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Phytochemistry and anti-bacterial activity of thirteen plants used in traditional medicine to treat typhoid fever in Benin

Akomoun Blandine KAKPO[1-2], Eléonore YAYI[1], Bruno N. LENTA[2],* Fidèle M. ASSOGBA[1], *Placide M. TOKLO[1], Fabrice Fekam BOYOM[3], Lamine BABA-MOUSSA[4], and Joachim GBENOU[1]

[1]Department of Chemistry, University of Abomey-Calavi, Faculty of Sciences and Techniques, Laboratory of Pharmacognosy and Essential Oils, 01 Po. Box 918 Cotonou, Benin

[2]Department of Chemistry, University of Yaoundé 1, Higher Teacher Training College, P.O. Box 47, Yaoundé, Cameroon

[3]Department of Biochemistry, University of Yaoundé 1, Faculty of Sciences and Techniques, Antimicrobial Agents Unit, Laboratory for Phytobiochemistry and Medicinal Plants Studies, P. O. Box 812, Yaoundé, Cameroon

[4]Department of Biochemistry, University of Abomey-Calavi, Faculty of Sciences and Techniques, Laboratory of Biology and Molecular Typing in Microbiology, 05 BP 1604 Cotonou, Benin

ABSTRACT: The objective of this study is to study the phytochemistry of the anti-bacterial activity of thirteen plants used in traditional medicine to treat typhoid fever in Benin. For this fact, we carried out the phytochemical screening, then the antibacterial activity was carried out by the micro-dilution method. The dosage of polyphenols was made on the extracts having exhibited good *anti-salmonella* activity. Phytochemical screening has revealed the presence of alkaloids, tannins (gallic and catechic) and flavonoids for all plants, as for other secondary metabolites, they vary from one plant to another. For the forty-five [H2O, EtOH (96%), CH_2Cl_2-MeOH (V / V)] extracts, the extraction yield ranged from 3.3% to 23.78%; the CH_2Cl_2-MeOH extracts have the best yields followed by ethanolic extracts. The evaluation of the *anti-salmonella* activity of the forty-five extracts on seven strains of *Salmonella* (clinical isolate and reference) made it possible to determine the minimum inhibitory concentration (MIC) of the active extracts following the biological screening. The ethanolic extract of *Zanthoxylum zanthoxyloides* and CH_2Cl_2-MeOH from *Azadirachta indica* are the most active in inhibiting four types of salmonella with MICs ranging from 250 to 500 µg / mL. The determination of the polyphenol contents showed the richness of these plants in these compounds and we noticed that the activity of the extracts varies according to their content of flavonoids. The results obtained confirmed the *anti-salmonella* potential of certain plants at the concentration tested and constitute a scientific database for the research of phytomedicines.

KEYWORDS: secondary metabolites, active extracts, MIC, polyphenols, phytomedicines.

1 INTRODUCTION

Foodborne illness is a major public health problem in the world and especially in Africa. Typhoid fever is a serious disease caused by bacteria of the genus Salmonella, present in water and food contaminated with feces.

Each year, typhoid affects between 11 and 20 million people and causes 128,000 to 161,000 deaths [1]. This prevalence is higher in Asia, followed by Sub-Saharan Africa and Latin America. The Benin is moving from highly endemic areas despite the significant progress made over the past two decades in the area of sanitation of localities. Antibiotic resistance by Salmonella strains is an emerging and important problem [2].

Corresponding Author: Eléonore YAYI

Nowadays, many herbal recipes are used in traditional African medicine to treat typhoid fever, without prior knowledge of adequate doses and toxicity. Faced with this problem, it is therefore necessary to study the anti-Salmonella potential of traditional use plants in order to validate their use by populations or by traditional healers, but also with a view to finding new inexpensive effective active ingredients and accessible to all (improved traditional medicine). More specifically, it involves identifying secondary metabolites, evaluating polyphenol content and anti-Salmonella activities in vitro of crude plant extracts and plant combinations.

2 MATERIAL AND METHODS

2.1 MATERIAL

2.1.1 PLANT MATERIEL

The plant material use is present in the table below :

Table 1. List of plant material

Species	Family	Party used
Acanthospermum hispidum	*Asteraceae*	leaves
Azadirachta indica	*Meliaceae*	leaves
Combretum micranthum	*Combretaceae*	leaves
Corchorus olitorius	*Tiliaceae*	leaves
Citrus aurantifolia	*Rutaceae*	fruits
Dichapetalum madagascariense	*Dichapetalaceae*	leaves
Jatropha multifida	*Euphorbiaceae*	leaves
Khaya senegalensis	*Meliaceae*	leaves
Momordica charantia	*Cucurbitaceae*	leaves
Morinda lucida	*Rubiaceae*	leaves
Ocimum canum	*Lamiaceae*	leaves
Ocimum gratissimum	*Lamiaceae*	leaves
Zanthoxylum zanthoxyloides	*Rutaceae*	roots

In addition to plants, incense resins have been used in combination in a recipe.

The plants were harvested in June 2017 in Ouidah in southern Benin. All these plants have been identified in the National Herbarium of Benin.

2.1.2 BIOLOGICAL MATERIAL

The biological material consists of the reference bacterial strains of *Salmonella typhi* (R 3095140), *Salmonella enterica* (NR 4294) and *Salmonella enterica* NR 431 and clinical bacterial strains of *Salmonella typhi*, *Salmonella paratyphi A, Salmonella typhimurium and Salmonella choleraesius*. These strains were provided by the National Health Laboratory of Benin and the Laboratory for Phytobiochemistry and Medicinal Plants Studies of Cameroon.

2.2 METHODS

2.2.1 CHOICE OF PLANTS

The plants were chosen following an ethnopharmacological survey conducted in southern Benin to identify the plants used by the population for the traditional treatment of typhoid fever. The plants studied are among the most cited and least studied.

2.2.2 PHYTOCHMICAL SCREENING

Phytochemical screening was done by coloring and precipitation reactions described by Houghton [3] and cited by Assogba [4] with some modifications.

Alkaloids: the alkaloids were highlighted by the Meyer test;
Tannins: they were determined by Stiasny's test;
Flavonoids: Flavonoids were identified by the cyanidin test;
Anthocyanins: anthocyanins were identified by the hydrochloric acid and ammonia test at 50%;
Leuco anthocyanins: they were highlighted by the shinoda test;
Quinone derivatives: Quinone derivatives were determined by the Born-Trager test;
Saponosides: they were determined by the foam index test;
Triterpenoids: triterpenoids were highlighted by the Liebermann-Buchard test;
Steroids: they were determined by the Kedde test;
Cyanogenic derivatives: they were highlighted by the Gugnard test;
Mucilages: mucilages were identified by the absolute alcohol test;
Reducing Compounds: The reducing compounds were evidenced by the hot Fehling Liquor test.
Coumarins: The identification of coumarins was performed by the ammonia test at 25% and revealed at UV 365 nm.

2.2.3 PREPARATION OF CRUDE EXTRACT

After collecting the plant material, the samples were dried at laboratory temperature (16 ° C) for about two to three weeks depending on the part and type of the plant, and then reduced to powder.

For each plant, three types of extracts were prepared.

The aqueous extract was prepared by decoction: a quantity of 50 g of powder is boiled for 30 minutes in 500 ml of distilled water. After cooling, the decoction obtained is filtered using wattman paper and then evaporated using a rotary evaporator under reduced pressure at 50 ° C.

The ethanolic extract was obtained by maceration of the powders in 96% ethanol for 72 hours. The mixture was stirred twice a day and the macerate obtained was filtered using wattman paper and then evaporated under reduced pressure at 40 ° C.

The CH 2 Cl 2 / MeOH extract was prepared by maceration of the powders in CH 2 Cl 2 / MeOH (V / V) for 24 hours. The macerate obtained was filtered using wattman paper and then evaporated under reduced pressure at 40 ° C.

The extracts obtained were stored in a refrigerator at 4 ° C.

The yield of the extracts was calculated by the following formula:

$$\text{yield } (\%) = \frac{Mass\ of\ the\ crude\ extract\ powder}{\text{Mass of the powder of vegetable matter}} * 100$$

2.2.4 EVALUATION OF ANTIBACTERIAL ACTIVITY

2.2.4.1 PREPARATION OF INOCULUM

A young colony of one day was taken and introduced into a tube containing physiological saline (Nacl 0.9%) sterile, the turbidity of this tube was compared to standard 0.5 Marc Farland 1.5 108 cells / mL and then adjusted to 106 cells / mL, load required for the test.

2.2.4.2 PREPARATION OF STOCKS SOLUTIONS

The aqueous extracts were dissolved in sterilized distilled water to obtain a stock solution of 100 mg/mL, the ethanolic and methylene chloride/methanol extracts were dissolved in pure DMSO and sterilized distilled water to that the proportion of DMSO is equal to 1% at the end.

Ciprofloxacin was dissolved in the 0.1 N aqueous hydrochloric acid solution to obtain a stock solution of 1000 µg/mL.

These stock solutions were diluted in liquid medium (Muller Hinton Broth) to have the concentrates tested.

2.2.4.3 BIOLOGICAL SCREENING OF EXTRACTS

The tests were carried out in the 96-well microplates. The culture medium used is Muller Hinton Broth (MHB), prepared according to the manufacturer's instructions. In fact, 50 µL of a sterile solution of each extract concentrated at 1000 µg / mL was deposited in three wells of the microplate so that the test was repeated three times and that the final concentration of the extract in each well was equal. at 500 µg / mL. Each microplate consists of extracts, the positive control at 128 µg / mL (ciprofloxacin), sterility controls (culture medium alone) and negative controls. Then 50 .mu.l of a suspension of bacteria, prepared at 10.sup.6 Cells / ml are introduced into the wells of the microplate, except those of the wells containing the controls of sterility of the culture medium. The microplates are covered and incubated at 37 ° C for 24 hours.

2.2.4.4 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS (MIC)

The micro-dilution method using the M07-A10 protocol [5] was used, with some adjustments.

The tests were carried out in the 96-well microplates. Indeed, 50 .mu.l of Muller Hinton broth culture medium are introduced into all the wells of the microplate; then, 50 µL of a sterile solution of a concentrated extract at 1000 µg / mL in the first wells. A series of 7 2-fold dilutions was made in each column; finally, 50 µL of a bacterium suspension prepared at 106 cells / mL are introduced into the wells of the microplate, except those of the wells of line H - column 1 to 6 which contain only the culture medium and serve as a blank ; the wells of line H - column 7 to 12 containing the culture medium and the inoculum serves negative control. Trials were performed in triplicate. The microplates are covered and incubated at 37 ° C for 24 hours. At the end of the incubation time, the smallest concentration where there is no visible growth marked by the absence of turbidity corresponds to the MIC of the extract concerned.

2.2.5 QUANTIFICATION OF POLYPHENOLIC COMPOUNDS

The determination of the polyphenolic compounds was carried out in the 96-well microplate

2.2.5.1 QUANTIFICATION OF PHENOLIC CONTENT

The content of total phenolic compounds was determined by the method of Folin-Ciocalteu [6], [7], [8] with some modifications. 20 µL of extract solution (3 mg / mL) were added to 100 µL of Folin-Ciocalteu reagent (0.1N). After 5 min of incubation in the ambient air in the dark, 80 µl of sodium carbonate solution (Na_2CO_3, 75 g / l) were added and incubated for 15 min at room temperature and at room temperature. shelter from the light. Absorbance was measured at 765 nm. White is the extract and distilled water. Gallic acid (0.195 to 100 µg / mL) was used as a standard reference for the calibration curve. All tests were done in triplicate. The concentration was obtained from a calibration curve and expressed in microgram equivalent of gallic acid per milligram of extract (µg EAG / mg).

2.2.5.2 QUANTIFICATION OF TOTAL FLAVONOIDS

The quantification of flavonoids was carried out according to the aluminum trichloride method [7], [8], [9]. 100 µL of extract solution (3 mg / mL) was added to 100 µL of methanolic solution of aluminum trichloride (2%) and incubated for 15 min. White is the extract and methanol; the absorbance was read at 415 nm. All measurements are done in triplicate. Quercetin is the reference used. The flavonoid content was calculated using the standard calibration curve and expressed in micrograms equivalent of quercetin per milligram of extract (µg EQ / mg).

2.2.5.3 QUANTIFICATION OF CONDENSED TANNINS

The tannin content was determined according to the vanillin method [7], [8], [10]. 100 µL of sulfuric vanillin solution (1%, 7M H2SO4) were mixed with 50 µL of the extract solution (3 mg / mL). The mixture was incubated for 15 min at 25 ° C in the absence of light. The absorbance was read at 500 nm against its white. The reference used was catechin. All measurements were done in triplicate. The tannin content was calculated using the standard calibration curve (catechin) and expressed in micrograms equivalent of catechin per milligram of extract (µg ECat / mg).

2.2.5.4 QUANTIFICATION OF ANTHOCYANINS

The determination of anthocyanins was carried out according to the differential pH method [7], [8], [11]. Two reaction mixtures, one consisting of 100 µL of extract solution (3 mg / mL) and 100 µL of buffer solution [pH = 1 (0.2 M KCl - 0.2 M HCl)] and another 100 µL of the extract solution (3 mg / mL) and 100 µL of another buffer solution [pH = 4.5 (0.2 M acetic acid - 0.2 M sodium acetate)] were incubated for 15 minutes at room temperature and protected from light. Then, the absorbance of each mixture was measured at 510 nm and 700 nm. All assays were performed in triplicate. The anthocyanin content was calculated according to the equation:

$$TAC(\mu g/mg) = \frac{A * M * DF}{\varepsilon * L} * 10^3$$

A = absorbance of the sample = [(abs510-abs700) pH = 1 - (abs510-abs700) pH = 4.5; M = molar mass of cyanidin-3-glucoside = 449.2 g / mol; FD = dilution factor of the sample; = = Molar absorption coefficient of the majority anthocyanin = 29,600 L.mol-1cm-1; The optical path = 0.52 cm; 103 = conversion factor from mg to µg

3 RESULTS AND DISCUSSIONS

3.1 PHTHOCHEMICAL SCREENING

The secondary metabolites of the plants investigated are shown in Table 2.

Table 2. Results of the phytochemical screening of the powder of the investigated plants

	PLANT												
Secondary Metabolites (MS)	**A. hi**	**A. in**	**C. ol**	**C. mi**	**D. ma**	**J. mu**	**K. se**	**O. ca**	**O. gr**	**M. ch**	**M. lu**	**Z. za**	**% of each secondary metabolites**
alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	**100**
tannins	+	+	+	+	+	+	+	+	+	+	+	+	**100**
Catechism tannins	+	+	+	+	+	+	+	+	+	+	+	+	**100**
Gallic tannins	+	+	+	+	+	+	+	+	+	+	+	+	**100**
Flavonoids (Flavones)	+	+	+	+	+	+	+	+	+	+	+	+	**100**
anthocyanins	+	+	+	+	+	+	+	+	+	+	+	-	**91,66**
Leuco-anthocyanins	+	+	-	+	-	+	+	+	+	-	+	+	**75**
Quinone derivatives	+	+	+	+	-	-	+	-	-	-	+	+	**58,33**
saponosides	+	+	+	+	+	+	+	+	+	-	+	+	**91,66**
triterpenoids	+	+	-	+	-	-	-	-	-	-	+	+	**41,67**
steroids	+	+	+	+	-	+	-	+	+	+	+	+	**83,33**
Cyanogenic derivatives	-	-	-	-	-	-	-	-	-	-	-	-	**0**
mucilage	+	+	-	-	+	+	+	+	+	+	+	-	**75**
coumarins	-	+	-	+	-	-	-	-	-	-	+	+	**33,33**
Reducing compounds	+	+	+	-	+	+	+	+	+	+	+	+	**91,66**
Anthracene free	-	+	-	-	+	-	-	+	+	+	+	+	**58,33**
O-glycosides	-	+	-	-	-	-	-	-	-	-	+	+	**25**
C-glycosides	-	-	-	-	+	-	-	-	-	-	-	+	**16,66**
Number of each Secondary Metabolites per plant	**13**	**16**	**10**	**12**	**11**	**11**	**12**	**12**	**12**	**12**	**16**	**15**	**-**

Legend: - Absence + Presence

A. hi : A. hispidum ; A. in : A. indica ; C. ol : C. olitorius ; C. mi : C. micranthum ; D. ma : D. madagascariense ; J. mu : J. multifida ; K. se : K. senegalensis ; O. ca : O. canum ; O. gr : O. gratissimum ; M. ch : M. charantia ; M. lu : M. lucida ; Z. za : Z. zanthoxyloides.

The plants investigated all contain alkaloids, tannins (gallic and cathechic) and flavonoids, as for the cyanogenic derivatives, they are absent in all the plants. The presence of the other compounds varies according to the plant.

Our results on the leaves of Momordica charantia are in agreement with those of [12], likewise for the roots of Zanthoxylum zanthoxyloides by [13]. The results from Ocimum gratissimum leaves are not in agreement with those obtained by [14] and [15].

The results of the leaves of Dichapetalum madagascariense differ from those of [16].

The differences in the presence of the chemical groups of a plant worked by several authors at the level of the results discussed may be due to several geographical factors.

Some of these groups of identified compounds such as tannins, flavonoids, saponosides, steroids and terpenoids have anti-bacterial activity [17].

According to [18] polyphenols are responsible for the antibacterial activity of plants.

All these secondary metabolites were found in the plants studied; this may be at the origin of the anti-bacterial activity revealed by the population and traditional healers.

3.2 EXTRACTION YIELD

The extraction yield of the plants with the various solvents are illustrated in the figures below:

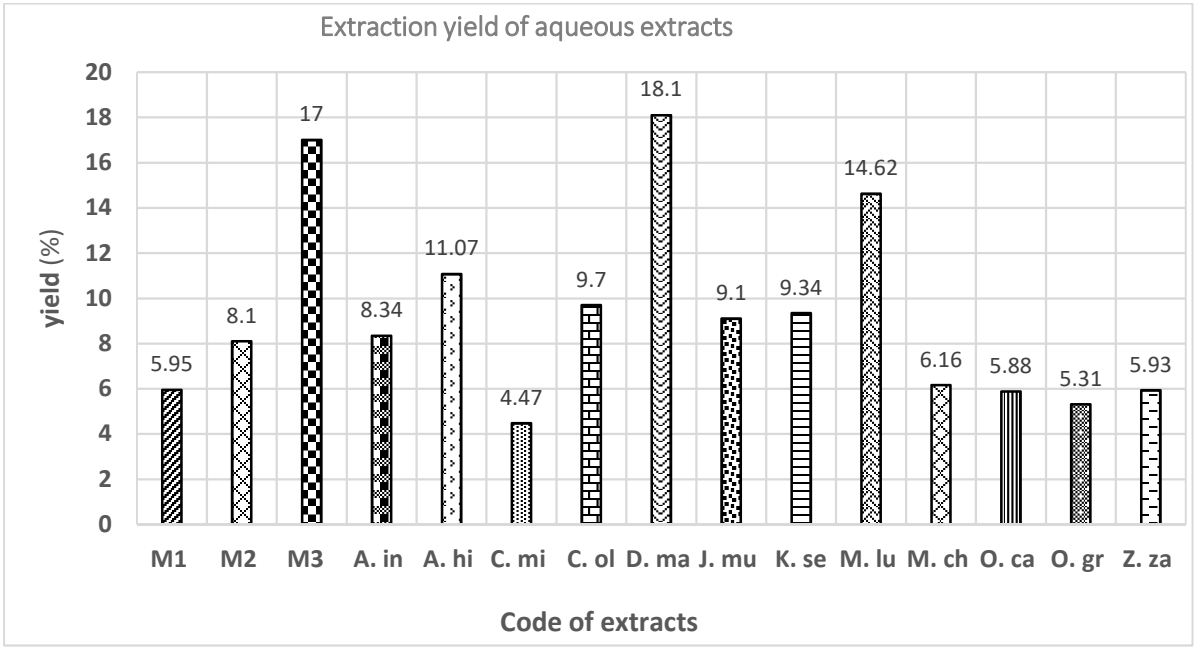

Fig. 1. Extraction yield of aqueous extracts

Legend: *M1: plant association recipe (C. micranthum, M. charantia, O. canum and O. gratissimum); M2: recipe for association of C. olitorius and C. aurantifolia; M3: recipe of association D. madagascariense and Incense; A. hi : A. hispidum ; A. in : A. indica ; C. ol : C. olitorius ; C. mi : C. micranthum ; D. ma : D. madagascariense ; J. mu : J. multifida ; K. se : K. senegalensis ; O. ca : O. canum ; O. gr : O. gratissimum ; M. ch : M. charantia ; M. lu : M. lucida ; Z. za : Z. zanthoxyloides.*

The extraction yield of the aqueous extracts ranges from 4.47% to 18.1%. The leaf extract of Dichapetalum madagascariense shows the best yield followed by the mixture of Dichapetalum madagascariense-frens and Morinda lucida leaf extract.

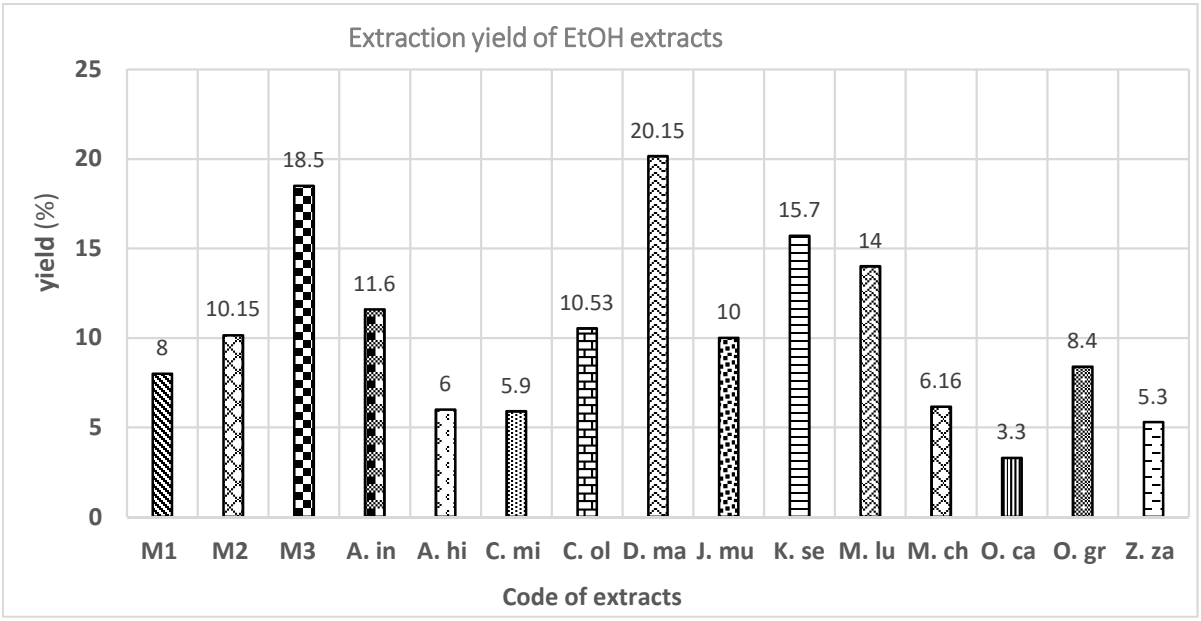

Fig. 2. Extraction yield of EtOH extracts

Legend: *M1: plant association recipe (C. micranthum, M. charantia, O. canum and O. gratissimum); M2: recipe for association of C. olitorius and C. aurantifolia; M3: recipe of association D. madagascariense and Incense; A. hi : A. hispidum ; A. in : A. indica ; C. ol : C. olitorius ; C. mi : C. micranthum ; D. ma : D. madagascariense ; J. mu : J. multifida ; K. se : K. senegalensis ; O. ca : O. canum ; O. gr : O. gratissimum ; M. ch : M. charantia ; M. lu : M. lucida ; Z. za : Z. zanthoxyloides.*

The analysis of FIG. 2 reveals that the extraction yield of the ethanolic extracts varies from 3.3% to 20.15% respectively for the leaf extract of Dichapetalum madagascariense and Ocimum canum.

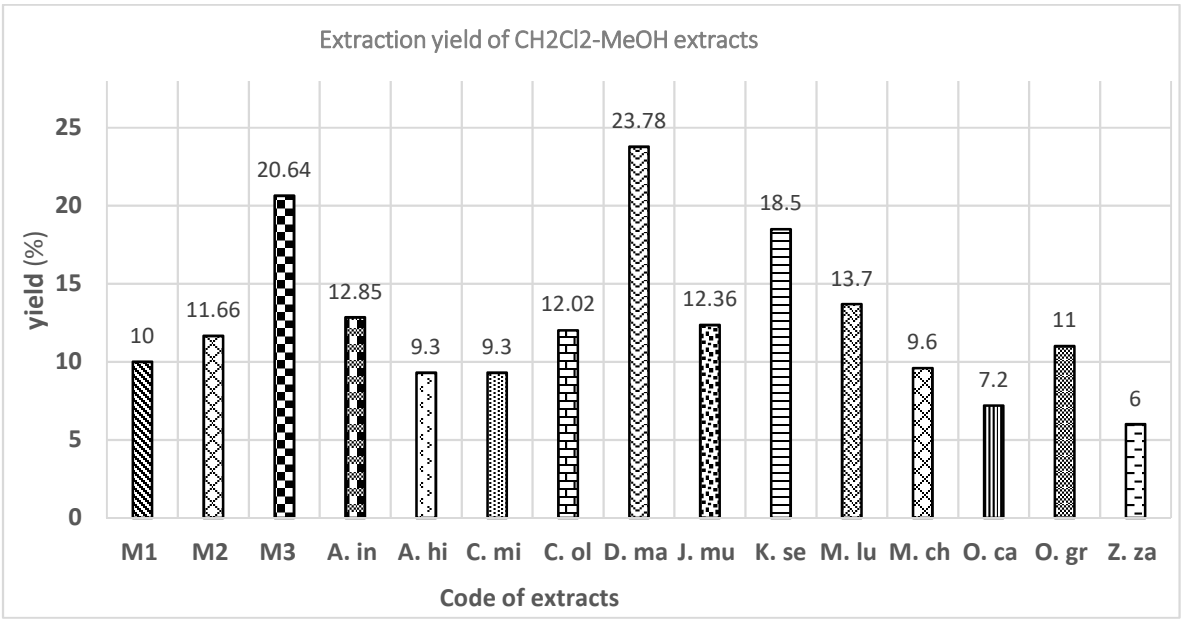

Fig. 3. Extraction yield of CH2Cl2-MeOH extracts

Legend: *M1: plant association recipe (C. micranthum, M. charantia, O. canum and O. gratissimum); M2: recipe for association of C. olitorius and C. aurantifolia; M3: recipe of association D. madagascariense and Incense; A. hi : A. hispidum ; A. in : A. indica ; C. ol : C. olitorius ; C. mi : C. micranthum ; D. ma : D. madagascariense ; J. mu : J. multifida ; K. se : K. senegalensis ; O. ca : O. canum ; O. gr : O. gratissimum ; M. ch : M. charantia ; M. lu : M. lucida ; Z. za : Z. zanthoxyloides.*

In Figure 3, we accept that the extraction efficiency of CH_2Cl_2-MeOH extracts is between 6% and 23.78%.

The general analysis of the three figures allows us to conclude that the alcoholic solvents have a better extraction yield than that of water for the majority of plants. To the authors' knowledge, there is no data on the extraction yields of the various extracts.

3.3 MICROBIOLOGICAL SCREENING

A total of forty-five extracts including fifteen aqueous extracts, fifteen ethanolic extracts (96%) and fifteen CH2Cl2-MeOH extracts (V / V) were tested on seven different strains of *salmonella*. The results are shown in the table below.

Table 3. Result of the screening of extracts on salmonella strains

	BACTERIA						
extracts	***S. typhi***	***S. typhi R 30951401***	***S. paratyphi A***	***S. typhimurium***	***S. choleraesius***	***S. enterica NR 4294***	***S. enterica NR 4311***
M1 EtOH	-	-	-	-	-	-	-
M2 EtOH	-	-	-	-	-	-	-
M3 EtOH	-	-	-	-	-	-	-
A. in EtOH	-	-	-	-	-	-	-
K. se EtOH	-	-	-	-	-	-	-
M. lu EtOH	-	-	-	-	-	-	-
C. mi EtOH	-	-	-	-	-	-	-
C. ol EtOH	-	-	-	-	-	-	-
M. ch EtOH	-	-	**+**	-	-	**+**	-
O. ca EtOH	-	-	-	-	-	-	-
O. gr EtOH	-	-	-	-	-	-	-
A. hi EtOH	-	-	-	-	-	-	-
D. ma EtOH	-	-	-	-	-	-	-
J. mu EtOH	-	-	-	-	-	-	-
Z. za EtOH	-	**+**	-	**+**	**+**	**+**	-
M1 CH_2Cl_2-MeOH	**+**	-	-	-	-	-	-
M2 CH_2Cl_2-MeOH	-	-	-	-	-	-	-
M3 CH_2Cl_2-MeOH	-	-	-	-	-	-	-
A. in CH_2Cl_2-MeOH	**+**	**+**	-	**+**	**+**	-	-
K. se CH_2Cl_2-MeOH	-	-	-	-	-	-	-
M. lu CH_2Cl_2-MeOH	-	-	-	-	-	-	-
C. mi CH_2Cl_2-MeOH	-	-	-	-	-	-	-
C.ol CH_2Cl_2-MeOH	-	-	-	-	-	-	-
M. ch CH_2Cl_2-MeOH	**+**	-	-	-	-	-	**+**
O. ca CH_2Cl_2-MeOH	-	-	-	-	-	-	-
O. gr CH_2Cl_2-MeOH	-	-	-	-	-	-	-
A. hi CH_2Cl_2-MeOH	-	-	-	-	-	-	-
D. ma CH_2Cl_2-MeOH	-	-	-	-	-	-	-
J. mu CH_2Cl_2-MeOH	-	-	-	-	-	-	-
Z. za CH_2Cl_2-MeOH	-	**+**	-	-	**+**	-	-
M1 Aq	-	-	-	-	-	-	-
M2 Aq	-	-	-	-	-	-	-
M3 Aq	-	-	-	-	-	-	-
A. in Aq	-	-	-	-	-	-	-
K. se Aq	-	-	-	-	-	-	-
M. lu Aq	-	-	-	-	-	-	-
C. mi Aq	-	-	-	-	-	-	-
C. ol Aq	-	-	-	-	-	-	-

M. ch Aq	-	-	-	-	-	-	-
O. ca Aq	-	-	-	-	-	-	-
O. gr Aq	-	-	-	-	-	-	-
A. hi Aq	-	-	-	-	-	-	-
D. ma Aq	-	-	-	-	-	-	-
J. mu Aq	-	-	-	-	-	-	-
Z. za Aq	-	-	-	-	-	-	-
Ciprofloxacine 128 µg/ml	**+**	**+**	**+**	**+**	**+**	**+**	**+**

Legend: - no total inhibition at 500 µg / mL; + total inhibition at 500 µg / mL

EtOH: Ethanol (96%); CH2Cl2-MeOH: dichloromethane-methanol (V / V); Aq: Aqueous; M1: plant association recipe (C. micranthum, M. charantia, O. canum and O. gratissimum); M2: recipe for association of C. olitorius and C. aurantifolia; M3: recipe for association of D. madagascariense and Incense; A. hi : A. hispidum ; A. in : A. indica ; C. ol : C. olitorius ; C. mi : C. micranthum ; D. ma : D. madagascariense ; J. mu : J. multifida ; K. se : K. senegalensis ; O. ca : O. canum ; O. gr : O. gratissimum ; M. ch : M. charantia ; M. lu : M. lucida ; Z. za : Z. zanthoxyloides.

The results of the microbiological tests of the different extracts at 500 µg / mL on the Salmonella strains show that none of the aqueous extracts showed inhibition on *Salmonella* germs. The inhibitions were obtained with some ethanolic extracts and CH_2Cl_2 -MeOH as shown in Table 2. The comparative analysis of the results of the recipes in the form of a combination of plants and extracts obtained with single plants makes it possible to conclude that the antibacterial activity does not depend on the synergistic effect created by the association of plants but rather of the plant possessing the active principle.

3.4 MINIMUM INHIBITOR CONCENTRATION (MIC)

The minimum inhibitory concentrations of the extracts that inhibited at least one of the *Salmonella* strains at the concentration of 500 µg / mL were determined by the micro-dilution method in a liquid medium. These results are noted in Table 3.

Table 4. MIC (µg / mL) crude extracts from Salmonella

	BACTERIES						
EXTRAITS	***Salmonella typhi***	***Salmonella typhi R 30951401***	***Salmonella paratyphi A***	***Salmonella typhimurium***	***Salmonella choleraesius***	***Salmonella enterica NR 4294***	***Salmonella enterica NR 4311***
Z. za E_{EtOH}	> 500	**250**	> 500	**500**	**500**	**500**	> 500
Z. za $E_{CH2Cl2-MeOH}$	> 500	**250**	> 500	> 500	**500**	> 500	> 500
A. in $E_{CH2Cl2-MeOH}$	**500**	**500**	> 500	**500**	**500**	> 500	> 500
M. ch E_{EtOH}	> 500	> 500	**500**	> 500	> 500	**500**	> 500
M. ch $E_{CH2Cl2-MeOH}$	**500**	> 500	> 500	> 500	> 500	> 500	500
M1 $E_{CH2Cl2-MeOH}$	**500**	> 500	> 500	> 500	> 500	> 500	> 500
Ciprofloxacine	4	64	4	4	4	4	4

Legend: *> 500 no inhibition at 500 µg / mL; MIC = 500 µg / mL active extract;*
MIC = 250 µg / mL very active extract

Table 3 shows that the ethanolic extract of Z. zanthoxyloides at a MIC ranging from 250 to 500 µg / mL and inhibits four out of seven strains while its CH2Cl2-MeOH extract inhibited two of the strains.

The CH2Cl2-MeOH extract of *A. indica* inhibited four strains of Salmonella with a MIC of 500 µg / mL.

The ethanolic extract of *M. charantia* inhibited strains *of S. paratyphi* A and *S. enterica* NR 4294 with a MIC of 500 µg / mL.

The CH_2Cl_2-MeOH extracts of the leaves of *M. charantia* and the association of *C. micranthum, M charantia, O. canum and O. gratissimum*. (M1) inhibited *S. typhi* with a MIC of 500 µg / mL. .

Our results of extracts with MICs ranging from 250 to 500 µg / mL are better than those of [19] that worked on *Paullinia pinnata* leaves with MICs of 390 to 781 µg / mL on Salmonella strains.

Our results on the sheets of *A. indica* whose activity varies according to the extraction solvent are in agreement with those of [20] and [21] which worked on different extract of bark *A. indica*.

The results obtained with the *M. charantia* EtOH and CH2Cl2-MeOH extract are better than those of [22] who reported that the seeds and flowers of *M. charantia* have MIC of 5.63 to 22.5 mg / mL on strains of *S. enteritidi*s ATCC 13076.

The anti-salmonella activity of the extract of EtOH and CH2Cl2-MeOH from the roots of Z. zanthoxyloides obtained is in agreement with the results of [23] and [24] s which both worked on the essential oils of different parts. of Z. zanthoxyloides.

The work of [25] has shown that extracts from the root of *Z. zanthoxyloides* possess inhibitory activity on *Escherichia coli* strains, which further demonstrates the antibacterial property of the *Z. zanthoxyloides* root.

According to the analysis of the MIC results, the most active extracts are: the ethanolic extract of *Z. zanthoxyloides* and the CH2Cl2-MeOH extracts of *A. indica and Z. zanthoxyloides*. These extracts are potential candidates for a thorough scientific study to identify and isolate the active ingredients.

3.5 TOTAL POLYPHENOL CONTENT

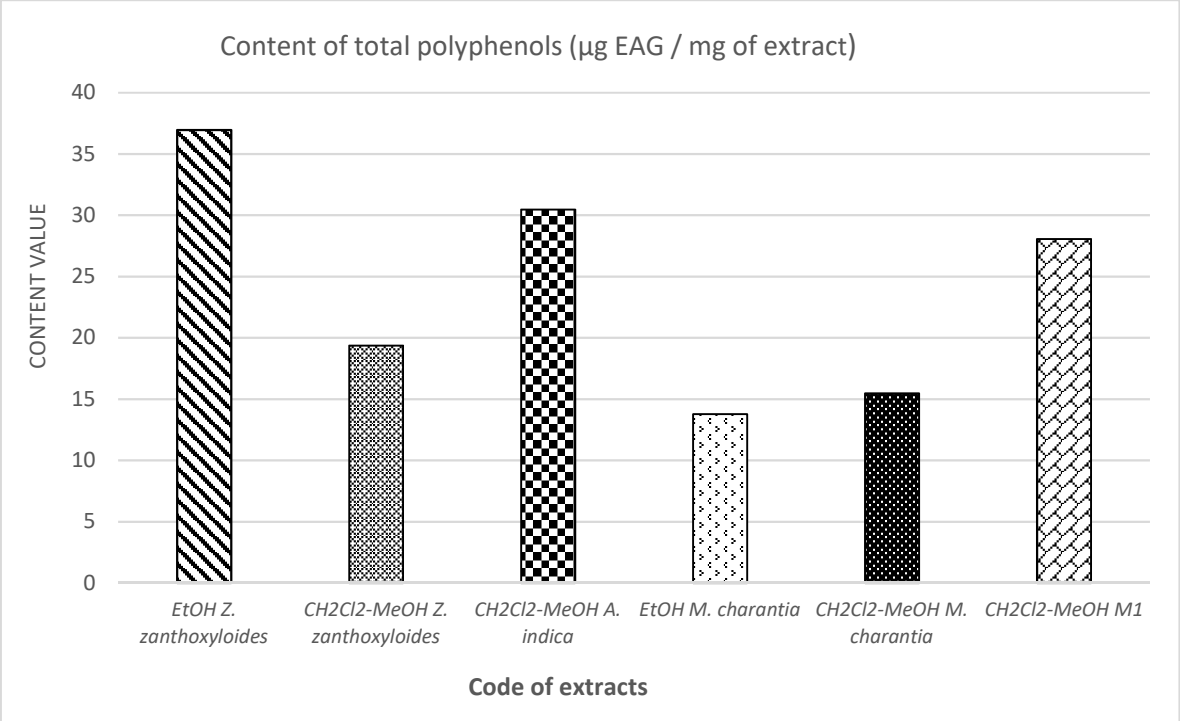

Fig. 4. Content of total polyphenols (µg EAG / mg of extract)

Figure 4 shows the total polyphenol contents (expressed in microgram equivalent of gallic acid per milligram of extract) of the active extracts on Salmonella bacteria. The levels range from 15.921 ± 0.017 µg EAG / mg extract to 36.952 ± 0.899 µg EAG / mg extract respectively for ethanolic extracts of Z. zanthoxyloides roots and leaves of *M. charantia*. We find that all extracts that inhibit salmonella growth are rich in polyphenolic compounds. Our results are in agreement with those of [18] who showed that leaf extracts, flowers and roots of *T. diversifolia* have antibacterial activities due to the presence of polyphenols.

3.6 FLAVONOID CONTENT

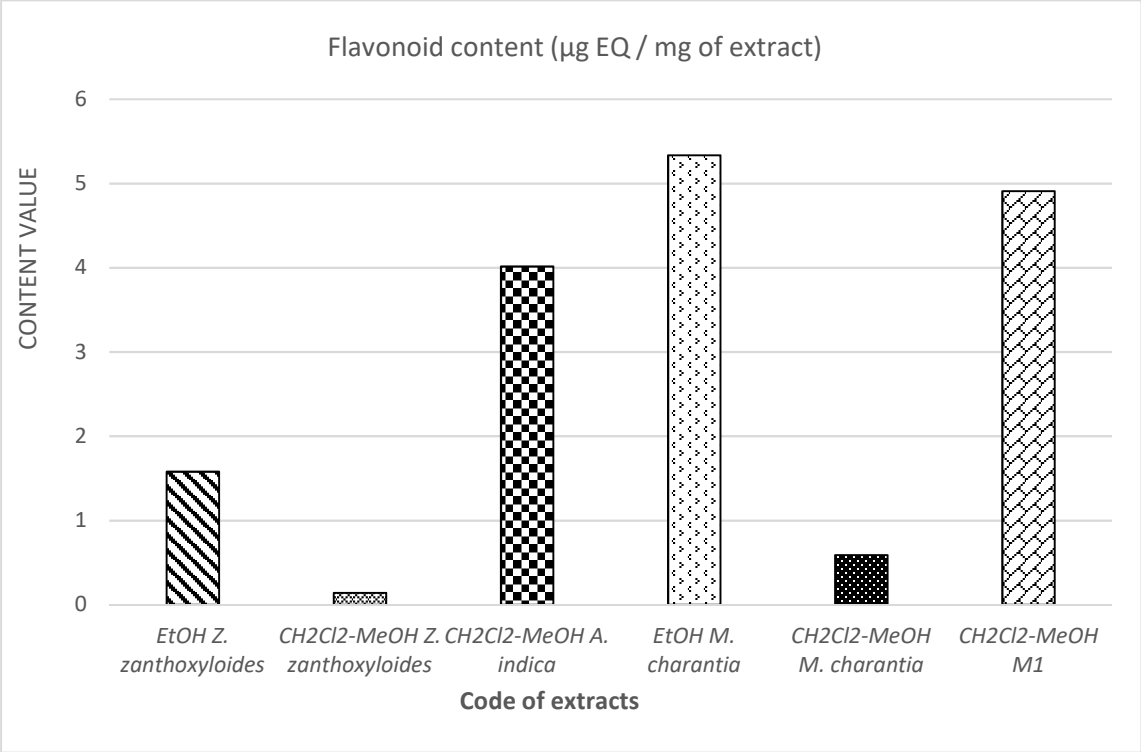

Fig. 5. Flavonoid content (µg EQ / mg of extract)

The flavonoid content of the ethanolic extract of *Z. zanthoxyloides* roots is 0.139 ± 0.001 µg EQ / mg of extract and 1.579 ± 0.043 µg EQ / mg of extract for its CH_2Cl_2-MeOH extract. This difference was also observed in their *anti-Salmonella* activity, the same remark was made in the ethanolic extracts and CH_2Cl_2-MeOH leaves of *M. charantia*. Thus, we can deduce that the flavonoids could be responsible for the anti-Salmonella activity of the roots of Z. zanthoxyloides and the leaves of *M*. charantia. Similarly, other studies have shown that bacterial growth decreases with the lowering of the concentration of flavonoid fractions the anti-bacterial activity of flowering heads of *Origanum glandulosum* [27].

In addition, it has been shown that the mechanism of toxicity of flavonoids to microorganisms is either by deprivation of metal ions such as iron, or by non-specific interactions such as the establishment of hydrogen bridges with the proteins of the cell walls of microorganisms (adhesins) or enzymes [28]. Therefore, flavonoids known for their potent antioxidant potency could potentially have an effect in iron chelation and thus prevent the intracellular entry of the Ca^{2+} cofactor into the bacterial cell, thereby inhibiting their activity [29].

3.7 TANNINS CONTENT

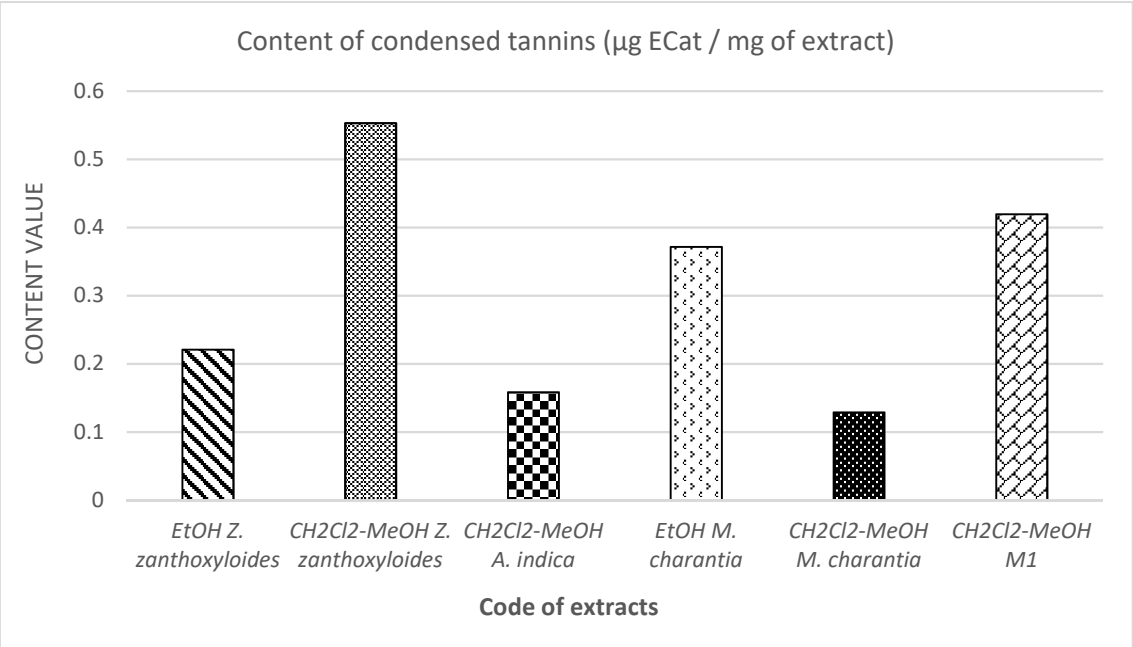

Fig. 6. Content of condensed tannins (µg ECat / mg of extract)

Figure 6: shows that the content of condensed tannins which varies according to the plant and the extraction solvent and this variation is not a function of their anti-salmonella activity.

3.8 ANTHOCYAN CONTENT

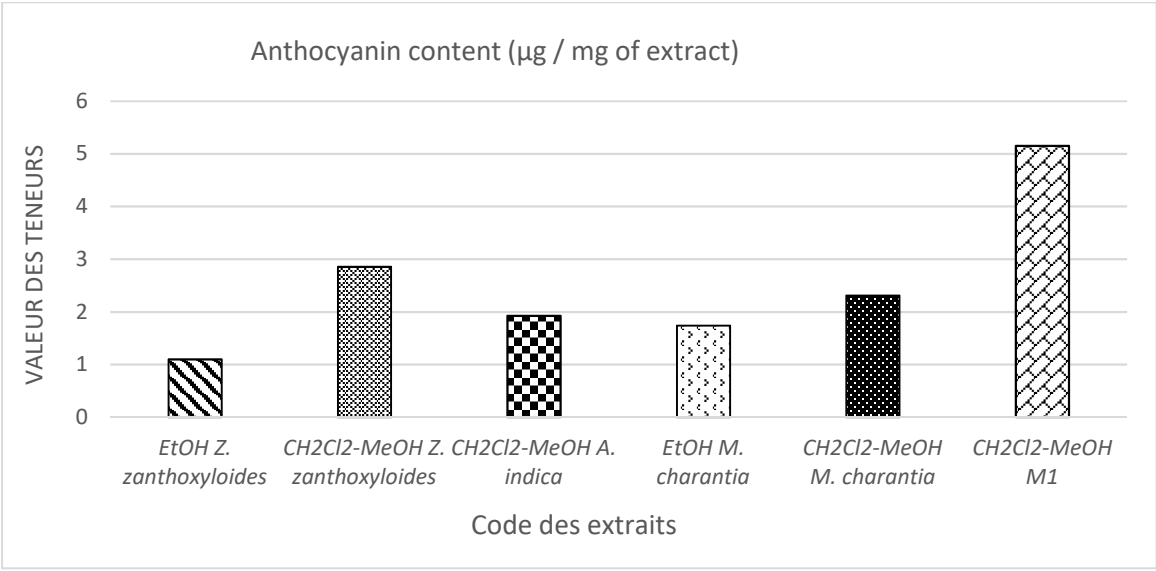

Fig. 7. Anthocyanin content (µg / mg of extract)

As regards the anthocyanin content, the highest is obtained at the level of the CH_2Cl_2-MeOH extract of the M1 association of plants (5.151 ± 0.341 µg / mg of extract) and the lowest content with the ethanolic extract of *Z. zanthoxyloides* (1.097 ± 0.052 µg / mg of extract). There is no significant difference in comparing the anthocyanin content of the extracts except for the content of the CH_2Cl_2-MeOH extract of the M1 association of plants.

4 CONCLUSION

This study aimed to study the *anti-salmonella* potential of medicinal plants used to treat typhoid fever in Benin. More specifically the realization of the chemical and biological study of thirteen plants.

Phytochemical screening revealed that all plants contain chemical groups suspected of having antibacterial activities. With regard to the extraction yield, the CH_2Cl_2 -MeOH (V / V) solvent mixture gave a better extraction yield.

The bioassay revealed that of the forty-five extracts, only six inhibited the growth of Salmonella bacteria. It should be noted that of the three types of extracts tested, it is the ethanolic extract that concentrates the active ingredients, so for the use of these plants for the treatment of typhoid fever we could recommend the ethanolic form after the study of toxicity. The results of the MIC led to the conclusion that the most active extracts are the ethanolic extract of Z. zanthoxyloides and the CH_2Cl_2-MeOH extracts of *A. indica and Z. zanthoxyloides.* These extracts are potential candidates to be investigated by bio-guided splits in order to identify and isolate the active ingredient (s).

The result of the polyphenol assays confirmed the richness of the polyphenol compound extracts and it was found that the activity of the extracts varied according to its flavonoid content. We could therefore conclude that flavonoid compounds are responsible for the *anti-salmonella* activity of plants.

Our results have confirmed the anti-bacterial activity of certain plants and justify their use by the population and traditional healers.

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