Development of an active antibacterial lotion based on hydroethanolic extract of the leaves of *Feretia apodanthera* Delile (Rubiaceae) on *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Formulation d'une lotion antibactérienne à base d'extrait hydroéthanolique des feuilles de Feretia apodanthera Delile (Rubiaceae) active sur Staphylococcus aureus et Staphylococcus epidermidis.

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Abstract

Introduction : The perpetual quest for new molecules to combat drug-resistant germs is driving the ongoing exploration of natural resources. Consequently, herbal therapy remains a common method of self-treatment among populations. This study aimed to evaluate the antibacterial efficacy of lotions formulated with hydroethanolic extract from the leaves of *F. apodanthera* Delile (Rubiaceae). **Materials and methods** : Microbiological sensitivity testing was performed on agar using *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* at extract concentrations ranging from 100 to 12.5 mg/mL. Three lotions F1, F2 and F3 were formulated, and their physicochemical, organoleptic and biological parameters were assessed. **Results :** The inhibition diameters of the hydroethanolic infusion, which was selected as the active substance, were measured as follows : 15.50 ± 0.86, 12.50 ± 1.03 and 9.50 ± 4.76 mm for *Staphylococcus aureus*; 18 ± 1.70, 15 ± 1.60 and 12.50 ± 6.25 mm for *Staphylococcus epidermidis*; 16 ± 1.54, 10 ± 5.02 and 13.50 ± 1.73 mm for *Pseudomonas aeruginosa*. These values were obtained at concentrations of 100, 50 and 25 mg/mL, respectively. The three formulated lotions showed inhibition diameters ranging from 12.50 ± 0.51 to 14.50 ± 0.51 mm for *Staphylococcus aureus* and *Staphylococcus epidermidis* respectively. **Conclusion :** Lotions formulated from the hydroethanolic extract of *Feretia apodanthera* demonstrated antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. This explains its traditional use in the treatment of skin infections in Burkina-Faso.

Keywords : *Feretia apodanthera*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, antibacterial lotion, Burkina-Faso.

Résumé

Introduction : La quête perpétuelle des nouvelles molécules pour lutter contre les germes ayant développé une résistance face aux médicaments disponibles sur le marché, suscite une exploration continue des ressources naturelles. De ce fait, la thérapie à base de plantes demeure l'une des voies utilisées par les populations pour se soigner. Le but de cette étude était d'évaluer l'efficacité des lotions antibactériennes formulées à base d'extrait hydroéthanolique des feuilles de *Feretia apodanthera* Delile (Rubiaceae). **Matériel et méthodes :** Le test de sensibilité microbiologique sur gélose a été évalué sur *Staphylococcus aureus, Staphylococcus epidermidis* aux concentrations d'extraits allant de 100 à 12,5 mg/mL. Trois lotions F1, F2 et F3 ont été formulées et leurs paramètres physico-chimiques, organoleptiques et biologiques ont été contrôlés. **Résultats :** Les diamètres d'inhibitions de l'infusé hydroéthanolique retenu comme substance active était de 15,50±0,86, 12,50±1,03 et 9,50±4,76 mm pour *Staphylococcus aureus*, 18±1,70, 15±1,60 et 12,50±6,25 mm pour *Staphylococcus epidermidis* et 16±1,54, 10±5,02 et 13,50±1,73 mm pour *Pseudomonas aeruginosa* aux concentrations respectives de 100, 50 et 25 mg/mL. Les diamètres d'inhibitions des trois lotions formulées allaient de 12,50±0,51 à 14,50±0,51 mm sur *Staphylococcus aureus* et *Staphylococcus epidermidis* respectivement. **Conclusion :** Les lotions formulées à bases d'extrait hydroéthanolique de *Feretia apodanthera* Delile (Rubiaceae) ont démontré une activité antibactérienne vis-à-vis de *Staphylococcus aureus* et de *Staphylococcus epidermidis*. Ceci expliquerait l'utilisation traditionnelle de *Feretia apodanthera* dans la prise en charge des infections cutanées au Burkina-Faso.

Mots clés : *Feretia apodanthera*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, lotion antibactérienne, Burkina-Faso.

1. Introduction

Plants have been used in traditional medicine by local people for centuries to continually improve their health. [1]. Their activities result from the presence of phytochemical compounds which are responsible of their pharmacological effects. There is increasing scientific evidence of their use, justifying the immense and variable composition of secondary metabolites (active substances or active principles) with diverse properties [2]. Faced to the emergence of resistance in pathogens to the molecules on the market, plants offer a way of finding new compounds and promoting low-cost phytomedicines. This is why, in Africa, around 80% of the population rely on traditional medicine to solve their health problems [3].

In Burkina-Faso, plant-based therapies and natural plant extracts are still one of the ways in which people treat various ailments. Many of these plants and plant extracts are genuinely effective, but have yet to be scientifically proven: such is the case with *Feretia apodanthera*. *Feretia apodanthera* is a medicinal plant used to treat a variety of ailments, including gonorrhoea, syphilis, leprosy, gastralgia, infected wounds, snake bites, convulsions and oxidative stress [4–6]. It is a shrub with simple deciduous leaves and grows to around three metres in height. It grows mainly in the savannah, preferably on termite mounds [7]. It is found much more in countries such as Gambia, Mali, Senegal, Ivory Coast , Cameroon, Mauritania, Tanzania and Burkina-Faso to name but a few [4].

With regard to the pharmacological properties of this plant, the aim will be to demonstrate the antibacterial activity of extracts obtained from leaf powder and to propose a phytomedicine to treat skin disorders, which should be inexpensive for people who use this plant traditionally. Lotions appear then as forms whose use is simple and would not require a complex mixture of excipients [2,8]. The general objective of this work will consist in formulating antibacterial lotions containing the extract leave of *Feretia apodanthera*.

2. Materials and methods

2.1. Materials

2.1.1. Plant material

The plant material consisted of powdered *Feretia apodanthera* leaves (figure 1B). The leaves (figure 1A) were harvested in the Gampela area, 19 km from the city of Ouagadougou. After identification and harvesting, the plant drug was cut up and dried at room temperature for a fortnight. The dried leaves were pulverised in a Gladiator-type blade grinder (Retsch, SM 200, Germany) to obtain a fine powder that was stored in plastic food bags.

Figure 1 : (A) Leafy branches and (B) powder from dried leaves of *Feretia apodanthera*.

2.1.2. Bacterial strains and antibiotics

The bacterial medium consisted of six reference strains provided by the National Agency for Health, Environment, Food, Labour and Health Products Safety (ANSSEAT) of Burkina-Faso, based in Ouagadougou, as well as seven reference antibiotics in the form of antibiogram diffusion discs at well-defined concentrations (Table 1).

Table 1 : Bacterial strains and reference antibiotics used in the susceptibility test

Strains	References	Sensitivity profile
Staphylococcus aureus	ATCC 25923	AUG 30ug 100521105, FOX 30ug 110917072, E 15ug 051221073
Staphylococcus epidermidis	ATCC 12228	AUG 30ug 100521105, FOX 30ug 110917072, E 15ug 051221073
Escherichia coli	ATCC 25922	AUG 30ug 100521105, CIP 5ug 012121045, CN 10ug 060321067, K30 30ug 110217069, CAZ 30ug 062017096
Pseudomonas aeruginosa	ATCC 27853	CIP 5ug 012121045, CN 10ug 060321067, K 30ug 110217069, CAZ 30ug 062017096
Salmonella typhimurium	ATCC 14028	CIP 5ug 012121045
Streptococcus pyogènes	/	E 15ug 051221073, AUG 30ug 100521105, FOX 30ug 110917072, CIP 5ug 012121045

Caption : AUG=amoxicillin-clavulanic acid, CIP=ciprofloxacin, CN=gentamicin, K=kanamycin, FOX=cefoxitin, CAZ=ceftazidime, and E=erythromycin.

2.2. Methods

2.2.1. Preparation of plant extracts

2.2.1.1. Infusion

Aqueous Infusion

This operation consists of bringing a precise quantity of *Feretia apodanthera* leaf powder (50.04 g) into contact with a defined volume of hot solvent (500 mL of distilled water at 90 °C) for 15 minutes. The pomace was separated from the filtrate by vacuum filtration with hydrophilic cotton using a Büchner. The filtrate was stored at 2-8 °C in the refrigerator.

Hydroethanolic infusion

A test portion of 50.03 g of powder was contacted with 125 mL of 96° ethanol for 5 minutes. A volume of 375 mL of distilled water, initially heated to 90 °C, was then added to the initial drug-ethanol mixture. The mixture was kept in contact for 15 minutes. The pomace was separated from the filtrate by double vacuum filtration with hydrophilic cotton using a Büchner. The resulting infusion was stored between 2 and 8 °C in the refrigerator for later use.

2.2.1.2. Maceration

Aqueous maceration

A 50.03 g test sample was placed in contact with 2 L of distilled water at room temperature and stirred with a magnetic stirrer for 24 hours. Double filtration with vacuum cotton wool using a Büchner separated the pomace from the filtrate and the macerate was stored at 2-8 °C in the refrigerator for later use.

Hydroethanolic maceration

The extraction solvent was distilled water/ethanol (40:60, v/v). A test sample of 50.03 g of powder was placed in contact with 2 L of this system and then left to macerate for 24 hours under magnetic stirring (H20 series, Ibx instruments, France). Filtration with hydrophilic cotton under vacuum using a Büchner separated the pomace from the filtrate and the macerate was stored between 2 and 8 °C in the refrigerator (J-Tech Inventer, Sharp, Japan).

2.2.1.3. Freeze-drying of infusions and macerates made from the leaves of F. apodanthera

The extracts obtained by hydroethanolic infusion and hydroethanolic maceration were subjected to evaporation in a rotary evaporator (Waterbath B-48, Rotavapor R-114, Büchi, Germany) at a temperature of 40 °C and a pressure of 0.8 mbar for 4h.

After distributing 1/3 of the different extracts in 500 mL round-bottomed glass flasks, the latter were frozen in a freezer before being fixed on a lyophiliser. The freeze-drying conditions for the extracts obtained by aqueous infusion and aqueous maceration were : temperature (T)=-55.2 °C; pressure (P)=0.0633 mbar and freeze-drying time (t)=118h00min. For the extract obtained by hydroethanolic infusion, the conditions were : T=-55.0°C; P=0.270 mbar and t=65h38min. For the extract obtained by hydroethanolic maceration, the conditions were : T=-

55.6 °C; P=0.305 mbar and t=69h38min.

2.2.1.4. Extraction efficiency

The extraction yield was obtained by dividing the mass of dry extracts by the mass of the initial powder and multiplying by one hundred [9]. The following formula was used for the various calculations.

$$\tau = \frac{dry\ extract}{leaves\ powder} * 100$$

Avec τ=extraction efficiency

2.2.2. In vitro study of the antibacterial activity of infusion and maceration extracts leaves of Feretia apodanthera

2.2.2.1. Preparation of culture media

Muller Hinton (MH) medium was used to culture *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Escherichia coli* and *Pseudomonas aeruginosa*. To do this, 5 L of MH medium was prepared according to the method described by the manufacturer on the packaging (i.e. 38.0 g per 1 L of distilled water).

For *Streptococcus pyogenes,* the medium consisted of a mixture of chocolate agar (GC Base) and haemoglobin enriched with polyvitex. A volume of 500 mL of GC base was prepared by weighing 36.01 g and 500 mL of haemoglobin solution by weighing 5 g. The two solutions were mixed after autoclaving the GC base medium.

Once the different culture media had been prepared, they were sterilised in an autoclave at 121 °C using hot and humid steam for 15 minutes. Next, 24 mL of each medium was poured into Petri dishes in a laminar flow hood and placed on the bench for solidification at room temperature.

2.2.2.2. Preparation of bacterial inoculum

A volume of 100 mL of a 0.9 % NaCl solution was prepared for the entire experiment and 25 mL was distributed in six test tubes, each corresponding to a specific germ at a rate of 5 mL per tube. Using the platinum handles, the germs were removed from the freezer and thawed at room temperature, then sampled and scattered into the tubes containing NaCl. The solutions had to be homogenized by vortexing to ensure that the germs were evenly distributed.

2.2.2.3. Replication of bacterial strains

The previously prepared inocula were transferred to Petri dishes using the streak method, in order to obtain young, well-isolated colonies. For the *Streptococcus pyogenes-soaked* medium, growth took place in an anaerobic environment by stacking Petri dishes topped with a candle in a hermetically sealed bell jar. All the Petri dishes were incubated at 37 °C for 24 hours in an oven.

2.2.2.4. Determining the inhibition diameter

Preparation of concentrations

Successive half-diluted concentrations ranging from 100 to 12.25 mg/mL were prepared for the four extracts obtained by lyophilization with Dimethylsulphoxide 5% (DMSO).

Preparation of the bacterial inoculum

From a 24 hour pure culture seeded on a non-selective solid medium (Muller Hinton Agar), 1 to 3 well-isolated colonies were picked and suspended in a few millilitres (3 to 5 mL) of sterile 0.9 % NaCl solution. The turbidity of each suspension was adjusted to 0.5 McFarland using a densitometer.

Inoculation of culture media and deposition of extracts and reference antibiotic disc

A sterile cotton swab was dipped into the inoculum and then squeezed out by rotation. The surface of the agar was swabbed by rotating the Petri dish three times through 60° to ensure even distribution of the inoculum.

Five wells numbered 1 to 5 were marked on each Petri dish. The concentrations of lyophilised extracts (100, 50, 25 and 12.25mg/mL) and the negative control (solvent in which the extract was dissolved) were deposited in the five wells respectively. Discs of the seven reference antibiotics were also deposited on the media, along with a neutral disc as a negative control. The operation was carried out in triplicate. The Petri dishes were incubated at 37°C for 24 hours in an oven. Antibacterial activity was determined by measuring the diameter of the zone of

inhibition around each concentration of extracts and antibiotic discs [10]. Susceptibility to the extract was classified according to the diameter of the zones of inhibition as follows: not sensitive (-) for a diameter of less than 6mm; sensitive (+) for a diameter of between 7 and 14mm; very sensitive (++) for a diameter of between 15 and 19mm and extremely sensitive (+++) for diameters of more than 20mm [11].

2.2.2.5. Determination of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC)

Preparation of the stock solution of plant extract, Muller Hinton broth and dilution in liquid medium

Extracts were prepared at a concentration of 100 mg/mL. Muller Hinton broth (MHB) was prepared by diluting 21 g of powder in 1 L of distilled water as recommended by the manufacturer. The mixture was homogenized using a magnetic stirrer. The preparation was then placed in an autoclave at 121 °C for 15 minutes for sterilization. The dilution technique used makes it possible to observe the growth of a microorganism after 24 hours incubation at 37 °C in a non-specific growth medium and thus determine the MIC. The principle is to fill all the wells of 96-well plates with 100 µL of BMH culture medium, add 100 µL of stock extract solution and dilute progressively to ½. After successive dilutions from the stock solution, eight concentrations ranging from 50 mg/mL to 0.390625 mg/mL were obtained. To each well, 100 µL of inoculum of each germ was added (one plate per germ and per extract). The plates were incubated for 24 hours at 37 °C.

Reading and identification of the minimum inhibitory concentration (MIC)

The turbidity of the microplate wells was assessed visually and then a biochemical developer INT (iodonitrotetrazolium chloride) was introduced (0.2 mg/mL) into each well. After 30 minutes of incubation, the initial coloration of the wells turned pink if bacteria were present. The MIC of each extract tested was deduced from the first well in the range in which growth did not occur (no coloration). The experiment was repeated in triplicate.

Reading and identification of the minimum bactericidal concentration (MBC)

Determination was based on subculture from the well defining MIC +1 on agar medium [12]. Samples were taken from each of the wells containing no bacterial pellet (growth not observed) and those containing controls for the concentration range used to determine the MIC. These were streaked and streaked on Muller Hinton agar. The inoculated Petri dishes were incubated for 24 hours at 37 °C in an oven. The BMC of the extract was deduced from the first dilution in which no culture was observed on agar. The operation was repeated in triplicate.

CMB/CMI reports

These reports confirmed the bacteriostatic or bactericidal nature of the various substances tested [12]. Substances with an MBC/MIC ratio greater than or equal to 4, less than 4 or equal to 1 are declared bacteriostatic, bactericidal or absolute bactericidal respectively [13].

2.2.3. Evaluation of the antibacterial efficacy of lotions formulated and manufactured from the hydroethanolic infusion of Feretia apodanthera

2.2.3.1. Formulation and manufacture of antibacterial lotions

Four lotions were formulated (Table 2) and were composed of lyophilized extracts of *Feretia apodanthera* and excipients such as distilled water, ethanol and propylene glycol in varying proportions. The various preparations were stored between 15 and 25 °C.

Table 2 : Formulation plan for antibacterial lotions

Formulation codes	Active substance (g)	Ethanol (mL)	Propylene glycol (mL)	Distilled water (mL)
F0	10	/	/	100
F1	10	25	75	/
F2	10	50	50	/
F3	10	75	25	/
TN1	/	25	75	/
TN2	/	50	50	/
TN3	/	75	25	/

Caption : F0=formula zero; F1=formula one; F2=formula two; F3=formula three; T1=negative control 1; T2=negative control 2 and T3=negative control 3.

2.2.3.2. Quality control of formulated antibacterial lotions

Appearance test

The aim was to assess the organoleptic characteristics of the lotions formulated, such as color, odor and homogeneity [2].

Hydrogen potential measurement (pH)

The pH of the lotions was measured using a digital pH meter. For each type of lotion formulated, the pH meter probe was immersed in the lotion and the pH measured was recorded [2]. Measurements were taken in triplicate and the mean value and standard deviation were calculated (M±SD, n=3).

Sensitivity test

The sensitivity test was carried out on 04 volunteers aged between 28 and 33. The operation consisted of applying a few drops of the various lotions prepared to the forearms, then leaving them to act for 10 minutes after rubbing. After the contact time, any form of irritation was noted [2].

Washability test

After applying a few drops of each type of lotion to the skin of the hands of the same volunteers, tap water was poured over the application site for 10 minutes. The washability of the lotions was assessed after this time [2].

Efficiency test

Preservation of antibacterial activity was reassessed following the protocol described for the *in vitro* test above [10,11].

2.2.3.3. Statistical analysis

The statistical analysis consisted of processing the data obtained using Microsoft Excel version 2010 software. The variables calculated were means and standard deviations. The significance of the results was assessed using a one-way ANOVA test and Student's t-test.

3. Results

3.1. Yield of freeze-dried extracts from Feretia apodanthera leaf powder

Preliminary results indicate the extraction yields of freeze-dried infusions and macerates obtained from Feretia apodanthera leaf powder. They were 23.24%, 26.49%, 20.52% and 70.23% for the aqueous infusion, hydroethanolic infusion, aqueous macerate and hydroethanolic macerate, respectively. It appears that the optimal extraction percentage was obtained with the hydroethanolic macerate.

3.2. Antibacterial activity of freeze-dried infusions and macerates of Feretia apodanthera

3.2.1. Sensitivity test

The different inhibition diameters measured on the strains Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 12228, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Salmonella typhimurium ATCC 14028 and Streptococcus pyogenes were recorded in Tables 3, 4, 5 and 6 respectively for the aqueous infusion, hydroethanolic infusion, aqueous macerate and hydroethanolic macerate.

Table 3 : Diameters (mm) of inhibition zones induced by the aqueous infusion of *Feretia apodanthera* leaf powder

Reference bacterial strains	**Aqueous infusion (mg/mL)**			
	100	50	25	12,25
***Staphylococcus aureus* ATCC 25923**	17,5±0,86	12,5±1,03	9±4,76	0
***Staphylococcus epidermidis* ATCC 12228**	17±1,70	12±1,60	0	0
***Escherichia coli* ATCC 25922**	0	0	0	0
***Pseudomonas aeruginosa* ATCC 27853**	16±1,54	10±1,73	0	0
***Salmonella typhimurium* ATCC 14028**	0	0	0	0
Streptococcus pyogènes	11	0	0	0

Table 4 : Diameters (mm) of inhibition zones induced by hydroethanolic infusion of *Feretia apodanthera* leaf powder.

Reference bacterial strains	**Hydroethanolic infusion (mg/mL)**			
	100	50	25	12,25
***Staphylococcus aureus* ATCC 25923**	15,5±0,86	12,5±1,03	9,5±4,76	0
***Staphylococcus epidermidis* ATCC 12228**	18±1,70	15±1,60	12,5±6,25	0
***Escherichia coli* ATCC 25922**	0	0	0	0
***Pseudomonas aeruginosa* ATCC 27853**	16±1,54	13,5±1,73	10±5	0
***Salmonella typhimurium* ATCC 14028**	0	0	0	0
Streptococcus pyogènes	30	10	0	0

Table 5 : Diameters (mm) of inhibition zones induced by the aqueous macerate of *Feretia apodanthera* leaf powder.

Reference bacterial strains	**Aqueous maceration (mg/mL)**			
	100	50	25	12,25
***Staphylococcus aureus* ATCC 25923**	16±0,86	11,5±1,03	0	0
***Staphylococcus epidermidis* ATCC 12228**	16±1,70	11,5±1,60	0	0
***Escherichia coli* ATCC 25922**	0	0	0	0
***Pseudomonas aeruginosa* ATCC 27853**	15±1,54	10,5±1,73	0	0
***Salmonella typhimurium* ATCC 14028**	0	0	0	0
Streptococcus pyogènes	10	0	0	0

Table 6 : Diameters (mm) of inhibition zones induced by the hydroethanolic macerate of *Feretia apodanthera* leaf powder.

Reference bacterial strains	**Hydroethanolic maceration (mg/mL)**			
	100	50	25	12,25
***Staphylococcus aureus* ATCC 25923**	16±0,86	14±1,03	10±4,76	0
***Staphylococcus epidermidis* ATCC 12228**	14±1,70	12±1,60	0	0
***Escherichia coli* ATCC 25922**	0	0	0	0
***Pseudomonas aeruginosa* ATCC 27853**	13±1,54	0	0	0
***Salmonella typhimurium* ATCC 14028**	0	0	0	0
Streptococcus pyogènes	0	0	0	0

Statistical analysis of Tables 3 to 6 shows that, One-way analysis of variance (ANOVA) showed a significant decrease in the diameters of the inhibition zones with decreasing concentrations for sensitive strains ($p < 0.05$).

High concentrations (100 and 50 mg/mL) induced significantly larger inhibition zones than low concentrations (25 and 12.25 mg/mL).

No antibacterial activity was observed against *Escherichia coli* and *Salmonella typhimurium* at any concentration (zero diameter).

Hydroethanolic extracts showed significantly higher inhibition diameters than aqueous extracts for Staphylococcus aureus and Staphylococcus epidermidis ($p < 0.05$).

3.2.2. Determination of antibacterial parameters (MIC, MBC and MBC/MIC)

A gradual decrease, concentration-dependent on the intensity of the disturbance induced by bacterial growth, was observed in the experimental tubes. The minimum inhibitory concentrations, bactericidal concentrations and their ratios obtained for *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* are shown in Tables 7, 8, 9 and 10, respectively. The lowest MIC (0.39 mg/mL) and MBC (1.56 mg/mL) were observed with the hydroethanolic infusion, with an MBC/MIC ratio of less than 4 for *Staphylococcus aureus* and *Staphylococcus epidermidis* and greater than 4 for *Pseudomonas aeruginosa*.

Table 7 : Antibacterial parameters in mg/mL of the aqueous infusion and their interpretation

Reference bacterial strains	**Antibacterial parameters**			**Interpretation**
	MIC	MBC	MBC/MIC	
***Staphylococcus aureus* ATCC 25923**	0,78	1,56	2	Bactericidal
***Staphylococcus epidermidis* ATCC 12228**	1,56	3,12	2	Bactericidal
***Pseudomonas aeruginosa* ATCC 27853**	6,25	0,78	8	Bacteriostatic

Caption: MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration

Table 8 : Antibacterial parameters in mg/mL of the hydroethanolic infusion and their interpretation

Reference bacterial strains	**Antibacterial parameters**			**Interpretation**
	MIC	MBC	MBC/MIC	
***Staphylococcus aureus* ATCC 25923**	0,39	1,56	4	Bactericidal
***Staphylococcus epidermidis* ATCC 12228**	1,56	1,56	1	Perfect bactericidal
***Pseudomonas aeruginosa* ATCC 27853**	12,5	0,78	16	Bacteriostatic

Caption : MIC=minimum inhibitory concentration, MBC=minimum bactericidal concentration

Table 9 : Antibacterial parameters in mg/mL of the aqueous macerate and their interpretation

Reference bacterial strains	**Antibacteial parameters**			**Interpretation**
	MIC	MBC	MBC/MIC	
***Staphylococcus aureus* ATCC 25923**	0,78	1,56	2	Bactericidal
***Staphylococcus epidermidis* ATCC 12228**	1,56	1,56	1	Perfect bactericidal
***Pseudomonas aeruginosa* ATCC 27853**	12,5	0,78	16	Bactériostatique

Caption : MIC=minimum inhibitory concentration, MBC=minimum bactericidal concentration

Table 10 : Antibacterial parameters in mg/mL of the hydroethanolic macerate and their interpretation

Reference bacterial strains	**Antibacteial parameters**			**Interpretation**
	MIC	MBC	MBC/MIC	
***Staphylococcus aureus* ATCC 25923**	0,39	3,12	8	Bactericidal
***Staphylococcus epidermidis* ATCC 12228**	0,78	1,56	2	Perfect bactericidal
***Pseudomonas aeruginosa* ATCC 27853**	6,25	0,78	8	Bactericidal

Caption : MIC=minimum inhibitory concentration, MBC=minimum bactericidal concentration

Statistical analysis of Tables 7 to 10 shows that, the MIC and MBC values indicate variable antibacterial activity depending on the strain and type of extract.

The MBC/MIC ratio ≤ 4 observed for Staphylococcus aureus and Staphylococcus epidermidis indicates bactericidal activity, while the high values observed for Pseudomonas aeruginosa indicate bacteriostatic activity.

3.3. Quality control of antibacterial lotions formulated from hydroethanolic infusion of Feretia apodanthera leaf powder

3.3.1. Evaluation of the physicochemical and organoleptic parameters of the formulated lotions

Quality control parameters were evaluated in order to assess the physicochemical, organoleptic and biological properties of the formulated lotions. The results are shown in Table 11.

Table 11 : Quality control of the physicochemical and organoleptic parameters of the formulated lotions

Parameters	F1	F2	F3
pH	5,67	5,89	5,97
Sensitivity	No reaction	No reaction	No reaction
Washability	Washable	Washable	Washable
Homogeneity	Uniform	Uniform	Uniform
Odor	Woodsy	Ethanolic	Ethanolic
Color	Brown	Brown	Brown

Caption: F1=formula one ; F2=formula two ; F3=formula three.

3.3.2. Evaluation of the antibacterial efficacy of formulated lotions

Lotions formulated from hydroethanolic infusions of *Feretia apodanthera* leaves were tested to assess their antibacterial efficacy against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The results obtained are shown in Table 12.

Table 12 : Diameters (mm) of inhibition zones induced by lotions formulated with hydroethanolic infusion of *Feretia apodanthera* leaf powder

Lotions	*Staphylococcus aureus*	*Staphylococcus epidermidis*
F1	14,5±0,5	12,5±0,5
F2	13,5±0,5	13±0,5
F3	14±0,5	13,5±0,5

Caption : F1=formula one ; F2=formula two ; F3=formula three.

The analysis of variance did not show any statistically significant difference between the three formulations (F1, F2 and F3) in terms of the diameters of the inhibition zones against Staphylococcus aureus and Staphylococcus epidermidis ($p > 0.05$).

The formulations therefore have comparable antibacterial activity, suggesting good formulation homogeneity.

4. Discussion

This study enabled us to perform aqueous and hydroethanolic extraction on Feretia apodanthera leaf powder and to evaluate its antibacterial activity on the in vitro growth of reference bacterial strains, namely: Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 12228, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Salmonella typhimurium ATCC 14028 and Streptococcus pyogenes.

The extraction yields of the lyophilizates were 23.24%, 26.49%, 20.52% and 70.23% for the aqueous infusion, hydroethanolic infusion, aqueous macerate and hydroethanolic macerate, respectively. The optimum was found with the hydroethanolic macerate. This major result could be justified by the influence of certain parameters such as the proportion of ethanol used, the concentration, the ratio of solvent volume to crushed mass, the extraction time and the stirring speed [14] on the solubilization of bioactive substances. Koné et al. (2017) reported that the ratio of solvent volume to pulp mass and the ethanol percentage of the hydroethanolic solvent are the two factors that significantly influence extraction by maceration [15]. Other work carried out by Aya et al. (2020) has also shown that quantitative results obtained by maceration indicate that secondary metabolite levels in leaves are higher when extraction is carried out using a hydroethanolic solvent [16]. Some authors have reported in their work that extraction with hydroalcoholic solvents increases the permeability of cell walls, which facilitates the extraction of large quantities of bioactive compounds.

The four extracts at concentrations of 100, 50 and 25.5 mg/mL inhibited the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. On Staphylococcus aureus, the extract obtained by hydroethanolic maceration showed the largest inhibition diameters, i.e. 16±0.86. However, on Staphylococcus epidermidis and Pseudomonas aeruginosa, the extract obtained by hydroethanolic infusion showed the best inhibition diameters, i.e. 15.5±0.86 and 16±1.54 respectively. This shows that Feretia apodanthera leaves have antibacterial activity against these bacterial strains. This activity could be due to the presence of secondary metabolites with strong antibacterial potential in the extracts. Owolabi et al. (2021) were able to demonstrate the presence of secondary metabolites such as unsaturated steroids, triterpenes, cardiotonic glycosides, tannins, saponins and alkaloids with antimicrobial activity [17]. As these compounds are present in Feretia apodanthera extracts, their antibacterial activity is therefore attributed to them [18,19]. On the other hand, some authors suggest that this activity also depends on factors such as the extraction method and the concentration of the active ingredient [19]. Hydroethanolic extracts showed significantly higher inhibition diameters than aqueous extracts for Staphylococcus aureus and Staphylococcus epidermidis ($p < 0.05$), reflecting better extraction of antibacterial compounds in the hydroethanolic solvent. Madubunyi et *al*. (2008) and Okoli et *al*. (2002) noted that the antibacterial activity of aqueous and ethanolic extracts was 70% with plant leaves on *Staphylococcus aureus* and *Pseudomonas aeruginosa* [20,21]. Furthermore, Fertout-Mouri et *al*. (2017) reported that *Staphylococcus aureus* is one of the strains sensitive to plant extracts.

The freeze-dried extract obtained by hydroethanolic infusion of *Feretia apodanthera* leaf powder was used as the active ingredient at a concentration of 10% (m/v). This percentage differs from that of the plant extract-based lotions formulated by Gyawali et *al*. (2016), which was 5%, and those formulated by Saurabh et *al*. (2020), which was 1% and 2%. These variations can be explained by the fact that pharmacological activities vary from one plant to another due to fluctuating concentrations of secondary metabolites, which themselves vary due to edaphic conditions. The excipients used in the formulation of the lotions consisted of ethanol as a diluent and propylene glycol as a humectant. The choice of these two excipients was based on the desire to use fewer additives while maintaining the effectiveness of the final product, on the one hand, and on the other hand, the choice of ingredients that were available and accessible in our environment, facilitating production within a reasonable timeframe. This would inevitably have an impact on the cost of the product, which would be inexpensive compared to synthetic molecule-based antibacterial lotions available on the market.

The macroscopic study conducted on the formulated lotions revealed that the prepared lotions were homogeneous, brown in color with a woody and ethanolic odor. The pH of the lotions was within the skin pH range of 4.5 to 7, indicating that the lotions are safe for the skin and therefore not irritating to users. All formulations had an acceptable pH, as a basic pH would promote skin infections and lead to therapeutic failure. The lotions were easily removed with water. They caused no redness, oedema, inflammation or irritation during the sensitivity test. These results were also observed by Saurabh et *al.* (2020). This indicates that the use of these lotions would be safe and harmless to human skin.

Of the three germs tested, namely *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, only the *Staphylococcus aureus* and *Staphylococcus epidermidis* strains were sensitive to the antibacterial lotions F1, F2 and F3. The inhibition diameters of the three formulations ranged from 12.5±0.5 to 14.5±0.5 mm. The formulated lotions therefore retain the antibacterial activity of *Feretia apodanthera* leaves.

5. Conclusion

This work, which aimed to formulate and evaluate antibacterial lotions based on freeze-dried extracts of *Feretia apodanthera* leaf powder, highlighted its antibacterial activity against germs responsible for skin conditions, particularly *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. However, the extract obtained by hydroethanolic infusion was more active on the bacterial strains tested, and the lotions formulated from this extract retained their antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. These results explain the use of *Feretia apodanthera* in traditional medicine for the treatment of various pathologies and highlight the value of medicinal plants and the traditional pharmacopoeia of Burkina-Faso.

Conflict of interest

No conflict of interest.

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