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Evaluation of Python Fat-Based Topical Formulation for Enhanced Furuncles (Boils) Management

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ABSTRACT

Boils are common skin infections that can be painful and unsightly and contemporary treatments for it involves, use of oral and topical antibiotics, incision and drainage. In this study, Python fat-based topical formulation was made and evaluated for its efficacy, stability and general properties in the treatment and management of boils for a period of time. The study dwelt on formulation of four batches of creams comprising of, the control (C1) without raw material (python fat), the standard containing Gentamicin only (C2), (C3) formulated product with python fat and C4 (the raw python fat alone). Randomized study was conducted with involvement of twelve wistar rats where three rats were apportioned to each batch. Induction of furuncles was carried out after shaving a section of the skin around the thigh using a shaving cream then with aid of syringe, 0.1ml of isolated ***S. aureus*** isolate was injected through a hair pore. The animals were left for 4days to allow for prominent growth of boils and the level of indurations measured. Upon successful induction of boils, animals in various groups were treated accordingly using the cream from each batch every 12 hours for 9 days and then monitored for reduction in the diameter of the growth. The formulated products reduced the diameter of the boil faster and the median time for complete healing was shorter with results almost same in group treated with batches of C2, C3, and C4 than in C1. The formulated cream was found to be washable, non irritant, light brown in color, pleasing odor with srpeadability of 6.7gm.cm/sec. Stability studies for a three weeks duration under varied storage conditions (5 and 25°C) show the product stored at 5°C to maintain a mean pH of 5.42 and viscosity of 5811cp while that at 25^{O}C, the mean pH was 5.37 with viscosity of 5716cp. The python fat-based topical formulation is a safe and effective treatment for boils however; its stability at room temperature is still an issue of concern

Keywords*:* Python fat, Boils, Topical formulation, Stability

BACK GROUND OF STUDY

For ages, nature has whispered secrets of healing to mankind and these has evolved into remedies which have been passed down through generations. From the willow's bark containing salicin, the precursor of aspirin used for soothing fevers to ginseng, a precious herb in traditional Chinese medicine, used for its purported benefits in boosting energy, improving cognitive function, and promoting overall health (Sambi et al; 2023) (Singh et al; 2013). Nature has continually blessed man with secrets for treatments. Amongst these also lies the python fat, a potent ingredient in West African folk traditions, long rumored to possess potent healing properties. This study embarks on a study to clarify the potential of this traditional treatment, focusing specifically on its ability to alleviate the painful plight of boils through a novel cream formulation.

Boils also termed as furuncles, are common skin infections distinguished by the painful inflammation of hair follicles or sweat glands and surrounding tissues. They are reddish swellings containing pus and can

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occur anywhere on the body. However, the face, neck, armpits, and buttocks are the most common sites. Boils are a prevailing skin condition, which affects individuals of all ages and demo graphs but most times are usually waded off as unserious but they can be very painful and progress to something more serious if left untreated . Various researches suggest an annual boil incidence of around 2% in the general population, with much more higher rates seen in specific groups like adolescents, individuals with diabetes, and those with immune suppression (Collier et al., 2019).

The discomfort and pain accompanied with boils can notably impact on individual's daily activities and quality of life. Furthermore, inappropriate or delayed treatment can lead to complications like abscess formation, cellulitis, and even septicemia (Cunha et al., 2015), which might require more aggressive interventions, including hospitalization and intravenous antibiotics, further escalating the economic burden of boil management..

Current boil treatment employs several methods, such as warm compresses, topical and oral antibiotics, and, in severe cases, surgical drainage (Andrews et al 1926). Though these methods provide relief, they have limitations and draw backs which may include:

Inappropriate use of antibiotics contributing to the development of antibiotic-resistant bacterial strains, posing a significant public health threat (Spellberg and Gilbert, 2014).

Invasive process of surgical drainage which might have the risk of scarring, pain, and potential for secondary infection

Lack of targeted delivery of semisolid formulations where medication may not reach the intended site of infection effectively and in addition, could be messy on application, especially on hairy areas, and may irritate surrounding healthy skin.

These limitations, therefore requires investigation and study to search for alternative remedies.

Python fat

Fig 1: Python fat Sacs

This is a semi solid fat gotten from various species of python example, Python sebae, Python molurus, Python tigris, etc (Ugwudike et al., 2013). The fat has always been used in folk medicine as a bio resource with interesting antimicrobial, anti-inflammatory, and wound healing properties. Studies have shown its efficacy against a broad spectrum of bacteria, including Staphylococcus aureus, the primary culprit behind boil infections (Okokon et al., 2015). Furthermore, python fat exhibits anti-inflammatory activity, which can

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help reduce pain and swelling associated with boils (Ogundipeet al., 2012). Also, it is used for the treatment of keloids, rheumatism, psoriasis, wound healing etc.(Mishra et al., 2020).Research suggests that it stimulates fibroblast proliferation and collagen synthesis, and these two are crucial processes in tissue repair and regeneration (Latti et al., 2018) and these characteristics suggest that python fat-based formulations could potentially accelerate boil healing and minimize scarring.

This research focuses on investigating the fascinating potential of python fat-based cream as a novel remedy for enhanced boil healing and by leveraging the unique properties of python fat, this studies offers a promising avenue for a more effective, targeted, and potentially safer alternative of boil management.

AIM OF THE STUDY

This research was motivated by the issue of increasing microbial resistance and insufficient literature on the use of **python fat** for the treatment of boils hence aims to formulate a cream and provide an evidence-based insight (animal study) into the effectiveness and safety of python fat use in treatment of boils.

SIGNIFICANCE/OBJECTIVE OF THE STUDY

This study could;

Provide a concise review and lay a foundation for further research on the effectiveness and safety of python fat in treatment of boils.

Prove python fat as a viable, evidence-based ingredient for topical creams, offering a natural alternative or complement to conventional treatments.

Lead to the development of novel, natural, and effective cream formulations for boils and abscesses benefitting numerous individuals seeking gentle and effective topical solutions.

Introduction

The use of python fat for boils has been documented in ethno medicinal practices, particularly in Africa, however the scientific evidence supporting its efficacy is limited and inconclusive. Python fat is a semi-solid fat (at room temperature) with a distinct characteristic odour and yellow to off white color. It is obtained from the abdominal region or skin of pythons. It is used for the treatment of boils, keloids, rheumatism, psoriasis etc.(Mishra et al., 2020).It contains oleic acid (49.47%), palmitic acid (28.51%), stearic acid (9.38%), linoleic acid (5.85%), palmit oleic acid (3.80%), myristic acid (1.30%), gadoleic acid (0.72%), a-linolenic acid (0.36%), arachidonic acid (0.25%), γ -linolenic acid (0.19%), and valcenic acid (0.15%)(Quyen & Quoc, 2023). It contains also phospholipids, Vitamins A, D and E with heavy presence of saponins, flavonoids, steroids and terpenoids, moderate presence of phenols and little presence of alkaloids, tannin and cardiac glycosides (Offurum et al., 2019).

Boils are hard painful swellings or inflammation on a localized part of the skin, filled with pus and are usually caused by bacterial infection of the hair follicle or sweat gland. Many causes have been attributed to boil formation but bacterial infection is the most prominent with *S.aureus* which has accounted for up to 90% of cases being the most implicated organism (James et al.,2019)(Wilson et al., 2021). *S.aureus* is a normal micro flora of the skin which causes no deleterious effect provided all things are normal. However, in the case of boils, the organism living on the epidermis of the skin is introduced into the skin through cuts, abrasions, injection needles e.t.c. Other causes of boils may include; coagulase negative Staphylococci, Streptococcus. pyogenes, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus spp and Escherichia coli (Al-Shuaib et al., 2022). Also, underlying medical conditions such as diabetes predisposes one to

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reoccurrence of boils (Shallcross et al., 2015). This is because, such conditions impairs the body's ability to ward off infections thereby making individual more prone to it.

Often times, boil is used interchangeably with abscess though they may have similar symptoms of swelling, pain, redness and pus filled. It is worthy to note that they are different in formation size and their location and treatment pattern differs as boils are shallower and smaller in size while abscesses are much larger and deeper. Also boils mainly occurs around a hair follicle or sweat gland whereas, abscess occurs anywhere including internal organs (skin abscess, brain, dental region etc.).

The treatment option for each differs, with abscess needing invasive methods such as surgery(depending on severity), drainage and strict use of antibiotics, boils might need warm compresses, drainage and little or no use of antibiotics and in some cases, are self-healing.

Over the years, man has continually sorted for methods and therapeutic procedures for the management of boils and this search is necessitated by the need to deliver the most efficient, safe and cost-effective treatments. However, emergence of new microbes and resistant to existing antibiotic treatments has become the main drive towards this study.

MATERIALS AND METHOD

MATERIAL: Python fat, Nutrient Agar, isolated, Wistar rats, *S.aureus* (Department of Biology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education Iwofe, Rivers State.

CHEMICALS: Ethanol, potassium hydroxide, phenolphthalein, ethanolic potassium hydroxide, hydrochloric acid, Dimethylsulfoxide (DMSO), shaving cream, Cruset dye, Gentamicin,

EQUIPMENT AMD APPARATUS: Brookfield viscometer, pH meter,25g weight bar, Wire mesh cages(locally sourced),beakers(Pyrex, England),water bath (Techmel & Techmel,U.S.A), Electronic hand mixer(Bosch), Cork borer, incubator, Syringes (Agray Nigeria), thermometer, sterile bottles.

METHODS

The sample material was sourced from a traditional medicine home at Choba, Obio-Akpor LGA, Rivers State.

IDENTIFICATION OF SAMPLE MATERIAL (PYTHON FAT)

Determination of the saponification value, Acid value, viscosity and organoleptic properties of the sample was performed using standard procedures described by the International Organization of Standardization (IOS) and methods of Moussounga et al., 2018

SAPONIFICATION VALUE DETERMINATION:

Five gram (5 g) of sample was saponified by boiling gently with excess ethanolic potassium hydroxide solution(0.5 M, 25 mL) for 85 minutes . Afterwards, 1ml of phenolphthalein was added to the resultant hot solution and titrated with hydrochloric acid solution (HCL) until the pink colour of the indicator disappeared.

Furthermore, a blank test was carried out following the procedures as mentioned above.

The Saponification value (SV) of the text sample (Python fat) was determined using the formula;

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SV = (V0-V1) × C × 56,100/M

Where;

V_0 = Volume of the standard volumetric hydrochloric acid solution used for the blank test;

V1 = Volume of the standard volumetric hydrochloric acid solution used for the determination;

C = The exact concentration, in moles per litre, of the standard volumetric hydrochloric acid solution,

M = Mass, in grams, of the test portion

DETERMINATION OF ACID VALUE

This was determined using the titrimetric method. The sample (10 g) was dissolved in 100 mL of alcohol/toluene (ratio of 1/1, v/v) by boiling gently. Then,2 drops of phenolphthalein was added to the solution and titrated with potassium hydroxide (KOH) solution while swirling gently, to neutralize the free fatty acids in fat. The endpoint was marked by a slight but definite color change, which persisted for about 15secs.following the addition of a drop of the alkali.

Acid value (AV) was calculated using the formula;

AV = 56.1 × cV/M

where ;

c = the exact concentration, in moles per litre, of the standard volumetric sodium or potassium hydroxide solution used;

V = Volume, in millilitres, of standard volumetric sodium or potassium hydroxide solution used;

M = mass, in grams, of the test portion.

DETERMINATION OF VISCOSITY.

The Python fat was gently melted in a beaker using a water bath and the Viscosity was determined using the **Brookfield viscometer** set at a temperature of 25°C and 100rpm.(Moussounga et al., 2018). This determination was also applied to the formulated cream.

ORGANOLEPTIC TESTS

VISUAL ANALYSIS: The formulated creams and raw material were, visually observed for color.

SENSORY EVALUATION: The Samples were gently warmed and assessed for aroma, taking note of any rancidity, nutty, grassy, or other characteristic odors. Texture was evaluated by pressing between fingers, gauging for firmness and elasticity.

ANTI-MICROBIAL TESTS

Tests for antibacterial activity and minimum inhibitory Concentration (MIC) was performed following the

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procedures described by Awestruck Research consult, office B4 Lagfog plaza ,East-West Road, Port-Harcourt, Nigeria.

DETERMINATIONS OF ANTI-MICROBIAL ACTIVITY USING THE INHIBITION ZONE TEST.

0.1ml of. Isolated *S.*aureus culture was introduced into 15ml of sterile molten Nutrient Agar, gently swirled to mix and transferred into a sterile Petri dish to solidify. After solidification of the agar, a sterile Cork borer was used to bore a whole in the center of the plate. Then few drops of the sample (Python fat) was added to the hole. This procedure was repeated for comparison with standard (Gentamicin) and in triplicates. All plates were incubated at 37°C for 24hours.

DETERMINATION OF THE MIC.

A Two-fold serial dilution of the sample was made using Dimethylsulfoxide as the diluting solvent. Afterwards, using a sterile syringe,0.1ml of isolated *S. aureus* solution introduced into each bottle of molten Nutrient Agar(5 bottles),swirled gently to mix and poured into sterile Petri dishes to solidify. After solidification, a sterile Cork borer was used to make holes in the plates. Two to three drops of each dilution was introduced into the corresponding plates. The plates were incubated at 37°C for 24hours.

FORMULATION OF TOPICAL PRODUCT.

Table 1: Formulae for Cream formulation

INGREDIENT	QUANTITY (%w/w)
Python Fat	40
Emulsifying wax	15
Liquid Parraffin	5
Tween 80	5
Ascorbic Acid	2
Vitamin E	2
Fragrance	4
Aqua to	100

Preparation of Cream

This involved two phase preparation involving – aqueous and non aqueous phases.

The aqueous phase involved, (Tween 80, Ascorbic acid + Water) and the oil phase (Python fat, emulsifying wax and Liquid parraffin) were heated to 80°C in separate vessels. Upon attainment of same temperature for both phases, the mixtures were homogenized using an electronic hand mixer by gradually addition of the aqueous phase to the oil phase. At about 31°C, Vit E and the frangrance were added, while mixing continued until a stable cooled cream was formed. The cream was labeled and properly packaged in the appropriate container.

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CREAM EVALUATION.

The cream was evaluated for general properties and stability at different temperatures for a period of three weeks.

EVALUATION OF GENERAL PROPERTIES.

Organoleptic tests were performed for texture , odour and color using sensory cells.

Washability: A pea amount of the formulation was applied on the back of the palm. The hand surface was placed under running water and the rate and time at which it was washed off was noted.

Irritancy: A pea sized amount of the formulation was applied on the arm and allowed for about 5minutes then observations were made for reactions such as rash, redness e.t.c.

Spreadibility: One gram(1g) of formulation was placed between two horizontal glass slides and a standard weight of 25g was applied to the upper plate for one minute. After which spreadibility was calculated using the formulae

$$\text{Spreadibility (S)} = \frac{M \times L}{T}$$

Where; M= Standard mass applied L = Distance moved by the slides T= Time allowed.

pH EVALUATION

The pH of the formulation was tested for 22days at 5days intervals and it involved making a 1:10 solution of the sample then the pH was tested using the pH meter in triplicates.

VISCOSITY EVALUATION

This evaluation was done for 22days at 5days intervals. Brookfield viscometer set at 26.7°C and 60rpm was used to measure the viscosity of the formulation

ANIMAL STUDIES.

The animals (Wistar rats) used for this study were housed in cages of wood shavings as a bedding and maintained at room temperature. The animals were provided with pellet chow and water given ad libitum and acclimatized for a period of 14 days before the commencement of the treatment. The experimental animals were treated humanely in accordance with the rules of Laboratory Animal Care of the National Institute of Health (NIH,1985).The animals were randomly grouped and treated as already described.

INDUCTION OF BOIL/FURUNCLES

A section of the skin around the thigh of the animal in each group was shaved using a shaving cream. Using a syringe, 0.1ml of isolated *S.* aureus culture was injected into the shaved skin through a hair pore. The animals were left for 4days to allow for prominent growth of the boils.

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TREATMENT OF ANIMALS

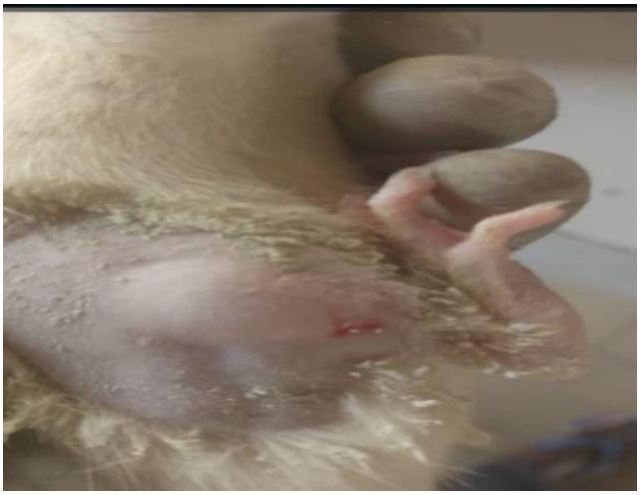

Fig 2: Induced boil size

After successful induction of boils, animals were treated with the test samples every 12 hours for 7 days and monitored for reduction in the diameter of the growth.

STATISTICAL ANALYSIS OF RESULTS

All the experimental results were analyzed using the Microsoft Excel software (Version). Analysis of variance (ANOVA) was used to determine the significant difference between the groups.

RESULTS

Table 2: Evaluation Results

Evaluation Parameter	**Result**
Color	**Light brown with tiny particles**
Odor	**Pleasing**
Washability	**Easily washable**
Irritability	**Non irritant**
Spreadability	**3.67**) $\pm$ 0.12 **gcm/s**
Saponification value	**166.6mg.KOH/g**
Acid value	**44.88mg.KOH/g**
Viscosity	**749.7cp**
Appearance	**Gritty with visible tiny particles**

Table 3 : pH evaluation of Python fat-based topical formulation

DAYS	pH at (27^{O} C)	Standard Deviation	pH at 5^{O} C	Standard Deviation
1	5.53	±0.13	5.53	±0.13
6	5.47	±0.05	5.43	±0.05
11	5.53	±0.05	5.37	±0.04

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17	5.20	±0.08	5.47	±0.05
22	3.40	±0.17	5.53	±0.09

Table 4 : Viscosity Evaluation of Topical Formulation

Days	Viscosity at ($27^{O}C$)	Standard deviation	Viscosit at $5^{O}C$	Standard Deviation
1	**5687.93**	**±1.13**	**5858.07**	**±2.06**
6	**5697.13**	**±2.10**	**5799.60**	**±1.11**
11	**5791.45**	**±1.30**	**5824.51**	**±2.54**
17	**5775.31**	**±3.73**	**5733.48**	**±0.90**
22	**4108.36**	**±0.16**	**5772.92±**	**±1.72**

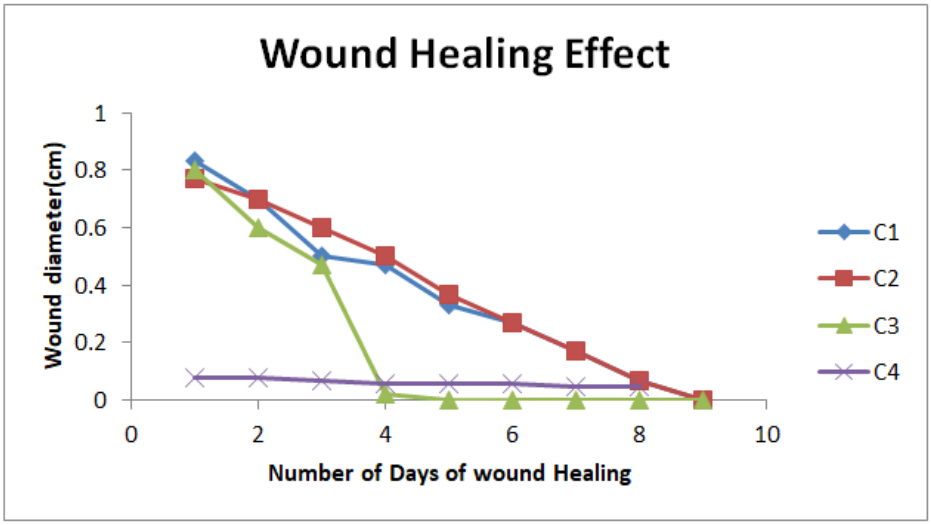

Fig. 3: **Boil treatment and Wound Healing Evaluation**

Table 5: Weight of Rats and Inhibition zone diameter

Batches	Weight of Rat	Inhibition zone Diameter (IZD)	
		Python Fat	Gentamicin
C1	129±5.15	5 ± 0.5	15.33 ± 0.58
C2	112.6±7.57		
C3	123.6±16.01		
C4	96.7±9.29		

Table 6 : Minimum Inhibitory Conce6ntration of Python fat

Concentration Of Sample (python fat)	Observations
50%	++
25%	++
12.50%	+
6.13%	+

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3.03%	–

KEY: ++= Total Absence of microbial growth, += partial absence of microbial growth,- = presence of microbial growth

DISCUSSION

Organoleptic, viscosity, saponification and acid value tests were carried out for the identification of the sample (Python fat) and results were compared with the standard as characterized by Moussounga & Dzondo,2018 . The viscosity was found to be 7497 centipoise while the saponification value was 166.6mg/KOH/g. This high saponification value depicts that the sample has long chains of carbon and can be applied in the field of soap making (Ordu & Abraham 2023). The acid value as determined was found to be 44.88mg/KOH/g signifying high quality of the oil with the low acid value as the acid value determines the amount of free fatty acids in the fat and all values corresponded with the standards from literature hence the sample was ascertained to be from *Python sebae.* More so, the higher the acid value the higher the free fatty acid content and the lower the quality of the oil as a result of deterioration of oil fats (Ayanlowo et al; 2022). Therefore at room temperature and upon prolonged storage under such condition, the acid value was assumed to additionally increase with the age of the oil as triglycerides decomposes into fatty acids and glycerol as an effect of time temperature.

Microbial tests were carried out on the sample (Python fat) to confirm if it has antimicrobial activity and the minimum inhibitory concentration. The sample inhibited microbial growth within a diameter of 5mm while the comparative standard, gentamicin, inhibited microbial growth within a diameter of 15.33mm within same duration. This confirms that the sample has lower antimicrobial activity when compared to Gentamicin. For the minimum inhibitory concentration test, the sample, at 25 and 50% concentrations completely inhibited microbial growth while 6.25% and 12.5% concentrations partially inhibited growth and there was absence of inhibition at 3.125%. Thus, the minimum inhibitory concentration is 6.25% and from the test result, it could be inferred that the inhibitory activity of the python fat increases with increase in concentration.

The pH of the formulation was studied for 22days at room temperature (37°C) and 5°C respectively. The pH at 5°C was found to be 5.53, 5.43, 5.37, 5.47 and 5.43 for days 1 to 22 respectively as in Table 2. At room temperature, the pH was found to be 5.53, 5.47, 5.53, 5.20 and 3.40 for days 1 to 22 respectively as shown in Table 2. Both at room temperature and 5°C, the pH showed a slight decrease over the 22-day period. At room temperature, the drop was from 5.53 to 3.40, while at 5°C, it was from 5.53 to 5.37.The decrease in pH was significantly more pronounced and started earlier at room temperature compared to 5°C. The decrease in pH of formulation stored at room temperature can be due to various factors such as exposure to air, light, and heat. This decrease in pH affected the stability of the formulation, causing it to cream. Also, the decrease in pH could be due to decomposition or hydrolysis reactions of certain components in the sample which could cause, release of acidic products, hydrolysis or oxidative reaction. Also the pH change could lead to increase of acid value as triglycerides decompose into fatty acids and glycerol as an effect of time and temperature

The Viscosity of the formulation was also studied for 22days at room temperature and 5°C respectively. The viscosity values at room temperature were 5687.93cp, 5697.13cp, 5791.45cp, 5775.31cp and 4108.36cp for days 1 to 22 respectively. While the viscosity values at 5°C were 5858.07cp, 5799.60cp, 5824.51cp, 5733.48cp and 5772.96cp for days 1 to 22 respectively as in Table 3. Both at room temperature and 5°C, the viscosity decreased over the period of study. At room temperature, the drop was from 5687.93 cp to 4108.36 cp, while at 5°C, it was from 5858.07 cp to 5772.96cp.The decrease in viscosity could be due to the instability issues leading to degradation or interaction of components in the formulation over time and also

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resulting to a decrease in the internal resistance to flow. The lower viscosity at room temperature compared to 5°C suggests that temperature plays a role in the flow properties and integrity of the formulation. Higher temperatures generally increase the kinetic energy of molecules, making them to be relatively mobile and move more freely thus reducing the resistance to flow. Furthermore, slight fluctuations in the measured viscosity values could also be due to experimental errors or instrument limitations.

In the overall, the observed decrease in pH and viscosity, particularly at room temperature, suggests that the formulation undergoes some changes during storage as a result of some environmental factors.

Animal studies aimed at comparing the boil healing/reducing rate using different formulations was carried out for a period of 9 days. In group C1 and C2 (the group receiving the raw python fat, and that receiving the python fat-based topical formulation), there was observed similar reduction rate of the boil diameter. On the first day, the boil sizes were 0.83cm and 0.77cm for groups C1 and C2 respectively while on day 5, the sizes were 0.33cm and 0.37cm. The slight difference in reduction rate suggests that both formulations have similar efficacy. However, the standard (Gentamicin) proved to be more efficacious as the boil sizes were significantly reduced to 0.00cm on day 5. The control group which received no treatment, had no healing rate with the boil size at same diameter (0.5cm) even up to day 9.

CONCLUSION

Python fat is effective in the treatment of boil even when formulated as cream for topical applications however, its efficacy was observed to be lesser than Gentamicin used as standard. Also, the sample and the topical formulation seems stable at low temperatures but not at room temperature. Based on the observation, there is an instability challenge with the use of this product therefore, further study will be needed on ways of improvement and enhancing stability of the fat.

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