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# Ethnobotanical study, antibacterial activity evaluation of the extracts of leaves *Aspilia africana* (Pers.) Adams var. *africana* (Asteraceae) on Multi-Resistant *Staphylococcus aureus* strains and phytochemical sorting.

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**Abstract**

*Aspilia africana* is a commonly used plant in traditional medicine in the treatment of many diseases. Bacterial resistance revealed the ineffectiveness of some conventional antibiotics against several diseases. The objective of this work was to evaluate the antibacterial activity of the crude extracts of leaves of *A. Africana* on *Staphylococcus aureus* strains Meti-R. The solid medium dilution method using Mueller-Hinton® agar was used to evaluate the extracts antibacterial activity. For the phytochemical sorting, tube characterization method was used. The results obtained show that the total aqueous extract of *A.africana* is bacteriostatic on all bacterial strains tested with inhibition diameters of 12.3 ± 0.6 to 18.3 ± 0.5 mm. The studied extracts principally contain secondary metabolites. This work provides a scientific basis for the traditional use of *Aspilia africana* especially in the treatment of bacterial diseases.

**Keywords**

Antibacterial, *Aspilia africana*, traditional pharmacopeia, medicinal plant, phytochimical sorting, *Staphylococcus aureus* Méti-R

## Introduction

In developing countries, infectious diseases are a major public health concern because of their frequency and severity (Bourgeois, 1999). Infectious diseases are responsible for over 17 million deaths per year worldwide of which more than half comes from African continent alone (OMS, 2006).There is in the therapeutic arsenal, drugs that control these diseases. Nowadays, these drugs are no longer evidence of their effectiveness due to resistance developed by these pathogens (Aubrey, 2015). Various germs are responsible of these diseases as it happens pathogenic bacteria. In many countries of sub-Saharan Africa as in Ivory Coast, many cases of multi-resistant bacteria have been reported (Akoua-Koffi *et al*., 2004;

Akinyemi *et al*., 2005; Guessennd *et al*., 2008). That is why it was declared the thirtieth of April 2014 under the aegis of the World Health Organization (OMS), that antibiotic resistance was no longer a threat, but a reality (OMS, 2014). Moreover, because of their high costs, these pharmaceutical specialties are no longer accessible. Faced with this situation, people are turning towards the plants of their pharmacopoeia. It is therefore urgent to initiate multidisciplinary scientific research in order to obtain a real benefit from the use of medicinal plants. To respond to this worry as the example of researchers around the world, our laboratories are oriented towards research of new plant based therapies that would be effective against bacterial infections, cheaper and accessible to all. So after ethnobotanical survey, the plant species *Aspilia africana*, medicinal Asteraceae, was selected to evaluate its antibacterial activity. Also, many chemical, pharmacological and toxicological research work were conducted by researchers to verify and justify the traditional use of the plant. Thus, *A. africana* has been studied for its antimicrobial (Boka *et al*., 2014), anti-ulcer (Ubaka *et al*., 2010), anti-inflammatory (Okoli *et al*., 2007), parasitic (Waako *et al*., 2005;Chono *et al*., 2009 ; Akuodor *et al*., 2012), reproductive (Oyesola *et al*., 2010) activities. This plant has also been the subject of chemical (Usman *et al*., 2010;Boka*et al*., 2014) and toxicological (Oluyemi *et al*., 2007;Oko *et al*., 2011) study. For this study, the interest was focused on the antibacterial properties and selected biological target is methicillin-resistant *Staphylococcus aureus* (SARM). It is a multiresistant bacteria to antibiotics (BMR) frequently found in sub-Saharan area and has a broad clinical impact. It is involved in various diseases and often responsible for nosocomial infections (Mesaros *et al*., 2007;Mirabaud, 2003). These reasons justify the choice of this bacterial strain to evaluate the antibacterial extracts of the leaves of this plant and to carry out a phytochemical sorting.

## Materials and Methods

## Material

### Vegetal material

The plant material is the leaves of *Aspilia africana* (Pers.) D.C. Adams var. *africana*, one Asteraceae collected in the autonomous Abidjanin August2014. The identification was performed at the National Floristic Centre(CNF) from the University Félix Houphouët-BoignyAbidjan-Cocody where a sample is preserved.

### Bacterial strain

Made up of a reference strain (ATCC 25923) and five multiresistant strains of *Staphylococcus aureus* (MRSA)obtained from biological products (Table I). They are 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 (Kouamé, 2005). 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 Cote d'Ivoire (IPCI).

**Table I:**List of strains studied

| Strains | Codes | Profile | Biological products |
|---|---|---|---|
| *Staphylococcus aureus* | ATCC 25923 | Sensitive to methicillin | - |
| | 680Y/14 | Methicillin resistant | Suppuration |
| | 583C/14 | Methicillin resistant | Suppuration |
| | 1325Y/14 | Methicillin resistant | Suppuration |
| | 590Y/14 | Methicillin resistant | Blood |
| | 1690C/14 | Methicillin resistant | Blood |

## Methods

### Botanical description and traditional utilization of this plant

The botanical description hang into account: the general appearance of the plant; the size, shape, and arrangement of the leaves; the type and arrangement of inflorescences on the stem, the section of the stem; the type, shape and appearance of the fruit. As for the traditional use of this plant, it will focus on its use in traditional medicine.

### Preparation of plant extracts

The leaves of this species were dried in the Laboratory for two weeks and powder using an electric grinder type IKA Labortechnik (MFC type).

**Preparation of total aqueous extract (ETA)** : the preparation of this extracts was performed using the method described by Zirihi *et al*. (2007) which consists in macerating 100 g of plant powder of species in 1L of sterile distilled water using a blender Blinder type 7 SEVEN STAR. The homogenate were filtered over hydrophilic cotton and then on filter paper Whatman 3 mm. The aqueous filtrate thus obtained are evaporated in an oven type Med Center Venticell at 50°C to obtain powders that constitute the total aqueous extract (ETA).

**Preparationof70 % ethanolic fraction (FE70 %) and aqueous residual fraction (FRA)** : these fractions were obtained by dissolving 5 g of ETA 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 FE70 % extract (FE70 %). Likewise the aqueous residual deposit was collected and evaporated in an oven at 50 °C. The obtained constitutes the FRA extract.

### 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 :

$$Yd = (m \times 100) / M.$$

(Yd : Extraction yield in percentage ; m : mass in grams of the dry extract ; M : mass in grams of the drug powder).

### Sterility test of extracts

This test aimed to verify that the extract didn't contain any bacteria and fungus. For this, 0.1 g of the extract to be tested was placed in 10 mL of thioglycholate broth and incubated at 37 °C for 24 h. After that, the turbidity of the broth is appreciated with eyes. The broth was then plated on a Petri dish containing nutrient agar and another containing Sabouraud agar and incubated under the same conditions for three days with an observation every 24 hours to check whether any germs have grown in boxes dish. The substance is called sterile if no colonies are visible on the agar box.

### Antibacterial study

### Preparation of the *inoculums* to solid culture medium test

The inoculum was prepared from a young colony of 24 hours. It was emulsified in 2 mL of 85% NaCl suspension. Then the optical density was adjusted to 0.5 McFarland using a densimat. A volume of 1000 µL of the resulting solution was introduced into 10 mL of physiological water (0.9% NaCl) and constituting the bacterial inoculum estimated at $10^6$ bacteria / mL.

### Activity test

This test was performed by the solid medium diffusion method (Dosso and Faye-Kette, 2000;Kon*éet al*., 2004). To do this, a solution with a concentration of 100 mg / mL of extract was prepared. Petri plates containing Mueller-Hinton agar were cultured by swabbing with the prepared inoculum. Then the wells were dug by pushing the butt end of a Pasteur pipette into the agar and filled with 50 µL of the solution of prepared extracts. The set was incubated at 37 ºC for 24 hours. After that, the diameter of inhibition around each well was measured using a caliper. The assessment of extracts activity was made according to the criterion of Ponce *et al*. (2003). Thus, a bacterial strain is said to be non-responsive to a given substance if the diameter of inhibition of the substance is less than 8 mm then it is said to be sensitive if the diameter is between 9 mm and 14 mm. On the other hand, the

bacterial strain is considered to be very sensitive when the diameter of inhibition of substance is between 15 mm and 19 mm and extremely sensitive when the diameter exceeds 20 mm. The activity test allowed the selection of the most active extracts for the determination of antibacterial parameters. And extracts the diameters of inhibition higher than 10 mm were retained (Ponce *et al*., 2003).

## Preparation of the inoculums to liquid culture medium test

Two bacterial colonies of 24 hours were taken using a Pasteur pipette and emulsified in a test tube containing 10 mL of sterile Muller-Hinton broth. The mixture was incubated at 37 °C for three hours. After this incubation, a suspension of 0.3 mL of this pre-culture was taken and diluted in 10 mL of sterile Mueller-Hinton broth then homogenized.

## Preparation of the concentration rang of plant extracts

A solution of concentration of 100 mg/mL of extracts obtained was prepared. The method of half dilution, was performed from this solution in order to obtain, ranges of concentrations from 100 mg/mL to 3.156 mg/mL.

## Determination of the antibacterial parameters

Determination of antibacterial parameters was carried out by the liquid medium dilution according to the method used by Konan *et al*. (2013). Thus, in 10 experimental tubes hemolysis, 1 mL of each concentration range of plant extract was contacted with 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 only received 2 mL of sterile Mueller-Hinton Broth (BMH). The tubes were incubated for 24 hours at 37 °C. After this incubation time, an observation with the naked eye was performed and the lowest concentration for which no bacterial growth was observed with the naked eye is the minimum inhibitory concentration (MIC). As for the minimal bactericidal concentration (MBC) which is the concentration of a substance for obtaining, after 24 hours incubation at 37 °C, 0.01% of viable bacteria. His determination started with numeration. This was to dilute the initial inoculum from $10^{-1}$ to $10^{-4}$ and inoculate these dilutions using a calibrated loop of 2 µL streak of 5 cm long, on Mueller Agar -Hinton (GMH) then incubated for 24 hours. These petri dishes were named A. After reading the MIC, the contents of the tubes in which there was no visible growth was used to inoculate the Mueller-Hinton agar streaks on 5 cm. This series of petri dishes is named B. The MBC was determined by comparing bacterial growth boxes A and B. Thus, the lowest concentration of the tube that has less than 0.01% of viable bacteria compared to the initial *inoculum* is MBC. The MBC toMIC clarified the mode of action of a substance (Fauchere and April, 2002). If the MBC to MIC is less than or equal to 2, the substance is called bactericide. On the other hand, if it is higher than 2, the substance is classified as bacteriostatic.

## Phytochimical sorting

The identification of different chemical compounds in the extracts was done by tubes characterization reactions. This method consists in detecting the different families of chemical compounds that may exist in plant extracts on the basis of characteristic colorations or precipitation reactions.

## Alkaloids characterization

The characterization of alkaloids was made from Bouchard (iodo-iodide) and Dragendorff (tetraiodo potassium bismuthate)reagent. 6 mL of each plant extract solution was evaporated to dryness. The residue was taken up in 6 mL of alcohol at 60 °C. The filtrate thus obtained was divided into two test tubes. In the first tube, two drops Dragendorffreagent were added. The presence of alkaloids was characterized by observing orange-coloured precipitates. In the second tube, there was added two drops of Bouchard reagents. The appearance of a reddish-brown color indicates the presence of alkaloids. A control test was made with quinine.

## Characterization of polyphenols

The polyphenols colorimetry forms colored precipitates with a solution of ferric chloride (FeCl3). Thus, in 2 mL of solution of each plant extract was added one drop of alcoholic solution of ferric chloride 2%. The formation of blue-black or green colouring more or less dark testifies to the presence of polyphenols. A control test was performed with a solution of phenol.

### Characterization of flavonoids

Flavonoids have been characterized by the reaction to cyanidin. Thus, 2 mL of each plant extract were evaporated to the dry sand bath. The residue thus obtained was taken up in 5 mL dilute hydrochloric alcohol 2 times. The mixture was collected in a test tube, in which 3 meta chip of magnesium were added pink-orange or violet. The addition of 3 drops of isoamyl alcohol intensifies this coloring and confirms presence of flavonoids. An alcoholic solution of quercetin was used as a control.

### Tannins characterization

The Stiasny reagent (Formalin 30%, concentrated HCl 1/0.5) helped to distinguish the catechin tannins (by precipitation) of gallic tannins (by saturation).

Tannins cathéchiques: 10 mg of each plant extract, were added 10 mL of Stiasny reagent. The mixture was heated in a water bath at 80 °C for 30 minutes. After cooling in a stream of water, observation of precipitate in the form of clear-brown flakes characterizes catechin tannins. An alcoholic solution of catechin was used as a control.

Gallic tannins: For this test, the filtrate obtained from the reaction of catechol tannins characterization was saturated with sodium acetate. To this mixture was added a few drops of a dilute aqueous solution of $FeCl_3$ at 1% (approximately 1 mL). The appearance of an intense blue-black coloration indicates the presence of gallic tannins not precipitated by Stiasny reagent. An alcoholic solution of gallic acid was used as a control.

### Terpenes characterization

Sterols and terpenes characterizationwas made by the Liebermann-Burchard reaction. To 0.2 g of each plant extract, were added 5 mL of ethyl ether, then the mixture was macerated for 30 minutes. The solution obtained after the maceration was filtered and then evaporated to dryness. The residue was then dissolved in 0.5 mL of acetic anhydride.

Using a pipette, 2 mL of concentrated sulfuric acid were laid down at the bottom of the test tube without stirring. The appearance of brownish red or purple ring reflects the two liquid contact zone. The upper liquid turns green or purple to green or purple indicates the presence of sterols and terpenes. A control test was performed with progesterone.

### Coumarins characterization

For the detection coumarins, 2 mg of each plant extract was added to 2 mL of warm water and then homogenized. The homogenate thus obtained was divided into two test tubes. Thereafter, 0.5 mL of diluted ammonia at 25% was added to the contents of one of the tubes. After observation under UV 365 nm, the presence of fluorescence in the tube where it was added ammoniac indicates the presence of coumarins.

### Saponins characterization

For the detection of saponins, it was introduced 10 mL of each plant extract in the test tubes. Each tube were strongly stirred in a vertical position for 15 seconds, and thenleft to set 15 minutes. The height of persistent foam is higher than 1 cm, testifies to the presence of saponins.

## Results and Discussion

## Results

### Ethnobotanical study

The plant species studied is qualified weed. It is common to the Guineo-Congolese region and the Sudano-Zambian region. The general appearance of this plant is presented in figure 1 and in table II are summarized the morphological details description of the different organs. Table IIIare summarized some therapeutic uses of *Aspilia africana* in traditional african medicine. *Aspilia africana*,is calledzeu-nanhinAkye(Southern ethnic group of Côte d'Ivoire) and Soumadibrouin Malinke(Northern ethnic group of Côte d'Ivoire). It growsin cultivated fields. It is also found in fallow, especially in the forest area.


**A :**drawing **B :** photo of ground

**Figure 1 :**General appearance of the leaves and flower stalks of *Aspilia africana*

**Table II :**Description of the plant

| **Plant species** / **Described parts** | ***Aspilia africana* (Pers.) C.D. Adams var *africana*** |
|---|---|
| General appearanceof the plant | Hardy herb withramification<br>Stem with regular section, 60 cmto 1.5m high |
| Section of the stem | Circular |
| Size of the leaves | Length : 6 to 15 cm ; width : 3 to 7 cm |
| Form of the Leaves | Oval-lanceolate |
| Arrangementof the leaves | Opposite |
| Types of inflorescences | Terminals andsolitarycapitules<br>Colorof the floret : bright yellow |
| Type of fruit | Akènes |
| Fruit size | Length about5 cm ; width : 0,5 to 2 mm |
| Appearance fruit | Quadrangularand covered withhard and thin hairs |

**Table III :**Some uses of *Aspilia africana* in traditional african medicine

| Diseases, Symptoms | Parts of the plant used | Methods of préparation | Medicinal forms | Methods of administration | Way of administration |
|---|---|---|---|---|---|
| Whitlow | Leaves | Kneading | Paste | Dressing | Externway |
| Furuncle | Leaves | Softening | Juice | Massage | Externway |
| Shingles | Leaves | Toasting, Spray | Powder | Locale appl | Externway |
| Dermics spots | Leaves | Kneading | Paste | Locale appl | Externway |
| Surface large wound | Leaves | Décoction/ Kneading | Décocté/Paste | Locale appl. / Dressing | Externway |
| Eyepains | Youngfreshleaves | Expression | Juice | Ocular instillation | Ocular tract |
| Cough | Freshleaves | Softening+ expression | Juice | Drink | Orally |
| Stomachpains | RFF | Décoction/ Kneading | Décocté/Paste | Drink/Purge | Orally/Anal tract |
| Paludism | RF | Décoction | Décocté | Bath | Externway |
| Infantilediarrhoea | Youngfreshleaves | Kneading | Paste | Purge | Anal tract |

Appl : application ; RF : branch with leaves ; RFF : branch with leaves and flowers

## Yield for extracts

Maceration with distilled water the leaves of *A. Africana* gives a blackish powder 11 g (ETA) is a yield of 11% per 100 g of drugs. The partition of 5 g of ETA dissolved in 100 mL of ethanol 70% solution gives 1.44 g of FE70% representing a yield of 28.8% and 3.10 g of FRA is a yield of 62%.

## Antibacterial activity

The total aqueous extract is active against all studied strains with diameters inhibition between 12.3 ± 0.6 and 18.3 ± 0.5 mm. The 70% ethanol extract is active only on the reference strain with andiameter inhibition of 10.3 ± 0.1 (Table IV). Besides the reference strain, the total aqueous extract is more active on the strain 1690C / 14 (Figure 2) and 583C / 14 which are *S. aureus* isolates resistant to methicillin with cross-resistant to fluoroquinolones. Conversely, the residual aqueous extract had no activity studied strains. These first two extracts were then extracts that have been used to determine antibacterial parameters. As regards antibacterial parameters, turbidity induced by the growth of decreased bacteria inversely with the concentration of extracts into the wells of the microplate. It emerged from this experience that the total aqueous extract has minimal inhibitory concentrations (MIC) ranging from 6.25 mg/mL and 25 mg/mL, while the 70% ethanol extract has an MIC of 25 mg/mL for the reference strain. As for the minimum bactericidal concentrations (MBC), it ranges from 50 mg / ml to more than 100 mg/mL for aqueous total extract. For the reference strain, 70% ethanol extract has a MBC 50 mg / mL (Table V). The total aqueous extract of leaves of *Aspiliaafricana* var. *africana* is bacteriostatic 100% of strains tested, while 70% ethanolic is bactericidal against the standard strain extract.

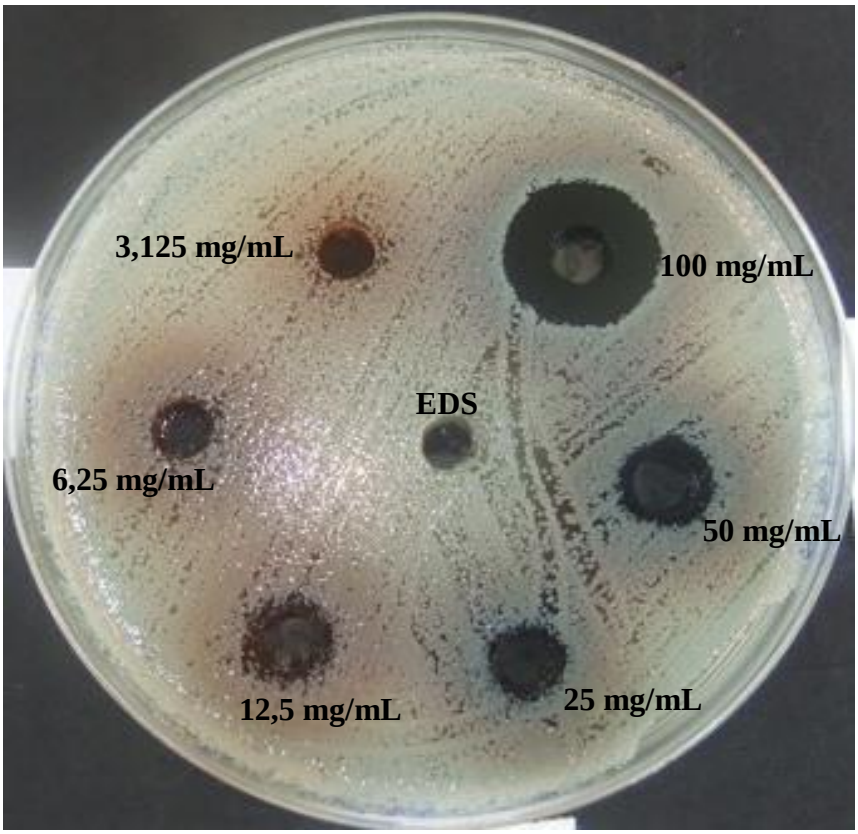


**Figure 2 :**Effect dose-response of aqueous concentrations of *Aspilia Africana* on *Staphylococcus aureus* Méti-R (SARM) 1690C/14

(EDS : sterile distilled water)

**Table IV :**Diameters of the zones of inhibition obtained with the extracts of leaves *A. africana* var. *Africana* and of antibiotics on strains of SARM and reference strain

| Bacterium | Codes | Diameter of inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | ETA | FE70 % | FRA | EDS | FOX |
| *S. aureus* | 680Y/14 | 12,3±0,6 | 6,0±0,0 | 6,0±0,0 | 6±0,0 | 21,3±0,3 |
| | 583C/14 | 16,1±0,3 | 6,0±0,0 | 6,0±0,0 | 6±0,0 | 20,1±0,6 |
| | 1325Y/14 | 15,3±0,2 | 6,0±0,0 | 6,0±0,0 | 6±0,0 | 17,0±0,1 |
| | 590Y/14 | 15,1±0,5 | 6,0±0,0 | 6,0±0,0 | 6±0,0 | 21,3±0,3 |
| | 1690C/14 | 17,0±0,6 | 6,0±0,0 | 6,0±0,0 | 6±0,0 | 11,1±0,2 |
| | ATCC25923 | 18,3±0,5 | 10,3±0,1 | 6,0±0,0 | 6±0,0 | 19,3±0,3 |

Included diameter of the wells (6 mm), ETA : total aqueous extract ; FE70 % :70%ethanolicextract ;FRA :aqueous residual extract ;EDS : sterile distilled water(control) ; ATCC : American Type Culture Collection ; FOX :Céfoxitine.

**Tableau V :**Antibactérial parameters of the extracts of leaves of *A. africana* var. *Africana* on strain of *S. aureus*(SARM) and the strain of reference

| Bacteria | Extract ETA (mg/mL) | | | | Extract FE70 % (mg/mL) | | | | FOX µg/mL |
|---|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | MBC/MIC | Pv | MIC | MBC | MBC/MIC | Pv | MIC |
| 690Y/14 | 25 | >100 | >4 | bt | 25 | 50 | 2 | bc | MIC>32 |
| 583C/14 | 6,25 | 50 | 8 | bt | nd | nd | nd | nd | MIC>32 |
| 1325Y/14 | 12,5 | 100 | 8 | bt | nd | nd | nd | nd | MIC>32 |
| 590Y/14 | 12,5 | >100 | >8 | bt | nd | nd | nd | nd | MIC>32 |
| 1690C/14 | 12,5 | 100 | 8 | bt | nd | nd | nd | nd | MIC>32 |
| ATCC25923 | 25 | >100 | >4 | bt | nd | nd | nd | nd | MIC<32 |

MIC : Minimum Inhibitory Concentration ; MBC : Minimal Bactericidal Concentration ; Pv : power ; bc : Bactericidal ; bt : Bacteriostatic ; FOX : Céfoxitine ; nd : not détermined.

### Phytochimical sorting

The phytochemical sorting performed with the extracts of leaves of *A. Africana* allowed to detect the presence of various chemical groups (Table VI). They are the polyphenols, catechin tannins, flavonoids, terpenes,saponins, sterols and steroids, coumarins and quinones in both 70%, aqueous extracts and ethanol. Except for two extracts, alkaloids were detected only in the 70% ethanol extract as trace.

**Tableau VI :** Chemical compounds contained in the leaves of *A. africana* var. *africana*

| Chimical compounds | Extracts | | |
|---|---|---|---|
| | ETA | FE70 % | FRA |
| Alkaloids | - | ± | - |
| Polyphénols | ++ | - | ++ |
| Tanins catéchiques | ++ | ± | ++ |
| Tanins galliques | - | - | - |
| Flavonoïdes | + | ++ | + |
| Terpènes | +++ | ++ | +++ |
| Saponosides | +++ | - | ++ |
| Stérols et stéroïdes | ++ | ++ | + |
| Coumarines | - | ++ | - |
| Quinones | - | ++ | - |

- :negative reaction ; ± : rough reaction ; + : positive reaction

## Discussion

Analysis of the results of ethnobotanical study revealed that *A. africana* is a plant with many therapeutic properties. It is frequently used in the treatment of dermatoses. N'Guessan (2008) also showed that *A. africana* is traditionally used to treat skin diseases. This study aimed to evaluate the antibacterial activity of 70 % ethanol extract and total aqueous extract of the leavesof *A.africana* var. *africana* on the in vitro growth of multiresistant bacteria.This study showed that the total aqueous extract was bacteriostatic active and 100% of tested strains while the ethanol extract was active and bactericidal only on the reference strain ATCC 25923 tested. The residual aqueous extract showed no activity on strain that is to say there is not a difference between the activity of the extract and the negative control that is sterile distilled water. This lack of activity observed for the residual aqueous extract and ethanol extract reveal that the active compounds in these extracts would act in synergy.

Total aqueous extract of *A.africana* shows inhibitory activity on the in vitro growth of the studied bacteria. However, the 1690C/14 strains, 583C/14 as well as the reference strain ATCC25923, the totalaqueous extract is very active total that70%ethanol extract. This could be justified by a synergy between an extractable compound in water and another extractable in ethanol (Kouadio, 2013).

The total aqueous extract of this plant is bacteriostatic on most studied strains with MICs between 6.25 mg/mL and 25 mg / mL. Bako *et al* (2014) had also shown that the methanol extract of *A.africana* has an inhibitory activity on *S. aureus*. However, the MIC values obtained in this work are clearly superior. This difference can be justified by the plant parts used and the method because it's the cups method that was used in place of the disk impregnated blotting method. According to Kamanzi (2002), the whole amount of extract diffuse in the case of wells while a portion is retained in the case blotters discs.

Traditionally, this plant is used in the treatment of various bacterial diseases such as paronychia, furuncle and urinary diarrhea (N'Guessan, 2008 ;Yapi*et al*, 2015). Similarly, *A. africana* is used in traditional medicine against large wound (ulcer Burili). However, Konein (2001) showed that the *Staphylococcus aureus*are involved in the surinfectionin the disease.

In addition, the phytochemical screening performed on the different extracts of these plants revealed the presence of chemical groups such as tannins, flavonoids, terpenes, saponins and sterols, whose antibacterial properties are known (Morel, 2011 ;Daglia, 2012). Tannins for example, are known for their ability to inhibit the growth of many microorganisms including bacteria (Sepúlveda*et al*., 2011). In addition to this property, the tannins are endowed with astringent and healing power. This provides a scientific rationale for the use of this plant in the treatment of ulcer Burili. As for the flavonoids, they are endowed with antifungal and antibacterial properties. The biological properties of these compounds to justify the antimicrobial activities expressed in this study and to link these activities and the traditional use of *A. africana* in the treatment of bacterial diseases.

## Conclusion

This study allowed the multiple uses of *A. africana* var. *africana* in ivorian traditional medicine. This study also demonstrated antibacterial activity of leaves of *A. africana*on *Staphylococcus aureus* strains resistant to methicillin (SARM) and also to determine the MIC and MBC. This activity is primarily bacteriostatic in nature. Moreover, the groups of chemical compounds, probably responsible for this activity have been identified and are more concentrated in the distilled water. This work must continue in order to prove the safety of the plant and produce a bio-guided fractionation in order to isolate the molecules responsible for this biological activity.

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