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Evaluation of the Antimicrobial Effectiveness of Topical Gels Containing *Acalypha wilkesiana*

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ABSTRACT

Traditional medicines used in developing countries in the treatment and management of diseases are mostly used in their crude forms, consequently, users are exposed to unwanted toxic effects due to no dosing standards and/or poor packaging. This study aims to formulate A*calypha wilkesiana* leaves known for its antimicrobial properties into an appropriate conventional topical gel and standardize the formulation for possible use in the treatment of superficial skin infections. The minimum inhibitory concentration and antimicrobial susceptibility tests of methanol extracts of the red and green variants of *Acalypha wilkesiana* leaves were determined against select bacteria and fungi. The physical and microscopic properties, stability and compatibility of the extracts in different topical gels such as hydrogels, bigels and organogels were investigated and the effective strength of the extract for incorporation into the appropriate gel form was determined using antimicrobial sensitivity studies. The organogel was the most appropriate gel form for incorporation of the methanolic extract of *Acalypha wilkesiana*. Both variants of *Acalypha wilkesiana* showed antibacterial activity against *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus and Staphylococcus albus* and antifungal activity against *Microsporum canis* and *Malassezia furfur*. Both were inactive against *Candida albicans* and *Trichophyton rubrum.* The red *Acalypha* variant showed antibacterial activity against *Klebsiella oxytoca* at a concentration of 125 mg/mL in the organogel. A topical organogel compatible with the two variants of *Acalypha wilkesiana* was developed and the antimicrobial assay showed potential therapeutic efficacy in the treatment of microbial skin infections caused by common skin pathogens.

***Keywords*:** *Acalypha wilkesiana*, Topical gels, Skin infections, Organogel.

Introduction

Herbal remedies are used considerably in developing cultures for the treatment of diseases ranging from infections to physiological anomalies. Eighty percent of Africans use herbal treatment in some form.[1] These remedies handed down through the years have been shown to be effective and affordable hence their continued use in these cultures. Unfortunately, these remedies despite their assumed effectiveness lack the purity and standardization that characterize conventional pharmaceutical dosage forms. This consequently leads to adverse effects that complicates the diseases that they were initially set to heal.[1,2]

Acalypha wilkesiana (Euphorbiaceae) is one of the several herbs used in Southwestern Nigeria for the treatment of a myriad of diseases. The effectiveness of this plant has been substantiated by various peer reviewed publications.

It is known to have shown pharmacological effect against fungal skin infections[2,3] and bacterial infections including methicillin-resistant *Staphylococcus aureus*.[4,5] It has also been used as a cardioprotective agent as well as in the management of diabetes.[6] Other reported uses of *A. wilkesiana* include treatment of cold, headache, malaria[4], inflammation and tumours in the breast.[6]

Most publications on *A. wilkesiana are* based on the investigation of its pharmacological action or the support of its use in traditional health through phytochemical analysis of the aqueous, ethanolic or powder extracts. To the best of our knowledge, apart from Oyelami *et al*, who investigated the efficacy and safety of *Acalypha wilkesiana* ointment in the treatment of superficial fungal skin infection[2], none have investigated the formulation of this medicinally active plant into a dosage form for optimized dosing and therapeutic effect.

In this study, the antimicrobial (antibacterial and antifungal) properties of *A. wilkesiana* were determined, the most appropriate gel dosage form for its therapeutic effectiveness was investigated and the effective strength in the dosage form ascertained through minimum inhibitory and zone of inhibition studies.

Materials and Methods

Solvents and Reagents

Tween 80 was purchased from BDH Chemicals, England. Clotrimazole powder BP (> 98%) purity and Levofloxacin powder BP (>98%) were obtained from Drugfield Pharmaceutical company, Ogun State, Nigeria. Carbopol 940 (Shree organics, India), methylparaben, propyl paraben (Sigma–Aldrich St. Louis, USA), triethanolamine (DBS Chemicals, India), propylene glycol (Sigma–Aldrich St. Louis,

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USA), Absolute ethanol (Sigma–Aldrich St. Louis, USA), and transcutol was obtained as a gift from Gattefosse (Cedex, France). Other materials used include Refined palm oil (Raffles oil LFTZ Enterprise Lagos, Nigeria), Mueller Hinton agar (Sigma–Aldrich St. Louis, USA), Sabouraud Dextrose agar (Sigma–Aldrich St. Louis, USA).

Preparation of acalypha wilkesiana extract
Two leaf variants of *Acalypha wilkesiana* (green and red leaf variant) were separately collected in Abuja at the coordinates corresponding to latitude 9.0637° N and longitude 7.3382° E and stored under herbarium numbers NIPRD/H/ 6788 and NIPRD/H/ 6789 respectively. 500 g of the leaves were washed, dried, and pulverized. The resultant powder was weighed and macerated with 2 L of methanol for 48 h. The methanolic extract was filtered and concentrated via evaporation in an oven at 40^{o}C. Solubility test was carried out by dissolving the extract in water. The mixture was shaken to assess the suitability of water as diluent.

Topical gel Formulation
Hydrogels, organogels and bigels were formulated and the *A. wilkesiana* methanolic extract were incorporated into the formulation to determine the compatibility of each of the preparations with the extract.

Preparation of Hydrogel
Purified water 100 mL, accurate weight of methyl and propyl paraben were measured and mixed. Carbopol 940 was weighed and added to the purified water-preservatives mixture and stirred on a magnetic stirrer for a period of 30 mins until a homogenous mixture was achieved and gel formed (Mix A). Propylene glycol, transcutol and extract dissolved in 70 mL of distilled water were measured and mixed in a separate beaker. For red *A. wilkesiana*, 90% ethanol was used to dissolve the extract before adding to the Propylene glycol and water mixture (Mix B). Mix A was then added to Mix B and stirred consistently. Few drops of triethanolamine was added to aid gelation and the pH of the gel was determined (Table 1).

Preparation of Organoge
This was done based on the method described by Sagiri *et al*.[11] with a few modifications. Tween 80 and refined Palm oil were each measured into two separate beakers. A range 6.25% to 25% w/w of extract for each formulation was weighed and added to the oil (Table 2). Tween 80 and the oil-extract mix were heated in a water bath to 80 ^{o}C with continuous agitation for 5 minutes. The mixture was then transferred to a beaker and placed on magnetic stirrer at 50 rpm for 20 min, while maintaining the temperature at 80 ^{o}C. Sterile distilled water heated to 80 ^{o}C was added drop wise with continuous stirring until the organogel formed.

Preparation of Bigel
The bigel was formulated by making the hydrogel and organogel and mixing both in equal proportions at 30 ± 0.5 ^{o}C in the following order; BG 1 (OG 1 + F1), BG 2 (OG 2 + F2), BG 3 (OG 3 + F1), BG 4 (OG 4 + F2), BG 5 (OG 5 + F1), BG 6 (OG 6 + F2).

Characterization of organogels
The following physical and microscopic properties of the formulated gels were carried out -

Organoleptic evaluation
The freshly prepared gels were subjected to organoleptic evaluation by observing the color, odor, appearance and texture of the gels.

Microscopic study
The gels microstructure was observed under microscope S6 Basic Stereo Zoom LED Microscope. CA, USA. The samples were prepared by mounting a drop of the samples on the glass slides and enclosed with a cover slip. An average of 5 fields were examined and microstructure was classified based on presence or absence of clusters of spherical, granular, droplets or tubular structures having 3-dimensional network structure.

pH measurement
The pH of the gels was measured with a pH meter (Mettler Toledo S975 Multiparameter probe with Stirrer USA). The measurement was done by immersing the electrode of the pH meter in the samples and values recorded accordingly.

Spreadability Studies
The spreadability of the gels was determined by placing a predetermined weight of samples (0.5g) in between two glass slides of equal weight and area. The initial spreading diameter before placing the weight was noted. Thereafter, varying weights of 10, 20, 50 and 100 g were placed over the upper slide. The final diameter was noted over a period of 60 secs. The percentage spreadability of the formulations were determined using Equation 1

$$\%\, Spreadability = \frac{D_1 - D_2}{D_1} \times 100 \quad \text{..... Eq.1}$$

Where D_1 and D_2 are the initial and final spreading diameter before loading and after loading, respectively.

Rheological Study
Rheological studies of the gel samples were carried out using Brookfield Viscometer DV-1 with a concentric cylinder spindle LV-4 at 100 rpm. A temperature of 25 ^{o}C was maintained throughout the experiment. The samples were subjected to a shear rate sweep starting from 10 to 60 sec^{-1}.

Sol-to-gel transition study
The organogels were heated in a temperature-controlled water bath. The experiment was started at 30 ^{o}C and increased to 80 ^{o}C with an increment of 5 ^{o}C. The samples were maintained at the specified temperature for 5 min. and observed by inverted test tube method. The temperature at which the gel started to flow was regarded as gel-sol transition temperature.[12] The study was done in triplicates.

Accelerated stability testing and draize test.
The ICH guidelines (40ºC/75%RH) were followed in the accelerated stability testing of the hydrogel and organogel formulation. The gels were packed in amber colored jars and kept in a stability chamber with set temperature and relative humidity. The formulations were subjected to accelerated stability testing at both room temperature and at 40^{o}C and parameters such as pH, spreadability, sol-to-gel temperature and viscosity were recorded on day 0 and 90.[12-14] Skin sensitivity test i.e., Draize test was carried out. Organogel 5 ml, was applied on intact skin of five human volunteers and left for 24 h. The applied part of the skin was observed for any adverse reactions. Dermoscopy was done to objectively document the erythema or skin changes after formulation application. Physical indications such as redness, inflammation, swelling, or a rash were observed for and noted using a dermoscope. Ethical approval was obtained from the Human Research and Ethics Committee of Lagos University Teaching Hospital, Idi-araba Lagos, with Health Research Committee assigned No. ADM/DCST/HREC/APP/3148.

Media preparation
Media were prepared according to the manufacturer's prescriptions. They were heated to melt in the water bath and then autoclaved at 121^{o}C for 15 min. All procedures were carried out under laminar flow to prevent microbial contamination.

Mueller Hinton Agar
Mueller Hinton agar was prepared by dissolving 3.8g of the agar into 100 mL of distilled water. The mixture was stirred and heated to melt in water bath at 100 ^{o}C after which it was autoclaved at 121 ^{o}C for 15 min. This was used for all bacteria assay.

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Sabouraud Dextrose Agar (SDA)
This agar was prepared by dissolving 65 g into one liter of distilled water. This was stirred and heated to melt in a water bath and then autoclaved at 121 ^{o}C for 15 min. This was used for all fungi assay.

Preparation of Antibacterial standard: Levofloxacin
Four (4) working concentrations (50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL) of standard drug were used. The above concentrations were obtained from a stock solution of 0.05 g of Levofloxacin powder in 10 mL of sterile distilled water to get a concentration of 5000 µg/mL. The working concentrations were obtained using Equation 2.

$C_1V_1 = C_2V_2$…….. Eq. 2
Where C_1 and C_2 are the initial and final concentrations respectively; and V_1 and V_2 are the initial and final volumes, respectively.

Preparation of antifungal standard: Clotrimazole
Four (4) working concentrations (160 µg/mL, 80 µg/mL, 40 µg/mL and 20 µg/mL) of standard drug were used. The above were obtained from a stock solution of 0.016g of Levofloxacin powder in 10 mL of absolute ethanol to get a concentration of 1600 µg/mL. The working concentrations were obtained using Equation 2.

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing (AST) was carried out by Kirby-Bauer disk diffusion method[15-16] using standard antimicrobial disks, Mueller Hinton agar and Sabouraud dextrose agar plates.

Determination of Minimum inhibitory concentration of extract
Using the method prescribed by the Clinical and Laboratory Standard Institute Codex[13], 2 mg/mL (intermediate concentration) and 256 mg/mL (final concentration) concentrations of primary stock of extract were prepared. These were diluted with sterile agar to obtain concentrations of 0.01 to 163.84 mg/mL *Acalypha wilkesiana* extract. These were then poured into sterile petri dishes labeled A to O and allowed to solidify. Thereafter, 0.1 mL from the 0.5 Mcfarland standards of the micro-organisms were used to inoculate the petri dishes. The plates were then incubated at 37 ^{o}C for 24 -72 h. The lowest concentration of extract that inhibited the growth of the test organisms was recorded as the minimum inhibitory concentration (MIC).

In vitro antibacterial activity study
The antibacterial activity of the extract alone and gel containing extract were determined and compared against that of levofloxacin. The agar-well diffusion method was used to determine the zone of inhibition. The clinical strains of cultured bacteria (*Pseudomonas aeruginosa, Staphylococcus aureus, Stapyhylococcus albus, Klebsiella oxytoca, Bacillus subtilis* (obtained from the Nigerian Institute of Medical Research, Lagos, Nigeria) were calibrated using 0.5 McFarland Turbidity standard to achieve an approximate cell density of 1.5 x 10^8 CFU/mL.
Mueller Hinton agar plate was seeded with 1 mL (concentration of 10^8 CFU/mL) of the test micro-organisms in sterile petri-dishes and the media allowed to set at room temperature. Three wells of 6 mm diameter were created with a sterile cork borer per agar plate. Each well was filled with 0.1 mL of the extract at a concentration of 250 mg/mL, 125 mg/mL and 62.5 mg/mL. The plates were left to stand for 1 hour at room temperature to allow for diffusion of the extract or standard drugs into the surrounding medium. The plates were finally incubated at 37 ^{o}C for 24 hours and the diameter of inhibition zone (DIZ) was read to the nearest millimeter. This measurement was done in triplicate. The same method was used in evaluating the zone of inhibition of the formulated organogels (red and green variants of the extract) at varying concentrations of 250 mg/mL, 125 mg/mL and 62.5 mg/mL.

In vitro antifungal activity Study
The antifungal activity of the extract was done by agar-well diffusion method. The cultured fungi were calibrated using 0.5 McFarland Turbidity standard to achieve an approximate cell density of 1.5 x 10^8 CFU/mL. Sabouraud dextrose agar plate was seeded with 1 mL (concentration of 10^8 CFU/mL) of the test fungi (*Malassezia furfur, Trichophyton rubrum, Microsporum cannis, Candida albicans, Aspergillus flavus and Aspergillus niger)* in sterile petri-dishes and allowed to set. Wells (4.6 mm in diameter) were cut into the agar using a sterile cork borer and the formulations/standard drug were added into the wells. The plates were incubated at 28 °C for 3 days after which DIZ were measured.

Statistical Analysis
Statistical data analysis was carried out using Microsoft Excel Office software. Significant differences ($p \leq 0.05$) of mean values were determined by student t-test.

Results and Discussion

Characterization of gels
Organoleptic evaluation
The formulated gels were assessed for texture, consistency and aroma. Incorporation of the extract into the hydrogel led to breaking of the gel structure shown by reduced viscosity (Table 3). The bigels were found to be unstable on addition of the extract; there was separation of the aqueous and oil phases of the bigel. The organogel, however, was stable and the extract was well incorporated into the formulation (Table 2). It had a brownish colouration and showed a homogenous appearance as shown in Figure 1.

Microscopic study
The micrographs of organogels containing two variants of extract (Red and Green *A. wilkesiana*) are shown in Figure 1. The micrographs show the size of the disperse phase as bubbles dispersed in the continuous phase. The smaller the size of the bubbles, the more thermodynamically stable the organogel is. The microstructures of the organogels were studied under light microscope (Figure 1) and the micrographs reveal that the gels contained clusters of water filled spherical, granular, droplets and tubular structures which led to the formation of a 3-dimensional network structure. The stability of the organogel increases with reduction of the globule size. This was ensured by increasing the speed of mixing during formulation. The homogeneity of the formulation on storage confirmed that the product was stable.

pH measurements
The pH of the organogel ranged from 6.31 to 6.57 (Table 4). This implies that the developed organogel may not cause skin irritation. Hence may be applied over skin without any side effect. The pH of the skin is approximately between 5.2 – 6.8 while the pH of the organogel fell between 6.31 to 6.57. Accelerated stability testing of the organogels showed no significant change in the physicochemical characteristics of all the formulations (Table 5). Compared to the organogel formulations the presence of the acalypha plant extracts altered the pH of hydrogels (Table 3) to a neutral pH of approximately 7. This shows that the formulation would not protect the acid mantle of the skin due to its unstable nature. The developed organogel showed compatibility with the skin pH (slightly acidic) reflecting no risk of skin irritation. Draize tests carried out on the developed formulations showed that there was no irritation experienced when applied to intact skin. Figure 1 highlights dermoscopy pictures of the skin surface after the use of the formulations showing skin devoid of irritation and erythema.

Rheology and sol-gel transition studies
The formulated organogel with extract showed shear thinning properties. This means that the formulation exhibited the appropriate consistency on storage, easily pourable at low shear and spread easily on the skin on application of high shear. This non-newtonian flow exhibited by the organogel also ensures the formulation does not run off the skin surface on application. The sol-gel transition temperatures were found to be between 41.4^{o}C and 42.7^{o}C (Table 4). As the temperature is increased, there is a corresponding increase in the

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surface free energy with a subsequent increase in the mobility of the self-assembled structures of the gelators. Further increase in temperature beyond the reported values, diminished the interaction among the self-assembled structures and consequently led to disruption of the networked structure causing the system to flow freely.[8]

Spreadability studies

From the results obtained, all the formulations possess good spreadability (Table 4). This explains the fact that the formulations will be able to penetrate its site of action easily. Generally, there was an increase in percentage spreadability as the weight of the applied load was increased. Spreadability denotes the extent of area to which a gel readily spreads on application to the skin and it depends on the viscosity of the formulations and physical characteristics of the excipients used in the formulation. The therapeutic efficacy of a formulation also depends on its spreading value.[9, 10] A more viscous formulation would have poor spreadability while good spreadability ensures proper wetting, uniform application of incorporated medicament and hence good permeation into the skin.

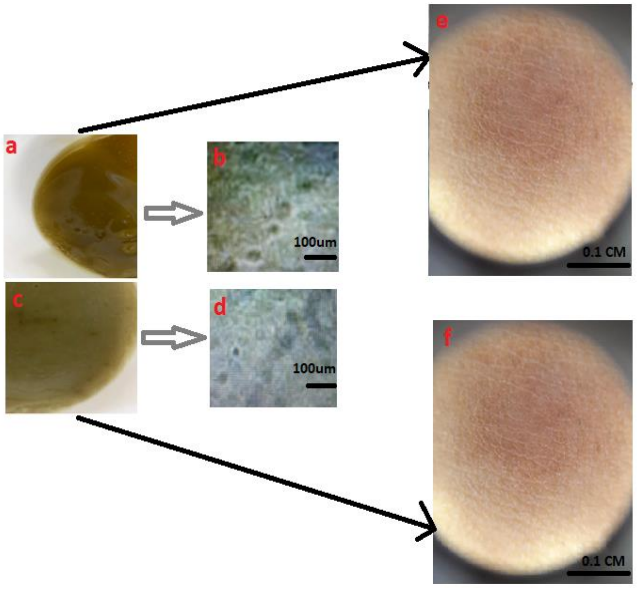

Figure 1: (a) Organogel containing red *Acalypha wilkesiana* extract (b) Organogel micrographs containing red *Acalypha wilkesiana* extract (c) Organogel containing green *Acalypha wilkesiana* extracts (d) Organogel micrographs containing green *Acalypha wilkesiana* extracts (e) Dermoscope picture post application of Organogel containing red *Acalypha wilkesiana* extract after application on healthy skin(f) Dermoscope picture post application of Organogel containing red *Acalypha wilkesiana* extract after application on healthy skin.

Rheological Studies

The viscosity of the formulated organogels ranged from 10872 to 12528 mPas (Table 4). The viscosity of the bigel was much lower than that of the organogels 7354 to 7992 mPas. The bigel formulations exhibited poor stability and progressively lost its rheological property on storage at 25^{o}C and 45% RH (Relative Humidity) for 14 days with resultant viscosity being 3799 to 5923 mPas. The hydrogels were also shown to lose their rheological property after accelerated stability tests at 40 ^{o}C for 90 days were carried out as shown in Table 3. The organogel formulation with the lowest viscosity is OG3 while that with the highest viscosity is OG4.

Sol-gel transition studies

The organogels were subjected to increasing temperatures starting from 30^{o}C. An increment of 5^{o}C was made after 5 mins and the samples were considered to have undergone gel-to-sol transition when they started flowing (Tables 4 and 5).

Minimum inhibitory concentration

The minimum inhibitory concentration study of *Acalypha wilkesiana* and zone of inhibition study informed the appropriate strength of extract to be incorporated into the different topical dosage forms (Tables 6 and 7). Similar studies were done using standard discs of clotrimazole and levofloxacin which served as controls for the acalypha extracts (Tables 7 and 8).

Antimicrobial activity screening

The antimicrobial properties of the gel were evaluated against some fungi organisms *(Trichophyton rubrum, Microsporum canis, Malassezia furfur and Candida albicans)* and bacterial organisms *(Staphylococcus aureus, Staphylococcus albus, Klebsiella oxytoca, Pseudomonas aeruginosa* and *Bacillus subtilis)* using Sabouraud dextrose agar and Mueller Hinton agar plates respectively.

Candida albicans and Trichophyton rubrum showed no zone of inhibition with the extract as a result, they were not evaluated for minimum inhibitory concentration (MIC). The MIC result in Table 9 showed that *Klebsiella oxytoca* was susceptible to the extracts of the red variant but resistant to the extracts of the green variant of *A. wilkesiana* up to the maximum concentration tested (163.84 mg/mL).

All bacteria except *Klebsiella oxytoca* and fungi (*Microsporum canis* and *Malassezia furfur*) were sensitive to the organogel formulations of the green and red leaf variants of the extract (Tables 9). The antimicrobial study of the green leaf extract formulation showed that *Klebsiella oxytoca* was resistant at all formulated concentrations (250 mg/mL, 125 mg/mL and 62.5 mg/mL) and showed no zone of inhibition while the red leaf extract showed activity against *Klebsiella oxytoca* (Table 9) at a concentration of 250 mg/mL and 125 mg/mL.

The antibacterial standard drug, levofloxacin, activity showed zones of inhibition against all tested bacteria except *Klebsiella oxytoca* (Table 8). This was the case for the organogels containing the green and red variants of the herbal extract though the zones of inhibition were observed at a much higher concentration of the extracts. However, a clear zone of inhibition was observed with the organogel containing the red acalypha variant against *Klebsiella oxytoca* at 125 mg/mL concentration and above (Table 9).

The antifungal standard clotrimazole showed some activity against all tested fungi but the acalypha extract showed none against *Candida albicans* and *Trichophyton rubrum.* The red and green variants of *Acalypha* showed antimicrobial activity against most of the tested organisms and there were no significant differences in their antimicrobial activity ($p = 0.29$).

Herbs are a good source of medicinal products. Conventional medicine has argued that the lack of standardization of herbal products have limited their use or acceptability in modern times. This is mostly true because most herbal products especially in Nigeria are used in their crude forms. Studies have shown that patients living with chronic diseases use herbal products concurrently with conventional drugs.[7] Some of the challenges associated with the use of these products include no definite dosing, lack of documented side effects and toxicity. Dosage form stability ensures that the medicinal content of the formulation is homogenously dispersed in the formulation vehicle which ensures that the medicine diffuses through the formulation to the skin on application. *Acalypha wilkesiana* has been used in various countries for management of different topical conditions. Laut *et al*., [17] developed medicated ointment containing extract of *A. indica* leaves obtained from Indonesia which exhibited wound healing process in the incision wound models by enhanced wound contraction and shortened epithelization period. Fawehinmi *et al.,*[18] showed that *Acalypha wilkesiana* ethanol extract obtained from Nigeria, has high potential as an anti-dermatophyte agent when formulated as cream for topical application. The cream formulation however was not very stable at elevated temperatures. There was a noticeable change in odor and

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color of the product since the elevated temperature degenerates the components of the products. This result is in consonance with our research. Based on the physicochemical properties and formulation studies, it was determined that of the three gel forms investigated – hydrogel, organogel and bigel, organogel was the most appropriate gel form for *Acalypha wilkesiana*. The stability of the formulations is dependent on the activity of the plant extract within the microstructure of each of the formulations. The bigel formulations exhibited the least stability whilst the hydrogel formulation did not have the ability to keep the plant extract suspended within its aqueous matrix.

Topical formulations like organogel are used to treat various skin and systemic diseases and are also utilized in delivering anti-HIV agents vaginally for HIV prophylaxis.[8] They help avoid hepatic first-pass metabolism and minimize serum absorption. The developed organogel were pale white to light brown colored viscous preparations with smooth homogenous appearance. They were pleasant to sight and feel, oily to touch, non-gritty in nature, and having slight odor. The organogel formulations OG1 to OG6 had similar properties; this implies that the two variants of *Acalypha wilkesiana* did not confer different physical properties on the formulation. Oyelami *et al*., [19] studied the efficacy and safety of *Acalypha wilkesiana* ointment in superficial fungal skin diseases. Clinical cure was achieved in 73.3% of the patients showing the efficacy of the herbal product. This is in consonance with our research, however the superiority of the novel organogel formulation which encapsulated the liquid organic phase containing the *Acalypha wilkesiana extract* within a three-dimensional, cross-linked network enable the organogel to remain stable and ensure a sustained release of the extract from the self-assembly of the organogel network.

Dermoscopy pictures show the organogels were safe and did not cause any erythema or adverse reaction, this serves as an advantage over ointment and creams which have been documented to cause adverse reaction such as excoriation which led to discontinuation of such products[18]. The developed organogels containing *Acalypha wilkesiana* extract were seen to be stable after accelerated stability testing was carried out. This ensures that the formulation would endure the rigors of storage in tropical regions whilst maintaining its physicochemical activities and anti-infective properties. The organogel formulation containing the red *Acalypha wilkesiana* extract had the highest antimicrobial effect on the test organisms, comparable with clotrimazole against *Candida albicans, Malassezia furfur, Microsporum canis and Trichophyton rubrum*. *Klebsiella oxytoca* was sensitive to only high concentrations of the red *Acalypha wilkesiana* extract (250 mg/mL and 125 mg/mL) in the organogel formulation containing the red *Acalypha wilkesiana* extract. The green *Acalypha wilkesiana* did not show any activity against this organism. The absence of inhibitory effect on *Klebsiella oxytoca* was not surprising as it had been earlier reported by Alade and Irobi [20] with an aqueous leaf extract. Both the red and green extracts when formulated as organogels showed excellent activity against all other bacteria and fungi tested. This is in consonance with previous research [21-23], where it was observed that the leaf extract possessed a broad spectrum of activity on both fungi and bacteria with Gram negative bacteria being more resistant than Gram positive bacteria.[14]

Table 1: Composition of formulated hydrogels

Batch no	Red A.W ($\%\frac{w}{v}$)	Green A.W ($\%\frac{w}{v}$)	Car. 940 ($\%\frac{w}{v}$)	P.Gly ($\%\frac{v}{v}$)	90% Eth. ($\%\frac{v}{v}$)	T.E.A ($\%\frac{v}{v}$)	Trans cutol ($\%\frac{v}{v}$)	M.Pb ($\%\frac{w}{v}$)	P.Pb ($\%\frac{w}{v}$)	Water to ($\%\frac{v}{v}$)
F1	0.2	-	0.25	10	5	0.4	0.1	0.05	0.01	100
F2	-	0.2	0.25	15	-	0.4	0.1	0.05	0.01	100

*A.W = *A. wilkesiana* Car. 940 = Carbopol 940, P.Gly = Propylene glycol, 90% eth. = 90% ethanol, T.E.A = Triethanolamine, M.Pb = Methyl paraben, P.Pb = Propyl paraben

Table 2: Composition of formulated Organogels

Batch no	Red *Acalypha wilkesiana* (%)	Green *Acalypha wilkesiana* (%)	Oil (%)	Tween 80 (%)	Water to (%)
OG1	25	-	23.3	46.7	100
OG2	12.5	-	23.3	46.7	100
OG3	6.25	-	23.3	46.7	100
OG4	-	25	23.3	46.7	100
OG5	-	12.5	23.3	46.7	100
OG6	-	6.25	23.3	46.7	100

*OG - Organogel

Table 3: Accelerated stability testing on formulated hydrogels ($p \leq 0.05$)

	pH (day 0)	pH (day 90)	Dynamic viscosity mPas (day 0)	Dynamic viscosity mPas (day 90)	Stability after 90 days
F1	6.97 ± 0.04	7.05 ± 0.01	1990 ± 4.04	785 ± 10.8	Unstable
F2	6.58 ± 0.04	7.10 ± 0.05	11005 ± 6.07	699 ± 11.4	Unstable

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Table 4: Physical properties of formulated organogels

Formulations	pH	Spreadability %	Sol gel Temperature (^{o}C)	Viscosity (mPas)
OG1	6.57 ± 0.03	85.40 ± 1.11	42.5 ± 0.3	12113 ± 3.45
OG2	6.44 ± 0.01	82.00 ± 1.07	42.7 ± 0.2	11557 ± 7.54
OG3	6.38 ± 0.11	78.50 ± 0.93	41.4 ± 0.5	10872 ± 1.99
OG4	6.31 ± 0.04	81.90 ± 2.41	41.6 ± 0.3	12528 ± 9.23
OG5	6.35 ± 0.03	79.40 ± 1.54	43.2 ± 0.1	11741 ± 6.77
OG6	6.47 ± 0.02	81.70 ± 1.27	42.4 ± 0.6	11236 ± 8.21

*OG - Organogel

Table 5: Accelerated Stability testing on the formulated organogels for 90 days ($p \leq 0.05$)

Formulations	pH	Spreadability %	Sol gel Temperature (^{o}C)	Viscosity (mPas)
OG1	6.58 ± 0.03	85.51 ± 0.99	42.5 ± 0.3	12116 ± 3.55
OG2	6.46 ± 0.05	81.97 ± 0.97	42.7 ± 0.2	11559 ± 7.06
OG3	6.38 ± 0.09	79.00 ± 1.01	41.4 ± 0.5	10872 ± 1.89
OG4	6.33 ± 0.02	81.80 ± 1.32	41.6 ± 0.3	12527 ± 8.78
OG5	6.35 ± 0.03	79.50 ± 1.60	43.2 ± 0.1	11745 ± 5.99
OG6	6.47 ± 0.02	81.69 ± 1.11	42.4 ± 0.6	11237 ± 8.14

*OG - Organogel

Table 6: Minimum inhibitory concentration of *Acalypha wilkesiana* (mg/mL)

	0.01	0.02	0.04	0.08	0.16	0.32	0.64	1.28	2.56	5.12	10.24	20.48	40.96	81.92	163.84
BACTERIA															
B. subtillis	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
P. Aeruginosa	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
K. Oxytoca	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. Aureus	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
S. Albus	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
FUNGI															
M. Furfur ATCC 14521	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
M. Canis ATCC 11621	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-

"+" indicates growth, "-" indicates no growth

Table 7: Zone of Inhibition (mm) for Clotrimazole

Bacteria	160 µg/mL clotrimazole	80 µg/mL clotrimazole	40 µg/mL clotrimazole	20 µg/mL clotrimazole
Microsporum canis	18.3 ± 0.10	15.5 ± 0.06	-	-
Malassezia furfur	18.0 ± 0.08	17.0 ± 0.08	-	-
Candida albicans	22.8 ± 0.10	21.8 ± 0.10	19.8 ± 0.10	-
Trichophyton rubrum	25.0 ± 0.08	22.8 ± 0.13	17.0 ± 0.14	-

Zone of Inhibition ± S.D.

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Table 8: Zone of Inhibition (mm) for Levofloxacin

Bacteria	50 µg/mL Levofloxacin	25 µg/mL Levofloxacin	12.5 µg/mL Levofloxacin	6.25 µg/mL Levofloxacin
Bacillus subtilis ATCC6633	24.0 ± 0.08	22.3 ± 0.10	20.3 ± 0.10	18.0 ± 0.08
Klebsiella oxytoca	-	-	-	-
Pseudomonas aeruginosa ATCC10105	22.8 ± 0.10	21.8 ± 0.10	19.8 ± 0.10	17.5 ± 0.06
Staphylococcus aureus ATCC 25923	25.3 ± 0.10	23.0 ± 0.08	21.3 ± 0.10	19.8 ± 0.10
Staphylococcus albus	24.5 ± 0.10	21.3 ± 0.10	20.5 ± 0.10	18.0 ± 0.10

Zone of Inhibition ± S.D.

Table 9: Zone of inhibition (mm) for *Acalypha wilkesiana*

	Zone of inhibition (mm) for red *Acalypha wilkesiana*			Zone of inhibition (mm) for green *Acalypha wilkesiana*		
BACTERIA	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**
Bacillus subtilis ATCC6633	22.8 ± 0.10	17.3 ± 0.10	-	17.0 ± 0.10	15.3 ± 0.06	12.7 ± 0.06
Klebsiella oxytoca	17.3 ± 0.10	14.0 ± 0.08	-	-	-	-
Pseudomonas aeruginosa ATCC 10105	23.0 ± 0.14	19.8 ± 0.10	17.8 ± 0.10	19.0 ± 0.10	17.7 ± 0.06	14.7 ± 0.06
Staphylococcus aerus ATCC 25923	17.8 ± 0.13	16.3 ± 0.10	15.0 ± 0.08	19.0 ± 0.10	17.0 ± 0.10	14.6 ± 0.06
Staphylococcus albus	10.0 ± 0.01	16.7 ± 0.05	14.7 ± 0.06	18.0 ± 0.10	16.3 ± 0.06	14.0 ± 0.10
FUNGI						
Microsporum canis	21.0 ± 0.10	18.0 ± 0.10	16.0 ± 0.10	20.0 ± 0.17	17.3 ± 0.06	14.6 ± 0.15
Malassezia furfur	22.0 ± 0.10	20.0 ± 0.10	18.0 ± 0.10	21.3 ± 0.06	19.0 ± 0.10	18.3 ± 0.06
Candida albicans	-	-	-	-	-	-
Trichophyton rubrum	-	-	-	-	-	-

Values are mean ± S.D.

Conclusion

This study reports the successful development of a topical organogel compatible with the two variants of *Acalypha wilkesiana.* Dermoscope readings have shown that the formulations are safe on intact skin showing no evidence of erythema. The antimicrobial assay of the organogel showed potential therapeutic efficacy in the treatment of microbial skin infections hence a potential formulation for management of topical fungal and bacterial infections.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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