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Chemical Composition, Cytotoxicity and Antibacterial Activity of Selected Extracts of *Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia* on Enteropathogenic Bacteria

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Abstract

Euphorbia hirta, Citrus aurantifolia and *Heterotis rotundifolia* are some plants traditionally used in Benin in the treatment of gastrointestinal diseases. To confirm the virtue of these plants, the antibacterial activity of their aqueous and ethanol extracts was carried out in vitro on 10 enteropathogenic strains by the diffusion method in agar medium. Inhibitory Minimal Concentrations (MIC) and Bactericides (CMB) were determined by macrodilution and inoculation methods on a solid medium, respectively. The cytotoxicity test previously carried out on the larvae of *Artemia salina* shows that these extracts have no toxic effect (LD50 > 0.1 mg/ml for all extracts). Moreover, the presence of these secondary metabolites in the extracts of these plants in varying proportions is revealed by the polyphenols and Flavonoids dosage. The two types of extracts of these plants inhibited the growth of the strains studied and the inhibition diameters varied according to the strains, extracts and plants. The CMB/CMI ratios obtained showed that the extracts of these plants have much more bactericidal effect on the strains tested. In view of these results, we can say that these plants are a good candidate for the development of an improved traditional medicine for the control of certain gastrointestinal diseases such as bacillary dysentery, diarrhea and salmonellosis.

Keywords: *Euphorbia hirta; Citrus aurantifolia; Heterotis rotundifolia; Antibacterial Activity; Physico-Chemical Composition; Cytotoxicity*

Introduction

Plants are vital components of biological diversity. They essentially serve in human well-being [1]. Humans in their environment have given increasing attention to the study of medicinal plants and their traditional use in different regions of the world [2]. According to the World Health Organization (WHO), medicinal plants are valuable resources for the majority of rural populations in Africa, with nearly 80 per cent using them for basic care [3].

Considerable economic benefits have been reported regarding the use of medicinal plants for the treatment of various diseases [2]. Medicinal plants are recognized to have many biologically active chemical substances that have different pharmacological activities: antioxidant, anti-inflammatory, analgesic, antibacterial, antifungal, antiviral [4-8]. Moreover, in traditional medicine, many plants successfully treat diseases against which modern medicine has failed [9].

In Benin, for several years, various ethnobotanical studies have focused on identifying medicinal plants, as a significant source of new medicines [10-12]. Moreover, it is reported that plants have fewer side effects [9].

In addition, the shortage of health infrastructures, the ever-increasing prices of pharmaceuticals and health services, and the inaccessibility of generic drugs are a major problem for the poor. Traditional medicine therefore, appears to be the most appropriate alternative to fill the gaps in health needs that populations aspire to [14]. The promotion of natural plant resources also become a necessary and an important concern in the research field for the development of new drug molecules [15]. Among the scientifically less known plants, which are widely used in the traditional pharmacopoeia in Benin, against enteric infections, are *Euphorbia hirta, Citrus aurantifolia* and *Heterotis rotundifolia* [16-18].

Euphorbia hirta is a small creeping plant but can be found sometimes erected up to 25 cm high. The stems and leaves bear many yellowish hairs. The species is widespread in all intertropical countries of the world. It is a ruderal plant, growing in various places, especially along roads, on wastelands and in old crops. The decoction of the whole plant administered orally fights dysentery and amibia in adults and children. It is also known to have great anti-asthmatic (inhalation) and galactogenic properties [19,20].

Citrus aurantifolia (Christm) belongs to the family of *Rutaceae*, it is a well-known medicinal and food plant. It is widely cultivated throughout the world and almost all of its parts are used in traditional medicine like astringent, diuretic, antiseptic, antimicrobial and especially in the treatment of gastrointestinal infections, coughing, colds and sore throats [21].

Heterotis rotundifolia is a medium-sized perennial herbaceous plant with slaying stems rooted at the nodes. The leaves are ovate lanceolate and sub-orbicular [22]. It is a plant of wet places, streams, hydromorphic fields, roadsides, crop fields, and empty spaces. It is widespread in rice fields [23]. In Africa, it is used in traditional medicine to treat conditions such as rheumatism and diarrhea [24]. The extracts of the leaves and stems of *Heterotis rotundifolia* have an antimicrobial effect [25].

The present study aims to contribute to the scientific knowledge of these plants through the evaluation of their cytotoxicity and their chemical compound as well as their extract efficacy *in vitro* on enteropathogenic bacterial strains.

Materials and Methods

Materials

Biological Materials

Larvae of *Artemia salina* were used for larval cytotoxicity tests and ten clinical enteropathogenic bacterial strains (*Klebsiella pneumoniae, Pseudomonas oryzihabitans, Citrobacter freundii, Salmonella choleraesuis*, *Escherichia coli, Pseudomonas aeruginosa, Escherichia coli* ATCC 25922, *Klebsiella rhinoscleromatis, Shigella flexneri, Klebsiella oxytoca*) were used for antibacterial testing.

Plant material

The plants were harvested at Kpomassè (*Euphorbia hirta*) and Abomey-Calavi (*Citrus aurantifolia* and *Heterotis rotundifolia*) and then dried in the laboratory at 25°C for two weeks. After drying, they were crushed and the powders were used for the extractions. Identification of the plants was carried out at the national herbarium of the University of Abomey-Calavi.

Table 1: *Plants used.*

Plants	Parts used
Euphorbia hirta	Whole plant
Citrus aurantifolia	Leaves
Heterotis rotundifolia	Whole plant

Methods

Artemia larvae production

Live larvae were obtained by hatching 10 mg of *Artemia salina* eggs (ARTEMIO JBL GmbH D-67141 Neuhofem) under slight continuous agitation in 1L of seawater for 72h.

Production of the extracts

Aqueous extracts

To do aqueous extracts, 50 g of plant powder were macerated in 500 ml of distilled water on a magnetic stirrer for 48 hours at room temperature. The homogenate obtained was filtered twice on hydrophilic cotton and once on Whatman No. 1 paper. This filtrate was then dried at 50°C. in the oven and the obtained powder constituted the total aqueous extract [26].

Ethanol extracts

About, ethanolic extracts, 50g of powder of the plants were macerated in a 1000 ml Erlenmeyer flask containing 500 ml of 96% ethanol, and agitated for 48 hours. After two successive filtrations on hydrophilic cotton and filtration on wattman paper No. 1, the filtrate was concentrated under vacuum at 50°C. After concentration, the filtrates were placed in an oven at 50° C until a dry mass was obtained. This constitute ethanolic extract [27,28].

All the powders obtained after various extractions were stored at 4°C in sterile glass vials.

Evaluation of larval toxicity

To evaluate the cytotoxicity of the extracts, serial dilutions were made out with sterile distilled water following ½-fold dilutions in test tubes numbered from T1 to T10, from a concentration of 40 mg/ml of extracts. Then 1 ml of seawater containing 16 live larvae was added to all the tubes. The percentage of larval mortality was determined after 24h of incubation [29].

Dosage of polyphenols and flavonoids

To do polyphenol dosage, we proceeded as follows: To 200 µl of extract or standard (prepared in methanol) with suitable dilutions is added 1 ml of Folin's reagent (10 times diluted). After 4 minutes, 800 µl of a sodium carbonate solution (75 mg/ml) was added to the reaction medium. After 2h of incubation at ambient temperature, the absorbance was measured at 760 nm. The concentration of the total polyphenols was calculated from the regression equation of the calibration range established with gallic acid and is expressed in µg of equivalent gallic acid per 100 milligram of extract (µg EAG/100 mg of extract) [30].

To quantify the flavonoids in the plant extracts, the method of aluminum trichloride was used. 1 ml of the AlCl3 solution (2% in methanol) was added to 1 ml of sample or standard (prepared in methanol). After 10 minutes of reaction, the absorbance was read at 415 nm. The concentration of flavonoids was deduced from a calibration range established with rutin and expressed in micrograms of equivalent Rutin per 100 milligrams of extract (µg EQ/100 mg of extract) [31].

Antibacterial activity of plant extracts

A sensitivity test of the extracts on enteropathogenic bacterial strains was carried out followed by a determination of the antibacterial parameters, namely the minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC) with an extract concentration equal to 100 mg/ml previously sterilized in an autoclave at 121°C for 15 minutes.

Sensitivity test

The *in vitro* antibacterial activity of the various extracts was performed by the Mueller Hinton disc diffusion method whereby Whatman No. 1 papers impregnated with plant extracts were used as antibiotic disc [32].

A bacterial suspension of 10^6 CFU/ml of the test strain was inoculated with a swab on MH agar. The bacterial suspension (10^6 CFU/ml) was obtained by diluting an inoculum of 10^8 CFU/ml with sterile distilled water. The inoculum was obtained by homogenizing fresh colonies of the strain in sterile distilled water and then adjusting the turbidity to 0.5 Mc Farland scale of 10^8 CFU/ml [33].

After plating, sterile Whatman No. 1 paper of 5 mm diameter were impregnated with 50 µl of plant extract of 100 mg/ml concentration were placed on the cultured plates. Synthetic antibiotic discs were used as positive control and the negative control was sterile distilled water.

The plates were then left for 15 to 30 minutes at room temperature (25°C ± 2°C) for pre-diffusion of the substances before being incubated at 37°C. The diameters of the zones of inhibition were measured using a graduated scale after 24 h of incubation [34-37]. For each extract, the experiment was duplicated.

Determination of Minimum Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) was determined by the method of macrodilution in liquid medium with visual appreciation of the growth of microorganisms [38]. The 48-well plates (6 rows of 8 wells) were used.

From a 100 mg/ml concentration of sterile extract, serial concentrations were made following ½-fold dilutions. With eight wells (P0 to P7), 500 µl of sterile distilled water were distributed in all the wells except the first (P0). To achieve the concentration range, 500 µl of plant extract with a concentration of 100 mg/ml was dispensed in the well P0. Then, 500 µl of plant extract with a concentration of 100 mg/ml was also added to the contents of the well P1. After homogenization, 500 µl of the mixture (extract + distilled water) of the well P1 was taken and added to the well P2. Then, 500 µl taken from the well P2 was transferred into the well P3. This operation continued until the well P6 of which 500 µl was taken and discarded.

At the end of the serial dilutions, we obtained a series of concentrations ranging from 100 to 1, 5625 mg/ml in wells P0 to P6. The well P7 received only sterile distilled water to serve as a negative growth control well.

An inoculum of 10^6 CFU/ml of each bacterial strain was prepared in MH broth. 1 ml of inoculum was added to each well (P0 to P7). After 24h of incubation, bacterial growth which results in turbidity was examined in each well. The MIC of an extract with respect to a given strain corresponds to the lowest concentration showing no visible growth to the naked eye.

Determination of minimum bactericidal concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined on the basis of the results of the MIC. After identifying the MIC, we have inoculated all the other tubes from the MIC to the higher concentrations on MH agar. These plates were examined after 24 h incubation at 37°C. On observation, the concentration of the extract where bacteria do not grow corresponds to the MBC [39].

Results

Cytotoxicity of the plant extracts

The mortality of the larvae subjected to plant extracts follows a dose-response relationship because the number of dead larvae increases when the concentration of extract increases (Figure 1). The concentrations that cause the death of half (8) of the 16 introduced larvae (LD 50) for each extract determined using the Y = aX + b regression equation (Figure 1) are shown in table 2.

The LD50 values of ethanol extracts are higher than those of the aqueous extracts for *Euphorbia hirta* and *Citrus aurantifolia*, whereas for *Heterotis rotundifolia* the LD50s are almost the same (6.41 mg/ml and 6.22 mg/ml respectively for aqueous extract and ethanol extract) (Table 2).

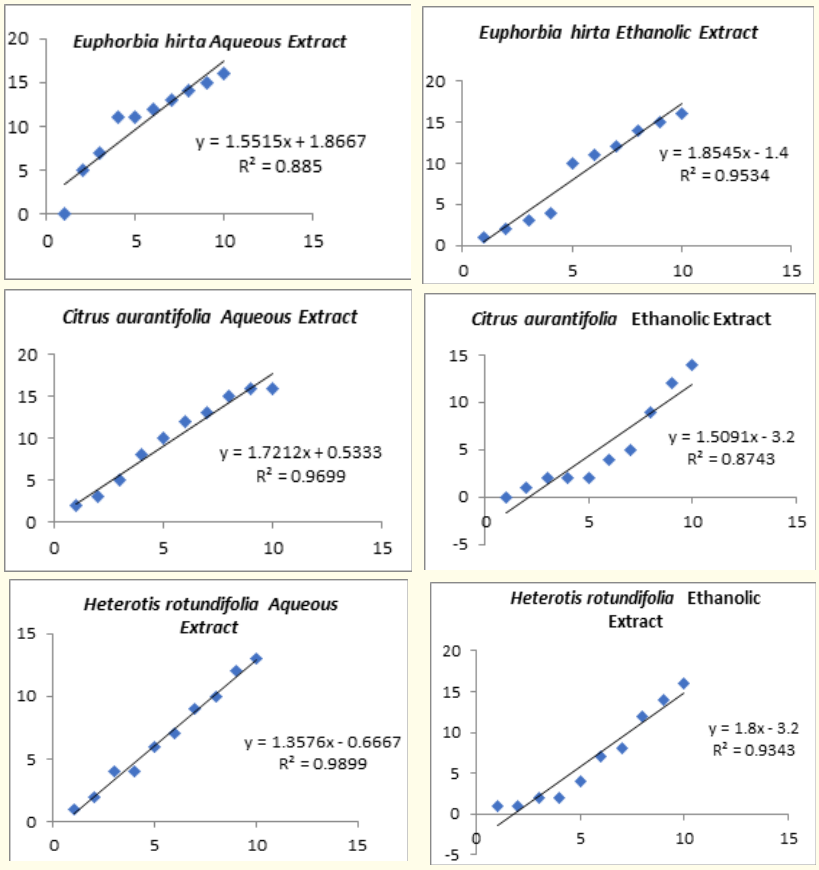

Figure 1: *Tendency curve of the number of surviving larvae in relation to the concentration of each extract.*

Table 2: *LD50 of plant extracts on Artemia salina larvae.*

	LD50 in mg/ml		
Extracts	*Euphorbia hirta*	*Citrus aurantifolia*	*Heterotis rotundifolia*
Aqueous extracts	3.96	4.34	6.41
Ethanol extracts	5.08	7.56	6.22

Dosage of Polyphenols and Flavonoids

Tables 3 and 4 present respectively the polyphenols and flavonoids contents of the aqueous and ethanol extracts of the plants. These values were determined from the regression equations (Figure 2a and 2b) of the calibration curves of gallic acid (polyphenols) and rutin (flavonoids). It is found that the aqueous extracts have higher contents of polyphenol compared to the ethanol extracts except for the ethanol extract of *Heterotis rotundifolia*, which has a polyphenol content higher than that of its aqueous extract. The aqueous extract of *Euphorbia hirta* has the highest level of polyphenols (201.72 ± 0.27 µgGAE/100 mg of extract) among all the aqueous extracts whereas in ethanol extracts it is *Heterotis rotundifolia* that had the highest content (189.08 ± 0.34 µgGAE/100 mg extract) (Table 3). In contrast to polyphenols, ethanol extracts have a higher flavonoid content than aqueous extracts except for *Citrus aurantifolia*, which has higher flavonoid content in its aqueous extract compared to the ethanol extract. The highest flavonoid level was 10.7 ± 0.35 µgER/100 mg of extract

obtained with the ethanol extract of *Euphorbia hirta*, while the lowest flavonoid content was 0.65 ± 0.42 µgRE/100 mg obtained with the ethanol extract of *Citrus aurantifolia* (Table 4).

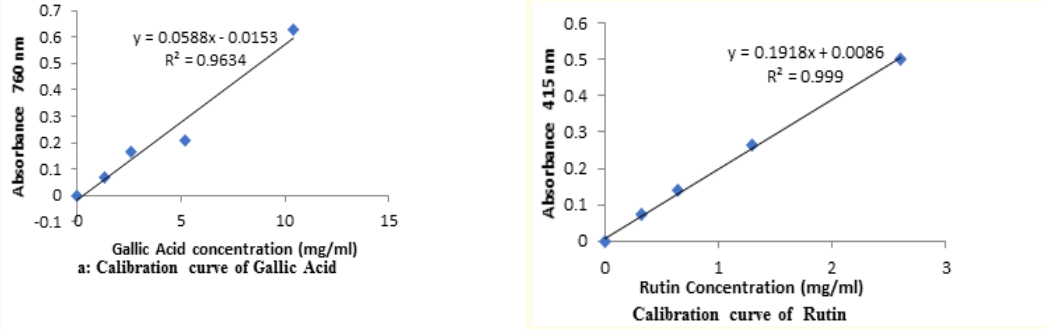

Figure 2: *Calibration curve of the reference molecules.*

Table 3: *Level of Polyphenols in µgGAE/100mg of extract.*

Extract	*Euphorbia hirta*	*Citrus aurantifolia*	*Heterotis rotundifolia*
Aqueous extract	201.72 ± 0.27	25.57 ± 0.3	189.08 ± 0.34
Ethanol extract	15.51 ± 0.25	34.48 ± 0.27	370.11 ± 0.29

Table 4: *Level of Flavonoids in µgRE/100mg of extract.*

Extract	*Euphorbia hirta*	*Citrus aurantifolia*	*Heterotis rotundifolia*
Aqueous extract	2.17 ± 0.33	2.35 ± 0.35	3.3 ± 0.33
Ethanol extract	10.7 ± 0.35	0.65 ± 0.42	6.38 ± 0.35

Antibacterial activity of plant extracts

Susceptibility of the entropathogenic bacterial strains to the plant extracts

Figure 3 shows the inhibition zone diameters of the aqueous and ethanol extracts of *Euphorbia hirta* on the studied strains. It is noted that only four strains (*cholereasuis Salmonella, Escherichia coli, Pseudomonas aeruginosa, Klebsiella rhinoscleromatis*) were sensitive to the aqueous extract and two strains (*Pseudomonas oryzihabitans, Escherichia coli*) to the ethanol extract. The inhibition diameters vary according to the strains and extracts. The highest inhibition diameter (10 mm) was obtained on *Salmonella choleraesuis* and *Pseudomonas oryzihabitans* respectively with the aqueous and the ethanol extracts and the smallest diameter (7 mm) was recorded in *Escherichia coli* (clinical strains) with ethanol extract.

Figure 4 shows the inhibition zone diameters of the aqueous and ethanol extracts of *Citrus aurantifolia* on the tested strains. Only *Salmonella choleraesuis, Escherichia coli* ATCC 25922 and *Klebsiella oxytoca* were sensitive to both extracts. *Citrobacter freundii* and *Escherichia coli* were susceptible to the ethanol extract only, whereas *Shigella flexneri* was only sensitive to the aqueous extract.

Figure 5 shows the inhibition zone diameters of the extracts of *Heterotis rotundifolia* on the tested strains. Only two (2) strains (*Salmonella choleraesuis* and *Klebsiella oxytoca*) were resistant to both extracts.

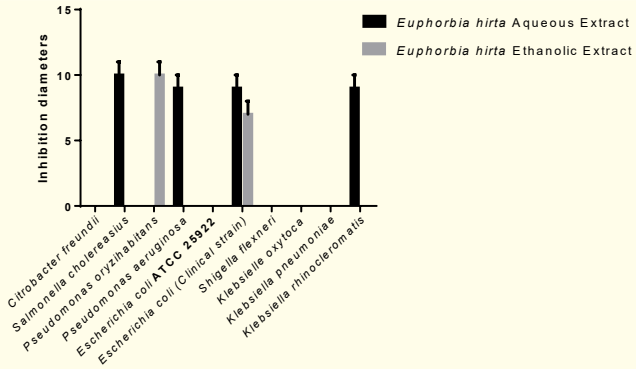

Figure 3: *Euphorbia hirta extracts on clinical strains.*

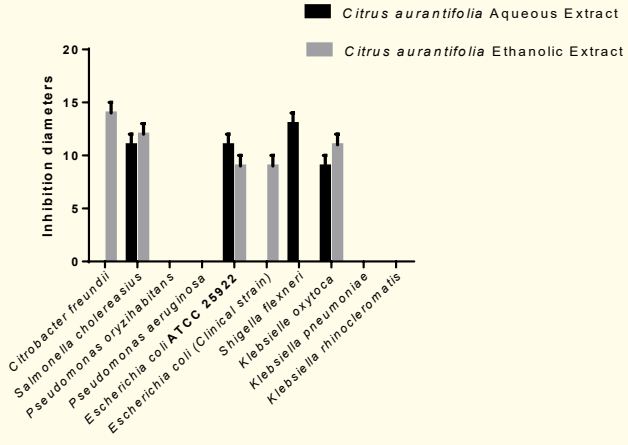

Figure 4: *Citrus aurantifolia extracts on clinical strains.*

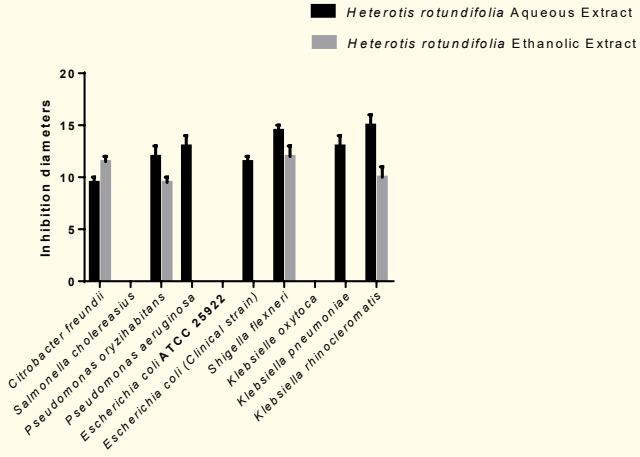

Figure 5: *Heterotis rotundifolia extracts on clinical strains.*

Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MBC) of the three plant extracts on the tested strains

Table 5 presents the Minimal Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of the extracts of *Euphorbia hirta* on clinical strains. MICs range from 25 mg/ml to 100 mg/ml, while MBCs range from 50 mg/ml to 100 mg/ml for the two extracts (Aqueous and Ethanolic). The lowest MIC (25 mg/ml) is obtained with the aqueous extract on *Salmonella choleraesuis* and the largest MIC (100 mg/ml) is obtained on both *Klebsiella rhinoscleromatis* (Aqueous Extract) and *Pseudomonas oryzihabitans* (Ethanol Extract). The values of the MBCs are the same (100 mg/ml) for all extracts and strains except for the aqueous extract, which gives 50 mg/ml on *Pseudomonas aeruginosa*.

Table 5: *Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MBC) of the extracts of Euphorbia hirta on clinical bacteria.*

Bacterial strains	MIC and MBC in mg/ml of Euphorbia hirta			
	Aqueous extract		Ethanol extract	
	MIC	MBC	MIC	MBC
Citrobacter freundii	0	0	0	0
Salmonella choleraesuis	25	100	0	0
Pseudomonas oryzihabitans	0	0	100	100
Pseudomonas aeruginosa	50	50		0
Escherichia coli ATCC 25922	0	0	0	0
Escherichia coli (Clinical strain)	50	100	50	100
Shigella flexneri	0	0	0	0
Klebsiella oxytoca	0	0	0	0
Klebsiella pneumoniae	0	0	0	0
Klebsiella rhinoscleromatis	100	100	0	0

Table 6 shows the Minimal Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of *Citrus aurantifolia* extracts. This table shows that MICs vary between 12.5 mg/ml and 100 mg/ml, whereas MBCs range from 50 mg/ml to 100 mg/ml for the two extracts (Aqueous and Ethanolic). The MICs of the two extracts vary according to the strains and extracts. In the aqueous extract, the MBCs are the same (50 mg/ml) except for *Salmonella choleraesuis*, which has a MBC greater than 100 mg/ml with this extract. The same was observed for the ethanol extract with a concentration of 100 mg/ml for all strains except *Citrobacter freundii* and *Salmonella choleraesuis* that have a MBC greater than 100 mg/ml.

Table 7 presents the Minimal Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of the extracts of *Heterotis rotundifolia*. From the analysis of this table, it comes out that the MIC values of the two extracts (Aqueous and Ethanolic) are between 12.5 mg/ml and 100 mg/ml whereas those of the MBC are between 25 mg/ml and 100 mg/ml. MICs and MBCs varied between strains and extracts. For the ethanol extract, MICs are equal to MBC (100 mg/ml for *Citrobacter freundii* and 50 mg/ml for *Shigella flexneri* and *Klebsiella rhinoscleromatis*) except for *Pseudomonas oryzihabitans* that has a MBC beyond 100 mg/ml. For the aqueous extract, only the MIC of *Pseudomonas aeruginosa* was equal to the MBC (100 mg/ml).

Table 6: *Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MBC) of the extracts of Citrus aurantifolia on clinical bacteria.*

Bacterial strains	MIC and MBC in mg/ml of Citrus aurantifolia			
	Aqueous extract		Ethanol extract	
	MIC	MBC	MIC	MBC
Citrobacter freundii	0	0	50	MBC > 100
Salmonella choleraesuis	25	MBC > 100	25	MBC > 100
Pseudomonas oryzihabitans	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0
Escherichia coli ATCC 25922	50	50	12.5	100
Escherichia coli (Clinical strain)	0	0	12.5	100
Shigella flexneri	50	50	0	0
Klebsiella oxytoca	50	50	100	100
Klebsiella pneumoniae	0	0	0	0
Klebsiella rhinoscleromatis	0	0	0	0

Table 7: *Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MBC) of the extracts of Heterotis rotundifolia on clinical bacteria.*

Bacterial strains	MIC and MBC in mg/ml of Heterotis rotundifolia			
	Aqueous extract		Ethanol extract	
	MIC	MBC	MIC	MBC
Citrobacter freundii	25	50	100	100
Salmonella choleraesuis	0	0	0	0
Pseudomonas oryzihabitans	12.5	25	100	CMB > 100
Pseudomonas aeruginosa	100	100	0	0
Escherichia coli ATCC 25922	0	0	0	0
Escherichia coli (Clinical strain)	50	100	0	0
Shigella flexneri	50	CMB > 100	50	50
Klebsiella oxytoca	0	0	0	0
Klebsiella pneumonia	25	50	0	0
Klebsiella rhinoscleromatis	50	100	50	50

Bactericidal and bacteriostatic effects of the plant extracts on clinical bacterial strains

The ratio MBC/MIC revealed that the three extracts have bactericidal and bacteriostatic effects on the tested strains. The extracts of *Heterotis rotundifolia* have only bactericidal effects on all strains except *Shigella flexneri* and *Pseudomonas oryzihabitans* respectively for the aqueous and the ethanol extract (Table 8).

Table 8: *Bactericidal and bacteriostatic effects of the plant extracts (Euphorbia hirta, Citrus aurantifolia, Heterotis rotundifolia) on the tested clinical bacterial strains.*

Strains	MBC/MIC					
	Euphorbia hirta		*Citrus aurantifolia*		*Heterotis rotundifolia*	
	Aqueous extracts	Ethanol extracts	Aqueous extracts	Ethanol extracts	Aqueous extracts	Ethanol extracts
Citrobacter freundii	0	0	0	NA	2*	1*
Salmonella choleraesuis	4	0	NA	NA	0	0
Pseudomonas oryzihabitans	0	1*	0	0	2*	NA
Pseudomonas aeruginosa	1	0	0	0	1*	0
Escherichia coli ATCC 25922	0	0	1*	8	0	0
Escherichia coli (Clinical strain)	2*	2*	0	8	2*	0
Shigella flexneri	0	0	1	0	NA	1*
Klebsiella oxytoca	0	0	1*	1*	0	0
Klebsiella pneumonia	0	0	0	0	2	0
Klebsiella rhinoscleromatis	1*	0	0	0	2*	1*

Discussion

In this study, two types of extracts (Aqueous and Ethanolic) of three plants (*Euphorbia hirta, Citrus aurantifolia* and *Heterotis rotundifolia*) were assessed.

According to the toxicity scale established by some researchers and authors, all LD 50 values of our extracts are greater than 0.1 mg/ml (Table 3), above which the extract is considered non-toxic [40,41]. Therefore, the aqueous and ethanol extracts of the tested plants (*Euphorbia hirta, Citrus aurantifolia* and *Heterotis rotundifolia*) are not toxic. Similar results have already been reported [42,43].

A study conducted elsewhere on several species of the genus *Euphorbia* on *Artemia salina* larvae demonstrated that these plants are not toxic [42]. Moreover, some authors concluded that the aqueous, methanol and ethyl acetate extracts of *Citrus aurantifolia* have no toxic effect on the larvae of *Artemia salina* [43].

The dosage of polyphenols and flavonoids revealed the presence of these secondary metabolites in our extracts with varying proportions.

The polyphenol content of the aqueous extract of *Euphorbia hirta* is 201.72 ± 0.27 µgEAG/100 mg of extract, while its flavonoid content is 2.17 ± 0.33 µgER/100 mg of extract. These contents are less than 120.97 ± 7.07 mg EAG/g and 41.4 ± 0.5 mg EQ/g respectively for the polyphenols and flavonoids obtained by with the aqueous extract of *Euphorbia hirta* in other study (Table 3 and 4) [44].

In *Citrus aurantifolia*, the polyphenol content was 25.57 ± 0.3 µgEAG/100mg extract and 34.48 ± 0.27 µgEAG/100 mg extract respectively for the aqueous, and the ethanol extracts. This plant has a low flavonoid content, especially in its ethanol extract (0.65 ± 0.42 µgER/100 mg extract) (Table 3 and 4). The presence of flavonoids in leave extracts of *Citrus aurantifolia* has been demonstrated [45]. However, others have reported the absence of flavonoids in the methanol extract of the leaves of *Citrus aurantifolia* [46].

For the two extracts (aqueous and ethanolic) of *Heterotis rotundifolia*, there was a higher polyphenol level in the aqueous extract (370,11 ± 0,29 µgEAG/100 mg of extract) whereas ethanol extracts had the highest flavonoids content (6.38 ± 0.35 µgER/100 mg extract) (Table 3 and 4).

Furthermore, the two types of extracts of the three plants at a dose of 100 mg/ml inhibited the growth of the tested clinical bacteria strains and the inhibition diameters varied according to the strains, extracts and plants. The sensitivity of bacteria to plant extracts based on the diameters of the zones of inhibition is a function of strain and bacterial species [47]. The same observations were made in this study.

The diameters of the zones of inhibition of the aqueous extract of *Euphorbia hirta* vary between 9 mm and 10 mm (Figure 3). These values are lower than those indicated in other study, where they ranged between 17 mm and 21 mm on enteropathogenic strains at the same dose [48]. In addition, at 100 mg/ml the aqueous extract of *Euphorbia hirta* has no effect on *Klebsiella pneumoniae*. This is contrary to other results in other country of, in which the aqueous extract of the dry leaves of *Euphorbia hirta* at the same dose (100 mg/ml) inhibits the proliferation of this microorganism with an average inhibition diameter of 6.67 mm [16]. The differences observed in our work and those authors ([16,48]), could be because our extracts were sterilized prior to use or attributable to the source of the tested bacterial strains. During sterilization, our extracts could lose some antimicrobial active ingredients under the effect of heat. Moreover, the source of the strains must be taken into account, since it could influence the selective pressure that the strains would have undergone.

The ethanol extract of *Euphorbia hirta* had an effect on only two strains, namely *Pseudomonas oryzihabitans* and *Escherichia coli* with respective inhibition diameters of 10 mm and 7 mm (Figure 3). These results are similar to 6.5 mm and 10.2 mm respectively on *Pseudomonas aeruginosa* and *Escherichia coli* with the ethanol extract of the leaves of *Euphorbia hirta* revealed during the past investigations [49].

The ethanol extract of *Citrus aurantifoli*a is more active on our strains with diameters of inhibition that vary between 9 mm and 14 mm than the aqueous extract of this plant whose inhibition diameters are between 9 mm and 13 Mm (Figure 4). Some authors made similar observations and reported that at the same dose, the ethanol extract of the leaves of *Citrus aurantifolia* is more active than their aqueous extracts on bacteria strains [50]. Moreover, the two extracts have no effect on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Figure 4), which corroborates the findings of some studies in which the aqueous and ethanol extracts of *Citrus aurantifolia* have no effect on strains of *Klebsiella* spp. and *Pseudomonas aeruginosa* [51].

The aqueous and ethanol extracts of *Heterotis rotundifolia*, are active on certain tested strains with diameters varying between 9.5 mm and 15 mm (aqueous extract) on the one hand and between 9.5 mm and 12 Mm (ethanol extract) (Figure 5). These values are close to 8 mm, 9 mm and 11 mm obtained respectively on *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* with the ethanol extract of *Dissotis rotundifolia* in a previous study [25].

Moreover, the aqueous extracts of *Euphorbia hirta* and *Heterotis rotundifolia* are more active on our strains than the ethanol extracts of the same plants. (Figure 3 and 5). These results are justified by the reports of Brama thesis who showed that antibacterial substances are more soluble in water than in organic solvents [52]. A similar result in the anti-amoebic study of *Euphorbia hirta* concluded that the active substances are more soluble in water than in methanol or ethyl acetate [19].

The Minimum Inhibitory Concentrations (MICs) varied according to the strains of extracts and plants. In the extracts of *Euphorbia hirta*, the smallest MIC is 25 mg/ml obtained on *Salmonella choleraesuis* (aqueous extract) and the largest MIC is 100 mg/ml obtained on *Klebsiella rhinoscleromatis* (aqueous extract) and *Pseudomonas oryzihabitans* (extract Ethanol) (Table 5). These values are much lower than those of some authors who found MIC values between 62.5 mg/ml and 250 mg/ml with the methanol extract of the root of *Euphorbia condylocarpa* on bacteria strains [53].

The smallest MIC for the extracts of *Citrus aurantifolia*, is 12.5 mg/ml obtained on *Escherichia coli* (clinical strains) with the ethanol extract and the largest MIC is 100 mg/ml obtained on *Klebsiella oxytoca* with the same extract (Table 6). According to a previous study, results found MIC values between 12 µg/ml and 25 µg/ml on some Gram-negative bacteria using concentrated Citrus fruit juices [54].

However, higher values (64 ≤ MIC ≤ 256) of MIC compared to those reported in the present study using extracts of the fruit of *Citrus aurantifolia* on Gram-negative bacteria has been recorded [55].

For *Heterotis rotundifolia*, the lowest MIC is 12.5 mg/ml with *Pseudomonas oryzihabitans* (aqueous extract) and the largest MIC is 100 mg/ml from *Citrobacter freundii* (ethanol extract) *Pseudomonas oryzihabitans* (ethanol extract) and *Pseudomonas aeruginosa* (aqueous extract) (Table 7).

The extraction method, the solvents used and the organ of the plant could explain the differences observed between the MIC values of this study and those of the authors mentioned above. When different extraction methods, the solvents and even plant organs are used, the antimicrobial active ingredients will not have the same concentrations in the extracts. Moreover, depending on the location and the nature of the field or climate, plants may have different "chemical profiles" [56].

MBC values recorded in this study also varied according to the strains, the extracts and the plants. The smallest MBC obtained with extracts of our plants is 25 mg/ml on *Pseudomonas oryzihabitans* with the aqueous extract of *Heterotis rotundifolia*. Moreover, the majority of the MBCs of our extracts on the tested strains is 100 mg/ml (Table 5, 6 and 7).

The ratio of CMI and MBC shows that the majority of our extracts have bactericidal effects on most strains (Table 8). Similar observations were made in some a study, who indicated that the dichloromethane extract of Erythrina senegalensis has a bactericidal activity on several microorganisms (*Candida albicans, Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus, Proteus mirabilis, Escherichia coli and Pseudomonas aeruginosa*) [57].

The extracts of *Heterotis rotundifolia* are the most active extracts with bactericidal activity on the majority of tested bacterial strains. These results may justify the frequent use of this plant in the treatment of several ailments.

The antibacterial effect observed with the extracts of these plants can be explained by the presence in the extracts of major chemical groups such as flavonoids and polyphenols. The antibacterial properties of these chemical compounds were previously demonstrated by several authors [58-60].

Conclusions

This study describes the antibacterial properties of extracts (aqueous and ethanol) of three plants namely *Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia*. It revealed that these extracts had various effects on the enteropathogenic strains. Moreover, among these plants, the extracts of *Heterotis rotundifolia* were found to be more active than others and the activity of its aqueous extracts was very remarkable on the tested strains. The antibacterial activity observed with our extracts can be attributed to the presence of polyphenols and flavonoids in these extracts. The cytotoxicity tests carried out with our extracts showed that the extracts have no toxic effect.

These results may justify the traditional use of these plants for the treatment of gastrointestinal infections such as dysentery and diarrhea.

These plants can therefore be considered as good candidate for the development of traditional medicine to improve the fight against certain enteric diseases such as bacillary dysentery, diarrhea, salmonellosis.

Conflicts of Interest

Authors declare no conflict of interests.

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Bibliography

1. Adjanohoun E. "La biodiversité face au développement des industries pharmaceutiques africaines". In: Réseau des « espèces ligneuses médicinales », EyogMatig O, Adjanohoun E, de Souza S et Sinsin B (eds). Compte rendu de la première réunion du réseau tenue 15-17 décembre 1999 à la station IITA Cotonou, Bénin (2000): 88-103.

2. Muthu C., *et al*. "Medicinal plants used by the traditional healers in Kancheepuram District of Tamil Nadu, India". *Journal of Ethnobiology and Ethnomedicine* 2 (2006): 43.

3. World Health Organization (WHO). "WHO Traditional Medicine Strategy 2002-2005" (2002): 74.

4. Martínez MJ., *et al*. "Screening of some Cuban medicinal plants for antimicrobial activity". *Journal of Ethnopharmacology* 52.3 (1996): 171-174.

5. Essawi T and Srour M. "Screening of some Palestinian medicinal plants for antibacterial activity". *Journal of Ethnopharmacology* 70.3 (2000): 343-349.

6. Voravuthikunchai SP and Kitpipit L. "Effective medicinal plant extracts against hospital strains of methicillin-resistant Staphylococcus aureus". *European Journal of Clinical Microbiology and Infectious Diseases* 11.6 (2005): 510-512.

7. Kawshik KC., *et al*. "Analgesic and anti-inflammatory activities of Desmodium triflorum DC". *International Journal of Biological Sciences* 5 (2005): 581-583.

8. Fei Q., *et al*. "Antioxidant properties of Chinese yellow wine, its concentrate and volatiles". *LWT - Food Science and Technology* 39 (2006): 111-117.

9. Millogo-Koné H., *et al*. "Etude de l'activité antimicrobienne d'extraits de Parkia biglobosa (JACQ.) BENTH. sur des souches de Staphylococcus aureus". *Pharmacopée et Médecine Traditionnelle Africaine* 15 (2008): 1-5.

10. Biecke B. "Etnobotanishe studie van geneeskrachtige planten in Manigri en Igbére, Benin". Universiteit Gent, Bioingenieurin hetland, En Bos Beheer (2004): 420.

11. Sopkon N and Ouinsavi, C. "Utilisations du Khaya senegalensis en médecine traditionnelle au Bénin". *Revue de Médecine et Pharmacopée Africaines* 16 (2002): 9-19.

12. Adjanohoun E., *et al*. "Contribution aux Etudes Ethnobotaniques et Floristiques en République Populaire du Bénin". 2nd édition. ACCT: Paris (1989): 895.

13. Maghrani M., *et al*. "Antihypertensive effect of Lepidium sativum L. in spontaneously hypertension rats". *Journal of Ethnopharmacology* 100.1-2 (2005): 193-197.

14. Mpondo Mpondo E., *et al*. "Les plantes à phénols utilisées par les populations de la ville de Douala". *Journal of Animal and Plant Sciences* 15.1 (2012): 2083-2098.

15. Awono A., *et al*. "Mobilisation et renforcement des capacités des petites et moyennes entreprises impliquées dans la filière des produits forestiers non ligneux en Afrique Centrale". CIFOR Cameroun (2008).

16. Kareem KT., *et al*. "In-vitro antimicrobial activities of Euphorbia hirta against some clinical isolates". *Agriculture and Biology Journal of North America* 3.4 (2012): 169-174.

17. Kirtikar KR and Basu BD. "Indian Medicinal Plants, Periodical Experts Books Agency, 2nd edition". New Delhi 3 (1991).

18. Dougnon TV., *et al*. "Traditional treatment of human and animal salmonelloses in Southern Benin: Knowledge of farmers and tradi-therapists". *Veterinary World* 10.6 (2017): 580-592.

19. Ndir O and Pousset JL. "Plantes médicinales africaines: Contribution à l'étude pharmacologique et chimique d'Euphorbia hirta". *Medecine d'Afrique Noire* 29.7 (1982): 503-518.

20. Adjanohoun EJ, *et al*. "Revue de Médicine et Pharmacopées Africaines". Editions GRIPT 10 (1996): 138.

21. Enejoh OS., *et al*. "Ethnomedical Importance of Citrus Aurantifolia (Christm) Swingle". *Pharma Innovation* 4.8 (2015): 01-06.

22. Wagner WL., *et al*. "Manual of the flowering plants of Hawaii". Revised edition. Honolulu, Hawaii, USA: University of Hawaii Press/ Bishop Museum Press (1999): 1919.

23. Pier, Pacific Islands Ecosystems at Risk. Honolulu, Hawaii, USA: HEAR, University of Hawaii (2013).

24. Prota4U, PROTA4U web database. Grubben GJH, Denton OA, eds. Wageningen, Netherlands: Plant Resources of Tropical Africa (2013).

25. Abere TA., *et al*. "Antidiarrhoea and toxicological evaluation of the leaf extract of Dissotis rotundifolia triana (Melastomataceae)". *BMC Complementary and Alternative Medicine* 10 (2010): 71.

26. Guede-Guina F., *et al*. "Activité antibactérienne d'un extrait végétal contre les germes opportunistes au cours du SIDA". *Rev Med Pharm* 9 (1995): 13-19.

27. Sanogo R., *et al*. "Activité antibactérienne et antalgique de deux recettes traditionnelles utilisées dans le traitement des infections urinaires et la cystite au Mali". *Mali Médical* 21.1 (2006): 18-24.

28. N'Guessan JD., *et al*. "In vitro assays for bioactivity-guided isolation of anti-Salmonella and antioxidant compounds in Thonningia sanginea flowers". *African Journal of Biotechnology* 6.14 (2007): 1685-1689.

29. Kawsar SMA., *et al*. "Cytotoxicity assessment of the aerial part of Macrotyloma uniflorum Lim". *International Journal of Pharmaceutics* 4 (2008): 297-300.

30. Talbi H., *et al*. "Evaluation of antioxidant activity and physico-chemical composition of methanolic and aqueous extracts of Nigella sativa L". *Journal of Materials and Environmental Science* 6.4 (2015): 1111-1117.

31. Bahorun T., *et al*. "Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations". *Arzneimittelforschung* 46.11 (1996): 1086-1089.

32. Anani K., *et al*. "Investigation of medicinal plants of Togo for antiviral and antimicrobial activities". *Pharmaceutical Biology* 38.1 (2000): 40-45.

33. Société Française de Microbiologie (SFM), Recommandations du Comité de l'Antibiogramme de la Société Française de Microbiologie, SFM, France (2012): 59.

34. Bauer AW., *et al*. "Antibiotic susceptibility testing by a standardized single disk method". *American Journal of Clinical Pathology* 45.4 (1966): 493-496.

35. Carbonnelle B., *et al*. "Bactériologie medical". Techniques usuelles, SIMEP, Paris (1987): 141-144.

36. Adesokan AA., *et al*. "Antibacterial potentials of aqueous extract of Enantia chlorantha stem bark". *African Journal of Biotechnology* 6.22 (2007): 2502-2505.

37. Doughari JH., *et al*. "Antibacterial effects of Balanites aegyptiaca L. Drel. and Moringa oleifera Lam. on Salmonella typhi". *African Journal of Biotechnology* 6.19 (2007): 2212-2215.

38. Delarras C. "Microbiologie. 90 heures de travaux pratiques". Gaétan Morien (1998): 169-178.

39. Moroh JLA., *et al*. "Étude de l'activité antibactérienne de l'extrait acétatique (EAC) de Morinda morindoides (Baker) milne-redheat (Rubiaceae) sur la croissance in-vitro des souches d'Escherichia coli". *Bulletin de la Société Royale des Sciences de Liège* 77 (2008): 44-61.

40. Mousseux M. "Test de toxicité sur les larves d'Artémia salina entretien d'un élevage de balanes". Rapport de stage de 2ème année, Université française de Pacifique, Centre universitaire de Nouvelle Calédonie, Deutsch Aquaculture (1995): 20.

41. Moshi JM., *et al*. "Testing Beyond Ethnomedical Claims : Brine Shrimp Lethality of some Tanzanian Plants". *Journal of Pharmaceutical Biology* 42.7 (2004): 547-551.

42. Meyer BN., *et al*. "Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents". *Journal of Medicinal Plants Research* 45.5 (1982): 31-34.

43. Bapna S., *et al*. "Brine shrimp toxicity and in vitro antimalarial activity of Citrus aurantifolia (Christm.) Swingle against Plasmodium falciparum 3D7". *Journal of Pharmaceutical and Biological Sciences* 9.5 (2014): 24-27.

44. Fofie NBY., *et al*. "Minerals salt composition and secondary metabolites of Euphorbia hirta Linn., an antihyperglycemic plant". *Pharmacognosy Research* 7.1 (2015): 7-13.

45. Fasola TR and Iyamah PC. "Comparing the Phytochemical Composition of Some Plant Parts Commonly Used in the Treatment of Malaria". *International Journal of Pure and Applied Sciences and Technology* 21.1 (2014) : 1-11.

46. Abdallah EM. "Preliminary Phytochemical and Antibacterial Screening of Methanolic Leaf Extract of Citrus aurantifolia". *Pharmaceutical Biotechnology: Current Research* 1.2 (2016): 1-5.

47. Karou D., *et al*. "Antibacterial activity of alkaloids from Sida acuta". *African Journal of Biotechnology* 5.2 (2006): 195-200.

48. Abubakar El MM. "Antibacterial activity of crude extracts of Euphorbia hirta against some bacteria associated with enteric infections". *Journal of Medicinal Plants Research* 3.7 (2009): 498-505.

49. Upadhyay B., *et al*. "Pharmacognostical and antibacterial studies of different extracts of Euphorbia hirta L". *Journal of Phytology* 2.6 (2010): 55-60.

50. Akinnibosun FI and Edionwe O. "Evaluation of the Phytochemical and Antimicrobial potential of the Leaf Extracts of Bryophyllum pinnatum L. and Citrus aurantifolia Sw. and their Synergy". *Journal of Applied Sciences and Environmental Management* 19.4 (2015): 611-619.

51. Oboh PA and Abulu EO. "The antimicrobial activities of extracts of Psidium guajava and Citrus aurantifolia". *Nigerian Journal of Biotechnology* 8.1 (1997): 25-29.

52. Brama SO. "Activité antibactérienne d'extraits d'Euphorbia hirta (Linn), une plante utilisée traditionnellement dans le traitement des infections urinaires". Thèse de Doctorat. Université de Ouagadougou, Burkina-Faso (2002): 110.

53. Mohammadi S., *et al*. "Antimicrobial activity of methanolic root extract of Euphorbia Condylocarpa against pathogenic bacteria". *Advanced Studies in Biology* 7.2 (2015): 55-64.

54. Oikeh E., *et al*. "Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates". *Food Science and Nutrition* 4.1 (2016): 103-109.

55. Aibinu I., *et al*. "Evaluation of the antimicrobial properties of different parts of Citrus aurantifolia (lime fruit) as used locally". *African Journal of Traditional, Complementary, and Alternative Medicines* 4.2 (2007): 185-190.

56. Badiaga M. "Etude ethnobotanique, phytochimique et activités biologiques de Nauclea latifolia Smith, une plante médicinale africaine récoltée au Mali". Thèse de Doctorat, Université de Bamako, Mali (2011): 184.

57. Dramane S., *et al*. "Evaluation des Activités Antimicrobiennes et Anti-Radicaux Libres de Quelques Taxons Bioactifs de Côte d'Ivoire". *European Journal of Scientific Research* 40 (2010): 307-317.

58. Amvam ZPH., *et al*. "Aromatic plants of Tropical Central Africa, Part XXXl1 Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon". *Flavour and Fragrance Journal* 13 (1998): 107-114.

59. Cowan MM. "Plants products as anti-microbial activity". *Clinical Microbiology Reviews* 12.4 (1999): 564-582.

60. Kolodziej H., *et al*. "Evaluation of the anti-microbial potency of tannins and related compound using the microdilution broth method". *Planta Medica* 65 (1999): 444-446.

Volume 12 Issue 4 October 2017