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Ethnobotanical study, antistaphylococcic activity of the 70 % ethanolic extract of *Ecliptaprostrata* (L.) L leaves andcytotoxicitystudy on humancells HFF

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ABSTRACT: *Ecliptaprostrata* 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 extract of leaves of *E. prostrata* on *Staphylococcus aureus* strains Meti-R as well as its cytotoxic activity. The solid medium dilution method using Mueller-Hinton® agar was used to evaluate the extract antibacterial activity and the Mossman method was used to evaluate the toxicity test. The results obtained show that the 70 % ethanolic extract of *E. prostrata* is principally bactericidal on all bacterial strains tested with inhibition diameters of 10.0 to 17.3 mm. At the concentration of 1000 µg/mL, ethanolic extract of *E. prostrata*is not cytotoxic. This work provides a scientific basis for the traditional use of *Ecliptaprostrata*especially in the treatment of bacterial diseases.

KEYWORDS: **Ecliptaprostrata**, medicinal plant, anti bacterial activity, *Staphylococcusaureus*Méti-R,humancells HFF, toxicity

I. INTRODUCTION

The increased prevalence of antibiotic-resistant bacteria due to the extensive use of anti biotics may render the current antimicrobial agents inefficient to control some bacterial diseases[29-30]. In addition, Renaltoxicity presented by some modern drugs of references and the inaccessibility of thesedrugs in our population due to their high costs, are for developing country a real concern [29]. Herbal medicine is frequently a part of a larger therapeutic system such as traditional and folk medicine. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens[3]. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases. They can also be a possible source for new potentantibiotics to which pathogenstrains are not resistant.

Ecliptaprostrata(Astraceae)is a plant distributed in the tropical and subtropical regions of the world. The herb has been used in the treatment of snakevenompoisoning in Brazil[31] and infective hepatitis in India[32].It has been reported that the leaves of this herb are used in the case of gastritis and respiratory disorders like cough and asthma[33]. In addition, the crude form of the plant is reported to have anti-inflammatory, antimicrobial and anti hepatotoxic properties in India[34]. But thereis as yet no report concerning the antibacterial and cytotoxic activity of this plant in IvoryCoast.

Therefore, the present study is intended to evaluate the anti bacterial and cytotoxic activities of the ethanolic extract of leaves of *E. prostrata*.For this study, the interest was focused on the antibacterial properties and selected biological target is methicillin-resistant *Staphylococcus aureus* (SARM). It is multi-resistant bacteria to antibiotics (BMR)

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International Journal of Advanced Research in Science, Engineering and Technology
Vol. 5, Issue 3 , March 2018

frequently found in sub-Saharan area and has a broad clinical impact. It is involved in various diseases and often responsible for nosocomial infections [35]. These reasons justify the choice of this bacterial strain to evaluate the antibacterial extracts of the leaves of this plant.

II. RELATED WORK

The reciprocal relationship between human beings and the plant, for the rapeutic purposes, led to ethno botany[1]. And according to [2], ethno medicine is a discipline of ethno botany. For example, in many countries all over the world, medicine using plants plays a major role in health systems[3].In China, traditional herbal preparations account for 50% of total drug consumption[4] cited by [5].Moreover, if one refers to the estimates of [6] and [7], nearly 50% of the drugs prescribed in conventional medicine are of plant origin .This is the case of maprouneacin, [8] and artemisinin[9].For this reason, ethno botanical studies have proven to be one of the most reliable approaches to the discovery of new drugs. Therefore, several ethno botanical surveys have been conducted in various countries, particularly in Africa[10-11] and especially in Côte d'Ivoire [12-13].
Moreover, the assistance to repeated therapeutic failures despite the progress made by the industrialized countries, for the rise of modern medicine, atypical pathologies such as bacterial infections, lead to the search for multiple bacteria resistances.For example, in many countries in sub-SaharanAfrica, several studies have revealed cases of multidrug-resistant bacteria[14]. In Côte d'Ivoire, numerous cases of multi-resist antbacteria have also been reported[15-16].This is why, under the auspices of OMS, it was declared on April 30, 2014, that anti microbial resistance was no longer a threat, but a reality [17].Thus, many researches have been done everywhere in the world to find plants that can effectively treat bacterial infections. We have the works of [18] in France, those of [19] in Thailand. In Africa, for example, the work of [20] in Congo, those of [21] in Burkina Faso, those of [22] in Cameroon, those of [23] in Senegal.In Côte d'Ivoire, so many researches have been done. They are those of [24-25-26-27-28].

III. MATERIAL AND METHODS

A. MATERIAL

1. Vegetal material

The plant material is the leaves of *Ecliptaprostrata* (L.) L (Asteraceae), one Asteraceae collected in the autonomous district of Abidjanin August2015. The identification was performed at the NationalFloristicCentre(CNF) from the University Félix Houphouët-BoignyAbidjan-Cocody where a sample is preserved.

2. Bacterialstrain

Made up of a reference strain (ATCC 25923) and four methicillin resistant strains of *Staphylococcus aureus* (MRSA) obtained from biological products (urine and suppuration). 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 Ivory Coast (IPCI).

3. Cellules HFF

The cells HFF (HumanForeskinFibroblasts) used for this work were provided by the Adaptation and Pathogenesis of MicroorganismsLaboratory (LAPM) at Grenoble (France).

B. METHODS

1. Botanical description and traditionalutilization 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.

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 5, Issue 3 , March 2018

2. Preparation of plant 70 % ethanolic extract

The leaves of this species were dried in the Laboratory for two weeks and powder using an electric grinder type IKA Labortechnik (MFC type).
The 70 % ethanolicextract was obtained by dissolving 5 g of total aqueous extract (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. It should be notedt hat the ETA was performed using the method described by[36].

3. Sterility test of extract

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.

4. Preparation of the concentration rang of plant extract

A solution of concentration of 100 mg/mL of extract 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.

5. Evaluation of antibacterial activity

The antibacterial activity of the extract of *Ecliptaprostrata* (L.) L was evaluated by the method of diffusion in solidmedium. This test was performed by the solid medium diffusion method [37-38].The wells were made using a Pasteur pipette in agar gel previouslyseeded and 50µl of the substance to be tested is deposited. The agar was incubated at 37 °C for 18 to 24 hours. The reading was made by measuring the diameter of inhibition around each well using a slidingcaliper. The diameters of inhibition zone were expressed in mm according to the criteria expressed in [39]. The strainisthensaid resistant, sensitive, very sensitive and extremely sensitive respectively for a diameter less than 8mm,between 9 and 14mm, between 15 and 19 mm and equal to 20 mm respectively.

6. Determination of the antibacterial parameters

Determination of antibacterial parameters was carried out by the liquid medium dilution according to the method used by [40]. 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 to MIC clarified the mode of action of a substance [41]. 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.

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 5, Issue 3 , March 2018

7. Toxicity test

The HFF cells used for toxicity test, were cultured at 37 °C, 5 % CO_2 in D10 (Dulbecco Minimum Essential Medium,Gibco, supplemented with 10 %fetal calf serum; 1 %glutamine, 50U.mL^{-1}penicillinand50µg.µL^{-1}streptomycin). To measure the toxicity of this ethanol extract (FE 70 %), the cells HFF (Human Foreskin Fibroblasts) were sowed in 96-well plates (CellStar) at a rate of 3000 to 5000 cells per well in 100µL of medium D10. These cells are maintained in culture for 24 hours (dividing cells) or 96 hours (confluent cells). Subsequently they were exposed for 24 hours at different concentrations (0-1000 mg/mL) in the plant extract solubilized in PBS buffer. This was done in three parts. Viability was determined using the 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium (MTT). In each well, the MTT is added to a concentration of 500 µg/mL and incubated for 3 hours at 37 °C. The formazan crystals are solubilized in dimethylsulfoxide (DMSO) 10 mM. The Measurement of the optical density at 544 nm was made using a Safir spectrophotometer (Tecan). This absorbance measurement was used to determine the relative amount of living and metabolically active cells. The results were expressed as percentage of viability compared to the control without plant extract (control) [42].

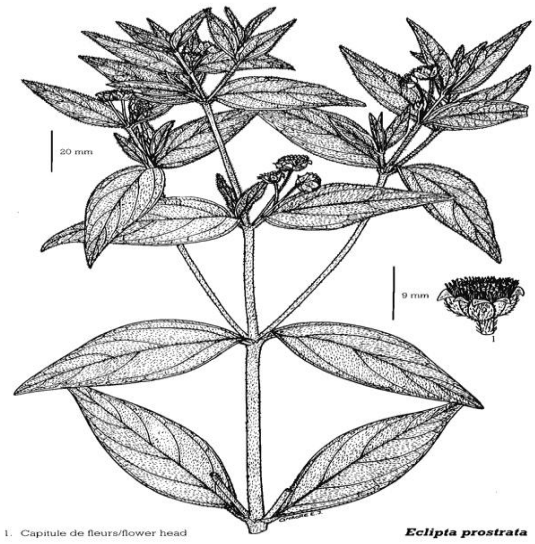
Viability rate = (Abs_{544} extract nm / nm Abs_{544} control) × 100.

IV. RESULTS AND DISCUSSION

A. RESULTS

1. Ethnobotanicalstudy

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 I are summarized the morphological details description of the different organs. Table II are summarized some therapeutic uses of *Ecliptaprostrata*in traditional african medicine. *E.prostrata*, is called GnikièinAkye(Southern ethnic group of Ivory Coast), N'da-loublé in Baoulé(Center ethnic group of Ivory Coast)andMissofiinMalinke(Northern ethnic group of Ivory Coast). It grows on the moist and badly drained soils, in flooded rivers, irrigated canal and fields.



A :drawing (Source [43])

B : photo of ground

Figure 1 : General appearance of the leaves and flowers talks of *Ecliptaprostrata*

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 5, Issue 3 , March 2018

Table I : Description of the plant

Plant species / Described parts	*Ecliptaprostrata* (L.) L
General appearanceof the plant	Erect form an ramification annual herb Stem slightly fleshy, more or less woody at the base, rough and generally reddish, 50 cmto 60cm high
Section of the stem	Circular
Size of the leaves	Length : 2 to 10 cm ; width : 1 to 3 cm
Form of the Leaves	Oval-lanceolate
Arrangement of the leaves	Opposite, simple, sessile
Types of inflorescences	Terminals or axilesandsolitarycapitules Colorof the floret :white
Type of fruit	Akènes
Fruit size	Length about3 mm ; width : 0,5 to 1 mm
Appearance fruit	Warty and cuneiform, Brown or black

Table II : Some uses of *Ecliptaprostrata* in traditional ivorian medicine

Deseases, Symptoms	Parts of the plant used	Methods of préparation	Medicinalforms	Methods of administration	Way of administration
Asthma	Leaves	Softening	Juice	Drink	Orally
Paludism	Leaves	Décoction	Décocté	Drink	Orally
Diabetes	Leaves	Décoction	Décocté	Drink	Orally
Deepwound	Leaves	Décoction/ Kneading	Décocté/Paste	Locale application/ Dressing	Externway
Infantile diseases	Leaves	Décoction/ Kneading	Décocté/Paste	Locale application/ Dressing	Externway
Skin diseases	Young freshleaves	Expression/ Kneading/ Décoction	Juice/Paste/ Décocté	Locale application/ Dressing	Externway

2 Antibacterialactivity

Thisethanolic extract is active all studied strains with diameters inhibition between 10.0 ± 0.1 and 17.3 ± 0.4 mm (Table III).At the highest concentration studied, the diameters of the inhibition zones ranged from 12 mm to 17 mm. Besides the reference strain, the ethanolic extract of *E. prostratata* is more active on the strain 1325Y/14and 680Y/14 which are *S. aureus* isolates resistant to methicillin with cross-resistant to fluoroquinolones. It emerged from this experience that this extract has minimal inhibitory concentrations (MIC) ranging from 6.25± 0.0 mg/mL and 12.5 ± 0.0mg/mL.As for the minimum bactericidal concentrations (MBC), it ranges from 25 ± 0.0 mg / mL to 50 ± 0.0mg/mL (Table IV). The 70 % ethanolic extract of leaves of *Ecliptaprostrata* is bactericidal 60 % of strains tested.

Table III : Diameters of the zones of inhibition obtainedwith the FE 70 % of leaves*E. prostrata* and of antibiotics on strains of SARM and referencestrain

Bacterium	Codes	Diameter of inhibition (mm)				
		FE 70 %		EDS	FOX	OXA
		100 mg/mL	50 mg/mL		30 µg/mL	5 µg/mL
S. aureus	680Y/14	13.3±0.3	10.0±0.3	6.0±0.0	20.3±0.4	16.3±0.2
	1325Y/14	14.3±0.3	12.0±0.3	6.0±0.0	17.0±0.3	16.0±0.1
	590Y/14	12.1±0.5	10.0±0.2	6.0±0.0	20.3±0.3	15.3±0.3
	485Y/14	12.0±0.6	10.0±0.0	6.0±0.0	17.1±0.2	15.1±0.2
	ATCC25923	17.3±0.4	11.3±0.1	6.0±0.0	19.3±0.1	20.0±0.3

Includeddiameter of the wells (6 mm) ; FE70 % : 70 % ethanolicextract ; EDS : sterile distilled water (control) ; ATCC : American Type Culture Collection ; FOX :Céfoxitine ; OXA : oxacilline

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 5, Issue 3 , March 2018

Tableau IV : Antibactérialparameters of the 70 % ethanolicextract of leaves of *E. prostrata* on strain of *S. aureus* (SARM) and the strain of reference

Bacteria	Extract FE70 % (mg/mL)			
	MIC	MBC	MBC/MIC	Power
690Y/14	6.25±0.0	25±0.0	4	bacteriostatic
1325Y/14	12.5±0.0	25±0.0	2	bactericidal
590Y/14	12.5±0.0	25±0.0	2	bactericidal
485Y/14	12.5±0.0	25±0.0	2	bactericidal
ATCC25923	6.25±0.0	50±0.0	8	bacteriostatic

MIC : Minimum Inhibitory Concentration ; MBC : Minimal Bactericidal Concentration ; FOX : Céfoxitine ; OXA : oxacilline

3 Toxicity test

The result of toxicity test on HFF cells is summarized in figure 2.This result reveals that the 70 % ethanolicextract of *E. prostra* shows littletoxiceffect on the human cell studied compared to controls. We observe a decrease of the drop of HFF cells viability rate when the concentration of the plant extract increased (from 125 µg/mL to 1000 µg/mL). Thus for confluent cells, lower viability rate is between 34 % (125 µg/mL) and 9 % (1000 µg/mL). As for dividing cells, this decrease is between 32 % (125 µg/mL) and 23 % (1000 µg/mL).

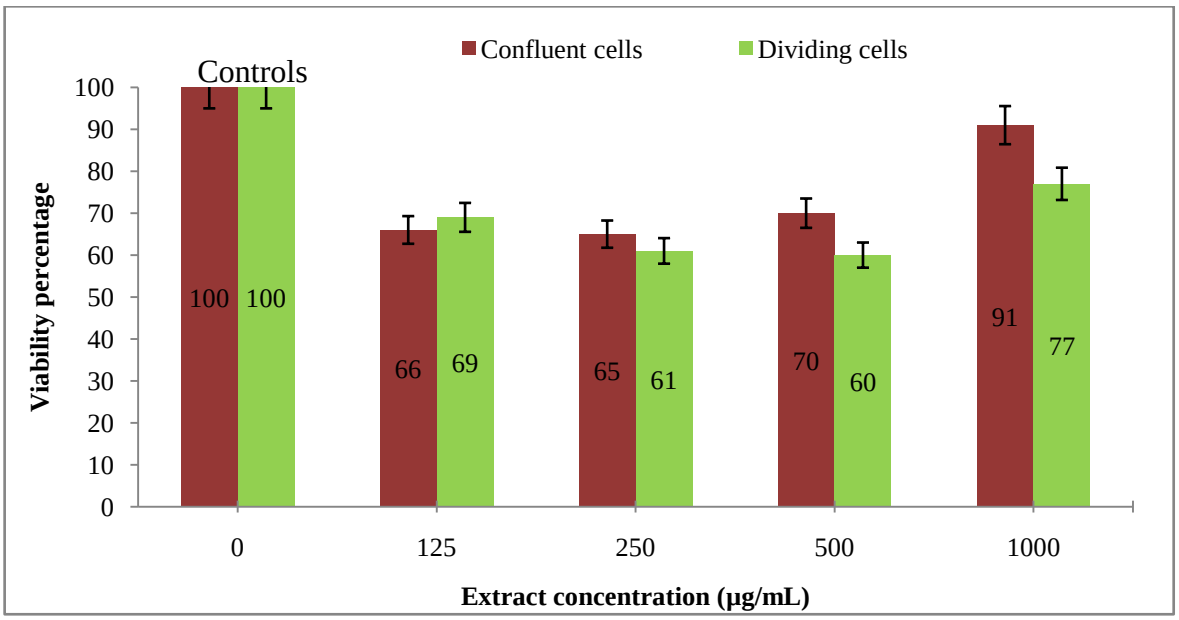


Figure 2: Viability rate on HFF cells of 70 % ethanolicextract of *E. prostrata*

B. DISCUSSION

Analysis of the results of ethnobotanical study revealed that *Ecliptaprostrata* is a plant with many therapeutic properties. These results are confirmed by [44] who showed several pharmacologicalproperties of this plant.
As regards antibacterial parameters, turbidity induced by the growth of decreased bacteria inversely with the concentration of extract into the wells of the microplate.This shows that this 70 % ethanolicextract of *E.*

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International Journal of Advanced Research in Science, Engineering and Technology

Vol. 5, Issue 3 , March 2018

*prostrata*exhibits antibacterial activity by inhibiting the *in vitro* growth of strains of *Staphylococcus aureus* according to a dose-response relationship. All the tested bacterial strains showed different sensitivities to this extract. Thus, *S. aureus*1325 Y/14 is the most sensitive with a diameter of inhibition of 14.3 mm.Comparison of the activity of this ethanolic extract of the leaves of *E. prostrata* on the basis of the MBC relative to the strains of infections and reference shows that this extract is twice as active on all strains of infections studied resistant to methicillin.We deduce that the 70 % ethanol extract exerts different inhibitory actions on the tested bacterial strains at different concentrations. Our results are in agreement with those of [34-45]which showed that *E. prostrata* had an antibacterial effect on *S. aureus*. Sixty percent of activity reports (CMB / MIC) are equal to 2. According to [46], this fraction has a bactericidal activity with regard to the tested germs.

In terms of toxicity, analysis of the results reveals that the 70% extract of *E. prostrata*presents little toxicity effect on confluent cells. Moreover, the toxicity effect decreases with further increase in the concentration of the extract. This can be caused by the presence in the extract molecules stimulating cell division. According [44], *E. prostrata* has has anti-hepatotoxic properties. In addition, the extract of this plant protects against the myotoxiceffect of snakevenom[47]. Which confirms these results.

V .CONCLUSION

This study allowed the multiple uses of *Ecliptaprostrata* in ivorian traditional medicine. This study also demonstrated antibacterial activity of leaves of *E. prostrata* on *Staphylococcus aureus* strains resistant to methicillin (SARM) and also to determine the MIC and MBC. This activity is principally bactericidal in nature.

Besides, this study reveals that the use of *E. prostrata*for therapy would be safe. The extract of the leaves of *E.prostrata* could therefore be a less costly alternative for the treatment of urinary tract and skin infections.

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