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Formulation and evaluation of topical gels containing *Phyllanthus muellerianus* leaf extract using various gelling agents

Osei-Asibey Antwi[1,2], Mariam El Boakye-Gyasi[1], Yaw Duah Boakye[1], Raphael Johnson[1], Frederick William Akuffo Owusu[1], Aboagye Agyei Eugene[3], Michael Lawrence Obeng[1], Kofi Asamoa Mensa Acheampong[1], Winifred Naa Adoley[1]

[1] *Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[2] *Department of Pharmaceutics, School of Pharmacy, University of Health and Allied Sciences, Ho, Volta Region, Ghana.*

[3] *Pathology Department, Manhyia Government Hospital, Kumasi, Ghana*

Abstract

Introduction: The high expense of current pharmaceuticals used to treat wounds, as well as some of their adverse effects, has spurred the quest for alternatives, particularly those derived from natural sources that have minimum side effects, less microbial susceptibility and are less expensive. *Phyllanthus muellerianus* leaf extract incorporated in creams and ointments greatly decreased wound closure time and increased epithelialization at the wound site. This study aims to formulate and evaluate a gel made from *P. muellerianus*. **Methods**: Leaves of *P. muellerianus* were extracted using water. Phytochemical screening for tannins, flavonoids, alkaloids and reducing sugars was performed on the extract. The water extract was used to formulate twenty gels with varying gelling agents. Physicochemical analysis, toxicity, wound healing and stability studies were performed on the gels. **Results:** The extraction of *P. muellerianus* leaves yielded 13.1 %w/w. Only tannins, glycosides, saponins, sterols and triterpenoids were present. *P. muellerianus* gels (1 %w/v) were formulated with five different concentrations of each of four different gelling agents. The gels had satisfactory physicochemical properties, and the microbial load and drug content were within the acceptable range for herbal formulations. There was no indication of chemical interactions between the extract, polymer, and other excipients in Fourier transform infrared spectroscopy investigations. There were no significant changes in the pH, spreadability, viscosity and drug content of the gels throughout the stability assay period. Dermal toxicity studies revealed that the *P. muellerianus* gels were not toxic to the skin (acute and repeated dose dermal toxicity tests). Wounds treated with formulations A4 and C5 showed significantly decreased wound area from the fifth day to day 15 post-injury compared to the positive and negative control groups, with an increased rate of re-epithelialization, fibroblast proliferation, collagen deposition and neovascularization. **Conclusion:** Ultimately, *P. muellerianus* gels (A4 and C5) showed tremendous wound healing activity, stability and safety.

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*Corresponding author's email: oaantwi@uhas.edu.gh

Introduction

Damages to the normal anatomical state and form, which may or may not affect bodily functions, are classified as wounds. There could be a breach in the skin's epithelial structure, or it may be more significant, expanding into subcutaneous tissues and injuring relevant cells like muscles, arteries, parenchymal organs, tendons and nerves (Gushiken *et al.*, 2021). Underlying connective tissues, such as muscles, bones, or nerves, may be lost (Ding *et al.*, 2021). Wounds may be characterized in several approaches, such as location, level of contamination, wound depth, duration of the wound, type of injury, and presenting symptoms and tissue loss. The body has its ways and cascades of dealing with wounds; however, when it becomes infected, the wound healing process is aided by pharmaceuticals. Synthetic agents having antimicrobial, anti-inflammatory and analgesic properties are incorporated in topical dosage forms for wound cleansing, dressing and treatment. The high expense of current pharmaceuticals used to treat wounds, as well as some of their adverse effects, has spurred the search for new innovative drugs, particularly those derived from natural sources that have minimum side effects and are relatively less expensive (Bhuyan *et al.*, 2021).

Many plants with known wound healing activities exist, and such a plant is *P. muellerianus,* which has shown tremendous activity when formulated into various topical dosage forms such as ointments and creams. Wounds treated with 0.25, 0.5, and 1% w/w *P. muellerianus* extract significantly ($p < 0.001$) reduced wound area from day 5 to 11 post-injury compared to the untreated wounds. Furthermore, the area under the curve (AUC) revealed that 0.25, 0.5, and 1% w/w *P. muellerianus* extract significantly ($p < 0.001$) reduced wound area compared to the untreated wounds (Boakye *et al.*, 2018). Transdermal systems are convenient, inexpensive, self-administered and can provide a steady drug concentration profile for an extended period. They include patches, creams, ointments, lotions, etc., of which gels are the most preferred (Apriani *et al.*, 2023). Gels are made by capturing large volumes of water or hydroalcoholic liquid in a mesh of colloidal solid particles (Nayak and Bera, 2019). Gels are known to be soluble, do not retain sweat and dry faster than ointments and creams and are ideal for people with hairy skin. Cosmetically, they are acceptable and have a bigger aqueous component than an ointment or cream base, which allows for better drug solubility and easier drug migration in a vehicle that is almost a liquid. In terms of ease of use and patient acceptability, these are superior (Bhuyan *et al.*, 2021; Kabiri *et al.*, 2018). The current study aims to develop an effective gel formulation with the aqueous extract of the leaves of *P. muellerianus* and evaluate its wound healing activity. Its wound healing properties if found comparable to standard wound healing agents, would serve as a good option for wound treatment and ultimately, reduce the cost of producing topical wound healing medications for manufacturers in Ghana.

Materials and methods

Materials

The leaves of *Phyllanthus muellerianus* were acquired in Ghana in July 2021 from Kwahu in the Eastern Region. They were authenticated at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, with specimen voucher number KNUST/HMI/2021/L022. Carbopol 940, carboxymethyl cellulose (CMC), hydroxypropyl methyl cellulose (HPMC) and triethanolamine were obtained from UK chemicals, Kumasi. Calcium chloride, glycerol, propyl paraben, propylene glycol, methyl paraben and xanthan gum were also obtained from the stores of the Department of Pharmaceutics.

Method

Extraction of P. muellerianus leaves

Extraction was done as described by Boakye et al. (2018). Powdered *P. muellerianus* leaves (0.5 kg) were heated in 5 L of distilled water at 90 ^{o}C for fifteen (15) minutes. A vacuum rotary evaporator was used to concentrate the filtrate under decreased pressure at 45 ^{o}C after a Buchner funnel and Whatman no. 10 filter paper were used to filter the

extract. The extract was freeze dried, yielding a powdered substance which was stored in the desiccator until use.

Phytochemical analysis of P. muellerianus extract

The powdered extract of *P. muellerianus* was screened qualitatively for secondary metabolites following standard procedures according to Evans, (2009).

Test for saponins

A mass of 1 g of powdered *P. muellerianus* extract was mixed with 10 mL of water in a test tube. The mixture was filtered, and the filtrate was vigorously agitated and placed aside for five minutes and observed for froth formation.

Test for tannins

A mass of 0.5 g of powdered *P. muellerianus* extract was combined with 25 mL of boiling water and was allowed to stand for 5 minutes. The mixture was filtered and chilled. Ten drops of 1 % Lead acetate solution were added to 1 mL of filtrate. The presence of tannins was revealed by the development of a precipitate.

Test for flavonoids

Twenty milliliters (20 mL) of water were added to 1 g of powdered *P. muellerianus* extract, and the mixture was then filtered. After being dipped into the filtrate, a white filter paper strip was dried and subjected to hydrochloric acid vapors. The appearance of a bright yellow colour confirms the presence of flavonoids.

Test for alkaloids

A mass of 1 g of powdered *P. muellerianus* extract was weighed and dissolved in 1 % H_2SO_4. The solution was filtered. One drop of Dragendorff's reagent (potassium bismuth iodide solution) was added to 1 mL of the solution, and the appearance of an orange-red precipitate indicates the presence of alkaloids

Test for sterols

A mass of 1 g of powdered *P. muellerianus* extract was extracted with chloroform to obtain a $CHCl_3$ extract. Concentrated H_2SO_4 was carefully drizzled down the side of the test tube after two drops of acetic anhydride were added to 5 mL of the extract. A layer formation was observed in the sample.

Test for triterpenoids

A chloroform ($CHCl_3$) extract was obtained by extracting 5 g of powdered *P. muellerianus* extract with $CHCl_3$. A volume of 5 mL of $CHCl_3$ extract was gently poured down the edge into a test tube containing 1 mL concentrated H_2SO_4. The sample was observed for layer formation.

Test for reducing sugars

A mass of 0.5 g of *P. muellerianus* extract was reheated in 5 mL of diluted H_2SO_4 on a water bath for two minutes before being filtered. To the filtrate, four (4) drops of a 20% sodium hydroxide solution were added. The filtrate was mixed with 1 mL of Fehlings solutions A and B, which were then heated over a water bath for approximately two minutes. The appearance of a red precipitate confirms the presence of reducing sugars.

Formulation of P. muellerianus gels

Different masses (Table 1) of the gelling agents (Carbopol 940, CMC, HPMC and xanthan gum) respectively were carefully weighed and dispersed in 50 mL of a suitable solvent (deionised water for Carbopol and xanthan gum, distilled water heated to 70–90 ^oC for HPMC and 3 %w/v calcium chloride solution for CMC) in separate beakers. The beakers were set aside to allow the gelling agent to swell for half an hour and then stirred using a mechanical stirrer for 30 minutes. In 5 mL of propylene glycol, 1 g of the extract was dissolved, and in a separate beaker, 5 mL of glycerol was mixed with methyl and propylparaben. After all of the gelling agents had dispersed, 1 g of extract and preservative solutions were added to each of the gelling agent dispersions while constantly being stirred. Finally, the volumes were increased to 100 mL by adding more of the solvent. In the case of the Carbopol-based gels, triethanolamine was added in drops to the formulations to adjust the pH and consistency (Jamadar and Shaikh, 2017).

Table 1: Composition of *Phyllanthus muellerianus* gel formulations

Composition	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5
Extract/g	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Carbopol 940/g	0.25	0.40	0.50	0.75	1.0	-	-	-	-	-	-	-	-	-	-
HPMC/g	-	-	-	-	-	1.00	1.50	2.00	2.50	3.00	-	-	-	-	-
CMC/g	-	-	-	-	-	-	-	-	-	-	2.00	2.50	3.00	3.50	4.00
Propylene glycol/mL	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Glycerol/mL	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Methyl paraben/g	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben/g	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Triethanolamine/mL	qs	qs	qs	qs	qs	-	-	-	-	-	-	-	-	-	-
Deionised Water / mL	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Physicochemical evaluation of P. muellerianus gels

Physical appearance

The colour, homogeneity, and phase separation of the produced gel base and gel formulations containing the *P. muellerianus* leaf extract were visually evaluated. The texture of the gel was determined by rubbing it between the thumb and the middle finger.

Measurement of pH

Using a digital pH meter, the pH of the gel base and gel formulations was measured. One gram of gel was dissolved in one hundred millilitres of distilled water and allowed to stand for two hours. Each formulation's pH was measured in triplicate, and the mean was determined (Jamadar and Shaikh, 2017).

Spreadability

On two sets of standard-sized glass slides, a sphere with a diameter of 2.4 cm was produced. One gram of gel was sandwiched between the two slides and uniformly pressed to generate a thin layer in the centre of the sphere on the slide. The upper slide was loaded with a 100 g weight. Over one minute, the distance (new circumference to old circumference) generated by the gel spreading out under the impact of the weight was measured. The experiment was done in triplicate, with the average distance being used to determine the gel's spreadability (Helal et al., 2012).

Extrudability

The gel formulations were packed in a conventional collapsible aluminium tube with a capped end and crimped shut. The tubes' weights were recorded. The tubes were secured between two panes of glass. The slides underwent compression using a 500 g weight before the removal of the cover. After extrusion, the gel was gathered and weighed. The percentage of the extruded gel was then determined by using equation 1 (Jamadar and Shaikh, 2017):

$$\frac{wt\ of\ gel\ extruded}{wt\ of\ gel\ and\ tube - wt\ of\ empty\ tube} \times 100\% \qquad (\mathbf{1})$$

Drug content determination

One gram of *P. muellerianus* extract was dissolved in 50 mL of phosphate buffer, and after a series of suitable dilutions, the absorbance spectrum was scanned with a UV-visible spectrophotometer. The highest absorbance (peak) was determined at 279 nm and was used as a mark for the active constituent in the extract. One milliliter of each formulation was dissolved in 50 mL of phosphate buffer solution at pH 7.2. The resultant solution was adjusted to 100 mL in a volumetric flask with phosphate buffer at pH 7.2 after being filtered through Whatman number 1 filter paper. The resulting solution was appropriately diluted and using phosphate buffer with a pH of 7.2 as a blank, the absorbance was determined at 279 nm using a UV-visible spectrophotometer (Bhuyan *et al.*, 2021).

FT-IR analysis

At a scan resolution of 4 cm^{-1} and over a wave number range of 400 – 4000 cm^{-1}, IR spectra were developed for the dried powdered aqueous extract of *P. muellerianus* leaves and each of the *P. muellerianus* gel formulations with an ALPHA II FTIR Spectrometer (Amponsah et al, 2016).

Microbial content by pour plate method

Aseptically, 1 mL of the *P. muellerianus* gel was transferred into 9 mL sterile distilled water to achieve a 10-fold dilution. One millilitre (1 mL) of the 10-fold dilution was pipetted into sterile petri dishes labelled for viable aerobic bacterial and fungal counts, including pathogenic microorganisms such as *Staphylococcus aureus*, *Salmonella* species, *Pseudomonas aeruginosa* and *Escherichia coli*. Fifteen millilitre (15 mL) of stabilized Cetrimide agar, Sabouraud Dextrose agar, Mannitol Salt agar, MacConkey agar, Nutrient agar and Bismuth Sulphite agar were placed separately into their appropriate designated plates and swirled to mix. Aerobic viable bacterial and pathogenic organisms were cultured at 37 ^{o}C for 48 hours, while fungi were incubated at 25 ^{o}C for 5 days. The determination was performed in duplicates. The colonies were counted to determine the mean, then calculated for colony-forming units (cfu/ml or cfu/g).

Toxicity studies on dermal application

Test animals

Male Sprague Dawley rats (130-300 g) were purchased from the "Animal House" at the University of Ghana in Accra, Ghana. They were housed in steel cages where they were exposed to room temperature (25 °C), light, and 50 – 60 % relative humidity. Throughout the toxicity testing, they were fed rat meal and water from clean bottles on an ad libitum basis. The animals were given a week to adapt before each trial.

Skin irritation test

As specified in Organisation for Economic Co-operation and Development (OECD) standard 404, Sprague Dawley rats were used for the skin irritation test (OECD, 2015). Before each rat was caged separately, hair covering approximately 10 % of the entire back of each rat was cut with a razor blade. Skin abrasion was prevented by using only animals with intact skin. Twenty-eight (28) rats were used in this experiment. The treatment group consisted of twenty-four of the twenty-eight subjects, with each group of four getting one of the six optimal formulations, while the control group consisted of the remaining four. After that, the rats were given 72 hours to adjust to their new surroundings without being disturbed. In the treatment group, A uniform 1 mL of the gel was applied to the area that had been shaved. (about 6 cm^2). Gauze and non-irritating adhesive tape were used to keep it in place. In the control group, sterile water was applied to the shaved area, which was then secured with gauze and non-irritant tape. After a 4-hour exposure period, the covers were removed, distilled water was used to clean the test area, and the OECD scoring system was used to detect oedema and erythema symptoms at 1, 12, 24, 48, and 72-hour intervals (Nayeem *et al.*, 2021; Pedrosa *et al.*, 2017).

Repeated dose dermal toxicity test

This test was performed following the OECD guidelines 410 (OECD, 2015). About ten percent (10%) of the total surface area of the back of the rat, where the incisions will be made, was shaved. The animals were put into 7 groups of 4 rats each. These groups consisted of six (6) *P. muellerianus* gel-treatment groups and one (1) control group to which only sterile distilled water was applied. Each rat weighed between 100 and 200 g. The rats were then left alone for 24 hours before the application of the *P muellerianus* gel formulation. The test ingredient (1 mL from each formulation) was applied to the shaved region and secured with a porous gauze bandage and non-irritant adhesive tape twice a day for 21 days. The skin and fur, eyes and mucous membranes, and respiratory and behavioural patterns of the animals were observed daily. The test animals were reweighed every week. The histology of skin tissues was analysed after 21 days of the test.

Histological examination

Skin tissues were removed from two rats in each group and promptly fixed in a 10 % buffered neutral formalin solution. The tissues were placed into tissue cassettes immediately after fixation. Water was removed from the fixed tissues by concentrated ethanol solution treatment. The tissues were subsequently cleared by immersing them in various xylene concentrations to displace the ethanol. The tissues were then infiltrated with paraffin wax, which displaced the xylene in the tissues. After they had solidified, they were sliced into 5 µm thick sections with a Leica rotary microtome. The tissues were placed on cleaned glass slides and stained with haematoxylin and eosin after the paraffin was removed. After that, a light microscope was used to assess the glass slides to determine the extent of cell regeneration, re-epithelialization, and granular tissue formation. At a magnification of x40, photomicrographs were obtained (Talekar et al., 2012; Boakye et al., 2018).

Wound healing assay using P. muellerianus gel

The excision wound healing model in Sprague Dawley rats, as stated by Boakye et al. (2018), was employed to investigate the wound healing properties of the formulated *P. muellerianus* gel. Eight sets of five rats each received topical treatments of *P. muellerianus* gel and silver sulphadiazine cream (1 % w/w), while the untreated group's wounds were just cleansed with 0.9% w/v normal saline solution. The wounds were cleansed with 0.9 % w/v saline solution daily before the

topical application of the formulated gels (0.5 mL of gel per daily application). Any rat that exhibited signs of wound infection was excluded from the experiment. Wound scar tissues were harvested on day 15 post-injury and used for histological studies. Wound treatment began at twenty-four hours (24 hr) post-wounding. The wound diameter was measured with a millimeter rule on days 1, 3, 5, 7, 9, 11, 13, and 15 post-injuries. The percentage of wound contractions was determined and noted accordingly using Equation 2:

$$\% \text{ WC} = (\text{IWS} - \text{SDWS}) / \text{IWS} \times 100 \% \quad (2)$$

% WC = Percentage wound contraction

IWS = Initial wound size

SDWS = Specific day wound size

Histological examination

The histological examination was performed as described by Talekar et al. (2012) and Boakye et al. (2018). Two rats from each group on day 15 were anaesthetized with pentobarbitone, and tissues from the wound sites were taken. These tissues were fixed in 10 % buffered neutral formalin. Tissue preparation followed by immersion in ethanol, xylene and paraffin. Five micrometer (5 μm) thick sections were cut from the prepared tissue, washed in sterile distilled water and stained with haematoxylin and eosin stain. These were viewed with a light microscope. Photomicrographs were taken at x40 magnification.

Stability studies on P. muellerianus gels

The optimized formulations (A3, A4, A5, C3, C4, C5) were subjected to stability tests by the International Conference on Harmonisation (ICH) guidelines. The formulations were tested for short-term stability for three months. The samples were kept at various temperatures, including refrigeration (4 - 8 °C), room temperature (25 °C), and a temperature of 40 °C in the oven. Every month, a sample was taken and analysed for visual appearance, pH, spreadability, viscosity, and drug concentration.

Statistical Analysis

Irritation data was simply reported as visual ratings using the Draize erythema and oedema grading system, and PII was computed. GraphPad Prism was used for statistical analysis. In every analysis, a p-value of less than 0.05 was deemed statistically significant. To compare the mean of each formulation group to the control, data on wound healing were evaluated and represented as mean ± SEM, whilst data on body weight measures were expressed as mean ± SD and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

Results and discussion

Extraction and determination of physicochemical properties of extract

The percentage yield for the extracted sample was 13.1% w/w, which is close to that of the aqueous extraction yield of 14.1 %w/w reported by Boakye et al, 2018. A phytochemical examination of the milled leaves of *P. muellerianus* revealed the presence of compounds with medicinal and physiological properties. The presence of tannins, saponins, alkaloids, glycosides, and triterpenoids were detected in the milled leaves (Table 2). Saponins are known to have anti-inflammatory properties. They can precipitate and coagulate red blood cells as well (Kumari *et al.*, 2017). Triterpenoids are anti-inflammatory, antiviral, antibacterial, and antitumoral compounds that are implicated in the mechanisms of action of many therapeutic plants (Rios, 2010). Tannins are recognized to aid the healing of wounds by binding to proteins and other organic substances and precipitating them because of their astringent polyphenolic biomolecules such as geraniin which has been reported to be the major isolate of the aerial part of the plant known for its wound healing activity (Boakye et al., 2018; Li, 2011).

Table 2: Chemical constituents of the leaf extract of *Phyllanthus muellerianus*.

Phytoconstituents	Powdered Sample
Tannins	+
Glycosides	+
Saponins	+
Alkaloids	—
Flavonoids	—
Coumarins	—
Triterpenoids	+
Phytosterols	+

Keys :(+) Detected **(—)** Not Detected

Physicochemical evaluation of P. muellerianus gel

FT-IR analysis revealed the compatibility between the extract alone and a mixture of the extract with excipients. There were no interactions between the extract and the excipients, as indicated by the intact primary peaks and stretches of all the different functional groups in the gels (Fig 2).

The Carbopol-based gel bases were colourless and transparent, whereas the gel bases of HPMC and CMC were translucent and odourless. Saroha et al. (2013) reported that the gel base in which the active ingredients(s) are incorporated should possess the physicochemical parameters expected of the final gel (including ease of spread, no lumps, non-gritty and smooth to feel) (Fig 1). The Xanthan gum gel bases were hazy, grainy, and odorous and were thus omitted from the optimized gels. All of the gel formulations developed were homogeneous and free of lumps.

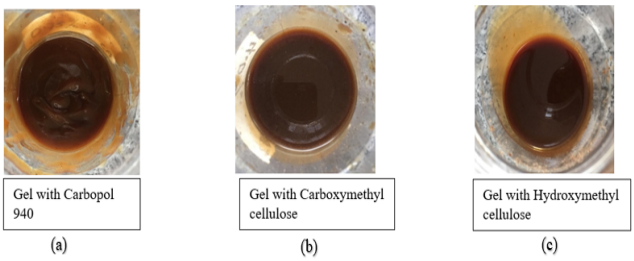

Fig 1: P. muellerianus gel with (a) Carbopol, (b) CMC and (c) HPMC

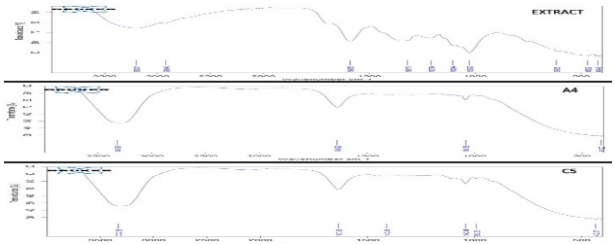

Fig 2: FT-IR of optimized gels (A4, C5) with *P. muellerianus* extract

The pH of human skin ranges from 5.5 to 6.8. To be evaluated for industrial usage, the pH of a formulation should be within or slightly above this range. Too acidic or alkaline pH levels can cause itching, redness, and scaly skin (Smaoui et al., 2017). The data suggests that as the gelling agent concentration increases, the pH of the resultant gel for the Carbopol bases increases (Table 3). The situation was unique with Carbopol-based gels since the gelling agent is naturally acidic and must be neutralized before the gel can be formed (chemical gelation). The concentration of the gelling agent employed affects the amount of neutralizers (TEA) required, which in turn influences the viscosity of the gel formed. Except for the xanthan-based gels, all of the formulated *P. muellerianus* gels had pH values that were within or slightly beyond the range of normal human skin, making them safe for usage (Table 3).

The spreadability of gels is important because it shows how the gel acts once it is removed from the packaging unit. The results obtained indicate that all of the polymers examined resulted in gels spreading by a modest amount of shear. Increasing the concentration of any of the gelling agents caused the spreadability to decline, as assessed by the smaller diameter of the spread circle (Table 3). The *P. muellerianus* gels were easily spreadable, according to the spreadability parameters of 15 – 20 gcm/s (Helal et al., 2012).

The extrudability of the gel formulations informs us of the ease with which the gels are removed from the packaging unit, which usually consists of tubes, with the application of a minimum force. More than ninety percent (90 %) of the packaged gels were extrudable, indicating excellent extrudability (Jamadar and Shaikh, 2017). Some had greater

Table 3: pH, Viscosity, spreadability, extrudability and drug content of *P. muellerianus* gels

Formulation	pH	Viscosity (centipoise)		Spreadability (gcm/s)	Extrudability	Percentage drug content
		Speed 3	Speed 6			
A1	6.55 ± 0.40	952.00±0.37	533.00 ±0.85	18.81	Excellent	91.51 ± 1.44
A2	6.31 ± 0.40	1121.00 ±0.19	772.00 ±0.23	18.11	Excellent	91.57 ± 1.47
A3	6.31 ± 0.39	2854.00 ±0.12	1768.00 ±0.66	17.87	Excellent	96.18 ± 1.38
A4	6.33 ± 0.36	3852.00 ±0.49	2631.00 ±0.02	17.76	Good	98.77 ± 1.83
A5	6.46 ± 0.31	6808.00 ±0.52	4715.00 ±0.50	17.53	Good	97.64 ± 1.32
B1	7.15 ± 0.27	711.00 ±0.21	205.00 ±0.12	23.03	Excellent	88.77 ± 0.15
B2	7.11 ± 0.29	1121.00 ±0.92	522.00 ±0.18	21.94	Excellent	89.17 ± 0.24
B3	7.12 ± 0.30	2010.00 ±0.61	1595.00 ±0.54	19.14	Excellent	91.19 ± 1.24
B4	7.18 ± 0.32	3190.00 ±0.53	2214.00 ±0.63	18.72	Good	85.90 ± 0.79
B5	7.21 ± 0.33	6401.00 ±0.18	3489.00 ±0.24	15.96	Good	87.22 ± 0.20
C1	6.62 ± 0.28	412.00 ±0.35	145.00 ±0.10	20.72	Excellent	99.22 ± 0.53
C2	6.77 ± 0.27	842.00 ±0.49	303.00 ±0.28	19.73	Excellent	101.17 ± 0.37
C3	6.69 ± 0.29	1770.00 ±0.70	900.00 ±0.71	18.94	Excellent	101.91 ± 2.97
C4	7.18 ± 0.02	2114.00 ±0.27	1150.00 ±0.18	18.34	Excellent	100.80 ± 1.72
C5	7.21 ± 0.03	3179.00 ±0.52	1276.00 ±0.24	18.17	Good	100.86 ± 2.14

Key: A1 – A5 = Carbopol Concentrations, B1 – B5 = HPMC Concentrations and C1 – C5 = CMC Concentrations

Table 4: The average weight of rats over the 21-day study period

Group	Weights of rats over 21-day study period			
	Day 1	Day 7	Day 14	Day 21
A3	138 ± 12.52	143 ± 12.17	151 ± 14.62	155 ± 12.34
A4	143 ± 13.85	151 ± 14.48	156 ± 14.29	163 ± 13.16
A5	133 ± 14.35	138 ± 13.24	145 ± 15.47	151 ± 12.72
C3	155 ± 15.31	162 ± 13.49	166 ± 15.94	168 ± 14.19
C4	141 ± 13.12	149 ± 15.62	153 ± 15.75	157 ± 12.86
C5	152 ± 13.78	155 ± 14.11	162 ± 15.51	169 ± 13.19
Control	159 ± 14.41	164 ± 13.87	168 ± 15.22	172 ± 15.25

than eighty percent (80 %) of the packaged gel extruded, indicating good extrudability. A few had greater than seventy percent (70 %) of the packaged gel extruded, indicating fair extrudability (Aiyalu et al., 2016). Some (0.8 %, 1.0 % and 1.2 % xanthan gel base) had less than seventy percent, indicating poor extrudability (Table 3).

The release of the active ingredient is also heavily influenced by numerous physical parameters, mainly the viscosity of the gel preparation. This is mostly related to the concentration of the gelling agents utilized in this study, such as Carbopol, CMC, HPMC, and Xanthan. Gels have a non- Newtonian fluid behaviour. Most non-Newtonian fluids undergo shear-thinning, which means that when shear stress increases, viscosity drops and could exhibit thixotropy (recoverable decreases in viscosity with stress over time) (Baviskar *et al*., 2013). In the study, increasing the concentration of the gelling agents resulted in a rise in viscosity for all gelling agents used (Table 3). However, increasing the speed (rate of shear) and concentration of active componentsresulted in a drop in viscosity. This demonstrates that the gels had a distinct shear-thinning feature, which makes it easy to apply.

The drug content of the gels was tested, and some of the Carbopol-based and all of the CMC-based gels ranged between 96.18 % and 101.91 % which was within the USP regulatory limits for herbal drugs (95 % – 105 %). The HPMC-based gels had the drug content below the acceptable limits (Table 3). The FT-IR of the HPMC-based gels did not reveal any interaction between the base and extract, or any other excipient and no evidence of physical interaction was observed. The data, however, suggests that the quantity of *P. muellerianus* extract decreased when incorporated in an HPMC gel base and therefore warrants further studies. The drug content analysis indicated that some of the gels contained acceptable amounts of the active *P. muellerianus* extract (Table 3) (Shiva *et al*., 2021; USP38/NF33, 2015).

Topical medications formulated with a herbal extract have specifications of the quantity of both pathogenic and non-pathogenic organisms permitted to be in them. For pathogenic organisms, none should be present in the formulation, while the quantities of non-pathogenic organisms allowed are specified (BP, 2018). No pathogenic organism was present in all the *P. muellerianus* gel formulations, and the non-pathogenic organisms were within the acceptable range for herbal formulations. Therefore, the gels can be used as a wound healing agent without the fear of wound infection and suppuration.

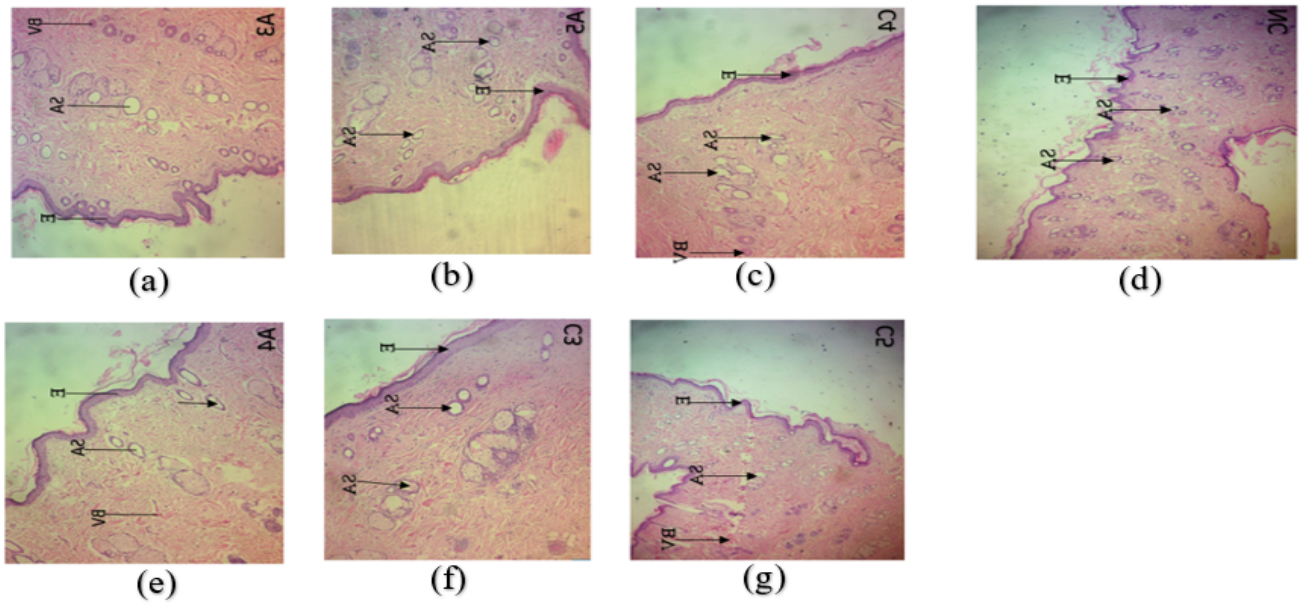

Fig 3: Histological images (x40) of excised skin tissues after 21-day treatment with *P. muellerianus* gels; (a) A3, (e) A4, (b) A5, (f) C3, (c) C4, (g) C5 and (d) negative control (NG)

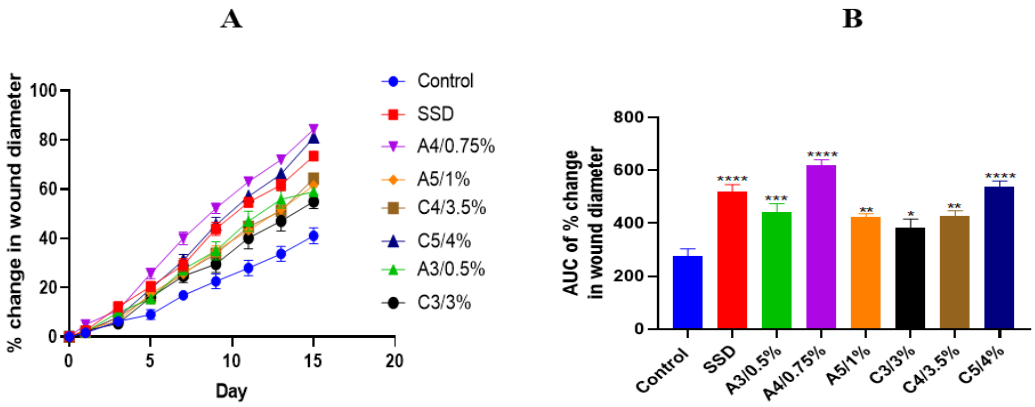

Fig 4: Influence of *Phyllanthus muellerianus* gel on the rate of wound closure

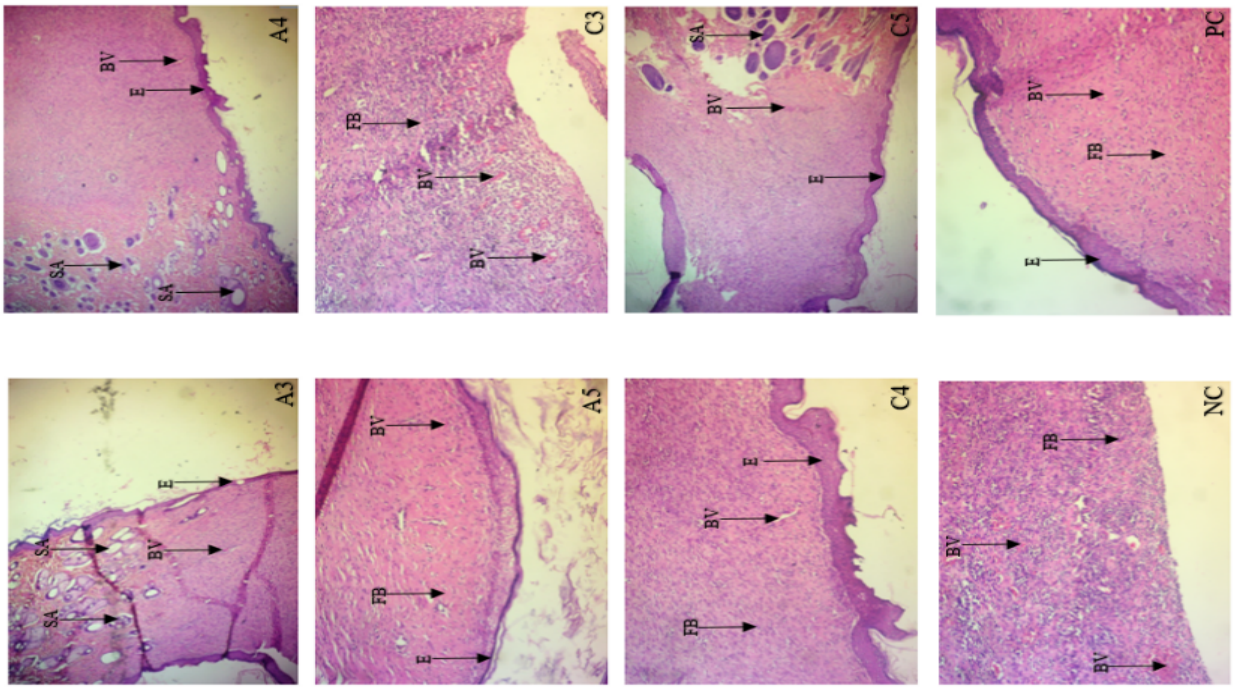

Fig 5: Histological images (x40) showing P. muellerianus gel activity in excised wound tissues from both treated and untreated rat wound

Dermal toxicity studies of P. muellerianus gel

The pharmacological ingredient present in topical formulations may have a negative impact on the skin, causing irritation and abrasion. As a result, toxicity studies on such medications are required in order to determine their safety. The gel formulations produced no oedema and redness on the rat skin 72 hours after it was applied, hence safe to use on the skin. Similarly, Ofokansi et al. (2018) found no irritation from *P. muellerianus* extract after it was applied externally to male Sprague-Dawley rats for 72 hours. Also, after the 21-day repeated dose assay, it was revealed that the relative organ weights of the test and control animals were statistically (p=0.999) similar (Table 4). Tissue histopathology after the study period revealed normal morphology of the skin (Fig 3). The epidermal layers of typical skin appendages and dermis were found to be intact in the skin tissues. In both the control and treatment groups, the hair follicles, sebaceous glands, and other skin appendages remained normal.

Wound Healing assay of P. muellerianus gel

In excised rat wounds, P. muellerianus gel demonstrated rapid cutaneous wound healing. The findings are comparable to those of an in vitro study by Boakye (2018), in which P. muellerianus showed increased fibroblast proliferation, angiogenesis, and granulation tissue formation, as well as

Table 5: pH, spreadability, viscosity and percentage drug content of optimized *P. muellerianus* gels for 3 months at room temperature

Gel Formulation	pH		Spreadability (gcm/s)		Viscosity (Centipoise)		Percentage Drug Content	
	Initial	Month 3	Initial	Month 3	Initial	Month 3	Initial	Month 3
A3	6.86 ± 0.19	6.72 ± 0.33[a]	17.87	17.69[a]	2854.00 ±0.12	2921.00±0.52[a]	96.18± 1.38	96.10±0.24 [a]
A4	6.99 ± 0.08	6.81 ± 0.61[a]	17.76	17.71[a]	3852.00 ±0.49	3885.00 ±0.73[a]	98.77± 1.83	98.49±0.13 [a]
A5	6.69 ± 0.10	6.69 ± 0.12[a]	17.53	17.60[a]	6808.00 ±0.52	6822.00 ±0.61[a]	97.64± 1.32	97.47±0.49 [a]
C3	6.73 ± 0.21	6.69 ± 0.49[a]	18.94	18.90 [a]	1770.00 ±0.70	1834.00 ±0.80[a]	101.91±2.97	101.72±0.15 [a]
C4	6.82 ± 0.30	6.87 ± 0.52[a]	18.34	18.39[a]	2114.00 ±0.27	2149.00 ±0.28[a]	100.80±1.72	100.50±0.35 [a]
C5	6.98 ± 0.43	6.98 ± 0.18[a]	18.17	18.28[a]	3179.00 ±0.52	3195.00 ±0.86[a]	100.86±2.14	100.78±0.05 [a]

Values are means ± SD (n = 3). Values were not significantly different from Initial at ([a]$p \geq 0.05$), and were significantly different from the Initial at ([c]$p < 0.01$) and ([d]$p < 0.001$).

Key: A3 - A5: Carbopol Concentrations, C3 - C5: CMC Concentrations

Table 6 pH, spreadability, viscosity and percentage drug content of optimized *P. muellerianus* gels for 3 months at refrigeration temperature

Gel Formulation	pH		Spreadability (gcm/s)		Viscosity (Centipoise)		Percentage Drug Content	
	Initial	Month 3	Initial	Month 3	Initial	Month 3	Initial	Month 3
A3	6.86 ± 0.19	6.80 ± 0.62 [a]	17.87	17.77 [d]	2854.00 ±0.12	2888.00±0.42[e]	96.18± 1.38	96.16±0.48[a]
A4	6.99 ± 0.08	6.91 ± 0.08 [a]	17.76	17.59 [d]	3852.00 ±0.49	3863.00±0.63[e]	98.77± 1.83	98.49±0.61[a]
A5	6.69 ± 0.10	6.52 ± 0.51 [a]	17.53	17.42 [d]	6808.00 ±0.52	6834.00±0.72[e]	97.64± 1.32	97.47±0.22[a]
C3	6.73 ± 0.21	6.64 ± 0.17 [a]	18.94	18.84 [d]	1770.00 ±0.70	1784.00±0.15[e]	101.91±2.97	101.72±0.05[a]
C4	6.82 ± 0.30	6.57 ± 0.62 [a]	18.34	18.3 [d]	2114.00 ±0.27	2179.00±0.75[e]	100.80±1.72	100.50±0.27[a]
C5	6.98 ± 0.43	6.71 ± 0.12 [a]	18.17	18.04 [d]	3179.00 ±0.52	3194.00±0.48[e]	100.86±2.14	100.78±0.21[a]

Values are means ± SD (n = 3). Values were not significantly different from Initial at ([a]$p \geq 0.05$) and were significantly different from the Initial at ([c]$p < 0.01$) and ([d]$p < 0.001$).

Key: A3 - A5: Carbopol Concentrations, C3 - C5: CMC Concentrations

Table 7: pH, spreadability, viscosity and percentage drug content of optimized *P. muellerianus* gels for 3 months at oven temperature

Gel Formulation	pH		Spreadability (gcm/s)		Viscosity (Centipoise)		Percentage Drug Content	
	Initial	Month 3	Initial	Month 3	Initial	Month 3	Initial	Month 3
A3	6.86 ± 0.19	6.59 ± 0.39 [a]	17.87	17.96 [e]	2854.00 ±0.12	2801.00±0.34[a]	96.18± 1.38	96.16±0.11[a]
A4	6.99 ± 0.08	6.46 ± 0.24 [a]	17.76	17.95 [e]	3852.00 ±0.49	3810.00±0.55[c]	98.77± 1.83	98.49±0.45[a]
A5	6.69 ± 0.10	6.43 ± 0.18 [a]	17.53	17.82 [e]	6808.00 ±0.52	6745.00±0.13[e]	97.64± 1.32	97.47±0.84[a]
C3	6.73 ± 0.21	6.50 ± 0.11 [a]	18.94	19.04 [e]	1770.00 ±0.70	1745.00±0.25[a]	101.91±2.97	101.72±0.61[a]
C4	6.82 ± 0.30	6.61 ± 0.15 [a]	18.34	18.47 [e]	2114.00 ±0.27	2092.00±0.11[a]	100.80±1.72	100.50±0.48[a]
C5	6.98 ± 0.43	6.57 ± 0.08 [a]	18.17	18.4 [e]	3179.00 ±0.52	3150.00±0.65[b]	100.86±2.14	100.78±0.50[a]

Values are means ± SD (n = 3). Values were not significantly different from Initial at ([a]$p \geq 0.05$), and were significantly different from the Initial at ([c]$p < 0.01$) and ([d]$p < 0.001$).

Key: A3 - A5: Carbopol Concentrations, C3 - C5: CMC Concentrations

significant re-epithelization and collagenation in the wound bed when compared to the untreated group, indicating that a P. muellerianus gel is an outstanding agent for wound care. Topical application of P. muellerianus gel (A3, A4, A5, C3, C4 and C5) resulted in a significant reduction ($p<0.001$) in wound size of excised rat wounds (Fig 4). This indicates a high rate of wound contraction. Histopathological examination confirmed the significant wound area reduction shown by P. muellerianus gel (A3, A4, A5, C4 and C5), which revealed increased neovascularization, fibroblast proliferation, and granulation tissue formation, as well as substantial collagen deposition and epithelial regeneration in comparison to the control (Fig 5). P. muellerianus gel-treated wounds produced fibroblast secretion that improved collagen deposition and crosslinking more than the untreated group. This aided in the faster healing of wounds and hence enhanced the tensile strength of the recovered skin.

Accelerated stability studies

The primary purpose of testing the stability of pharmaceuticals is to provide confidence that medicines will retain an acceptable level of quality during their duration on the market, and that they will be safe to use until the patient exhausts the product (Sengupta *et al.*, 2018). Organoleptic examinations of the formulations revealed no *muellerianus* gels in terms of colour, smell, texture, and homogeneity over the three-month stability study period. The gels remained clear, odourless, and uniform. The gels retained their clear, transparent, and translucent appearance, were smooth and homogeneous, and had no bad odour. Throughout the accelerated stability study, *P. muellerianus* gels remained stable and showed no significant alterations in pH, drug content, spreadability and viscosity when stored at room temperature (25 ^{o}C) (Table 5). However, there were statistically significant changes in pH, spreadability and viscosity from the second month when stored at higher (40 ± 2 ^{o}C) and lower (4-8 ^{o}C) temperatures (Tables 6 and 7). This suggests that storage at highly elevated and decreased temperatures, respectively, for a period of more than a month, will cause a marked decrease in product quality.

Conclusion

P. muellerianus gels with Carbopol 940 (0.75%w/v) and CMC (4 %w/v) as gelling agents had acceptable homogeneity, optimum pH values, good extrudability, viscosity and stability. The gels had no toxic effect on the skin after prolong usage, decreased wound closure time and promoted epithelisation and vascularization of the dermis. *P. muellerianus* gels could serve as an alternate wound healing agent and offer a scientific insight into future topical gel formulations.

Authors contributions

Study conception and design: OAA, MEBG, YDB, RJ
Data Collection: OAA, MLO, KAMA, WNA
Analysis and interpretation of results: OAA, MEBG, FWAO, AAE, RJ, YDB. Draft manuscript preparation: OAA, MEBG, FWAO

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Ethical approval statement

The animal study protocols were approved by the Animal Research Ethics Committee (AREC) of Kwame Nkrumah University of Science and Technology (KNUST 0041) and approved on August 02, 2023).

Conflict of interest

The authors declare that they have no conflict of interest with regards to publication of this paper.

Declaration of generative AI and AI-assisted technologies in the writing process

No AI (e.g. ChatGPT, Gemini and others) was used to improve readability and language during the preparation of this work.

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