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BIOLOGICAL AND CHEMICAL ACTIVITIES OF SOME BENINESE PLANT'S EXTRACTS

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ABSTRACT

Purpose: Treatment failures and increasing costs of treatment of infections due to enteric pathogens call to find other alternative treatments.

Methods: This study was initiated to evaluate the biological and chemical activities of aqueous and ethanolic extracts of some plants of Southern Benin namely *Moringa oleifera* (leaves), *Carica papaya* (leaves and seeds), *Ocimum graticimum* (leaves), *Cajanus cajan* (leaves), *Persea americana* (leaves), *Vernonia amygdalina* (leaves) and *Psidium guayava* (Leaves and roots)on multidrug-resistant bacterial strains. Agar diffusion method was used for sensitivity testing and liquid microdilution method was used to determine the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC).

RESULTS: All selected plants showed high content in flavonoids and polyphenols and are non-toxic at the concentration of 100 mg / ml. Aqueous and ethanol extracts *of Moringa oleifera* (leaves), *Carica papaya* (leaves and seeds), *Ocimum graticimum* (leaves) did not show antibacterial activity on the tested strains. The MIC and MBC of active extracts varied depending extracts and bacterial strains. Lowest MIC and MBC were obtained at a concentration of 25 mg/ml.

Conclusion: The study has confirmed the potential of Beninese pharmacopoeia plants. Some of these plants can be used in the fight against enteropathogens

Keywords: Aqueous and ethanol extract, phenolic compounds, Minimal Inhibitory Concentration, Minimal Bactericidal Concentration, enteropathogens, Beninese plants

INTRODUCTION

Currently microbial infections are on first rank in human pathology. Known for a long time, they have gradually changed the face putting the clinic in a situation of therapeutic impasse (Cornaglia *et al*, 2011). Antibiotics are widely used in treatment and prevention of bacterial infections. However, because of uncontrolled, inappropriate and abusive uses of antibiotics in human and animal health, the emergence of multi-resistant bacteria is increasingly criticized (Savard 2003). The emergence and spread of these multi-resistant bacteria in human populations have become very worrying public health problems (Lozniewski and Rabaud 2010). The progress of multi-resistance and the absence of real prospects for discovery of new antibiotics in the coming years, led to study the effectiveness of therapeutic plants in order to isolate the active ingredients. The use of ancient therapeutic plants is experiencing a surge of interest from the public. It is possible to use the whole plant or mining products they provide (Mark *et al*., 2001). The therapeutic use of plants for the treatment of all human diseases evolves the history of humanity. Traditional medicine remains the main use of a vast majority of people (Kone, 2009). Worldwide, 80% of people have used medicinal plants for treatment.

Today, the skills of traditional healers, is less transmitted and tends to disappear. This is why ethnobotany and ethnopharmacology working to identify, worldwide, reputable active plants and it belongs to the modern research to specify the properties and validate uses. In addition, chemical diversity of secondary plant metabolites that result from plant evolution is equal or superior to that found in synthetic combinatorial chemical libraries (Cosa *et al*., 2006). Such plants should be investigated to better understand their properties, safety and efficiency. Crude extracts of plants begin to have a lot of interest as a potential source of natural bioactive molecules. They are studied for their possible use as an alternative for the treatment of infectious diseases. This study was initiated to provide more knowledge of the therapeutic values of Beninese plants. 07 plant species common in South Benin were selected. The objective of this study is to evaluate the biological and chemical

activities of aqueous and ethanolic extracts of the selected plant species.

The selection of these plants was based on several criteria. They are not only among most popular found in traditional medicine in Benin but also very popular in the culinary field.

METHODS

Sample collection and treatment

The plant material consisted of fresh *Moringa oleifera* leaves, *Carica papaya* (leaves and seeds) *Ocimum gratissimum* (leaves), *Cajanus cajan*(leaves), *Persea americana* (leaves), *Vernonia amygdalina* (leaves) and *Psidium guajava* (leaves and roots). These leaves were harvested in South-Benin region in February 2017. They were authenticated by the National Herbarium of the University of Abomey-Calavi. The leaves of each plant were carefully washed in water containing bleach (1 / 100) and dried at roomtemperature for three weeks. The dried leaves were crushed and the resulting powders were sieved through a mesh of 0.2 mm. They were then stored in clean containers at laboratory temperature.

Extraction and preparation of extracts

Aqueous extracts

The aqueous extracts were obtained by an adaptation of the method developed by Guede-Guina *et al*. (1995). Fifty (50) grams of powder was macerated in 500 ml of distilled water on a shaker"Stuart Fisher Bioblock Scientific" for 72 hours at laboratory temperature. The mixture was filtered three times on hydrophilic cotton and once on paper Whatman No. 1. This filtrate was then dried at 45 ° C in the oven. The obtained powder was aqueous total extract used.

Ethanol extracts

The extraction method used is an adaptation to the protocol used by Sanogo *et al*. (2006) and N'Guessan *et al*. (2007). It has the advantage of putting the powder correctly in contact with the solvent with continuous stirring. A mass of 50 g leaf powder was soaked in 500ml of ethanol 96 ° with continuous stirring for 72 hours. The mixture was filtered three times on hydrophilic cotton then once on Whatman No. 1. The filtrate obtained with ethanol was evaporated at the temperature of 40 ° C in an oven to obtain a dry mass which represents the ethanol extract.

Preparation of extracts

Aqueous and ethanolic extracts of each plants were taken up in distilled water in an amount of 100 mg per 1 ml. The stock solutions and concentrated to 100 mg / ml were prepared. They were then sterilized by autoclaving at 121 ° C for 15 min. The sterility of the stock solutions of extracts was verified by plating aliquots of each solution on Mueller Hinton medium and

incubated at 37 ° C for 24 hours. The absence of colonies on the medium Mueller Hinton after 48 hours confirmed the sterility of the whole extracts solutions.

Yield of extraction

The yield of the crude extract is defined as the ratio between the mass of the dry extract obtained and the mass of the treated plant material (Harborne, 1998). This efficiency was calculated with the equation:

$$\mathbf{R}(\%) = \frac{\mathbf{Me}}{\mathbf{Mv}} \times \mathbf{100}$$

R (%): Yield in%
Me: Mass of the extract after solvent evaporation
Mv: mass of plant material used for extraction

Determination of Chemical substances (flavonoids and phenolic compounds)

Content of total polyphenols

Principle

The determination of total phenols was performed by a method adapted from Singleton (1998) using the commercial Folin-Ciocalteu reagent Folin consisting of a mixture of phosphotungestique acid (H3PW12 O40) and phosphomolybdic acid (H3PMO12 O40) is reduced during the oxidation of phenols, a mixture of blue oxides of tungsten and molybdenum. The blue coloration produced, whose maximum absorption is at 760 nm is proportional to the amount of polyphenols in the various extracts. The rate of total polyphenols in the different extracts was calculated from a linear calibration curve (y = ax+b), established with specific gallic acid as the reference standard concentrations.

Determination of Phenolic componentin samples

Each test sample was dissolved in ethanol so as to obtain a concentration of 10 mg / ml and then diluted 1/100 with distilled water. A volume of 125 µl of diluted solution was then mixed with 625 µl of Folin-Ciocalteu reagent 10% (diluted 10th in distilled water) and incubated for 5 min. 500 µl of an aqueous solution of sodium carbonate (Na2CO3) at 75 g/l were then added and mixed by vortexing and incubated for 2 h. After incubation, the optical densities (OD) were read at 760 nm using a spectrophotometer. Three readings were taken per sample. The reading was taken against a blank consisting of a mixture of 0.5 ml of FCR and 1 ml of Na_2CO_3. The total phenolic contents were determined using a calibration curve Gaelic acid (0-200 mg / l).

The calibration curve of gallic acid

From an aqueous stock solution of gallic acid of 10 mg / ml mass concentration, a standard range of aqueous test solutions was prepared. Using a micropipette, 125 ml of each working solution were put into test tube and then 625µl of Folin-Ciocalteu reagent at 10% (diluted 1/10 with distilled water) is added. After 5 minutes of incubation, 500 µl of sodium carbonate

(Na_2CO_3)at 75 mg / ml and 4.75 ml of distilled water was added. The tubes were then stirred and placed in the dark for 30 minutes at laboratory temperature. Absorbance of each solution prepared was read using a spectrophotometer at a wavelength of 760 nm against a blank prepared in the same manner except that it contains distilled water rather than gallic acid. The absorbance values for each concentration enabled to draw the calibration curve of gallic acid (Singleton *et al*., 1999)

The contents expressed as milligram gallic acid equivalent to 100 mg of extract or fraction (mg / EAG / 100 mg of extract) were determined by the following formula:

$$C = \frac{c \times D}{Ci} \times 100$$

Avec

C = total phenolics concentration in mg EAG / 100 mg dry extract

c = concentration of the sample read on the standard curve

D = sample dilution factor under assay

Ci = initial concentration of the sample solution to be assayed

Determination of Flavonoids

Flavonoids contents were measured by a method adapted from Zhishen *et al.* (1999) and Kim *et al.* (2003) using aluminum trichloride ($AlCl_3$) as a reagent. The presence of a free space in the reagent $AlCl_3$ forms a dative bond with the lone pairs of the oxygen of the OH groups of flavonoids, producing a yellow colored complex, whose maximum absorbance is recorded at 415 nm. The amounts of flavonoids in our extracts were calculated from the calibration curve of a standard flavonoid (rutin).

The calibration curve of rutin

A stock solution of rutin with mass concentration 10 mg / ml was prepared in ethanol. From this stock solution, a standard range of aqueous working solution was prepared. A volume of 500 µl of each working solution was placed in test tube completed by addition of 500 µl of aluminum trichloride at 2%and 3 ml of ethanol. The tubes were then slightly shaken and incubated in the dark for 10 min at room temperature. Absorbance of each solution prepared was measured in the same spectrophotometer at a wavelength of 415 nm against a blank. The absorbance values obtained have allowed to draw the calibration curve of rutin.

Determination of Flavonoids in the extracts

500 µl of $AlCl_3$solution (2%) were collected and 500 µl of the sample was added. 3 ml of ethanol to this mixture was added. Blank consisted of 500 µl of $AlCl_3$ and 3.5 ml of ethanol. The reading of absorbance was done on a spectrophotometer at 415 nm after incubation for 10 min.

Cytotoxicity testing

Hosts freshwater and brackish, brine shrimp (*Artemia salina*) survive extreme salinity levels (up to about 350 g / l) prohibiting the development of any other animal body. These small shrimp larvae do not exceed 13 mm. The cytotoxic effect of the extracts was assessed following an adaptation of the method described by Kawsar *et al*., (2008). The tests were conducted on the larvae hatch obtained by 10 mg of Artemia salina eggs (ARTEMIO JBL GmbH D-67141 Neuhofem) with continuous stirring in 1 liter of sea water for 72 hours. To 1 ml of each dilution in geometrical series reason½ , extract prepared from a stock solution of 20 mg.ml-1, was added 1 ml of seawater containing 16 larvae. The number of surviving larvae was counted after 24 hours of incubation. The LC50 was determined from the regression line obtained from the representative curve of the number of surviving larvae on the basis of the concentration of the extracts. Each test was performed in duplicate.

To interpret these results, correlation grids associating the degree of toxicity LC50 have been proposed (Moshi *et al*;. 2004; Sparkling, 1995).

LC50≥ 0.1 mg / ml, the extract is not toxic.

0.1 mg / ml> LC50 ≥ 0.050 mg / ml, the extract has a mean toxicity.

LC50 <0.01 mg / ml, the extract shows strong toxicity.

Bacterial species

Bacterial medium was composed of 09 clinical strains namely *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas oryzihabitans*, *Citrobacter freundii, Escherichia coli*, *Klebsiella rhinocleromatis*, *Shigella flexneri*, *Klebsiella oxytoca* and *Salmonella cholereasius* and a reference strain *Escherichia coli* ATCC 25922

These bacterial strains were provided by the National Laboratory of Benin.

Antimicrobial activity test using the agar well diffusion method

Bacterial preculture (1 colony of 18h in 5 ml of sterile distilled water) was diluted to obtain a turbidity of 0.5 McFarland (or 108 CFU / mL) and reduced to 106 CFU / ml in sterile distilled water.Each inoculum was plated with swabs on petri dishes containing Mueller Hinton agar (CA-SFM, 2012). Using the tip of sterile Pasteur pipette, wells of 6 mm in diameter were drilled. Then using a sterile cone and a micropipette, 50 µl of each sample was deposited in the previously dug wells. A well containing sterile distilled water was used as negative control. Antibiotic discs standards were also used to serve as positive controls. The Petri dishes were left for 1 hour at laboratory temperature to a

pre-diffusion of the substances before being incubated at 37 ° C in an oven for 18 hours to 24 hours. After incubation period, the dishes were examined to note zones of inhibition (diameter measured in mm). All tests were performed in duplicate.

Table 1: Standard used for reading the results of susceptibility testing plant extracts.

Diameter of the inhibition's area (Δ)	Level of sensibility	Symbol
Δ < 7mm	insensitive	-
7mm ≤ Δ < 8mm	sensitive	+
8mm ≤ Δ < 9mm	Quite sensitive	++
Δ ≥ 9mm	Very sensitive	+++

Source: OMS., 2002 ;Tsirinirindravo et Andrianarisoa, 2009.

Determination of Minimum Inhibitory Concentration (MIC) by micro dilution and minimum bactericidal concentration (MBC)

A sample stock solution was prepared at a concentration of 100 mg / ml in distilled water. 100 µl of Mueller-Hinton Broth (MHB) was deposited in each well of the micro plate (1 to 8 wells). 100 µl of the extract stock solution was deposited into the first well. After homogenization by suction-discharge using a micropipette, 200 µl of extract solution at 100 mg / ml is obtained. 100 µl of this new solution were collected and mixed with MHB contained in the 2nd well and continues this ½ dilution from well to well until 6th wells. Finally, 100 µl of the bacterial suspension were added to each well. The 7th and 8th wells were respectively the positive control and the negative control and contain 100 µl of MHB + 100 µl of the bacterial suspension to a positive control and 100 µl of MHB to the negative control. The microplates were coated placed for 24 hours in an oven at 37 ° C. MICs were estimated with the naked compared to controls and each well was plated eye on the MH agar and placed at 37 ° C for 24 hours. The MBC corresponded to the lowest concentration of extract for which there was no bacterial colonies.

Processing and analysis of data

The file Managed data and encoded Were Recorded in an Excel database. The graphs have been realized with the Graph Pad Software. Descriptive statistics were Performed using SPSS 20.

RESULTS

- **Yield extraction and Sterility test**

The ethanol extracts showed the best yield with the exception of *Moringa oleifera* aqueous extract of which has performed better yield than the ethanol extract. Figure 1 shows the results of performance of aqueous extracts and ethanol extracts sterility tests revealed no contamination at all sample.

Determination of Chemical substances (flavonoids and phenolic compounds)

Determination of Phenolic Compounds

Chemical test to determine polyphenol content of plant extracts was used to determine the polyphenol content of

aqueous and ethanolic extracts of selected plants. A variation in the polyphenol content of plants extracts was observed from the regression line obtained from the curvecalibration of Gallic acid. Figure 2 shows the calibration curve of Gallic acid.

Table 2 shows polyphenol contents of aqueous and ethanolic extracts of the plants tested.

The polyphenol content is EAG microgram / extract 100milligramme. The ethanolic extracts in general are more concentrated in polyphenols than the aqueous extracts with the exception of ethanolic extracts of *Psidium guajava* (leaves and roots), *Vernonia amygdalina*, *Cajanus cajan* and *Moringa oleifera* which are less concentrated in polyphenols that aqueous extracts of these plants.

Determination of Flavonoids

The biochemical test to determine the flavonoid content of plant extracts was used to determine the flavonoid content of aqueous and ethanolic extracts of selected plants. A variation of the content of flavonoid was observed from the equation of the regression line obtained from the calibration curve of rutin. Figure 3 shows the calibration curve of rutin. Table 3 shows the flavonoid content of aqueous and ethanolic extracts of the plants tested.

The content in flavonoids is ER microgram / 100 mlliigramme extract. Flavonoids contents of ethanolic extracts are generally higher than aqueous extracts except ethanolic extracts of *Psidium guajava* (roots), *Carica papaya* (seeds) and *Vernonia amygdalina* that are less concentrated in flavonoids the aqueous extracts.

The contents of all sample polyphenols exceed the flavonoid content.

Cytotoxicity of extracts

The test to determine plant extracts lethal effect on crustaceans (*Artemia salina*) was used to assess cytotoxicity all previews. Tables 7 and 8 show the average lethal concentrations (LC50) by extracts and regression coefficients. *Artemia salina* larvae were sensitive against extracts of all plants at a dose of 20 mg / ml. Change in LC50 was observed from the regression line obtained from the representative curve of the number of surviving larvae on the basis of the concentration of the extracts. Figures from 4 to 12 show the regression lines which expresses the percentage of larvae killed as a function of the concentration of aqueous and ethanolic extracts.

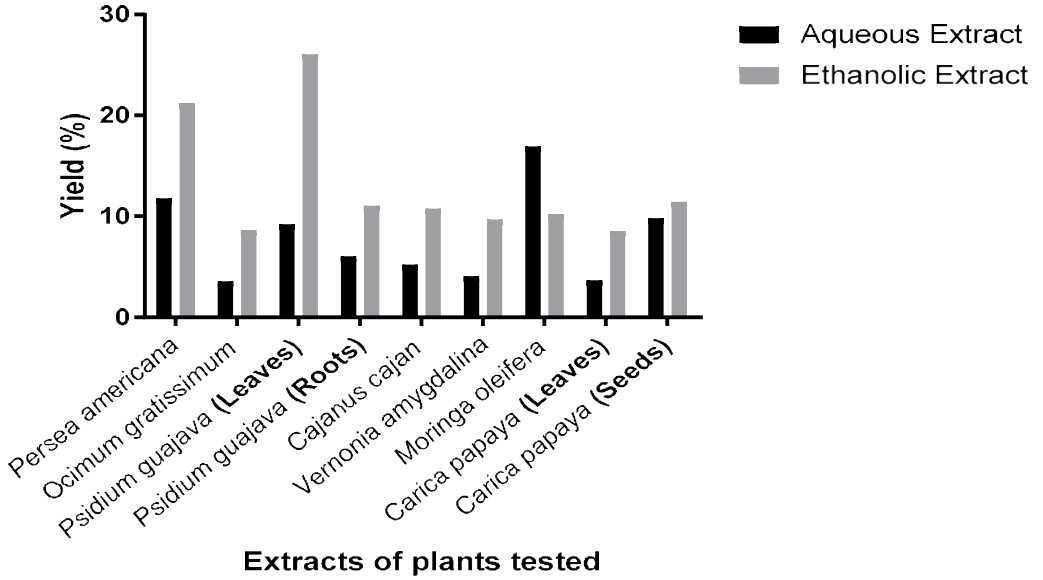


Figure 1: Yield extraction

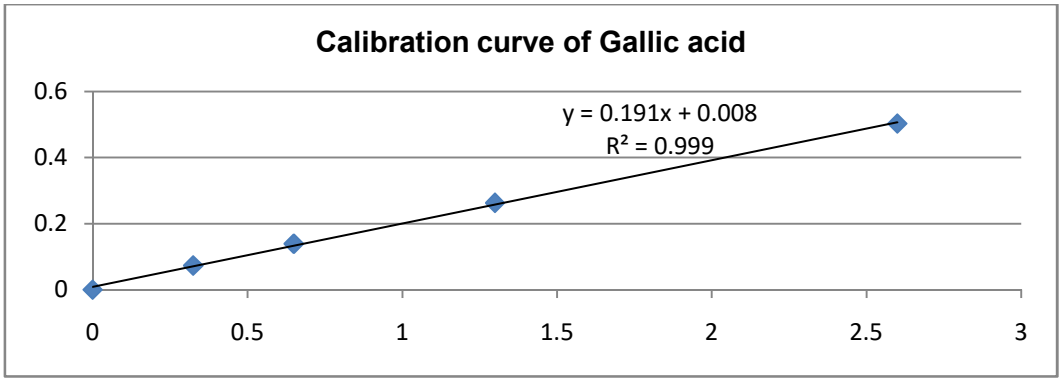


Figure 2: Calibration curve of the Gaelic acid

Table 2: Levels of Polyphenols aqueous and ethanolic extracts

extracts	Clue	Uncertainty	Vr	Vp	Cex	Content
P.a EA	1.40804598	0.268575	6.25	0,125	0.1	70.4022989
P.a EE	2.53448276	0.27586207	6.25	0,125	0.1	126.724138
O.g EA	0.55172414	0.27586207	6.25	0,125	0.1	27.5862069
O.g EE	2.24712644	0.268575	6.25	0,125	0.1	112.356322
P.g(f) EA	9.98275862	0.27586207	6.25	0,125	0.1	499.137931
P.g(f) EE	2.84482759	0.25862069	6.25	0,125	0.1	142.241379
P.g(r) EA	4.64367816	0.268575	6.25	0,125	0.1	232.183908
P.g(r) EE	3.55747126	0.268575	6.25	0,125	0.1	177.873563
C.c EA	2.95977011	0.268575	6.25	0,125	0.1	147.988506
C.c EE	0.56896552	0.25862069	6.25	0,125	0.1	28.4482759
V.a EA	7.50574713	0.30839226	6.25	0,125	0.1	375.287356
V.aEE	0.92528736	0.268575	6.25	0,125	0.1	46.2643678
M.o EA	3.90804598	0.268575	6.25	0,125	0.1	195.402299
M.o EE	2.35057471	0.268575	6.25	0,125	0.1	117.528736
C.p(f) EA	0.54022989	0.30201054	6.25	0,125	0.1	27.0114943
C.p(f) EE	6.45402299	0.28495733	6.25	0,125	0.1	322.701149
C.p(g)EA	0.54022989	0.30201054	6.25	0,125	0.1	27.0114943
C.p(g)EE	6.45402299	0.28495733	6.25	0,125	0.1	322.701149

P.a = *Persea americana*; O.g = *Ocimum gaticimum*; P.g (f) =*Psidium guajava (Leaves);* P.g (r) =*Psidium guajava (Roots)*,C.c = *Cajanus cajan*; V.a = *Vernonia amygdalina*; M.o = *Moringa oleifera*; C.p (f) = *Carica papaya* (leaves); C.p (g) = *Carica papaya* (seeds)

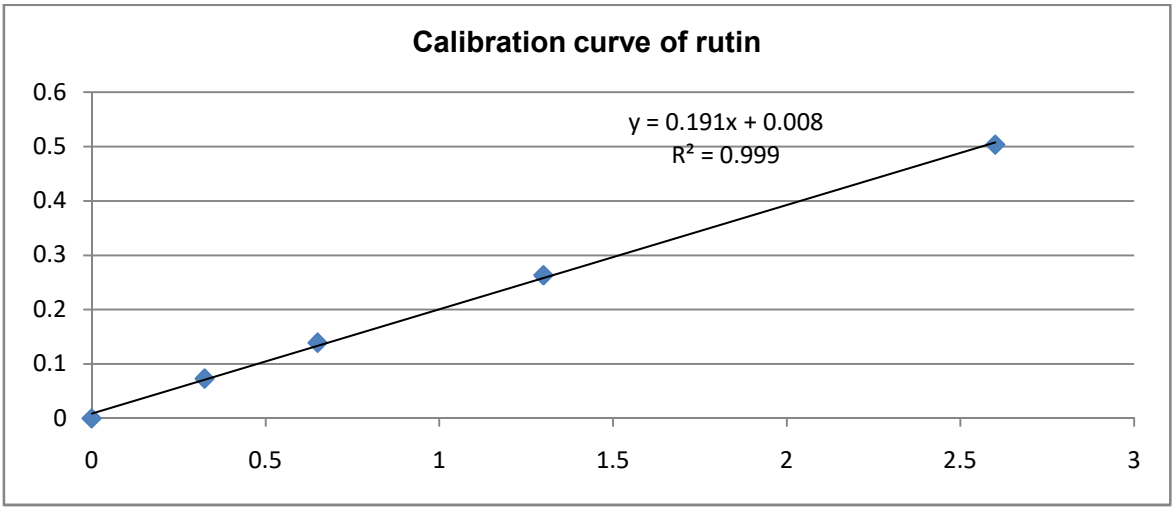


Figure 3: calibration curve rutin

Table 3: Levels of flavonoids of aqueous and ethanolic extracts

extracts	Clue	Uncertainty	Cex	Vr	Vp	Content
P.a EA	0.34554974	0.04188482	0.1	4	0.5	2.76439791
P.a EE	1.15357766	0.04490759	0.1	4	0.5	9.22862129
O.g EA	0.47120419	0.04712042	0.1	4	0.5	3.76963351
O.g EE	1.53577661	0.04490759	0.1	4	0.5	12.2862129
P.g(f) EA	0.66317627	0.04490759	0.1	4	0.5	5.30541012
P.g(f) EE	1.2530541	0.04793037	0.1	4	0.5	10.0244328
P.g(r) EA	0.07678883	0.04490759	0.1	4	0.5	0.61431065
P.g(r) EE	0.05061082	0.04490759	0.1	4	0.5	0.40488656
C.c EA	0.94938918	0.04490759	0.1	4	0.5	7.59511344
C.c EE	2.92321117	0.04988233	0.1	4	0.5	23.3856894
V.a EA	2.60383944	0.04988233	0.1	4	0.5	20.8307155
V.a EE	0.7521815	0.04490759	0.1	4	0.5	6.01745201
M.o EA	0.29842932	0.04188482	0.1	4	0.5	2.38743455
M.o EE	1.98080279	0.04490759	0.1	4	0.5	15.8464223
C.p(f) EA	0.87260035	0.04490759	0.1	4	0.5	6.98080279
C.p(f) EE	1.83246073	0.04188482	0.1	4	0.5	14.6596859
C.p(g) EA	2.38045375	0.55841705	0.1	4	0.5	19.04363
C.p(g) EE	2.10122164	0.04793037	0.1	4	0.5	16.8097731

P.a = *Persea americana*; O.g = *Ocimum graticimum*; P.g (f) =*Psidium guajava (Leaves);* P.g (r) =*Psidium guajava (Roots),*C.c = *Cajanus cajan*; V.a = *Vernonia amygdalina*; M.o = *Moringa oleifera*; C.p (f) = *Carica papaya* (leaves); C.p (g) = *Carica papaya* (seeds)

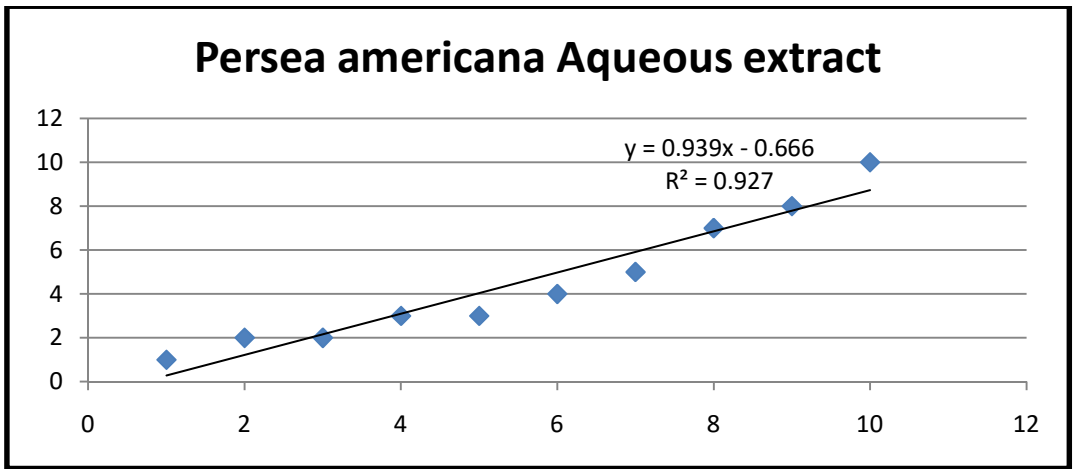
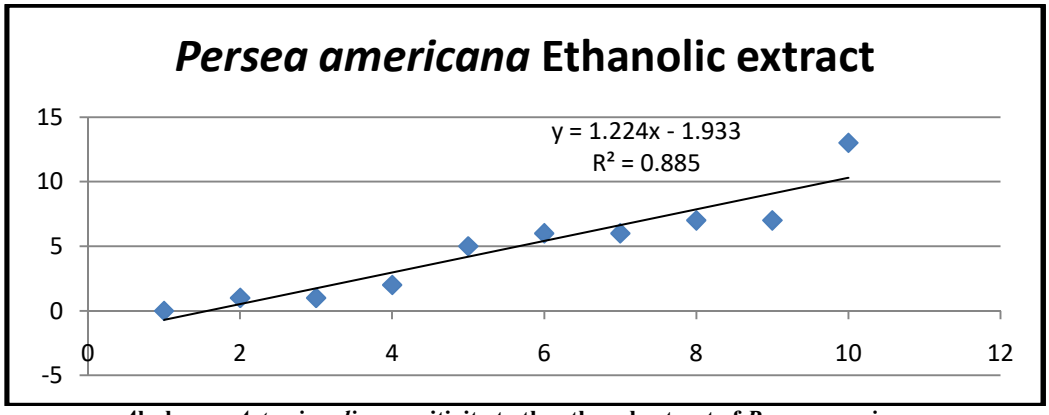
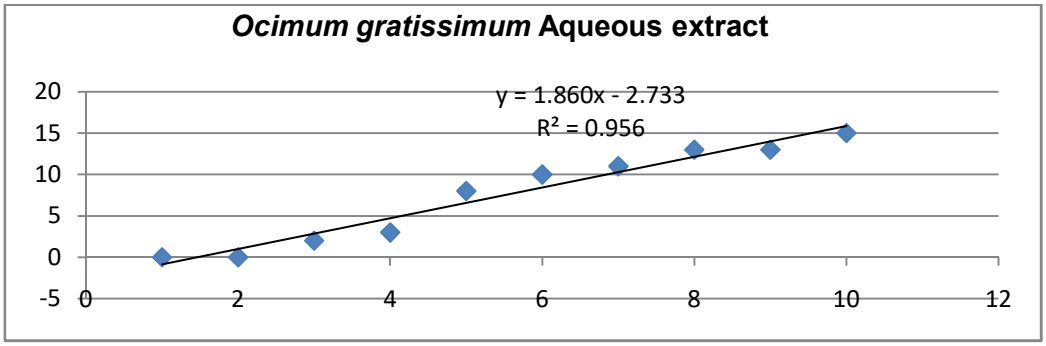


Figure 4: Sensitivity larvae of *Artemia salina* to the aqueous extract of *Persea americana*



4b: larvae *Artemia salina* sensitivity to the ethanol extract of *Persea americana*



5a: Sensitivity larvae of Artemia salina to the aqueous extract of *Ocimum gratissimum*

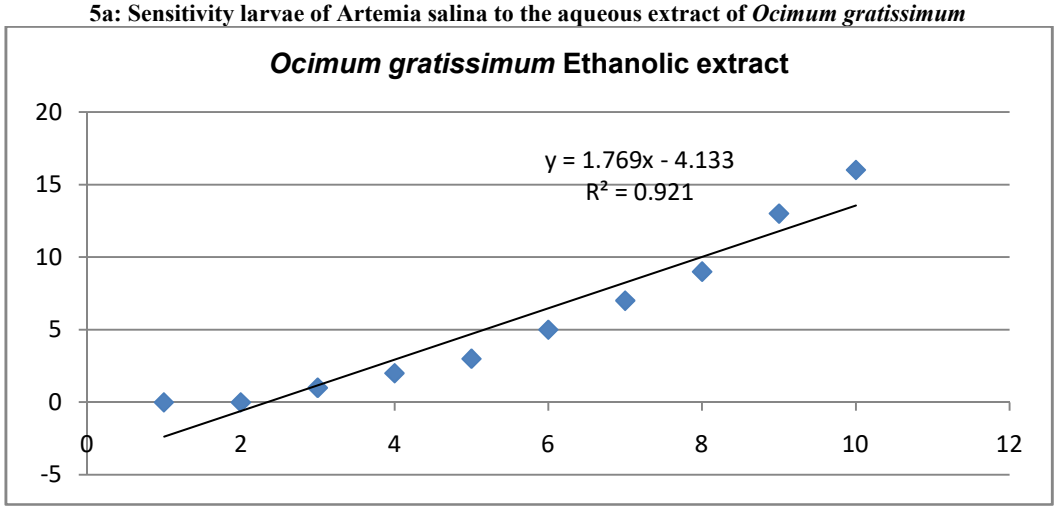


Figure 5b: Larvae *Artemia salina* sensitivity to the ethanolic extract of *Ocimum gratissimum*

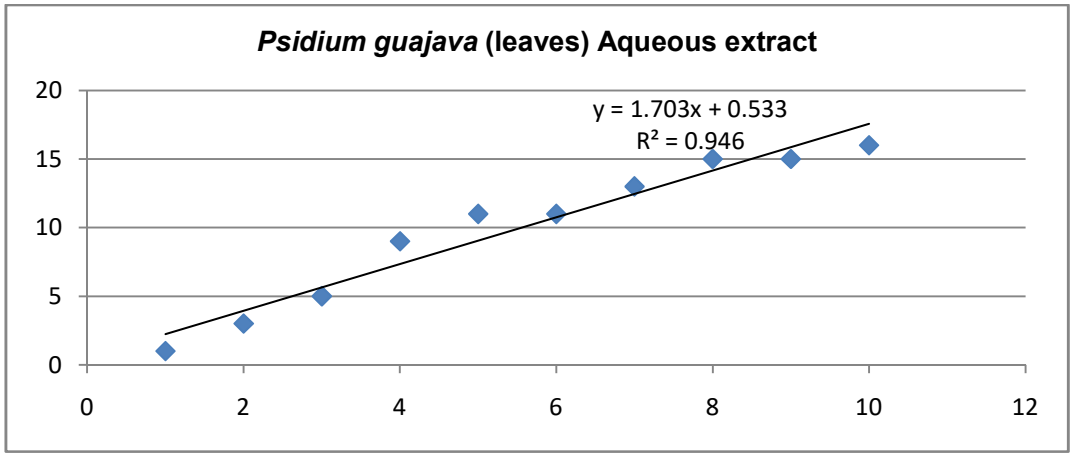


Figure 6a: Sensitivity larvae of *Artemia salina* in *Psidium guajava* aqueous extract (leaves)

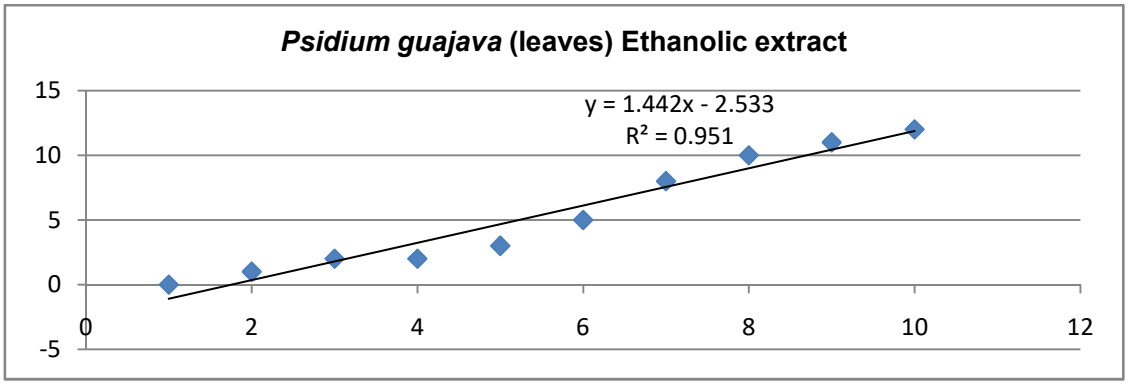


Figure 6b: Sensitivity larvae *Artemia salina* in ethanolic extract of Psidium guajava (leaves)

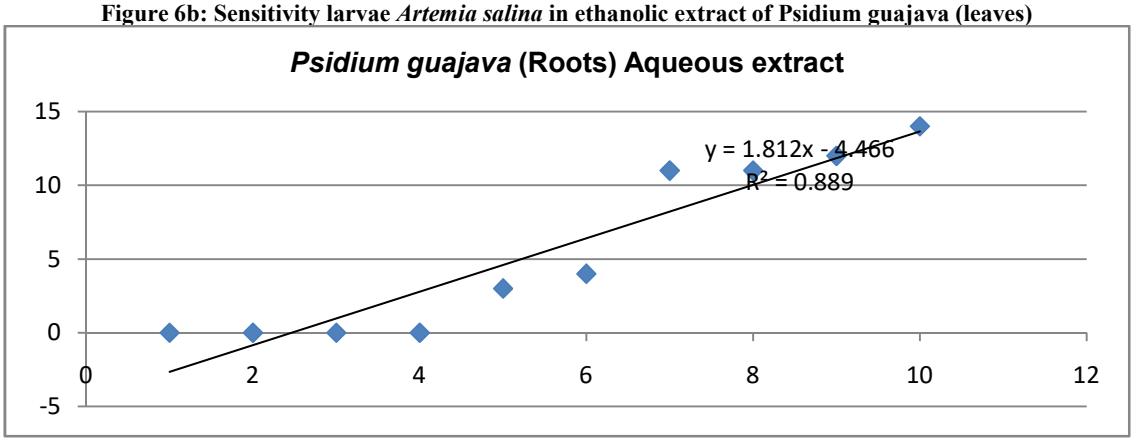


Figure 7a: Sensitivity larvae *Artemia salina* in ethanolic extract of *Psidium guajava* (Roots)

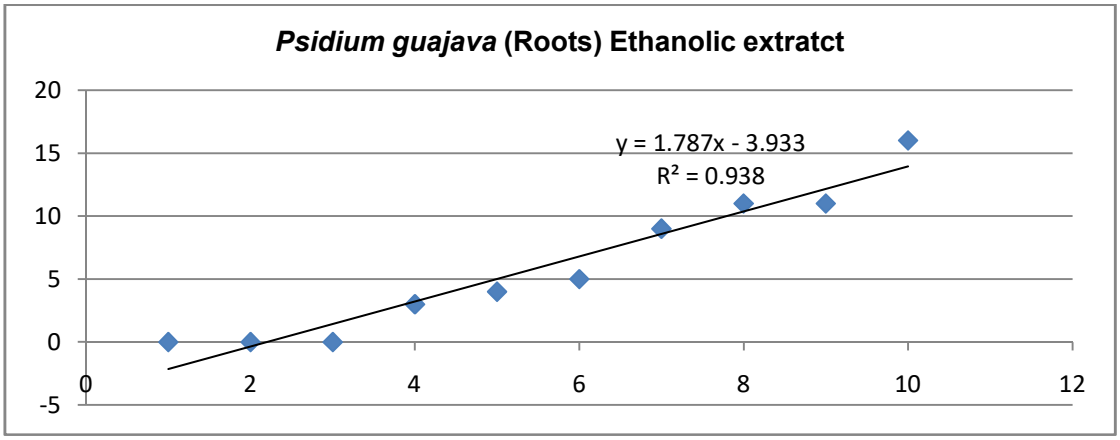


Figure 7b : Sensitivity larvae *Artemia salina* in ethanolic extract of *Psidium guajava* (Roots)

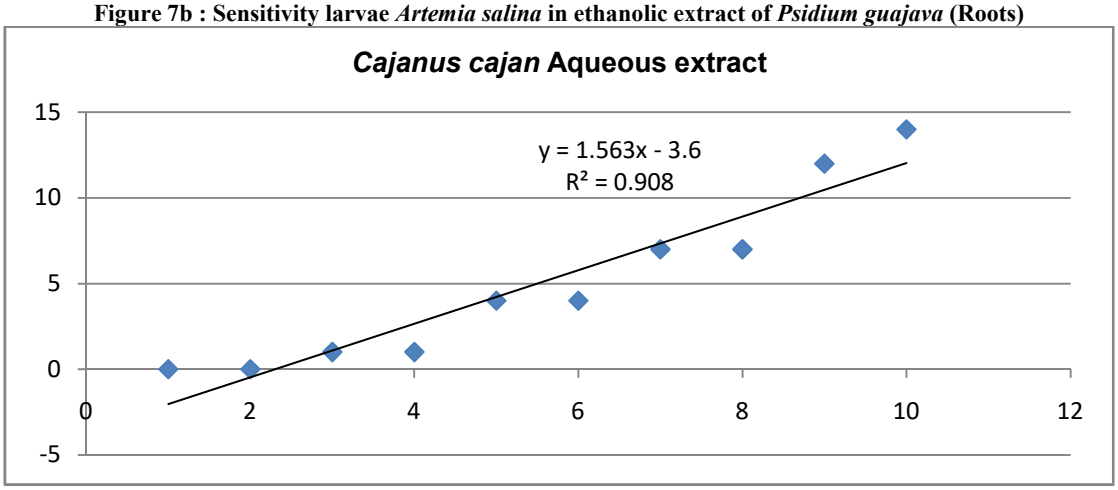


Figure 8a Sensitivity larvae of *Artemia salina* to the aqueous extract of *Cajanus cajan*

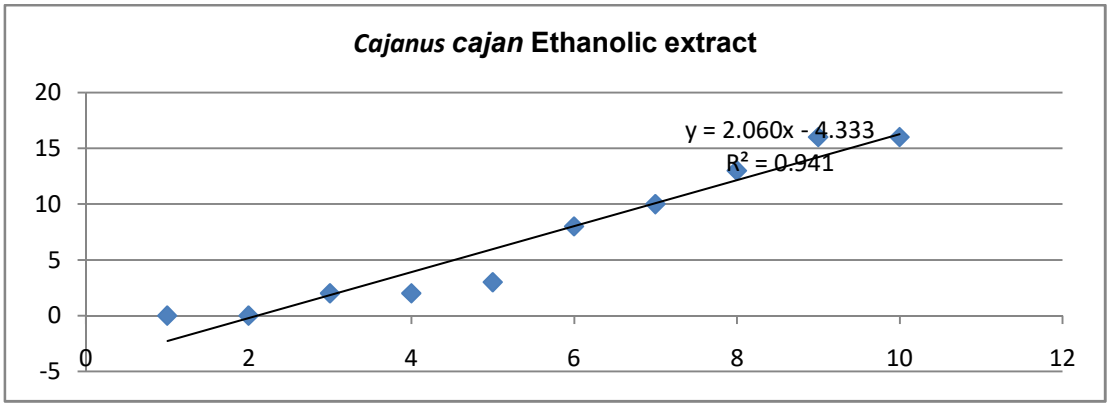


Figure 8b : Sensitivity larvae *Artemia salina* in ethanolic extract of *Psidium guajava* (leaves)

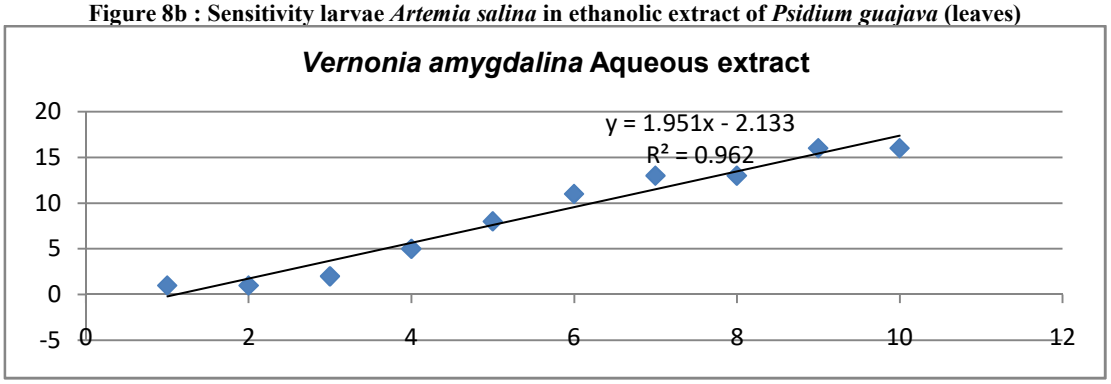


Figure 9a Sensitivity larvae of *Artemia salina* to the aqueous extract of *Vernonia amygdalina*

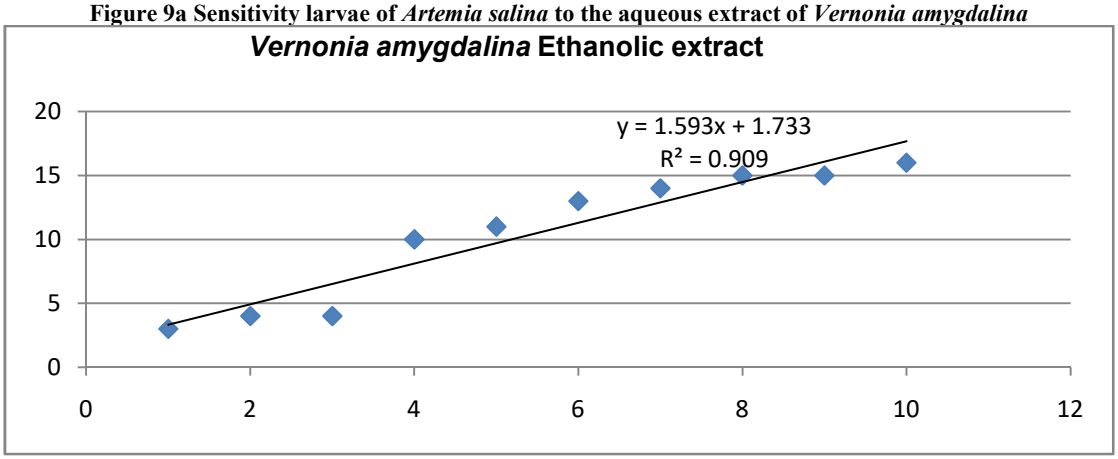


Figure 9b : Sensitivity larvae *Artemia salina* in ethanolic extract of *Vernonia amygdalina*

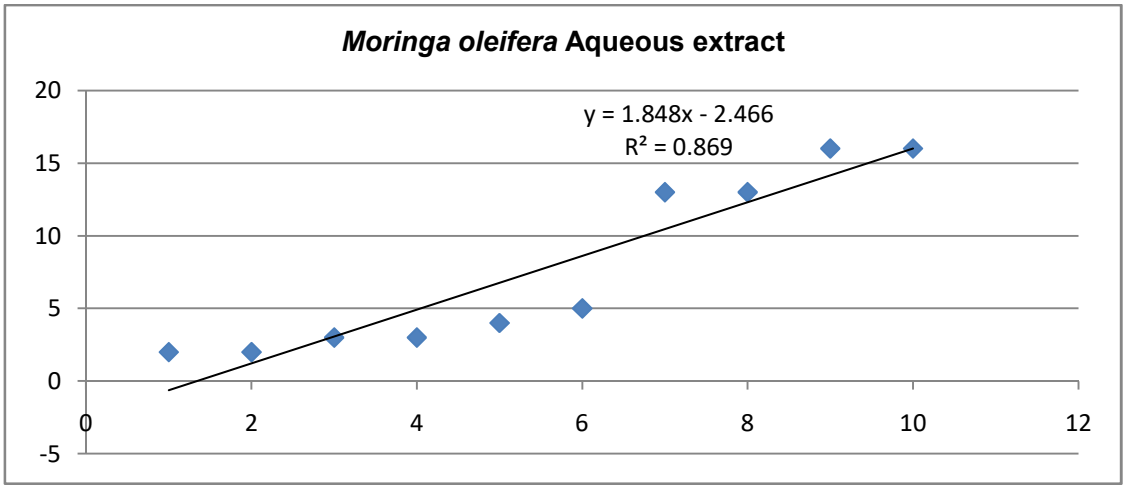


Figure 10a Sensitivity larvae of *Artemia salina* to the aqueous extract of *Moringa oleifera*

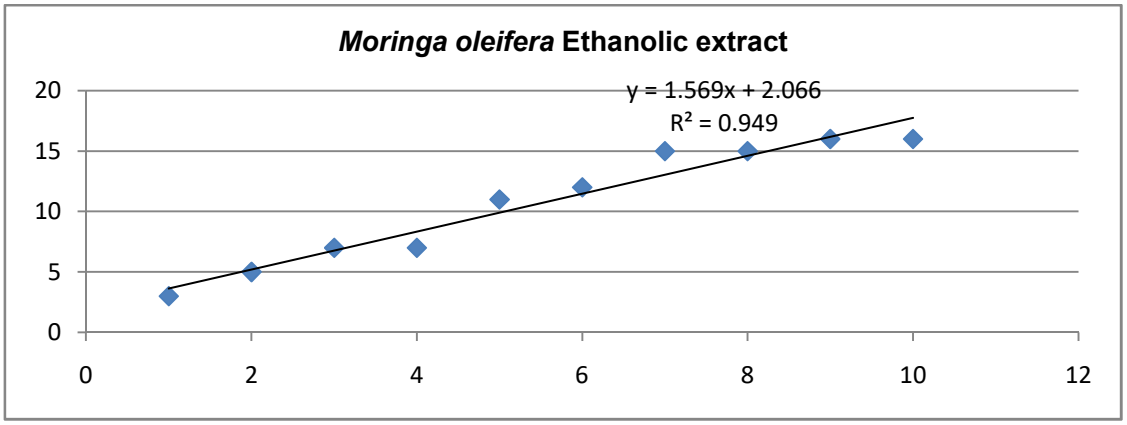


Figure 10b : Sensitivity larvae *Artemia salina* in ethanolic extract of *Moringa oleifera*

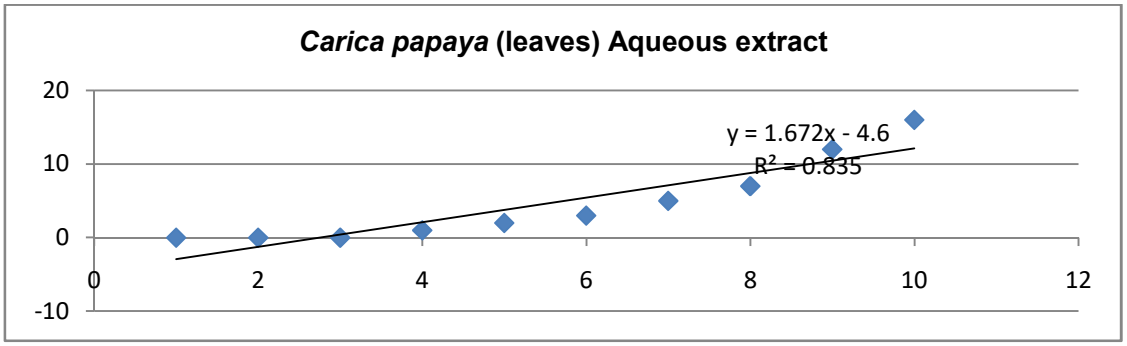


Figure 11a : Larvae of *Artemia salina* Sensitivity to the aqueous extract of *Carica papaya* (leaves)

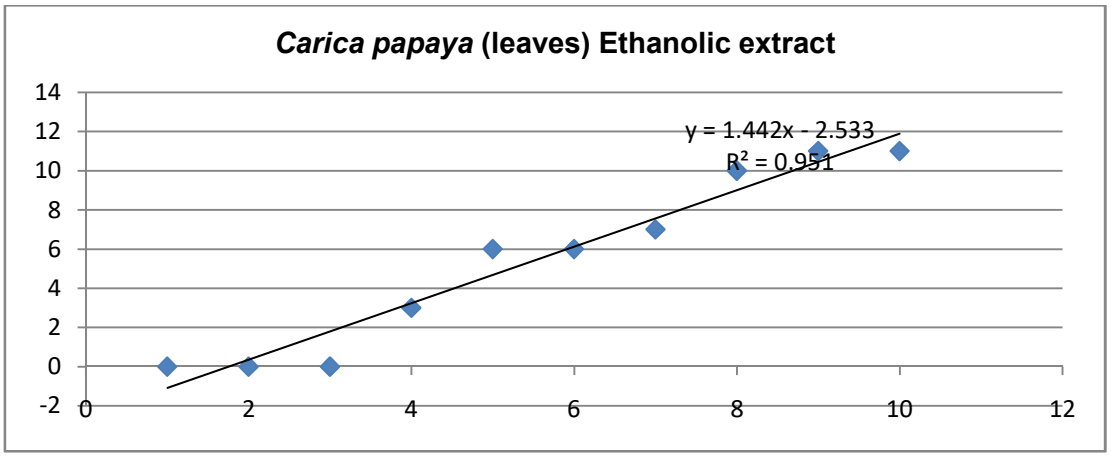


Figure 11b: Sensitivity larvae *Artemia salina* in ethanolic extract of *Carica papaya* (leaves)

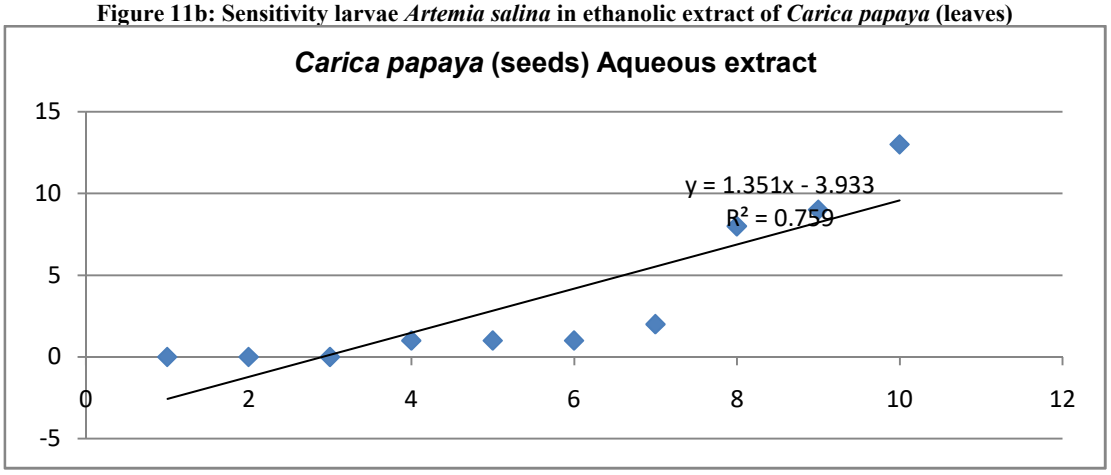


Figure 12a: Larvae of *Artemia salina* Sensitivity to the aqueous extract of *Carica papaya* (seeds)

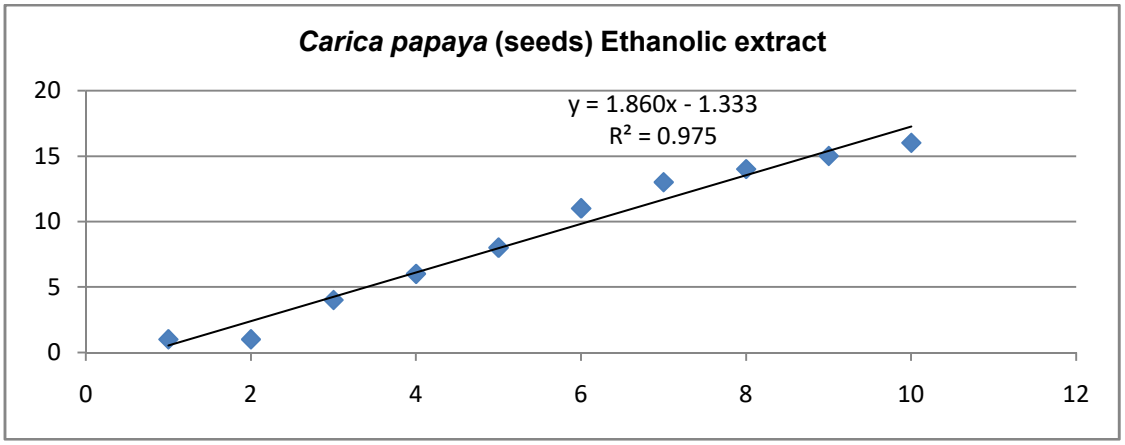


Figure 12b: Sensitivity larvae *Artemia salina* in ethanolic extract of Carica papaya (seeds)

Table 4: Correspondence between LC50 and toxicity

LC50	Toxicity
LC50 _ 0.1 mg / ml	- (Non-toxic)
0.1 mg / ml> LC50 _ 0050 mg / ml	+ (Low toxicity)
0.050 mg / mL> LC50_0.01 mg / ml	++ (Moderate toxicity)
LC50 <0.01 mg / ml	+ + + (High toxicity)

The LC50 lethal concentration was determined from the regression line that expresses the percentage of larvae killed depending on the concentration of the extracts. Table 4 shows the correspondence table between the LC50 and toxicity.

All LC50 obtained with the linear regression lines were above 0.1 mg / ml. The aqueous and ethanol extracts of all plants are therefore non-toxic.

Antibacterial activity

- **Sensitivity test**

All extracts were prepared at a concentration of 100mg / ml. The tested strains showed varying sensitivity to the tested extracts. Inhibition diameters of extracts ranged between 7 and 20 mm. All bacterial strains were resistant to aqueous and ethanolic extracts of *Ocimum gratissimum*, *Carica papaya* (leaves and seeds) and *Moringa oleifera*. The most active extracts were extracts *Psidium guajava* (Root and leaves), the aqueous and ethanolic extracts of leaves of *Cajanus cajan,* and *Vernonia amygdalina*. The less active extracts were those of aqueous and ethanolic *Persea americana* that have been active on a bacterial strain. *Klebsiella oxytoca* was sensitive to two extracts of 18 namely aqueous leaf extract of *Psidium guajava* and the ethanol extract of leaves of *Cajanus cajan*. The active ethanol extract showed a better inhibition than the aqueous extract. The ethanolic and aqueous extracts of roots of *Psidium guajava*, the ethanolic extracts of leaves of *Cajanus cajan* and *Vernonia amygdalina* were active on *Klebsiella pneumoniae* strain. The ethanol extracts showed better inhibition on this strain except ethanolic extract of *Cajanus cajan* that showed a lower activity than the aqueous extract of root of *Psidium guajava*.

Aqueous and ethanolic extracts of leaves of *Psidium guajava*, ethanolic extracts from *Psidium guajava*roots,ethanolic extract of leaves of *Cajanus cajan* and the aqueous extract of leaves of *Vernonia amygdalina* were active extracts on *Klebsiella rhinocleromatis*. The most active extracts were aqueous extract of the leaves of *Vernonia amygdalina* followed by ethanolic root extract of *Cajanus cajan* and aqueous leaf extract of *Psidium guajava*. *Pseudomonas aeruginosa* was sensitive to leaf extracts of *Persea americana*, aqueous and ethanolic extracts of leaves and roots of *Psidium guajava* and the ethanolic extracts of *Vernonia amygdalina*. The extracts that are the best activity are ethanolic extract of leaves of *Vernonia amygdalina* and aqueous extract of *Psidium guajava* root. Ethanolic extracts of leaves and roots of Psidium guajava were the only active extracts of the strain of *Pseudomonas oryzihabitans* with root ethanolic extract which had the best inhibition. Aqueous and ethanolic extracts of leaves of*Psidium guajava*, aqueous extracts of leaves of *Cajanus cajan* and leaves of *Vernonia amygdalina* were active on the strain of *Salmonella cholereasius*. The aqueous extract of leaves of *Psidium guajava* presented the lowest inhibitory activity. The ethanolic extracts of roots *Psidium guajava* and leaves of *Cajanus cajan* were the only active extracts on clinical *Escherichia coli* strain with the best antibacterial activity for the ethanolic extract of root *Psidium guajava*. Ethanolic extract of leaves of *Psidium guajava* and aqueous extract of roots of *Psidium guajava* were the only active extracts on the reference strain of *Escherichia coli*. The aqueous extract presented the lowest inhibitory activity and all extracts from this this strain (7mm). Both extracts of leaves and roots *Psidium guajava* and the ethanolic extract of *Cajanus cajan* sheet have summers active on the bacterial strain of *Citrobacter freundii*. Ethanolic extracts from root of *Psidium guajava* and *Cajanus cajan* ethanolic extracts were the most active. The most active aqueous extract was leaves extract of *Psidium guajava*. Both extracts of leaves and roots of *Psidium guajava* followed by the two extracts from the leaves of *Cajanus cajan* and *Vernonia amygdalina* and the ethanolic extract of *Persea americana* have been active on the strain of *Shigella flexneri*. The ethanolic extracts generally showed the best inhibitory activities. The results of the sensitivity tests showed that both extracts of active plants did not exhibit exactly the same effectiveness of the same bacterial strain. Figure 5 shows the different diameters of inhibition extracts on the bacterial strains.

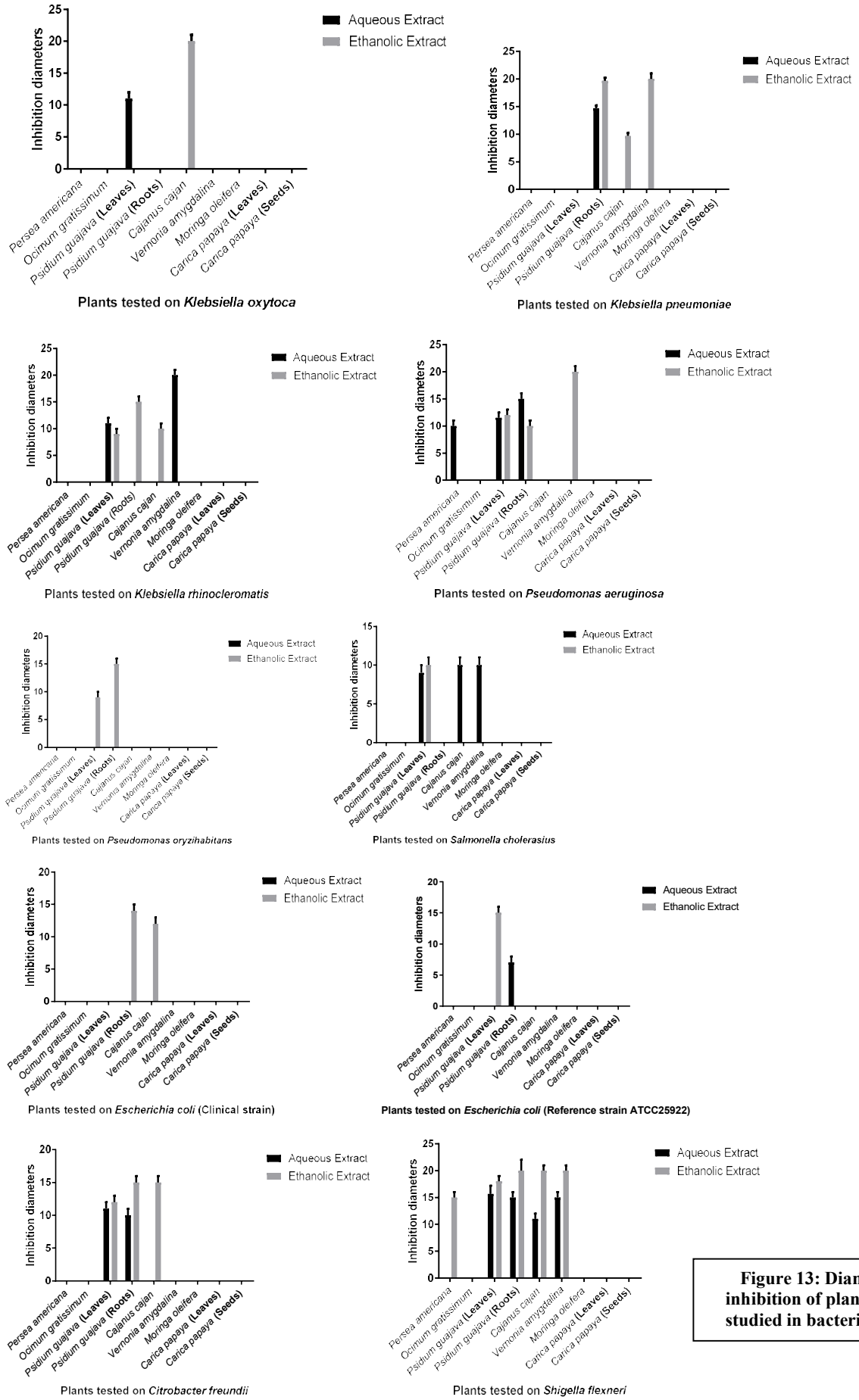


Figure 13: Diameter of inhibition of plant extracts studied in bacterial strains

- **Minimum Inhibitory Concentration, concentration and minimum bactericidal antibiotic to excepts**

The minimum inhibitory concentrations (MICs) obtained are variable depending on the types and strains of the extract. The lowest MICs were obtained at the concentration of 25mg. Minimum bactericidal concentrations (MBC) also varied depending on the type of extract and susceptible strains. The antibiotic potency (a.p) was determined through the ratio MBC / MIC. Tables 5 and 6 show the minimum inhibitory concentrations, bactericides and power antibiotic aqueous and ethanol extracts.

The report MBC / MIC of the aqueous extract of the leaves of *Psidium guajava* on*Pseudomonas aeruginosa*, *Citrobacter freundii* and *S. flexneri* is less than 4. The extract therefore has a bactericidal activity on these strains. The ratio MBC / MIC of the aqueous extract of the roots of *Psidium guajava* on *Pseudomonas aeruginosa* is less than 4. The extract therefore has a bactericidal activity on the strain. In addition, this sample has a bacteriostatic action on *Shigella flexneri with* a report MBC / MIC equal to 4. The ratio MBC / MIC of aqueous extract of *Persea americana* leaves against *Pseudomonas aeruginosa* is higher than 4. This extract therefore has a power bacteriostatic on this strain. The aqueous extracts of *Vernonia amygdalina* and *Cajanus cajan* gave good inhibition diameters of some strains with good minimum bactericidal concentrations but any MBC was not observed. These extracts therefore have not any power on antibiotic strains that were sensitive to them.

The ethanolic extract of the leaves of *Psidium guajava* presented bactericidal activity on *Pseudomonas aeruginosa*, *Salmonella cholereasius* and *Shigella flexneri* with ration MBC / MIC less than 4. Ethanolic extract of *Psidium guajava* root presented a bactericidal power on *Pseudomonas aeruginosa* strains, *Escherichia coli* (clinical strain) and *Shigella flexneri* with a ratio MBC / MIC of less than 4. Ethanolic extract of leaves of *Persea americana* presented a bactericidal power on the strain of *Shigella flexneri* with a ratio MBC / MIC less than 4. Ethanolic extract of *Cajanus cajan* leaves presented a bactericidal power on *Shigella flexneri* strains with MBC / MIC less than 4.Ethanolic extract of *Vernonia amygdalina* leaves is bactericidal for the strain of *Shigella flexneri* with a ratio MBC / MIC of less than 4.

Table 5: MIC, MBC and power antibiotic aqueous extracts

Extracts	parameters	*K. pneumoniae*	*K.rhinocleromatis*	*K.oxytoca*	*P.oryzihabitans*	*P. aeruginosa*	*E.coli*	*E.coli*ATCC 25922	*C.freundii*	*S.cholerasius*	*flexneri*
P.a	MIC	-	-	-	-	25	-	-	-	-	-
	MBC	-	-	-	-	50	-	-	-	-	-
	a.p	-	-	-	-	2	-	-	-	-	-
P.g(f)	MIC	-	100	50	-	50	-	-	25	50	25
	MBC	-	-	50	-	50	-	-	25	-	25
	a.p	-	-	1	-	1	-	-	1	-	1
P.g(r)	MIC	50	-	-	-	50	-	50	25	-	25
	MBC	-	-	-	-	50	-	-	-	-	100
	a.p	-	-	-	-	1	-	-	-	-	4
C.c	MIC	-	-	-	-	-	-	-	-	50	50
	MBC	-	-	-	-	-	-	-	-	-	-
	a.p	-	-	-	-	-	-	-	-	-	-
Go	MIC	-	-	100	-	-	-	-	-	-	100
	MBC	-	-	-	-	-	-	-	-	-	-
	a.p	-	-	-	-	-	-	-	-	-	-

P.a = *Persea americana*; P.g (f) =*Psidium guajava* (Leaves); C.c = *Cajanus cajan*; V.a = *Vernonia amygdalina*; P.g (r) =*Psidium guajava* (Roots), K. pneumoniae =*Klebsiellapneumoniae, K.rhinocleromatis = rhinocleromatis Klebsiella, Klebsiella oxytoca K.oxytoca =, P. oryzihabitans = Pseudomonas oryzihabitans, P.aeruginosa = Pseudomonas aeruginosa, E. coli = Escherichia coli*(Clinical strain), E. coli ATCC 25922 Escherichia coli ATCC25922 = (reference strain), C.freundii = *Citrobacter freundii*, S. *cholereasius= Salmonella cholereasius*, *S. flexneri = Shigella flexneri*

Table 6: MIC, MBC and power antibiotic ethanolic extracts

Extracts	parameters	*K. pneumoniae*	*K.rhinocleromatis*	*K.oxytoca*	*P.oryzihabitans*	*P. aeruginosa*	*E.coli*	*E.coli*ATCC 25922	*C.freundii*	*S.cholerasius*	*flexneri*
P.a	MIC	-	-	-	-	-	-	-	-	-	50
	MBC	-	-	-	-	-	-	-	-	-	100
	a.p	-	-	-	-	-	-	-	-	-	2
P.g(f)	MIC	-	100	-	50	50	-	100	100	50	25
	MBC	-	-	-	-	50	-	-	-	50	25
	a.p	-	-	-	-	1	-	-	-	1	1
P.g(r)	MIC	100	-	-	50	100	25	-	50	-	25
	MBC	-	-	-	-	100	50	-	-	-	25
	a.p	-	-	-	-	1	2	-	-	-	1
C.c	MIC	50	50	100	-	-	-	-	-	-	50
	MBC	-	-	-	-	-	-	-	-	-	50
	a.p	-	-	-	-	-	-	-	-	-	1
V.a	MIC	50	-	-	-	25	-	-	-	-	50
	MBC	-	-	-	-	-	-	-	-	-	50
	a.p	-	-	-	-	-	-	-	-	-	1

P.a = *Persea americana*; P.g (f) =*Psidium guajava* (Leaves); C.c = *Cajanus cajan*; V.a = *Vernonia amygdalina*; P.g (r) =*Psidium guajava* (Roots), K. pneumoniae =*Klebsiella pneumoniae, Klebsiella K.rhinocleromatis = rhinocleromatis, K.oxytoca = Klebsiella oxytoca, P. oryzihabitans = Pseudomonas oryzihabitans, P.aeruginosa = Pseudomonas aeruginosa, E. coli = Escherichia coli*(Clinical strain), *E. coli* ATCC 25922 = *Escherichia coli* ATCC25922 (reference strain), C.freundii = *Citrobacter freundii*, *S. cholereasius= Salmonella cholereasius*, *S. flexneri =Shigella flexneri*

DISCUSSION

This study aimed to assess the biological potential of aqueous and ethanolic extracts of some plant parts (leaves, roots or seeds) commonly used in traditional medicine in southern Benin for the treatment of gastric disorders. Thus, the concentrations of polyphenols and flavonoids in differents extracts were determined by conventional methods. All plant extracts have been relatively rich in these components. The ethanolic extracts of certain plants were

richer in these components that aqueous extracts. This can be explained by the fact that ethanolic has a higher polarity and therefore extract more molecule thanwater. Then, the extracts were tested on nine strains Clinics and on one reference strain. All aqueous and ethanolic extracts of *Ocimum gratissimum*, *Moringa oleifera*, *Carica papaya* (seeds and leaves) did not showed any efficacy against bacterial strains used. The results of this study on *Carica papaya* are discordant with the work of Kayalvizhi *et al*. done on 2015 in India who reported that the ethanolic extract of the leaves of *Carica papaya* does not contain phenolic compounds but only flavonoids. The present results are also contrary to those of their antibacterial tests that showed good efficiency of the ethanolic extract of the leaves of *Carica papaya*on *Klebsiella pneumonia*, *Vibrio chloreae*, *Enterobacter aerogenes*, *Escherichia coli* and *Salmonella paratyphi*. This discordance in the results observed may be due to the difference between climate and the nature of the Indians and Benin soils. This difference may also be due to the difference in pathogenicity of the bacterial strains used as self-medication is a practice in vogue in Benin and which causes the appearance of a multitude multiresistants strains. The results of chemical activity of *Carica papaya*leaves are similar to those of Arumugam *et al.* on 2014 , which have achieved good levels of flavonoids and polyphenols extracts from *Carica papaya*leaves. The results of this study of*Carica papaya* are also discordant with those of Saravana Singh *et al*, 2016 who have had good results with the ethanolic extract of the leaves on *Escherichia coli* and *Salmonella Typhi*. The results of this study on *Ocimum gratissimum* arenear with those of Ogundare done in 2011which have had negative results with the ethanolic extract on *Salmonella Typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*. This results are discordant with those of Mbajiuka *et al*. done on 2014 which have obtained positive results with the aqueous and ethanolic extracts of *Ocimum gratissimum*. The bacteria used in this study could be less resistant than that used in this study. These differences could also be due to the age of the plants used in both studies or the type of soil on which the plants were grown. The results of the work of Dalukdeniya *et al*. on 2016 showed a sensitivity of *Salmonella enteritica* food strains, *Vibrio parahaemolyticus*, *Escherichia coli* with aqueous and ethanolic extracts of leaves of *Moringa oleifera*. Considering that the bacterial strains isolated from foods are less resistant than those isolated from human biological products, it can easily understand

the discrepancy between the results of these two studies. The chemical potential of leaves of *Moringa oleifera* obtained in this study are confirmed by the work of Okumu *et al*, 2016 which showed very good levels of polyphenols and flavonoids of the aqueous extract of leaves of *Moringa oleifera*.

The active extrcats were those of *Psidium guajava* (feuiles and roots), *Vernonia amygdalina*, *Cajanus cajan* and *Persea americana*. The aqueous extract of *Psidium guajava* leaves had a antibiotic power on *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Citrobacter freundii* and *S. flexneri*. On the other hand,the ethanolic extract had an antibiotic effect on *Pseudomonas aeruginosa*, *Salmonella cholereasius* and *Shigella flexneri*. The two leaves extracts have not therefore the same activities on bacterial strains. This observation confirms that the solvent used may influence the effectiveness of the extract to be obtained. At least one of two strains of *Escherichia coli* (clinical and reference) were sensitive to extracts of leaves of *Psidium guajava*. This partial result is contradictory to those of Biswas *et al*. on 2013 which achieved the opposite results with the ethanol extract of the leaves of *Psidium guajava*. *Psidium guajava* root extracts have also been active on several bacterial strains. These findings are confirmed by those of Neviton *et al.* on 2005 which have good results with ethanol extract of *Psidium guajava* root. Other authors found the antimicrobial activity of *P. guajava* extracts (Gonçalves, 2008; Okamoto, 2010)

Some studies such as Anibijuwon *et al*. on 2012 with *Vernonia amygdalina* revealed that the aqueous and ethanolic extracts of this plant are active against oral bacteria. The results of this study come prove that extracts of leaves of *Vernonia amygdalina* may have a bactericidal effect on some Gram-negative bacteria. These results are contrary to those of Agbankpe *et al*, 2016 and Ogundare, 2011 that had no results with extracts of *Vernonia amygdalina* on Gram negative bacteria. The results of the study of chemical properties of leaves of *Vernonia amygdalina* are comparable to those obtained by Festus *et al*. on 2016 with ethanol extract of these leaves.

Very few research works show antibacterial extracts of leaves of *Cajanus cajan* and *Persae americana*. However, the results of this study demonstrate that aqueous and ethanolic extracts of the leaves of these plants may contain significant levels of polyphenols and flavonoids, and may also have antibacterial activity on strains of enteric pathogens. The presence of polyphenols and flavonoids in every plant extracts confirm the work of Takin *et al.*,

(2014) which showed that the plants are naturally rich in phenolic components which are natural compounds known for their antioxidant properties.

CONCLUSION

This study has reaffirmed that plant field are unfamiliar land for scientific research as part of the search microbial inhibitors as an alternative to conventional antibiotics. Results obtained from this work achieved that the plants in the South Benin pharmacopoeia can be effective in the fight against certain enteric pathogens. Note that all the selected plants did not showed any toxicity at the dose of 100 mg / ml but awareness is still needed on the appropriate use of plants in traditional medicine especially for applying doses for each patient to prevent overdoses.

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Conflict of interest

No conflict of interested associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be done by the authors.

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