

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).

ABSTRACT :

Objective : To evaluate the antibacterial power of extracts of : *Combretum paniculatum* Vent leaves (Combretaceae) ; *Trema orientalis* L (Cannabaceae) and *Duguetia staudtii* stem bark (Engl & Diels) (Annonaceae), 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 two solvents : osmosed water and ethanol 96° and the tri-phytochemistry of the extracts was carried out by colouring reactions. The aqueous and ethanolic plant extracts were tested in vitro against *Staphylococcus aureus* ATCC 1292 and *Pseudomonas aeruginosa* ATCC 29213. The Muller-Hinton agar diffusion and dilution method was used.

Results : Tri-phytochemistry 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 on all the strains tested, with MICs ranging from (0.048 to 06.25 mg/ml) and MBCs from (1.56 to 06.25 mg/ml). Of the two bacterial strains studied, *Pseudomonas aeruginosa* ATCC 29213 (Gram (-) bacterium) was the most sensitive to all the extracts. The antibacterial potentials thus highlighted by the values of the ratio (CMB/CMI) obtained indicated that all the extracts showed a high bactericidal activity (100%) on *Pseudomonas aeruginosa* ATCC 29213 compared with *Staphylococcus aureus* ATCC 29213.

Conclusion : These results provide a database for in-depth phytochemical and pharmacological studies that could lead to the discovery of new antibiotics and the formulation of an antimicrobial drug effective against inflammatory dermatoses.

Key words: Phytochemical study; extracts; medicinal plants; antibacterial.

1- Introduction

Cutaneous and subcutaneous staphylococcosis are increasingly common skin diseases, especially in developing countries [1]. They are caused mainly by the golden staphylococcus,

also known as ‘ *Staphylococcus aureus* ’ [2]. This bacteria colonises various parts of the human body, including the skin, and can cause various infections, such as folliculitis, boils and acne [3]. Some types of these bacteria have become resistant to several antibiotics, making it increasingly difficult to treat skin infections [4]. 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 [5].

To face these growing challenges of antibiotic resistance, the search for new molecules from medicinal plants has become a priority for the scientific community [6]. 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.

2.2.1. Technical material

For this study, technical material was used to gain access to the plants and to take plant samples. For phytochemical screening, 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 hydroethanol).

- Reagents : Phytochemical screening required various reagents (Stiasny's reagent, sodium acetate and ferric chloride ; acetic anhydride and sulphuric acid, etc;).

2.2.2. Biological material

2.2.2.1 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 in February 2023 in Alépé, 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.

2.2.2.2 Drying and pulverisation

At around 5pm, the cleaned fresh leaves of *Combretum paniculatum* and *Trema orientalis* were spread out and dried at low air conditioning at 16°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), 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. 1) with different characteristics (colours and granulometries).

- **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 triphytochemistry.

- **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 (ethanol70%).

2.2.1.1. Extraction by decoction

The modified protocol described by **Odoh et al. [7]** 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 air-conditioning of the Medicinal Sciences Laboratory of the Pharmaceutical and Biological Sciences Research Unit of the Université Félix-Houphouët-Boigny until use.

2.2.1.2. Extraction by maceration

The chemical compounds of each plant were extracted using the method of **Zirihi [8]** 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. [9]**.

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 : $Rd (\%) = (m \times 100) / M$ (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 Houphouët-Boigny. The characterisation of the phytochemicals 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 [10]**, 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 T_1 to T_{15} , using the modified liquid double dilution method of **Ouattara et al [11]**. 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 T_1 and 5 ml in all other tubes.

A mass of 0.5 g of plant extract was then dissolved in tube T₁ and homogenised completely to give a concentration of 50 mg/ml. Half the volume of tube T₁ (5 ml) was then transferred to tube T₂ and homogenised. This operation was repeated up to tube T₁₅, after which half the volume was discarded. This resulted in concentrations C₁=50 mg/ml ; C₂=25 mg/ml ; C₃= 12.5 mg/ml ; C₄= 06.25 mg/ml ; C₅= 03.125 mg/ml ; C₆=01.56 mg/ml ; C₇= 0.78 mg/ml ; C₈= 0.39 mg/ml ; C₉= 0.195 mg/ml ; C₁₀= 0.097 mg/ml ; C₁₁= 0.048 mg/ml ; C₁₂= 0.024 mg/ml ; C₁₃= 0.012 mg/ml ; C₁₄= 0.006 mg/ml and C₁₅= 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 corresponds 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 [12].

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. 1).

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. 1).

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. 1).

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. 1).

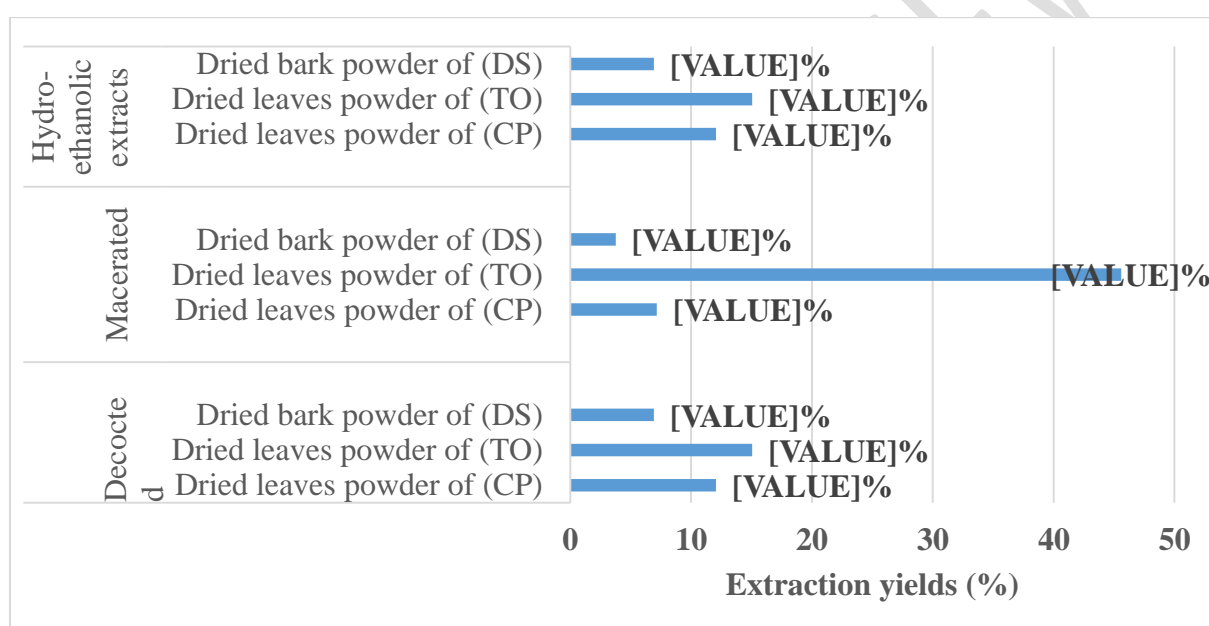


Fig. 1 : Histogram of percentage (%) yields of aqueous and hydro-ethanolic extracts of three Ivorian medicinal plants used in the Mé region (South-East, Ivory Coast).

CP : *Combretum paniculatum* ; **TO :** *Trema orientalis* et **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 I).

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 I).

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

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

Medicinal plants		<i>Combretum paniculatum</i>			<i>Trema orientalis</i>			<i>Duguetia staudtii</i>		
Extractions		Decoction	Maceration	Hydro-Éthanolique	Decoction	Maceration	Hydro-Éthanolique	Decoction	Maceration	Hydro-Éthanolique
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.

4.3 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 II to IV).

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 IV).

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 III).

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 ($MBC/MIC \leq 4$). 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 III).

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 II).

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 III).

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 II : 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)	<i>S. aureus</i> ATCC 1292	06,25 mg/ml	50 mg/ml	CMB/CMI= 8	Bacteriostatic
Cp. / Macerated extract (C2)	<i>S. aureus</i> ATCC 1292	12,5 mg/ml	>100 mg/ml	ND	ND
Cp. / Hydro-ethanolic extract 70 % (C3)	<i>S. aureus</i> ATCC 1292	06,25 mg/ml	50 mg/ml	CMB/CMI= 8	Bacteriostatic
Cp. / Decocted extract (C1)	<i>P. aeruginosa</i> ATCC 29213	06,25 mg/ml	25 mg/ml	CMB/CMI= 4	Bactericide
Cp. / Macerated extract (C2)	<i>P. aeruginosa</i> ATCC 29213	25 mg/ml	50 mg/ml	CMB/CMI= 2	Bactericide
Cp. / Hydro-ethanolic extract 70 % (C3)	<i>P. aeruginosa</i> ATCC 29213	06,25 mg/ml	06,25 mg/ml	CMB/CMI= 1	Bactericide
Legend : C.p : <i>Combretum paniculatum</i> Vent. (Combretaceae) ; Differents extracts of the leaves of the plant.	Two multi-resistant bacteria : <i>S. aureus</i> : <i>Staphylococcus aureus</i> and <i>P. aeruginosa</i> : <i>Pseudomonas aeruginosa</i> ATCC : American Type Culture Collection	MIC=Minimal Inhibitory Concentration	MBC = Minimal Bactericide Concentration	ND = Not Determinated	

Table III : 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)	<i>S. aureus</i> ATCC 1292	06,25 mg/ml	25 mg/ml	MBC/MIC = 4	Bactericide
TO. / Macerated extract (T2)	<i>S. aureus</i> ATCC 1292	01,56 mg/ml	50 mg/ml	MBC/MIC = 32,05	Bacteriostatic
TO. / Hydro-ethanolic extract 70 % (T3)	<i>S. aureus</i> ATCC 1292	0,78 mg/ml	25 mg/ml	MBC/MIC = 32,05	Bacteriostatic
TO. / Decocted extract (T1)	<i>P. aeruginosa</i> ATCC 29213	3,125 mg/ml	10,5 mg/ml	MBC/MIC = 3,36	Bactericide
TO. / Macerated extract (T2)	<i>P. aeruginosa</i> ATCC 29213	12,5 mg/ml	25 mg/ml	MBC/MIC = 2	Bactericide
TO. / Hydro-ethanolic extract 70 % (T3)	<i>P. aeruginosa</i> ATCC 29213	3,125 mg/ml	06, 25 mg/ml	MBC/MIC = 2	Bactericide
Legend : TO : <i>Trema orientalis</i> (L.) (Cannabaceae); Different extracts of the leaves of the plant.	Two multi-resistant bacteria : <i>S. aureus</i> : Staphylococcus aureus and <i>P. aeruginosa</i> : Pseudomonas aeruginosa ATCC : American Type	MIC =Minimal Inhibitory Concentration	MBC = Minimal Bactericide Concentration	ND = Not Determinated	

Culture Collection

Table IV : 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)	<i>S. aureus</i> ATCC 1292	0,048 mg/ml	12,5 mg/ml	MBC/MIC = 266	Bactériostatic
DS. / Macerated extract (D2)	<i>S. aureus</i> ATCC 1292	0,097 mg/ml	06,25 mg/ml	MBC/MIC = 64,43	Bactériostatic
DS. / Hydro-ethanolic extract 70 % (D3)	<i>S. aureus</i> ATCC 1292	0,048 mg/ml	01,56 mg/ml	MBC/MIC = 33,19	Bactériostatic
DS. / Decocted extract (D1)	<i>P. aeruginosa</i> ATCC 29213	06,25 mg/ml	12,5 mg/ml	MBC/MIC = 2	Bactericide
DS. / Macerated extract (D2)	<i>P. aeruginosa</i> ATCC 29213	06,25 mg/ml	25 mg/ml	MBC/MIC = 4	Bactericide
DS. / Hydro-ethanolic extract 70 % (D3)	<i>P. aeruginosa</i> ATCC 29213	0,78 mg/ml	03,125 mg/ml	CMB/CMI= 4	Bactericide
Legend : DS : <i>Duguetia staudtii</i> (Engl & Diels) (Annonaceae) ; Differents extracts of the leaves of the plant.	Two multi-resistant bacteria : <i>S. aureus</i> : <i>Staphylococcus aureus</i> and <i>P. aeruginosa</i> : <i>Pseudomonas aeruginosa</i>	MIC =Minimal Inhibitory Concentration	MBC = Minimal Bactericide Concentration	ND = Not Determinated	

**ATCC : American Type
Culture Collection**

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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 phytochemicals, 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 phytochemicals, resulting in better extraction.

This observation was made by **Tahou** [13] 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 mass 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 [14, 15]. 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 [16].

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 [17]. Photosynthesis is a fundamental metabolic process for plants, allowing them to produce organic matter and oxygen [18].

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 [19]. 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. [20], 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.) because water is a polar solvent that attracts more substances or molecules of increasing polarity. This is because 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 [16] 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. [21] 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. [22] admit that in addition to water, its combination with other polar solvents such as ethanol separately has an extracting power in phytotherapy.

Several researchers, such as Mahamoudi et al. [23], 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 [24]. 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 and Oudjedi [25] who find that secondary metabolites are active compounds found in all parts of plants, but unevenly.

For example, in Ivory Coast, Zirihi et al. [26] 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. [27], 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. [28], 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. [29] confirmed that *Combretum paniculatum* had a higher content of total polyphenols ($14426.684 \pm 2502.747 \mu\text{g AG / g DM}$) identified in the chloroform extract of the plant's leaves.

Furthermore, Bonazaba et al. [30] confirmed the presence of polyphenols and flavonoids in the hydroethanolic extract of *Trema orientalis* leaves, which are responsible for an anti-hypertensive 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 [31]. 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 [31]. 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. [32], 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. [33], 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 [34].

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. [28], after phytochemical analyses carried out on two plants in the Ivorian pharmacopoeia : *Combretum paniculatum* Vent and *Nymphaea lotus* Linn, detected the presence of several categories of tannins in the leaves of *Combretum paniculatum* Vent. (Combretaceae), namely : 4 ellagitannins (casuarictine, H₂DP-glucose, trigalloyl H₂DP-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 [35]. 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⁺ ; Ca²⁺ ; Mg²⁺ ; Fe²⁺ ; etc.) to more complex mechanisms involving hydroxylation reactions.

5.2- Antibacterial activities of plant extracts

The sensitivity of the bacterial strains tested (*Staphylococcus 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 phytochemicals 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 *Staphylococcus aureus* ATCC 1292 [36].

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 [37] and Mbajiuka et al [38], 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 [39]. *Trema orientalis* leaves are also used to treat scabies [40],

Finally, Yapi et al. [41] 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. [42] 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. [43] and Paevez et al. [42], 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. [44], 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 (H₂O) and/or ethanol (CH₃-CH₂OH) 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 [45], 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 and al [46] 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 hydro-ethanol extracts on the strains studied. This explanation is consistent with that given by Ouattara et al. [11] 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*.

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 hydro-ethanolic 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, 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.

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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