ORIGINAL ARTICLE

DIVERSITY OF MEDICINAL ASTERACEAE OF THE DISTRICT OF ABIDJAN (COTE D'IVOIRE): ANTIBACTERIAL AND PHYTOCHEMICAL SCREENING

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ABSTRACT

Introduction: At the end of an ethnobotanical survey in the district of Abidjan, ten medicinal Asteraceae frequently used in primary health care among poor people and especially to treat infectious diseases, were selected to carry out an antibacterial screening. **Objectives:** This study is part of the search for new natural therapeutics through plants of our immediate environment. **Methods:** Twenty extracts including ten aqueous total extracts (ATE) and ten ethanolic fractions (EF70 %) were tested *in vitro* on fifteen *Staphylococcus aureus* (MRSA) and ten *Pseudomonas. aeruginosa* (CRPA). **Results:** Ten extracts showed antibacterial activities with inhibition diameters from 9 to 19 mm on Gram + bacteria and from 9 to 12 mm on Gram – bacteria. These activities are comparable to those of certain antibiotics such as FOX, OXA, CAZ, IPM whose inhibition diameters respectively ranged from 11 to 19 mm, 14 to 20 mm, 15 to 18 mm and 13 to 15 mm on the same bacteria. The most active plants are *Eclipta prostrata*, *Acanthospermum hispidum*, *Aspilia africana*, *Ageratum conyzoides* and *Erigeron floribundus*. They were each either bacteriostatic or bactericidal. The activity of these weeds could be justified by the presence of numerous secondary metabolites in the different tested extracts. **Conclusion:** These results show that some of the studied plants could be potential sources of new antibacterial agents against antibiotic-resistant strains.
Keywords: *Antibacterial activity, ethnobotany, secondary metabolites, medicinal plant.*

1. INTRODUCTION

In many countries of the world, and especially in developing countries, infectious diseases are a public health problem because of their frequency and severity due to immunodepressing diseases. Indeed, they are responsible for more than 17 million deaths per year worldwide, more than half of which comes from Africa alone [1]. Thus, the discovery of antibiotics has been a real relief for humanity because these remedies have significantly reduced the incidence of infectious diseases especially in developed countries. But, senseless and often uncontrolled use of these molecules has caused a phenomenon of resistance in many infectious agents [2]. Despite the progress made by these industrialized countries, in the field of chemistry, for the development of modern medicine, the populations in large numbers cure themselves with plants. The reasons for this use of herbal medicine are multiple. In developing countries, for cultural reasons and economic difficulties, plant medicine still occupies a prominent place in the health system and remains an essential resort for people in primary health care [3]. Also, nowadays, with the constantly high cost of available drugs, combined with the emergence of multidrug-resistant pathogens, there is renewed interest in the African pharmacopoeia [4, 5, 6]. In front of these difficulties of a socio-economic order that are shabby, the progress the diseases and the repeated failures of the treatments due to the resistance of the microorganisms against drugs which cure infections, it is essential to look for new effective substances for the effective cure of patients especially those who are immunodepressing. To do so, the main field to explore is that of medicinal plants. Thus, to derive a real benefit from the use of medicinal plants, it becomes imperative to initiate scientific research for the rational exploitation of the indisputable virtues of the plants of our immediate environment in order to further identify substances possessing. Antibacterial properties and streamline their use. The interest was therefore focused on the medicinal plants of the Asteraceae family marketed in the district of Abidjan (south part of Côte d'Ivoire). These plant species, most often abundant, are frequently used in primary health care in Africa. The present work aims to carry out an antibacterial screening of the leaves of ten medicinal Asteraceae selected during an ethnobotanical survey carried out in the district of Abidjan on Meticillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) strains that have been isolated in cases of diarrhea, urinary tract infections, wound pus, and carry out phytochemical screening.

2. MATERIAL AND METHODS

2.1. Selection of plants

The plants are selected after an ethnobotanical survey conducted in the autonomous district of Abidjan [7]. This region is located in the south of Ivory Coast. The uses of selected plants in traditional medicine are summarized in table 1. These Asteraceae are used both by the local population and by the traditional healers for the treatment of various diseases including bacterial infections. The identification of these Asteraceae was authenticated with the Botanical Technician ASSI Yapo Jean, of the National Floristic Center (N.F.C), of the Felix HOUPHOUET-BOIGNY University of Cocody (Côte d'Ivoire) where samples are kept.

Table 1: The table presents the some traditional uses of medicinal and selected Asteraceae from the district of Abidjan for antibacterial screening.

Plant species	Plant parts used	Indications
Aspilia africana (Pers.) C.D. Adams var. *africana*	Leaves	Eyeaches, cough, wounds, stomachache, infantile diseases, skin diseases, malaria
Ageratum conyzoides L.	Leaves	Malaria, ovarian cyst, whites, stomachache, furuncle, cervix diseases, hemorrhoid, pregnancy, skin diseases
Acanthospermum hispidum DC.	Whole plant	Malaria, jaundice, ovarian cyst, white losses, Hypertension, stomachache, intestinal parasites, pregnancy, infantile diseases, skin diseases
Bidens pilosa L.	Leaves	Whitlow, infantile diseases, cough, wounds, ulcers, , skin diseases, hypertension, pregnancy
Erigeron floribundus (H.B. et K.) Sch. Bip.	Leaves	Skin diseases, ovarian cyst, whitlow, whites, snake bite
Eclipta prostrata (L.) L.	Leaves	Asthma, malaria, diabetes, wounds, infantile diseases, skin diseases
Mikania cordata var. *chevalieri* C.D. Adams	Whole plant	Malaria, jaundice, fever, skin diseases, infantile diseases, intestinal parasites, pregnancy
Microglossa pyrifolia (Lam.) O. Ktze	Leaves/Roots	Anaemia, infantile diseases, pregnancy, furuncle
Synedrella nodiflora Gaertn	Leaves	General tiredness, pregnancy, abortion, kidney failure, heart failure, wounds
Struchium sparganophora (L.) O. Ktze	Leaves	Malaria, sinusitis, pregnancy, ovarian cyst, wounds

2.2. Plant material extraction

The leaves of these ten species were dried separately in the Laboratory for two weeks and reduced to a fine powder using an electric grinder type IKA Labortechnik (MFC type).

Preparation of aqueous total extracts (ATE): the preparation of these extracts was performed using the method described by Zirihi (2007) which involves macerating 100 g of each plant powder in 1 L of sterile distilled water using a blender Blinder type 7 SEVEN STAR [8]. The homogenates were filtered over hydrophilic cotton and then on filter paper Whatman 3 mm. The aqueous filtrates thus obtained were evaporated using an oven type Med Center Venticell at 50 °C to give powders that constitute ATE.

Preparation of ethanolic fractions 70 % (EF70 %): these fractions were obtained separately by dissolving 5 g of each ATE in 100 mL of a ethanol 70 % solution and then homogenized. After decantation and filtration of the alcoholic fraction on hydrophilic cotton and on filter paper Whatman 3 mm, the filtrate collected is evaporated in an oven at 50 °C. The powder obtained constitutes the EF70 % extract.

2.3. Yield Calculation

The yield is the amount of extract obtained from the plant powder. It is expressed as a percentage or without any unit. In practice, it is determined by the ratio of weight of the solids content after evaporation by the weight of the dry powder of the plant material used for the extraction, multiplied by 100. This gives the following formula:

$$Yd = (m \times 100) / M \quad (1)$$

Yd: Extraction yield in percentage;
m: mass in grams of the dry extract;

M: mass in grams of the drug powder).

2.4. Antibacterial screening

The microbial carrier is composed of 25 multidrug-resistant bacterial isolates of which 23 clinical strains including 14 strains of *Staphylococcus aureus* (MRSA) and nine strains of *Pseudomonas aeruginosa* (CRPA) obtained from different biological products. Quality control is ensured by two reference strains: *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 (Table 2). They are provided by the Antibiotics Unit, Natural Substances and Monitoring of Microorganisms for Anti-Infective (ASSURMI) and the Department of Bacteriology and Virology of the Pasteur Institute of Côte d'Ivoire (IPCI). Susceptibility testing performed previously briefed us on the profile of each strain used in this study.

S. aureus is Gram-positive bacteria (Gram+), spherical, with a diameter of 1 µm, in diplococcior small cluster (cluster of grapes) motionless, spore, not encapsulated. This bacterium is an aero-anaerobic germ respiratory and fermentative metabolism, catalase positive [9]. As for *P. aeruginosa*, it is a Gram- bacillus in the form of straight rods and considered as a germ of secondary infection. It is a ubiquitous germ that is found in a privileged way in wet or aqueous environments, soils and plants [10].

Table 2: The table presents the list of studied strains.

Name of germs	Code	Resistance phenotypes	Biological products	Types of Bacteria
Clinical strains				
Staphylococcus aureus	680Y/14	MRSA ; Méti-R ; MLST06 ; RCFQ	Suppuration	Gram+
	583C/14	MRSA ; Méti-R ; MLST06 ; RCFQ		
	1325Y/14	MRSA ; Méti-R ; MLSb (i) ; KTG ; RCFQ		
	5229C/15	MRSA ; Méti-R	Pus	
	590Y/14	MRSA ; Méti-R	Blood	
	1690C/14	MRSA ; Méti-R ; MLST06 ; RCFQ		
	1541C/14	MRSA ; RCFQ	Urine	
	485Y/14	MRSA ; Méti-R; RCFQ; MLSbi; KTG	Probe tip	
	039C/11	MRSA ; Méti-R		
	1532C/13	MRSA ; Méti-R	Pus	
	408C/15	MRSA ; RCFQ ; KTG ; MLbc+S		
	754Y/15	MRSA ; Méti-R	Urine	
	446C/14	MRSA ; RCFQ		
	1000C/14	MRSA ; RCFQ	Pus	
Pseudomonas aeruginosa	255C/12	CRPA by porin deficiency D2	Wounds	Gram-
	489C/11	CRPA by porin deficiency D2		
	1742C/14	CRPA ; RCFQ	Urine	
	1605C/14	CRPA ; RCFQ		
	1175C/14	CRPA ; RCFQ	Faeces	
	1591C/14	CRPA ; RCFQ	Pus	
	1060Y/14	CRPA ; RCFQ		
	1780C/14	CRPA by porin deficiency D2	Urine	
	1076C/11	CRPA by porin deficiency D2		
Reference strains				
Staphylococcus aureus	ATCC 25923			
Pseudomonas aeruginosa	ATCC 27853			

A sterility test was first performed in order to verify that the studied plant extracts do not contain any bacteria or fungus. Thus, 0.1 g of each tested extract was placed in 10 mL of thioglycholate culture medium and incubated at 37 °C for 24 hours at the end of which the turbidity of the culture medium is assessed with the naked eye. This culture medium was then cultured on nutrient agar and Sabouraud agar solidified in petri dishes and incubated under the same conditions for 3 days with an observation every 24 hours. The substance is called sterile, if no colony is visible on the agar plate.

To evaluate the antibacterial activity, two methods were used : the antibiogram by diffusion in a solid medium [11, 12, 13] and the liquid microdilution method for the determination of MIC and MBC [6-14]. They make it possible to carry out several serial dilutions without risk of error and to test several antibiotics at the same time.

For the antibiogram by diffusion in solid medium, an inoculum was prepared from a 24 hours young colony emulsified in 2 mL of 85 % NaCl suspension and adjusted to an optical density of 0.5 Mac Farland using a densimat. This bacterial suspension was diluted in physiological saline (0.9 % NaCl) according to the 1/100 multiplication rate for *Staphylococcus aureus* and 1/1000 for *Pseudomonas aeruginosa* in order to obtain a new suspension of 10^6 to 10^5 bacteria/mL. This new microbial suspension constitutes the definitive bacterial *inoculum*.

For the realization of this method, a solution of 100 mg/mL concentration of each of the extracts was prepared. Petri dishes containing Mueller-Hinton agar were inoculated by swabbing with the prepared *inoculum*. Then, wells were dug by pressing the big end of a Pasteur pipette into the agar and filled with 50 µL of the prepared extracts solution. The whole was incubated at 37 °C for 24 hours after which the inhibition diameter around each well was measured using a vernier caliper. The evaluation of the activity of the extracts was made according to the reference of [15]. Thus, a bacterial strain is said to be insensitive to a given substance if the inhibition diameter of the substance is less than 8 mm whereas it is said to be sensitive if the diameter is between 9 and 14 mm. On the other hand, it is considered very sensitive when the inhibition diameter is between 15 and 19 mm and then extremely sensitive if the diameter is greater than 20 mm. The activity test allowed the selection of the most active extracts for the determination of antibacterial parameters.

For the second method, the *inoculum* was prepared from an 18 hours bacterial culture on Mueller-Hinton agar. Two colonies of this culture were taken and emulsified in 10 mL of sterile Mueller-Hinton broth. This mixture is then incubated at 37 °C. for 3 hours at the end of which a suspension of 0.3 mL of this pre-culture was removed and diluted in 10 mL of sterile Muller-Hinton broth and then homogenized (turbidity ≈ 10^6 bacteria/mL). This solution constitutes the bacterial *inoculum* used for the entire study.

For the determination of antibacterial parameters, in 10 experimental hemolysis tubes, 1 mL of each concentration range (100 mg/mL to 3.125 mg/mL) of plant extract was added to 1 mL of bacterial *inoculum*. The growth control tube received 1 mL of sterile distilled water in addition to the *inoculum* while the sterility control received only 2 mL of the sterile Mueller-Hinton broth. The tubes were incubated at 37 °C for 24 hours at the end of which an observation with the naked eye was made and the lowest concentration for which no bacterial growth was observed corresponds to the Minimum Inhibitory Concentration (MIC). As for the Minimal Bactericidal Concentration (MBC), its determination is made by counting the *inoculum*. This consists in diluting the *inoculum* from 10^{-1} to 10^{-4} and inoculating these different dilutions in 5 cm long streaks, on Mueller-Hinton agar solidified in Petri dishes using a loop calibrated 2 µL and incubates for 24 hours. These petri dishes have been named A. After reading the MIC, the contents of tubes in which there was no visible growth were used to culture Mueller-Hinton agar on 5 cm long streaks. This series of petri dishes is named B. MBC was determined by comparing bacterial growth in A and B boxes. Thus, the lowest concentration of the tube that has less than 0.01 % viable bacteria compared to the initial *inoculum* is MBC. The MBC/MIC ratio clarified the modality of action of each substance [16]. If the MBC/MIC ratio is less or equal than 2, the substance is said to be bactericidal. On the other hand, if it is greater than 2, the substance is called bacteriostatic.

2.5. Phytochimical sorting

A phytochemical sorting was carried out in order to detect some large groups of secondary metabolites contained in the studied plant extracts. The summaries of the reactions are contained in table 3. The color reaction screen was used [17].

Table 3: The table presents the synthetic table of chemical groups, identification reagents and indicators.

Chemical groups		Identification reagents	Indicator (positive reaction)
Sterols and Polyterpenes		Acetic anhydride Concentrated sulfuric acid	Appearance at the interphase of a purple and violet loop, turning blue then green
Polyphenols		Ferric chloride $FeCl_3$ (2 %)	Appearance of a blackish or greenish blue colouring more or less dark
Flavonoids		Hydrochloric alcohol, Magnesium chips, Isoamyl alcohol	Heat release then pink-orange or purplish coloration
Tanins	Catechiques	Formaldehyde Concentrated hydrochloric acid	Gelatinous precipitate (in large flakes)
	Gallic	Sodium acetate, Ferric chloride	Intense blue-black coloration
Quinones		Ammoniac	Appearance of a coloration ranging from red to purple
Saponins		Foam index	Appearance of a persistent foam
Alkaloids		Dragendorff (Potassium iodo-bismuthate solution) Burchard (Iodine-iodinated reaction)	Precipitate of reddish-brown coloration

Solutions with these indicators have a positive reaction and that indicates the presence of chemical groups in the drugs.

3. RESULTS

3.1. List of studied medicinal Asteraceae

At the end of the ethnobotanical investigations carried out on the markets of the autonomous district of Abidjan, ten plant species of the Asteraceae family were selected for an antibacterial screening. This selection was motivated by the frequency of use of these plants and especially the diseases (infectious diseases) for which they are solicited. Thus, figures 1 to 10 refer to the different medicinal Asteraceae selected. These are all herbs usually called invasive plants (weeds). They are mostly Therophytes (60 %) and the same species to the Guineo-Congolese region and the Sudano-Zambian region (GC-SZ) are the most represented with six species (60 %). They are all wild plants whose ruderal plants are the majority (50 %) (Table 4).

Figure 1: *Aspilia africana* (Pers.) C.D. Adams var. *africana*

Figure 2: *Ageratum conyzoides* L.

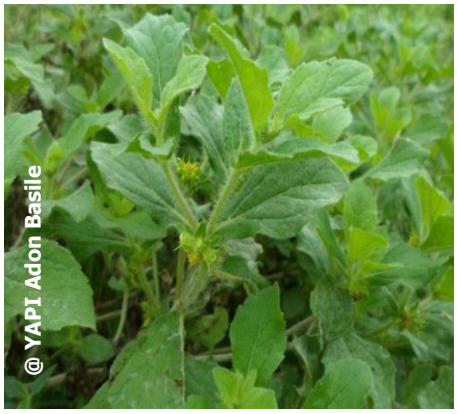

Figure 3: *Acanthospermum hispidum* DC.

Figure 4: *Bidens pilosa* L.

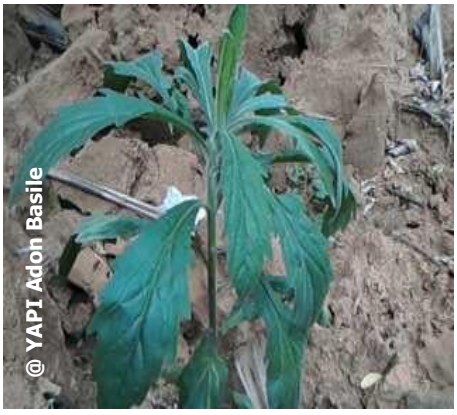

Figure 5: *Erigeron floribundus* (H.B. and K.) Sch. Bip.

Figure 6: *Eclipta prostrata* (L.) L.

Figure 7: *Mikania cordata* var. *chevalieri* C.D. Adams

Figure 8: *Microglossa pyrifolia* (Lam.) O. Ktze

Figure 9: *Synedrella nodiflora* Gaertn.

Figure 10: *Struchium sparganophora* (L.) O. Ktze

Table 4: Botanical and ecological characteristics of medicinal Asteraceae studied.

Plant species	Morphological types	Biological types	Phytogeographic types	PTHM
Aspilia africana	Herb	Nanophanerophyte	GC-SZ	Ruderal
Ageratum conyzoides	Herb	Therophyte	GC-SZ	Spontaneous antropophile
Acanthospermum hispidum	Herb	Therophyte	GC-SZ	Ruderal
Bidens pilosa	Herb	Therophyte	GC-SZ	Spontaneous antropophile
Erigeron floribundus	Herb	Therophyte	GC-SZ	Spontaneous antropophile
Eclipta prostrata	Herb	Therophyte	GC-SZ	Ruderal
Mikania cordata	Liana	Microphanerophyte	GC	Wild spontaneous
Microglossa pyrifolia	Herb	Nanophanerophyte	GC	Wild spontaneous
Synedrella nodiflora	Herb	Therophyte	GC	Ruderal
Struchium sparganophora	Herb	Nanophanerophyte	GC	Ruderal

PTHM : Plant Type by Harvest Medium ; **GC** : Guinéo-Congolaise ; **SZ** : Soudano-Zambienne.

3.2. Yield extractions

The extraction yields of the ten Asteraceae that were the subject of this study were calculated. The obtained results are summarized in table 5 for the aqueous total extracts (ATE) and in table 6 for the ethanolic fractions (EF70 %). From the analysis of these results, it was observed that for ATE extracts, *Acanthospermum hispidum* gave the best yield (17 %), whereas *Eclipta prostrata* had the lowest yield (8 %) (Table 5). As for EF70 % extracts, *Bidens pilosa* provided the lowest yield (21 %). However, *A. hispidum* yielded the highest yield at 52.2 % (Table 6). Of the two types of obtained extracts, ethanolic fraction showed the highest yields. And in general, it was observed that *A. hispidum* gave the best yields.

Table 5: Values of yied of ten aqueous total extracts (ATE)

Plant extract	ATE Aa	ATE Ac	ATE Ah	ATE Bp	ATE Ef	ATE Ep	ATE Mc	ATE Mp	ATE Sn	ATE Ss
Yield (%)	11	15	17	12.5	9.5	8	15	8.5	9	15

ATE : Aqueous Total Extract ; Aa : *Aspilia africana* ; Ac : *Ageratum conyzoides* ; Ah : *Acanthospermum hispidum* ; Bp : *Bidens pilosa* ; Ef : *Erigeron floribundus* ; Ep : *Eclipta prostrata* ; Mc : *Mikania cordata* ; Mp : *Microglossa pyrifolia* ; Sn : *Synedrella nodiflora* ; Ss : *Struchium sparganophora.*

Table 6: Values of yied of ten ethanolic fractions 70 % (EF70 %)

Plant extract	EF70 % Aa	EF70 % Ac	EF70 % Ah	EF70 % Bp	EF70 % Ef	EF70 % Ep	EF70 % Mc	EF70 % Mp	EF70 % Sn	EF70 % Ss
Yield (%)	28.8	50.4	52.2	21	23	43.6	25	39	46	31

EF70 %: Ethanolic Fraction 70 %.

3.3. Antibacterial activities

Among the twenty tested extracts, ten extracts (50 %) showed some inhibitory activities against the studied bacteria. From these ten extracts with antibacterial activity, four showed inhibitory actions only against Gram- bacteria (*Pseudomonas aeroginosa*). In contrast, two extracts exclusively inhibited the growth of Gram+ bacteria (*Staphylococcus aureus*). However, only two ATE extracts showed true inhibitory actions on bacterial growth with inhibition diameters from 12 to 18 mm for *Aspilia africana* on *S. aureus* strains, from 9 to 10 mm and from 11 to 12 mm for *Eclipta prostrata* respectively on *S. aureus* and *P. aeroginosa* strains (Table 7). As for the EF70 % extracts, four also showed true inhibitory actions on bacterial growth with inhibition diameters from 9 to 19 mm for *Acanthospermum hispidum* and from 12 to 16 mm for *E. prostrata* on *S. aureus* strains. On the other hand, on the growth of *P. aeroginosa* strains, true inhibitory actions were observed with EF70 % extracts of *Ageratum conyzoides* and *Erigeron floribundus* with respectively from 9 to 10 mm and from 11 to 12 mm as inhibition diameter (Table 7).

On *S. aureus* strains, three extracts showed bacteriostatic powers while an extract (EF70 % Ep) was both bacteriostatic on some germs and bactericidal on others. On the *P. aeroginosa* strains, two extracts showed bactericidal powers (Table 8). The active extracts were all bacteriostatic on *S. aureus* strains whereas on *P. aeroginosa* strains, they showed bactericidal powers. *E. prostrata* was the most active medicinal Asteraceae, followed by *A. hispidum, A. africana, A. conyzoides* and *E. floribundus*.

Table 7: Diameters (mm) of the zones of inhibition obtained with the extracts of the most active plants and the antibiotics on MRSA and CRPA strains and references strains

Bacteria \ Plant species		*A. africana*		*A. conyzoides*		*A. hispidum*		*E. floribundus*		*E. prostrata*		EDS	FOX	OXA
		ATE	EF70 %	ATE	EF70 %	ATE	EF70 %	ATE	EF70 %	ATE	EF70 %			
S. aureus	ATCC 25923	18±0.5	10±0.3	6±0.0	6±0.0	9±0.7	13±0.0	6±0.0	6±0.0	10±0.3	16±0.4	6±0.0	19±0.1	20±0,2
	680Y/14	12±0.7	6±0.0	6±0.0	6±0.0	8±0.0	10±0.3	6±0.0	6±0.0	8±0.4	13±0.3	6±0.0	20±0,4	16±0,2
	583C/14	16±0.3	6±0.0	6±0.0	6±0.0	8±0.0	9±0.7	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	20±0,2	17±0,0
	1325Y/14	15±0.5	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	9±0.2	14±0.3	6±0.0	17±0,3	16±0,3
	5229C/15	6±0.0	6±0.0	6±0.0	6±0.0	9±0.3	13±0.7	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	18±0,0	17±0,0
	590Y/14	15±0.2	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	8±0.0	12±0.4	6±0.0	20±0,1	15±0,2
	1690C/14	17±0.7	6±0.0	6±0.0	6±0.0	9±0.7	13±0.0	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	11±0,2	19±0,1
	1541C/14	6±0.0	6±0.0	6±0.0	6±0.0	9±0.0	19±0.5	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	16±0,2	14±0,2
	4885Y/14	8±0.3	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	9±0.3	12±0.0	6±0.0	17±0,2	17±0,2
P. aeruginosa	ATCC 27853	6±0.0	6±0.0	9±0.7	10±0.5	9±0.3	9±0.3	9±0.5	12±0.0	11±0.0	6±0.0	6±0.0	18±0.3	15±0.3
	1742C/14	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	8±0.4	6±0.0	11±0.0	12±0.0	6±0.0	6±0.0	15±0.2	13±0.5
	1605C/14	6±0.0	6±0.0	8±0.0	10±0.2	9±0.3	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	15±0.0	14±0.2
	1591C/14	6±0.0	6±0.0	9±0.0	8±0.1	8±0.2	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	nd	nd
	1060Y/14	6±0.0	6±0.0	8±0.0	9±0.3	6±0.0	6±0.0	9±0.3	6±0.0	9±0.1	6±0.0	6±0.0	nd	nd
	1076C/11	6±0.0	6±0.0	6±0.0	6±0.0	8±0.1	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	6±0.0	nd	nd

Values are expressed as mean ± standard deviation (n=3), Included diameter of the wells (6 mm), MRSA : Methicillin-Resistant *Staphylococcus aureus* : **CRPA** : Carbapenems Resistant *Pseudomonas. aeruginosa* ; **ATE** : Aqueous Total Extract ; **EF70 %** : Ethanolic Fraction 70 % ; **EDS** : Sterile Distilled Water (control) ; **ATCC** : American Type Culture Collection ; **FOX** : Cefoxitin ; **OXA** : oxacillin ; **CAZ** : ceftazidime ; **IPM** : imipenem ; nd : not determined.

Table 8: Antibacterial parameters of the most active plant extracts on strains of *S. aureus* (MRSA), *P. aeruginosa* (CRPA) and reference strains

Plant extracts / Bacteria		MIC and MBC (mg/ml)												MBC/MIC report and Power					
		ATE Aa		ATE Ep		EF70 % Ah		EF70 % Ac		EF70 % Ep		EF70 % Ef							
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	ATE Aa	ATE Ep	EF70 % Ah	EF70 % Ac	EF70 % Ep	EF70 % Ef
S. aureus	ATCC25923	25	>100	6.25	50	6.25	100	nd	nd	6.25	50	nd	nd	>4 bt	8 bt	16 bt	nd	8 bt	nd
	680Y/14	25	>100	nd	nd	12.5	100	nd	nd	6.25	25	nd	nd	>4 bt	nd	8 bt	nd	4 bt	nd
	583C/14	6.25	50	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8 bt	nd	nd	nd	nd	nd
	1325Y/14	12.5	100	nd	nd	nd	nd	nd	nd	12.5	25	nd	nd	8 bt	nd	nd	nd	2 bc	nd
	5229C/14	nd	nd	nd	nd	12.5	50	nd	nd	nd	nd	nd	nd	nd	nd	4 bt	nd	nd	nd
	590Y/14	12.5	>100	nd	nd	nd	nd	nd	nd	12.5	25	nd	nd	>8 bt	nd	nd	nd	2 bc	nd
	1690C/14	12.5	100	nd	nd	12.5	50	nd	nd	nd	nd	nd	nd	8 bt	nd	4 bt	nd	nd	nd
	1541C/14	nd	nd	nd	nd	6.25	50	nd	nd	nd	nd	nd	nd	nd	nd	8 bt	nd	nd	nd
	4885Y/14	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
P. aeruginosa	ATCC27853	nd	nd	25	50	nd	nd	25	50	nd	nd	25	50	nd	2 bc	nd	2 bc	nd	2 bc
	1742C/14	nd	nd	25	100	nd	nd	nd	nd	nd	nd	12.5	25	nd	4 bt	nd	nd	nd	2 bc
	1605C/14	nd	nd	nd	nd	nd	nd	25	50	nd	nd	nd	nd	nd	nd	nd	2 bc	nd	nd

Values are expressed on average (n=3), **MRSA:** Methicillin-Resistant *Staphylococcus aureus.* **CRPA** : Carbapenems Resistant *Pseudomonas. Aeruginosa* ; **Aa** : *Aspilia africana* ; **Ac** : *Ageratum conyzoides* ; **Ah :** *Acanthospermum hispidum* ; **Ef :** *Erigeron floribundus* ; **Ep** : *Eclipta prostrata* ; **EF70 %** : Ethanolic Fraction 70 % ; **ATCC** : American Type Culture Collection ; **MIC** : Minimum Inhibitory Concentration ; **MBC :** Minimal Bactericidal Concentration ; **bc :** bactericidal ; **bt :** bacteriostatic ; **nd:** not détermined ; nd : not determined.

3.4. Phytochimical sorting

Phytochemical sorting revealed the presence of flavonoids, terpenes, sterols and steroids in the ten active extracts on bacteria. On the other hand, no trace of gallic tannins has been revealed in these extracts (Table 9). However, the presence of catechin tannins was revealed in all studied extracts except in the *A. conyzoides* ones. The presence of saponins has been noted in all ATE extracts except in the *E. prostrata* one as in all studied EF70 %. As for coumarins and quinones, they are present only in the extracts EF70 % of *A. africana* and *A. hispidum*. However, the presence of coumarins in EF70 % extract of *E. floribundus* was noted. It was also noted the presence of flavonoids in almost all the studied extracts except in the EF70 % extracts of *A. africana*, *A. hispidum* and *E. floribundus*. Concerning alkaloids, they are present or in trace in almost all the studied extracts with the exception of the extracts of *A. hispidum* and the ATE extract of *A. africana*.

Table 9: Chemical compound contained in the most active plants extracts.

Plant species / Chimical compounds	*A. africana*		*A. conyzoides*		*A. hispidum*		*E. floribundus*		*E. prosrata*	
	ATE	**EF70 %**	**ATE**	**EF70 %**	**ATE**	**EF70 %**	**ATE**	**EF70 %**	**ATE**	**EF70 %**
Alkaloids	-	±	+	+	-	-	+	+	+	±
Polyphenols	+	-	+	+	+	-	+	-	+	+
Tanins catechiques	+	±	-	-	+	±	+	+	±	+
Tanins gallic	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+	+
Terpenes	+	+	+	+	+	+	+	+	+	+
Saponins	+	-	+	-	+	-	+	-	-	-
Sterols et steroids	+	+	+	+	+	+	+	+	+	+
Coumarins	-	+	-	-	-	+	-	+	-	-
Quinones	-	+	-	-	-	+	-	-	-	-

- : negative reaction; **±:** rough reaction; **+**: positive reaction; **ATE:** Aqueous Total Extract; **EF70 %**: Ethanolic Fraction 70 %

4. DISCUSSION

In this study, we note a high proportion of therophytes (44.45 %) and species common to the Guineo-Congolese region and the Sudano-Zambian region (GC-SZ) (60 %) among the studied Asteraceae. According to [18], therophytes represent a large proportion of herbaceous Asteraceae. Herbs account for more than 75 % of medicinal plants traditionally used in primary health care coverage in Africa and particularly in Côte d'Ivoire [19]. According to [20], the massive use of herbs is due, on the one hand, to the fact that these plants are frequently found in the immediate environment of users and, on the other hand, by the easy access to different organs.

Antimicrobial investigations have found that 10 of the 20 crude extracts obtained from Asteraceae marketed in the district of Abidjan have been identified as having *in vitro* antibacterial activity. These plants are locally used in primary health care and especially for the treatment of various bacterial diseases. These results are consistent with herbalists' statements about the traditional uses of these plants. The most active extracts are those of *Eclipta prostrata*, *Acanthospermum hispidum*, *Aspilia africana*, *Ageratum conyzoides* and *Erigeron floribundus*. In view of test results, bacteria are sensitive to plant extracts in a dose-response relationship, but with a lower sensitivity for *P. aeruginosa*. For the aqueous extracts, the highest activity was obtained with that of *A. africana*. On the other hand, at the level of ethanolic fractions, the best activity was obtained with *A. hispidum* extract. It follows from these results that the inhibitory activity of plant extracts observed on *P. aeruginosa* strains is low compared to that induced by antibiotics. However, these extracts could exert a better antibacterial activity to the extent that they are not pure products, but crude extracts [21].

According obtained MIC results, all the extracts are active to different degrees. This is related to the contents of extracts in substances with antimicrobial activity.

[22] have shown that the aqueous extract of *A. hispidum* does not show any antibacterial activity on *S. aureus*. On the other hand, its crude ethanolic extract and its ethanolic fraction gave a lower activity to obtained one. This difference in activity can be justified by the methods used because it is the cup method that is used in place of the impregnated blotting disc method. According to [12], all the quantity of extract diffuses in the case of the cups whereas a part is retained in the case of the blots discs. However, the work done by [23] on *S. aureus* with hydro-ethanolic extracts of *Pyrenacantha staudtii* and *Harrissonia abyssinica* gave significantly lower inhibition diameters

than those obtained with the ethanolic extracts of *E. prostrata* and *A. hispidum*. Bako *et al*. (2014) by their work have shown that the methanolic extract of *A. africana* flowers has activity on *S. aureus*.

It follows from the results of the different ratios (CMB/CMI) that, unlike the aqueous *A. africana* and ethanolic *A. hipidum* extracts, which showed a bacteriostatic effect on *S. aureus*, the extract EF70 % of *E. prostrata* and *Erigeron floribundus*, exerted a bactericidal power. The difference in antibacterial activity between these extracts can be explained by the fact that they come from different species despite their belonging to the same botanical family. This analysis shows that ethanolic extracts have the best activity on *S. aureus*. Ethanol, an organic solvent, thus improved the antibacterial activity of the aqueous extracts of *E. prostrata* and *A. hispidum*, on the studied *S. aureus* germs by a better concentration of the chemical constituents. Thus, the phytochemical screening carried out, has shown the presence of numerous chemical groups such as polyphenols, tannins, flavonoids, terpenes, sterols, steroids, saponins, coumarins and alkaloids.

Flavonoid, their presence has been identified in all extracts. This could give them anti-inflammatory properties [25]. Given the pharmacological interest of flavonoids, many studies seem to indicate that they have anti-oxidant, anti-HIV, antiviral, antibacterial and antiallergic properties [26].

Terpenes, sterols and steroids were also detected in all the studied extracts. The presence of these chemical compounds gives the plants an antipyretic and antifungal property [27]. They also help to fight against inflammations. Thus, many authors report the presence of terpene compounds in the organs of *A. hispidum* and in the leaves of *A. Africana* [28, 29]. This observation has just been confirmed in this work, and that in agreement with the traditional use of these Asteraceae in the treatment of dermatoses, gastroenteritis and swelling. The industrial use and the therapeutic interest of the triterpenes and steroids represent a capital stake in the field of the research of the natural substances [30].

This work, performing a microbiological and chemical screening, has a great importance. They contribute to the orientation of populations towards plants with a real healing power, thus offering them low-cost remedies at a time when financial realities no longer make antibiotics sold in pharmacies accessible to all. However, one must be careful about the use of some medicinal plants as they can be very toxic.

5. CONCLUSION

This study demonstrates that many of the ten Asteraceae commonly used by the local population and traditional healers in the Abidjan district for bacterial diseases showed antibacterial activity when tested *in vitro* in laboratory. The presence of many secondary metabolites in the different extracts of these plants could justify this activity. This work also shows that plants considered as bad weeds can contain very interesting therapeutic molecules. The results of this study indicate that some of these plants could become a source of new antibacterial agents. Further phytochemical researcher on the most active plants is planned to identify and characterize the active principles and to evaluate the toxicity by laboratory tests.

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6. REFERENCES

[1] OMS. Maladies infectieuses en Afrique. Situation et perspectives d'action. 7ème Réunion du forum pour le partenariat avec l'Afrique. Moscou, Russie. 2006 ; 19 p.
[2] Guessennd KN, Gbonon VC, Tiékoura KB, Kakou-N'douba A, Ouattara DN, Boni- Cissé C, Dosso M. Le GER-BMR : Evolution de la résistance bactérienne à l'imipénème en Côte d'Ivoire de 2005 à 2009. Colloque scientifique de l'Institut Pasteur de Côte d'Ivoire: pathologies émergentes et biologie intégrative. 2009 ; p.17
[3] Konan A. Place de la médecine traditionnelle dans les soins de santé primaires à Abidjan (Côte d'Ivoire). Thèse de Doctorat d'Etat de médecine, Université Toulouse III Paul Sabatier et Université Henri Poincaré Nancy 1. 2012 ; 96 p.
[4] Akoua KC, Guessend N, Gbonon V, Fayekette AYH, Dosso M. Methicilliniresistant of *S. aureus* activity in Abidjan (1998-2001): A new hospital problem. *Medecines Maladies Infectieuses*. 2004 ; 34 (3) : 132-136. Doi : 10.1016/j.medmal.2003.12.001
[5] Guillemot D, Maugendre P, Vhauvin STC.. Consommation des antibiotiques en France. *BEH*. 2004 ; 3233 :141-147.
[6] Konan KF, Guessennd KN, Oussou KR, Bahi C, Coulibaly A, Djaman AJ, Dosso M. Effet antibactérien de l'extrait aqueux de l'écorce de *Terminalia glaucescens* Planch ex Benth (Combretaceae) sur la croissance *in vitro* des entérobactéries productrices de bêtalactamases à spectre élargi (EBLSE). *Int. J. Biol. Chem. Sci*. 2014 ; 8(3) : 1192-1201. DOI: http://dx.doi.org/10.4314/ijbcs.v8i3.30
[7] Yapi AB, Kassi NJ, Fofié NBY, Zirihi GN. Etude ethnobotanique des Asteraceae médicinales vendues sur les marches du district autonome d'Abidjan (Côte d'Ivoire). *Int. J. Biol. Chem. Sci*. 2015 ; 9(6) : 2633-2647. Available on : https ://dx.doi.org/10.4314/ijbcs.v9i6.10

[8] Zirihi GN, Kra AKM, Dibie ET. Etude botanique et évaluation des activités antifongiques de *Mitracarpus villosus* (MV) (Rubiaceae) et *Spermacoce verticillota* (SV) (Rubiaceae) sur la croissance *in vitro* de *A. fumigatus*. *Rev. Med. Pharm. Afr*. 2007 ; 9-17.
[9] Kouamé D. Travaux Pratiques de Microbiologie alimentaire (BCH 44), Abidjan (Côte d'Ivoire). 2005 ; 60 p.
[10] Boffi EAE. Traitement et pronostic des bactériémies à *Pseudomonas aeruginosa*. Thèse de Doctorat en médecine. Université de Genève. 2004 ; 43 p.
[11] Dosso M, Faye-Kette H. Contrôle de qualité de l'antibiogramme en pratique courante : Expérience du laboratoire de bactériologie de l'Institut Pasteur de Cote d'Ivoire. *Bactériolo. Int*. 2000 ; nº spécial, 53 p.
[12] Kamanzi AK. Plantes médicinales de Côte d'Ivoire : Investigations phytochimiques guidées par des essais biologiques. Thèse, Université de Cocody, Abidjan. 2002 ; p.176.
[13] Koné WM, Kamanzi AK, 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
[14] Méité S, Konan KF, Guessennd KN, Bahi C, Djaman A, Coulibaly A, Dosso M. Antioxidant and antibacterial activity of the extracts of stem bark of *Terminalia glaucescens* Planch Ex Benth (Combretaceae) on enterobacteriaceae Extended-Spectrum Beta-Lactamase Producing (ESBL). *Ijppr.Human*. 2015 ; 4(4) : 148-160. Available on: www.ijppr.humanjournals.com
[15] Ponce AG, Fritz R, Del AC, Roura SI. Antimicrobialactivity of essential oil on the native microfloraof organic Swisschard. *Lebensmittel-WissenschaftundTechnologic*. 2003 ; 36 : 679-684. Doi : 10.1016/S0023-6438(03)00088-4
[16] Fauchere LL, Avril JL. Bactériologie Générale et Médicale. Editions Ellipses : Paris. 2002 ; 122 p.
[17] Mangambu MJDD, Mushagalusa KF, Kadima NJ. Contribution à l'étude phytochimique de quelques plantes médicinales antidiabétiques de la ville de Bukavu et ses environs (Sud-Kivu, R. D. Congo). *Journal of Applied Biosciences*. 2014 ; 75 : 6211-6220. Available on : http : //dx.doi.org/10.4314/jab.v75i1.7
[18] Aké-Assi L. Flore de la Côte d'Ivoire : étude descriptive et biogéographique avec quelques notes ethnobotaniques. Thèse de Doctorat Es-Sciences Naturelles. Université d'Abidjan, F.A.S.T. ; 1984. 176 p (Tome IA).
[19] Ake-Assi L. Abrégé de médecine et pharmacopées africaines : quelques plantes employées traditionnellement dans la couverture des soins de santé primaire. Edition NEI/CEDA (Côte d'Ivoire). 2011 ; 157 p.
[20] N'Guessan K, Kadja B, Zirihi GN, Traoré D, Aké-Assi L. Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte d'Ivoire). *Sciences & Nature*. 2009 ; 6 (1) : 1-15. Available on : https ://dx.doi.org/10.4314/scinat.v6i1.48575
[21] Sanogo R, Diallo D, Diarra S, Ekoumou C, Bougoudougou F. Activité antibactérienne et antalgique des deux recettes traditionnelles utilisées dans le traitement des infections urinaires et la cystite au Mali. *Mali Medical*. 2006 ; 1 : 18-24.
[22] Fleischer TC, Ameade EP, Sawer IK. Antimicrobial activity of the leaves and flowering tops of *Acanthospermum hispidum*. *Fitoterapia*. 2003 ; 74 : 130-132. Doi:10.1016/S0367-326X(02)00290-3
[23] Biyiti LF, Meko'o DJL, Tamze V, Amvam ZPH. Recherche de l'Activité Antibactérienne de Quatre Plantes Médicinales Camerounaises. *Pharmarcologie et Médecine Traditionnelle Africaine*. 2004 ; 13 : 11-20.
[24] Bako SS, Usman OE, Justina MI, Ngbede OP, Lajide L. Antimicrobial activity and Chemical Composition of the flowers of *Aspilia africana*. *Advances in Life Science and Technology*. 2014 ; 16 : 54-57.
[25] Read MA. Flavonoids : naturally occurring anti-inflammatory agents Vascular. *American Journal of Pathology*. 1995 ; 147(2) : 235-237.
[26] Van DBR, Haenen G, Van DBH, Van DVW, Bast A. The predictive value of antioxidant capacity of structurally related flavonoids using the Trolox equivalent antioxidant capacity (TEAC) assay. *Food Chemistry*. 2000 ; 70 : 391. DOI: 10.1016/S0308-8146(00)00092-3
[27] Cowan MM. Plants products as antimicrobial agents. *Clinical Microbiology Reviews*. 1999 ; 12 : 564-582.
[28] Harekrishna R, Anup C, Satyabrata B, Bhabani SN, Sruti RM, Ellaiah P. Preliminary phytochemical investigation and anthelmintic activity of *Acanthospermum hispidum* DC. *Journal of Pharmaceutical Science and Technology*. 2010 ; 2(5) : 217-221.
[29] Usman LA, Oladosu IA, Olawore NO, Fakunle CO, Hamid AA, Lawal A. Chemical composition of leaf oil of Nigerian grown *Aspilia africana* C.D. Adams. *Journal of Medicinal Plants Research*. 2010 ; 4 (4) : 346-348. Available on : Available on : http: //www.academicjournals.org/JMPR
[30] Bruneton J. Pharmacognosie, Phytochimie, Plantes médicinales. Lavoisier Technique et Documentation, 3ème Ed. Lavoisier. Paris, France. 1999 ; 1120 p.

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