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Study of the *in vitro* Antibacterial Activity of the Aqueous and Hydroethanolic Extracts from the Leaves of the *Erythrococca anomala (*Euphorbiaceae*)* on Six Bacterial Strains

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Abstract The problems of the resistance of pathogenic bacteria to the common antibiotics in hospitals and the high costs of the treatments of pathologies due to those resistant bacteria have brought about innovation in the search for alternative treatments: plants. The purpose of this study is to assess the antibacterial activities of the aqueous and hydroethanolic extracts from the leaves of *Erythrococca anomala* on the *in vitro* growth of six multiresistant bacterial strains such as: *Pseudomonas aeruginosa*, *Escherichia coli Klebsiella pneumoniae*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*. They were isolated from patients. The results of the studies showed that the aqueous extract, being more active than the hydroethanolic extract, contains Minimal Inhibitory Concentrations (MICs) which vary between 0.78 mg/mL and 12.5, whereas the Minimal Bactericidal Concentrations (MBCs) vary between 0.78 mg/mL and 25 mg/mL. Concerning the hydroethanolic extract, the MICs and the MBCs vary between 3.125 mg/mL and 25 mg/mL respectively. *Streptococcus pneumoniae* and *Staphylococcus aureus* are more susceptible because they need 0.78 mg/mL and 1.56 mg/mL respectively before they are completely inhibited by the aqueous extract. Compared with the Ciprofloxacin, a pure molecule, those extracts have clearly better antibacterial activity. That may justify why those leaves are used in traditional areas as anti-infectious treatment. *Erythrococca anomala* is a glimmer of hope for the treatment of infectious diseases in Côte d'Ivoire.

Keywords *Erythrococca anomala,* Antibacterial Activity, *In Vitro*

1. Introduction

Antibiotics have always been man's most effective means against infectious diseases since they were discovered. Among these antibiotics, the beta-lactam antibiotics are used widely today and especially in developing countries. This is due to the large extent of their spectrum of activity, their harmlessness, their efficiency and above all they are affordable [1].

Unfortunately, since the antibiotics are used extensively [2], incorrectly and inappropriately for treatments, we can note the emergence of multi resistant bacteria [3] in many sub-Saharan countries today [4].

As the major success of modern medical science has its origin in the discovery and use of antibiotics, the emergence and expansion of the multi resistant bacteria are a major public health issues today [5].

Because of the emergence of resistant bacterial strains, those antibiotics failed to prove their efficiency. Moreover they are so expensive that the poor among the populations cannot afford [6].

Besides, the side effects of these pharmaceutical molecules are another problem [7]. Consequently, it is expedient to search for potential anti-bacterial agents [8, 9].We then turn towards plant based drugs which are considered as "danger free" drugs, compared with other products which are said to be "risky" for men and for the environment [10].

That justifies why we chose *Erythrococca anomala*, a plant used by Ivorian traditional doctors.

Besides, according to the World Health Organization (WHO), plants are the best sources in the quest for new

drugs. This institution and other Author reported that about between 60 % and 80% of the world population use traditional medicine against various diseases [11, 12].

In the developing countries, about 80% of populations depend on traditional medicine [13, 14]**.** The developed countries are not to be outdone since about 80% of the population use traditional medicine for their primary health care needs [15]. In developed countries, adapting traditional medicine sometime, called additional or alternative drugs plays an important role in the health system of populations [16].

In Côte d'Ivoire the leaves of that plant are macerated and used against meningitis and malaria [17, 18], meanwhile in Cameroon, macerated and in decoction, they are used to treat dental pain, and also used as laxative and purgative to help evacuate worms. In Nigeria, the bark is used to treat arthritis and rheumatisms [19].

As complement, the objective of this study is to assess the antibacterial proprieties of the aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* on the *in vitro* growth of six multi resistant bacterial strains, pathogenic with men.

2. Material and Methods

2.1. Material

2.1.1. Plant Material

The plant material consists of the leaves of *Erythrococca anomala* collected in the area of Yakassé-mé, department of Adzopé. They were washed and dried up at ambient temperature, in the shade of the sun. That plant was identified at the Côte d'Ivoire National Center of Floristic. A sample (OAT-ErAn) is available in that centre.

2.1.2. Microbial Material

The microbial support consists of clinic bacterial strains (Gram negative Bacilli: *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*; Gram negative cocci: *Neisseria meningitidis*; Gram positive cocci: *Streptococcus pneumoniae*, *Staphylococcus aureus*), isolated from patients from the University Hospital Centre of Treichville in Abidjan (Côte d'Ivoire).

Those bacteria are involved in various human pathologies which are more or less serious. They are often multi resistant to common antibiotics.

2.2. Methods

2.2.1. Preparation of the Plant Extracts

- **The aqueous extract**

The leaves of *Erythrococca anomala* were ground with a mechanic crusher (IKA® LabortechniK Staufen; Germany: Janke & Kunkel.)

One hundred (100) grams of that powder were boiled in one liter of distilled water for fifteen minutes. The subsequent substance was filtered with wool cotton, then under vacuum with ordinary filter paper. The collected filtrate was taken into the oven at 40°C to produce a dry darker brown powder: that was the crude aqueous total extract of *Erythrococca anomala* [20].

- **The hydroethanolic extract 70 %**

It was obtained from the method by Guédé-Guina [21]: One hundred (100) grams of plant powder were extracted by maceration in one (1) liter of absolute ethanol solution - water in a ratio 70: 30 (V/V), under magnetic stirring for 24 hours, on a RTC B IKA® agitator (LabortechniK Staufen; Germany: Janke & Kunkel). The subsequent homogenate was filtered on wool cotton then, under vacuum. The filtrate collected was condensed with a rotary evaporator (RVO5-ST, IKA®). The subsequent condensed dark green product was taken into an over under a temperature of 40°C for a complete drying. Thus, we had the hydroethanolic 70 % of *Erythrococca anomala*.

2.2.2. Bacteriological tests with plant extracts

- **Preparation of the inoculum**

The bacterial inoculum was prepared from the stock cultures of bacterial strains, transplanted by the technics of stria, on a Mueller Hinton®) Agar (MHA) (BIO-RAD, Marnes-la coquette, France). An isolated colony of the bacterial culture was taken by means of platinium loop homogenized in 10 mL of Mueller Hinton® Broth (MHB) (BIO-RAD, Marnes-la coquette, France), then incubated for 5 hours at a temperature of 37 °C to get a pre-culture. One (1) mL of this pre-culture was collected for every bacterial strain and added to 10 mL of sterile MHB. That bacterial suspension developed was assessed at about 10^6 cells/mL thus making up the inoculum.

- **Determining the diameters of the inhibition areas**

The susceptibility of the strains to the extracts of plants was carried out by means of the agar diffusion test. The MHA, in the glass Petri dishes® (Petri dishes® in glass: 12O × 20, JENA^{ER}, GLASWERK SCHOTT& GEN., MA Germany) were sown by flooding. Control wells using a sterile punch, 8 mm diameter well were carried in the agar. Every well received 50 µl of plant extract to be tested with concentrations of 200 ml and 100 mg/mL. Comparatively to the test boxes, the wells received 50 µl of Ciprofloxacin (5µg), a reference antibiotic. The control wells which received 50 µl of distilled water were also developed.

Thirty minutes after the diffusion at the temperature of the laboratory, all the petri dishes were incubated at a temperature of 37 °C for 18 to 24 hours. The presence or the absence of an inhibition area around the wells with control wells was noted [22].

The interpretation was done according to Duraffourd and *al*., Ponce and *al*., [23, 24].

- **Determining the minimal inhibitory concentration (MIC) and the Minimal Bactericidal Concentration (MBC)**

The quest for these two parameters was done in liquid medium in MHB, according to De Paiva and *al*. [25].

The aqueous and hydroethanolic extracts from the leaves of *Erythrococca anomala* (*E. anomala*) were tested to determine the MIC and the MBC for each bacterial strain by the broth dilution method.

For that purpose, we use series of eight hemolysis tubes numbered started from C_0 to C_7.

- **Minimal Inhibitory Concentration (MIC).**

This antibacterial parameter is defined as the lowest concentration of substance for which there is no visible growth with naked eye after 18 to 24 hours of incubation.

Thus, these hemolysis tubes numbered C_0 to C_7, received, by means of a micropipette with sterile protective cover (PRO Accumex®), 1mL of crude inoculum. Then it was added into the tubes, 1 mL of plant extract according to the range of the prepared concentration. This share of plant extracts was done in such a way that 1 mL of plant extract can be transferred into tube C_0. Tube C_1 received 1 mL of 100 mg/mL and so on until C_6 in which 1mL of the solution was put with 3.125 mg/ml. Tube C_7 received 1mL of sterile BMH instead of plant extract and was used as growth control.

The final (intermediate) concentrations that resulted vary between 100 mg / mL and 1.56 mg/mL.

At last, 1mL of the mixture in each tube (C_0 to C_6) was removed, then 1mL of BMH was added into each tube just like in tube C_7.

Considering the volume/volume thus carried out, the concentration in the tube was reduced by half, i.e. a geometric suit of reason ½.

The subsequent final concentrations vary from 50 mg / mL to 0.78 mg/mL. These tubes were incubated at 37 °C for 24 hours, and then observed with naked eyes. The growth was observed on the basis of their turbidity using the series of tubes without bacterial suspension as control.

- **Numbering the inoculum**

On the same day, we developed control boxes by numbering the inoculum from dilutions. The dilutions (d) are : d = 1/10, 1/100, 1/1000, 1/10000. The last dilution d = 1/10000, corresponds to 0.01%, and the inoculum corresponds to 100% of survivors, i.e (d = 10°). The various dilutions and the inoculum are cultured by the use of a calibrated loop of 5µl on the MHA, following 5 cm lines, carried out on the reverse of glass petri boxes®. The boxes were incubated at a temperature of 37°C for 18 to 24 hours, and then kept at a temperature of 4°C till the end of the tests.

On the second day, (from 18 to 24 hours of incubation of test tubes and control boxes), The MIC of the various extracts was determined by comparing the contents of the tubes (C_0 to C_6), from the macroscopic point of view, with the content of well C_7, as growth control reference with no extract.

Soon after determining the MIC on the second day, the content of the tubes, that showed no trouble at naked view (with that of tube of MIC), was cultured just like those of control boxes and incubated at 37 °C for 18 to 24 hours : These were test boxes.

- **The Minimal Bactericidal Concentration (MBC)**

The Minimal Bactericidal Concentration (MBC) is considered as the lowest concentration of plant extract for which there is more than 0.01% of surviving bacteria. MBC is determined, on the third day, by comparing the number of surviving bacterial germs from test box or boxes, with the control box.

After 24 hours of incubation with a drier at 37 °C, the number of colonies on the stria was compared with those of the numbering boxes of the inoculum (controlled boxes). Thus, the first test tube whose germs were on the stria is inferior or equal to that of the dilution 10^{-4} , corresponds to the MBC.

The antibacterial effect is said to be bactericidal or bacteriostatic compared with the ratio : MBC/MIC

Actually, if MBC/MIC=1 to 2, the effect is bactericidal, and if MBC/MIC= 4 to 16, the effect is bacteriostatic [26, 27].

3. Results

The results of the various anti-bacterial tests carried out on the culture mediums of Mueller-Hinton have been grouped in Table I and in Table II.

Table I delineates the inhibition diameters (mm) of the aqueous and hydroethanolic extracts from the leaves of *E. anomala* and of Ciprofloxacin compared with the concentrations.

Table II delineates the values of MIC, MBC and the ratio MBC/MIC of the aqueous and hydroethanolic extracts from the leaves of *E. anomala.*

These results were collected following several tests.

- Concerning the quest for the inhibition diameters of the aqueous and hydroethanolic extracts, the results stemmed from the averages of three tests.
- for the quest for antibacterial parameters MIC and MBC, following at least three tests, the values remained invariable.

Table I. Inhibition diameters (mm) of the aqueous and hydroethanolic extracts from the leaves of the *Erythrococca anomala* and of Ciprofloxacin compared with the concentrations.

Souches	Ciprofloxacin(µg/mL)	Aqueous extract (mg/mL)		Hydroethanolic extract (mg/mL)	
	5	200	100	200	100
S. aureus	19±2,85	21±1,3	13±0,58	18±0,37	14±0,44
S. pneumoniae	10±0,34	18±0,7	15±0,54	23±0,33	20±0,34
P. aeruginosa	23±0,47	21±3,5	19±0,83	15±0,77	13±0,58
N. meningitidis	00	22±0,83	15±0,34	25±3,57	21±0,55
K. pneumoniae	00	18±0,58	13±0,74	13±0,39	11±0,38
E. coli	00	22±0,73	20±0,34	19±0,97	16±0,83

Table II. Values of MIC, of MBC and the ratio MBC/MIC of the aqueous and hydroethanolic extracts from the leaves of the *Erythrococca anomala*.

Strains	Aqueous Extracts			Hydroethanolic Extracts			
	CMI (mg/mL)	CMB (mg/mL)	CMB/CMI = r	CMI (mg/mL)	CMB (mg/mL)	CMB/CMI	Interpretation
Staphylococcus aureus	0.78	1.56	2	3,125	3.125	1	bactericidal
Streptococcus pneumoniae	0.78	0.78	1	25	25	1	bactericidal
Pseudomonas aeruginosa	3.125	6.25	2	6.25	6.25	1	bactericidal
Neisseria meningitidis	3.125	6.25	2	6.25	6.25	1	bactericidal
Klebsiella pneumoniae	12.5	25	2	12.25	12.25	1	bactericidal
Escherichia coli	6.25	12.5	2	3.125	3.125	1	bactericidal

r = 1 to 2

4. Discussion

This study helped assess the antibacterial of the aqueous and hydroethanolic extracts, on the *in vitro* growth of the multi resistant pathogenic bacterial strains such as: *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae, Neisseria meningitidis*, *Streptococcus pneumoniae, and Staphylococcus aureus*).

Actually, the first part of this study that deals with search for the inhibition diameters (mm) of the aqueous and hydroethanolic extracts from the leaves of ***E. anomala*** and the Ciprofloxacin according to the concentrations, delineates the influence those substances have on the *in vitro* growth of those six bacterial strains, by using the agar diffusion method.

The analysis of the experimental data shows that the extracts from the leaves of this plant, have an effective antibacterial activity for 200 mg/mL and 100 mg/mL, for 50 µL of the extracted substance, 10 mg and 5 mg respectively of the substance contained in the volume extracted.

Thus, 200 mg/mL of extract have the best inhibitory activity on the *in vitro* growth of the six bacterial strains compared with 100 mg/mL because the inhibition diameters are higher at 200 mg/mL than at 100 mg/mL.

But, generally speaking, the aqueous extract has the best inhibitory activity, just like 200 mg/mL of aqueous extract with better anti-inflammatory activity on rats, thus explaining why we choose to divide the concentrations from 100 to 200 mg/mL [28].

In fact, the inhibition diameters at 200 mg/mL of aqueous extract for the following bacteria are higher when compared with those for 200 mg/mL for the hydroethanolic extract: Thus, we associated each bacterial germ with its inhibition diameter:

For 200 mg/mL of aqueous extract:

- *Staphylococcus aureus*: 21 mm
- *Pseudomonas aeruginosa*: 21mm
- *Klebsiella pneumoniae*: 18mm
- *Escherichia coli*: 22mm

For 200 mg/mL of hydroethanolic extract:

- *Staphylococcus aureus*: 18 mm
- *Pseudomonas aeruginosa*: 15mm
- *Klebsiella pneumoniae*: 13mm
- *Escherichia coli*: 19mm

Generally speaking, the data analysis shows that the bacteria are not totally resistant to the extracts; that could result in null inhibition diameters.

Therefore the germs have different susceptibility revealed through different inhibition diameters.

However, *Neisseria meningitidis*, *Klebsiella pneumoniae* and *Escherichia coli* resist totally to the Ciprofloxacin, that proved by null inhibition diameters.

The anti-bacterial activity of the two types of plant extracts on the *in vitro* anti-bacterial growth of the six bacterial strains improved clearly, compared with that of the Ciprofloxacin, the antibiotic under test. That may be the reason behind its therapeutic use in traditional medicine by some people.

The second part of our study consisted in determining the MICs and the MBCs of the aqueous and the hydroethanolic

extracts from the leaves of *E. anomala*.

The analysis of those results reveals that the strains under test are all susceptible to extract of plants. However, the observation of the antibacterial parameters shows that the values of the MBC vary between 0.78 and 25 mg/mL concerning the aqueous extract, whereas those of the hydroethanolic extract are between 3.125 and 25 mg/mL. It appears that the total aqueous extract has the best anti-bacterial activity.

In fact, a drug is more active against a bacterial germ when the MBC is low. Therefore, here is, in order of decreasing susceptibility to the total aqueous extract, the following germs along with respective MBCs: 0.78; 1.56; 6.25; 12.5; 25 mg/mL

Streptococcus pneumoniae
Staphylococcus aureus
Pseudomonas aeruginosa and Neisseria meningitidis,
Escherichia coli
Klebsiella pneumoniae

The antibacterial activity of the various extracts can be justified by the existence of various compounds including the tannin, flavonoids, polyphenols and saponins in the extracts from the leaves of that plant [29].

According to Harbonne and Williams[30], one of the basic funcions of flavonoids is the protection against bacterial infection. Consequently, it is not surprising that those plants rich in flavonoids, as decribed by Cushine and Lamb[31], have been used for ages by traditional doctors to treat infectious diseases. These same compounds groups, according to Erasto and *al*. [32], have antibacterial properties also. Indeed, Erasto and *al*. worked on pure isolated flavonoids. The results of those preceding studies corroborate this study, and confirmed by those of Tona and *al*., Cimanga and *al*. [33, 34]. Cimanga and *al* even isolated other flavonoids of another plant species which are luteolin and its glycosides, whose antibacterial activities have already been proved by the work of Basile et *al*. [35]; Zhu, Zhang and Lo[36]. These authors also worked on purified and isolated flavonoids.

.That may justify the noted antibacterial properties because these plants have acknowledged antibacterial properties [37]. But the noticeable presence of the chemical groups like the saponins, the flavonoids, and the alkaloids in the total aqueous extracts [29], may justify why that extract is very active, as Bragré and *al*. noted in their studies [37].

The susceptibility of the enterobacteria like *E. coli,* to the various extracts may justify why the leaves of *E. anomala* are used to treat diarrhoea. The inhibitory activities of extracts from the leaves of this plant on *N. meningitidis* may justify its use against meningitis. The susceptibility of *S. aureus* to the extracts, as discussed in this study, may justify the use of the leaves of *E. anomala* to treat whitlow and furunculosis. Besides, the susceptibility of *K. pneumoniae* to extracts may be the reason behind its use in the treatment of pneumonia.

The susceptibility of *P. aeruginosa* to extracts from the leaves of *E. anomala* is of great importance because the strains of Pseudomonas show resistance to the common antibiotics. Also, we devote a special interest in every antibacterial agent it is susceptible to.

The activity of a plant substance depends on several factors including the mode of extraction and the concentration of active ingredients [38, 39].

All extracts of this plant have a bactericidal anti-bacterial activity with regard to all bacterial strains according to Berche and *al*. and Marmonier [26, 27] and water contains the active ingredients better.

Considering the results of these studies, the use of the leaves of the *E. anomala* as an anti-infectious drug in traditional areas is expedient since the aqueous and hydroethanolic extracts from the leaves of this plant have an effective activity on the *in vitro* growth of bacterial strains under test.

5. Conclusions

Through this study of the antibacterial activity of the aqueous and hydroethanolic extracts from the leaves of *Erythrococca anomala* with regard to pathogenic and multi resistant bacterial strains, it appears that those extracts truly have a bactericidal antibacterial activity on the *in vitro* growth of the bacterial strains under test. The anti-bacterial activity of the extracts vary according bacterial species, and according to the concentrations of extracts.

Unlike the ciprofloxacin, which has its spectrum of action decreased, reflected by resistances on some bacteria tested, all the bacterial strains tested are sensitive to the extracts of this plant.

These results confirm that extracts from that plant may compete with synthetic chemicals and the antibiotics used for the treatment of infectious diseases.

These tests contribute to the scientific validation of the use of that plant species in traditional areas.

Perspective

It would be worth carrying out a deeper study on a larger number of bacterial strains and identify the active components responsible for antibacterial activities.

REFERENCES

[1] D. M. Livermore. ß-lactamase mediated resistance: past, present and future, *J Infect. Dis. Soc*. (6), 75-83, 1995.

[2] C.T. Ndour, N. M. Manga, R. Kâ, N.M. Dia/Badiane, L. Fortez, M. Seydi and *al.* L'épidémie de choléra de 2004 à Dakar: Aspects épidémiologiques, cliniques et thérapeutiques. *Med Trop*. (66), 33-38, 2006.

[3] P. Y., Savard. Caractérisation structurale et dynamique de la bêta-lactamase TEM-1 de la bactérie *Escherichia coli* par RMN liquide, Philosophiae Doctor de Biochimie et de

Microbiologie, Faculté des Sciences et de Génie, Université Laval, Québec, 224 p .2003

[4] K. O. Akinyemi, O. Olopado, C. E. Okwara, C.C. Ibe and K. A. Fasure. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity. *BMC complemen-tary* and *alternative Medicine*, (5), 1-6, 2005.

[5] A. Lozniewski, C. Rabaud. Résistance bactérienne aux antibiotiques, Fiches conseils pour la prévention du risqueinfectieux–Infections associées aux soins, CCLIN, Sud-Est, Nancy, 4 p. 2010.

[6] K. Schnaubelt. Advanced aromatherapy: the sciences of essential oil therapy. Healing Art Press (Rochester, Vermont). 138 p. 1998

[7] I. Hamad, Z. Mehmood, F. Mohammad. Screening of some Indian medical plants for their antimicrobial properties. *Journal of Ethnopharmacology*. 62: 183- 193, 1998..

[8] M. C. Recio, J. L. Rios, A. Villar. A review of some antimicrobial compounds isolated from medicinal plants reports in literature. *Phytotherapy Research* 3: 117-25, 1989.

[9] T. Essawi, M. Srour. Screening of some Palestinian medicinal plants for antibacterial activity. *Journal of Ethnopharmacology* .70: 343-9, 2000..

[10] P. P. Joy, J. Thomas, S. Matthew, B. P. Skaria. In Bose TK et al (eds) Medicinal plants. Naya Prokash publishers, Calcutta, *Tropical*. *Horticulture*. 2: 449-632, 1998.

[11] World Health Organization (W.H.O.), 2002. .Traditional Medicine Strategy 2002–2005. Geneva: World Health Organization.

[12] X. Zhang. Traditional medicine: its importance and protection. In: Twarog S, Kapoor P (eds). Protecting and Promoting Traditional Knowledge: Systems, National Experiences and International Dimensions. Part 1. The Role of Traditional Knowledge in Healthcare and Agriculture. New York: United Nations. 3–6, 2004.

[13] G. C. Kirby,. Medicinal plants and the control of parasites. *Transaction of the Royal Society of Tropical Medicine and Hygiene* 90: 605-609, 1996.

[14] K. Hostettmann, A. Marston. Twenty years of research into medicinal plants: results and perspectives. *Phytochemistry Reviews* 12: 75-285, 2002.

[15] N. R. Farnsworth, D. D. Soejarto ,. Potential consequence of plant extinction in the United States on the current and future availability of prescription drugs. *Economic Botany* 39: 231-40, 1985.

[16] World Health Organization (W.H.O.), 2003. Traditional medicine. Available from: http://www.who.int/mediacentre/factsheets/fs134/en/print.html.

[17] E. J. Adjanohoun, L. Ake- Assi. Contribution au recensement des plantes médicinales de Cote d'Ivoire. Centre National de Floristique de l'Université Nationale de Côte 8d'Ivoire, Tome1, P. 23-30. 1979.

[18] Z. N. Guédé, K. N'guessan, E. T. Dibié, P. Grellier. Ethnopharmagicological study of plants used to treat malaria, in traditional medicine, by bete populations of Issia (Côted'Ivoire). *J Pharm Sci Res*, 2 (4): 216 - 227. 2010.

[19] E. J. Adjanohoun, N. Aboubakar, K. Dramane, M. E. Ebot, J. A. Ekpere, E. G. Enow-Orock, D. Focho, Z. O. Gbile, A. Kamanyi, K. J. Kamsu, A. Keita, T. MBenkum, C. N. Mbi, A. L. Mbiele, I. L. Mbome, N. K. Mubiru, W. L. Nancy, B. Nkonmgneeck, B. Satabie, A. Sofowora, V. Tamze, and C. K. Wirnum. Contribution to ethnobotanical and floristic studies in Cameroon. CSTR/OUA, Cameroon. 641 pp. 1996

[20] I. Bagré, C. Bahi, S. Méité, A. J. Djaman, G. F. Guédé. Evaluation et amélioration *in vitro* de l'activité antifongique de *Morindamorindoides* (Baker) Milne-Redh (Rubiaceae) sur *Cryptococcusneoformans*, un champignon responsable de mycose humaine. *J Sci Pharmabiol* (7), 37-46, 2006.

[21] F. G. Guédé. Extraction of mansonin from *Mansonia altissima* as cardiovascular agent (patent application). Ministère de la Recherche Scientifique, Côte d'Ivoire. 35 pp. 1990.

[22] F. Bssaibis. N. Gmira and M. Meziane. Activité antibactérienne de Dittrichia viscoa (L.) W. Greuter. *Rev. Microbiol. Ind. San et Environn*, 3 (1), 44-45. 2009.

[23] C. Duraffourd, L. D'Hervicourt and J. C. Lapraz. Cahiers de phytothérapie clinique. Examen de laboratoire galénique, Eléments Thérapeutiques Synergiques. 2ème Edition, Masson (Paris), 87 p. 1990.

[24] A. G. Ponce, R. Fritz, C. del Valle, and S. I. Roura, Antibacterial activity of essential oils on the native microflora of organic Swiss chard. Society of Food Science and Technology (Elsevier), 36, 679-684. 2003.

[25] S. R. De paiva, M. R. Figueiredo, T. V. Aragao, M. A. Kaplan. Antibacterial activity *in vitro* of Plambagin isolated from Plumbayo species. *Memórias do InstitutoOswaldo Cruz* 98 : 959-61. 2003.

[26] P. Berche, J. L. Gaillard, M. Simonet. Les bactéries des infections humaines. Editeur: Flammarion, Médecine & Sciences. 660 p. 1991.

[27] A. A. Marmonier. Introduction aux techniques d'études des antibiotiques. *In*: *Bacteriologie Médicale, Techniques usuelles*. Doin, Paris, France.227-236. 1990.

[28] M. B. A. Patrice, H. F. Yapi, T. T. OPkekon, G. Kouakou Sirancy and Y. K. F. Kouassi. Evaluation of anti-inflammatory activities of *Erythrococca anomala* aqueous and ethanolic extracts from leaves in rat *IJBCR*, 14, (14): 1-7, 2016

[29] B.A.P. Miezan, A.T. Okpekon, H. F. Yapi, F. N. Bony, G. Gbassi, Y. J. Assi. Chemical component and acute toxicitystudy of *Erythrococca anomala* (Euphorbiaceae). *Asian Journal of Biomedical and Pharmaceutical Sciences*. 6 (57) :4-8.2016.

[30] J. B. Harborne et C. A. Williams . Advances in flavonoïds research since 1972. *Phytochemistry*. 55: 481-504, 2000.

[31] T. P. Cushinie, et A. J. Lamb. Antimicrobial activity of flavonoïdes. *International Journal of Antimicrobial Agents.* 26: 243-56, 2005.

[32] P. Erasto, G. Bojase-m, R. R. T. Majinda. Antimicrobial and antioxidant flavonoids from the roots wood of *Bolusanthus spesiosus*. *Phytochemistry* 65: 875-880, 2004.

[33] L. K. Tona, N. Kambu, K. Ngimbi, A.J. Cimanga, Vlietinck. Antiamoebic and phytochemical screening of some

Congolese medicinal plants. *Journal* of *Ethno pharmacology*. 61: 57-65, 1998.

[34] R. K. Cimanga, K. Kambu, L. TONA, N. Hermans, S. Apers, J. Totte, L. Pieters, A. J. Vlietinck,. Cytotoxicity and *in vitro* susceptibility of *entomoeba histolytica* to *Morinda morindoides* leaf extracts and its isolated constituents. *Journal of Ethnopharmacology* 107: 83-90, 2006.

[35] A. Basile , S. Giordano, J. A. Lopez-s, B. C. Cobianchi. Antibacterial activity of pure flavonoïds isolated from mosses. *Phytochemistry*, 2 (8): 1419-82, 1999.

[36] X. Zhu, H. Zhang, R. Lo,. Phenolic compounds from the leaf extract of artichoke(*Cynara scolymus L.*) and their antimicrobial activities. *Journal of Agricultural and Food Chemistry*. 52: 7272- 8, 2004.

[37] I. Bagré, C. Bahi, G. Gnahoué, A. J. Djaman, G. F. Guédé. Composition phytochimique et Evaluation *in vitro* de l'activité antifongique des extraits des feuilles de *Morinda morindoides* (Baker) Milne-Redh (*Rubiaceae*) sur *Aspergillus fumigatus* et *Candida albicans*. *J Sci Pharm Biol* 8 : 15-23. 2007.

[38] A. Scalbert. Antimicrobial properties of tannins. *Phytochemistry*, 30, 3875-3883. 1991.

[39] H. Wagner. Pharmazeutische Biologe. Drogen und irheinhaltsstaffe, Gustav Fisher Verfag. Sturtgart-New-York, 50 p. 1993.