ejpmr, 2024, 11(4), 24-35

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

IN-VITRO STUDY OF THE ANTIBACTERIAL PROPERTY FROM THE HYDROETHANOLIC EXTRACT OF *SPERMACOCE OCYMOIDES BURM* (RUBIACEAE) PLANT ON ISOLATES OF MICROBIAL STRAINS RESPONSIBLE FOR IMPETIGO IN INFANTS

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Article Received on 14/02/2024 **Article Revised on 04/03/2024** **Article Accepted on 24/03/2024**

ABSTRACT

The pursuit of safe and effective therapeutic alternatives for treating impetigo in infants remains a persistent focus in pharmaceutical research. This study explores the in-vitro antibacterial activity of *Spermacoce ocymoides Burm* (Rubiaceae) against microbial strains associated with infantile impetigo. The methodology encompassed phytochemical screening to identify secondary metabolites, solid medium diffusion sensitivity tests, and determination of inhibition parameters through macro dilution in a liquid medium, along with subculture on solid agar. Oxacillin served as the reference antibiotic. Phytochemical screening revealed the presence of phenolic compounds (flavonoids, coumarins, tannins, and phlobatannins), sterols, anthocyanins, saponins, and terpenoids, while alkaloids were absent. *Staphylococcus aureus* was the sole identified pathogen in our isolates. Antibacterial assays demonstrated the efficacy of our extract against Staphylococcus aureus. Gradual concentration sensitivity assays at 500 mg/mL, 250 mg/mL, 125 mg/mL, and 62.5 mg/mL produced average inhibition zone diameters of 15 ± 0.71 mm, 7 ± 0.71 mm, 4 ± 0.71 mm, and 3 ± 0.71 mm, respectively. These values were lower than the 20 mm diameter of oxacillin at 5 µg/mL. Inhibition parameter determination yielded a Minimum Inhibitory Concentration (MIC) of 1.95 mg/mL and a Minimum Bactericidal Concentration (MBC) of 3.9 mg/mL, with an MBC/MIC ratio of 2, affirming the bactericidal effect of *Spermacoce ocymoides Burm* on *Staphylococcus aureus* isolates from impetigo pustules in infants. This research highlights the potential of *Spermacoce ocymoides Burm* as a promising antibacterial agent for impetigo treatment, laying the groundwork for further exploration and pharmaceutical development.

KEYWORDS: *Spermacoce ocymoides*, Infant, Impetigo, *Staphylococcus aureus*.

INTRODUCTION

An infant is a child aged between their first and 23rd month of life.[1] Due to the fragility of their skin and the immaturity of their defense system, infants are often affected by various skin diseases of viral, fungal, and bacterial origin. In health centers, skin diseases are the third most common reason for consultation after presumed malaria fevers and diarrheal diseases.[2] Studies indicate that skin conditions account for 67% of dermatoses in children aged 0 to 4 years.[3] Regarding bacterial skin conditions, studies have found that impetigo is more prevalent in children and infants, estimating over 12.2% of all dermatoses.[3,4] Indeed, it is a bacterial, superficial, epidermal skin infection, non-immunizing, caused by *Staphylococcus aureus* in 70% of cases and/or Group A β-hemolytic Streptococcus in 30% of cases.[5,6]

Impetigo is a major public health problem in low-resource countries, particularly affecting young children. Its global prevalence is estimated at 12.3%.[6] It is generally more common in tropical and poor regions than in temperate climate regions. This bacterial infection remains highly contagious and can occur in the form of small family epidemics or within the community. The follow-up of impetigo relies mainly on antibiotics through local and/or systemic application.[7,8] However, conventional treatments in infants require great caution due to the fragility of their skin and the immaturity of their organs.[7] Additionally, there is a growing resistance

of microorganisms to conventional treatments, along with secondary toxic manifestations.[7,8] Therefore, the search for new effective and non-toxic therapeutic alternatives is a constant concern in pharmaceutical research, leading to renewed interest in traditional medicine through plant research.

In traditional medicine, prescribed ingredients are often of plant origin because plants provide therapy with highly original active molecules with varied structures. Our study focuses on the plant *Spermacoce ocymoides*, which is widespread in Africa. Ethnobotanical studies have highlighted its anti-inflammatory, antibacterial, and anti-anemic properties.[9,10,11,12,13] Another study demonstrated its significant antioxidant and anticandidal properties against *Candida parapsilosis*.[14] Following these findings, our curiosity led us to understand the traditional use of its leaves as a poultice in East region of Cameroon to treat skin infections. Hence, the general objective of our study was to determine in vitro the antibacterial activity of the hydroethanolic extract of *Spermacoce ocymoides Burm* (Rubiaceae) on clinical isolates responsible for impetigo in infants.

MATERIALS AND METHODS

Materials

During our work, the various materials we used were grouped as follows

Didactic Material

Didactic material included a computer, USB drive, ballpoint pens, pencils, A4-sized paper pads, ruler and eraser.

Plant Material

Plant material consisted of leaves and stems of *Spermacoce ocymoides* collected in the locality of AFANEYO (Central Region, Cameroon).

Screening Reagents

Chemical screening using colorimetric tests required reagents such as Mayer's reagent (1.36 g $HgCl_2$ and 5 g KI for a final volume of 100 mL), Hager's reagent (saturated solution of picric acid), Wagner's reagent (1.27 g I_2 and 2 g KI for a final volume of 100 mL), a diluted solution of iron perchloride ($FeCl_3$), lead acetate at 10%, acetic anhydride, sulfuric acid, chloroform, anhydrous acetic acid, NaOH 2N, concentrated sulfuric acid (and 10%), diluted NH4OH, iso-amyl alcohol, 1% hydrochloric acid, 10% $FeCl_3$, a solution of copper sulfate 1% (1 g $CuSO_4$ in solution added to 65 mL distilled water shaken vigorously and made up to 100 mL), absolute ethanol, STIASNY reagent (10 mL of 40% formaldehyde plus 5 mL of concentrated HCl), $HN0_3$, and ammonia.

Extraction Material and Reagents

The extraction material and reagents consisted of 5L buckets, a flat-bottomed beaker, a sieve with a mesh size of 10, a stainless steel spatula, a stainless steel tray, No. 2 filter paper, distilled water, and 96° alcohol.

Sampling and Sample Processing Material

The sampling and sample processing material consisted of hydrophilic cotton, sterile physiological water, swabs, care gloves, and Brain Heart Infusion (BHI) media.

Microbiological Material

We used isolates from pus and crust samples from our patients, which were cultured and identified. After identification, *Staphylococcus aureus* (SA) and Group A β-hemolytic Streptococcus (SGA) were used as microorganisms.

Material for Microbiology Assays

The material used for microbiology assays included sterile Petri dishes with a diameter of 25 cm, test tubes, disposable platinum loops, Bunsen burners, an agitator, an autoclave, sterile empty discs, a vortex, micropipettes, sterilized forceps, a heating plate, a refrigerator, DNase, plasma, PolyViteX, an optical microscope, a stand, the gentian violet phenol solution, Lugol's iodine-iodide solution, alcohol-acetone solution, and a diluted Ziehl's fuchsin solution.

Culture Media

Culture media (Chapman, Muller Hinton, chocolate agar with PolyViteX, and blood agar) were prepared according to the manufacturer's instructions and sterilized in an autoclave at 121°C for 15 minutes.

Reference Antibiotic

In our work, we used oxacillin as the reference antibiotic due to its widespread availability and accessibility in terms of cost.

Methods

Period, Type, and Study Location

We performed an analytical study from 23th May 2022 to 30th October 2022 (approximately 6 months) in two laboratories: the Laboratory of Pharmacognosy and Pharmaceutical Chemistry at the Faculty of Medicine and Biomedical Sciences (FMSB) from the University of Yaounde I, for extraction and phytochemical screening, and the Bacteriology Laboratory of the Yaounde University Teaching Hospital (CHUY), for bacteriological analysis.

Collection and Identification of the Plant

The plant was collected in May 2020 in the locality of AHALA-AFANEYO, Mfoundi District, Mefou-et-Akono Department, Central Region, Cameroon. We dried the leaves and stems in a dry place shielded from sunlight for an average of 4 days, then ground, sifted, and stored the obtained powder in a glass bottle. Identification was done at the National Herbarium of Cameroon and confirmed by comparison with a botanical sample registered at the National Herbarium of H.

Jacquen-Felix No. 8389 (25099/SRF/HNC) collected on 28/09/1967.

Preparation of the Hydroethanolic Extract

Extraction was performed according to the protocol adapted and described in the study by Onawumi conducted in 2012.[15] After pulverization, we obtained a powder (weighing 150g), which was macerated in a water-ethanol mixture (30/70) for 48 hours. The macerates were filtered through N° 2 Filter Paper, and the filtrates were evaporated at 50°C in the oven. We obtained a crude extract after two days in the oven. We then stored and preserved it in a refrigerator at 20°C (Figure 1). The extraction yield was calculated using the formula below

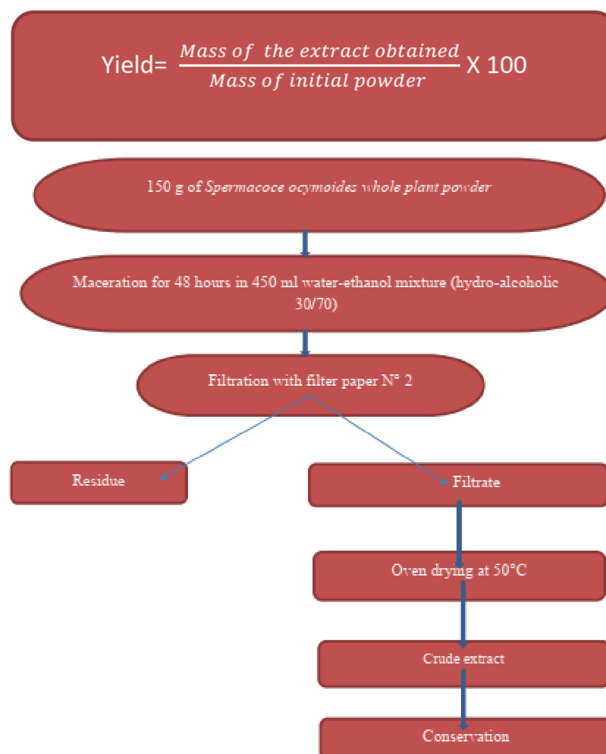

Figure 1: Hydroethanolic Extraction Protocol of *Spermacoce ocymoides* Plant.

Chemical Screening

Chemical screening was carried out using the method of colorimetric reactions. This allowed us to highlight secondary metabolites by obtaining specific colorations under defined experimental conditions. Various groups of secondary metabolites such as terpenoids, steroids, phenolic compounds including flavonoids, coumarins, polyphenols, tannins, alkaloids, and saponins were sought according to the methods described by PRAVAT Parhi in 2012.[8]

Free Flavonoids or Genins Search (cyanidine reaction)

5 mL of 1% aqueous extract and 5 mL of hydrochloric alcohol (95% alcohol + distilled water + concentrated HCl in equal parts by volume) were introduced into a test tube with a few shavings of magnesium and 1 mL of isoamyl alcohol. The appearance of a pink-orange coloration in the isoamyl alcohol supernatant layer indicated the presence of flavones, a rose-violet color indicated the presence of flavanones, and a red color indicated the presence of flavonols and flavanols.

Alkaloids Search

In a test tube, a given amount of extract was introduced, and then 10 mL of 10% sulfuric acid was added, and the mixture was shaken for 2 minutes. The obtained solution was divided into two tubes. In the first tube, two drops of Mayer's reagent were added, and in the second tube, two drops of Dragendorff's reagent were added. The appearance of an orange precipitate revealed the presence of alkaloids.

Polyphenols Search

To a given amount of plant extract, a drop of 2% alcoholic solution of ferric chloride was added. The appearance of a dark blue or greenish color signified the presence of polyphenolic derivatives.

Saponins Search

100 mL of 1% aqueous extract was distributed into 10 test tubes numbered from 1 to 10, corresponding to volumes of 1, 2 ...10 mL, respectively. The volume of each tube was adjusted to 10 mL with distilled water. Each tube was shaken for 15 seconds lengthwise and then left to rest for 15 minutes. The height of the foam was then measured for each tube. The tube for which the

foam height was 1 cm allowed us to calculate the foam index, which is the inverse of the dilution corresponding to that tube.

Sterols and Triterpenes Search
We placed 5g of plant powder in 20 mL of ether for 24 hours. After filtration, the filtrate was evaporated, and then 0.5 mL of acetic anhydride and 0.5 mL of chloroform with a little sulfuric acid were added. The appearance of a purple or violet ring at the interface indicated a positive reaction.

Anthocyanins Search
This assay was performed by introducing 2 mL of different extracts into four test tubes and 2 mL of distilled water into a fifth tube. Then, 2 mL of sulfuric acid and 2 mL of NH4OH were added to each tube. In the presence of anthocyanin, the color intensified upon acidification and turned violet-blue in a basic environment.

Tannins Search
A few drops of ferric chloride were added to the aqueous solution of the extract. The presence of tannins (catechins) was indicated by a change in color of the solution to dark blue.

Quinones Search
The quinone detection reaction was demonstrated by adding 2 mL of extract to 2 mL of distilled water plus 2 mL of concentrated sulfuric acid. The appearance of a red color indicated the presence of quinones.

Mucilage Search
In two test tubes, one containing 2 mL of plant extract and the other 2 mL of distilled water, 3 mL of ethanol was added. The formation of flakes confirmed the presence of mucilage.

Recruitment, Sampling, and Sample Processing
Recruitment and sampling were carried out in the external dermatology consultation in two hospital structures: the Gynecological-Obstetric and Pediatric Hospital of Yaoundé (HGOPY) and the District Hospital (HD) of Biyem-Assi. After explaining the study and obtaining consent from the infant's mother, we proceeded to sample lesions that were preferably not treated with antiseptics. In the absence of these lesions, sampling was done on typical yellowish crusts. Sampling was focused on the face, peri-oral area, and upper or lower limbs.

Sampling was done using a cotton swab soaked in sterile physiological water. The site to be sampled was delicately cleaned from the inside to the outside over a 2 cm surface, and then the sample was taken by making a slight rotating movement in the pustule using the swab. Immediately, the part that came into contact with the lesion (the cotton part) was introduced into the BCC medium bottle, ensuring that the part that came into contact with our hand was removed. The collected samples were routed to the laboratory within 2 hours of sampling for culture.

Identification of Strains
After culturing the samples in culture media, they were incubated at 37°C for 24 hours. When bacterial growth was observed by the appearance of colonies, two types of identification were performed. Bacteriological identification through Gram staining and biochemical identification through biochemical tests such as catalase, mannitol, coagulase, and DNASE.

Bacteriological Identification
This involved performing a Gram stain. The preparation was fixed on a slide, covered with gentian violet, and the reagent was poured after a 15-second incubation. The slide was briefly rinsed with water, and then a few drops of Lugol's solution were added, followed by another rinse with water after a one-minute incubation. A solution of decolorization (alcohol-acid) was added to the slide, which was rinsed again with water before air-drying. At the end of this staining, the slide was observed under an optical microscope at 100x magnification. The focus was on bacterial aspects such as bacterial shapes (Search for Gram-positive cocci), grouping mode (Search for the presence of clusters and chains), and the absence or presence of mobility.

Biochemical Identification
This involved highlighting the biochemical characteristics of the strains using tests such as

- **Catalase:** A drop of hydrogen peroxide was deposited on a slide, and then 1 to 3 colonies were added. The reaction was positive if characteristic boiling indicative of hydrogen peroxide degradation was observed.

- **Mannitol:** Mannitol consumption was characterized by a yellowing of the Chapman culture medium after 24 hours of incubation.

- **Coagulase:** This test involved mixing 500 µL of plasma taken from a gray tube with anticoagulant with 2 to 3 colonies of the strains to be studied in a hemolysis tube. The mixture was then placed in an incubator and incubated for 24 hours in a water bath. The reaction was positive when coagulation of the plasma was observed, usually within the first 4 hours after incubation.

- **DNASE:** This assay involved inoculating two colonies in the form of a cross on a DNASE medium, followed by a 24-hour incubation. After incubation, diluted hydrochloric acid was added. The test was positive when clear rings appeared around the cross or where the colonies were inoculated.

After identifying the strains, we proceeded to sub-culture to obtain fresh and pure colonies of the target species, with which we performed sensitivity tests using our hydroethanolic extract of Spermacoce Ocymoides plant (Rubiaceae).

Evaluation of Antibacterial Activity

Preparation of Extracts

Firstly, we prepared a mother solution at 500 mg/mL by mixing 500 mg of our crude extract with 1 mL of the DMSO-water mixture in proportions of 10:90. From our mother solution, we made intermediate solutions of different concentrations: 250 mg/mL, 125 mg/mL, and 62.5 mg/mL.

Preparation of Culture Media

All our culture media were prepared according to the manufacturer's recommendations. Firstly, we weighed the various powders constituting the culture media according to the corresponding proportions for 1 liter of distilled water. These solutions were homogenized in a flat-bottomed flask on a hot plate until boiling. Subsequently, these solutions were sterilized in an autoclave at 121°C for 15 minutes. After sterilization, they were poured into Petri dishes for Chapman and simple Mueller Hinton media. Finally, for fresh blood agar media, blood was added after sterilization in a proportion of 5%. However, it was also from the fresh blood agar medium that we obtained chocolate agar, by heating it under a flame until it showed a brown chocolate color as its name suggests. It should be noted that chocolate agar + polyvitex was obtained by adding polyvitex after preparing chocolate agar, respecting the proportion of one polyvitex vial for 500 mL of culture medium.

Sensitivity Assay of Bacterial Isolates and Measurement of Inhibition Diameters

The solid medium diffusion method was adopted to conduct this test. This test proceeded similarly to an ordinary antibiogram, but antibiotic disks were replaced by 6 mm filter paper disks impregnated with 30µL of our extract at different concentrations. This test was also considered a means of screening concentrations to initiate subsequent dilution tests. Oxacillin was used as our reference antibiotic.

Principle

Its principle was based on the diffusion of substances to be tested at a known concentration, deposited via filter paper disks on a Mueller Hinton medium previously seeded with a bacterial inoculum. After incubation, if the substance impregnated on the filter paper disks had antibacterial activity against our inoculated isolate, a zone of growth inhibition was observed around it.[11]

Experimental Protocol

From a culture of less than 24 hours of our bacterial isolate, the inoculum was prepared by suspending 10 mL of sterile physiological water, NaCl 0.9%, and a pure colony of bacteria to obtain a 0.5 McFarland suspension. Inoculation of the inoculum was carried out on the surface of the Mueller Hinton medium by flooding previously poured to a thickness of 4mm in each circular Petri dish with a diameter of 90 mm. The dishes were allowed to dry at room temperature. After 15 minutes, filter paper disks impregnated with our extract were placed. For this test, the disks, previously sterilized in an autoclave for 15 minutes at 121°C, were impregnated with our extract concentrated at different concentrations, namely 500 mg/mL, 250 mg/mL, 125 mg/mL, and 62.5 mg/mL.

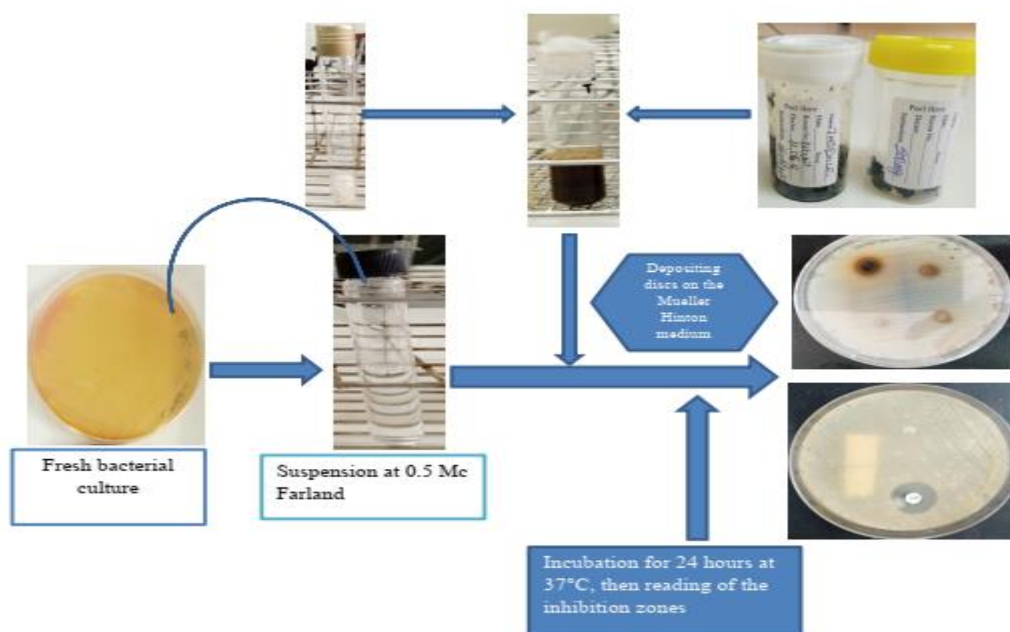

Figure 2: Experimental Protocol for the Sensitivity Test of *Spermacoce ocymoides* Extract.

Sensitivity to different extracts based on the inhibition zone diameter was classified as follows[14]: A diameter of less than 8 mm (non-sensitive), a diameter between 9-14 mm (sensitive), a diameter between 15-19 mm (very sensitive), and a diameter greater than 20 mm (extremely sensitive).

Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC)

The determination of Minimum Inhibitory Concentration (MIC) relied on evaluating the sensitivity of the identified isolate. It is the smallest concentration that inhibits any visible growth of a microorganism after incubation at 37°C for 18 to 24 hours. We used the macro-dilution technique in liquid medium.[15] It involved introducing 2 mL of Mueller Hinton Broth (MHB) into each of the 13 test tubes. Ten of the 13 tubes served as test tubes. These assay tubes were those in which successive serial dilutions of two were made, to obtain concentrations ranging from 25 mg/mL for the first tube to 0.049 mg/mL for the tenth. The remaining three tubes served as control tubes (a positive control consisting of broth and bacterial inoculum, a negative control consisting of broth and extract without inoculum for sterility control of the extract, and a tube containing only the broth for sterility control of the broth). Subsequently, 15 µL of bacterial inoculum was added to each tube, then incubated at 37°C. After 18 to 24 hours, turbidity was first evaluated, and then the tubes were centrifuged at a speed of 5.000 revolutions per minute for 5 minutes. The Minimum Inhibitory Concentrations (MIC) of the tested extracts were deduced from the first tube in the range inside which growth did not occur (absence of turbidity, absence of deposition of bacterial products compared to the negative control).[16] Visual turbidity reading and the absence of bacterial deposition after centrifugation should be in agreement to validate the obtained results.

Determination of Minimum Bactericidal Concentrations (MBC)

The Minimum Bactericidal Concentration (MBC) corresponded to the lowest concentration of a substance capable of killing more than 99.9% of the initial bacterial inoculum (i.e., less than 0.1% survivors) when optimal conditions were met to ensure the growth of the tested bacterial strain. From each tube devoid of bacterial pellet (no visible microbial growth compared to the positive control) made for the determination of Minimum Inhibitory Concentration (MIC), about 5 µL of the resuspended solution was taken and then reseeded on Mueller Hinton agar in a Petri dish and incubated at 37°C for 18 to 24 hours. Minimum Bactericidal Concentrations (MBC) were recorded from the first dilution for which no visible growth was obtained at the end of the incubation period. The MBC/MIC ratio was calculated, allowing us to confirm the bacteriostatic or bactericidal nature of the extracts. According to studies conducted by Fauchere in 2002 and Berche in 1991, when the MBC/MIC ratio is greater than 4, the substance is considered bacteriostatic; if the ratio is less than 4, the substance is bactericidal. If it is equal to 1, then the substance is considered absolute bactericidal.[17,18]

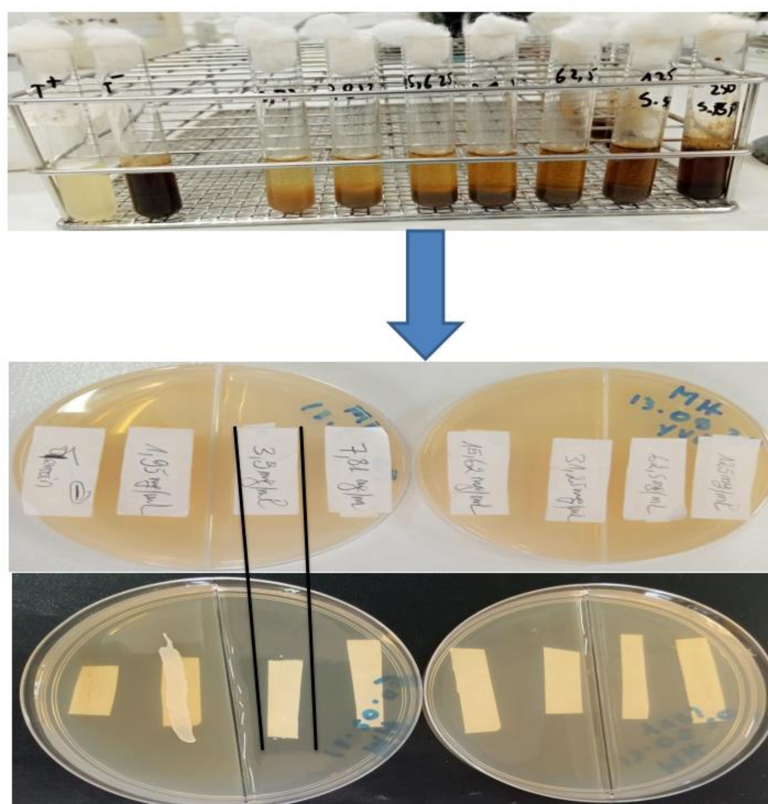

Figure 3: Determination of MIC and MBC.

Data Analysis
The data were recorded in a database created in Excel. Quantitative variables were expressed as mean ± Standard Error (SD), median, and measures of dispersion such as standard deviation with a 95% confidence interval. Qualitative variables obtained from qualitative assays in our study were expressed using alphanumeric signs such as positive (+) and negative (-).

II.2.9- Ethical Consideration
Ethical clearance was obtained from the Institutional Ethics and Research Committee of the Faculty of Medicine and Biomedical Sciences at the University of Yaoundé I. Information sheets were distributed to study participants identified in the dermatology departments of the Gyneco-Obstetric and Pediatric Hospital of Yaoundé (HGOPY) and the District Hospital (HD) of Biyem-Assi. Informed consent from these participants was obtained through the agreement of the parents of infants. The confidentiality and anonymity of the participants were preserved throughout the study and were not subject to any publication.

III- RESULTS

Extraction and Yield
After extraction using the Alcohol-water mixture (70/30) from 150g of *Spermacoe ocymoides* powder, the process yield and characteristics of the obtained extract were recorded in Table 1 below. The process resulted in a yield of 7.37%, and the consistency was pasty and blackish in color.

Table 1: Characteristics of the Extract Obtained.

Designation	Powder weight	Crude extract weight	Yield	Characteristics	
				Color	Consistency
HAE	150g	11.06g	7.37 %	Blackish	Pasty

HAE= Hydro-Alcoholic Extract.

Chemical Screening
To determine the secondary metabolite composition of *Spermacoce ocymoides* harvested in AFANEYO-AHALA (Cameroon), our extract underwent phytochemical targeting. The results obtained are presented in Table 2 and Figure 4 below. It appears that our extract was very rich in polyphenols and devoid of alkaloids.

Table 2: Results of Chemical Screening.

Metabolite	Hydro-Alcoholic Extract
Alkaloids	-
Total Polyphenols	+++
Flavonoids	++
Sterols	++
Coumarins	-
Quinones	++
Terpenoids	++
Anthocyanes	+
Phlobatannins	+
Tannins	++
Mucilage	++
Resins	+
Betacyanes	+
Saponosides	+

(-) Negative reaction, (+) Weakly positive reaction, (++) Moderate reaction, (+++) Very positive reaction.

These results provide valuable insights into the chemical composition of the extract, indicating significant presence of polyphenols, flavonoids, sterols, quinones, terpenoids, tannins, and mucilage, among others. The absence of alkaloids is noteworthy. This chemical profile can inform further investigations and potential applications of the extract.

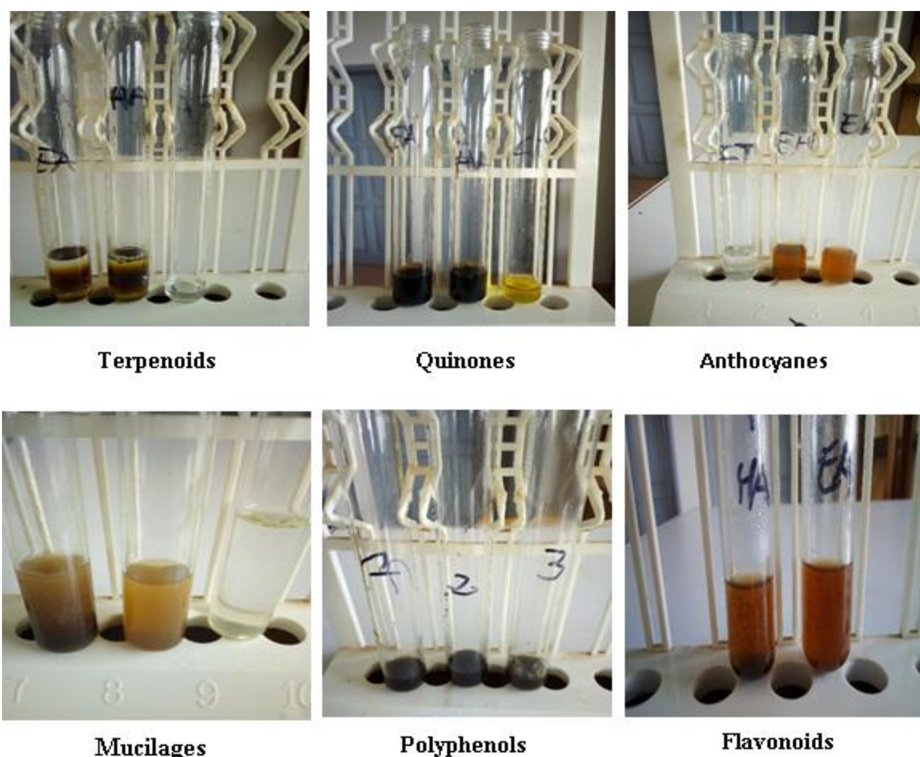

Figure 4: Illustration of the phytochemical screening results.

Antibacterial Activity

Sampling and Confirmation of Isolate Identification

Out of 15 samples collected, 11 were identified as containing the sought-after isolates. The results of the identification assays are recorded in Table 3 and Figures 5 and 6 below. It is evident that the 11 samples predominantly contained the *Staphylococcus aureus* species, and unfortunately, we couldn't isolate the *Streptococcus pyogenes β-hemolytic* group A species among the two targeted species.

Table 3: Results of Isolate Identification Assays.

Characteristics	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Gram strain	CG+	CG+	CG+	CG+	CG+	CG+CG+	CG+	CG+	CG+	CG+	CG+
Grouping mode	AM	DP	AM	AM	GRP	AM	AM	DP	AM	AM	GRP
Catalase assay	+	+	+	+	+	+	+	+	+	+	+
Mannitol assay	+	+	+	+	+	+	+	+	+	+	+
DNASE assay	+	+	-	+	+	+	+	+	+	+	+
Oxydase assay	-	-	-	-	-	-	-	-	-	-	-
Coagulase assay	+	+	+	+	+	+	+	+	+	+	+
Identified species	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA

Positive Reaction (+), Negative Reaction (-), Patient (P), Cocci Gram positive (GG+), Staphylococcus Aureus (SA).

Figure 5: Illustrative Photograph of Growth after Inoculation on Chapman, Chocolate Agar + Polyvitex, and Fresh Blood Agar following 24 hours of Incubation at 37°C.

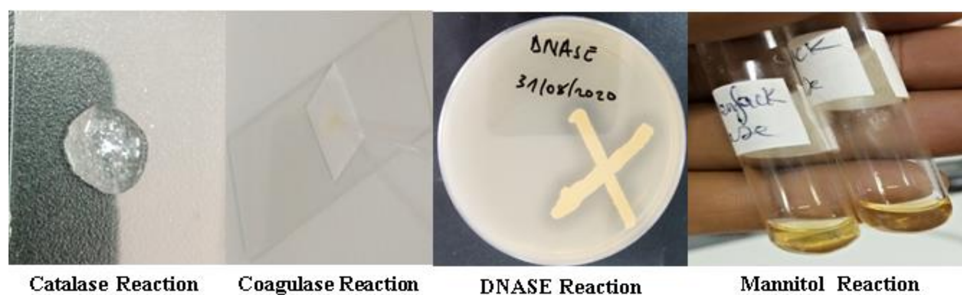

Figure 6: Illustrative Photograph of the biochemical identification assay result.

Sensitivity assay

The activity of the hydro-ethanolic extract of *Spermacoce ocymoides* and oxacillin (reference antibiotic) tested on the *Staphylococcus aureus* isolate obtained from the impetigo pustules of infants was revealed by the presence of inhibition zones around the paper discs soaked in the extract. It was observed that the diameter of inhibition was more significant when the extract concentration was at 500 mg/mL, although it remained lower than that of the reference antibiotic at 5 µg/mL, as depicted in Figure 7 below.

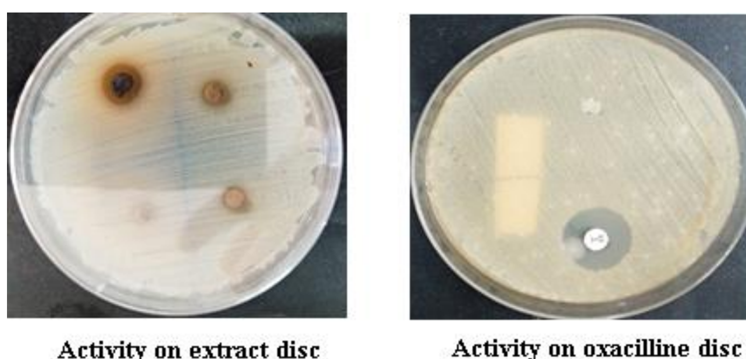

Figure 7: Comparison of Inhibition Zones of hydroethanolic extract with oxacilline on Staphylococcus aureus isolate.

The calculation of the mean diameters of inhibition and standard deviations for the activity of our extract and the reference antibiotic (ATB) was carried out, with results documented in Table 4 below. It was observed that the diameters of the inhibition zones ranged from 15±0.71 mm to 3±0.71 mm, correlating with the decrease in concentrations of the hydroethanolic extract.

Table 4: Distribution of Diameter Measurements of Inhibition Zones at Different Concentrations of the Hydroethanolic Extract.

Extract Concentration mg/mL	Diameter of inhibition zone obtained (mm)												
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	M
500	14	15	16	15	15	16	15	14	16	15	15	14	15±0.71
250	6	6	7	7	8	8	8	7	8	7	8	7	7 ±0.71
125	3	3	4	3	4	5	4	5	4	4	5	3	4±0.71
62.5	2	2	3	2	3	3	4	4	3	2	4	2	3±0.71
Concentration of reference ATB (Oxacilline) µg/mL	**Diameter of inhibition zone (mm)**												
5	20												

Parameters of Bacterial Growth Inhibition (MIC, MBC, and MBC/MIC Ratio)

Following the investigation of bacterial growth inhibition on solid medium, we determined the Minimum Inhibitory Concentration (MIC) of our extract against the Staphylococcus aureus (SA) isolate in liquid medium. Subsequently, the Minimum Bactericidal Concentration (MBC) was determined after subculture on agar. These two parameters allowed us to calculate the MBC/MIC ratio. The results obtained are presented in Table 5 below. From this table, we observed that the Minimum Inhibitory Concentration (MIC) was 1.95 mg/mL, while the Minimum Bactericidal Concentration (MBC) was 3.9 mg/mL, resulting in an MBC/MIC ratio of 2 for the

Staphylococcus aureus isolate collected from an infant's impetigo pustule.

Table 5: Distribution of Parameters of Inhibition for the Hydroethanolic Extract of *Spermacoce Ocymoides*.

Isolate assay	MIC Value (mg/mL)	MBC Value (mg/mL)	MBC/MIC Ratio
Staphylococcus aureus (SA)	1.95	3.9	2

***SA**: Staphylococcus aureus,*
***MIC**: Minimum Inhibitory Concentration,*
***MBC**: Minimum Bactericidal Concentration.*

DISCUSSION

This study aimed at assessing the *in-vitro* antibacterial activity of the hydroethanolic extract from *Spermacoce ocymoides* plant on bacteria isolates responsible for impetigo in infants.

Regarding the hydroethanolic extraction (30/70) (v/v), we obtained 11.06 g of extract from the leaves-stems of *Spermacoce ocymoides*, with a yield of 7.37%. This yield was lower than those reported by Théophile Mpaba in 2018 and Pravat in 2012, who found respective yields of 18.78% and 15.4% for hydroethanolic and methanolic extracts with the whole plant.[19,20] The variation in yield could be attributed to differences in parameters such as plant parts used, extraction protocol, harvest season, and drying methods.[21]

The phytochemical screening of the *Spermacoce ocymoides* plant was carried out using qualitative colorimetric methods on aqueous, mathanolic and hydro-ethalonic extracts. This screening revealed the presence of polyphenols, flavonoids, coumarins, terpenoids, anthocyanins, tannins, steroids and resins. On the other hand, we did not find saponosides and alkaloids. On the other hand, the phobatanins were present only in the hydro-ethanolic extract, as were the mucilages which were absent in the methanolic extract. This result differed from the one found in the studies carried out by Ilonlu and Felix which highlighted the presence of alkaloids and saponosides in extracts of this plant.[22,23] Our result also differed from those found in the studies carried out by Onawumi, and Nwachukwu Francis who did not highlight flavonoids, steroids, tannins and phobatanins in the extracts of this plant.[24,25] This discrepancy in results from previous studies, likely due to differences in geographical location, harvesting season, plant parts used, and solvents employed.

Sample collection from impetigo lesions in infants revealed primarily the presence of *Staphylococcus aureus* (SA). Streptococcus β-hemolytic group A (SBHGA) was not detected. This aligns with findings from Koning's study in 2012, indicating that impetigo in newborns and infants is mainly caused by single organism, *Staphylococcus aureus* (SA), belonging mainly to group II (80%), phage type 71 (60% of cases) and mainly presents in the bullous form.[26]

The antibacterial activity was evaluated using solid medium diffusion, liquid medium macro-dilution, and subculture on agar. Sensitivity assays demonstrated that all Staphylococcus aureus (SA) isolates were sensitive to the hydroethanolic extract of *Spermacoce ocymoides*. The inhibition zone diameters indicated antibacterial activity, with the highest inhibition observed at a concentration of 500 mg/mL, averaging 15 ± 0.71 mm. However, this was lower than the inhibition zone diameter of the reference antibiotic (Oxacillin) at a concentration of 5 µg/mL. This value of the inhibition diameter from our extract was lower than the one found in the study carried out by Onawumi oluwayemi in Nigeria, who found a diameter of 20 mm on Staphylococci isolates. This discrepancy in results could be explained by the difference in the geographical location of the plant's harvest area. Indeed, studies have shown that the presence and availability of secondary metabolites with antimicrobial activity varies depending on the geographical area as well as the harvest season of the plant.[27]

In terms of inhibition parameters, the liquid macro-dilution technique allowed the determination of Minimum Inhibitory Concentration (MIC) and Bactericidal Concentration (MBC). The MIC was 1.95 mg/ml, the MBC was 3.9 mg/ml, resulting in an MBC/MIC ratio of 2, which was less than 4, indicating the bactericidal activity of our hydroethanolic extract from *Spermacoce ocymoides* plant against *Staphylococcus aureus* isolates from infant impetigo pustules.

CONCLUSION

This study demonstrated the in-vitro antibacterial activity of the hydroethanolic extract from *Spermacoce ocymoides* plant against impetigo-causing bacteria in infants. The extract showed richness in polyphenols and other antimicrobial metabolites. *Staphylococcus aureus* dominated the impetigo isolates, and the hydroethanolic extract exhibited antibacterial activity against these isolates. Further research avenues include formulating ointments, creams, cosmetic lotions, antibacterial soaps, quantifying secondary metabolites, evaluating dermotoxicity, assessing antifungal activity against dermatophytes, and developing pharmaceutical formulations for active extracts.

ACKNOWLEDGEMENT

The authors are thankful to the Laboratory of Pharmacognosy and Pharmaceutical Chemistry at the Faculty of Medicine and Biomedical Sciences (FMSB) from the University of Yaounde I and Bacteriology

Laboratory of the Yaounde University Teaching Hospital (CHUY), which made the laboratories and work materials available to us for the successful completion of the study.

Competing interest

The authors have reported no competing interests.

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