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PHYTOCHEMICAL STUDY AND ANTIBACTERIAL ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF LEAVES OF *Myrianthus holstii* ENGL. (CECROPIACEAE)

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ABSTRACT

This study aims to perform the phytochemistry study and evaluate the antibacterial effects of aqueous extracts and ethanolic macerated leaves of *Myrianthus holstii* Engl. (Cecropiaceae) on five strains derived from biological products and three reference strains. Phytochemical analysis revealed the presence of polyphenols, flavonoids, quinones, tannins and sterols-polyterpenes in both extracts. The diffusion and dilution methods on Muller-Hinton made it possible to evaluate the antibacterial activity of the extracts. The results revealed that these extracts have a dose-dependent antibacterial activity on the bacterial strains used. However, the 70% ethanolic extract has a better antibacterial potential on the strains compared to the aqueous extract namely on *S. aureus* (MIC = 3.12 mg / mL), *E. coli* ATCC (MIC = 12.5 mg / mL) and *S. aureus* Meti-R (MIC = 6.12 mg / mL). The diameters of inhibition are between 08 and 18 mm for the ethanolic extract from 25 mg / ml and between 08 and 14 mm for the aqueous extract from 50 mg / ml. The MIC of the aqueous extract vary from 12.5 to 100 mg / mL and those of the ethanol extract range from 3.12 to 50 mg / mL. The extracts are bactericidal on all the strains after 24 and 48 hours of incubation. This study showed that *Myrianthus holstii* extract could be used in the treatment of infectious diseases.

KEYWORDS: *Myrianthus holstii*; Phytochemistry; Antibacterial Activity.

INTRODUCTION

Infectious diseases are the leading cause of death in the world. They account for almost half of all deaths in tropical countries.[1] The agents responsible for these infections are diverse and varied. To combat these microbial attacks, the scientific world has discovered numerous treatments to relieve patients.[2] However, the acquisition of these drugs is extremely difficult because of high costs and makes access to medical care obsolete for the poor. This has led people to always resort to traditional medicine.

Indeed, plants have been used since antiquity as remedies for the treatment of various diseases because they contain components rich in therapeutic principles.[3,4] According to the World Health Organization (WHO) nearly 80% of populations depend on traditional medicine.[5] They constitute a natural source of chemical molecules[6], such as secondary metabolites that intervene in several domains.[7] They are found in aromatherapy, pharmacy, perfumery, cosmetics and food preservation. Their use is linked to their broad spectra of recognized biological activities.[8] It is therefore important to direct research towards new pathways and especially towards plants that have always been the basis of new drugs.[7] Therefore, the present work has been undertaken to highlight the therapeutic virtues of *Myrianthus holstii*.

It is one of the plants that could be used to fight against bacterial infections. It is used by the population of western Côte d'Ivoire for the belly enema. Scientific studies have shown that a decoction of leaves, stem bark, trunk of this species is mixed with a twig for the enema and helps fight against diarrhea, dysentery, cholera, fatigue, miscarriage and strengthens the children.[9] It is widely used in traditional medicine because of its febrifuge, depurative, antipyretic, antispasmodic, fortifying, stimulant, antidiarrheal, laxative and antimalarial properties.[9,10] And studies have shown that leaves are often used in traditional pharmacopoeia to treat these previously listed conditions.

This work aims to determine on the one hand the chemical compounds of the aqueous and ethanolic extracts of the leaves of *Myrianthus holstii* and on the other hand to evaluate the antibacterial activity of these extracts.

MATERIALS AND METHODS

Plant material

The plant material consists of the leaves of *Myrianthus holstii* (Cecropiaceae) harvested in October 2018 on the site of the University of Man, located 7 km from the city of Man, on the Man-Danane axis, (Ivory Coast).

Microorganisms

The bacterial carrier used in this study consists of three (3) reference strains namely *Staphylococcus aureus* CIP 7625 (ATCC 25923), *Escherichia coli* CIP 7624 (ATCC 25922) and *Pseudomonas aeruginosa* CIP 76110 (ATCC 27853) and five (5) Isolated clinical strains of biological products that are: ESBL *E. coli* (1087C / 13 isolated from urine sample), *Salmonella typhi* (1585C / 13, isolated from blood sample), *Klebsiella pneumoniae* ESBL (1942C/13, isolated from pus sample), *S. aureus* Met-R (1532C / 10, isolated from pus sample) and *Klebsiella pneumoniae*. These are the components of the Antibiotics Natural Substances and Monitoring Microorganisms for Anti-Infective (ASSURMI) of the Department of Bacteriology and Virology of the Pasteur Institute in Ivory Coast (IPCI).

PREPARATION OF PLANT EXTRACTS

Preparation of aqueous extract

100g powder of the leave of *Myrianthus holstii* were macerated for 24 hours in 1L of distilled water.[11] The macerate has been wrung into a square of sterile tissue, filtered successively on cotton wool and one fold on filter paper (Whatman paper® 2mm). The filtrate was dried slowly in the stove at 50°C. The powder obtained was stored in a hermetically sealed jar and refrigerated at 4°C.[12]

Preparation of ethanolic 70% extract

It was carried out using modified.[11] method. A mass of 20g of plant powder was added in 100ml of ethanol 70% and subjected to maceration for 72 hours. The macerate was treated according to the same procedure like the aqueous extract.

Preparation of bacterial inoculum

Two isolated colonies from each bacterial culture for 18 hours were homogenized in 10 mL of Muller-Hinton broth and incubated for 3 hours at 37°C for preculture. A levy of 0.1 mL of the preculture broth was diluted in a tube containing 10 mL of Mueller-Hinton (MH). This

bacterial suspension was made consisting of 10^0 dilution of bacterial inoculum so as to obtain a bacterial load estimated to 10^6 Unit Format colonies per milliliter (CFU/mL).

Preparation of extracts concentration ranges

A range of concentration of each extract was prepared with a series of ten vice tubes through the method of double dilution an in medium liquid. This range of concentration is 200 mg / mL to 0.39 mg / mL numbered T1 to T10. For this, 10 mL of a mixture solution of DMSO / sterile distilled water (V / V) were placed in the tubes T1 and 5mL in all the other tubes. Two grams (2g) of each extract were dissolved in the tubes T1 to obtain a concentration of 200mg/mL. A 5mL volume of the tubes T1 was transferred into the tubes T2 and then homogenized. This operation was repeated until T10 tubes where 5 mL of T10 tubes are rejected. All tubes are kept refrigerated at 4°C.[13,14]

Determination of growth inhibition zones

The method of holes punch in the MH agar described by[15] has been accepted. Each pit or holes of 6 mm diameter was filled with 80 µL of extract concentrations of 200 and 100 mg / mL, taking care to separate two holes of at least 20 mm. A negative control wells was performed for each bacterial strain with 80 µL of the mixture of DMSO / sterile distilled water solution (V/V). After a pre-release of 45 minutes at laboratory temperature to 16 ° C, all the Petri dishes were incubated in an incubator at 37°C for 18-24h. Meanwhile, Ceftriaxone (CRO 30µg) for Enterobacteriaceae and oxacillin (OX 5µg) for staphylococci were used as positive controls. After incubation, the activities of the extracts were assessed by measurement of a growth inhibition area around the wells using a caliper. According to[16], a strain is called insensitive or resistant, sensitive and very sensitive if the diameters of inhibition are respectively less than 8 mm, between 9 and 14 mm and between 15 and 19 mm.

Determination of Minimum Inhibitory Concentration (MIC)

The macro dilution method in liquid medium described by[17] was used to determine these antimicrobials parameters. Thus, in a series of 10 hemolysis tubes numbered C1 to C10 for each extract was introduced 1 mL of the bacterial inoculum. Then 1 mL of each extract concentration well known by the range of prepared concentration was added in the same tubes. This distribution of plant extract is made so that 1 ml of plant extract of 200 mg / mL was transferred in the tube C1, that of 100 mg / mL in the tube so C2 to C9 tube receive 1mL plant extract of 0.78 mg / mL. C10 has been tube, received instead of plant extract, 1 mL of DMSO / Sterile distilled water (V/V), was used as a control. This distribution of plant extract

concentration is well known in each tube already containing 1 mL of inoculum reduced the concentration of plant extract in medium at its half. Tube and the concentration of C1 increased from 200 mg / mL to 100 mg / mL. 100 mg / mL to 50 mg / mL for C2 so on until a concentration of 0.39 mg / mL for T9. This experiment was performed identically for each sample tested. The first nine (9) tubes (C1 to C9) are called "experimental tubes" and the last tube (C10) is rated "growth control tube or TC." The loaded tubes were incubated at 37 ° C for 24 h. The MIC is the concentration of the first tube where it finds no trouble visible to the naked eye.

Determination of Minimum Bactericidal Concentration (MBC)

From the MIC, the lowest concentration that leaves no more than 0.01% survival of bacteria suspended starting 24 hours corresponds to the CMB. It is determined by plating by a streak on Mueller-Hinton agar by streaking 5 cm using a loop, beginning with the first and incubated undisturbed at 37°C for 24 h tube.

Antibacterial activity of the extracts tested

The antibacterial effect of different extracts tested was considered bactericidal or bacteriostatic depending on the MBC / MIC ratio. According[18] when this ratio is greater than 4, the extract has bacteriostatic and bactericidal, if the ratio is less than or equal to 4.

Phytochemical screening

Test for sterols and polyterpenes (reaction LIEBERMANN)

After evaporation to dryness 5mL of each solution in a capsule on a sand bath without charring, the residue was dissolved in hot acetic anhydride and 1 mL in a test tube, we poured cautiously with 0.5 mL of concentrated sulfuric acid along the tube wall to the solution. The applications to the interphase of a purple or purple ring, turning blue to green, indicate a positive reaction.[19]

Test for alkaloids (reactions Dragendorff and Bouchardat)

Six milliliters of plant extract were evaporated. The residue was taken up in six milliliters of alcohol at 60 ° and the alcoholic solution thus obtained was divided into two test tubes.

In the first tube was added two drops of Dragendorff reagent. The appearance of a precipitate or an orange color indicated the presence of alkaloids.

In the second tube was added two drops of reagent Bouchardat. The appearance of a reddish brown color indicated a positive reaction to the presence of alkaloids.[20]

Test for polyphenols

Two milliliter of extract was added a drop of alcoholic solution of ferric chloride at 2%. The appearance of a dark green or lighter or darker blue color indicated the presence of polyphenolic derivatives.[21]

Test for flavonoids

For this research, two milliliter of the extract was evaporated to dryness in a porcelain dish on a sand bath. The residue was taken after cooling in five milliliter hydrochloric alcohol half. The successive addition of three magnesium shavings and three drops of isoamylic alcohol showed an intense pink or violet in the presence of flavonoids.[22]

Test for saponosides

A volume of two milliliters of each extract was evaporated and taken up in five milliliters of water. After vigorous stirring, the foaming of more than one centimeter, stable and persistent high for 30 minutes indicated the presence of saponins.[23]

Test for catechol or condensed tannins (reaction Stiasny)

A volume of five milliliter of each extract was evaporated and an amount of 10 ml of a reagent solution Stiasny was added to the residue. This mixture was placed in a water bath at 80°C for 30 minutes and was cooled to room temperature. Positive feedback had resulted in the formation of large flakes brown clear or dirty precipitates.[19]

Quinonic substances research

For this research, 2 mL of each extract solution is first evaporated to dryness in a sand-bath capsule without charring, then the residue is triturated in 5 mL of 1: 5 hydrochloric acid. Then the solution obtained is brought to the boiling water bath for half an hour. Finally, after cooling on a current of cold water, the hydrolyzate is extracted with 20 ml of chloroform and the chloroform phase is collected in another test tube supplemented with 0.5 ml of ammonia diluted by half. The appearance of a color ranging from red to purple indicates the presence of quinones.[19]

Search anthocyanins

The presence of anthocyanins in an extract solution is indicated by a red color which increases with the addition of dilute HCl and turns purplish-blue-green by the addition of ammonia.[19]

Test for Gallic tannins

The above solution was saturated and one or two drops of alcoholic solution of iron chloride to 2 % have been added. The positive response has led to the appearance of blue-black coloration characteristic intense tannins Gallic.[19]

RESULTS

Yield

The values of the yields were calculated with respect to the initial mass of *M. holstii* powder for one test. The extraction yields of the plant are 12.60% for the ethanolic extract (EE) and 08.03% for the aqueous extract (EA). (Table I)

Table I: Extraction Yield of Extracts.

Extracts	Mass (g)	Yield (%)	Colors and Aspects
EEfe 70%	37,8	12,60	Black (oily)
EAfe	24,1	08,03	Black (tights)

Phytochemical study

Table II shows the large groups of chemical families contained in the two extracts of *Myrianthus holstii*. Phytochemical screening of Aqueous Extract (EA) and Ethanolic Extract (EE) of *Myrianthus holstii* leaves shows the presence of several families of chemical compounds. These are sterols/polyterpenes, alkaloids, gallic tannins and catechins, quinones, saponosides, anthocyanins, flavonoids and total polyphenols.

Tableau II: Phytochemical analysis of ethanolic 70% and aqueous extract of *m. Holstii* leaves.

Extracts	Alkaloids		Saponosides	Anthocyanins	Tannins		Flavonoids	Polyphenols	Quinones	Sterols and polyterpenes	
	B	D			Gal	Cat					
EAfe	-	-	-	++	+++	+++	++	++	++	+++	
EEfe 70%	-	+	+	+	+++	+	+++	+++	++	+++	

- : Absence *+: Presence* *++ means* *+ + +: abundant.*;

EE 70% Ethanol Extract**, EA :** Aqueous Extract; **Gal** : Gallic ; **Cat** : catechetical ; **Fe** : leaves **B** : Bouchardaf ; **D** : Dragendorff

Antimicrobial Activity

Tables III and IV show the antibacterial activity of EA and EE of *Myrianthus holstii* leaves. These results show that the best bacterial activity is obtained with EE with *S. aureus* (18 mm), followed by *S. aureus* Méti-R (16 mm), *Klebsiella pneumoniae* (14 mm), *Salmonella Typhi*, *E. coli* and *Pseudomonas aeruginosa* (12 mm).

For the aqueous extract (EA), the best activity is obtained in leaves with *S. aureus* (14 mm), *E. coli*, *Salmonella Typhi* and *K. pneumoniae* (12 mm). Then come *K. pneumoniae* ESBL, *S. aureus* Méti-R and *P. aeruginosa* (10 mm).

Tableau III: Inhibition zone diameters (mm) with Ethanolic Extract (70%) of *Myrianthus holstii* leaves and antibiotics on the strains tested (n = 3).

Tested strains	Concentrations (mg/ml)					Antibiotics	
	C1=200	C2=100	C3=50	C4=25	Ts	AMC/CRO /IPM	OXA/ FOX
Salmonella Typhi	12±0.6	10±1.3	06±0.0	06±0.3	06±0.0	06	Nd
K. Pneumoniae	14±1.3	12±0.3	10±0.3	08±0.3	06±0.0	06	Nd
***K. Pneumoniae* BLSE**	14±0.3	12±1.3	10±0.3	08±0.3	06±0.0	08	Nd
***E. coli* BLSE**	12±0.6	10±0.3	08±0.3	08±0.3	06±0.0	10	Nd
***S. aureus* Méti-R**	16±1.3	12±0.6	08±0.6	06±0.3	06±0.0	Nd	06
***E. coli* ATCC 25922**	12±0.3	10±0.3	08±0.3	06±0.3	06±0.0	08	Nd
***S. aureus* ATCC 25923**	18±0.3	16±0.6	14±0.3	08±0.3	06±0.0	Nd	08
***P. aeruginosa* ATCC 27853**	12±0.3	10±0.3	08±0.3	06±0.3	06±0.0	Nd	06

T = 0: *Including Control = the diameter of the wells (6mm) with DMSO / water (0.5: 0.5, V/V);* ***CRO****: Ceftriaxone (30µg),* ***OXA****: oxacillin (5µg);* ***Meti-R****: Methicillin -resistant;* ***IMIP -I****: Intermediate imipenem;* ***ESBL****: extended spectrum beta-lactamase.*

Tableau IV: Inhibition zone diameters (mm) with Aqueous Extract (EA) of *Myrianthus holstii* leaves and antibiotics on the strains tested (n = 3).

Tested strains	Concentrations (mg/ml)					Antibiotics	
	C1=200	C2=100	C3=50	C4=25	Ts	AMC/CRO/ IPM	OXA/FOX
Salmonella Typhi	12±1.3	10±0.3	06±0.3	06±0.3	06±0.0	06	Nd
K. Pneumoniae	12±0.3	10±0.3	08±0.3	06±0.3	06±0.0	06	Nd
***K. Pneumoniae* BLSE**	10±0.0	06±0.3	06±0.3	06±0.3	06±0.0	08	Nd
***E. coli* BLSE**	12±0.3	10±0.6	08±0.3	06±0.3	06±0.0	10	Nd
***S. aureus* Méti-R**	10±0.6	08±0.3	08±0.3	06±0.3	06±0.0	Nd	06
***E. coli* ATCC 25922**	14±0.3	10±0.3	08±0.3	06±0.3	06±0.0	08	Nd
***S. aureus* ATCC 25923**	14±0.3	12±0.3	10±0.3	06±0.3	06±0.0	Nd	08
***P. aeruginosa* ATCC 27853**	12±0.6	08±0.3	06±0.3	06±0.3	06±0.0	Nd	06

T = 0: Including Control = the diameter of the wells (6mm) with DMSO / water (0.5: 0.5, V/V); **CRO**: Ceftriaxone (30µg), **OXA**: oxacillin (5µg); **Meti-R**: Methicillin -resistant; **IMIP -I**: Intermediate imipenem; **ESBL**: extendedspectrum beta-lactamase

Tableau V: Antibacterial Parameters of Ethanol and Aqueous Extract Fractions of *m. Holstii* Leaves on in Vitro Growth of Test Organisms.

Extracts	Antibacterial parameters (mg/mL)	*Salmonella Typhi*	*K. pneumoniae* BLSE	*K. pneumoniae*	*E. coli* BLSE	*S. aureus* Méti-R	*E. coli* ATCC	*S. aureus* ATCC	*P. aeruginosa* ATCC
EEfe	MIC	50	12,50	25	50	6,25	12,5	3,12	50
	MBC	100	50	50	50	6,25	12,5	3,12	50
	MBC/MIC	2	4	2	1	1	1	1	1
	Effect	bactericidal	bactericidal	bactericidal	bactericidal	bactericidal	bactericidal	bactericidal	bactericidal
EAfe	MIC	50	>100	>100	25	50	50	12,5	>100
	MBC	100	>100	>100	50	100	50	25	>100
	MBC/MIC	2	Nd	Nd	2	2	1	2	Nd
	Effect	bactericidal	-	-	bactericidal	bactericidal	bactericidal	bactericidal	-

MIC: Minimum Inhibitory Concentration; **CMB:** Minimum Bactericidal Concentration **Nd :** Not determined

DISCUSSION

The aim of this work was to search for the most active phytochemicals of the extracts and to scientifically verify the therapeutic anti-infectious properties that are given to the leaves of *Myrianthus holstii*. To do this, our approach was to prepare Ethanol (EE 70%) and Aqueous (EA) extracts by the maceration method and then to evaluate their antibacterial activities.

Table 1 shows the extraction yields of various solvents used. The yield of the ethanolic extract is higher with 12.60% than the aqueous extract which has 08.03%. These yields depend on the extraction capacity of the solvent and the content of chemical compounds.

The extracts were then subjected to qualitative phytochemical analysis, the results of which are shown in Table II. The presence of the majority of the desired metabolites was detected in the 70% EE extract. In contrast, there is an absence of saponosides and alkaloids with the Dragendorff reagent in EA. This could be explained by the fact that ethanol concentrates more compounds than water.

Tables III and IV show the diameter values of the growth inhibition zones of the bacteria tested. The sensitivity test carried out with the extracts shows that the susceptibility of the microbial strains varies from one strain to another depending on the type of extract. Similarly, the results differ according to the extract concentrations used. It should be remembered that the activity of a plant substance depends on several factors, including the mode of extraction and the concentration of active ingredient[24] and the origin of the strains used.

For the antibacterial activity, the ethanolic extract has a well-defined activity on the growth of all the organisms studied. The diameters of inhibition are between 08 and 18 mm for ethanol and between 08 and 14 mm for the aqueous extract from 50 mg / ml. These diameters obtained with EE are comparable to those obtained by[15] on clinical strains producing beta-lactam with total extracts of *Terminalia glaucescens* Planch Ex Benth used in the treatment of various infections. Still in the same vein, our results are similar to those obtained by[19] with the same strains and solvent on *Cochlospermum planchonii*.

However, the strains *Staphylococcus aureus* ATCC, *S. aureus* Meti-R and *K. Pneumoniae* are sensitive to the ethanolic extract at 200 mg / ml. This could be explained by the presence of flavonoids, polyphenols, quinones and polyterpenes.[25] The aqueous extract of this plant had no activity on strains *K. Pneumoniae* ESBL, *Salmonella Typhi*, *S. aureus* and *P. aeruginosa*

at a dose of 100; 50 and 25mg / ml. Such a finding was made by[26] in Indonesia. Contrary to these results,[27] in Malaysia showed that the aqueous extract of some plants were active on pathogenic strains (*Streptococcus oralis*, *Staphylococcus aureus*). The observed difference can be explained by the variation in the concentration of active ingredients and the solubilization of these active ingredients in water from one plant to another. In addition, it should also be noted that *S. aureus* Meti-R, *E. coli*, *K. pneumoniae* and *Salmonella Typhi* strains showed resistance to both extracts at the concentrations 100, 50 and 25 mg / mL used. The explanation lies in the inefficiency of the active molecules in this plant and in relation to the membrane structure and the origin of the strains. This observation could be explained by the nature or the complexity of the structure and the nature of the bacteria (Gram- and Gram +). Indeed, the wall of Gram + bacteria is almost exclusively composed of peptidoglycan, which is associated with teichoic acid polymers.[28]

According to[16], an extract is judged as active if it induces a zone of inhibition greater than or equal to 10 mm. Thus, against the tested seeds, at 200 mg / mL, the 70% ethanolic extract was active on all the strains studied. It was more active, compared to the aqueous extract and antibiotics namely Ceftriaxone (30 micrograms), Cefoxitin (30 micrograms) and Oxacillin (5 micrograms). The analysis of these results shows that the MIC values are consistent with those of the diameters of the growth inhibition zones. This is the case of the ethanolic extract on the strain of *S. aureus* ATCC with a MIC = 03.12 mg / mL for 18 mm of zone of inhibition, on the *S. aureus* strain Méti-R for a MIC = 06.12 mg / mL for 16 mm or the case of the aqueous extract on ESBL *E.coli* (MIC = 25 mg / mL) for 12 mm and on *S. aureus* Méti-R for MIC = 50 mg / mL for 10 mm.

Overall, the greatest sensitivity is observed in the presence with the ethanolic extract for both *S. aureus*, *K. pneumoniae* and *P. aeruginosa* strains, both sensitive and resistant. It is difficult to compare our results with those of the bibliography because no scientific study has been conducted on this plant.

CONCLUSION

This work allowed us to highlight the presence of certain chemical compounds and antibacterial properties in the aqueous and ethanolic extracts of the leaves of *Myrianthus holstii*. On the other hand, the 70% ethanolic extract proved to be more active on most of the strains tested, in particular on strains of *S. aureus* and *K. pneumoniae* ESBL. This sensitivity is dose-dependent and varies with germs and extraction solvent and chemical compounds.

Indeed, ethanol concentrates the active ingredients better than distilled water. Our results demonstrate the use of the *M. holstii* plant in traditional medicine against various pathologies.

CONFLICT OF INTERESTS

The authors claim that there is no conflict of interest.

REFERENCES

1. N Ullah; M Khurram; F Ali Khan; S Umar Khayyam; MU Amin; S Ullah; U Najeeb; S Muhammad; J Hussain; MA Khan. *Journal of Applied Pharmaceutical Sciences,* 2011; 1(07): 81-84.
2. Y Traoré; K Ouattara; D Yéo; I Doumbia; A Coulibaly. *Journal of Applied Biosciences,* 2012; 58: 4234-4242.
3. A Khaldi; B Meddah; A Moussaoui; H Benmehdi. *European Journal of Scientific Research,* 2012; 80(3): 311-321.
4. G Nsi Akoué; LC Obame; JP Ondo; I Brama; ES Otogo N'Nang; SY Tapoyo; A Souza. *International Journal of Biomolecules and Biomedicine,* 2013; 3(3): 1-8.
5. C Moutinho. *International Journal of Indigenous Medicinal Plants,* 2013; 29(1): 1167-1174.
6. S Cornbrinck; GW Du Plooy; R Mccrindlc; BM Botha. *Annals of botany,* 2007; 6: 1111-1119.
7. KR Oussou; SF Yolou; B Tue Bi; C Kanko; JB Boti; C Ahibo; J Casanova. *European Journal of Scientific Research,* 2010; 40(1): 50-59.
8. F Amarti; B Satrani; M Ghanmi; A Farah; A Aafi; L Aarab; M El Ajjouri; A Chaouch. *BiotechnoLogie Agronomie Société et Environnement,* 2010; 14: 141-148.
9. M Baerts; J Lehmann. *Annal Sciences Ecology,* 1989; 18: 214.
10. I Balagizi; A Cihyoka; S Mapatano. *Plateforme Diobass au Kivu,* 2005; 118p.
11. G Wole Olakunle; F Myade Emmanuel. *International Journal of Biological and Chemical Sciences,* 2005; 8(4): 1570-1580.
12. G Zirihi; AKM Kra; F Guédé-Guina. *Revue Medicinal,* 2003; 17(3): 11-18**.**
13. K Ouattara; AJ Djaman; A Coulibaly; JD N'guessan; YJ M'baiasbe; F Guedé-Guina. *Revue de Médecines et Pharmacopées africaines,* 2007; 20: 1-8.
14. GEK Bolou; B Attioua; AC N'guessan; A Coulibaly; JD N'guessan; AJ Djaman. *Bulletin de la Société Royale des Sciences de Liège,* 2011; 80: 772-790.

15. KF Konan; KN Guessennd; D Ouattara; C Bahi; GK Julien; A Coulibaly; A Djaman; M Dosso. *International Journal of Pharmacology*, 2014; 26: 37-42.
16. AG Ponce; R Fritz; C Del Valle; SI Roura. *Lebensmittel-Wissenschaft und Technologic*, 2003; 36: 679-684.
17. M Dosso; H Faye-kette. *Le bactériologiste international*. 2000, n° spécial, 53.
18. P Berché; JL Gaillard; M Simonet. *Médecine et Sciences*, 1991; 660.
19. Yéo Sounta Ouma; Guessennd Kouadio Nathalie; Ouattara Karamoko; K Konan Fernique; Djaman Allico Joseph; Dosso Mireille; Coulibaly Adama. *Scholars Academic Journal of Biosciences*, 2014; 2(10): 663-670.
20. E Kpemissi Amana. Thèse de Doctorat, Université de Lomé, Togo, 2007.
21. Bidie Alain Dit Philippe; B N'guessan Banga; F Yapo Adou; N'guessan Jean David. *Sciences & Nature*, 2011; 8: 1-11.
22. Zakia Khanama; Chew Shwu Wen; Irshad Ul Haq Bhatb. *Science Direct*, 2015; 27: 23-30.
23. Munmi Borkatak; Bibhuti Bhusan Kakot; Lakhi Ram Saikia. *International Journal of Pharmaceutical Sciences Review and Research*, 2013; 23: 116-120.
24. JHS Thangara; O Adjei; BW Allen; F Portaels. *Journal of Antimicrobial Agents and Chemotherapy*, 2000; 45(2): 231-233.
25. P Franchomme; D Pénoël. *Revue Judiciaire Editeur Limoges*, 1990; 2: 73-227.
26. N Sharmila; N Gomathi. *International Journal of Phytomedicine*, 2011; 3: 151-156.
27. J Parveen; AK Ismail; A Erlina; AR Raha; ZH Yumi. *African Journal of Biotechnology*, 2011 ; 10(81): 18795-18799.
28. E Guinoiseau. *Thèse de Doctorat soutenue le 6 Décembre 2010*. Université de Corse-Pasquale Paoli, 2011; 149.