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Research Article

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Formulation of an Antimicrobial Ointment Based on Senna alata (L.) Leaves Roxb (Fabaceae) for the Topical Treatment of Ringworm and Tinea

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ABSTRACT

Fungal skin infections such as ringworm and tinea capitis represent a public health concern due to resistance and side effects of conventional treatments. This study aims to formulate an antimicrobial ointment based on the leaves of Senna alata harvested along the banks of the Benue River in the northern region of Cameroon and to evaluate the in vitro activity of this extract against the causative agents of ringworm and tinea capitis. Chemical group screening was performed using a basic qualitative phytochemical analysis based on staining tests. The reference clinical microbial strains consisted of Malassezia. furfur (ATCC 14521), Trichophyton rubrum (ATCC 28188), Microsporum *M. canis* (ATCC 36299) and *M. canis* (ATCC 25923) were acquired from certified collections. The fungal strains were cultured on Sabouraud Dextrose Agar (SDA) and the bacteria on Mueller-Hinton Agar (MHA). The results show that the crude ethanolic extract of the leaves revealed the presence of six chemical groups: flavonoids, tannins, anthraquinones, saponins, alkaloids, and steroids/ triterpenes. The extract demonstrated promising antimicrobial activity against all the tested strains. This extract proved effective by completely inhibiting the growth of *M. furfur* and *M. canis*. (MIC = 31.25 µg/ mL.); T. rubrum (MIC = 125 µg/ mL.) as well as S. aureus (MIC = 250 µg/ mL). Through its antimicrobial efficacy, ointments formulated with the ethanolic extract of S. alata leaves have shown significant dose-dependent antimicrobial activity in vitro. The P2 formulation (1%) demonstrated the largest zones of inhibition for all tested strains. Clinical observations revealed complete patient recovery after 19 days of treatment for tinea cruris and 26 days of treatment for ringworm. This preliminary work paves the way for dermatological safety research and in vivo clinical trials to validate this ointment as a natural and effective therapeutic option for targeted skin conditions.

Keywords: Senna Alata, Ringworm, Tinea, Ointment Antimicrobial, Topical Treatment, Pharmaceutical Formulation

Introduction

Human skin covers the entire body and is therefore the organ that forms the body's external protection (Dréno , 2009). With an average surface area of approximately two square meters, it is among the largest organs in the body. It is an organ composed of several layers of tissue and constitutes the main interface between the body and the environment [1]. These functions are fundamental to a person's balance and well-being [2]. It plays a dual role as an envelope, notably as a sensor and transmitter of sensations, as well as a protective barrier against harmful foreign substances and a regulator of body [3,4]. Furthermore, a link between the skin's immune system and the psyche has been established [5]. Thus, healthy skin is a major asset for facing all environmental challenges.

Skin infections, particularly superficial fungal infections such as ringworm caused by Malassezia furfur and ringworm caused by Trichophyton rubrum and Microsporum Fungal infections affect millions of people worldwide, with a high prevalence in hot and humid climates [6]. These conditions cause significant discomfort, cosmetic disfigurement, and, in some cases, can lead to stigmatization [5]. Conventional topical antifungal treatments are often effective, but prolonged use can lead to local side

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effects, resistance, and high costs, limiting their accessibility in many communities [7].

Senna alata (L.) Roxb . is a tropical shrub of the Fabaceae family used as an ornamental and medicinal plant for its therapeutic properties [8]. Native to Central America and Africa, this species has gradually spread to other tropical regions of the world [9]. The World Health Organization estimates that more than 80% of the world's population uses medicinal plants for primary healthcare [10].

In several regions of Cameroon, various preparations made from parts of this plant are commonly used by the population for their medicinal properties in treating certain conditions such as eczema, dermatitis, ringworm, asthma, and bronchitis. In the literature, extracts of S. alata have demonstrated antimicrobial and antiviral properties (Adedayo. et al 2008), antioxidants (Priya et al., 2021), strong antimicrobial activity against various species of dermatophyte fungi [11]. In this context, traditional medicine, rich in ancestral knowledge, offers promising prospects for the development of new therapies.

Despite its documented traditional use, the formulation of standardized topical products, such as ointments based on S. alata extract, and their scientific evaluation remain limited. The main objective of this work is to contribute to the formulation of an antimicrobial ointment based on the ethanolic extract of S. alata leaves, to evaluate the in vitro activity of this extract against the etiological agents of ringworm and tinea capitis, and to propose a natural, effective, and potentially more accessible alternative for the topical treatment of these dermatoses.

Materials and Methods

Plant Material

S. alata samples were collected along the banks of the Benue River in the North Region of Cameroon, and a herbarium was then created. Identification was carried out at the National Herbarium of Cameroon, where a specimen had been preserved. The leaves from these samples were carefully washed under running water to remove impurities and then dried in the shade in a well-ventilated area for 14 days to preserve the heat-sensitive compounds. The dried leaves were then ground into a fine powder using an electric grinder (Retsch-SK100) with a particle size of less than 250 µm. The resulting powder was stored in airtight jars in a refrigerator, protected from humidity (4°C), until use.

Extract Preparation

A 100g sample of S. alata leaf powder was macerated in one liter of ethanolic solution (70% v/v ethanol). This ethanol concentration was chosen based on literature indicating that it is optimal for the extraction of a wide range of secondary metabolites, including flavonoids and anthraquinones [12]. Maceration was carried out for 72 hours at room temperature with intermittent stirring using a magnetic stirrer. The extract was then separated by wringing in a clean muslin cloth, and the filtrate was subsequently filtered through absorbent cotton and finally through 1 mm Whatman filter paper to remove fine particles. The resulting clear filtrate was concentrated under reduced pressure at 50°C to avoid the degradation of heat-sensitive compounds using a rotary evaporator (Büchi R-210) to obtain a dry crude extract. The extraction yield was calculated to be 18.2 ± 0.5% (w/w) relative to the initial dry powder.

Phytochemical Screening

Qualitative phytochemical screening of the dry crude extract of S. alata was performed to identify the main classes of secondary metabolites, in accordance with standard methods [13]. The following tests were carried out:

- **Alkaloids:** Mayer's test (formation of a creamy-white precipitate) and Wagner's test (formation of a reddish-brown precipitate) were used.
- **Flavonoids:** The Shinoda test (reaction with magnesium and concentrated HCl, producing a pink, orange or red color) and the ferric chloride test (FeCl3, appearance of a green, blue or black color) were used.
- **Tannins:** The ferric chloride test (FeCl3, appearance of a blue-black or green-black color) and the gelatin test (formation of a white or yellowish-white precipitate) were carried out.
- **Saponosides:** The foam test (formation of a persistent foam after shaking) was used.
- **Steroids and Triterpenes:** The Liebermann- Burchard test (appearance of a blue-green to red-violet color) was carried out.
- **Anthraquinones:** The Bornträger test (extraction with benzene followed by the addition of ammonia, producing a pink or red color in the ammonia layer) was applied.

Evaluation of the Antimicrobial Activity of the Extract in Vitro

The antimicrobial activity of the S. alata extract was evaluated against reference microbial strains acquired from certified collections: Malassezia furfur (ATCC 14521), Trichophyton rubrum (ATCC 28188), Microsporum *Canis* (ATCC 36299) and *Staphylococcus aureus* (ATCC 25923) were used. Fungal strains were cultured on Sabouraud Dextrose Agar (SDA) and bacteria on Mueller-Hinton Agar (MHA). Microbial suspensions were prepared from fresh cultures and adjusted to a turbidity equivalent to 0.5 on the McFarland scale, corresponding to approximately 1.2×10^8 CFU / mL for bacteria and 1.5×10^6 CFU / mL for fungi. Two methods were used:

- **Disc Diffusion Method:** Sterile 6 mm diameter paper discs were impregnated with 20 µL of the extract diluted to various concentrations ranging from 250 to 2000 µg/ mL. These discs were placed on pre-inoculated agar plates. The plates were incubated at 30°C for fungi for 48 to 72 hours and at 37°C for bacteria for 24 hours. The diameters (mm) of the inhibition zones were measured using a transparent ruler.
- **Microdilution Method:** 96-well microplates were used. Serial dilutions of the extract (2000 to 7.8 µg/ mL) were prepared in a suitable broth. Each well was then inoculated with a standardized microbial suspension. The plates were incubated under the same conditions as for disk diffusion. The Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of extract that inhibited any visible growth. To determine the Minimum Fungicidal Concentration (MFC) or Bactericidal Concentration (MBC), 10 µL from each well without visible growth were subcultured onto extract-free agar and incubated. Ketoconazole (0.01–10 µg/ mL) served as a

positive control for fungi, and gentamicin (0.01–10 µg/ mL) for Staphylococcus aureus. One well containing only the culture medium and inoculum served as a growth control, and another well with the culture medium alone served as a sterility control.

Formulation of the Antimicrobial Ointment

The hydrophilic ointment base was prepared by melting at 70°C, according to a standardized formula to ensure good cosmetic quality and suitability for cutaneous application. The base consisted of cetostearyl alcohol (8.7%), white petrolatum (43.47%), liquid paraffin (39% v/v, average density 0.85 g/ mL), and polysorbate 80 (30.44%). The dry crude extract of S. alata was finely micronized using a ceramic mortar and pestle to ensure uniform dispersion, then incorporated at final concentrations of 0.5%, 1%, and 2% for formulations P1, P2, and P3, respectively, into the melted base, which was maintained at 60°C. The ointment was incorporated under constant stirring at 200 rpm for 15 minutes using a mechanical stirrer (IKA Eurostar 20 digital) until completely homogenized. The ointment was then allowed to cool gradually to room temperature while being stirred at a reduced speed (50 rpm) to prevent phase separation and ensure a uniform texture. A control ointment (Formulation PT) was prepared identically, but without the incorporation of the extract, serving as a negative control.

Treatment of Skin Conditions

This section describes a preliminary observational study, not a randomized clinical trial. Observations were conducted on patients with confirmed cases of ringworm or tinea capitis who had given their informed consent. The antimicrobial ointment was applied daily to the affected areas. This is consistent with the twice-daily application of existing modern topical antifungals [14]. The practical test took place on five individuals from May 1st to 30th, 2025. During this test, photos of the testers were taken regularly every Sunday to visually document the evolution of the lesions.

Physico-Chemical and Rheological Characterization of the Ointment

The formulated ointments underwent rigorous testing to evaluate their physicochemical and rheological properties, which are essential for their performance and stability. The measured parameters were performed in triplicate at 25°C.

- **Homogeneity:** This was assessed visually for each formulation to detect any particle aggregation or phase separation. In addition, a thin layer of ointment was spread on a glass slide and examined under a light microscope (100x magnification) to verify the uniformity of particle dispersion in the base.
- **pH:** The pH was measured on a 1% (w/v) suspension of each ointment in distilled water. A digital pH meter (Hanna Instruments HI2211 model), previously calibrated with buffer solutions of pH 4.0, 7.0 and 10.0, was used.
- **Spreading:** The spreading capacity was determined using the spreading diameter method. One gram of ointment was placed in the center of a lower glass plate. Another upper glass plate, weighing 100 g, was placed on top of the ointment. After one minute, the diameter of the spread was measured. Additional 100 g weights were added successively every minute until a total weight of 1 kg was reached, and the corresponding diameters were recorded [15].
- **Consistency:** This was determined using a cone penetrometer (Koehler Instrument Company, K23570) equipped with a standard cone (90° angle, 50g mass). The ointment was placed in a container and maintained at 25°C for at least two hours before the test. The penetration depth of the cone into the ointment (mm) was measured after five seconds of immersion [12].
- **Stability:** The formulated ointments and the control ointment underwent accelerated and long-term stability studies. Samples were stored in airtight containers under different conditions: 4°C (refrigerator), 40°C (oven) and at room temperature (25°C) for a period of three months.

Every two weeks during the first month and then monthly, the visual appearance (color, odor, homogeneity, presence of phase separation), pH and spreading were monitored to detect any signs of physical or chemical instability.

Evaluation of the Antimicrobial Activity of the Ointment

The antimicrobial activity of the formulated ointments was evaluated by the well diffusion method on agar plates. Agar plates were prepared and uniformly inoculated with standardized microbial suspensions (0.5 McFarland). Wells 6 mm in diameter were made in the agar using a sterile punch. 50 µL of each formulated ointment (P1, P2, P3) and the control ointment (PT) were added directly to the wells. Wells containing the positive controls (2% ketoconazole cream and 0.1% gentamicin cream) were also included for comparison. The plates were incubated at 30°C for fungi for 48–72 hours and at 37°C for bacteria for 24 hours. After incubation, the diameters of the inhibition zones (mm), including the well diameter, were measured using a ruler or calipers. The absence or presence of an inhibition halo was noted.

Statistical Analysis

All experiments, including physicochemical characterizations and antimicrobial activity tests, were performed in triplicate to ensure data reliability. Results are expressed as mean ± standard deviation(s). Statistical analysis was performed using STATGRAPHICS Centurion 18 software (version 2019). Analysis of variance (ANOVA) tests were used to compare means between different groups of formulations and extract concentrations. When significant differences were detected ($p < 0.05$), post-hoc tests (such as Tukey 's HSD test) were applied to identify pairs of significantly different groups.

Results

S. Alata Extract

The results of the qualitative phytochemical screening of the ethanolic extract of Senna alata revealed six chemical groups: flavonoids, tannins, anthraquinones, saponins, alkaloids, and steroids/ triterpenes (Table 1). This table illustrates that the S. alata extract reacted weakly to the Mayer, Wagner, and Liebermann- Burchard tests, suggesting a low presence of alkaloids and steroids/ triterpenes. The foam test showed stable and persistent foam, while the Shinoda, ferric chloride, gelatin, and Bornträger tests revealed a high presence of flavonoids,

tannins, and anthraquinones in the extract of this legume. The diverse and significant presence of secondary metabolite classes confirms its richness in bioactive compounds potentially responsible for its antimicrobial properties. Table 1 summarizes the results relating to the qualitative phytochemical screening of the ethanolic extract of S. alata.

Table 1 : Qualitative phytochemical screening of the ethanolic extract of S. alata

Classes	Tests performed	Reactions	Observations
Flavonoids	Shinoda $FeCl_3$	+++ +++	Strong pink-orange coloration Strong green-black coloration
Tannins	$FeCl_3$ Gelatin	+++ +++	Strong blue-black coloration strong yellowish-white precipitate
Anthraquinones	Bornträger	+++	Strong pink-red coloration
Saponosides	Foam test	++	Stable and persistent foam
Alkaloids	Mayer Wagner	+ +	Slight white-cream precipitate Faint brown-red precipitate
Steroids/ Triterpenes	Liebermann- Burchard	+	Slight green-blue coloration

Antimicrobial Activity of the Extract in Vitro

The ethanolic extract of S. alata leaf demonstrated promising antimicrobial activity against all tested microorganisms, as evidenced by the significantly observed inhibition zones in Table 2. The mean diameters of the inhibition zones of the S. alata extract were 19.2 mm (n = 3; s = 1.1) for M. furfur, 17.8 mm (n = 3; s = 0.9) for T. rubrum, 18.5 mm (n = 3; s = 1.0) for M. Canis, and 16.9 mm (n = 3; s = 0.8) for S. aureus. Statistical analysis showed no significant difference between the mean diameters of the inhibition zones against the different microbial strains ($F = 2.57$; $df = 3.8$; $P < 0.001$). The MIC and CMF/CMB values confirm the extract's efficacy, particularly against fungi, where concentrations of 31.25 µg/ mL were achieved for M. furfur and M. canis , and 125 µg/ mL for T. rubrum. These values are competitive with those reported for some traditionally used plant extracts. The extract also showed significant activity against S. aureus (MIC of 250 µg/ mL), which is relevant for secondary skin infections.

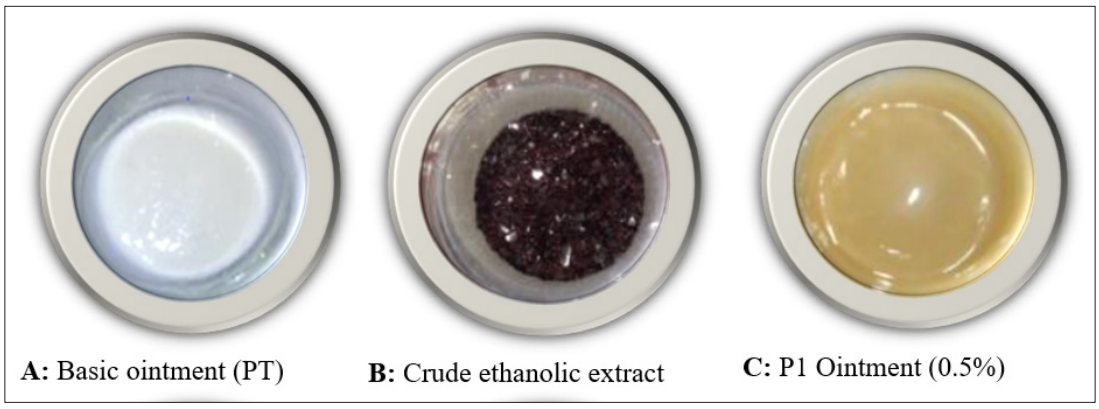
Table 2 : Minimum inhibitory concentration, minimum fungicidal concentration, minimum bactericidal concentration, mean diameter of inhibition zones of S. alata extract and Positive Control

Microorganism	N	MIC (µg/ mL)	CMF/CMB (µg/ mL)	MDZI (mm)	CP (MIC in µg/ mL)
Malassezia furfur	3	31,25	62,5	19,2 ± 1,1	Kétoconazole (1,0)
Trichophyton rubrum	3	125	250	17,8 ± 0,9	Kétoconazole (2,0
Microsporum canis	3	31,25	62,5	18,5 ± 1,0	Kétoconazole (1,0)
Staphylococcus aureus	3	250	500	16,9 ± 0,1	Gentamicin (0.5)

MIC: Minimum Inhibitory Concentration; CMF: Minimum Fungicidal Concentration; CMB: Minimum Bactericidal Concentration MDZI: Mean Diameter of Inhibition Zones (mm); CP: Positive Control; means are followed by standard deviations.

Formulation of the Antimicrobial Ointment

Senna alata leaf extract. All formulations showed excellent visual and microscopic homogeneity, without agglomeration or phase separation, indicating good dispersion of the extract in the base. From Figure 1A, we conclude that the base ointment is characterized by an off-white color, a smooth and homogeneous texture, and a creamy appearance. The crude extract (Figure 1A), on the other hand, is a fine, dark brown, granular powder. The various antimicrobial ointments formulated from a mixture of the base ointment and the crude extract at different concentrations (0.5%, 1%, and 2%) are illustrated in Figures 1C, 1D, and 1E. The color of the formulated ointments ranges from light yellow P1 (0.5%) to dark brown P3 (2%), depending on the concentration of the incorporated extract, which is expected given the extract's inherent color.



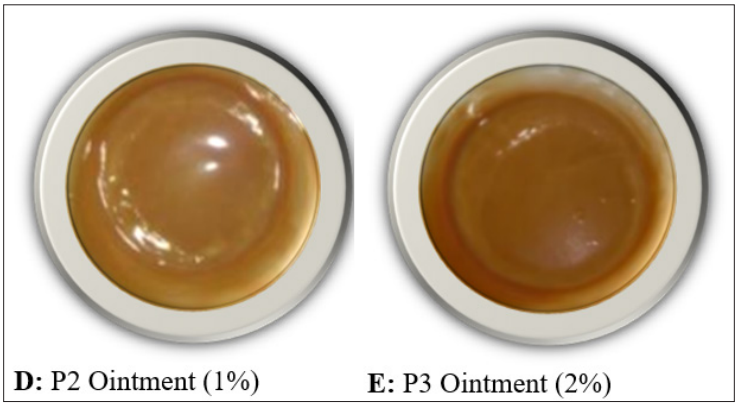


Figure 1: Different formulations of the antimicrobial ointment based on Senna alata leaf extract

Physico-Chemical and Rheological Characterizations of the Formulated Ointment

Table 3 summarizes the physicochemical and rheological characteristics of the formulated ointments. The pH ranged from slightly acidic to neutral, from 5.5 (n = 3; s = 0.1) in P1 (0.5%) to 6.2 (n = 3; s = 0.1) in P3 (2%). The difference between the pH values was significant (P = 0.0023). A pH within this range minimizes the risk of irritation and promotes skin barrier integrity. Spreading was satisfactory for all ointments, with diameters ranging from 3.5 cm (n = 3; s = 0.1) in P3 (2%) to 3.8 cm (n = 3; s = 0.2) in P1 (0.5%). Furthermore, there was no significant difference between the different concentrations (P = 0.2329). These values indicate good applicability and ease of spreading on the skin, which is crucial for patient compliance and ensuring uniform coverage of the affected areas. The consistency measured by penetrometer also varied depending on the formulation, from 42 mm (n = 3; s = 2) to 45 mm (n = 3; s = 2) for formulations P3 (2%) and P1 (0.5%), respectively. Analysis of variance revealed no significant difference between the different concentrations (P = 0.2885). This confirms a soft texture that is easy to apply without being too runny.

These rheological properties are essential for good adhesion of the ointment to the skin and controlled release of the active ingredients. The three-month stability study revealed no significant changes in appearance, pH, odor, or spreadability for the ointments stored at 4°C and 25°C, suggesting good stability at room temperature and under cold conditions. At 40°C, a slight color change was observed for the P3 formulation, but homogeneity and consistency were maintained, indicating that the ointment base is stable even under heat stress. However, this slight color change at 40°C for the highest extract concentrations suggests that there may be minor degradation of some heat-sensitive phytochemicals. The results of the physicochemical and rheological characterization of the formulated ointments are presented in Table 3.

Table 3: Physico-chemical and rheological characterizations of the formulated ointment

Features		pH			Spread (cm)			Consistency (mm)		
	N	m ± s	min	Max	m ± s	min	Max	m ± s	min	max
PT	3	5.7 a ± 0.1	5.50	5.9	4.0 a ± 0.3	3.60	4.4	48 a ± 2.00	44.00	52.00
P1 (0.5%)	3	5.5 a ± 0.1	5.30	5.7	3.8 a ± 0.2	3.40	4.2	45 a ± 2.00	41.00	49
P2 (1%)	3	5.8 a ± 0.1	5.70	6.10	3.6 a ± 0.1	3.40	3.80	43,5a ± 2,00	39,50	47,50
P3 (2%)	3	6,2b ± 0,1	6,4	6,6	3,5a ± 0,1	3,4	3,6	42a ± 2,00	40,00	44,00
Test ANOVA		F = 12,22 ; Ddl = 3, 8			F = 1,76 ; Ddl = 3, 8			F = 1,49 ; Ddl = 3, 8		

N: number of measurements taken; min: minimum; max: maximum; m: mean; s: standard deviation

Antimicrobial Activity of the Formulated Ointment

Ointments formulated with the ethanolic extract of S. alata leaves demonstrated significant dose-dependent antimicrobial activity in vitro. The mean inhibition zones obtained are shown in Table 4. The P2 formulation (1%) showed greater inhibitory activity for all microbial strains, with diameters of 16.5 mm (s = 1.1; max = 17.6) for M. furfur, 15.2 mm (s = 1.05; max = 16.2) for T. rubrum, 16.24 mm (s = 0.96; max = 17.00) for M. canis, and 14.8 mm (s = 0.88; max = 15.6) for S. aureus. Overall, the difference was highly significant between the mean diameter of the inhibition zone of the control ointment (PT) and formulations P1 (0.5%), P2 (1%), and P3 (2%) in M. furfur (F = 185.09; df = 3.8; P < 0.001); T. rubrum (F = 217.32; df = 3.8; P < 0.001); M. canis (F = 251.34; df = 3.8; P < 0.001); and S. aureus (F = 282.60; df = 3.8; P < 0.001). This confirms the antimicrobial efficacy of the incorporated extract.

By comparing the diameter of the inhibition zone of the ointment formulated P2 (1%) to formulations P1 (0.5%) and P3 (2%) against the different microbial strains, statistical analysis reveals that there is no significant difference in M. furfur (P = 0.0656), in T. rubrum (P = 0.138), in Mr. canis (P = 0.0759) and S. aureus (P = 0.138). Positive controls showed comparable or slightly superior zones of inhibition, ranging from 18–22 mm for ketoconazole cream (2%) to 16–20 mm for gentamicin cream (0.1%), favorably positioning the S. alata ointment compared to clinical standards. The P3 formulation (2%) showed slightly lower activity than P2 (1%), suggesting an optimal concentration of approximately 1% for the ointment.

Table 4: Diameter of inhibition zones (mm) of ointments formulated against different microbial strains

Microorganisms		Malassezia furfur			Trichophyton rubrum			Microsporum canis			Staphylococcus aureus		
	N	m ± s	min	Max	m ± s	min	max	m ± s	min	max	m ± s	min	max
PT	3	2,01a ± 0,1	2,1	1,9	1,00a ± 0,05	1,00	0,8	1,00a ± 0,05	1,0	0,8	1,05a ± 0,09	1,1	0,9
P1 (0,5%)	3	14,25b ± 0,72	14,9	13,50	13,54b ± 0,60	14,1	12,90	14,05b ± 0,83	14,8	13,20	13,13b ± 0,52	13,6	12,60
P2 (1%)	3	16,57c ± 1,14	17,6	15,4	15,22c ± 1,05	16,2	14,1	16,24c ± 0,96	17,0	15,1	14,81b ± 0,88	15,6	13,9
P3 (2%)	3	15,85bc ± 0,83	16,6	15,00	14.95 bc ± 0.70	15.6	14.20	15.54 bc ± 0.62	16.1	14.90	14.04 b ± 0.62	14.6	13.40

N: number of diameters measured; min: minimum; max: maximum; m: mean; s: standard deviation

Comparison of the diameter of the inhibition zones (mm) of the control ointment (PT) to formulations P1 (0.5%), P2 (1%), P3 (2%) versus: Malassezia furfur : ($F = 185.09$; $df = 3.8$; $P < 0.001$); Trichophyton rubrum : ($F = 217.32$; $df = 3.8$; $P < 0.001$); Microsporum canis :($F = 251.34$; $ddl = 3.8$; $P < 0.001$); Staphylococcus aureus: ($F = 282.60$; $ddl = 3.8$; $P < 0.001$)

Comparison of the inhibition zone diameter of formulation P2 (1%) to formulations P1 (0.5%), P3 (2%) versus: Malassezia furfur: ($F = 4.44$; $ddl = 2.6$; $P > 0.05$); Trichophyton rubrum: ($F = 2.81$; $ddl = 2.6$; $P > 0.05$); Microsporum canis :($F = 4.09$; $ddl = 2.6$; $P > 0.05$); Staphylococcus aureus: ($F = 2.01$; $df = 2.6$; $P > 0.05$).

Preliminary Clinical Observations (Ringworm and Tinea)

Preliminary observations made on patients have shown encouraging results for the topical treatment of ringworm and tinea with the ointment formulated at P2 (1%).

Treatment of eczema (Pityriasis Alba or Eczematous Rash)

Figure 2 shows the condition of the skin on the back of a patient with tinea cruris before the start of treatment. This skin was hairless and covered with multiple depigmented lesions. After 15 days of daily application of P2 ointment (1%), a marked improvement was observed in the patient's skin (Figure 2B), with a reduction in the extent and visibility of the spots. After 19 days of treatment (Figure 2C), the patient's skin had regained a healthy appearance, without residual spots, suggesting near-complete healing.

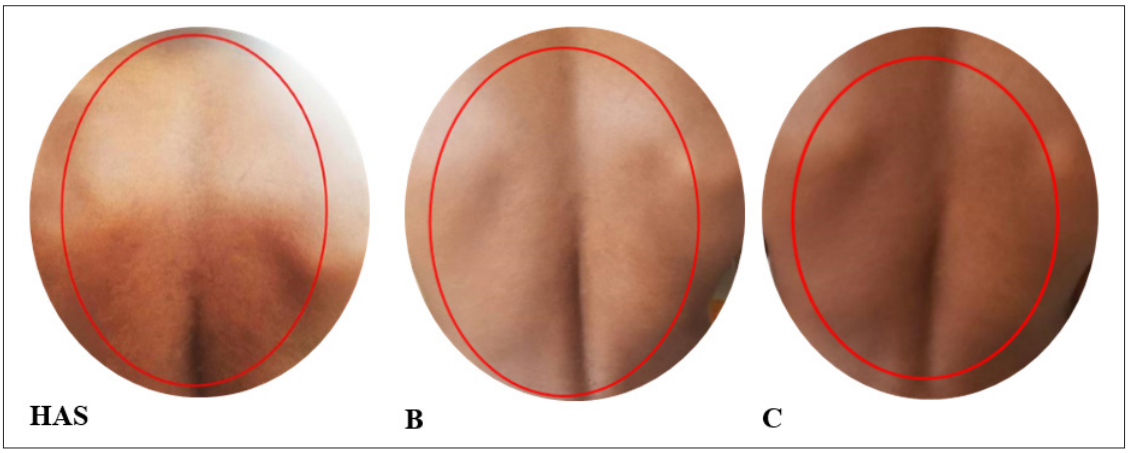


Figure 2: Patient's skin affected by eczema before treatment (A), Patient's skin after 15 days of treatment (B) and Patient's skin after 19 days of treatment (C)

Treatment of ringworm (Dermatophytosis)

Figure 3 illustrates the case of a patient with tinea capitis on the scalp before treatment. The scalp is crusted, with areas of alopecia and scaly, inflamed lesions. After 13 days of daily application of P2 ointment (1%), encouraging signs were noted (Figure 3B), including slight hair regrowth in the affected areas and a visible reduction in the tinea capitis and crusting. After 26 days of treatment (Figure 3C), complete hair regrowth was observed on the patient's head, and the tinea capitis had virtually disappeared.

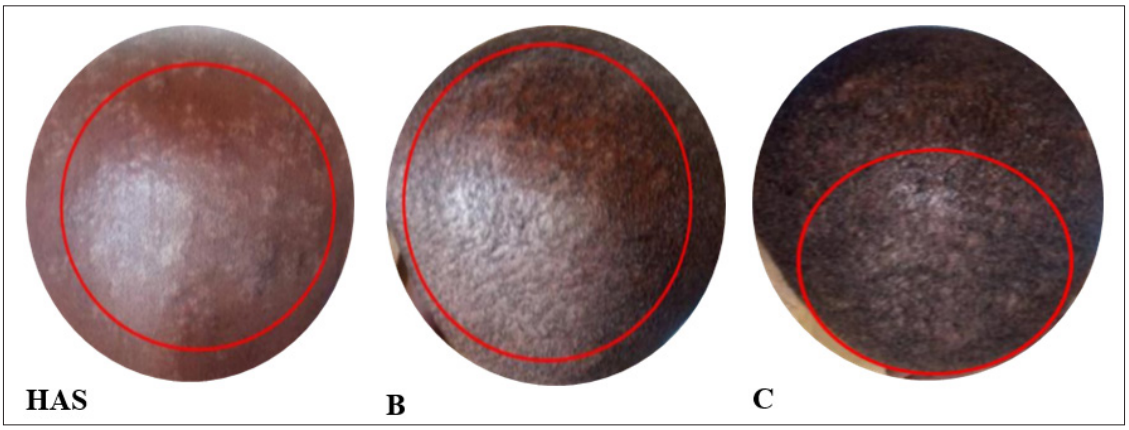


Figure 3: Skin of a patient with ringworm before treatment (A), Skin of a patient after 13 days of treatment (B), Skin of a patient after 26 days of treatment

Discussion

This study focuses on the formulation of an antimicrobial ointment based on senna extract. alata shows that the ethanolic extract is notably rich in secondary metabolites, particularly flavonoids, tannins, and anthraquinones. Several studies have reported similar results on the chemical constituents of this Fabaceae and that these secondary metabolites exhibit numerous biological activities [16-18]. The presence of flavonoids, tannins, phenols, alkaloids, saponins, and anthraquinones in the extract of this plant was also confirmed by Wuthi-udomlert et al [19]. Flavonoids, tannins, and saponins were identified by Passimna et al. (2016) as the main chemical compounds of the crude ethanolic extract of S. alata leaves. Similarly, the work of Oladeji et al. showed that the leaves of S. alata contain these active principles, thus corroborating the metabolic richness and pharmacological potential of the plant species studied with those of the present study [20]. This high proportion of these bioactive metabolites in the extract studied fully justifies the observed antimicrobial activity, both for the crude extract and for the formulated ointment.

S. alata extract against Malassezia furfur, Trichophyton rubrum, Microsporum Staphylococcus aureus is particularly noteworthy. The low minimum inhibitory concentrations (MICs) obtained demonstrate its ability to inhibit the growth of these pathogens at relatively low concentrations. The MIC of 31.25 µg/ mL observed against M. furfur is very promising, especially when compared to ketoconazole (1.0 µg/ mL), suggesting strong bioactivity and a broad spectrum of activity against yeasts of the genus Malassezia, which are often resistant to some conventional antifungals [21,22]. The detection of anthraquinone and flavonoid glycosides in the S. alata extract significantly inhibited the growth of S. aureus (Oluwale et al., 2020) as well as that of T. rubrum [19,23-25]. Furthermore, efficacy against S. aureus is a major advantage because this bacterium is frequently involved in superinfections of fungal skin lesions, complicating clinical management. Strong inhibitory effects have been shown on S. aureus with the decoction of S. alata leaves (Oluwole. et al, 2020). Work on the methanoic- water extraction of S. alata leaves by Makinde et al indicate that phenolic compounds, terpenoids, the alkaloid salt, and the alkaloid base of this plant species induced inhibition of 10 to 30 mM against M. canis [26]. The antimicrobial effect of S. alata leaves using the agar diffusion method demonstrated antifungal activity against S. aureus which required a higher concentration of approximately 25 to 50 mg/ml for inhibition [27]. Channa et al noted that the last 8 mm inhibition zone (located between 8 and 34 mm) was observed against S. aureus, thus showing antibacterial activity of the ethanolic extract of S. alata leaves against this pathogen [28].

After incorporating the ethanolic extract of S. alata leaves into the control ointment (PT), the results showed that the resulting antimicrobial ointment exhibited characteristics similar to those of the control ointment. The observed homogeneity resulted not only from the fineness and uniformity of the dispersed particles but also from proper mixing of the ingredients. The properties of the resulting ointment are consistent with those reported by Adiaffi, Toé, and Adelowo and Oladeji [29-32]. These authors used the same technique and obtained high-quality formulations with very fine particles (1 to 4 µm). The homogeneity of the preparation justifies the method employed: the gradual incorporation of small amounts of the dispersed phase into the dispersing phase. Exposing the antimicrobial ointment to a high temperature loosens the interparticle interactions, leading to a decrease in viscosity. This phenomenon has also been reported by several authors [21,32]. However, this increase in fluidity remains reversible when the temperature drops back to 25°C, which confirms the thermosensitive stability of the formulation. At very low temperatures, the ointment tends to harden due to its water content; however, it regains its initial homogeneity, smoothness, and stability after a temperature increase between 25 and 40°C. This type of physicochemical evaluation, commonly used to control the quality of pharmaceutical emulsions, was described by Jouanny-Bouyer [30]. All of these stability tests show that the antimicrobial ointment is a stable preparation, comparable to the control ointment used as a base.

Furthermore, all the formulated ointments have a pH ranging from 5 to 6, a favorable range for cutaneous application, respecting the skin's physiological pH, which is between 4.7 and 5.7 and likely to prevent any skin intolerance reactions [31]. This value is close to the pH of the control ointment (5.7) formulated by Adelowo and Oladeji but below the pH range of the hydrogel prepared by Iraqui et al. [32,33]. The spreadability of the formulated ointment also determines its therapeutic efficacy, as it determines the ointment's behavior when it is removed from the storage tube [34]. The antimicrobial ointment obtained is characterized by stability over time, hydrophilicity,

homogeneity, smoothness to the touch and a pH of 5.8, making it conceivable to formulate a stable ointment from the control ointment used as a base.

The P2 formulation (1%) showed the largest zones of inhibition for all tested strains, with diameters of 16.5 ± 0.9 mm for M. furfur, 15.2 ± 0.8 mm for T. rubrum, 16.0 ± 0.8 mm for M. canis, and 14.8 ± 0.7 mm for S. aureus. Ibrahim's work showed that ethanolic extracts of the leaves had strong antimicrobial activity against various dermatophyte fungi, with weak activity against non- dermatophyte fungi [11]. This was particularly true for the methanolic extract of S. alata leaves, which showed inhibition diameters of 15 mm and 17 mm, respectively, against Staphylococcus aureus. and Escherichia coli for 200 mg/ml of extract (El- Mahmood et al. 2008). Similarly, Iraqui et al. noted a large inhibition zone of 20.33 mm on S. aureus [33]. These authors further clearly indicated through the in vitro antibacterial assay that the antimicrobial efficacy of a gel based on S. alata leaf extract was higher than Renicol, an antibacterial formulation available on the market. Oliver-Bever (1986) reported that the leaves of this Fabaceae were used for the treatment of dermatitis, eczema, ringworm.

Complete healing of the ringworm was achieved 19 days after daily application of P2 ointment (1%). This result is identical to that reported for the control ointment formulation developed by, from which the present antimicrobial formulation is derived [35]. This shows that modifying the formula by adding water did not alter the vehicle's ability to transport the active ingredient or its antimicrobial activity. It is therefore possible to state that, for ringworm, the antimicrobial ointment maintained the therapeutic efficacy of the control ointment (PT). Regarding the duration of treatment, the result obtained is significantly better than that of the usual medications used to treat ringworm, for which complete healing is generally only achieved after three months [36]. Furthermore, the observed treatment duration of 19 days is slightly shorter than that reported by Bene et al, who achieved a cure in 23 days using Bersama extract [37]. Abyssinica extract was incorporated into shea butter, a fattier excipient. This difference could be attributed to the nature of the extract used as well as the type of excipient. Thus, it appears that the evaluated antimicrobial ointment retained the pharmacological activity of the control ointment.

For the treatment of ringworm, daily application of the P2 (1%) antimicrobial ointment resulted in a cure after 26 days. This timeframe is comparable to that observed with the control ointment formulated with a cream based on Terminalia extract. Catappa [35]. However, it is slightly lower than that reported by Bene et al, who achieved complete healing after 28 days [37]. This difference could be due to both the nature of the extract and the type of excipient used. Indeed, these authors used a B. abyssinica extract incorporated into shea butter, a fattier excipient, which could slow the release of the active ingredient. This difficulty could be explained by the reduced capacity of the fatty medium to ensure rapid transport of the extract through the different layers of the skin. This assertion is confirmed by who demonstrated that products with a high lipid content penetrate the skin barrier with greater difficulty [4,38-42].

Conclusion

The primary objective of this study was to formulate an antimicrobial ointment based on an ethanolic extract of S. alata leaves and to evaluate the in vitro activity of this extract against the causative agents of ringworm and tinea capitis. The results of the phytochemical screening confirmed the extract's richness in flavonoids, tannins, and anthraquinones, secondary metabolites known for their antimicrobial properties, which underlie the observed activities. The in vitro evaluation clearly demonstrated that the extract, and more specifically the P2 (1%) ointment, exhibits promising antimicrobial activity against a broad spectrum of clinically relevant microorganisms, including the causative agents of ringworm and tinea capitis, as well as opportunistic bacteria such as S. aureus. Preliminary clinical observations also highlighted a rapid and significant improvement in skin lesions in patients with ringworm and tinea capitis. These results confirm the potential of S. alata as a major active ingredient for the development of natural dermatological products. However, it would be worthwhile to optimize the ethanolic extraction by testing other solvents and to identify secondary metabolites using mass spectrometry and nuclear magnetic resonance.

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