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IN VITRO EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF 70% ETHANOLIC EXTRACT OF PIPTADENIASTRUM AFRICANUM HOOK. F. (FABACEAE) STEM BARK

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Abstract

Introduction: Treatments for microbial infections are numerous, but for the most part unaffordable and increasingly ineffective. The aim of this study was to investigate the antimicrobial power of *Piptadeniastrumafricanum* stem bark, a medicinal plant used to treat microbial infections in traditional settings. Methods: Phytochemical screening of the 70% ethanolic extract was carried out to identify the major groups of chemical molecules responsible for activity. The antimicrobial activity of the 70% ethanolic extract was then determined by evaluating and determining the sensitivity of nine clinical strains of bacteria and two fungi heavily involved in several types of infection. Results: Phytochemical screening revealed the presence of six groups of bioactive compounds in the 70% ethanolic extract (flavonoids, tannins, saponins, coumarins, polyphenols and alkaloids). Antifungal activity revealed the high sensitivity of *Trichophyton mentagrophytes* (MFC= 0.195 mg/mL; IC50= 0.2 mg/mL). The 70% ethanolic extract of *Piptadeniastrumafricanum* was bactericidal against all clinical strains of Staphylococcus aureus. Conclusion: *Piptadeniastrumafricanum* stem bark is a promising alternative for combating infectious diseases.

Keywords: stem bark, bacteria, fungi, *Piptadeniastrumafricanum*, 70% ethanolic extract.

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1. Introduction

Infectious diseases alone account for 50-60% of morbidity in Côte d'Ivoire [1]. The appearance of strains resistant to standard antibiotics, the emergence of pathogenicity in strains that are usually saprophytic in our environment, and immunodepressions caused by HIV infection are the main factors behind this sharp upsurge [2,3]. Despite the wide range of therapies available (antibiotics, antiseptics, dressings, agents active on bacterial biofilms, negative pressure therapy, hyperbaric oxygen therapy), these may prove ineffective or materially unaffordable. In a context of low purchasing power, combined with the phenomena of microorganism resistance to anti-infectives, and the side effects of certain powerful antibiotics, it is appropriate to look for new natural molecules derived from plants [4].Plants possess chemical compounds endowed with pharmacological activities that would enable them to play a beneficial role in terms of preventive and curative action of great importance to human health [5].

Several studies based on biological properties and classes of chemical compounds have substantiated this information [6]. This is why recent years have seen a renewed interest in medicinal and aromatic plants. The aim of our study is therefore to assess the antimicrobial activity of *Piptadeniastrumafricanum* stem bark, a medicinal plant traditionally used for a variety of microbial ailments.

2. Materials and methods

2.1.Plant material

It consists of all the stem barks of *Piptadenistrumafricanum*.

2.2.Fungal strains

Microbiological tests were carried out on two fungal species (*Candida albicans* and *Trichophyton mentagrophytes*). All fungal strains were supplied by the Parasitology Laboratory of the Unité de Formation et de Recherche (UFR) des Sciences Médicales, Université Félix Houphouët-Boigny, Côte d'Ivoire.

2.3. Bacterial strains

The bacterial strains used came from biological products derived from several infectious pathologies. All strains were supplied by the Laboratoire de Bactériologie-Virologie of the Institut Pasteur de Côte d'Ivoire. The bacterial support is composed of :

- three reference strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853);
- three strains of methicillin-resistant *Staphylococcus aureus*;
- three strains of intermediate-resistant *Pseudomonas aeruginosa* and
- three strains of enterobacteria (*E.coli*, *Salmonellasp*, *Klebsiella pneumoniae*).

2.4. Methods

2.4.1.Preparation of extracts

2.4.1.1.Total aqueous extracts (TAE)

Extracts were prepared using the method developed by [7]. This method can be summarized as follows: 100 grams of plant powder were extracted with one liter of distilled water by homogenization in a blender for 5 to 10 minutes. The resulting homogenate was wrung out in a square of white cloth, then filtered three times on absorbent cotton and once on 3 mm Wattman paper. The filtrate obtained was dried in an oven at 50°C, and the powder thus obtained constituted the total aqueous extract, ETA.

2.4.1.2.Preparation of 70% ethanol extract

Five (5) grams of each total aqueous extract were dissolved in 10 mL of a hydroalcoholic solution comprising 70% ethanol and 30% distilled water (V/V). After total exhaustion of the substance with the solvent in a mixer, a hydroalcoholic upper phase and a deposit were obtained using a separating funnel [8]. The hydroalcoholic phase was collected, filtered through 3 mm Wattman filter paper and oven-dried at 50°C. The powder obtained is the 70% hydroethanol extract EE 70%.

2.4.1.2.Phytochemical screening of the 70% ethanolic extract

Phytochemical screening was carried out using the tube staining method with reference to the techniques described in the work of [9,10]. The presence or absence of the various chemical species was determined using appropriate reagents.

2.4.2.Evaluation of antifungal activity

2.4.2.1.Preparation of culture medium

The culture medium was prepared by dissolving 42 g of agar powder in 1 liter of distilled water. This mixture was heated and stirred until completely homogenized, on an IKAMAG-RTC magnetic stirrer. The medium thus prepared was distributed in series of 12 tubes, 20 mL in tube no. 1 and 10 mL in the other tubes (from no. 2 to no. 12), using the method of [11].

2.4.2.2. Incorporation of extracts into the culture medium

Plant extracts were incorporated into the culture medium using the double dilution method in tilted tubes [11]. Each series comprises 10 test tubes containing the plant extract incorporated into the culture medium, plus two control tubes, one without plant extract for germ growth control and the other without plant extract or germ for culture medium sterility control, for a total of 12 tubes. Test tubes contain concentrations ranging from 50 to 0.098 mg/mL. To achieve double dilution, 1 g of plant extract was homogenized in tube no. 1, which contained 20 mL of Sabouraud agar (for the highest concentration 50 mg/mL). Half of this homogeneous content was then transferred to the next tube (n°2), containing 10 mL Sabouraud agar, and homogenized. This operation was repeated successively for the other tubes up to tube n°10, to achieve the lowest concentration (0.098 mg/mL). For the latter tube, half the volume of the mixture was discarded. The 12 prepared tubes were autoclaved at 121°C for 15 minutes, then tilted with small pellets at laboratory temperature to cool and solidify the agar [11]. All plant extracts and ketoconazole were prepared under the same conditions using the tilted-tube double dilution method.

2.4.2.3. *Inoculum* preparation

The*inoculum* was prepared from young cultures of the various fungal strains, aged 48 hours for *Candida albicans* and 5-10 days for *Trichophyton mentagrophytes*. This was done by homogenizing one or two well-isolated fungal colonies taken from sabouraud medium, using a Koch's loop, in 10 mL of sterilized distilled water (each fungal species taken separately). This gave the mother suspension, known as suspension10^0, with a load of10^6 cells/mL. Suspension 10^{-1} was then prepared by diluting the mother suspension to the 10th, by transferring 1 mL of the latter into 9 mL of sterile distilled water, thus reducing the load to10^5 cells/mL. This latter suspension will be used for antifungal testing [11].

2.4.2.4. Antifungal tests in the presence of plant extract

The previously prepared culture media were seeded with 10 µL of 10^{-1} suspension per tube (tubes n°1 to n°11). This corresponds to 1000 seeded cells. Each tube was cultured in transverse streaks until the 10 µL was used up. After this step, all 12 tubes in each series were incubated in an oven at 30°C for a period of three days for *Candida albicans* and 10 days for *Trichophyton mentagrophytes* [11,12]. Tests were repeated six times for each extract.

2.4.2.4.1.Colony counting

At the end of the incubation period, colonies were counted by direct counting using a colony counting pen (Science Ware: serial no. 23283). Growth in the 10 test tubes was expressed as percentage survivorship, calculated relative to 100% growth in the growth control tube [12]. The method for calculating survivorship can be summarized by the following formula:

$$S = \frac{n}{N} X\ 100$$

n = number of colonies in test tube

N = number of colonies in control tube

S = survival expressed in %

Antifungal parameters tested

The activity of extracts is assessed by determining the values of antifungal parameters (MIC, MFC, CI_{50}) and the shape of activity curves. Antifungal parameters can be defined as follows:

- MFC (Minimum Fungicidal Concentration) is the lowest concentration of extract in the tube that gives 99.99% inhibition compared with the growth control, or it is the concentration of extract in the tube that leaves 0.01% survival compared with the growth control [12];
- MIC (Minimum Inhibitory Concentration) is the lowest concentration of extract in the tube for which there is no growth visible to the naked eye [12];
- IC_{50} (Concentration for 50% inhibition) is the concentration that inhibits 50% of the number of colonies compared with the growth control.

The $_{IC50}$ is determined graphically from the antifungal chart, which corresponds to the curve representing the evolution of survivorship as a function of plant extract concentration [12].

2.4.2.4.2.Determination of fungicidal activity

A subculture from the MIC tube is performed on fresh agar without plant extract. Thus, after three or ten days of incubation, the surface of the agar contained in the test tubes is lightly picked, inoculated with a platinum loop on neutral agar, then incubated for 72 hours at room temperature[3]**.** Two cases are possible:

- if colonies are present, the extract is said to be fungistatic;
- if no colonies are present, the extract is said to be fungicidal.

2.4.2.4.3.Criteria for comparing extract activities

Extract performance is compared on the basis of several criteria. The values of the antifungal parameters (MFC, IC_{50} and the shape of the activity curves). The lower the MFC and IC_{50} values, the more active the extract. Thus, an extract X1 is considered more active than another extract X2 if and only if the MFC value of X1 is lower than that of X2. But when two extracts X1 and X2 have the same MFC value, then the more active extract is the one with the lower IC_{50} value.

As for the activity curve, its general shape (decreasing, regular or irregular) and the relative value of its slope (strong, medium or weak) provide information on the antifungal activity potential of the extract in question. The most active extract is the one with the steepest slope of the activity curve [7].

The activity ratio determines how many times a given extract is more active than another. It is calculated by dividing the value of the highest MFC by the value of the lowest MFC. For example, if CMF (X1) / MFC(X2) = k, then this means that the extract (X2) with the lowest MFC value is k times more active than the extract (X1) with the highest MFC value. According to the classification of [13], the activity levels of the extracts were classified according to MFC values into five classes:

-very low activity: MFC values > 50 mg /mL ;
-low activity: MFC value = 50 mg / mL ;
-medium activity: 50 mg / mL > MFC values ≥ 6.25 mg / mL ;
-strong activity: 6.25 mg / mL > MFC values ≥ 0.780 mg / mL ;
-very strong activity: 0.780 mg / mL > MFC values ≥ 0.001526 mg / mL.

2.4.2.5. Evaluation of the antibacterial activity of the 70% ethanolic extract

Two methods were used to assess the antibacterial activity of plant extracts. The solid-state diffusion method and the liquid-state dilution method were used to determine MICs and BMCs.

2.4.2.5.1.Efficacy test

2.4.2.5.1.1.Preparation of the*inoculum*

The*inoculum* is an essential factor likely to influence the results. It must therefore be standardized. Using a Pasteur pipette fitted with a bulb, two colonies isolated from a 24-hour Petri dish culture on selective medium were picked. The sample is used to make a suspension with an optical density of 0.5 on the Mac Farland scale in 0.85% NaCl. The suspension is diluted according to the rate of multiplication of the germ present. Thus, a dilution was performed by adding a bacterial suspension of 1000 µL for *Staphylococcus aureus*, 100 µL for Enterobacteriaceae and 10 µL for *Pseudomonas aeruginosa* in 10 mL of physiological water [14].This new bacterial solution is the finalinoculum with a concentration of10^6 germs/mL**.**

2.4.2.5.1.2.Preparation of the concentration range for each plant extract

The concentration range for each plant extract is prepared by the liquid double dilution method in a series of labeled test tubes. To do this, 10 mL sterile distilled water is added to tube t1 and 5 mL to all other tubes. Next, 1 g of plant extract is dissolved in tube t1, then thoroughly homogenized to give a concentration of 100 mg/mL. Half the volume of tube t1 (5 mL) is transferred to tube t2, then homogenized. This operation is repeated until the last tube, half the volume of which is discarded. The concentration range of plant extracts is then sterilized by filtration on a 0.45 µm millexgv membrane and stored in a refrigerator.

2.4.2.5.2.Efficacy testing of plant extracts

The efficacy test eliminates extracts that show no antibacterial activity at the concentrations studied, and retains only those that are active. It is performed using the solid-state diffusion method. This involves diffusing the extract or antibiotic from a point of deposition into agar previously seeded with the germ to be tested. The efficacy of the extract is assessed according to the diameter of the zone of inhibition measured with a caliper [15]. MH media poured into Petri dishes are swabbed with the prepared*inoculum*. Petri dishes are left at room temperature for 15 min. 6 mm-diameter wells are dug into the agar by inserting the large end of a sterile Pasteur pipette, and 50 µL of the extract is poured into the wells. A control cup receives 50 µL of distilled water. For preparations with DMSO added, the control is prepared by adding 1 mL DMSO to 10 mL distilled water.

The inoculated plates are left at room temperature (26°C) for 15 minutes to pre-diffuse the extracts. They are then incubated for 24 hours, after which the diameter of the zones of inhibition is measured. If there is a zone of inhibition, the extract is active. Otherwise, it is considered inactive on the bacterial strain tested. The extract which induces a zone of inhibition is used for the rest of the study. To assess strain sensitivity, there are four types of diameter according to [16]**:**

- diameter less than 8 mm: non-susceptible or resistant strain;
- diameter between 9 and 14 mm: sensitive strain;
- diameter between 15 and 19 mm: highly susceptible strain;
- diameter greater than 20 mm: extremely sensitive strain.

Steps for determining the Minimum Inhibitory Concentration

2.4.2.5.3. *Inoculum* preparation

Bacterial*inoculum* was prepared from young colonies and Mueller-Hinton broth (MHB). Two colonies isolated from the bacterial culture were picked with a 2 µL calibrated platinum loop and homogenized in 10 mL MHB, then incubated for three hours at 37°C to obtain a pre-culture. Next, 0.1 mL of the pre-culture broth for Enterobacteriaceae and 0.3 mL for *Staphylococcus* and *Pseudomonas* were taken and introduced into a tube containing 10 mL MHB. This bacterial suspension is evaluated at around10^6 cells/mL and constitutes the*inoculum*.

2.4.2.5.4. *Inoculum* count

The bacterial*inoculum* was diluted from 10 to 10 up to dilution 10^{-4}. Four successive dilutions were obtained: from 10^{-1} to 10^{-4}. The initial bacterial*inoculum* and the four successive dilutions

were inoculated with a 2 µL calibrated loop onto Mueller-Hinton agar plates along the 5 cm ridges, making up plate A.

2.4.2.5.5. Determination of Minimum Inhibitory Concentration

To determine the minimum inhibitory concentration, the liquid dilution method was used[17]. In a series of seven hemolysis tubes numberedT1 toT7(experimental tubes) and one growth control tube (Tc), 1 mL of the previously prepared inoculum was poured. 1 mL of plant extract was added. The plant extract was distributed by transferring 1 mL of 200 mg/mL extract into tubeT1, 1 mL of 100 mg/mL extract into tubeT2, 1 mL of 50 mg/mL extract into tubeT3 and so on up to tube T7, which received 1 mL of 3.12 mg/mL plant extract. Tube Tc received 1 mL distilled water instead of plant extract, and tube Ts received 2 mL sterile MH broth. The final concentration in the tubes changes from double to single. This test is repeated three times, and the results reported represent the averages obtained. The Minimum Inhibitory Concentration (MIC) is the lowest concentration of antibiotic inhibiting any visible growth after an incubation time of 18 to 24 hours [18]. It corresponds to the lowest concentration of extract for which no growth (cloudiness) is visible to the naked eye.

2.4.2.5.6. Determination of the Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) is the antibiotic concentration required to obtain 0.01% viable bacteria after 24 hours incubation at 37°C [18]. It was determined according to the method used by [19]. After incubation, plate A was stored in a refrigerator at 4°C. The following day, after reading the MIC, each of the tubes whose contents showed no cloudiness visible to the naked eye was inoculated along the 5 cm ridges. For each extract, the seeded Petri dishes are incubated at 37°C for 24 hours and marked B. The MBC is determined by comparing plates A and B through observation with the naked eye. The results are interpreted using the ratio (MBC/MIC). The ratio MBC/MIC allows us to conclude whether the substance is bactericidal or bacteriostatic. When the MBC/MIC ratio ≤ 4 mg/mL, the extract is said to be bactericidal. On the other hand, when this ratio > 4 mg/mL, the extract is said to be bacteriostatic[20].

3. Results

3.1. Phytochemical screening

The results of the phytochemical screening are summarized in Table 1. Analysis of this result revealed the presence of six groups of bioactive compounds in the 70% ethanolic extract of *Piptadeniastrumafricanum*. The ethanolic extract showed the presence of flavonoids, tannins, saponins, coumarins, polyphenols and alkaloids, with an absence of sterols and triterpenes.

Table 1: Phytochemical sorting of 70% ethanolic extract of *Piptadenistrumafricanum*

Species	Extract	Chemical groups							
		Sap	Flav	Terp/ster	Tanins		Coum	Alc	Poly
					Gall	Cathé			
Piptadenistrumafricanum	EE 70 %	-	+	-	+	+	+	+	+

EE 70%: 70% ethanolic extract

+: presence of chemical group

- : absence of chemical group

Sap: saponins ; Flav: flavonoids ; Terp / Ster: Terpenes / Sterols ; Gall: gallic ; Cathé: catechic ; Coum: coumarins ;Alc: alkaloids ;Poly: polyphenol

3.2. Antifungal activity

3.2.1.Effect of 70% ethanolic extract of *P. africanum*

The inhibitory activity of 70% ethanolic extract of *Piptadeniastrumafricanum* was observed on both fungal species tested. After 48 hours incubation, the sterility control tube (TS) was germ-free, demonstrating the sterility of the agar and the aseptic conditions under which the experiments were carried out.The growth control tube (Tc) without extract showed normal growth for both fungal species. In the experimental tubes, compared with the control, there was a progressive decrease in the number of colonies as the concentration of ethanolic extract in the tubes increased.

The 70% ethanolic extract of *Piptadeniastrumafricanum*exerted a fungicidal effect on the two fungal species tested (Figures 1 and 2). Figure 3 shows the activity curve of the 70% ethanolic extract, obtained from data relating to the survival percentages of germs and growth controls, on the maturation of the two strains, according to a dose-response relationship. The curve was used to determine IC_{50} values. The antifungal parameters (.M.IC;.MFC. and IC_{50}) summarized in the table confirm these data.

According to Table 2, the.M.ICvaries according to the fungal species. It is high in the case of *Candida albicans* (.M.I C= 25 mg/mL), which means that *Candida albicans* is less sensitive to the 70% ethanolic extract of *Piptadeniastrumafricanum*.

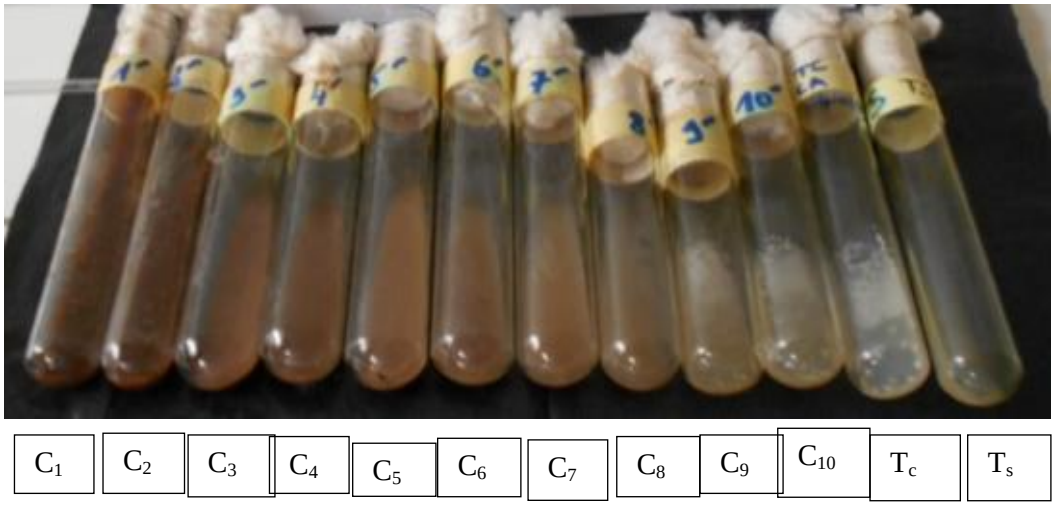

Figure 1: Dose-response action of 70% ethanolic extract of *Piptadeniastrumafricanum* on *in vitro* growth of *C.albicans*after 3 days incubation.

(C1= 50 mg/mL to C10 = 0.098 mg/mL)**; Tc**: control; **Ts**: sterility control

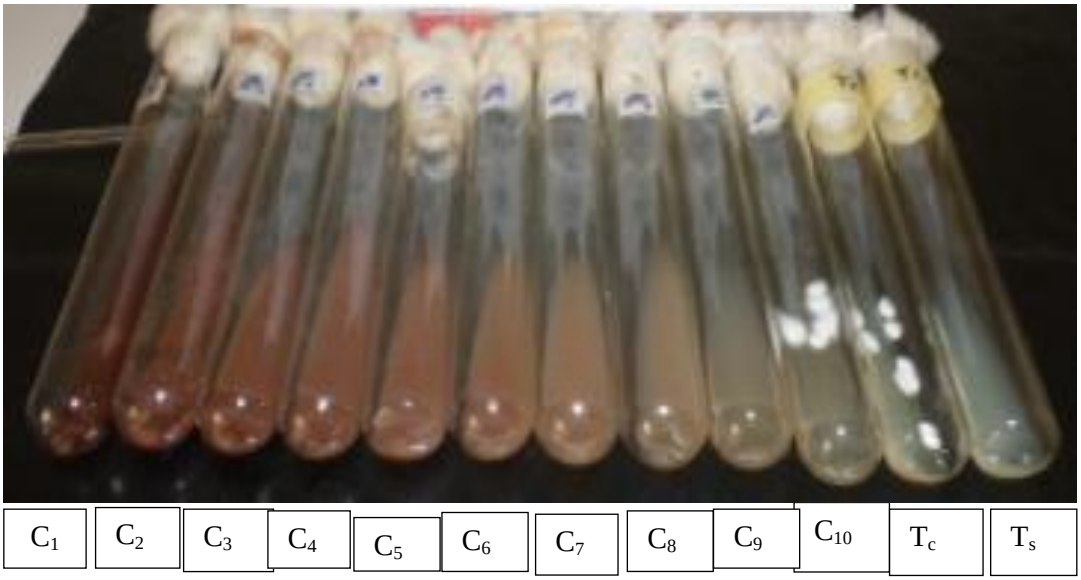

Figure 2: Dose-response action of 70% ethanolic extract of *Piptadeniastrumafricanum* on *in vitro* growth of *T.mentagrophytes* after 10 days incubation.

(C1= 50 mg/mL to C10 = 0.098 mg/mL);**Tc**: control; **Ts**: sterility control

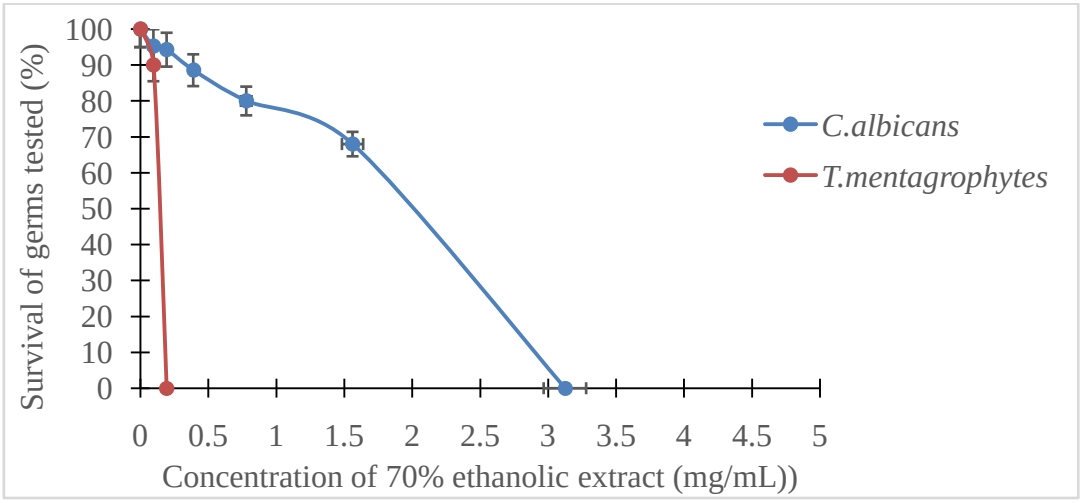

Figure 3: Sensitivity of *C. albicans* and *T. mentagrophytes* to ethanolic extract 70% of *Piptadeniastrumafricanum*

Table 2**:** Values (mg/mL) of antifungal parameters of ethanolic extract *70% Piptadeniastrumafricanum*

Extracts	Antifungalparameters	Strains	
		C. albicans	*T. mentagrophytes*
PIP (EE 70 %)	M.I. C	3,125	0,195
	M.C.F	25	0,195
	.I C_{50}	2	0,2

PIP (EE 70%): 70% ethanolic extract of *Piptadeniastrumafricanum*
EE: 70% ethanolic extract; PIP: *Piptadeniastrumafricanum*;

3.2.2.Antibacterial activity

3.2.2.1.Bacterial susceptibility to 70% ethanolic extract of *Piptadeniastrumafricanum*

The 70% ethanolic extract was active on all strains except *Pseudomonas aeruginosa* 795C/15. The largest inhibition diameter was observed on *Staphylococcus aureus* 408 C/14. Inhibition zone diameters on Enterobacteriaceae ranged from 10 to 20 mm at the 100 mg/mL concentration (Table 3).

Antibiotics such as ceftazidime (CAZ) and imipenem (IMP) gave diameters of 6 and 23 mm respectively on *Pseudomonas aeruginosa* strain 255C/12. Amoxicillin + clavulanic acid (AMC), cefotaxime (CTX) and cefepime (FEP) gave diameters of 16 mm, 13 mm and 6 mm respectively on *E. coli* 549 PP/15, while ethanolic extract at concentrations of 100 mg/mL, 50 mg/mL and 25 mg/mL gave relatively higher inhibition diameters than CTX and FEP. Overall, the 70% ethanolic extract gave the best inhibition diameters on the different strains studied compared with synthetic antibiotics on the same strains (Figures 4,5, 6).

Table III: Inhibition zone diameters (mm) of 70% ethanolic extracts of *Piptadeniastrumafricanum* on 30 bacterial strains

Bacterialstrains		Concentration (mg / mL)			T= 6± 0	Antibiotics	
Code	Espèces	C_1= 100	C_2 = 50	C_3 = 25		FOX	OXA
1541 C / 14	*S. aureus*	24±0,5^{c,d}	23±0,5^{f,g}	19±0,5^{f}	6 ± 0,0^{a}	12± 0,0^{b}	10± 0,0^{b}
408 C / 14		27±0,5^{e,f}	20±0,5^{c,d}	17±0,5^{b,a}	6 ± 0,0 ^{a}	15± 0,0^{c}	10± 0,0b
446 UB / 15		23±0,5^{c}	21±0,5^{d,e}	16±0,5^{a,b,c}	6 ± 0,0 ^{a}	11± 0,0^{b}	8± 0,0^{a}
ATCC 25923		25±0,5^{d}	18±0,5^{a,b}	15±0,5^{a,b}	6 ± 0,0 ^{a}	20± 0,0^{d}	21± 0,0^{d}
						IPM	CAZ
261 C / 6	*P. aeruginosa*	13±0,5^{d,e}	10±0,5^{b,c}	9±0,5^{b}	6 ± 0,0 ^{a}	15± 0,0^{d}	10± 0,0^{b}
795 C / 15		6±0,0 ^{a}	6±0,0 ^{a}	6±0,0 ^{a}	6 ± 0,0 ^{a}	12± 0,0^{c}	12± 0,0^{b}
255 C / 12		10±0,5^{b}	9±0,5^{b}	6±0,0 ^{a}	6 ± 0,0 ^{a}	23± 0,0^{e}	6± 0,0^{a}
ATCC 27853		14±0,5^{e}	12±0,5^{d}	11±0,5^{b}	6 ± 0,0 ^{a}	15± 0,0^{d}	16± 0,0^{c}
						AMC	FEP
1091 C / 15	*Salmonella sp*	15±0,5^{d,e}	11±0,5^{b,c}	6±0,0^{a}	6 ± 0,0 ^{a}	12± 0,0^{c}	11± 0,0^{c}
549 PP / 15	*Echerichia coli*	15±0,5^{d,e}	14±0,5^{e,f}	13±0,5^{d}	6 ± 0,0 ^{a}	16± 0,0^{d}	6± 0,0^{a}
563 UB / 15	*Klebsiella pneumoniae*	20±0,5^{f}	19±0,5^{g}	17±0,5^{e}	6 ± 0,0 ^{a}	9± 0,0^{b}	6± 0,0^{a}
ATCC 25922	*Echerichia coli*	14±0,5^{c,d}	13±0,5^{d,e}	11±0,5^{c}	6 ± 0,0 ^{a}	16± 0,0^{d}	14± 0,0^{d}

Means with the same superscript lowercase letters in the same column are not different at 5% according to the turkey test.

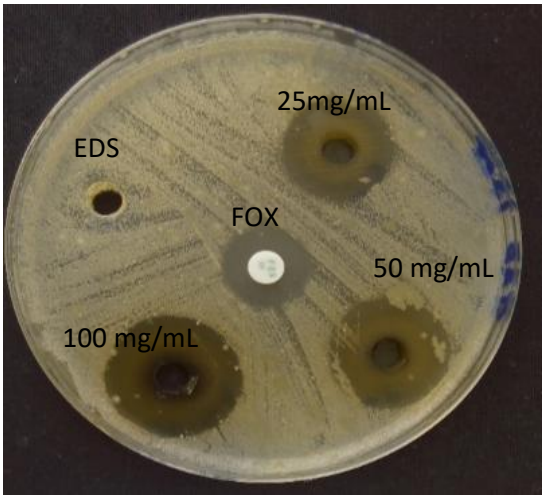

Figure 4: Inhibition diameters of ethanolic extract and antibiotic on *S.aureus*446 UB/15

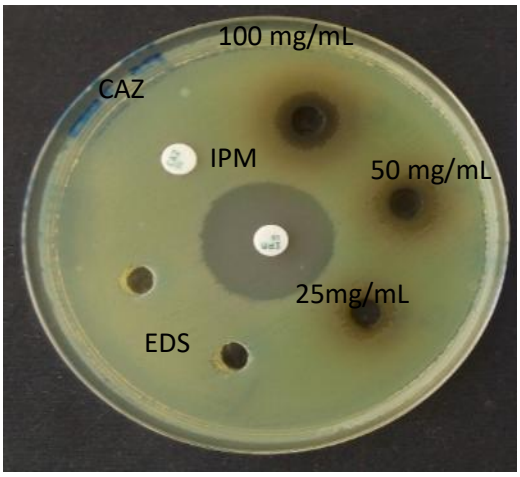

Figure 5: Inhibition diameters of ethanolic extract and antibiotics on *P.aeruginosa*255 C/12

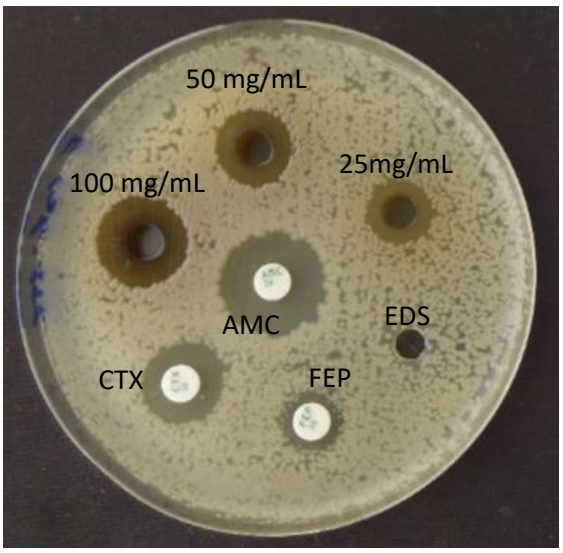

Figure 6: Inhibition diameters of ethanolic extract and antibiotics on *E.Coli*549 PP/15

3.2.2.2. *Evolution of MIC and MBC of Piptadeniastrumafricanum ethanolic extract*

Tables 4, V and 6 show the inhibitory action of 70% ethanolic extract (EE 70%) of *Piptadeniastrumafricanum*on strains *of Staphylococcus aureus*, *Pseudomonas aeruginosa* and Enterobacteriaceae. The 70% ethanolic extract was more active on *Staphylococcus aureus* than on *Pseudomonas aeruginosa* and Enterobacteriaceae, as it gave lower MICs and MBCs. This extract was therefore bactericidal on all 4 *Staphylococcus aureus* strains, unlike the 4 *Pseudomonas aeruginosa* and Enterobacteriaceae strains (Table 4, 5, 6).

Table 4: Antibacterial parameters of *Piptadeniastrumafricanum*ethanolic extract (EE 70%) on *in vitro* growth of *Staphylococcus aureus*

Code	EE 70 %			
	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Power
1541 C / 14	0,39	0,19	2,11	bc
408 C / 14	0,04	0,09	2,25	bc
446 UB / 15	0,09	0,19	2,11	bc
ATCC 25923	0,09	0,19	2,11	bc

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration**;** bt: bacteriostatic; bc: bactericidal; nd: not determined

Table 5: Antibacterial parameters of ethanolic extract (EE 70%) of *Piptadeniastrumafricanum*on in vitro growth of *Pseudomonas aeruginosa*

Code	EE 70 %			
	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Power
261 C / 6	12,5	> 100	nd	bt
795 C / 15	6,25	12,5	2	bc
255 C / 12	12,5	25	2	bc
ATCC 27853	12,5	25	2	bc

Table 6: Antibacterial parameters of *Piptadeniastrumafricanum*ethanolic extract (EE 70%) on in vitro growth of 4 enterobacteria

Codes	EE 70 %			
	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Power
1091 C / 15	25	50	2	bc
549 PP / 15	6,25	12,5	2	bc
563 UB / 15	12,5	100	8	bt
ATCC 25922	12,5	25	2	bc

4. Discussion

Phytochemical analysis of plant extracts is a preliminary step of great importance, since it reveals the presence of constituents known for their physiological activities and their possession of medicinal virtues. The phytochemical study revealed the presence of major groups of chemical compounds in *Piptadeniastrumafricanum*stem bark. The tannins and polyphenols present in *Piptadeniastrumafricanum*stem bark are substances recognized for their antioxidant properties [21]. These compounds are also recognized for their ability to bind to proteins, with a tendency to impermeability of the outer layers and protection of the underlying layers. Their antiseptic effects and tissue renewal properties could explain the traditional use of the plant's bark in the treatment of ulcers, skin superinfections and abscesses [22].

A study on the effects of the 70% ethanolic extract of *Piptadeniastrumafricanum*on the *in vitro* growth of *Candida albicans* and *Trichophyton mentagrophytes* revealed fungicidal activity of the extract at different concentrations. Several studies have been carried out with hydroalcoholic plant extracts on the *in vitro* growth of *Candida albicans* and *Trichophyton mentagrophytes*. A comparative analysis of the results obtained with those of [7,23]showed that the 70% ethanolic extract of *Piptadeniastrumafricanum*had better anticandidosis activity (twice as active) than the hydroalcoholic extracts of *Microglossapyrifolia*(MFC= 50 mg/mL) and *Harrisoniaabyssinica*(MFC=50 mg/mL) but less activity than the ethanolic extract of *Mitracarpusvillosus*(MFC=3.125 mg/mL) [14]. This 70% ethanolic extract of *Piptadeniastrumafricanum,* is 32 times more active than the ethanolic extract of *Ecliptaprostrata*(MFC=6.25 mg/mL) and eight times more active than the ethanolic extract of *Acanthospermumhispidum* (MFC=1.56 mg/mL). Finally, analysis of the results shows that *Trichophyton mentagrophytes* is more sensitive to the 70% ethanolic extract (MFC=0.195 mg/mL and CI_{50} = 0.2 mg/mL). These results are supported by the work of [24] who showed that the effect of an extract is probably due to the synergy between the number of components, which when separated become individually inactive. Ethanol is therefore the solvent that best concentrates the active ingredients.

The inhibitory activity of bacterial strains was assessed using the solid-state diffusion method (Mueller-Hinton agar) for sensitivity testing, and liquid-state dilution (Mueller-Hinton broth) to determine antibacterial parameters. All these results obtained in solid media show that the activity of the 70% ethanolic extract on methicillin-resistant *Staphylococcus aureus* remains superior to that induced by synthetic antibiotics, even though this extract is still a raw product. Numerous studies have highlighted the antibacterial effect of natural active ingredients. Indeed, [25]report that the aqueous extract of *Marrubium vulgare* L leaves exerts a strong inhibitory activity on strains *of Staphylococcus aureus* MTCC 740, *Staphylococcus epidermidis* MTCC 435 and a lesser degree of activity on

Proteus vulgaris MTCC 426 and *E.coli* MTCC 443. These results corroborate the work of other researchers [26,27]who noted the antibacterial power of *Piptadeniastrumafricam*on Gram-positive and Gram-negative bacteria. Since the main target of these natural compounds is the bacterial membrane, the antibacterial activity of *Piptadeniastrumafricanum*is explained by the presence of large groups of chemical molecules in the 70% ethanolic extract. The MIC/MBC ratio demonstrated the bactericidal or bacteriostatic effect of the 70% ethanolic extract. The ethanolic extract of *Piptadeniastrumafricanum*proved bactericidal on almost all the strains studied. Given its spectrum of action on all the bacterial strains studied, this extract would appear to be richer in active ingredients. Our results are similar to those of [26], who showed that *Piptadeniastrumafricanum*had a bactericidal effect on *S. aureus.*

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Each author's contribution

YK: Writing the article
KB: Responsible for cultivating fungal strains. He participated in the manipulation.
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5. References

1. Alice K. Place de la Medecine Traditionnelle dans les soins de santé primaires a Abidjan (Côte d'Ivoire). Thèse d'Etat de Docteur en médecine, Université Toulouse III–Paul Sabatier (France), 2012, 79 p.
2. Chabasse D., Bouchara J.P., Gentile L.D., Brun S., Cimon B., Penn P. Les dermatophytes. Biologie médicale. Cahier de formation, 2004, 31 p.
3. Coulibaly K**.** Etude botanique, pharmacologique et exploration phytochimique des extraits de *Terminaliaivorensis* et *Terminaliasuperba*, deux espèces ligneuses commerciales, médicinales antimicrobiennes de la forêt de Mopri, Tiassalé (Sud de la Côte d'Ivoire). Thèse de Doctorat de Botanique de l'Université Félix Houphouêt-Boigny, Abidjan, Côte d'Ivoire, 2012, 200 p.
4. Konan K F**.** Activitesantibacteriennes (sur les entérobacteries productrices de beta lactamases a spectre élargi) et antioxydantes in vitro de*terminaliaglaucescens*Planch (combretaceae), une plante medicinale ouest-africaine. Thèse de Doctorat, Université Félix Houphouët Boigny, Côte d'Ivoire, 2015, 144 p.
5. Sabu M.,Kattan R. Antidiabetic activity of medicinal plants and. its relationship with their antioxidant property. *Journal of Ethnopharmacology,* 2002,81: 155-60. DOI: 10.1016/s0378-8741(02)00034-x
6. Soro D., Kone M.W.,Kamanzi K**.**Evaluation de l'activité antimicrobienne et antiradicale libres de quelques taxons bioactifs de Côte d'Ivoire. European journal of scientific research, 2010, 40(2), pp 307-317.
7. Zirihi G.N., Kra A.M., Guédé-Guina F. Evaluation de l'activité antifongique de *Microglossapyrifolia* (Larmarck) O. kuntze (Asteraceae) " pymi " sur la croissance *in vitro* de *Candida albicans*. *Revue de Médecine et de Pharmacopées Africaines*,2003, 17 : 11-19.
8. Zirihi G.N., Kra A., Dadié E.T**.** Etude botanique et évaluation des activités antifongiques de *Mitracarpusvillosus* (MV) (Rubiaceae) et *Spermacoceverticillota* (SV) (Rubiaceae) sur la croissance *in vitro* de *A. fumigatus*. *Revue de Médecine et de Pharmacopées Africaines*, 2007, 20 : 9-17.
9. Harborne J.B.A guide to modern techniques of plant analysis. Springer, 3rd Edn, India (New delhi), 1998, pp 5-32.
10. Raaman N., Sivaraj C., Tenzing, Antioxidant activities and phytochemical analysis of methanol extract of leaves of *Artocarpusheterophyllus* Lam. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2014, 6: 289-293.
11. Kra A.K.M.Recherche bioguidée de composés antifongiques à partir de plantes médicinales de Côte d'Ivoire. Thèse de Doctorat d'Etat, UFR Biosciences, Université Félix Houphouët Boigny, Abidjan, Côte d'Ivoire, 2016, 264 p.
12. Ackah J.A., Kra A.K., Zirihi G.N., Guédé-Guina F.Evaluation et essais d'optimisations de l'activité anticandidosique de *Terminaliacatappa* Linn. (TEKAM3), un extrait de Combretaceae de la pharmacopée ivoirienne. *Bulletin de la Société Royale des Sciences de Liège*, 2008, 77 : 120-36.https://popups.uliege.be/0037-9565/index.php?id=823&file=1&pid=811
13. Kra A.K.M., Ahon G.M., Djo-Bi D., Ouattara S., Coulibaly A., Djaman A.J.Anti-fungal activities of medicinal plants extracts of Ivorian pharmacopoeia. Journal of Intercult Ethnopharmacol*ogy*, 2014, 3(4) : 159-166. doi: 10.5455/jice.20140627125512

14. CA-SFM/EUCAS. Comité de l'antibiogramme de la Société Française de Microbiologie. Edition de mai, Paris (France), 2016, 114 p.
15. Guessennd K.N**.** Détermination de l'activité antibactérienne des substances naturelles issues des plantes de la pharmacopée de Côte d'Ivoire. Fiche technique N°2, Institut Pasteur de Côte d'Ivoire, Abidjan (Côte d'Ivoire), 2005, 18 p.
16. Ponce A.G., Fritz R., Del Alle C., Roura S.I**.**Antimicrobial activity of essential oil on the native microflora of organic Swiss chard. *Lebensmittel-wissenchaftund technologic*, 2003, 36 : 679-684.
17. Koné W.M., Kamanzi A.K., Terreaux C., Hostettmann K., Traore D., Dosso M.Traditional Medicine in North Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. *Journal of Ethnopharmacology*, 2004, 93: 43-49.DOI: 10.1016/j.jep.2004.03.006
18. Moroh J.L.A., Bahi C., Djè K., Loukou Y.G., Guédé-Guina F.Etude de l'activité antibactérienne de l'extrait acétatique (EAC) de *Morindamorindoides* (Baker) milne-redheat (Rubiaceae) sur la croissance *in-vitro* des souches d'*Escherichia coli*. *Bulletin de la Société Royale des Sciences de Liège*, 2008, 77 : 44-61. https://popups.uliege.be/0037-9565/index.php?id=518
19. Dosso M., Faye-Kette H. Documents Techniques Antibiotiques, Université Nationale de Côte d'Ivoire, 1995, 178 p.
20. Kamanzi A.K.Plantes médicinales de Côte d'Ivoire : investigations phytochimiques guidées des essais biologiques. Thèse de Doctorat d'Etat ès-Sciences en Biologie Végétale. UFR Biosciences, Université de Cocody, Abidjan, 2002, 190 p.
21. Traoré A., Datte Y.J., Offoumou A.M., Aka K.J., Sawadogo L.L.Effets antihypertensifs de l'extrait aqueux de *Jatropha gossypiifolia*L. (Euphorbiaceae) sur la pression artérielle de mammifères. *Revue de Médecines et Pharmacopées Africaines,*1999, 13 : 41-48.
22. Bruneton J. Pharmacognosie et phytochimie des plantes médicinales. Technique et documentation, 2ème édition, Paris, 1993, 914 p.
23. Bene K., Camara D., Fofie N. B. Y.,Zirihi G. N. Étude ethnobotanique, activité antifongique in vitro sur Candida albicans et toxicité sur les cellules HFF de HarrisoniaabyssinicaOliv. (Simaroubaceae), une plante de la pharmacopée ivoirienne. Journal of Applied Biosciences, 2015, 94:8815 – 8824, http://dx.doi.org/10.4314/jab.v94i1.4
24. Sarker S.D., Latif Z., Gray A.I. Natural products isolation. *HumanaPress*, 2005, 20:1-23.
25. Mubashir H.M., Iqbal Z.M., Bahar A.A., Saroor A.K., Shamshir K., Singh P**.**Evaluation of Antimicrobial Activity of Aqueous Extract of *Marrubium vulgare* L*. Journal ofResearch Development*,2009, 9 : 53-56.
26. Obame E.L.C**.**Etude phytochimique, activités antimicrobiennes et antioxydantes de quelques plantes aromatiques et médicinales africaines. Thèse de Doctorat unique en Biochimie-Microbiologie, Université de Ouagadougou, 2009, 277 p.
27. Onanga M., Ekouya E., Ouabonzi A., Itoua C.B**.** Etudes ethnobotanique, pharmacologique et chimique des plantes utilisées dans le traitement des dermatoses MWANDZA. Pharmacopée et Médecine Traditionnelle Africaine, 1997, 9 :85-93.