and fungi especially those that cannot thrive under such pH condition.

Spreadability: The values of the spread ability test indicated that the formulated creams were easily spreadable even with a little amount of shear and also easily moisturizes skin surfaces indicating good wetting property but without any occlusive effect.

Stability: The study reveals that the creams were stable at temperature conditions of 29-38˚C and hence this should be the acceptable storage condition for the formulated creams because at higher temperature liquefaction were observed. The stability study indicated that the creams were able to maintain their integrity for 12 weeks without showing signs of instability or separation and this was well observed when subjected to centrifugation at 3000rpm for 30minutes indicating good homogeneity and ranking the formulated creams as very stable. Additionally, the main function, that is, antibacterial activity of all the formulations, remained stable for the tested time period at the study condition, which is promising.

Ease of removal: The creams were easily washed off from the skin with tap water hence ranked as excellent in terms of ease of removal and this is possible since the continuous phase are composed of water.

Emolliency: The formulated creams from the dilution test were identified as oil-in-water types hence are observed to be non-occlusive because they do not leave a continuous film of water-impervious liquids and are termed to be non- greasy. Emollient creams can only result if they deposit lipids and other moisturizers on the stratum corneum, thereby preventing the skin from drying out and by restoring its hydration ability.[19]

Viscosity: The viscosity of the various formulations of the creams was within 523-613 Cp which is an indication of moderate thickness, good quality of cream with acceptable flow property since the thickness (viscosity) of the formation also is a major determinant in the rheological property of the product. All batches of the creams had a pseudo plastic behavior, as expected.

Thermal testing: This is an important part of qualification and characterization study for virtually all heat-sensitive products and materials. Whether preparing components for thermal extremes or developing heat-resistant materials. This test makes certain that products can function in any thermal environment but the formulated creams when subjected to thermal test, cream B even at room temperature (29-36^{O}C) had partial liquefaction while at 60^{O}C cream E was observed to maintain stability liquefying only after 65 seconds followed by Cream C at 62 seconds, cream D at 57 seconds, Cream A at 46 seconds then Cream B at 38 seconds. This observation could be as a result that the creams B have loosely packed structures which are not solid and stable enough hence could easily lose strength and bonding structure in response to slight temperature or heat elevation. The result of the order of liquefaction time for the batches is as shown in Table 4.

Antimicrobial susceptibility of the formulated creams: As shown in Figure 6, all the creams formulated inhibited the growth of all the test organisms. The extent of inhibition was comparable to that of the respective extracts as depicted in the previous study.[13] The similarity between the zone of inhibition produced by the extracts and that of the cream formulations showed that the antimicrobial activity of the extracts was not reduced when it was incorporated into the cream formulations. The excipients used in the cream formulation did not affect the extracts antimicrobial activity hence no physical or chemical interaction existed between the components in the formulation. It was also observed that the zone of inhibition produced by the control (gentamicin) and (ketoconazole) was slightly higher than that produced by the cream formulations for all the test organisms. However, increasing the concentration of the extract in the cream may result in creams that are equipotent to the control and especially refered to this, is that resulting from batch E, and consisting of leave extracts of *S.alata* + *P.guajava* (1:3).

CONCLUSION

A pharmaceutical cream having close to neutral pH, with appreciable viscosity, substantial antimicrobial activity and predictable stability can successfully be formulated from leave extracts of *S. alata* and *P. guajava*. Studies from the various formulations made reveals that batch E consisting of *S. alata* and *P. guajava* extracts (1:3), was the most effective and stable cream. Therefore, cream formulation consisting of such plant extracts and the probable ratio combination could be adopted and elaborated upon for effective topical formulations activity and performance. This could help to reduce the focus on consistent use of unpredictable synthetic materials as excipients for skin application.

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