African Journal of Pharmaceutical Research and Development
Available online at https://ajopred.com
Vol. 16 No.2; pp. 88-98 (2024)

Original Research Article

ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITIES OF THE LEAVES OF *ACALYPHA WILKESIANA* MUELL. ARG. (EUPHORBIACEAE) FORMULATED AS HERBAL CREAM

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ABSTRACT

The use of some traditional creams in Nigeria is associated with negative outcomes. In order to tackle this problem, we conducted a study on the antibacterial and anti-inflammatory characteristics of *Acalypha wilkesiana* leaves, which were utilized in the creation of a herbal cream. Standard methods were employed to conduct analytical procedures for the analysis of the phytochemical and chromatographic content of *A. wilkesiana* leaves. The anti-inflammatory efficacy of the unrefined ethanol extract was assessed using the egg albumin technique, throughout a dosage range of 15.6 to 500 mg/ml. The effectiveness of the herbal cream and the raw ethanol extract of *A. wilkesiana* in killing specific bacteria were evaluated. A comprehensive evaluation was carried out to determine the physicochemical characteristics of the herbal cream formulation using appropriate methodologies. The phytochemical examination of *A. wilkesiana* which gave a yield of 12.6 % indicated the existence of alkaloids, tannins, and saponins. The anti-inflammatory properties of the crude extract derived from *A. wilkesiana* had a comparable effect to that of aspirin, a commonly prescribed medicine at doses of 125, 250 and 500 mg/kg. The herbal cream (25.5 ± 0.22) and crude extract (19.5 ± 0.01) had the greatest antibacterial effectiveness against *Candida albicans* at 200 mg/ml. The physicochemical examination of the cream that was created: pH (5.6 ± 0.64); consistency (5 mm); viscosity (22 x 10^6 cps); spreadability (36 g.cm/sec); Extrudability (510 gm) showed similarities to the commercially available Gentamycin cream. The herbal cream containing *A. wilkesiana* has demonstrated substantial promise and could function as a practical alternative to traditional creams for treating skin infections.

ARTICLE INFO

Received 10 January, 2024
Accepted 11 July,, 2024
Published 17 July, 2024

KEYWORDS

Acalypha wilkesiana,
Euphorbiaceae,
Herbal cream,
Anti-inflammatory,
Antimicrobial

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https://doi.org/10.59493/ajopred/2024.2.9 ISSN: 0794-800X (print); 1596-2431 (online)

INTRODUCTION

The incidence of cutaneous diseases caused by bacterial and fungal species is increasing, especially in developing countries like Nigeria, characterized by high levels of humidity and insufficient hygienic conditions. The prevalence of drug resistance and the limited efficacy of conventional drugs in treating dermatological infections have prompted research into medicinal plants with anti-microbial properties against the primary organisms responsible for such infections. Medicinal plants, whether directly or indirectly, contribute to almost 25% of prescribed drugs in affluent nations [1].

The World Health Organisation (WHO) recognizes natural plant-based traditional medicine practices as reliable sources for therapeutic effects [2]. Researchers are redirecting their attention to the examination of traditional medicines, which are known for their safety and effectiveness. The World Health Organisation (WHO) has been promoting the use of medicinal plants since they are affordable and easily accessible, especially in impoverished countries [3].

Topical semi-solid dose (SSD) formulations are commonly found in the form of creams, gels, ointments or pastes. Pharmaceutical products comprise one or more active chemicals that are dissolved or evenly dispersed in a suitable base, along with other ingredients such as emulsifiers, viscosity-enhancing agents, antibacterial agents, antioxidants or stabilizing agents. Herbal creams have been successfully formulated by utilizing extracts derived from various plants like *Syzygium samarangense* [4], *Andrographis paniculata* [5], *Mangnifera indica* [6], *Hibiscus abelmoschus* [7], *Vernonia ambigua* [8] *and seed oil from Garcinia kola* [9].

In order to meet the demand for scientific knowledge on therapeutic plants, it has become essential to give comprehensive information on bioactive compounds, toxicological assessments, and pharmacological studies of these plants [10]. *Acalypha wilkesiana* Muell. Arg. (Euphorbiaceae), commonly referred to as Copper leaf, is a plant species native to the South Pacific Islands and widely cultivated in tropical and sub-tropical areas. It is also known as Jiwere in Hausa, awor-oso in Yoruba, and ogwu nra in Ibo [12]. In Southern Nigeria, the leaves are ingested as a vegetable for their medicinal properties in regulating hypertension [13]. In addition, they are employed for the treatment of infections and inflammatory conditions [14]. The indigenous communities in Ado town (Ekiti State) and Ilorin (Kwara State) in Nigeria use the leaves of *A. wilkesiana* as a natural remedy for nonspecific skin illnesses in neonates and children [12, 15]. The aqueous leaf extract of *A. wilkesiana* is commonly used in Western Nigeria as a temporary remedy for infant jaundice. It has the characteristic of inducing hyponatremia without any adverse impact on the liver. The extracted juice or infusion is used to treat gastrointestinal disorders and fungal skin infections, such as *Pityriasis versicolor, Impetigo contagiosa, Candida intetrigo, Tinea versicolor, Tinea corporis* and *Tinea apedis* [16]. Evaluating the capability of medicinal plants such as *Acalypha wilkesiana* Muell. Arg. (Euphorbiaceae) (Figure 1) which have established antibacterial properties and minimal negative impacts, as an alternative to conventional creams, would be highly advantageous for manufacturers, given the documented detrimental effects of orthodox creams. The research seeks to investigate the anti-inflammatory and antibacterial characteristics of the unrefined extract and formulated herbal cream derived from *Acalypha wilkesiana* leaves. Furthermore, its objective was to provide an overview of the physicochemical properties of the herbal cream that was created.

MATERIALS AND METHODS

Plant Collection and Processing

In June 2022, the leaves of *Acalypha wilkesiana* Muell. Arg. (Euphorbiaceae) were collected from the medicinal garden of the Faculty of Pharmacy at Madonna University, Elele Campus. Professor Henry Akinnobosun, a plant taxonomist from the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, authenticated the plant's identity. A voucher specimen labeled with the identification code UBH-A660 was deposited at that location. Following a 72-hour period of air-drying, the fresh leaves were pulverized using an electric grinder.

Preparation of Extracts

The 1.65 kg of powdered leaves from *A. wilkesiana* were extracted using the Soxhlet extraction method with 8 cycles of 2L of 95% ethanol. The solvent was removed by evaporation at decreased pressure using a rotary evaporator, resulting in a crude extract weighing 208 g. The extract was then stored in a refrigerator until it was needed.

Animals

The male Wistar rats weighing 212.00 ± 12.25g were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. All animals were treated in compliance with international guidelines for the use of animals in research and kept in standard environmental settings [17]. They were granted unrestricted availability of tap water and provided with regular pellets for sustenance. The study received ethical approval from the Animal Ethics Committee of Madonna University, Nigeria MAU/SREC/A/18.

Phytochemical Screening

Chemical and chromatographic techniques were employed during the initial phases of phytochemical screening to identify various types of phytochemicals [18]. These included alkaloids (alkaloidal salts and bases), tannins (both authentic and counterfeit tannins), and glycosides (cardiac, saponins, anthracenes, and cyanogenetic). The constituents of the extracts of *A. wilkesiana* were identified and separated via paper and thin-layer chromatographic methods [19]. Thin-layer chromatography (TLC) was utilized to analyze the crude ethanol extract of *A. wilkesiana* on silica gel-G. After being

heated at 110 degrees Celsius for 30 minutes, silica gel-G became active. A variety of solvent systems were employed in the TLC procedure. To ascertain the existence of alkaloids, UV light was applied to the chromatogram that was obtained, followed by the application of Dragendorff's spray reagent. Following this, R_f values were calculated. An ascending method was employed in conjunction with Whatman No. 3 mm paper for a paper chromatography procedure. Diverse proportions of the solvent system, which comprised acetic acid, water, n-butanol, were utilized. For sample evaluation, both natural light and UV light with a wavelength of 25 nm were utilized. To identify the existence of phenolic compounds, ferric chloride was employed to spray the samples. Further, the R_f values were calculated.

Determination of Anti-inflammatory Activity

Following a seven-day acclimatization period, 40 fully matured Wistar rats of both genders were placed into eight groups (A–H) of five animals each. Before the trial, the rats were starved for 12 hours but given water. Water and food were provided throughout the trial. Yunusa and co-worker's method was used to compare the *A. wilkesiana* extract to reference samples for anti-inflammatory activity [20]. Each group of rats received the extract or reference standards orally. Group A got normal saline as the negative control. Group B-G received 15.6, 31.3, 62.5, 125, 250, and 500 mg/kg *A. wilkesiana* extract. Group H received 100 mg/kg aspirin. After 30 minutes, each rat received 1 ml of fresh egg albumin intraperitoneally into its right hind paw sub-plantar [20]. The hind paw diameter was measured every 30 minutes with a Vernier Caliper. Measurements were taken progressively at 30, 60, 90, 120, 150, and 180 minutes. Using Equations 1 and 2, inflammation and inhibition percentages were calculated:

$$\% \, inflammation = \frac{\text{Ct X } 100}{\text{Co}} \text{.....Equation 1}$$

$$\% \, inhibition \, of \, inflammation = \frac{\text{Co} - \text{Ct X } 100}{\text{Co}}$$

.....Equation 2

where Ct is inflammation at the dose
Co is inflammation at normal saline

Formulation of Herbal Cream [21]

The ethanol extract of *A. wilkesiana* was used as the active ingredient in the herbal cream's formulation, along with additional non-bioactive substances listed in Table 1. Liquid paraffin and beeswax were heated to a temperature of 75 ^{o}C in a glass beaker, creating an oil phase. The process involved dissolving borax and methyl paraben in water by heating it to a temperature of 75 ^{o}C in a separate container known as the aqueous phase. The oil phase, which was heated to a temperature of 75 ^{o}C, was slowly combined with the aqueous phase. Upon the addition of the *A. wilkesiana* ethanol extract, thorough mixing ensued until a homogeneous paste was obtained.

Physicochemical Evaluation of the Formulated Herbal Cream

The commercial product and the cream containing *A. wilkesiana* extract were evaluated for their rheological and physical properties (irritancy, the pH level, uniformity, and consistency) [7, 21].

Calculation of Irritation

On the dorsal surface of the left hand, one centimeter was marked. Time recorded subsequent to lotion application. For twenty-four hours, irritation, erythema, and edema were observed.

Determining pH

A digital pH meter measured the pH of cream (0.5g) dissolved in 50 ml pure water.

To Test Consistency

The holding rod-attached cone was dropped from 10 cm to land in the middle of the measuring cylinder containing the made cream. Ten seconds later, the cone's distance was measured.

Homogeneity

The cream formulations' homogeneity was visually inspected and graded as +++ = Excellent, ++ = Very good, + = Good, and - = Poor.

Properties of Rheology Viscosity Measurement

A Brookfield viscometer with spindle number 4 at speed 6 and a coefficient of 1000 was used to measure the viscosity of the prepared cream. The cream was contained in a beaker, and the spindle was turned at a speed of thirty revolutions per minute. Following recording, the matching readings were multiplied by 1000.

Quick Stability Analysis

A stability chamber was used to store 5g of the cream at 45 ^{o}C, while another 5g of cream was stored at room temperature (27 ± 2 ^{o}C). After four weeks, the organoleptic qualities (color, odor, and texture), homogeneity, and after feel were evaluated.

Spreadability

An excess of the sample was sandwiched between two glass slides and squeezed for five minutes with a 100-gram weight to achieve a consistent thickness. The pan was filled with weight. Spreadability was determined by timing the movement of the upper glass slide across the lower plate, or the amount of time needed to separate the two slides (Equation 3).

S = (m X l)/t .....Equation 3

while m is the weight fastened to the top slide. l = length traveled on the glass slide. t = amount of time spent

Extrudability

The cream formulation was firmly squeezed into a closed, collapsible tube with a crimped end. The cream formulation extruded until the pressure subsided once the cup was removed. The weight in grams needed to extrude the cream formulation into a 0.5 cm ribbon in 10 seconds was calculated. It was stated how many grams the average extrusion pressure was.

Antimicrobial Test

The antimicrobial assay utilized clinical strains of Candida albicans (yeast) and four bacteria: *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*. These strains were obtained from the Madonna University Teaching Hospital (MUTH) Microbiology Laboratory in Elele, Rivers State. Charles Akwuroha, the Chief Laboratory Technologist at the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Madonna University, Elele Campus, ensured the purity of the culture via traditional morphological, cultural, and biochemical methods prior to use [22]. The microbial cultures were stored at 4 ^{o}C in Sabouraud Dextrose Agar for fungi and Nutrient Agar for bacteria.

Getting the Inoculum Ready

To create a microbiological suspension with a turbidity equal to 0.5 McFarland's criterion, an overnight culture was employed [23].

Diffusion Method using Agar Wells

For fifteen minutes, the media were prepped and sterilized at 121 ^{o}C. A bacterial culture was added to 30 milliliters of nutrient agar, allowed to solidify, and 10 mm-diameter wells were created on each plate. The prepared herbal cream was adjusted to 20 mg/ml and made in Dimethylsulfoxide (DMSO). Various concentrations of the herbal cream, ranging from 25 mg/ml to 200 mg/ml, were added to the open wells along with 10 mg/ml of gentamycin (a reference standard), and the mixture was incubated for 24 hours at 37 ^{o}C. In the antifungal experiment, Sabouraud agar was substituted for Nutrient agar, with amphotericin-B 10 mg/ml serving as the reference standard. The medium of choice was incubated at a temperature of 28 ^{o}C for duration of 48 hours. Every test was run in three duplicates. Measurements were made of the inhibitory zone diameters [24]. The experiment was conducted again with *A. wilkesiana* crude ethanol extract.

Analytical Statistics

The number of rats used is indicated by "n," and the data are presented as mean ± SEM. One-way analysis of variance was used to examine the variations in the means (ANOVA). P < 0.05 values were interpreted as suggesting statistical significance between the compared data.

RESULTS

Percentage of the Extract's Yield

The ethanol extract obtained from *Acalypha wilkesiana* powdered leaves was 12.6% w/w.

Screening of *A. wilkesiana* Leaves for Phytochemicals

Alkaloidal salts and bases were detected in the phytochemical screening of *A. wilkesiana* leaves (Table 2), as precipitates containing different alkaloidal reagents were present during extractions using polar and non-polar solvents. Both real (hydrolysable and condensed tannins) and pseudotannins were found. Among the glycosidal experiments, only cardiac glycosides were verified.

Thin-Layer Chromatography to establish chromatographic fingerprints using various solvent systems and detecting with Dragendorff (Table 3a) showed that Chloroform: Methanol: Hexane (3: 2: 1) was able to separate the alkaloids into two components with R_f values of 0.83 and 0.79.

Anti- Inflammation Activities

The effects of aspirin, a common medication, and the crude extract of *A. wilkesiana* on the inflammation of the hind paw oedema are displayed in Table 4 at 30-minute intervals over the course of three hours. The crude extract had a dose-dependent and time-dependent percentage reduction of inflammation up to 120 minutes. At doses of 125, 250, and 500 mg/kg, the percentage inhibition was comparable to that of aspirin. The crude extract exhibited anti-inflammatory activity at all concentrations. With the exception of 180 minutes, the activity was always greater than that of the reference medication at the 500 mg/kg dose.

Physicochemical Properties

Table 5 shows the physical and rheological properties of the herbal cream that was created, which contained *A. wilkesiana* crude extract and 1% w/v gentamycin cream.

Antimicrobial Efficacy

The inhibitory activity of *A. wilkesiana* against particular bacteria is displayed in Table 6a. Although it showed no effect on *E. coli,* the *A. wilkesiana* crude extract showed a wide range of actions. The developed herbal cream's inhibitory activity is displayed in Table 6b. The cream's profile was identical to that of the *A. wilkesiana* crude extract, although it showed more noticeable activity.

DISCUSSION

Even though inflammation is a normal and adaptive physiological response to pathogenic insults like microbial infection and tissue damage, the prevalence of chronic low-grade, systemic inflammation—the underlying cause of

Figure 1: Photograph of the leaves of *A. wilkesiana* growing at Madonna University, Elele Campus, Rivers State

Table 1: Composition of formulated herbal cream

Material Used	Quantity
Crude extract of *A. wilkesiana*	10g
Beeswax	7.5g
Liquid paraffin	25ml
Borax	0.5g
Methyl paraben	0.05g
Water	Q.S

Table 2: Phytochemical constituents of *A. wilkesiana* leaves

Classes of secondary metabolites	Inferences
Alkaloidal salts	+
Alkaloidal bases	+
True Tannins	+
Pseudo Tannins	+
Anthracene derivatives	-
Saponins glycosides	-
Cardiac glycosides	+
Cyanogenetic glycosides	-

Key: - = absent; + = present

Table 3a: *A. wilkesiana* leaf ethanol extract using thin-layer chromatography

Systems of solvents	Quantity of spots	Luminance during the day	Vibrance in UV Color	Color following Dragendorff spraying	R_f values
Methanol: Chloroform (3: 7)	1	Colourless	Light green	Reddish brown	0.81
Chloroform: Methanol: Hexane	2	Colourless	Light green	Reddish brown	0.83 & 0.79
Acetone: Water: Ammonia (90: 7: 3)	1	Colourless	Light green	Reddish brown	0.35
N-butanol: Acetic acid: Water (12: 3: 5)	-	Colourless	Colourless	Colourless	-
Ethylacetate: Methanol: Water (150: 26: 19)	1	Colourless	Light green	Reddish brown	0.77

Table 3b: Paper Chromatography of the ethanol crude extract of *A. wilkesiana*

Solvent systems	No. of spots	Colour in daylight	Colour in UV	Colour after spraying with ferric chloride	R_f values
N-butanol: Acetic acid: Water (12: 3: 5)	3	Colourless	Light fluorescent green	Blue black	0.41, 0.40 & 0.37
N-butanol: Acetic acid: Water (4: 1: 5)	2	Colourless	Light fluorescent green	Blue black	0.21 & 0.17

Table 4a: Results of average inflammation in diameter (mm) and percentage inflammation (in bracket)

Doses	30 mins		60 mins		90 mins		120 mins		150 mins		180 mins	
500 mg/kg	5.43±0.06	(76. 27±0.06)	5.17±0.28	(72. 61±0.28)	5.03±0.84	(70. 65±0.84)	4.71±0.05	(58. 57±0.05)	4.40±0.74	(61. 79±0.74)	4.21±0.34	(59. 13±0.34)
250 mg/kg	5.76±0.50	(80. 89±0.50)	5.48±0.16	(76. 96±0.16)	5.27±0.42	(74. 02±0.42)	5.03±0.64	(70. 64±0.64)	4.82±0.52	(67. 69±0.52)	4.28±0.94	(60. 11±0.94)
125 mg/kg	6.02±0.07	(84. 55±0.07)	5.78±0.25	(81. 18±0.25)	5.58±0.72	(78. 37±0.72)	5.16±0.84	(72. 47±0.84)	4.85±0.18	(68. 12±0.18)	4.52±0.06	(63. 48±0.06)
62.5 mg/kg	6.27±0.16	(88. 06±0.16)	6.08±0.00	(85. 39±0.00)	5.82±0.00	(81. 74±0.00)	5.54±0.82	(77. 81±0.82)	5.06±0.08	(71. 02±0.08)	4.60±0.04	(64. 61±0.04)
31.3 mg/kg	6.49±0.56	(91. 15±0.56)	6.33±0.44	(88. 90±0.44)	6.17±0.32	(86. 66±0.32)	5.88±0.02	(82. 58±0.02)	5.21±0.06	(73. 17±0.06)	5.02±0.38	(70. 51±0.38)
15.6 mg/kg	6.67±0.32	(93. 68±0.32)	6.54±0.12	(91. 85±0.12)	6.15±0.00	(86. 38±0.00)	5.81±0.08	(81. 60±0.08)	5.63±0.04	(79. 07±0.04)	5.22±0.28	(73. 31±0.28)
Normal* saline	7.12±0.03	(100±0.03)	7.04±0.11	(98. 88±0.11)	6.91±0.02	(97. 05±0.02)	6.83±0.07	(95. 92±0.07)	6.31±0.01	(88. 62±0.01)	6.00±0.07	(84. 26±0.07)
Aspirin (100 mg/kg)	5.95±0.07	(83. 57±0.07)	5.33±0.04	(74. 89±0.04)	5.17±0.12	(72. 61±0.12)	4.86±0.14	(68. 26±0.14)	4.37±0.02	(61. 38±0.02)	4.01±0.08	(56. 74±0.08)

Key: The data are presented as mean ± standard error of mean. The percentage of inflammation (oedema) caused by the different A. wilkesiana doses was significant throughout the assay period, with $p < 0.05$ when compared to normal saline (n = 5 per group). The values in brackets show the percentage of inflammation (oedema) at each time interval, with non-treated animals serving as the control group.

Table 4b: Results of percentage inhibition of inflammation (oedema) per time intervals

Doses	30 mins	60 mins	90 mins	120 mins	150 mins	180 mins
500 mg/kg	23.75±0.06	26.56±0.28	27.21±0.84	38.94±0.05	30.26±0.74	29.83±0.34
250 mg/kg	19±0.50	22.16±0.16	23.73±0.42	26.35±0.64	23.61±0.52	28.67±0.94
125 mg/kg	15.45±0.07	17.89±0.25	19.25±0.72	24.45±0.84	21.13±0.18	24.67±0.06
62.5 mg/kg	11.93±0.16	13.63±0.00	15.77±0.00	18.87±0.82	19.81±0.08	23.33±0.04
31.3 mg/kg	8.85±0.56	10.08±0.44	10.71±0.32	13.91±0.02	17.43±0.06	16.33±0.38
15.6 mg/kg	6.32±0.32	7.10±0.12	10.99±0.00	14.93±0.08	10.77±0.04	13.0±0.28
Normal * saline	0	0	0	0	0	0
Aspirin (100 mg/kg)	16.43±0.07	24.29±0.04	25.18±0.12	28.24±0.14	30.75±0.02	32.67±0.08

Key: The data are presented as mean ± SEM. Throughout the assay period, the percentage of inflammation (oedema) that was inhibited by the different doses of A. wilkesiana was significant, with a p-value of less than 0.05 when compared to normal saline. Each group has n = 5.

Table 5: Physicochemical properties of the formulated herbal and gentamycin creams

Parameter	Formulated herbal cream	Gentamycin cream
Irritancy	Nil	Nil
pH	5.6 ± 0.64	5.7 ± 0.02
Homogeneity	+++	+++
Consistency (60 sec)	5 mm	5 mm
Viscosity (cps)	22 x 10^6	20 x 10^6
Colour	Green	White
Odour	Agreeable	Agreeable
Texture	Absence of lumps	Absence of lumps
Spreadability (g.cm/sec)	36	37
Extrudability (gm)	510	510

Key: Homogeneity: +++ Excellent, ++ Very Good, + Good, - Unsatisfactory, Irritation: +++ Severe erythema, ++ Moderate erythema, + Slight erythema, - No irritation

Table 6a: Antimicrobial activity of *A. wilkesiana* crude ethanol extract

Test organisms	Diameter of zones of inhibition (mm)						
	25 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	Gm (10 mg/ml)	Ap (10 mg/ml)
B. subtilis	12.0±0.02	16.5±0.55	18.0±1.42	19.5±0.20	22.0±0.24	13.0±0.06	ND
S. aureus	11.5±0.20	13.0±1.08	13.5±0.55	14.0±0.01	16.0±0.25	15.0±0.50	ND
E. coli	G	G	G	G	G	13.0±0.45	ND
P. aeruginosa	8.5±0.26	10.0±0.01	12.0±0.04	12.5±1.54	13.5±0.44	17.0±0.26	ND
C. albicans	14.5±0.24	16.0±0.56	17.0±0.02	18.5±0.05	19.5±0.01	ND	18.0±0.42

Key: The values are presented as mean ± SEM, where (G) stands for no inhibitory zone, (ND) for not determined, (Ap) for amphotericin B, and (Gm) for gentamycin. There are three values in the set.

Table 6b: Antimicrobial activity of the formulated cream containing *A. wilkesiana*

Test organisms	Diameter of zones of inhibition (mm)						
	25 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	Gm (10 mg/ml)	Ap (10 mg/ml)
B. subtilis	12.5±0.05	18±1.35	19.0±0.75	21.0±0.05	22.5±0.24	13.0±0.06	ND
S. aureus	11.5±0.01	13.5±0.06	15.0±1.50	16.5±1.25	17.5±0.75	15.0±0.50	ND
E. coli	G	G	G	G	G	13.0±0.45	ND
P. aeruginosa	10.0±0.84	11.5±1.04	13.0±0.22	14.0±0.72	15.5±0.05	17.0±0.26	ND
C. albicans	17.0±0.52	20.5±0.04	22.0±0.04	23.5±1.25	25.5±0.22	ND	18.0±0.42

Key: The values are presented as mean ± SEM, where (G) stands for no inhibitory zone, (ND) for not determined, (Ap) for amphotericin B, and (Gm) for gentamycin. There are three values in the set.

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several extremely common chronic metabolic diseases—has made research into the role that inflammatory processes play in the pathogenesis of disease necessary [20, 25]. Acute inflammatory responses mediated by immune system cells are considered advantageous if they take place in a regulated and restricted way. This is due to the fact that they respond to pathogenic stimuli by coordinating the resolution and activation of pro-inflammatory leukocyte activity, which restores homeostasis to the afflicted tissue [25]. However, if the body is unable to start and control acute inflammatory responses properly, it can lead to a harmful long-term inflammatory tissue condition. Because of leukocyte-mediated tissue damage, this syndrome is characterized by pathological tissue remodeling, fibrosis and decreased function in addition to continuous infiltration and activation of inflammatory cells [26]. Eggs may induce inflammation since they are a high-protein and high-lipid food in the body [27]. Bioactive substances found in eggs include proteins, phospholipids, cholesterol, and lutein. These components have a broad variety of pro- and/or anti-inflammatory properties, which could have a big impact on how the body responds to injury and how many chronic disorders arise. According to the study's findings, the inflammatory response that appeared in the rats' paws was brought on by egg albumin.

Rats administered 500 mg/kg of *A. wilkesiana* extract showed a significant decrease in inflammation, according to the study's findings. Rats given 100 mg/kg of aspirin experienced a similar drop. Inflammation levels across all doses of *A. wilkesiana* extract were statistically significantly reduced as compared to normal saline. *A. wilkesiana* extract at its maximal dosage of 500 mg/kg was shown to peak in 120 minutes (38.94%), while aspirin reached its peak effect (32.67%) at 180 minutes. A 38.94% decrease in inflammation was the *A. wilkesiana* extract's greatest effectiveness. This suggests that the mechanism of action of aspirin is probably similar to that of the ethanol leaf extract of *A. wilkesiana* with respect to its anti-inflammatory properties. Prostaglandins and thromboxanes are two inflammatory mediators that are produced by the cyclooxygenase (COX) enzymes COX-1 and COX-2, which are inhibited by this process [27, 28].

The COX-1 and COX-2 cyclooxygenase enzymes are inhibited by aspirin. These enzymes generate thromboxanes and prostaglandins, two different kinds of inflammatory mediators. Aspirin is one of the most often used drugs in the world because it can prevent the production of prostaglandins and thromboxanes. Second, aspirins are part of the group of pharmaceuticals called nonsteroidal anti-inflammatory drugs (NSAIDS), which hinder the formation of prostaglandins, particularly PGE2, by blocking COX-2 [28].

By determining their pH values, the creams' suitability for topical use on skin was evaluated. Extremely low pH levels, which are too acidic for the skin, can cause hypersensitivity reactions and other problems [5]. A high pH, on the other hand, indicates alkalinity and can lead to negative skin reactions and irritations, including rashes. The pH value of the gentamycin cream was 5.7 ± 0.02, but the pH of the herbal cream that was produced was 5.6 ± 0.64. Given that a pH of 5.5 is thought to be the ideal range for medications used for topical application, our findings are within permissible bounds [5, 29]. In order to maintain a sufficient concentration at the site of action, topical creams' active ingredients must be released at the skin's surface and permeate the skin in a suitable and timely manner [29]. The creams containing gentamycin and herbal ingredients exhibited good spreadability and extrudability ratings, indicating that they needed fewer shears to spread. This made it possible for the creams to ensure that the right amounts of the active components were present at the site of action.

Further supporting the claim that *A. wilkesiana* has potent antibacterial qualities were the investigation's results. Our knowledge of *A. wilkesiana's* therapeutic potential has been substantially expanded by the methanol extract's demonstrated efficacy against most of the species under investigation [30]. This is especially true for infections that are treated within hospitals as well as those that are acquired outside of them. The fact that some organisms were impervious to it, according to Oluremi and co-worker's [31], is another proof that antimicrobial agent resistance needs to be controlled rather than completely eliminated. This is due to the fact that some species are resistant to the agents by nature.

The crude extract of *A. wilkesiana* showed only moderate antibacterial activity against *P. aeruginosa* and ineffectiveness against *E. coli*, but high antibacterial activity against Gram-positive bacteria (*S. aureus* and *B. subtilis*). This set of data is in line with the findings published by Haruna [30], who found that, in contrast to the activities

recorded against Gram-negative organisms, the crude extract of *A. wilkesiana* obtained from ethanol demonstrated a considerable degree of activity against Gram-positive bacteria. Furthermore, at all tested concentrations, the crude extract of *A. wilkesiana* reduced the growth of *Candida albicans*, indicating strong antifungal activity. Both Ogunjobi and Abiala [32] have demonstrated the plant's efficacy against fungi, lending credence to the theory that *A. wilkesiana* may be used to treat fungus-related skin diseases and disorders.

The unrefined ethanol extract did not yield the same benefits as the manufactured herbal cream containing *A. wilkesiana*. Higher levels of activity against the examined organisms were demonstrated by the cream. The increased antibacterial activity of the herbal cream formulation may be due to some constituents' established antimicrobial properties. For example, studies in the past have looked at the antifungal and antibacterial properties of beeswax [33, 35]. The profusion of phytochemicals present in *A. wilkesiana* leaves may be responsible for a variety of biological actions, such as antibacterial and anti-inflammatory properties. Alkaloids, which included salt and base, glycosides, which included saponins, and polyphenolic compounds, which included tannins, were found in the sample during the early screening of phytochemicals. Higher plants produce polyphenols, which are secondary metabolites. Their many functions as compounds that are antibacterial, anti-inflammatory, anti-allergic, anti-cancer, and antihypertensive, among others, greatly enhance plant physiology and may even be advantageous to people [36]. The largest single class of secondary metabolites in plants is comprised of species categorized as alkaloids. These chemicals often cause harm to people and have a wide spectrum of pharmacological effects, some of which can be fairly dramatic [37, 38]. Several pharmacological activities of alkaloids have been demonstrated, such as emetic, anti-cholinergic, anticancer, diuretic, sympatho-mimetic, antiviral, antihypertensive, hypno-analgesic, antidepressant, muscle-relaxant, antimicrobial, and anti-inflammatory qualities [37, 38].

CONCLUSION

The study has shown that the decorative plant *Acalypha wilkesiana*, which is grown throughout the world and in Nigeria, is a rich source of phytochemicals that give it antibacterial and anti-inflammatory qualities. The specifications of the herbal cream comply with the guidelines established by the Pharmacopoeia..

CONFLICT OF INTEREST

No conflict of interest is associated with this work.

AUTHORS' CONTRIBUTION

AAT designed and coordinated the research work; hence he is the Corresponding Author; OCA formulated the herbal cream and also carried out the physicochemical evaluations of the creams; AO carried out the anti-inflammatory experiments; EIF collected and processed the plant and also carried out the preparations of the extract; IW carried out the phytochemical and chromatographic experiments.

FUNDING

This research was funded by the Petroleum Technology Development Fund (PTDF).

ACKNOWLEDGMENT

The authors would like to express their sincere gratitude to Mrs. Ngozi E. Amuchie, who works in the Madonna University in Nigeria's Faculty of Pharmacy's Department of Pharmaceutics and Industrial Pharmacy.

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