PHYTOCHEMISTRY AND STUDY OF THE ANTIMICROBIAL ACTIVITY OF FRACTIONS OF THE HYDROETHANOLIC EXTRACT OF PUPALIA LAPPACEA (L.) JUSS (AMANRATHACEAE)

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Abstract: *Pupalialappacea (*Amanrathaceae*)* is a plant traditionally used in various ailments including febrile states and diarrhoea as a decoction and also in infertility. The control of bacterial infections is becoming complex due to the fact that many bacteria have developed resistance to most antibiotics, which is a major health problem worldwide. It is within this framework that we wanted to study the phytochemistry and the anti-microbial activity of the fractions of the hydro ethanolic extract of the plant used in traditional medicine it is *Pupalialappacea* (L.) JussAmanrathaceae
We prepared the hydro ethanolic extract from the dried powdered leaves of these plants and the yield of crude extract is 20%. We then fractionated the hydro ethanolic extract obtained by the liquid-liquid extraction method using successively the following solvents of increasing polarity: cyclohexane, dichloromethane, ethyl acetate, and methanol. We measured flavonoids and polyphenols by the colorimetric method and the FolinCiocalteu method in these different fractions and then tested the hydroethanolic extract and the fractions on four reference microbial strains (Escherichia coli ATCC 25922 ; Salmonella typhi ATCC 14028 ; Staphylococcus aureus ATCC 25923 Pseudomonas aeruginosa ATCC 27853) The results showed us that the polar fractions are very rich in polyphenols which confirms the results obtained during the phytochemical screening carried out; and these fractions have a more interesting bactericidal activity than the hydro ethanolic extract. This plant could be an alternative in primary care systems for microbial infections.

Keywords: *Pupalialappacea* Bactericidal Concentration Minimum Inhibitory Concentration

INTRODUCTION

Bacterial infections are caused by different microorganisms and are the cause of the most fatal diseases and widespread epidemics. Many antibiotics are developed to treat them. However, their misuse is the cause of the emergence of bacterial multidrug resistance. There is a dire need for renewal of active ingredients (Mwambete 2009). These sought-after molecules must possess various other chemical properties and use new mechanisms of action against pathogenic microbes (Mada et al 2013).The control of bacterial infections is becoming complex due to the fact that many bacteria have developed resistance to most of the antibiotics which has been a major health problem globally.

However, there is concern about the adverse effects of synthetic molecules intended to combat oxidative stress and bacterial infections. It therefore seems important to find an alternative to the use of conventional antibiotics. Herbal remedies are an alternative in primary care systems and therefore a promising avenue for the development of traditionally improved medicines. It is within this framework that we wanted to study the phytochemistry and the anti-microbial activity of the fractions of the hydro ethanolic extract of the plant used in traditional medicine, namely *Pupalia lappacea* (L.) Juss Amanrathaceae.

Pupalia lappacea (L.) Juss is a highly branched herbaceous perennial or annual (height: 1 m). The leaves are oval elliptic to oblong or subcircular (length: 20-100 mm, width: 10-50 mm), with a wedge-shaped base, acuminate apex, and glabrescent petiole (length: 20-25 mm). The flowers with oblong ovate or oval lanceolate tepals (length: 4-5 mm), grouped in terminal spiciform thyrse (length: 5-50 cm). The capsules are ovoid (length: 2-3 mm). It is a plant of tropical and subtropical Africa, of the Indian Ocean (east Madagascar), of tropical Asia (from India to the Philippines and New Guinea). It is traditionally used in febrile states in diarrhoea in the form of decoction and also in sterility.

MATERIAL AND METHOD

Plant material

The plant material consists of dried leaves of *Pupalia lappacea*harvested in December 2020 in Abomey-Calavi the plant was identified at the National Herbarium of the University of Abomey Calavi. The harvested leaves were washed and then dried at room temperature in a ventilated room of the Pharmacognosy laboratory for three weeks before being reduced to powder.

Extraction

The extraction was done for the hydro ethanolic extract by mixing 50g of powder in 500 ml of hydro ethanolic mixture (40V/60V respectively) for 48 hours. After respective filtration on Whatman paper N°1 the filtrates obtained were evaporated using a rotary evaporator at 40°C. The residues of this filtrate were dried in the oven for 48 hours at 40°C to obtain the dry extracts.

Liquid-liquid extraction method

The liquid-liquid extraction is carried out by the intimate contact of the solvent with the solution in a separating funnel. The separation of the phases is obtained by gravimetric or centrifugal decantation after stirring of the whole. The solution consists of the crude hydroethanolic extract dissolved in 50 mL of distilled water. We used successively during the extraction 500 mL of cyclohexane, dichloromethane, ethyl acetate and methanol. The different fractions collected were evaporated with a rotavapor.

Phytochemical Screening

The presence of the main chemical groups in the extracts was investigated using the tests described by Bassene (2012): flavonoids (Shibata test) tannins (Stiasny reaction followed by ferric chloride reaction), carotenoids (Carr-Price reaction), anthracenes (Dragendorff reagent), sterols (Libermann-Buchard reaction), cardiotonic heterosides 'Baljet, Kedde and Raymond-Marthoud reaction) and saponosides (**Foam** index**)**

Polyphenol content

The polyphenol content of the extracts is determined by the Folin - Ciocalteu method. 1 mL of Folin's reagent is added to 1 mL of the solution of each extract, then 3 min later 1 mL of 25% sodium carbonate. After 2 hours of incubation, the samples were centrifuged at 4000 rpm for 4 minutes. The absorbances were then read with a spectrophotometer at 670 nm. Three tests were performed for each concentration of product tested.

A calibration curve based on a dilution series of tannic acid (0.005-0.01-0.015-0.02-0.025-0.03-0.025-0.03-0.035-0.04 mg/ml) was treated in the same way as the extracts. The results are expressed as milligram equivalent of tannic acid per gram of dry extract 'mg ETA/g).

Determination of Flavonoids

The flavonoid content of the extract was determined using the aluminium trichloride colorimetric method. A quantity of 100µL of the extract was mixed with 0.4 mL of distilled water and subsequently with 0.03 mL of 10% $ALCL_3$ solutionwas added. To the mixture, 0.2 µL of1M $NaNO_2$ solution and 0.25 mL of distilled water were added after a 5 min rest. The mixture was vortexed and the absorbance was measured at 510 nm. The results are expressed as milligrams of catechin equivalent per g of dry plant material

BACTERIAL MATERIAL

Consisting of four reference strains provided by the Research Unit in Applied Microbiology and Pharmacology of Natural Substances

Table 1: **Bacterial Material**

Strain	Origin
Pseudomonas aeruginosa	ATCC 27853
Salmonella Typhi	ATCC 14028
Staphylococcus aureus	ATCC 25923
Escherichia coli	ATCC 25922

DETERMINATION OF MIC IN LIQUID ENVIRONMENT

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of the substance for which there is no growth visible to the naked eye after an incubation time of 18 to 24 hours. Using a platinum loop, a quantity of bacterial strain previously preserved in Mueller Hinton agar was picked by simple scraping and then transferred by quadrant onto a plain agar plate and incubated at 37°C for 18 to 24 hours to obtain isolated colonies. After this incubation time, 3 to 5 colonies were picked, inoculated in 10 mL of broth and incubated at 37°C for 3 to 5 hours. During this incubation time and in parallel, the concentration ranges of each plant extract were prepared using the liquid double dilution method with a geometric progression of extract concentrations. They generally range from 0.781 mg/mL to 100 mg/mL. For each concentration range, 0.2 mL was taken and placed in a specific tube of a series of experimental tubes. In this series called the test series, one tube served as a growth control (containing 0.2 mL of sterile distilled water). After 3 to 5 hours of incubation, 0.2 mL of the inoculated broth was removed and homogenized with a "VLEP Scientifica" vortex mixer in 20 mL of sterile Mueller Hinton broth. Then, 1.8 mL of the latter broth was taken to complete the volume (0.2 mL) of the tubes of the 2 mL test series. Next to the test series, a reference series was prepared. In the latter, the experimental tubes each contained 0.2 mL of each concentration of plant extract previously prepared and the control tube 0.2 mL of sterile distilled water. To all the tubes of the reference series, 1.8 mL of sterile broth was added. The set of experimental tubes of the test series and the experimental tubes of the reference series were homogenized using a "VLEP Scientifica" type vortex shaker and then incubated at 37°C for 18 to 24 hours (Nassif et al., 1990; Okou et al., 2015). One day after incubation, the minimum inhibitory concentration (MIC) was determined by direct reading, by eye, in daylight. For the determination of this parameter, we compared concentration by concentration, the tubes of the test series with those of the reference series in search of absence of turbidity (Marmonier, 1990; Okou, 2012). This MIC determination was repeated during three successive experimental tests.

DETERMINATION OF MBC IN SOLID ENVIRONMENT

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of the substance that leaves no more than 0.01% surviving germs.

After the MIC determination, the growth control tube of a given bacterial strain was diluted from 10 to 10^{-4}in a geometric progression of reason 10^{-1}. The various dilutions were then plated on a Mueller Hinton agar plate, on 5 cm strips using a calibrated loop (Box A). To better appreciate the evolution of the sensitivity of the bacterial strains used in the presence or absence of plant extract, inocula obtained from a given bacterial strain were plated on a Mueller-Hinton agar plate on 5 cm strips using a calibrated loop. The inocula plated were the inoculum from the growth control tube, the inocula where turbidity was not visible, and some inocula preceding the tube that determined the MIC (high bacterial load) (Box B). Finally, Boxes A and B were incubated at 37°C for 18 to 24 hours. After this incubation time, comparison of the number of colonies on the streak at dilution $10^{-4}$of Box A with that of each streak of Box B allowed the determination of the minimum bactericidal concentration. According to Marmonier(1990):

- if the MBC/MIC ratio ≤ 4, the test substance is bactericidal.
- if the MBC/MIC ratio is > 4, the test substance is bacteriostatic.

RESULTS AND DISCUSSION

Table 2: Fractionation yield of the crude extract of *Pupalialappacea*

Plant material	Retrieved from	Mass	Performance
Crude extract (20g)	Extract C_6H_{12}	0.25 g	1.25%
	Extract CH_2CL_2	0.29 g	1.45%
	AcOEt extract	3.58 g	17.9%
	MeOH extract	3.4 g	17%
	Aqueous Extract	9.25 g	46.2%

Phytochemical screening

Phytochemical screening revealed the presence of flavonoids, tannins and saponosides in the hydro ethanolic extract of the plant. Reducing compounds, anthracenes, steroids, coumarins are also present in the plant extract; however, alkaloids, triterpenes, cardiotonic heterosides, quinones, anthocyanins, were not found in the plant extract which is the subject of the current study.

Total polyphenol content

The determination of the total polyphenol content in the extract was done by the Folin-Ciocalteux method. The content was reported in mg gallic acid equivalent/g dry plant material. It is an extract rich in total polyphenols. This is confirmed by phytochemical screening which reveals the presence of flavonoids, tannins and saponosides in the extract.

Table 3: Total Flavonoid Concentration; Tannin; Total Polyphenols

	Phenolic compounds dosage			**Antiradical activity**
	PT (AGE)	**FLA (EQ)**	**TAC (EC)**	**DPPH (IC_{50})**
Pupalia lappacea	170.0 ± 1.01	82.76 ± 3.42	43.61 ± 6.62	0.03
Standards				
AG				0.03
BHA				0.09
Q				0.1

Table 4: Total polyphenol concentration (mg GAE/L) of the fractions of the hydroethanolic extract of *Pupalia lappacea*

Extracts	Total polyphenol concentration (mg GAE/L)
Raw extract	93.7 ± 3.2
Extract C_6H_{12}	15.9 ± 0.3
CH_2Cl_2 extract	62.9 ± 2.0
AcOEt extract	289.7 ± 4.3
MeOH extract	212.7 ± 1.8
Aqueous Extract	147.7 ± 2.8

Table 5: Phytochemical screening of the hydro ethanolic extract

Compounds		***Pupalia lappacea***	**Compounds**	***Pupalia lappacea***
Tannins	Gallic	+	Reducing compounds	+
	Catechin	+	Quinonic	-

Flavonoids	+	Mucilage	+
Anthocyanins	-	Free anthracene	+
Leuco-anthocyanins	+	O-heterosides	-
Saponosides	+	C- heterosides	+
Cyanogenic derivative	-	Cardiotonic derivatives	-
Triterpenes	-	Alkaloids	-
Steroids	+	Coumarins	+

Determination of the Minimum Inhibitory Concentration (MIC) in liquid environment

In liquid environment the absence of turbidity was observed for the different strains studied from the concentrations of:
- 6.25mg/L; 4.25mg/L; 3.125mg/mL; 6.5mg/L for the ethyl acetate fraction of the hydroethanolic extract *Pupalia lappacea*on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* respectively;
- 0.39 mg/L; 3.12mg/L; 0.78mg/L; 156mg/L for the methanolic fraction of the hydroethanolic extract of *Pupalia lappacea*on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* respectively
- 6.25mg/L; 6.25 mg/L; 3.125mg/ L; 3.125mg/L for the hydroethanolic extract of *Pupalia lappacea* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa*respectively;
We observed turbidity for the dichloromethane fraction of the hydroethanolic extract for all concentrations.

Determination of the Minimum Bactericidal Concentration (MBC) in solid environment

Comparison of the number of colonies on the streak at dilution 10^{-4} of box A with that of a streak of box B allowed determining the concentrations of:

- 6.5mg/L; 6.25mg/L; 12.5mg/L; 12.5mg/L for the action of the ethyl acetate fraction of the hydroethanolic extract *Pupalia lappacea* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus*strains respectively;
-1.56mg/L; 6.25mg/L; 1.56mg/L; 3.52mg/L for the action of the methanolic fraction of the hydroethanolic extract of *Pupalia lappacea* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* strains respectively:
- 25mg/L; 25mg/L; 12.5mg/L; 12.5mg/L for the action of the hydroethanolic extract of *Pupalia lappacea* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa*; *Staphylococcus aureus* respectively;

DISCUSSION

1-Phytochemical screening and determination of total polyphenols

We had polar fractions rich in polyphenols compared to the apolar fractions of the extract, which confirms their presence in the hydroethanolic extract.

2-Determination of the antibacterial activity of different plant extracts

2-1 Determination of the minimum inhibitory concentration (MIC) in liquid environment

Insofar as the absence of turbidity was observed for the different strains studied from the:
- 6.25mg/L; 4.25mg/L; 3.125mg/mL; 6.5mg/L for the ethyl acetate fraction of the hydro ethanolic extract of *Pupalia lappacea* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* respectively;
- 0.39 mg/L; 3.12mg/L; 0.78mg/L; 1,56mg/L for the methanolic fraction of the hydroethanolic extract of *Pupalia lappacea*on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* respectively;
- 6.25mg/L; 6.25 mg/L; 3.125mg/L; 3.125mg/L for the hydroethanolic extract of *Pupalia lappacea*on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa*; *Staphylococcus aureus* respectively;
It is possible to deduce that these concentrations constitute the minimum inhibitory concentrations (MIC) of these tested substances.

2-2-Determination of the minimum bactericidal concentration (MBC) in solid environment

The results in the table show that these different fractions tested have activity on these strains. On the basis of MBC, the ethyl acetate fraction of the hydro ethanolic extract is more active on *Pseudomonas aeruginosa* (MBC equal to 12,25mg/L) and *Staphylococcus aureus*strains than on *Escherichia coli* (MBC equal to 6,25mg/L) and *Salmonella typhi*strains

The methanolic fraction of the hydro ethanolic extract of *Pupalia lappacea*is more active on *Salmonella typhi*(MBC equal to 6,25mg/L) and *Staphylococcus aureus*strains and less active on *Escherichia coli*and *Pseudomonas aeroginosa*strains (MBC equal to 1,56mg/L).

The hydroethanolic extract of *Pupalia lappacea* is more active on*Escherichia coli*(MBC equal to 25mg/L) and *Salmonella typhi*strains than on and *Pseudomonas aeroginosa*strains (MBC equal to 12,5mg/L) and *Staphylococcus aureus.*

On the basis of the comparisons of the MBC of the various extracts tested with those of the hydro ethanolic extracts (MBC_{crude}/ $MBC_{ethyl\ acetate;}$ MBCcrude/ $MBC_{methanolic}$) and on the in vitro growth of the various strains studied, it is possible to say that: the ethyl acetate fraction is 4 times more bactericidal than the hydro ethanolic extract of the plant on the *Escherichia coli* and *Salmonella typhi* strains.

The ethyl acetate fraction of the hydroethanolic extract of *Pupalia lappacea*is not more bactericidal than the crude (hydroethanolic) extract of *Pupalia lappacea*on *Pseudomonas aeruginosa and Staphylococcus aureus strains.*

The methanolic fraction of *Pupalialappacea* is 16 times more bactericidal on *Escherichia coli*strains, 4 times more bactericidal on *Salmonella typhi,* and 8 times more *Pseudomonas aeruginosa* and 3 times more bactericidal on *Staphylococcus aureus* than the crude extract of *Pupalia lappacea.*

The dichloromethane extract of the hydroethanolic extract of the plant is neither bactericidal nor bacteriostatic on the different strains.

To the extent that the reports MBC/MIC in the table as noted less than or equal to 4. It's possible to deduce that the action of this extract tested on the various bacterial strains studied is bactericidal (Marmonier 1990)

CONCLUSION

The phytochemical study of the crude extract of *Pupalia lappacea*and its fractions from the liquid-liquid extraction showed a high content of total polyphenols; especially for the polar fractions. The results of the study of antibacterial activity showed that the different fractions of the hydro ethanolic extract of *Pupalia lappacea* have an antibacterial activity on the studied strains. It is mainly the polar fractions.

This bactericidal action is dose-dependent because it is linked to the increase in the concentrations of the extract studied.

Table 6: Summary of the antibacterial parameters of the effects of the different extracts of *Pupalialappaceae*on the in vitro growth of the strains studied

		MICROBIAL STRAINS STUDIED			
		E. coli	*S. typhi*	*P. aeruginosa*	*S. aureus*
Ethyl acetate fraction	MIC (mg/mL)	6.25	4.25	3.125	6.5
	MBC (mg/mL)	6.5	6.25	12.5	12.5
	MBC/MIC	1.04	1.5	4	2
Methanol fraction	MIC (mg/mL)	0,39	3,12	0,78	1,56
	MBC (mg/mL)	1,56	6,25	1,56	3,52
	MBC/MIC	4	2	2	2
Crude extract	MIC (mg/mL)	6.25	6.25	3.125	3.125
	MBC (mg/mL)	25	25	12.5	12.5
	MBC/MIC	4	4	4	4

BIBLIOGRAPHIC REFERENCES

1. Aboya M. J., 2013. Résistance bactérienne etphytomolécule antimicrobienne issues deMorindamorindoïdes. Thèse de Doctorat del'Université Félix Houphouët-Boigny, Abidjan,Côte d'Ivoire, 184 p.
2. Bassène E. 2012. Initiation à la Recherche sur les Substances Naturelles : Extraction- Analyse-Essais Biologiques. Presse Universitaires de Dakar : Dakar ; 17, 94-96,140 p.

3. Candan F., Unlu M., Tepe B., Daferera D., PolissiouM., Sökmen A., Akpulat H. A., 2003. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. MillefoliumAfan. (Asteraceae). Journal ofEthnopharmacology, 87(2-3): 215-220.
4. Cheurfa M., Allen R., Sebaihia M., Belhireche S., 2013.Effet de l'huile essentielle de Thymus vulgarissur les bactéries pathogènes responsables degastroentérite. Phytothérapie, 11 : 154-160.
5. Dinzedi M. R., 2015. Activités antibactériennes des extraits de *Terminaliacatappa* et*Thonningiasanguinea* sur *Escherichia coli*, *Klebsiella pneumoniae* et *Staphylococcus aureus*
6. multiresistantes d'origine humaine. Thèse deDoctorat de l'Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire, 133 p.
7. Dromer F. et Dupont B., 1996. The increasing problem of fungal infections in immunocompromised host. Journal of MycologyMedical, 6(I) : 1-6.INSPQ (Institut National de Santé Publique du Québec),2015. Mesures de prévention et de contrôle dela transmission des bacilles Gram négatif multirésistantsdans les milieux de soins aigus auQuébec, N° 2022 : 16 p.
8. Lagnika L., Amoussa M., Adjovi Y., Sanni A., 2012.Antifungal, antibacterial and antioxidant properties of *Adansoniadigitata* and *Vitex doniana* from Bénin pharmacopeia. Journal of
9. Pharmacognosy and Phytotherapy, 4(4) : 44-52.
10. Marmonier A. A,. 1990. Technique de diffusion engélose : Méthode des disques. Dans :Bactériologie Médicale, Techniques usuelles, pp237-244.
11. Krichen F., Karoud W., Sila A. D., Abdelmalek B. E., Ghorbel R., Ellouz-Chaaboumi S., Bougatef A., 2015 Extraction, characterisation and antimicrobialactivity of sulfated polysaccharides fromfish skins. International Journal of Biological Macromolécules, 75, 283-289
12. Nassif X., Marmonier A. A., Carbonelle B., 1990. Étude del'activité bactéricide des associations binairesd'antibiotiques. Dans : Bactériologie Médicale,Techniques usuelles, pp 253-260.
13. Okou OC., 2012. Efficacité et spectre d'activité desextraits de *Mitracarpus scaber*Zucc. Ex Schult+ Scult.f. (Rubiaceae) et de l'acide fusidique surles Bactéries Cocci Gram Positif. Thèse deDoctorat de l'Université Félix Houphouët-Boigny,Abidjan, Côte d'Ivoire, 229 p.
14. Okou OC., Ackah JAAB., Angaman DM., Djaman AJ., 2015.Activity of *Mitracarpusscaber* on *Enterococcus faecalis*. World Journal of PharmaceuticalResearch, 4(5) : 377-385OOAS (Organisation Ouest Africaine de la Santé),2013. Pharmacopée ouest africaine, Dans :Solanum torvum, 195-198.
15. Onzo Caroline Fifamé, Azokpota Paulin, Dah-Nouvlessounon Durand, Toure Halphane, Adjatin Arlette, Baba-Moussa Lamine 2015, Evaluation de l'activité antimicrobienne de quatre feuilles utilisées comme emballages dans l'artisanat agroalimentaire au Bénin .Journal ofApplied Biosciences 95 : 9015-9027
16. Ouattara A., 2014. Évaluation et essai d'optimisation desactivités antibacteriennes de Pericopsislaxiflora (Papillionaceae) et Vitex Doniana(Verbenaceae), plantes utilisées dans letraitement traditionnel des gastroentérites parles populations des régions Nord et Nord-Est dela Côte d'Ivoire. Thèse de Doctorat del'Université Félix Houphouët-Boigny, Abidjan,
17. Côte d'Ivoire, 119 p.
18. Ouattara SFS, D'almedia MA, Kouakou K, 2009.Identification d'entérovirus par la techniquecellulaire chez l'enfant de 0 à 5 ans à Boribana,quartier précaire d'Abidjan (Côte d'Ivoire).
19. European Journal of Scientific Research, 32 : 500-513.
20. Parashar UD, Gibson CJ, Bresee JS, Glass RI, 2006. Rotavirus and severe childhood diarrhea. EmergingInfectiousDiseases, 12 : 304-306.Sirot J, 1990. Évaluation de l'activité antibactériennedes antibiotiques in vitro. Dans : Bactériologiemédicale 2e édition/Flammarion, 297-315.
21. Soro D, Koné MW, Kamanzi K, 2010. Évaluation desactivités antimicrobiennes et antiradicaux libresde quelques taxons bioactifs de Côte d'Ivoire.European Journal of Scientific Research, 40 : 307-317.
22. Toty A A, Guessennd N, Bahi C, Kra A. M., Otokore D. A., Dosso M.2013. Evaluation in vitro de l'activité antibactérienne de l'extrait aqueux de l'écorce de tronc de Harunganamadagascariensis sur la croissance de souches multi-résistantes. Bulletin de la Société Royale des Sciences de Liège V82, 2013, p.12-21
23. Tsakala TM, Tona GL, Mena K, Mboma JC, VanguJM, Voso SM, Kanja GL, Kodondi KK, MabelaM, Walo R, 2005. Évaluation des prescriptionsdans le traitement du paludisme et de la gastroentérite en milieu hospitalier cas deshôpitaux de la République Démocratique du
24. Congo, Cahiers Santé, 15: 119-124.
25. Zirihi GN, Kra AKM, Guédé-Guina F, 2003. Évaluationde l'activité antifongique de Microglossapyrifolia(Lamarck) O. Kantze (Astéracées) « PYMI» surla croissance in vitro de Candida albicans,Revue de Médecine et Pharmacie Afrique, 17 :11-18.

26. Zirihi GN, Grenier P, Guédé-Guina F, Bodo B, Mambu L, 2005. Isolation, characterization and antiplasmodial activity of steroidal alkaloids from Funtumiaelastica (Preuss) Stapf. Bioorganicand Medicinal Chemistry Letters, 15 : 2637-2640.