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# Phytochemical Study and *In vitro* Antibacterial Activities of Extracts from Three Ivorian Medicinal Plants Traditionally Used to Treat Inflammatory Dermatoses in the Mé Area, South-East of Ivory Coast

**KOUASSI Kouassi Germain [a*], AKA ANY-GRAH Adjoua Sandrine Armelle [b], KONAN Gbê Kouakou N'dri Ange [c], ODOH Alida Edwige [b] and ZIRIHI Guédé Noël [a]**

*[a] Biology, Environment and Health Doctoral School, Natural Environments and Biodiversity Conservation Laboratory, Research Unit in Biosciences, University of Félix HOUPHOUËT- BOIGNY (UFHB), 22 BP 582 Abidjan 22, Ivory Coast.*
*[b] Pharmaceutical Sciences, Analytical Sciences and Public Health Laboratory, Research Research Unit Pharmaceutical and Biological Sciences, University of Felix HOUPHOUËT- BOIGNY (UFHB), 22 BP 582, Abidjan 22 BP 582, Ivory Coast.*
*[c] Biology and Health Laboratory, Research Unit in Biosciences, University of Felix HOUPHOUËT-BOIGNY (UFHB), 22 BP 582 Abidjan 22, Ivory Coast.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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**Corresponding author: E-mail: germainkouassik@yahoo.fr;*

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## ABSTRACT

**Background :** Many of the pharmaceutical drugs used to treat Bacterial dermatoses, are becoming less and less effective in the face of bacterial multi-resistance. The search for remedies based on medicinal plants is a necessity in the discovery of new antibiotics effective in the treatment of dermatoses, above all inflammatory dermatoses become very common.
**Objective :** To evaluate the antibacterial power of aqueous and ethanolics extracts of three medicinal plants used in traditional medicine by the Akyé people to treat inflammatory dermatoses.
**Methods :** Plant organs were harvested in the Mé area, dried to a constant weight and then ground to a fine powder. Extraction was then carried out using osmosed water and ethanol 96° The phytochemical screening of the extracts was carried out by colouring reactions. The aqueous and ethanolic plant extracts were tested *In vitro* against *Staphyloccocus aureus* and *Pseudomonas aeruginosa*.
**Results :** The phytochemistry screening revealed the presence of sterols and polyterpenes, polyphenols, alkaloids, flavonoids, tannins, quinones and saponosides. Antibacterial tests showed that the hydroethanol extracts were the most active, with Minimal Inhibitory Concentrations ranging from (0.048 to 06.25 mg/ml) and Minimal Bacterial Concentrations from (1.56 to 06.25 mg/ml). Of the two bacterial strains studied, *Pseudomonas aeruginosa* was the most sensitive to all the extracts. The antibacterial potentials thus highlighted by the values of the ratio between Minimal Bacterial Concentrations and Minimal Inhibitory Concentrations obtained indicated that all the extracts showed a high bactericidal activity (100%) on *Pseudomonas aeruginosa* compared with *Staphylococus aureus.*
**Conclusion :** The results provide a solid database for future pharmaceutical research and the development of dermatological treatments derived from natural sources. In addition, this work highlights the importance of traditional medicinal knowledge in modern healthcare and supports efforts to develop effective topical forms of dermatoses based on active plant extracts through galenic formulations.

*Keywords: Phytochemical study; extracts; medicinal plants; antibacterial.*

## 1. INTRODUCTION

Cutaneous and subcutaneous staphylococcosis are increasingly common skin diseases, especially in developing countries (Messad, 2016). They are caused mainly by the golden staphylococcus, also known as ' *Staphylococcus aureus* ' (DIPE, 2021). This bacteria colonises various parts of the human body, including the skin, and can cause various infections, such as folliculitis, boils and acne (Price et al., 2013). Some types of these bacteria have become resistant to several antibiotics, making it increasingly difficult to treat skin infections (Marisa et al., 2012). In addition to *staphylococcus aureus*, the bacteria ' *Pseudomonas aeruginosa*' is also an opportunistic pathogen that causes inflammatory dermatoses and is resistant to many antibiotics, further complicating the treatment of skin infections (Kanga, 2017).

To face these growing challenges of antibiotic resistance, the search for new molecules from medicinal plants has become a priority for the scientific community (Kipré et al., 2017). This study focuses on three medicinal plants used by the Akyé people of the Mé region of Ivory Coast (south-east), with the goal of discovering new antibiotics that are effective in treating inflammatory dermatoses.

The objective of this study is to identify the metabolites contained in extracts from the leaves of *Combretum paniculatum* Vent. (Combretaceae) and *Trema orientalis* (L.) (Cannabaceae), as well as from the stem bark of *Duguetia staudtii* (Engl & Diels) (Annonaceae). These extracts were evaluated for their antibacterial activity. The results suggest that these plants could be promising sources of new antimicrobial drugs for the treatment of

inflammatory skin infections and skin superinfections.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The material used in this study is mainly consisted of technical and biological materials : plant and bacteriological material.

#### 2.1.1 Technical material

For this study, technical material was used to gain access to the plants and to take plant samples. For phytochemical screning, we used a Memmert-type oven at 50°C to dry the wet samples before pulverising them using a Retch GM electric grinder. We used an electric balance for the various weighing operations and a 37°C water bath. A sand bath was used to evaporate the extracts in porcelain capsules. A water heater was available. This material also included spatulas for removing drug powders, cotton wool used as a filter, a hood to protect against powders ejected during drug spraying, a trituration rod, solvents and reagents.

- Solvents: Osmosed water and ethanol 96° were used for extractions by decoction and maceration. Detection tests for compound groups were carried out on these 3 types of crude extracts (decoction, maceration and hydroethanolic).
- Reagents: Phytochemical screening required various reagents (Stiasny's reagent, sodium acetate and ferric chloride; acetic anhydride and sulphuric acid, etc;).
  All the reagents used were of analytical grade.

#### 2.1.2 Biological material

*2.1.2.1 Harvest of plant organs*

After harvesting in the morning between 8 and 11 a.m., plant organs (fresh leaves and stem bark) preserved in 50kg bags, collected starting in February 2024 in area of Mé were transported until to the Sciences of Medicines Laboratory of Research Unit of Pharmaceutical and Biological Sciences of Félix-Houphouët-Boigny University. On the evening of the sampling day (between 3 and 4.30 pm), the plant organs were meticulously sorted to remove foreign bodies (dead insects and plant organs, etc) and impurities.

*2.1.2.2 Drying and pulverisation*

At around 5pm, the cleaned fresh leaves of *Combretum paniculatum* Vent (Combretaceae) (Fig. 1) and *Trema orientalis* (L.) (Cannabaceae) (Fig. 2) were spread out and dried at low air conditioning at 16±1°C for one week in the Drug Science Laboratory of the Pharmaceutical and Biological Sciences Reaserch Unit**.** Next, the bark of the stem of *Duguetia staudtii* (Engl. & Diels) (Annonaceae) (Fig. 3), cut into pieces, was dried at room temperature (between 20°C and 25°C) for approximately two weeks. The organs (leaves and stem bark) were stirred during drying using the fingers of the hand. The choice of this difference in drying temperature followed the drying conditions of these plants used by the practitioners encountered in traditional medicine. Each dried organ was ground and pulverised using a Retsch GM 300 propeller mill. After grinding, each plant powder obtained was passed through a fine sieve (nnumber 1 with a diameter of 1 mm) to obtain a fine powder of each plant organ (Fig. 1c, 2c & 3c) with different characteristics (colours and granulometries).

#### 2.1.3 Plant material

Dry leaf powders from *Combretum paniculatum* ; *Trema orientalis* and bark from the stem of *Duguetia staudtii* constituted the plant material used for extractions and the crude extracts obtained were used for phytochemistry screening.

Photographs of plant species (A) studied and medicinal plant parts (B and C) used for phytochemical studies and evaluation of antibacterial activities.

#### 2.1.4 Bacteriological material

It consisted of *Staphylococcus aureus* ATCC 1292 and *Pseudomonas aeruginosa* ATCC 29213 available at the Biology and Health Laboratory of the Biological sciences research unit of the University of Félix-Houphouët-Boigny. These clinical bacterial strains are isolated from human pathological products such as urine, pus and blood. They are resistant to gentamicin, penicillin and chloramphenicol.

### 2.2 Methods

#### 2.2.1 Extraction

The extracts obtained from plant powders were prepared using two classic extract preparation processes used in village environments, namely decoction and maceration, as well as another maceration process using a solvent other than water, in particular ethanol 96°. A total of nine (9) extracts were obtained, distributed as follows : six (6) aqueous extracts, including three (3) decoctions, three (3) macerations and three (3) hydro-ethanolic extracts (ethanol 70%) obtained by maceration.


**Fig. 1. Leafy twigs (A); Dry leaves (B) and powder (C) of *Combretum paniculatum* Vent (Combretaceae), field photo; Montézo, 02-2024**


**Fig. 2. Leafy twigs (A); Dry leaves (B) and powder (C) of *Trema orientalis* (L.) (Cannabaceae), field photo; Akoupé, 05-2024**


**Fig. 3. View of the skinned stem of *Duguetia staudtii* (Diels & Engl) (Annonaceae), field photo ; Bécédi-Brignan, 04-2024**

*2.2.1.1 Extraction by decoction*

The modified protocol described by Zirihi, (2006) was used. 200 g of dry leaf powder from each plant was boiled separately at 100°C for 30 minutes in a pan containing 2 litres of gas-osmosis water. The homogenate, left to cool for approximately 1 hour, was first filtered using two fine sieves of different diameters (200 µm and then 100 µm). The homogenate obtained was then filtered through a white filtration cloth and finally through cotton wool contained in a funnel.

Eventually, the decocted were placed in porcelain plates and dried by evaporation in a Memmert oven at 55°C. Each dry extract was scraped with a blade and weighed on an OHAUS balance to assess its yield. They were then stored in sterile glass dishes at 24°C in the airconditioning of the Medicinal Sciences Laboratory of the Pharmaceutical and Biological Sciences Research Unit of the Université Félix-Houphouêt-Boigny until use.

*2.2.1.2 Extraction by maceration*

The chemical compounds of each plant were extracted using the method of Zirihi, (2006) modified as follows : 200 g of fine powdered leaves (*Combretum paniculatum* ; *Trema orientalis)* and dry stem bark of *Duguetia staudtii* were macerated in 2 litres of osmosed water for 24 hours using a propeller shaker.

The macerate obtained was filtered using two fine sieves of different diameters (200 µm then 100 µm). The homogenate obtained was then filtered through a white filtration cloth and finally through hydrophilic cotton contained in a funnel. After filtration, the macerates were placed in porcelain plates and dried for 72 hours by evaporation in a Memmert-type oven at 55°C. Each dry plant extract was scraped and weighed to assess its yield.

*2.2.1.3 Hydroethanolic extraction*

Hydroethanol extraction was carried out using the method of Zirihi et al., (2003).

200 grams of drug powder were macerated in 2 litres of a mixture (water-ethanol, with 600 ml of osmosed water and 1400 ml of ethanol 96°C) contained in a drum for 24 h under constant stirring.

After removing the solvent, the dry extract obtained was scraped off and stored in a hermetically sealed jar at 24°C in the laboratory's air-conditioning unit.

**2.2.2 Calculation of yield**

It is expressed as a percentage. In practice, it is determined by the ratio of the weight of the dry extract after evaporation to the weight of the dry plant matter powder used for extraction, multiplied by 100.

This results in the following formula : Rd (%) = (m x 100) / M (Rd : extraction yield in percentage, m : mass in grams of dry extract, M : mass in grams of drug powder).

**2.2.3 Phytochemical study of extracts**

The tri-phytochemistry of the plant extracts studied was carried out under favourable conditions at the Laboratory of Botanical Pharmacognosy and Cryptogamy of the Pharmaceutical and Biological Sciences Research Unit of the University of Felix HouphouëtBoigny.The characterisation of the phytocompounds most involved in biological activities, namely polyphenols, alkaloids, tannins, flavonoids, saponosides, quinones, sterols and polyterpenes, has been carried out. However, their research and characterisation were carried out using the staining reactions described by Bruneton, (2016)**,** using reagents for each group of secondary metabolites.

## 3. EVALUATION OF ANTIBACTERIAL ACTIVITIES

### 3.1 Preparation of Bacterial Inoculum

The bacterial inoculum was prepared from a 24 hours young colony in Mueller Hinton broth (MHB). A colony isolated from the bacterial culture was removed using a platinum loop and homogenised in 10 ml of broth, then incubated for 3 to 5 h at 37°C to obtain a pre-culture. A volume of 1000 µL for S. *aureus* ATCC 1292 and 10 µL for P. *aeruginosa* ATCC 29213 were introduced into 10 ml of physiological water (0.9% NaCl), constituting the bacterial inoculum estimated at $10^6$ bacteria/ml.

### 3.2 Preparation of the Concentration Range

The concentration ranges were prepared in a series of fifteen (15) test tubes (T) numbered $_{T1}$ to $_{T15}$, using the modified liquid double dilution method of Ouattara et al., (2013). These concentration ranges vary from 50 mg/ml to 0.003 mg/ml. For this, 10 ml of sterile distilled water was put in tube $_{T1}$ and 5 ml in all other tubes.

A mass of 0.5 g of plant extract was then dissolved in tube $T_1$ and homogenised completely to give a concentration of 50 mg/ml. Half the volume of tube $T_1$ (5 ml) was then transferred to tube $T_2$ and homogenised. This operation was repeated up to tube $T_{15}$, after which half the volume was discarded. This resulted in concentrations $C_1$=50 mg/ml ; $C_2$=25 mg/ml ; $C_3$= 12.5 mg/ml ; $C_4$= 06.25 mg/ml ; $C_5$= 03.125 mg/ml ; $C_6$=01.56 mg/ml ; $C_7$= 0.78 mg/ml ; $C_8$= 0.39 mg/ml ; $C_9$= 0.195 mg/ml ; $C_{10}$= 0.097 mg/ml ; $C_{11}$= 0.048 mg/ml ; $C_{12}$= 0.024 mg/ml ; $C_{13}$= 0.012 mg/ml ; $C_{14}$= 0.006 mg/ml and $C_{15}$= 0.003 mg/ml.

### 3.3 Determining Antibacterial Parameters

To determine the antibacterial parameters, which are the Minimum Inhibitory Concentration (MIC), the Minimum Bactericidal Concentration (MBC) and the antibacterial potentials characterised by the values of the ratio (MBC/MIC) of the different extracts, the liquid dilution method described by Ouattara et al., (2013) with slight modifications, was used. Thus, a range of extract concentrations from 50 to 0.003 mg/ml was prepared using the double dilution technic and then autoclaved at 121°C for 15 minutes. Then, in haemolysis tubes, 1 ml of each plant extract concentration was inoculated with 1 ml of a 24 hours inoculum with a density of 5 ×$10^6$.

Two tubes were prepared, one without extract to serve as the growth control and the other without germ to serve as the sterility control. After incubation for 24 hours at 37°C, the MIC was determined. This correspond to the concentration of the first tube in which there is no visible growth of the germ tested. To determine the MIC, the usual protocol was slightly modified. New antimicrobial-free nutrient agar, poured into petri dishes, was streaked 5 cm long with 0.1 ml of the contents of all the tubes following the first tube in which there was no visible growth of the germ tested by eye.

After inoculation, the petri dishes were incubated for 24 hours at 37°C, after which the BMC was determined. It represented the lowest concentration greater than or equal to the MIC that completely inhibited the growth of the germ tested. The MBC/MIC ratio was used to determine the mode of action of each extract (Fauchere & Avril, 2002).

## 4. RESULTS

### 4.1 Extraction Yield

In this study, the leaves of *Combretum paniculatum* and *Trema orientalis* and the bark of *Duguetia staudtii* had extraction yields irrespective of the method used.

Yields varied between 07.16% and 12.05% for *Combretum paniculatum* leaves, between 15.06% and 45.6% for *Trema orientalis* leaves and between 3.76% and 6.93% for *Duguetia staudtii* bark (Fig. 4).

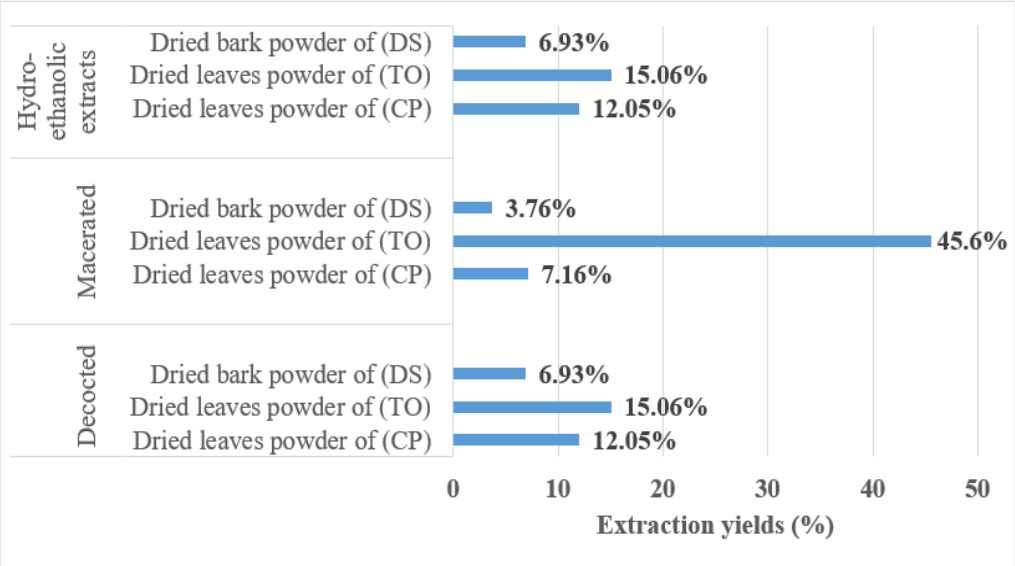


**Fig. 4. Histogram of percentage (%) yields of aqueous and hydro-ethanolic extracts of three Ivorian medicinal plants used in the Mé region (South-East, Ivory Coast)**

*Trema orientalis* leaves had the highest yield (45.6%) by aqueous maceration, approximately six (6) times higher than that of *Combretum paniculatum* leaves (7.6%) and twelve (12) times higher than that of *Duguetia staudtii* (Fig. 4).

The decoction and mixed solvent (water and 70% ethanol) extraction methods gave the highest yields for *Combretum paniculatum* leaves (12.05%) and *Duguetia staudtii* bark (6.93%) (Fig. 4).

Extractions of *Trema orientalis* leaves by decoction and by a mixture of solvents (water and ethanol) had a low yield (15.06%), while extraction by maceration of the plant's leaves had the highest yield (45.6%) (Fig. 4).

CP : *Combretum paniculatum* ; TO : *Trema orientalis* and DS : *Duguetia staudtii.*

The lowest yields were observed for extractions of *Duguetia staudtii* bark, whether by maceration (3.76%) or decoction, or by mixing solvents (osmosed water and ethanol 70%).

## 4.2 Phytochemical Analysis of the Studied Plant Extracts

In this phytochemical study, sterols and polyterpenes, polyphenols, flavonoids, alkaloids and saponosides were detected in the leaves of *Combretum paniculatum* and *Trema orientalis* as well as in the bark of the stem of *Duguetia staudtii* (Table 1).

From a quantitative point of view, the abundant presence of sterols and polyterpenes was detected in all the organs studied from these three plants.

On the other hand, polyphenols and flavonoids were more abundant in the leaves of *Combretum paniculatum* and *Trema orientalis* and less abundant in the bark of *Duguetia staudtii.* Alkaloids were more abundant in the leaves of *Combretum paniculatum* and in the stem bark of *Duguetia staudtii*, while they were present in small quantities in the leaves of *Trema orientalis.* Tannins (catechic and gallic) and quinones were only found in aqueous and hydro-ethanolic extracts of *Combretum paniculatum* leaves (Table 1).

The decoctate of *Combretum paniculatum* is therefore the extract richest in phytochemicals (Table 1).

## 4.3 Using Medicinal Recipes by the Cutaneous Route to Treat Inflammatory Dermatoses

The ethnobotanical surveys carried out previously showed that the recipes are generally aqueous extracts (decocted and macerated) and hydro-ethanol extracts which are used in several dermatological forms in traditional medicine by the traditional practitioners interviewed. The majority were liquid forms (50.01%), followed by semi-solid (44.77%) and solid (5.22%) forms (Fig. 5). The main route of administration of these remedies for better treatment of dermatoses was the cutaneous route (77.38%) by simple bath or steam (15.76%), by massage or local application (58.70%) and by dressing (12.50%) to treat inflammatory dermatoses effectively.

The oral (7.61%) and anal (5.41%) routes are also used to combat dermatoses systemically.

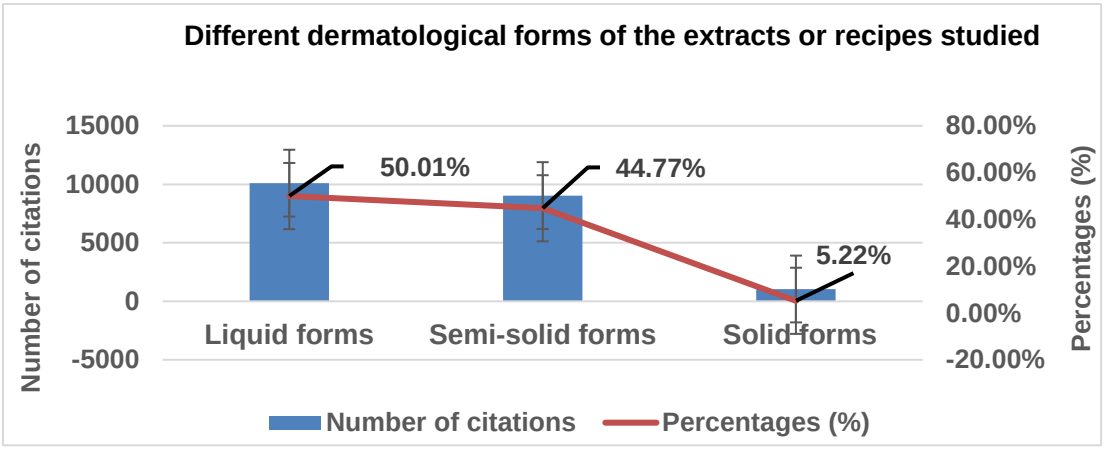


**Fig. 5. Histogram of different dermatological forms of the extracts of medicinal plants used to treat in traditional medecine inflammatory dermatoses in the Mé area (South-East, Ivory Coast)**

**Table 1. Results of tri-phytochemical tests on aqueous extracts and hydro-ethanol extracts of three Ivorian medicinal plants used to treat skin diseases in Mé region (South-east, Ivory Coast).**

| Medicinal plants | | ***Combretum panicutatum*** | | | ***Trema orientalis*** | | | ***Duguetia staudtii*** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Extractions | | Decoction | Maceration | Hydro-Éthanolic | Decoction | Maceration | Hydro-Éthanolic | Decoction | Maceration | Hydro-Éthanolic |
| Chemical compounds | | **C1** | **C2** | **C3** | **T1** | **T2** | **T3** | **D1** | **D2** | **D3** |
| Sterols and polyterpenes | | **++** | **++** | **++** | **++** | **++** | **++** | **++** | **++** | **++** |
| Polyphenols | | **++** | **++** | **++** | **++** | **++** | **++** | **+** | **+** | **+** |
| Flavonoids | | **++** | **++** | **++** | **++** | **++** | **++** | **+** | **+** | **++** |
| Alkaloids | D | **++** | **+** | **++** | **++** | **++** | **++** | **++** | **++** | **++** |
| | B | **++** | **++** | **++** | **+** | **+** | **+** | **++** | **++** | **++** |
| Tannins | Catechics | **++** | **+** | **-** | **-** | **-** | **-** | **-** | **-** | **-** |
| | Gallics | **++** | **+** | **-** | **-** | **-** | **-** | **-** | **-** | **-** |
| Quinones | | **++** | **++** | **+** | **-** | **-** | **-** | **-** | **-** | **-** |
| Saponosides | | **++** | **+** | **++** | **++** | **-** | **-** | **+** | **+** | **-** |

***NB : (++) :*** *More abundant* ***presence*** *of the chemical compounds ;* ***(+) :*** *Presence of the chemical compounds and* ***(-) :*** *Absence of the chemical compounds.*
***C1 :*** *Decocted extract of Combretum paniculatum ;* ***T1 :*** *Decocted extract of Trema orientalis ;* ***D1 :*** *Decocted extract of Duguetia staudtii ;*
***C2 :*** *Macerated extract of Combretum paniculatum ;* ***T2 :*** *Macerated extract of Trema orientalis* ***; D2 :*** *Macerated extract of Duguetia staudtii ;*
***C3 :*** *Combretum paniculatum hydroethanol extract ;* ***T3 :*** *Trema orientalis hydroethanol extract ;* ***D3 :*** *Duguetia staudtii hydroethanol extract*

**Table 2. Determination of antibacterial parameters of extracts of *Combretum paniculatum* (Vent) (Combretaceae) on the strains tested**

| **Medicinal plant / Extracts** | **Bacterial strains tested** | **MIC** | **MBC** | **MBC/MIC ratio** | **Antibacterial power of extract** |
|---|---|---|---|---|---|
| **Cp.** / Decocted extract (**C1**) | *S. aureus* ATCC 1292 | **06.25 mg/ml** | 50.00 mg/ml | CMB/CMI= **8** | **Bacteriostatic** |
| **Cp.** / Macerated extract (**C2**) | *S. aureus* ATCC 1292 | **12.50 mg/ml** | >100 mg/ml | **ND** | **ND** |
| **Cp.** / Hydro-ethanolic extract 70 % (**C3**) | *S. aureus* ATCC 1292 | **06.25 mg/ml** | 50.00 mg/ml | CMB/CMI= **8** | **Bacteriostatic** |
| **Cp**. / Decocted extract (**C1**) | *P. aeruginosa* ATCC 29213 | **06.25 mg/ml** | 25.00 mg/ml | CMB/CMI= **4** | **Bactericide** |
| **Cp.** / Macerated extract (**C2**) | *P. aeruginosa* ATCC 29213 | **25.00 mg/ml** | 50.00 mg/ml | CMB/CMI= **2** | **Bactericide** |
| **Cp.** / Hydro-ethanolic extract 70 % (**C3**) | *P. aeruginosa* ATCC 29213 | **06.25 mg/ml** | 06,25 mg/ml | CMB/CMI= **1** | **Bactericide** |

***Legend : Cp*** *:* ***Combretum paniculatum Vent. (Combretaceae) ;*** *Differents extracts of the leaves of the plant.*
*Two multi-resistant bacteria : S. aureus :* ***Staphylococcus aureus*** *and P. aeruginosa :* ***Pseudomonas aeruginosa***
*ATCC :* ***American Type Culture Collection***
***MIC****=Minimal Inhibitory Concentration ;* ***MBC*** *= Minimal Bactericide Concentration*
***ND*** *= Not Determinated*

**Table 3. Determination of antibacterial parameters of extracts of *Trema orientalis* (L.) (Cannabaceae) on the strains tested**

| Medicinal plant / Extracts | Bacterial strains tested | MIC | MBC | MBC/MIC ratio | Antibacterial power of extract |
|---|---|---|---|---|---|
| **TO.** / Decocted extract (**T1**) | *S. aureus* ATCC 1292 | **06.250 mg/ml** | 25.000 mg/ml | CMB/CMI= **4** | **Bactericide** |
| **TO.** / Macerated extract (**T2**) | *S. aureus* ATCC 1292 | **01.560 mg/ml** | 50.000 mg/ml | CMB/CMI= **32,05** | **Bacteriostatic** |
| **TO.** / Hydro-ethanolic extract 70 % (**T3**) | *S. aureus* ATCC 1292 | **00.780 mg/ml** | 25.000 mg/ml | CMB/CMI= **32,05** | **Bacteriostatic** |
| **TO**. / Decocted extract (**T1**) | *P. aeruginosa* ATCC 29213 | **03.125 mg/ml** | 10.500 mg/ml | CMB/CMI= **03,36** | **Bactericide** |
| **TO.** / Macerated extract (**T2**) | *P. aeruginosa* ATCC 29213 | **12.500 mg/ml** | 25.000 mg/ml | CMB/CMI= **2** | **Bactericide** |
| **TO.** / Hydro-ethanolic extract 70 % (**T3**) | *P. aeruginosa* ATCC 29213 | **03.125 mg/ml** | 06,250 mg/ml | CMB/CMI= **2** | **Bactericide** |

***Legend : TO** : **Trema orientalis (L.) (Cannabaceae) ;** Differents extracts of the leaves of the plant.*
*Two multi-resistant bacteria : S. aureus : **Staphylococcus aureus** and P. aeruginosa : **Pseudomonas aeruginosa***
*ATCC : **American Type Culture Collection***
***MIC**=Minimal Inhibitory Concentration ; **MBC** = Minimal Bactericide Concentration*

**Table 4. Determination of antibacterial parameters of extracts of *Duguetia staudtii* (Engl. & Diels). (Annonaceae) on the strains**

| Medicinal plant / Extracts | Bacterial strains tested | MIC | MBC | MBC/MIC ratio | Antibacterial power of extract |
|---|---|---|---|---|---|
| **DS.** / Decocted extract (**D1**) | *S. aureus* ATCC 1292 | **00.048 mg/ml** | 12.500 mg/ml | CMB/CMI= **266** | **Bacteriostatic** |
| **DS.** / Macerated extract (**D2**) | *S. aureus* ATCC 1292 | **00.097 mg/ml** | 06.250 mg/ml | CMB/CMI= **64,43** | **Bacteriostatic** |
| **DS.** / Hydro-ethanolic extract 70 % (**D3**) | *S. aureus* ATCC 1292 | **00.048 mg/ml** | 01.560 mg/ml | CMB/CMI= **33,19** | **Bacteriostatic** |
| **DS**. / Decocted extract (**D1**) | *P. aeruginosa* ATCC 29213 | **06.250 mg/ml** | 12.500 mg/ml | CMB/CMI= **2** | **Bactericide** |
| **DS.** / Macerated extract (**D2**) | *P. aeruginosa* ATCC 29213 | **06.250 mg/ml** | 25.000 mg/ml | CMB/CMI= **4** | **Bactericide** |
| **DS.** / Hydro-ethanolic extract 70 % (**D3**) | *P. aeruginosa* ATCC 29213 | **00.780 mg/ml** | 03.125 mg/ml | CMB/CMI= **4** | **Bactericide** |

***Legend : DS: Duguetia staudtii (Engl. & Diels). (Annonaceae) ;** Differents extracts of the leaves of the plant.*
*Two multi-resistant bacteria : S. aureus : **Staphylococcus aureus** and P. aeruginosa : **Pseudomonas aeruginosa***
*ATCC : **American Type Culture Collection***
***MIC**=Minimal Inhibitory Concentration ; **MBC** = Minimal Bactericide Concentration*

### 4.4 Antibacterial Tests

The antibacterial tests showed that the aqueous extracts (decocted and macerated) and the hydro-ethanolic extracts of the plants studied were all effective, with Minimum Inhibitory Concentrations (MICs) ranging from 0.048 to 25 mg/ml (Tables 2 to 4).

*Duguetia staudtii* stem bark extracts were found to be highly active against *Staphylococcus aureus* ATCC 1292, with very low MIC values (0.048 to 0.097 mg/ml) and active against *Pseudomonas aeruginosa* ATCC 29213, with low MIC values (0.78 to 6.25 mg/ml) (Table 4).

*Trema orientalis* extracts also showed significant activity against both bacterial strains, with low MIC values for *S. aureus* ATCC 1292 (0.78 to 6.25 mg/ml) and *P. aeruginosa* ATCC 29213 (3.125 to 12.5 mg/ml) (Table 3).

*Combretum paniculatum* extracts showed lower activity than *Trema orientalis* and *Duguetia staudtii*, with MIC values for *S. aureus* ATCC 1292 of 6.25 to 12.5 mg/ml and for *P. aeruginosa* ATCC 29213 of 6.25 to 25 mg/ml.

The hydro-ethanolic extracts of each plant showed the highest levels of antibacterial activity. *P. aeruginosa* ATCC 29213 was more sensitive to all extracts than *S. aureus* ATCC 1292. The MBC/MIC ratio values indicated bactericidal activity on *P. aeruginosa* ATCC 29213 for all extracts (MBC/MIC ≤ 4). On *S. aureus* ATCC 1292, all the extracts of *Duguetia staudtii* bark were bacteriostatic as well as the macerated and hydro-ethanolic extracts of *Trema orientalis* leaves were also bacteriostatic. However, the decocted extracts of *Trema orientalis* leaves showed bactericidal activity against *S. aureus* ATCC 1292 (Table 3).

For *Combretum paniculatum* alone, the MBC/MIC ratio remained indeterminate for the aqueous macerate, while the decocted and hydro-ethanolic extracts of the plant's leaves were bacteriostatic against *S. aureus* ATCC 1292 (Table 2).

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## 5. DISCUSSION

### 5.1 Extract Yield

According to our results, extraction of the leaves of *C. paniculatum* and *T. orientalis* gave the highest yields, ranging from 12.05% to 45.6%, while extraction of the bark of *Duguetia staudtii* produced low yields, ranging from 3.76% to 6.93%, whatever the extraction method used. These yields could be due to the solid/liquid ratio, as during these extractions, the volume of solvent used was large (2000 ml) because more the volume of the solvent is large, the degree of contact between the drug (200g) and the extraction solvents is also large.

This would have increased the solubilisation capacity of the phytocompounds, hence the penetration or diffusion of the solvents in the drug, thus allowing the extraction solvents to come into contact with a large number of phytocompounds, resulting in better extraction.

This observation was made by Tahouo, (2016) who showed in his research work on global solid-liquid extraction procedures that the volume of the large extraction solvent is a key factor that allows the increase in the solubilisation power of chemical compounds and the matter transfer properties of solvents. This difference in extraction yields obtained from one plant to another could be attributed to several factors in the natural environment. In fact, various studies have shown that external factors (geographical and climatic factors), genetic factors, but also the degree of ripening of the plant and storage time have a strong influence on the yield and content of chemical compounds, particularly phenolic compounds (Merouane et al., 2014 ; El Hazzat et al., 2015)**.** In addition, the drying time of the plant organs, the granulometry of the crushed material, the volume or percentage of extraction solvent (osmosed water, ethanol 96°C) used and the extraction time would also have influenced these yields (Koffi et al., 2010).

The high yields of *C. paniculatum* and *T. orientalis* leaves extracts can be explained by the fact that these plants are the seat of

photosynthesis, storing and synthesising numerous organic molecules of therapeutic interest (Feknous et al., 2014). Photosynthesis is a fundamental metabolic process for plants, allowing them to produce organic matter and oxygen (Kouwelton et al., 2017).

The higher yield (45.6%) of *Trema orientalis* leaves obtained by aqueous maceration would be linked to this extraction method, which appears to be better for *Trema orientalis* leaves than for *C. paniculatum* leaves (7.16%) and D. *staudtii* barks (3.76%). In fact, the use of the propeller shaker for the extractions by maceration of each plant powder would have resulted in better extractability with a high number of chemical compounds of various structures extracted respectively from the leaves of *Trema orientalis*, *Combretum paniculatum* and the barks of *Duguetia staudtii*.

In relation to the solvents used (osmosis water and ethanol 96°) for the extractions in this study, the solubility of some chemical compounds to be extracted from plant organs is affected by the polarity of the solvent used (Bourgou et al., 2016). In fact, water and ethanol are among the best solvents for extracting chemical compounds because they are protic polar solvents ; it is to say that they liberate protons ($H^+$) by ionisation with the difference that water is not selective and is capable of extracting a large number of polar chemical compounds while ethanol has the particularity of making a selection of chemical compounds.

These observations were made by Sani et al., (2019), following their work in various regions of Niger, who found that the rate of substances extractable by water from the seeds and pulps of *Lagenaria siceraria* (Molina) Standley used in Pharmacopoeia is higher than that of other solvents (ether, hexane, ethanol, etc.). In fact, water is a polar solvent which attracts more substances or molecules of increasing polarity, that is to say that molecules with sufficient polarised bonds and partial positive and negative electrical charges which are not confused. Otherwise, they are said to be apolar. These observations were also made by (Koffi et al., 2010) following their study carried out in Ivory Coast, who admit that water is one of the solvents most commonly used to extract a large number of active ingredients in phytotherapy.

Furthermore, these remarks were made by Bohui et al., 2018) in their work who find that extraction yield is influenced by the method used and the presence of interfering substances.

Outside Ivory Coast, Gacioui et al., (2014) admit that in addition to water, its combination with other polar solvents such as ethanol separately has an extracting power in phythotherapy.

Several researchers, such as Mahamoudi et al., (2013), have found that the best extraction yields are achieved by decoction, with an average of 17.34%, compared with 15.64% for maceration. However, water is a better solvent for both hot and cold extraction than apolar solvents such as acyclic hydrocarbons (n-hexane, petroleum ether, etc.) and alicyclic hydrocarbons (cyclohexane ; benzene, etc.).

The presence of chemical compounds such as sterols and polyterpenes, polyphenols, flavonoids, alkaloids, tannins, quinones and saponosides in the leaves of *Combretum paniculatum* and *Trema orientalis* and in the stem bark of *Duguetia staudtii* can be explained by the fact that these are active compounds which form part of the variety of secondary metabolites with interesting biological activities produced naturally by plants in addition to primary metabolites (N'guessan et al., 2017). In fact, according to these authors, there is an uneven distribution of these compounds in drugs (leaves, flowers, fruits, root barks, etc.) which depends on their different places of synthesis and storage as well as their different functions ; that is, their biological activities. This observation made is similar to that of Benbarka & Oudjedi, (2014) who find that secondary metabolites are active compounds found in all parts of plants, but unevenly.

For example, in Ivory Coast, Zirihi et al., (2005) conducted phytochemical studies on alkaloids, a group of secondary metabolites widely distributed in the plant kingdom. They showed the various parts of plants where alkaloids are produced, depending on the species. In Cameroon, Yinyang et al., **(**2014), following their work on alkaloid plants, admit that these are secondary metabolites that are unevenly distributed in plants.

Thus, they are produced at particular stages in the development of the flower, fruit, seed or seedling. The importance or richness of *C. paniculatum* in sterols and polyterpenes and phenolic compounds (polyphenols, flavonoids, alkaloids, tannins, etc.) corroborates with the

results of Kabran et al., **(**2014), following their work carried out in Ivory Coast. Indeed, these authors showed the presence of ten (10) phenolic compounds in *Combretum paniculatum* leaves, including 1 phenolic acid (gallic acid) ; 4 ellagitannins (casuarictine, HHDPglucose, trigalloylHHDP-glucose, chebulinic acid) ; 2 condensed tannins (procyanidine dimer and trimer) and 3 flavonoids (catechin, myricetin, quercetin hexoside).

Following on from their earlier work, Kabran et al., (2011) confirmed that *Combretum paniculatum* had a higher content of total polyphenols (14426.684 ± 2502.747 µg AG / g DM) identified in the cloroform extract of the plant's leaves.

Furthermore, Bonazaba et al., (2019) confirmed the presence of polyphenols and flavonoids in the hydroethanolic extract of *Trema orientalis* leaves, which are responsible for an antihypertensive action. The abundance of alkaloids in the leaves of *Combretum paniculatum* and in the bark of the stem of *Duguetia staudtii* could be due to the drying conditions and the thermal sensitivity of certain chemical compounds, including alkaloids.

In fact, this result could be due to the difference in drying temperature with respectively 16°C for the leaves of C*. paniculatum ; T. orientalis* in air conditioning and room temperature (between 20°C and 25°C) for the bark of the stem of *D. staudtii* in order to better preserve the fragile or heat-sensitive constituents of these plants*.*
A low drying temperature in an ambient environment or in the open air (between 20°C and 25°C) accelerates the drying process of fragile plant drugs (leaves, flowers) and denser drugs (fruits, seeds, rhizomes, bark) and better preserves the properties of the phytoconstituents contained in them (Ouedraogo et al., 2021). In addition, active principles could be destroyed by enzymatic processes that continue during long drying at low or ambient temperature in plant drugs and during their conservation (Ouedraogo et al., 2021). This could explain the absence of tannins and quinones in the leaves of *Trema orientalis* and in the stem bark of *Duguetia staudtii*.

These explanations are in line with those of Kémajou et al.**,** (2012), who revealed in their work that the alkaloid content of *Alstonia boonei* barks decreases as the drying temperature increases. Thus, the extraction yield of total alkaloids obtained by these methods was 0.0436% for the fresh sample, then 0.0430% for drying in the open air and 0.0174% for a drying temperature of 60°C. Natural drying of bark in the open air at room temperature would therefore limit the loss of certain heat-sensitive chemical compounds such as alkaloids, one of the plant's active ingredients. For example, in Cameroon, Ngouonpe et al., (2018), following their pharmacological investigations, reported the presence of numerous natural products of *Duguetia staudtii*. Previous chemical studies of plants in the Duguetia genus have revealed several types of alkaloid (Cavé et al., 1980).

The tannins (catechic and gallic) detected only in the aqueous extracts of *Combretum paniculatum* leaves can be explained by the plant's high phenolic compound content. Of the three medicinal species studied, only *Combretum paniculatum* is a tanning species in the Ivorian flora. Thus, Kabran et al., (2014), after phytochemical analyses carried out on two plants in the Ivorian pharmacopoeia : *Combretum paniculatum* Vent and *Nymphea lotus* Linn, detected the presence of several categories of tannins in the leaves of *Combretum paniculatum* Vent. (Combretaceae), namely : 4 ellagitanins (casuarictine, $H_2$DP-glucose, trigalloyl $H_2$DP-glucose and chebulinic acid) and 2 condensed tannins (procyanidine dimer and trimer).

Finally, the presence of quinones only in the aqueous and hydro-ethanolic 70% extracts of *Combretum paniculatum* Vent (Combretaceae) is thought to be due either to the presence in the plant's leaves of aromatic compounds such as benzene, naphthalene or anthracene ; or to electron-donating substitutes such as phenols and catechols (Bolton & Tareisha, 2017). Indeed, quinones are oxidised derivatives of aromatic compounds formed by various mechanisms from simple catechol/phenol oxidations catalysed by a variety of oxidative enzymes and metal ions ($Na^+$; $K^+$ ; $Ca^{2+}$ ; $Mg^{2+}$ ; $Fe^{2+}$ ; etc.) to more complex mechanisms involving hydroxylation reactions.

## 5.2 Antibacterial Activities of Plant Extracts

The sensitivity of the bacterial strains tested (*Staphylococus aureus* ATCC1292 and *Pseudomonas aeruginosa* ATCC 29213) to all the aqueous and hydro-ethanolic extracts with MICs ranging from 0.048 to 25 mg/ml can be

explained by the antimicrobial properties of the chemical phytocompounds or secondary metabolites contained in the plant extracts studied. In fact, the abundant presence of sterols and polyterpenes in addition to phenolic compounds such as polyphenols, flavonoids and alkaloids contained in the plant drugs would therefore depend on these antibacterial activities against *Pseudomonas aeruginosa* ATCC 29213 and *Staphylococus aureus* ATCC 1292 (Kakou et al., 2020).

In fact, several research studies have demonstrated the antimicrobial activities of the plant organs of these three plants and the chemical compounds found in them. These include the work of Faga, (2007) and Mbajiuka et al., (2014), which showed that *Combretum paniculatum* is a medicinal plant that is much in demand in traditional medicine and has antimicrobial properties. In addition, to soothe general itching of the body, the Malinkés recommend the decoction of *Trema orientalis* leaves as a drink and/or in baths (Adjanohoun & Aké-Assi, 1979). *Trema orientalis* leaves are also used to treat scabies (Malan, 2008).

Finally, Yapi et al., (2012) have shown that *Duguetia staudtii* bark is much sought after in traditional medicine by local Ivorian populations to treat a variety of diseases with antimicrobial activity. *Duguetia staudtii* bark decoctions, macerations, infusions and pastes are used to treat gastric ulcers, vomiting, tumours, pain, bodily inflammations and dermatitis.

Moreover, the recent work carried out by Parvez et al., (2019) on the crude extract of different parts of *Trema orientalis* showed antibacterial activity against gram-positive and gram-negative bacteria : *Staphylococcus aureus*, *Staphylococcus epidermidis, Staphylococcus saprophyticus, Plesiomonas shigelloides, Shigella dysenteriae, Vibrio cholerae, Shigella sonnei* and *Pseudomonas aeruginosa.*

*Staphylococcus aureus* ATCC 1292 was more sensitive than *Pseudomonas aeruginosa* ATCC 29213 to extracts of *Duguetia staudtii, Trema orientalis* and *Combretum paniculatum* respectively, with MIC values ranging from 0.048 to 12.5 mg/ml.

This could be due to the fragility of its membrane structure and outer cell wall. Indeed, for positive (+) Gram bacteria, such as *S. aureus*, there is an absence of an outer membrane (barrier) that allows direct contact of isolated phenolic compounds with the phospholipid bilayers of the cell membrane. This explanation is similar to that given by Kontiza et al., (2008) and Parvez et al., (2019), who confirmed that natural extracts of marine origin have an interesting antibacterial activity against Gram (+) bacteria due to the structure of their cell wall, which does not limit the diffusion of certain antimicrobial substances into the bacterial cell.

Furthermore, the significant antibacterial activity of the plant extracts studied on *S. aureus* ATCC 1292 would be linked to certain abundant phenolic compounds such as polyphenols, flavonoids and alkaloids contained in each extract. These explanations are similar to those of Aragão et al., (2018), who confirm that the antibacterial activity of *X. americana* stem extracts on *S. aureus* is due to their high polyphenol content. The inhibitory activity of plant extracts on *Pseudomonas aeruginosa* ATCC 29213 ; negative (-) Gram bacteria with MICs ranging from 0.78 to 25 mg/ml could probably be linked to the structure of its outer membrane which surrounds the cell wall limiting the diffusion of hydrophobic compounds by the covering lipopolysaccharides. This causes an accumulation of non-water-soluble substances in bacterial cell membranes, reducing membrane permeability to ions and certain antibacterial substances or molecules. This constitutes a factor of resistance to antibacterial activity on *P. aeruginosa* ATCC29213. The higher levels of antibacterial activity recorded by the hydroethanol extracts of the medicinal plants studied can be explained by their abundance of chemical compounds that are highly active on the *In vitro* growth of the bacterial strains studied.

In fact, chemical reactions characterised by the release and binding of the hydroxyl group (OH) of water ($H_2O$) and/or ethanol ($CH3\text{-}CH_2OH$) on certain polar chemical compounds such as polyphenols are becoming increasingly toxic to *S.* aureus ATCC 1292 and *P*. aeruginosa ATCC 29213.

Several photochemical studies have shown the effectiveness of hydroethanol extracts and the phenolic compounds they contain against bacteria and fungi. This is consistent with the work of Cowan, (1999), who states that the significant hydroxylation of polyphenols (such as catechol and pyrogallol) results in a high level of toxicity that inhibits micro-organisms.

Pinho et al., (2014) add that phenolic acids such as gallic and caffeic acids formed by hydroxylation of phenolic compounds have good antibacterial activity against gram-positive (*S. epidermidis* and *S. aureus*) and gram-negative (*K. pneumoniae*) bacteria.

Analysis of our results also showed that the combination of solvents (water 30% and ethanol 70%) for extractions is one of the best extraction methods for concentrating the active ingredients of these three plants. This explains the strong antibacterial activity of the hydroethanol extracts on the strains studied. This explanation is consistent with that given by Ouattara et al., (2013) following their study conducted in Ivory Coast on the evaluation of the antibacterial activity of *Morinda morindoides* leaf extracts (Morinda, Rubiaceae) on *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two germs responsible for skin infections. In their study, the combination of solvents (ethanol 70%, water 30% and ethyl acetate, water) is the method that best concentrates the active ingredients of *Morinda morindoides*.

### 5.3 Medicinal Recipes Used by the Cutaneous Route to Treat Inflammatory Dermatoses

The most active extracts tested should be used as raw materials in the development of formulations intended for the cutaneous route. According to Aka Any-Grah et al (2015), the use of liquid, semi-solid or solid forms depends on the patient (infant, child, adult, etc), the type of dermatosis, the causal agent and the location of the lesion. The galenic forms obtained must enable the activity of the extracts to be preserved and increase their residence time at the site of action in order to ensure better availability of the bioactive compounds at the sites of action.

## 6. CONCLUSION

This study of medicinal plants used to treat inflammatory dermatoses in the Mé area of Ivory Coast yielded a number of significant results. Total extracts of plant material from *Combretum paniculatum*, *Trema orientalis* and *Duguetia staudtii* showed that water and alcohol (ethanol 96°) were effective extraction solvents. Extraction yields varied according to the methods used, with values of up to 45.6% for *Trema orientalis* using cold maceration. Chemical screening revealed the presence of numerous chemical compounds in the leaves of *Combretum paniculatum* and *Trema orientalis*, as well as in the bark of *Duguetia staudtii*, including sterols, polyterpenes, polyphenols, flavonoids, alkaloids and saponosides.

The bacteria tested (*Staphylococcus aureus* ATCC 1292 and *Pseudomonas aeruginosa* ATCC 29213) were sensitive to both aqueous and hydro-ethanolic extracts with minimum inhibitory concentrations (MICs) ranging from 0.048 to 25 mg/ml. *Duguetia staudtii* bark extracts were particularly effective against *Staphylococcus aureus* ATCC 1292, while hydroethanolic extracts showed the highest levels of antibacterial activity, particularly against *Pseudomonas aeruginosa* ATCC 29213. These results, which constitute a database for pharmacological and pharmaceutical research, showed that the hydroethanol extracts of these three medicinal plants were the most active on the studied strains, with Minimal Inhibitory Concentrations ranging from (0.048 to 06.25 mg/ml) and bactericidal at 100% against *Pseudomonas aeruginosa* compared with *staphylococcus aureus*. The results also confirm the therapeutic potential of these three medicinal plants and their promising contribution to the formulation of dermatological forms to combat inflammatory dermatoses that meet the health needs of populations.

## CONSENT AND ETHICAL APPROVAL

It is not applicable.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

We hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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