# Exploration of the antibacterial and chemical potential of some Beninese pharmacopoiea traditional plants

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## Summary

*Objectives.* This study aims to evaluate the antibacterial and chemical properties of some medicinal plants used in the fight against enteropathogens in Benin.

*Methods.* Aqueous and ethanolic extracts of *Senna siamea*, *Uvaria chamae, Lantana camara* and *Phyllantus amarus* were tested on 10 bacterial strains. Well diffusion technique, coupled with the microdilution determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (CMB) was used for antibacterial testing. The larval cytotoxicity was evaluated by using *Artemia salina* crustacean larvae. flavonoids and polyphenols were also assayed by the method using aluminum trichloride (AlCl3) and the method using the folin-Ciocalteu reagent, respectively.

*Results*. The results of the study revealed that extracts had an effective antibacterial activity at 100 mg/mL, with MIC between 100 and 25 mg/mL and CMB between 100 and 50 mg/mL. The inhibition diameters of the extracts varied between 7.5 and 21 mm. The ethanolic extract of *Phyllantus amarus* leaves showed the best antibacterial activity. None of the extracts tested was found to be cytotoxic at the dose of 20 mg/mL. The aqueous *Uvaria chamae* root extract has the highest polyphenol content (231.896552±0.27586207 in µg EAG/100 mg extract), whereas the aqueous leaf extract of *Uvaria chamae* is the richest in flavonoids (41.061082 0.43180737 in µg ER/100 mg of extract).

*Conclusions.* These interesting results can be used in the development of improved traditional medicines against enteropathogens.

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Key words: Enteropathogens, salmonellosis in Benin, beninese pharmacopoiea.

Acknowledgments: The authors are very grateful to The World Academy of Science for the Advancement of Science in Developing Countries (TWAS) and the United Nation Educational, Scientific and Cultural Organization (UNESCO). These two institutions have made this research possible through research funding allocated to the research team under the number: 487 RG/BIO/AF/AC_G-FR3240293303.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare that they have no conflict of interest.

Received for publication: 16 August 2017.
Revision received: 6 November 2017.
Accepted for publication: 19 January 2018.


*Microbiologia Medica 2017; 32:6998*
*doi:10.4081/mm.2017.6998*



## Introduction

Infections caused by enteropathogens are serious forms of infectious pathology. They are a major public health problem that causes millions of deaths a year. This is the case, for example, of foodborne illnesses, causing 17 million deaths a year worldwide, more than half of which come from the African continent (37). Diarrheal diseases are also the cause of 550 million patients each year, including 220 million children under 5 years of age (37). *Salmonella*, *shigella* and *klebsielles* are amongst others epidemiologically active enterobacteria involved in serious infectious diseases.

Health management of enteropathogenic diseases is achieved through the use of antibiotics, but their inadequate and often anarchic use has resulted in bacterial resistance (24, 36). Indeed, recent data from the bibliography abound with descriptions of bacteria that are multiresistant or even toto-resistant to antibiotics. The number of these bacteria is increasing in both industrialized and developing countries (32). This development of microbial resistance to antibiotics has led researchers to carry out alternative investigations to identify other effective natural remedies against various pathologies in the plant kingdom (18). It has therefore proved essential to look for new antibacterial substances that are effective and have a broad spectrum of action. One of the effective strategies for this research is to explore plants used in traditional

medicine (8). This position is further reinforced by the fact that herbal medicines are of considerable importance in international trade. More than 120 compounds from plants are now used in modern medicine and almost 75% of them are used according to their traditional use (11). The World Health Organization estimates that 80% of the African population still uses traditional medicine to treat themselves (31).

In Benin, several plants are used in the traditional treatment of various pathologies, including infectious diseases (3, 4). Infectious pathologies related to enteropathogens are well documented. An ethno-pharmacological survey identified 56 medicinal plants used in the treatment of salmonellosis in Benin, including *Senna siamea* (Lam.) H.S. Irwin & Barneby, *Phyllantus amarus, Uvaria chamea*, and *Lantana camara* (15). These plants have been the subject of few pharmacological studies and are still unexploited from both the antibacterial and chemical point of view, aiming at their traditional valorization. This is why this study was initiated. It aimed to evaluate the antibacterial, pytochemistry ans cytotoxic properties of these plants used against the bacteria responsible for infections.

## Materials and Methods

### Equipment

Laboratory consumables, microbiology and chemistry materials were used during the work. The biological material consists, on the one hand, of the larvae eggs of *Artemia salina* acquired at the Laboratory of the Benin Center for Scientific and Technological Research (CBRST). On the other hand, the clinical strains *Klebsiella pneumoniae, Klebsiella rhinocleromatis, Klebsiella oxytoca, Shigella flexneri, Pseudomonas oryzihabitans, Citrobacter freundi, Salmonella cholerasius, Escherichia coli, Pseudomonas aeruginosa* and the reference strain *Escherichia coli* ATCC25992 were acquired at the National Public Health Laboratory , Ministry of Public Health of Benin. Four (4) medicinal plants formed the vegetal material: leaves of *Senna siamea* (Lam.) H.S.Irwin & Barneby (Leguminosae Caesalpinioideae), *Lantana camara* (verbenaceae), *Uvaria chamae* (Annonaceae), leaves and roots of *Phyllanthus amarus* Schumach & Thonn.

### Methods

#### *Collecting plant samples and obtaining powders*

Selected plant organs were collected at Porto-Novo, Calavi and Adjarra in April 2017. The plants are identified at National Herbarium of Benin Of University of Abomey-Calavi. Reference numbers are AA6686/HNB for *Phyllantus amarus,* AA6687/HNB for *Uvaria chamae*, AA6688/HNB for *Lantana Camara*, AA6691/HNB for *Senna siamea*. The organs were dried in the laboratory at a temperature of 16°C for 10 days. The dried material was then powdered using a Retsch SM 2000/1430/Upm/Smf type mill. The powders obtained were identified and stored in plastic jars at room temperature in the laboratory.

#### *Production of aqueous and ethanolic extracts*

The aqueous and ethanolic total extracts were obtained by adaptation of the method developed by Guede-Guina *et al*. (17). Fifty grams of powder were macerated in 500 mL of distilled water or ethanol on a Stuart Bioblock Scientific Fisher stirrer for 72 hours at room temperature. The resulting homogenate was filtered three times on hydrophilic cotton followed by filtration on Wattman No. 1 paper. This filtrate was then dried at 45°C in the Pasteur oven. The powder thus obtained was the total aqueous or ethanolic extract.

#### *Larval cytotoxicity*

The cytotoxic effect of the extracts was evaluated according to an adaptation of the method described by Kawsar *et al.* (22). The tests were carried out on larvae obtained by hatching 10 mg of *Artemia salina* eggs (ARTEMIO JBL GmbH D-67141 Neuhofem) under continuous stirring in one liter of seawater for 72 hours. To one mL of each dilution in geometric series 2 of extract prepared from a stock solution of 40 mg/mL is added 1 mL of seawater containing 16 larvae. The number of surviving larvae was counted after 24 h of incubation. The LC50 was determined from the regression line obtained from the curve representative of the number of surviving larvae as a function of the concentration of the extracts. To interpret these results, correlation grids associating the degree of toxicity with LC50 have been proposed: $CL_{50} \geq 0.1$ mg/mL (the extract is non-toxic), 0.1 mg/mL > $CL_{50} \geq 0.050$ mg/mL (low toxicity), 0.050 mg/mL > $CL_{50} \geq 0.01$ mg/mL (medium toxicity), $CL_{50} < 0.01$ mg/mL (high toxicity) (27).

#### *Implementation of antibacterial tests*

*Preparation of extracts and sterility testing of extracts.* The aqueous and ethanolic extracts of each plant were taken up in distilled water at a rate of 100 mg per 1 mL. Stock solutions containing 100 mg/mL were thus prepared. They were then sterilized in an autoclave at 121°C. for 15 minutes. The sterility of the stock solutions of extracts was verified by inoculating aliquots of each solution on the Mueller Hinton medium according to the methodology described by Agbankpe *et al*. (5).

*Preparation of bacterial suspension.* A 24-hour pure colony portion from the Mueller Hinton medium of each strain was emulsified in 5 mL of physiological water to give a turbidity of 0.5 on the Mc Farland scale (CA-SFM, 2017).

*Realization of the antibiogram by the diffusion technique in wells.* Each inoculum was seeded by swab on Petri dishes containing Mueller Hinton agar (CA-SFM, 2017). With the aid of the sterile Pasteur pipette tip, wells of 6 mm diameter were hollowed out. Then, using a cone and a micropipette, 50 µL of each extract was deposited in the wells previously excavated. A well containing sterile distilled water served as a negative control. The Petri dishes were left for 1 hour at room temperature for pre-diffusion of the substances and incubated at 37°C in an oven for 18 hours (30). Standard antibiotic discs were also used to serve as positive controls. The test was repeated three times. After the incubation period, the dishes were examined for the measurements of the zones of inhibition. The antibacterial activity of the extracts was determined from the diameters of zones of inhibition around the wells. The standard used for reading the results of the antibiogram tests is presented in Table 1.

*Determination of minimum inhibitory concentration and minimum bactericidal concentration.* This step was performed using the 96-well plate method described by Houngbeme *et al*. (19). 100 µL of the stock solution of each extract prepared at 200 mg/mL were added to 100 µL of Mueller-Hinton Broth. A series of two-fold dilution from well to well was made then 100 µL of different bacterial suspensions were added. Positive and negative controls were prepared respectively by adding 100 µL of MH broth to 100 µL of bacterial suspension and 100 µL of MH broth to 100 µL of the extracts. The microplates were coated with parafilm paper and incubated at 37°C for 24 hours. The MIC was estimated with the naked eye compared to the controls and each well was cultured on Agar MH Agar and incubated at 37°C for 24 hours for the determi-

nation of CMB. CMB is the lowest concentration of extract to which no colony of bacteria can be observed. The antibiotic potency (a.p) of each extract was then calculated with the formula CMB/CMI.

### *Determination of bioactive compounds*

*Determination of polyphenols.* The total phenols were assayed by a method adapted from that of Singleton (35) using the commercial folin-Ciocalteu reagent. The total polyphenol content in the various extracts was calculated from a linear calibration curve (y=ax + b), established with precise gallic acid concentrations as a reference standard (0-200 mg/L). Each sample tested was dissolved in methanol so as to obtain a concentration of 10 mg/mL and then diluted to 1/100 with distilled water. A volume of 125 µL of diluted solution was then mixed with 625 µL of the 10% Folin-Ciocalteu reagent (10 times diluted in distilled water) and incubated for 5 min. 500 µL of an aqueous solution of sodium carbonate ($Na_2CO_3$) at 75 g/L are then added and mixed with the vortex and incubated for 2h. After incubation, the optical densities (OD) were read at 760 nm using a CECIL CE 2041 spectrophotometer. Three readings were made per sample. The reading was made against a blank consisting of a mixture of 0.5 mL of FCR and 1 mL of Na 2 CO 3. The total phenol contents are determined using a gallic acid calibration curve (0-200 mg/L).

*Determination of flavonoids.* The contents of the flavonoids were measured by a suitable method of Zhishen *et al*. (38) and Kim *et al*. (23) using aluminum trichloride (AlCl3) as reagent. The presence of a free cell in the reagent AlCl3 forms a dative bond with the oxygen-free doublets of the OH groups of the flavonoids, producing a yellow complex whose maximum absorbance is recorded at 415 nm. The quantities of flavonoids in our extracts were calculated from the calibration curve of a standard flavonoid (rutin) as a reference standard (0-200 mg/L). To 500 µL of a solution of AlCl3 (2%), 500 µL of the sample is added. 3 mL of methanol are added to this mixture. The blank consists of 500 µL of AlCl3 and 3.5 mL of methanol. Absorbance reading is done at the spectrophotometer at 415 nm after 10 min incubation.

### *Statistical analysis*

The sensitivity tests were repeated three times and the results analyzed using the Graph Pad 7 software. They were then presented on average ± SD. An analysis of variance (ANOVA single factor) was used to compare the means of the zones of inhibition between the two extracts of the same plant. The level of significance was set at 5%.

For larval cytotoxicity, the LD50 of the extracts tested will be determined with the Microsoft Excel 2010 software, starting from the regression line obtained from the curve representing the number of surviving larvae as a function of the concentration of the extracts. For easy manipulation of the results, the following coding was adopted: Ss=*Senna siamea* (Lam.) H.S. Irwin & Barneby; Ucl=*Uvaria chamae* leaf; Ucr=*Uvaria chamae* roots, Pa=*Phyllantus amarus*; Lc=*Lantana camara*; EA=Aqueous Extract; EE=Ethanolic Extract.

## Results

### Extraction yield and sterility test

The yields at extraction vary from one plant to another and from one extract to another. The highest yield is obtained with the ethanolic extract of *L. camara* (19.12%) while the lowest yield (5%) is obtained with the aqueous extract of *P. amarus* (Figure 1). Sterility tests revealed no contamination at all of the extracts.

### Larval cytotoxicity of extracts

The *Artemia salina* model was chosen in particular for its relatively quick and inexpensive implementation. The results of the larval cytotoxicity test revealed a sensitivity of larvae of *Artemia salina* to the extracts of all plants at a dose of 20 mg/mL. Figure 2 shows the regression curve which expresses the percentage of larvae killed as a function of concentration for the aqueous extract of *Senna siamea* (Lam.) H.S. Irwin & Barneby.

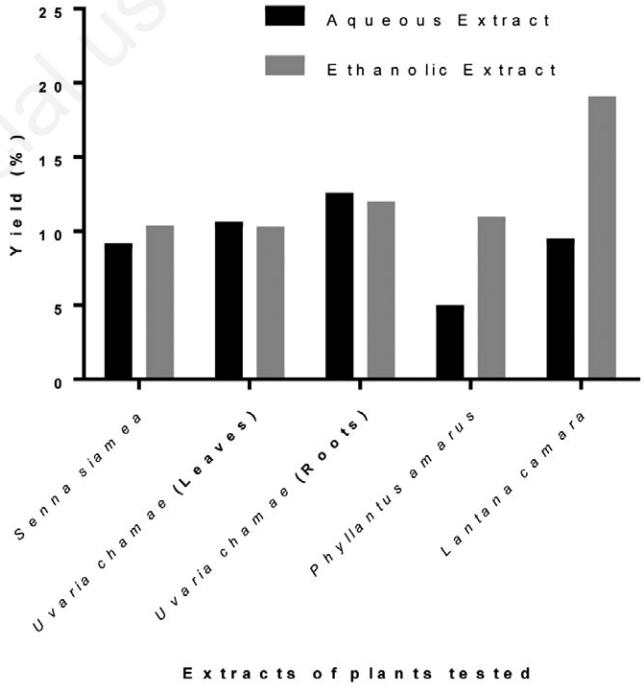


**Figure 1. Extraction yield.**

**Table 1. Standard values used to interpret the results of the susceptibility tests the plant extracts.**

| Diameter of the inhibition zone (D) | Degree of sensitivity of the germ | Symbol |
|---|---|---|
| Δ <7 mm | Resistant | - |
| 7 mm ≤ Δ <8 mm | Sensitive | + |
| 8 mm ≤ Δ <9 mm | Moderately sensitive | ++ |
| Δ ≥9 mm | Very sensitive | +++ |

The LC50 lethal dose is determined from the regression curve which expresses the percentage of larvae killed as a function of the concentration of the extracts. The lethal doses and regression coefficients of the extracts of all the plants studied are reported in Table 2.

All extracts have an LC50 greater than 0.1 mg/mL. All extracts are therefore non-cytotoxic with respect to larvae of *Artemia salina*.

## Antibacterial tests

### *CMI, CMB and AP of the plant extracts studied on the strains tested*

The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (CMB) and the antibiotic potency (AP) of the plant extracts studied on the tested strains are summarized in Table 3. The aqueous extract of *Senna siamea* (Lam.) HS Irwin & Barneby., The ethanolic root extract of *Uvaria chamae*, and the ethanolic extract of *Lantana camara* has no antibiotic power on the strains tested. With the exception of the ethanolic root extract of *Uvaria chamae*, which for the *K.oxytoca* strain has a MIC of 50 mg/m, these three extracts possess, on all the sensitive strains, a MIC of 100 mg/mL. The ethanolic extract of *Uvaria chamae* leaf and the ethanolic extract of the leaves of *P. amarus* have a bactericidal effect on 30% of the strains tested. The aqueous extract of *P. amarus* leaves has a bacteriostatic effect on *K.pneumoniae* and *P.oryzihabitans*, a bactericidal effect on *Sh.flexneri* (Table 3).

### *Sensitivity tests*

The results of the susceptibility tests of the strains used to the extracts of the plants studied are summarized in Figure 3. The tested strains exhibit a variable sensitivity with regard to the extracts tested. The inhibition diameters of the extracts vary between 7.5 and 21 mm. The ethanolic extract of leaves of *Phyllantus amarus* possesses the best antibacterial activity. The extract is very active (+++) on 50% of the strains and has the largest diameter of inhibition of the extracts on the strains (21 mm on *Sh.flexneri*). All extracts, except aqueous extract of *P. amarus*, are active on *K. oxytoca*. Only the ethanolic extract of *L.camara* is very active (+++) on *C.freundi*, with an inhibition diameter of 20.5±0.71.

Only the aqueous leaf extract of *U.chamae* is active on *E. coli* (reference strain) with an inhibition diameter of 14 mm. With the exception of *E.coli*, which is sensitive (+) to the ethanolic leaf extract of *U.chamae, S.cholerasius* and *P.aeruginosa* which are sufficiently sensitive (++) to the aqueous leaf extract of *U.chamae*, all strains which exhibit sensitivity to extracts showed an inhibition diameter greater than 9 mm, and therefore a very high sensitivity (+++).

Furthermore, there was a significant difference between the inhibition diameters of ethanolic extract and aqueous extract of all studied plants, on all tested strains ($P<0.05$).

## Total polyphenols and flavonoids content of extracts

The total phenolic compound contents of the extracts (Figure 4) were determined with reference to a standard curve ($y=0.058x - 0.015$) of gallic acid with y readout absorbance and x concentration (Figure 5). The total flavonoid contents of the extracts were determined with reference to a standard curve ($y=0.191x - 0.008$) of the rutin (Figure 6).

The aqueous root extract of *Uvaria chamae* has the highest total polyphenol content (231.896552±0.27586207 in µg EAG/100 mg of extract), whereas the aqueous extract of *P. amarus* leaves has the most Low (35.6321839±0.268575 in µg EAG/100mg). All extracts have a flavonoid content less than the content of polyphenols. The aqueous leaf extract of *U.chamae* had the highest flavonoid content (41.061082±0.43180737 in µg ER/100 mg extract) whereas the aqueous extract of *Senna siamea* leaf had the lowest Content of total polyphenols (1.59162304±0.33507853 in µg ER/100 mg of extract).

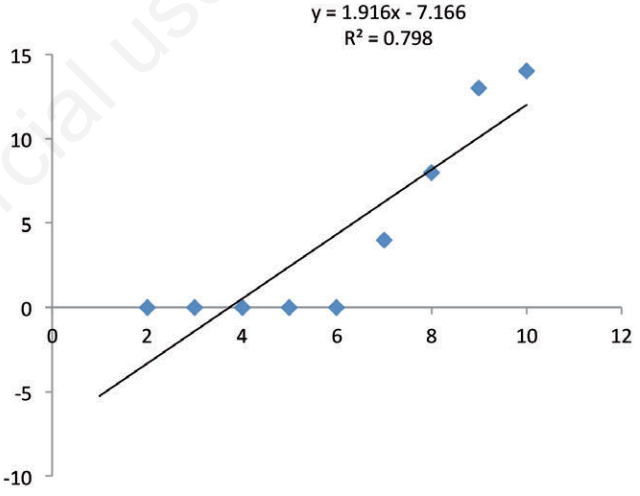


**Figure 2. Sensitivity of larvae of *Artemia salina* to aqueous extract of *Senna siamea* (Lam.) H.S. Irwin & Barneby.**

**Table 2. LC50 (mg/mL) and Regression coefficient ($r^2$) for all extracts of plants studied.**

| Extracts | $CL_{50}$ (mg/mL) | Regression coefficient ($r^2$) |
|---|---|---|
| Cs AE | 10.31 | 0.910 |
| Cs EE | 10.59 | 0.896 |
| Ucf AE | 3.04 | 0.938 |
| Ucf EE | 6.47 | 0.914 |
| Ucr AE | 8.689 | 0.818 |
| Ucr EE | 7.5231 | 0.946 |
| Pa AE | 5.9271 | 0.985 |
| Pa EE | 5.3254 | 0.990 |
| Lc AE | 5.12 | 0.959 |
| LcEE | 5.2498 | 0.986 |

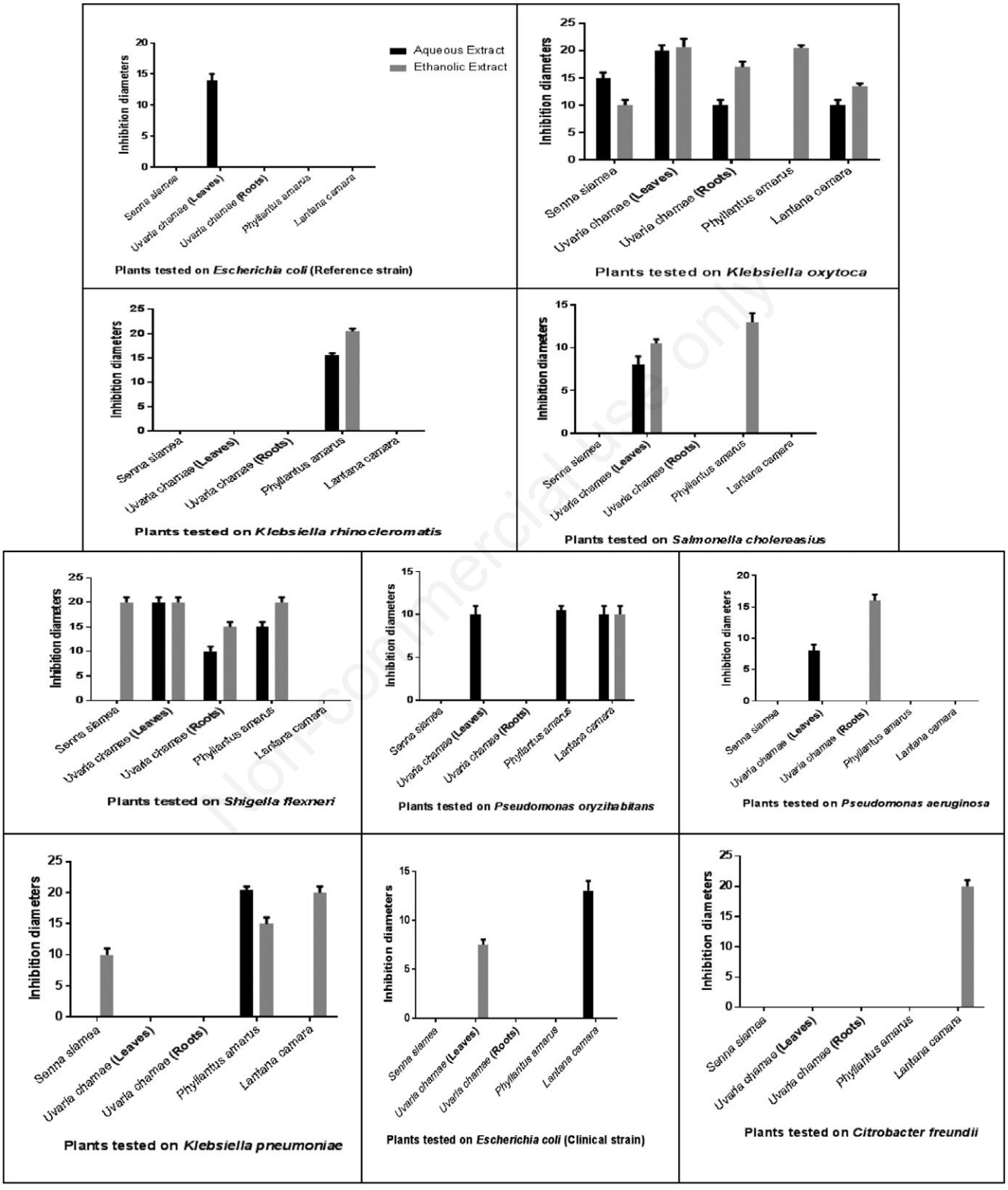


**Figure 3. Diameter of inhibition of the extracts of the plants studied on the bacterial strains.**

**Table 3. MIC (mg/mL), MBC (mg/mL) and a.p. of the aqueous and ethanolic extracts of tested vegetables on bacteria strains.**

| Extracts | Parameters | *K.pneumoniae* | *P.oryzihabitans* | *S.cholerasius* | *C.freundi* | *E.coli* | *P.aeruginosa* | *E.coli ATCC25992* | *K.Rhinocleromatis* | *Sh.flexneri* | *K.oxytoca* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cs AE | MIB | - | - | - | - | - | - | - | - | - | 100 |
| | MBC | - | - | - | - | - | - | - | - | >100 | |
| | a.p. | - | - | - | - | - | - | - | - | - | |
| Cs EE | MIB | 100 | - | - | - | - | - | - | - | 25 | 50 |
| | MBC | >100 | - | - | - | - | - | - | >100 | 100 | |
| | a.p. | - | - | - | - | - | - | - | - | 2 | |
| Ucf AE | MIB | - | 25 | 100 | - | - | - | - | - | 25 | 25 |
| | MBC | - | 50 | >100 | - | - | - | - | >100 | >100 | |
| | a.p. | - | 2 | - | - | - | - | - | | | |
| Ucf EE | MIB | - | - | 50 | - | 25 | - | - | - | 25 | 25 |
| | MBC | - | - | 100 | - | 50 | - | - | 50 | >100 | |
| | a.p. | - | - | 2 | - | 2 | - | - | 2 | | |
| Ucr AE | MIB | - | - | 50 | - | 25 | - | - | - | 12,5 | 50 |
| | MBC | - | - | 100 | - | 50 | - | - | >100 | >100 | |
| | a.p. | - | - | 2 | - | 2 | - | - | | | |
| Ucr EE | MIB | - | - | - | - | - | - | - | - | 100 | 50 |
| | MBC | - | - | - | - | - | - | - | >100 | >100 | |
| | a.p. | - | - | - | - | - | - | - | - | - | |
| Pa AE | MIB | 25 | 12,5 | - | - | - | - | - | 100 | 50 | - |
| | MBC | 100 | 50 | - | - | - | - | >100 | 100 | - | |
| | a.p. | 4 | 4 | - | - | - | - | - | 2 | - | |
| Pa EE | MIB | 12,5 | - | - | - | - | - | - | 50 | 25 | 50 |
| | MBC | >100 | - | - | - | - | - | 100 | 50 | 100 | |
| | a.p. | - | - | - | - | - | - | 2 | 2 | 2 | |
| Lc AE | MIB | - | - | - | - | 25 | - | - | - | - | 50 |
| | MBC | - | - | - | 50 | - | - | - | - | >100 | |
| | a.p. | - | - | - | 2 | - | - | - | - | - | |
| Lc EE | MIB | 100 | - | - | - | - | - | - | - | - | - |
| | MBC | >100 | - | - | - | - | - | - | - | - | |
| | a.p. | - | - | - | - | - | - | - | - | | |

## Discussion

The yield expressed as the percentage of active ingredients extracted by the solvent. It is an important parameter for discussing the biological activities of an extract. The variability of the yields obtained at the end of the extraction could be explained by the extraction capacity of each solvent. Indeed, the affinity of this solvent with respect to the phytomolecules and its polarity influences the yield (12). With the exception of the extracts of *U.chamae*, the yield of the ethanol extract of all the plants studied is higher than that of the aqueous extract. The high yields obtained with ethanol suggest that most of the active ingredients are soluble in ethanol and are therefore extractable by this solvent, which is normal since the alcohol is much more polar than water. It is the reverse for the extracts of *U.chamae* for which water manages to extract the largest number of active ingredients. This difference from the other extracts would be due to the extraction conditions. The aqueous extract of *Senna siamea* (Lam.) H.S. Irwin & Barneby. Yielded nearly 50% less than the 18.32% obtained by Ahonsou (7). This discrepancy may be due to several reasons. These include the level of maturity of the collected leaves, the extraction process, the geographical origin of the plant drug, leaf drying conditions (2), harvest time, degree of purity and quality of the solvents used.

All extracts have an LC50 greater than 0.1 mg/mL. The extracts of the plants studied at a dose of 20 mg/mL are therefore non-toxic to shrimp larvae cells according to the scale proposed by Sparkling (27). The cytotoxicity test according to the *Artemia salina* model constitutes a preliminary screening to determine not only the degree of cytotoxicity of a product, but also the presence of potential anti-cancer compounds. There is a positive correlation between the mortality of *Artemia larvae* and cytotoxicity against KB cells (26).This test therefore constitutes a preliminary anti-tumor screening of plant extracts. It can be said that the extracts of the plants studied are non-cytotoxic to human cancer cells at a dose of 20 mg/mL. This is true that some authors claim that there is no correlation between this test and The toxicological effects on a whole animal (33) but for 20 plant extracts tested using *in vivo* (mouse) and *in vitro* methods (25). A good correlation (r=0.85, P<0.05), suggesting that the Artemia test is a relatively useful alternative toxicity model.

The results of our study agree with those of Agbodjogbe et *al*. (6) who found an LC50 of 0.78 mg/mL for the aqueous extract of *Senna siamea* (Lam.) HS Irwin & Barneby. Cytotoxic effect of the extracts of this medicinal plant. All the extracts of the plants studied showed an interesting antibacterial activity on the strains studied, to varying degrees. This justifies the use of these plants in the treatment of infectious diseases (3, 4, 15). The aqueous extract of *Senna siamea* (Lam.) H.S. Irwin & Barneby was found to be very active on *K. oxytoca* and inactive on the other strains tested whereas the ethanolic extract of *Senna siamea* (Lam.) H.S. Irwin & Barneby was very active *on K. pneumonia* and *K. oxytoca*. These results are similar to those of De Souza *et al*. (13), which demonstrated the resistance of *E. coli* and *P. aeruginosa* to aqueous extracts of *Senna siamea* (Lam.) H.S. Irwin & Barneby. The resistance of the *S. cholerasius* strain calls into question the traditional use of this plant in the treatment of salmonellosis as reported by Dougnon *et al*. (15). However, this conclusion can not be made without first testing the extracts of this plant on other salmonella strains. The ethanolic extract of leaves of *Uvaria chamae* was found to be active on *S. cholerasius, E. coli, K. rhinocleromatis* with Minimal Inhibitory Concentrations (MIC) of 50 mg/mL; 25 mg/mL and 25 mg/mL respectively, lined with a bactericidal effect. As for the ethanolic root extract, it was active only on *Sh. Flexneri* and *K.oxytoca* with respective MICs of 100 mg/mL and 50 mg/mL. In Nigeria, Ogbulie made similar observations with respect to ethanolic extracts of *U. chamae* leaves on *E. coli* and *S. typhi* strains (28). Contrary results are obtained by Chika et *al*. (10) who observed the activity of the root-ethanolic extracts of *U. chamae* with a MIC of 72.44 mg mL-1 on *E. coli*. In this same study, the same extracts show a strong activity on *P. aeruginosa* and *S. typhi* whereas these strains are insensitive to our extracts. Moreover, for the same type of extract (ethanolic), the extracts of two different organs (roots and leaves) of *U. chamae* do not exhibit the same activity on the strains tested. This assumes that the active ingredients extracted at the level of the leaves are not the same at the level of the bark. The aqueous extract of *L. camara* is very active on *E. coli* with a bactericidal effect and a MIC of 25 mg/mL. This strong

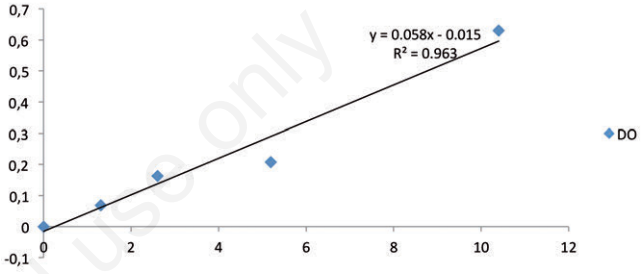


**Figure 4. Total polyphenols and flavonoids content of extracts.**

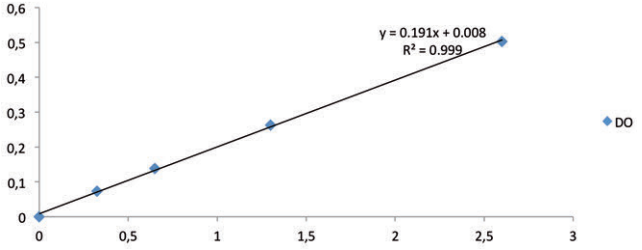


**Figure 5. Gallic acid calibration.**

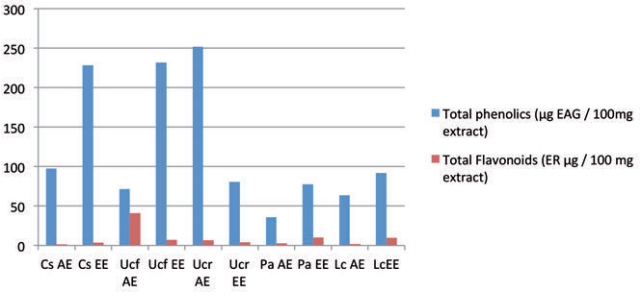


**Figure 6. Calibration of rutin.**

activity is already demonstrated in more recent studies such as that of Etchike *et al*. (16), which demonstrated the bactericidal effect on *E. coli* with a MIC of (1.47×10) − 2. The same observation was already made rather by Xaserra et Sharma (1999), but this time with the essential oils of the plant. The aqueous and ethanolic extracts of the leaves of *Phyllantus amarus* were inactive on *P. aeruginosa* and *E. coli*. These findings are in contrast to those of Adebo *et al.* (1), who found a minimum bactericidal concentration (CMB) of 9.4 mg/mL, a minimum inhibitory concentration (MIC) of 9.4 mg/mL and A concentration for 50% inhibition (IC50) of 1.5 mg/mL with *P. aeruginosa*. A CMB of 18.7 mg/mL, a MIC of 9.4 mg/mL and an IC50=0.9 mg/mL were revealed with *E. coli*. Differences in susceptibility of strains to extracts in comparison with other studies could have as explanations: the origin of the strains, the isolation techniques, the characteristics of the strains and the manipulation techniques.

All the extracts have a relatively high content of polyphenols and flavonoids, varying from one extract to another. This variability in the chemical composition of polyphenols in the extracts could be explained by the diversity of the plant species and the different solvents used during the extraction. With the exception of the particular case of root extracts of *Uvaria chamae*, it has been observed that the ethanolic extracts have a higher polyphenol content than the aqueous extracts. This reinforces the observation made during the raw extraction and suggests that the strong polarity of the ethanol makes it possible to extract a greater quantity of polyphenols than the water extracts from it. The interesting antibacterial properties of the plants involved in this study could be explained by their content of polyphenols and flavonoids. Indeed, the ability of an herbal remedy to exert microbial growth inhibitory effects is due to its different components (21). Most of these are plant metabolism products that can be chemically linked to a wide range of substances: phenolic compounds, tannins, anthocyanins, coumarins, alkaloids and flavonoids (9, 20). Bioactive compounds such as tannins, sterols and triterpenes, oses and holosides, coumarins and flavonoids have already been demonstrated (8, 25, 28). Similarly, flavonoids, alkaloids, tannins, phenols have been demonstrated in *Uvaria chamae* extracts.

## Conclusions

The traditional use of *Senna siamea* (Lam.) H. S. Irwin & Barneby., *Uvaria chamae*, *Lantana camara* and *Phyllantus amarus* in the treatment of enteropathogens is justified because of the interesting low-dose antibacterial properties revealed by our study. These biological activities can be explained by the richness of these plants in polyphenols, these secondary metabolites being known for their therapeutic properties including antibiotic potency. These interesting properties, reinforced by the non-cytotoxicity of the extracts, make these plants good candidates for the development of improved traditional medicines for the treatment of enteropathogens. However, additional *in vitro* and *in vivo* studies are needed to expand the database on antibacterial properties and toxicity of these plants.

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