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## Fight against the Resistance of *Salmonella* Spp. to Antibiotics: Case of the use of Plant Extracts from the Ivorian Pharmacopeia

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### Abstract

The importance of the emergence of antimicrobial resistance has led to the search for alternative solutions, including phytotherapy. The aim of this study is to find plants from the Ivorian pharmacopoeia with antibacterial powers on *Salmonella* spp. strains, with a view to their use in the fight against salmonellosis. The antibacterial activity of the aqueous and ethanolic (70%) extracts of the identified plants was carried out *In vitro* on 3 strains of *Salmonella* (multi) resistant, of avian and human origin, by the method of diffusion in agar medium. The minimal inhibitory (MIC) and bactericidal (MBC) concentrations were determined by macro dilution and inoculation methods on solid medium. The results obtained show that the aqueous and ethanolic extracts of *Mallotus oppositifolius* and *Kalanchoe crenata*, and the ethanolic extract of *Heterotis rotundifolia*, have the best antimicrobial activities, with inhibition zone diameters ranging from 12±1 mm to 31±1 mm, MICs between 6.25 mg/mL and 50 mg/mL and MBCs ≥ 25 mg/mL. In view of these results, these plants could constitute an alternative to the use of antibiotics for the control of salmonellosis.

**Keywords:** (Multi) resistant *Salmonella*, medicinal plants, antibacterial activities, phytotherapy

### Introduction

*Salmonella* is the most common cause of foodborne bacterial infections (Angulo *et al.*, 2000). Human contamination, which is of intrinsic or secondary origin, can occur mainly through the consumption of contaminated food (Bell & Kyriakides, 2002) [7]. *Salmonella* is an evolving hazard with a strong adaptive capacity. In the last decades, two major phenomena illustrate this (Rabsch & Tschäpe, 2001) [23]. Thus, in the 1980s, the Enteritidis serotype emerged in the United States and Europe, particularly associated with avian species and which has the capacity to cause a trans-ovarian infection and consequently to contaminate the eggs. The epidemic quickly becomes a global pandemic among human cases (Rabsch & Tschäpe, 2001) [23]. The significant incidence of salmonellosis as well as the emergence of multidrug resistance and resistance to critical antibiotics in human therapy, in a context where the danger of antibiotic resistance is the subject of all attention, make it a health issue first class (Ouattara *et al.*, 2013) [20].

To date, the only way to treat health and economic problems result to avian pathogens remain antibiotic treatment. However, the use of these molecules knows its limits, due to the appearance and the propagation of multidrug resistance bacterial strains.

An effective solution to this problem of antibiotic resistance would be to limit or avoid the use of antibiotics, by looking for alternative ways to the use of these molecules.

Recently, means of prevention have been proposed as alternatives to the use of antibiotic molecules, in order to reduce the impact of enteric pathogens. Among these means, the use of phytobiotics, such as medicinal plants, would seem to offer promising results (Mehrnaz *et al.*, 2010) [16] with a view to ensuring control of the host's digestive microbiota, whether animal or human, and to limit the appearance of enteropathogenic bacteria such as *Salmonella* sp. Medicinal plants are a potential source of antimicrobial compounds and / or inhibitors of antibiotic resistance mechanisms, acting according to several mechanisms: (i) formation of complexes with macromolecules such as proteins and polysaccharides, thus inhibiting their functions (polyphenols); (ii) rupture of microbial membranes (lipophilic

flavonoids, terpenoids, defensins) and (iii) inhibition of the adhesion of microbial proteins to the polysaccharide receptors of the host (polypeptides) (Okusa *et al.*, 2009) <sup>[18]</sup>. These compounds also act as efflux pump inhibitors (flavonoids, terpenoids, alkaloids), inhibitors of penicillin binding proteins (PLP), quinones, terpenoids, causing the permeability of bacterial membranes (terpenoids) and are also inhibitors of betalactamases (alkyl gallates). The active element of medicinal plants is grouped into several chemical families, namely sulfur compounds: thiosulfonates, aldehydes, phenols, terpenes and tannins. These substances work by blocking the multiplication of the microorganism or by destroying its wall (Rhayour *et al.*, 2003) <sup>[24]</sup>. If today the different properties of phyto-molecules observable *In vitro* are recognized (antibacterial, immunomodulatory, anti-oxidant action), none of these molecules has yet demonstrated a real potential to replace antibiotics. The purpose of this work is to select plant with high antimicrobial activity against *Salmonella* spp for their use against salmonellosis.

### Materials and Methods

The bacterial strains are composed of three (3) *Salmonella* sp. with known antibiotic profiles, (Table 1). These strains come from the Microbiology Unit of the Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources, the Biosciences Training and Research Unit of the Félix Houphouët-Boigny University of Côte d'Ivoire.

### Plant Collection

The plants used in this study were collected from traditional therapists at the medicinal plant sales market in the commune of Bingerville. Eight (08) species of plants were collected; these plants, known for their anti-diarrheal and/or anti-inflammatory properties, were sent to the herbarium of the National Floristic Center of the Félix Houphouët-Boigny University of Abidjan (Côte d'Ivoire), for their identification. They are *Abrus precatorius*, *Boerhavia diffusa*, *Heterotis rotundifolia*, *Kalanchoe crenata*, *Mallotus oppositifolius*, *Plectranthus monostachyus*, *Sida acuta* and *Tectona grandis*.

### Preparation of Aqueous and Hydroethanolic Extracts of the Different Plants Collected

The preparation of the aqueous and hydroethanolic extracts of the various plants was carried out according to the method described by Zirihi *et al.* (2003) <sup>[29]</sup>. To do this, the leaves of the various freshly harvested plants are washed, cleaned, weighed and then dried in the dark. After grinding the dried leaves, 100 g of each powder are dissolved respectively in 1000 ml of sterile distilled water and in 1000 ml of hydroethanolic solution (70%). The various solutions obtained are filtered successively through a fine mesh sieve, percol fabric and hydrophilic cotton. The filtrate obtained is dried in an oven at 55 ° C. for 48 hours, to obtain the total aqueous extract (ETA). As for the hydroethanolic extract (EE70), it is obtained after drying the filtrate in an oven at 40 ° C. for 48 hours. The various extracts obtained are stored at 4 ° C. for the antibacterial tests

### Yield

The yield, expressed as a percentage relative to the initial mass of the various plant powders subjected to extraction, was determined according to the following formula.

$$\text{Yield} = \frac{\text{mass of extract obtained}}{\text{mass of leaves used}} \times 100$$

### Preparation of Bacterial Inocula

The strains of *Salmonella* sp. are revived in Muller-Hinton broth, then an aliquot of each bacterial suspension is seeded on Muller-Hinton agar and incubated at 37 ° C for 24 H, to obtain young colonies. From a few young colonies, the turbidity of each bacterial suspension is adjusted so that its opacity is equivalent to Mac Farland's 0.5 control., estimated at 10<sup>8</sup> CFU / mL (Bauer *et al.*, 1966) <sup>[6]</sup>; Baker *et al.*, 1983) <sup>[4]</sup>; (Moosdeen *et al.*, 1988) <sup>[17]</sup>.

Subsequently, diffusion tests in agar medium are carried out as well as the determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (CMB), in order to assess the anti-bacterial power of each extract.

### Antibacterial Activity of Plant Extracts

#### Sensitivity Test

Stock solutions concentrated at 200 mg / mL are prepared from each plant extract. Whatman No. 1 papers of 9 mm diameters impregnated with plant extracts were used as antibiotic disc for the diffusion tests in agar medium as described by Bauer *et al.* (2003) <sup>[5]</sup>.

A bacterial suspension of 10<sup>6</sup> CFU/ml of the test strain was inoculated with a swab on Mueller-Hinton agar (Bio-Rad, France) agar. The bacterial suspension (10<sup>6</sup> CFU/ ml) was obtained by diluting an inoculum of 10<sup>8</sup> 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<sup>8</sup> CFU/ml. After plating, sterile Whatman No. 1 paper of 9 mm diameter 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 2 hours at room temperature (25°C ± 2°C) for pre-diffusion of the substances before being incubated at 37°C.

The antibacterial activity of the extracts is determined by measuring the diameter of the zone of inhibition around each disc (Doughari *et al.*, 2007) <sup>[9]</sup>. For each extract, the experiment was triplicated.

### Determination of the Minimum Inhibitory Concentration (MIC)

The determination of the antibacterial parameters (MIC) was carried out according to the method of dilution in liquid medium, as carried out by Kouadio *et al.* (2015) <sup>[15]</sup>. Thus, in hemolysis tubes, one (1) milliliter with a concentration range of 100 to 3.12 mg / mL was prepared by the double dilution method. For each bacterial strain, a bacterial inoculum whose turbidity corresponds to the opacity of 0.5 Mc Farland is prepared in sterile distilled water, then diluted to 1/100 in two times concentrated Mueller Hinton broth, for the obtaining a bacterial inoculum estimated at 10<sup>6</sup> CFU / mL. Subsequently, a ½ dilution by adding one (1) mL of different concentrations of extracts, ranging from 50 to 1.56 mg / mL, is carried out. After 24 h of incubation at 37 ° C, the turbidity of the medium reflects bacterial growth. The MIC of an extract for a given bacterial strain is the smallest concentration of extract, showing no visible growth to the naked eye of the bacteria.

### Determination of the Minimum Bactericidal Concentration (MBC)

After seeding the concentration range, a count of the viable colonies is carried out in order to determine the CMB. Using a 10 µL calibrated loop, a 5 cm strip seeding of the bacterial inoculum, used for inoculating the concentration range and its dilutions ranging from 10<sup>-1</sup> to 10<sup>-4</sup> is carried out on Mueller-Hinton agar (BioRad, France), to constitute the bactericidal control. Subsequently, subcultures of the tubes without visible growth are carried out on Mueller-Hinton agar (BioRad, France). After 24 hours of incubation at 37 ° C., the observable colonies are compared to the bactericidal control. MBC is the smallest concentration whose subculture shows germ growth less than or equal to 0.0001% of survivors.

### Determination of the Modality of Action of Different Plant Extracts

The antibacterial effect of the various plant extracts tested is judged to be bactericidal or bacteriostatic according to the MBC / MIC ratio (Fauchere & Avril, 2002), if the report:

- $MBC / CMI \leq 2$ , the substance is said to be bactericidal;
- $MBC / CMI > 2$ , the substance is said to be bacteriostatic.

### Statistical Analysis

The means of the inhibition diameters were calculated for each extract relative to each bacterial strain using the EXCEL 2013 software, at the end of the various tests. The STATISTICA 2019 software was used for the one-way analysis of variance (ANOVA 1). It was used to compare the means of the diameters of inhibition of the different extracts on each bacterial isolate, at the probability threshold  $p$  less than 5%. The results obtained were expressed in the form of the mean  $\pm$  Standard deviation.

### Results

#### The Yield

The yield depending on the plant species, the organ used, the drying conditions, the content of each plant species in metabolites and the nature of the solvent used in the extraction. The different yields of the extractions of the different plants are reported in Table 2.

#### Antibacterial Activity

The antibacterial activity of plant extracts differs from one plant to another and / or from one type of extract to another, on each of the strains studied (Photographs 1 and 2).

#### Antibacterial Activity of Plant Extracts on *Salmonella Enterica* Serogroup O: 8

Of all the 8 plants studied, only the ethanolic extracts of *Abrus precatorius* (Abr E), *Sida acuta* (Sid ac E), and the ethanolic and aqueous extracts of *Heterotis rotundifolia* (Het), *Kalanchoe crenata* (Kal), *Mallotus oppositifolius* (Mal), *Plectranthus monostachyus* (Plect), have shown antibacterial activity, with diameters of inhibition range from  $10.50 \pm 0.50^e$  mm (Het A) to  $21.66 \pm 0.57^a$  mm (Mal E). The greatest activity was observed in the ethanolic extract of *Mallotus oppositifolius* (Mal E ( $21.66 \pm 0.57^a$  mm)). Relatively high activities were observed in the aqueous and ethanolic extracts of *Kalanchoe crenata* ( $16.33 \pm 0.57^b$  mm (Kal A) and  $15.50 \pm 0.50^b$  mm (Kal E)). As for the aqueous extracts of *Mallotus oppositifolius* (Mal A) and

the ethanolic extracts of *Heterotis rotundifolia* (Het E), *Plectranthus monostachyus* (Plect E) and *Sida acuta* (Sid ac E), the inhibition diameters are relatively average with respective values of  $13.50 \pm 0.50^{cd}$  mm,  $14.33 \pm 0.57^c$  mm,  $13.33 \pm 0.57^{cd}$  mm and  $13.33 \pm 0.57^{cd}$  mm. Small diameters were observed in the aqueous extracts of *Abrus precatorius* (Abr A ( $13 \pm 2^d$  mm)), *Heterotis rotundifolia* (HetA ( $10.50 \pm 0.50^e$  mm)) and *Plectranthus monostachyus* (Plect A ( $10.55 \pm 0.50^e$  mm)). No antibacterial activity was observed in the aqueous and ethanolic extracts of *Boerhavia diffusa* (Boer A ( $00 \pm 00^f$  mm) and Boer E ( $00 \pm 00^f$  mm)), from *Tectona grandis* (Tect A ( $00 \pm 00^f$  mm) and Tect E ( $00 \pm 00^f$  mm)) and aqueous extracts of *Abrus precatorius* (Abr A ( $00 \pm 00^f$  mm)) and *Sida acuta* (Sid acA ( $00 \pm 00^f$  mm)).

#### Antibacterial Activity of Plant Extracts on *Salmonella Enterica* Serogroup O: 21

About this strain, only extracts (aqueous and ethanolic) of *Abrus precatorius* (Abr), *Heterotis rotundifolia* (Het), *Kalanchoe crenata* (Kal), *Mallotus oppositifolius* (Mal), *Sida acuta* (Sid ac), aqueous extracts of *Tectona grandis* (Tect), as well as the ethanolic extract of *Plectranthus monostachyus* (Plect), showed antibacterial activities with inhibition diameters range from  $10.66 \pm 1.15^d$  mm (Abr A) to  $27 \pm 1^a$  mm (Mal E). The greatest activity was observed in the ethanolic extract of *Mallotus oppositifolius* with an inhibition diameter of  $27 \pm 1^a$  mm. Relatively high activities were observed in the aqueous extract of *Mallotus oppositifolius* and ethanolic extract of *Sida acuta* with respective inhibition diameters of  $19 \pm 1^b$  mm and  $19.66 \pm 3.21^b$  mm; while the aqueous and ethanolic extracts of *Kalanchoe crenata* showed relatively average inhibition diameters (Kal A ( $16 \pm 1^c$  mm) and Kal E ( $16 \pm 2^c$  mm)). As for the aqueous and ethanolic extracts of *Abrus precatorius*, aqueous extracts of *Heterotis rotundifolia*, *Sida acuta* and *Tectona grandis*, as well as the ethanolic extract of *Plectranthus monostachyus*, these show small inhibition diameters range from  $10.66 \pm 1.15^d$  mm to  $13 \pm 2^{cd}$  mm (Abr A ( $10.66 \pm 1.15^d$  mm), AbrE ( $12.50 \pm 0.50^d$  mm), Het A ( $13 \pm 2^{cd}$  mm), Sid acA ( $11, 50 \pm 1.50^d$  mm), Tect A ( $12 \pm 1^d$  mm) and Plect E ( $13 \pm 2^{cd}$  mm)). Aqueous and ethanolic extracts of *Boerhavia diffusa*, aqueous extracts of *Plectranthus monostachyus* and ethanolic extract of *Tectona grandis* showed no inhibition zone ( $00 \pm 00^e$  mm).

#### Antibacterial Activity of Plant Extracts on *Salmonella Typhi*

About the human strain (*Salmonella Typhi*), the aqueous and ethanolic extracts of *Kalanchoe crenata*, *Mallotus oppositifolius*, *Tectona grandis*, the ethanolic extracts of *Abrus precatorius*, *Heterotis rotundifolia* and the aqueous extract of *Plectranthus monostachyus* showed antibacterial activity, with diameters of inhibition range from  $10.50 \pm 0.50^e$  mm to  $31 \pm 1^a$  mm. The greatest activity was showed by ethanolic extract of *Mallotus oppositifolius*, with an inhibition diameter of  $31 \pm 1^a$  mm. Relatively high activities were observed in the aqueous extract of *Kalanchoe crenata* (Kal A ( $16.66 \pm 1.15^{bc}$  mm)) and ethanolic extract of *Heterotis rotundifolia* (Het E ( $19.50 \pm 6.50^b$  mm)). As for the ethanolic extracts of *Abrus precatorius* and by *Kalanchoe crenata*, the inhibition diameters are relatively average (Abr E ( $14.50 \pm 0.50^{cd}$  mm), Kal E ( $14.33 \pm 2.30^{cd}$ )). The aqueous and ethanolic extracts of *Tectona grandis* and the aqueous extracts of *Mallotus oppositifolius*

and of *Plectranthus monostachyus*, showed small inhibition diameters (Tect A ( $10.50 \pm 0.50^e$  mm), Tect E ( $12.50 \pm 1.50$  mm), Mal A ( $12 \pm 1$  mm) and Plect A ( $11.50 \pm 0.50^{de}$  mm). The aqueous and ethanolic extracts of *Boerhavia diffusa*, of *Sida acuta*, as well as the aqueous extracts of *Abrus precatorius*, *Heterotis rotundifolia* and the ethanolic extract of *Plectranthus monostachyus*, showed no antibacterial activity ( $00 \pm 00^e$  mm).

*Heterotis rotundifolia* extracts (ethanolic), *Kalanchoe crenata* extracts (aqueous and ethanolic) and *Mallotus oppositifolius* (aqueous and ethanolic), are those which showed significant antibacterial activity on all the strains studied. On the other hand, the extracts (aqueous and ethanolic) of *Boerhavia diffusa* (Boer A and Boer E) show no antibacterial activity on all the strains studied.

The antibacterial activity of the various plant extracts is shown in Table 3.

### Minimum Inhibitory and Bactericidal Concentration of the Most Effective Plant Extracts

The minimum inhibitory and bactericidal concentrations of the most effective extracts of *Kalanchoe crenata*, *Mallotus oppositifolius* and *Heterotis rotundifolia* were determined respectively in liquid (Photo 3) and solid (Photo 4) medium, to evaluate their bacteriostatic and bactericidal activities.

On *Salmonella* serogroup O: 8 strain, ethanolic and aqueous extracts of *Mallotus oppositifolius*, *Kalanchoe crenata*, and ethanolic of *Heterotis rotundifolia* showed minimal inhibitory concentrations varying from 6.25 mg / ml to 50 mg / ml. The smallest minimum inhibitory concentration was observed with the ethanolic extract of *Mallotus oppositifolius* with a MIC of 6.25 mg / ml. The aqueous extracts of *Mallotus oppositifolius* and ethanolic extracts of *Kalanchoe crenata* both provided a MIC of 12.5 mg / ml. The greatest minimum inhibitory concentrations were observed with the aqueous extract of *Kalanchoe crenata* and the ethanolic extract of *Heterotis rotundifolia*, with concentrations of 50 and 25 mg / mL, respectively. Ethanolic extracts of *Mallotus oppositifolius*, *Kalanchoe crenata* and *Heterotis rotundifolia* killed *Salmonella* strain at 50 mg/ml (CMB = 50 mg/ml) whereas aqueous extract showed alive bacteria at the higher concentration tested (CMB > 50 mg/ml).

With *Salmonella typhi* strain, MICs were 6.25 mg / mL for the ethanolic extracts of *Mallotus oppositifolius* and *Heterotis rotundifolia*, and 12.5 mg / mL for the aqueous and ethanolic extracts of *Kalanchoe crenata* and the aqueous extracts of *Mallotus oppositifolius*. The extracts tested killed bacterial strain at 25 mg / mL (CMB = 25 mg / mL).

On *Salmonella enterica* O: 21 strains, of all the extracts tested, the aqueous and ethanolic extracts of *Mallotus oppositifolius*, the ethanolic extracts of *Kalanchoe crenata* and *Heterotis rotundifolia* showed no growth of strain at 50 mg / mL. Only the aqueous extract of *Kalanchoe crenata* showed no inhibition on growth of strain at 50 mg / mL (CMB > 50 mg / mL). The smallest minimum inhibitory concentration was 25 mg / mL and was observed with aqueous extracts of *Kalanchoe crenata* and ethanolic extracts of *Mallotus oppositifolius*. The aqueous extracts of *Kalanchoe crenata* and ethanolic extracts of *Heterotis rotundifolia* and *Mallotus oppositifolius* all had a minimum inhibitory concentration of 50 mg / mL (Table 4).

The MIC / CMB ratio made it possible to determine the bacterial power (bactericidal or bacteriostatic) of plant

extracts tested. Thus all the extracts tested showed a bactericidal power on the *Salmonella enterica* strain of serogroup O: 21, while with *Salmonella Typhi* strain, aqueous extracts of *Kalanchoe crenata* and *Mallotus oppositifolius*, and ethanolic extract of *Kalanchoe crenata* showed a bactericidal power (CMI / CMB = 2) against a bacteriostatic power (CMI / CMB = 4) of ethanolic extracts of *Mallotus oppositifolius* and *Heterotis rotundifolia*. Only ethanolic extract of *Heterotis rotundifolia* showed a bactericidal power (MIC / CMB = 2) against *Salmonella enterica* serogroup O: 8 strain (Figure 1).

### Discussion

The results of this study showed that an aqueous and/or ethanolic extracts of the selected plants have antibacterial activity against (multi) resistant strains of *Salmonella* from different origins. These medicinal plants are used in traditional medicine for the treatment of bacterial infections. Some of these plants, like many others, have been the subject of several studies in order to demonstrate their antibacterial activities. From these previous studies, we can quote Gupta *et al.* (2016) in India who demonstrated the antimicrobial activity of a selection of medicinal plants including *Tectona grandis* on Gram negative bacteria (*E. coli*, *Pseudomonas aeruginosa*) and Gram-positive bacteria (*S. aureus*, *Propionibacterium acnes*). Aibinu *et al.* (2007) [1] in Nigeria on *In vitro* antimicrobial activity of *Kalanchoe crenata* and *Bryophyllum pinnatum* against Gram positive bacteria, Gram negative bacteria including *Salmonella* paratyphi. Ouattara Karamoko *et al.* (2013) on ethanolic extracts of *Sida acuta* against bacteria responsible for nosocomial infection in Côte d'Ivoire. Saraka *et al.* (2019) [26] in Côte d'Ivoire on *In vitro* evaluation of the antifungal activity of extracts from *Mallotus oppositifolius* leaves against two phytopathogenic fungi. Dougnon *et al.* (2017) [10] on antibacterial activity of various plants including *Heterotis rotundifolia* against enteropathogenic bacteria in Benin. Kouadio *et al.* (2015) [15] in Côte d'Ivoire on activity of *Mallotus oppositifolius* leaves on multidrug-resistant bacteria. The antibacterial activity of these plants is thought to be due to the presence of certain chemical compounds contained within their cells. Indeed, the plants used in traditional medicine are an essential source of antimicrobial compound for human health. They have significant antibacterial properties for therapeutic treatments (Prakash *et al.*, 2013). Previous studies demonstrate the antibacterial activity of plant extracts against pathogenic microorganisms, based on ethnobotanical information. The variations in activity of the different extracts can be explained by the nature of the solvents used for the different extractions and the molecules contained in the cells of the plant (Traoré *et al.*, 2012) [28]. The extracts used in this study have an activity on at least one of the strains tested, with the exception of the aqueous and ethanolic extracts of *Boerhavia diffusa* which showed no activity on the strains tested. However, the largest diameters of inhibition were observed on all three bacterial strains with the aqueous and ethanolic extracts of *Mallotus oppositifolius* and *Kalanchoe crenata* ranging from  $12 \pm 0.50^{cd}$  to  $31 \pm 1a$  mm. These different diameters of inhibition zone are comparable to those of the diameters observed by Effah-Yeboah *et al.* (2020) for extracts of *Kalanchoe crenata*, on strains of *Salmonella* which was  $20 \pm 0.882$  mm and those of the work of Sahin *et al.* (2019) [25] on gram positive and gram-

negative bacteria whose diameters were between  $10 \pm 0.45$  and  $22 \pm 0.62$  mm. The good activity of *Kalanchoe crenata* observed in this study is also reported by Aibinu *et al.* (2007) <sup>[1]</sup>, on a strain of *Salmonella* Paratyphi whose inhibition diameter was 14 mm with the aqueous extract of *Kalanchoe crenata*. Also the efficacy of extracts of *Mallotus oppositifolius* observed in this work is similar with the extracts of *Mallotus oppositifolius* studied by Kouadio *et al.* (2015) <sup>[15]</sup> on *E. coli* producing ESBLs strains, methicillin-resistant *S. aureus*, whose diameters were between  $10.67 \pm 0.67$  and  $18.33 \pm 0.33$  mm for aqueous extracts and between  $11.00 \pm 0.57$  and  $24.33 \pm 0.67$  mm for the methanolic extract. In addition, the ethanolic extract of *Mallotus oppositifolius* has demonstrated excellent antifungal activity against fungi of the genus *Fusarium* and *Phytophthora* (Saraka *et al.*, 2019) <sup>[26]</sup>. The diameters of the zones of inhibition formed by the ethanolic extract of *Heterotis rotundifolia* were  $14.33 \pm 0.57$ ,  $15.66 \pm 3.51$ ,  $19.50 \pm 6.50$  respectively on the strains of *Salmonella* serogroup O: 8, O: 21 and *Salmonella* Typhi, while Dougnon *et al.* (2017) <sup>[10]</sup> reported diameters between 10 and 14 mm on enteropathogenic bacterial strains (*Citrobacter freundii*, *Pseudomonas oryzae*, *Shigella flexneri*, *Klebsiella rhinocleromatis*). Traditionally, these plants are used in the treatment of several pathologies of bacterial origin such as skin infections, diarrhea and urinary tract infections (Kouadio, 2013) <sup>[14]</sup>. The inhibitory power of the various extracts of *Heterotis rotundifolia*, *Mallotus oppositifolius* and *Kalanchoe crenata*, on the bacterial growth of the strains of *Salmonella* spp were demonstrated by the determination of the Minimum Inhibitory Concentration. Minimum Inhibitory Concentrations (MICs) vary from strain to strain and from extract to extract. With extracts of *Mallotus oppositifolius*, strain of serogroup O: 8, both with the ethanolic extract. The greatest MIC is located above 50 mg / mL obtained on the enterica strain of serogroup O: 21 (aqueous extract). These values are higher than those reported by certain authors, in particular those of Kouadio *et al.* (2015) <sup>[15]</sup> whose values were 0.78 and 1.56 mg / ml for the 70% methanolic extract and 3.12 mg / ml for the aqueous extract, on *E. coli* ESBL-producing strains. Orsot *et al.* (2015) also reported a better inhibition of the ethanolic extract of *Mallotus oppositifolius* compared to the aqueous extract of *Mallotus oppositifolius* on phytopathogenic strains of *Sclerotium rolfsii* with a MIC of 12.5 mg / ml for the

ethanolic extract and 100 mg / ml for the aqueous extract. The CMB / CMI ratio reveals that ethanolic extract for the most part has bacteriostatic power, unlike aqueous extract which mainly has bactericidal power. These different powers observed with the different extracts of *Mallotus oppositifolius* in this study are similar to the bacterial powers of the extracts of this same plant on the strains of phytopathogenic fungi studied by Saraka *et al.* (2019) <sup>[26]</sup>. As for *Kalanchoe crenata* extracts, the smallest MIC (12.5 mg / ml) is obtained with ethanolic extract on the Typhi and enterica strains of serogroup O: 8, also with the aqueous extract on the Typhi strain. While the greatest MIC (50 mg / ml) is obtained only with aqueous extract on the enterica strains of serogroup O: 21 and O: 8. The MIC values reported by Aibinu *et al.* (2007) <sup>[1]</sup> on *Salmonella* Paratyphi, by Dieye & Sarr (2020) on *Candida albicans* strains which were respectively 512 mg/ml and 256 mg/ml are clearly higher than the values observed in this work. However, the MIC values obtained in this study are higher than the values reported by Sahin *et al.* (2019) <sup>[25]</sup> obtained from natural compounds extracted from *Kalanchoe crenata* which were 50 µg / ml on a *E. coli* strain. About ethanolic extracts of *Heterotis rotundifolia*, the MICs were range between 6.25 mg / ml and 50 mg / ml, with the smallest MIC obtained on the Typhi strain (6.25 mg / mL) and the largest on the enterica strain of serogroup O: 21 (50 mg / mL). Previous works which have already been the subject of a study of the inhibitory effect of extracts of *Heterotis rotundifolia*, include those of Dougnon *et al.* (2017) <sup>[10]</sup> which offered MICs varying from 50 mg / mL to 100 mg / mL on enteropathogenic strains with bactericidal power (CMB / MIC  $\leq 2$ ). Extraction method, solvent and plant parts used on the microbial strain could explain the differences observed between the MICs obtained in this study and those of the above-mentioned authors. When extraction methods used, solvents and plant organs differ, the active elements responsible for antibacterial activity do not have the same concentrations whatever the nature of the extracts. The activity of plant substance therefore depends on its concentration of active ingredients (Thangara *et al.*, 2000). However, the harvest site and the type of local climate can influence the chemical profile of plants (Amvam, 1998), which could justify the frequent use of these plants for the treatment of certain infections.

**Table 1:** Salmonella strains used for the study

| Strain   | Coded    | Antibiotic profile                 | Origin of the strain         | Serogroup / Serotypes |
|----------|----------|------------------------------------|------------------------------|-----------------------|
| Strain 1 | C211 / A | A-AMC-Tic-Cf-CTX-GC-Te-SXT-Nal-Cip | Avian (raw chicken gizzards) | O: 8                  |
| Strain 2 | S30H3C   | A-Tic-TE                           | Avian (quail viscera)        | O: 21                 |
| Strain 3 | SH35     | AM-CRO-CAZ-FF-CFS                  | Human (Human Stool)          | Typhi                 |

A: amoxicillin; AM: ampicillin; AMC: amoxicillin / clavulanic acid; CRO: Ceftriaxone; CAZ : Ceftazidime; FF: Fosfomycin; CFS: Cefsulodine; Tic: ticarcillin; This f: cephalotine; Ctx: cefotaxime; G: gentamicin; C: chloramphenicol; Sxt: cotrimoxazol; Nal: nalidixic acid; Cip: ciprofloxacin; Te: tetracycline

**Table 2:** Yield of extractions

| Plants / | Extracts from of the study | Mass before extraction (g) | Mass after extraction (g) | Yield (%) |
|----------|----------------------------|----------------------------|---------------------------|-----------|
| Abr      | Abr A                      | 30g                        | 2.84g                     | 9, 46     |
|          | Abr E                      | 30g                        | 3.14g                     | 10, 46    |
| Boer     | Boer A                     | 30g                        | 4.5g                      | 15        |
|          | Boer E                     | 30g                        | 2.30g                     | 7, 6      |
| Het      | Het A                      | 17g                        | 1.34g                     | 7, 88     |
|          | Het E                      | 17g                        | 1.04g                     | 6;11      |

|        |         |     |       |        |
|--------|---------|-----|-------|--------|
| Kala   | kala A  | 30g | 1.68g | 5, 6   |
|        | kala E  | 30g | 2.04g | 6, 8   |
| Mal    | Mal A   | 30g | 4.12g | 13, 73 |
|        | Mal E   | 30g | 3.71g | 12, 36 |
| Plect  | Plact A | 35g | 2.10g | 06     |
|        | Plact E | 35g | 2.83g | 08, 08 |
| Sid ac | Sid acA | 30g | 3.92g | 10, 4  |
|        | Sid acE | 30g | 3.27g | 10, 09 |
| Tect   | Tect A  | 30g | 9.13g | 30, 43 |
|        | Tect E  | 30g | 6.35g | 21, 16 |

**Abr:** *Abrus precatorius*, **Boer:** *Boerhavia diffusa*, **Het:** *Heterotis rotundifolia*, **Kal:** *Kalanchoe crenata*, **Wrong:** *Mallotus oppositifolius*, **Plect:** *Plectranthus monostachyus*, **Sid ac:** *Sida acuta*, **Tect:** *Tectona grandis*, A: aqueous, E: ethanolic.

**Table 3:** Diameters of inhibition zones of the various plant extracts on *Salmonella* strains tested

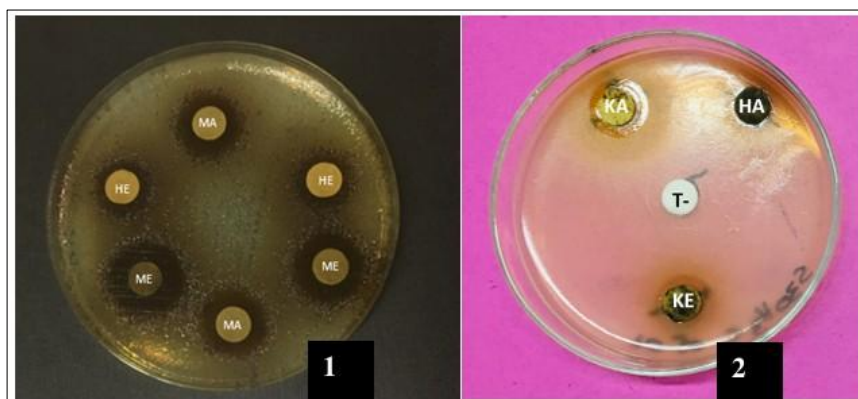
| Plants / | Extracts from of the study | Strains <i>Salmonella</i> spp |                            |                 |
|----------|----------------------------|-------------------------------|----------------------------|-----------------|
|          |                            | <i>S. enterica</i> (O: 8)     | <i>S. enterica</i> (O: 21) | <i>S. Typhi</i> |
| Abr      | Abr A                      | 00 ± 00f                      | 10.66 ± 1.15d              | 00 ± 00f        |
|          | Abr E                      | 13 ± 2d                       | 12.50 ± 0.50d              | 14.50 ± 0.50cd  |
| Boer     | Boer A                     | 00 ± 00f                      | 00 ± 00f                   | 00 ± 00f        |
|          | Boer E                     | 00 ± 00f                      | 00 ± 00f                   | 00 ± 00f        |
| Het      | Het A                      | 10.50 ± 0.50e                 | 13 ± 2cd                   | 00 ± 00f        |
|          | Het E                      | 14.33 ± 0.57c                 | 15.66 ± 3.51c              | 19.50 ± 6.50b   |
| Kala     | kala A                     | 16.33 ± 0.57b                 | 16 ± 1c                    | 16.66 ± 1.15bc  |
|          | kala E                     | 15.50 ± 0.50b                 | 16 ± 2c                    | 14.33 ± 2.30cd  |
| Mal      | Mal A                      | 13.50 ± 0.50cd                | 19 ± 1b                    | 12 ± 1de        |
|          | Mal E                      | 21.66 ± 0.57a                 | 27 ± 1a                    | 31 ± 1a         |
| Plect    | Plact A                    | 10.55 ± 0.50e                 | 00 ± 00f                   | 11.50 ± 0.50de  |
|          | Plact E                    | 13.33 ± 0.57cd                | 13 ± 2cd                   | 00 ± 00f        |
| Sid ac   | Sid acA                    | 00 ± 00f                      | 11.50 ± 1.50d              | 00 ± 00f        |
|          | Sid acE                    | 12.5 ± 0.50d                  | 19.66 ± 3.21b              | 00 ± 00f        |
| Tect     | Tect A                     | 00 ± 00f                      | 12 ± 1d                    | 10.50 ± 0.50e   |
|          | Tect E                     | 00 ± 00f                      | 00 ± 00f                   | 12.50 ± 1.50de  |

**Abr:** *Abrus precatorius*, **Boer:** *Boerhavia diffusa*, **Het:** *Heterotis rotundifolia*, **Kal:** *Kalanchoe crenata*, **Wrong:** *Mallotus oppositifolius*, **Plect:** *Plectranthus monostachyus*, **Sid ac:** *Sida acuta*, **Tect:** *Tectona grandis*, A: aqueous, E: ethanolic

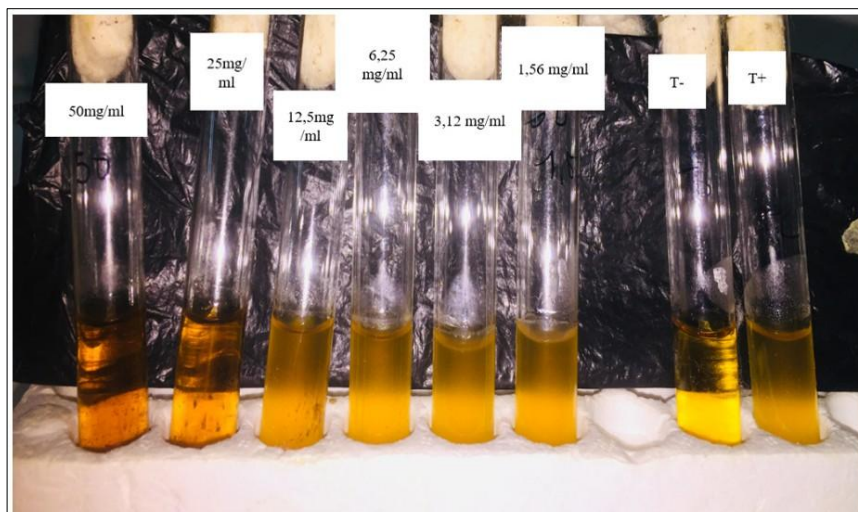
**Table 4:** Antibacterial parameters of extracts of *Mallotus oppositifolius* *Kalanchoe crenata*, and *Heterotis rotundifolia* leaves on (multi)resistant *Salmonella* strains

| Extracts from of the study | <i>S. enterica</i> (O: 8) | <i>S. enterica</i> (O: 8) | <i>S. enterica</i> (O: 21) | <i>S. enterica</i> (O: 21) | <i>S. Typhi</i> | <i>S. Typhi</i> |
|----------------------------|---------------------------|---------------------------|----------------------------|----------------------------|-----------------|-----------------|
|                            | MIC                       | BMC                       | MIC                        | BMC                        | MIC             | BMC             |
| Kal A                      | 50                        | > 50                      | 50                         | > 50                       | 12. 5           | 25              |
| Kal E                      | 12.5                      | 50                        | 25                         | 50                         | 12. 5           | 25              |
| Het E                      | 25                        | 50                        | 50                         | 50                         | 6.2 5           | 25              |
| Mal A                      | 12.5                      | > 50                      | 25                         | 50                         | 12. 5           | 25              |
| Mal E                      | 6.25                      | 50                        | 50                         | 50                         | 6.2 5           | 25              |
| Kal A                      | 50                        | > 50                      | 50                         | > 50                       | 12. 5           | 25              |

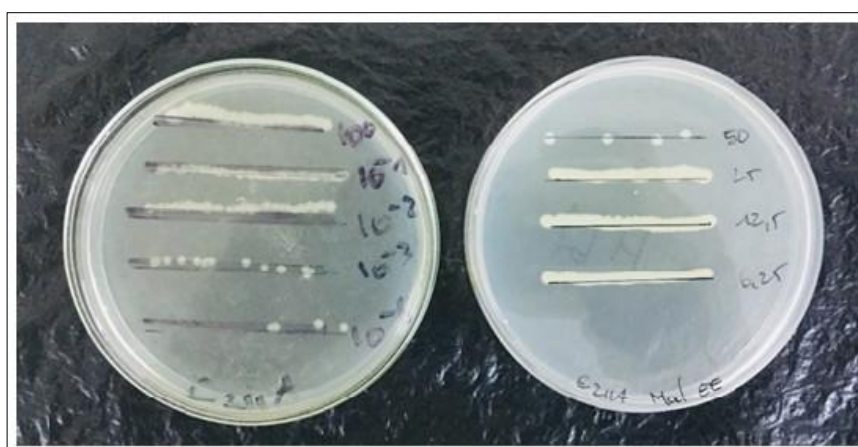
**Kal A:** aqueous extract of *Kalanchoe crenata*; **Kal E:** ethanolic extract of *Kalanchoe crenata*; **Mal A:** aqueous extract of *Mallotus oppositifolius*; **Mal E:** ethanolic extract of *Mallotus oppositifolius*; **Het E:** ethanolic extract of *Heterotis rotundifolia*.



**Photography 1:** Diameters of the growth inhibition zones of *Salmonella enterica* serogroup O: 2 1 strain by plant extracts (Kal A, Kal E, Mal A, Mal E, Het A and Het E)



**Photography 2:** Dilution range for the determination of the MIC of ethanolic *Kalanchoe crenata* on *Salmonella enterica* serogroup O: 21 strains



**Photography 3:** MBC of ethanolic extract of *Mallotus oppositifolius* leaves on *Salmonella* serogroup O: 8

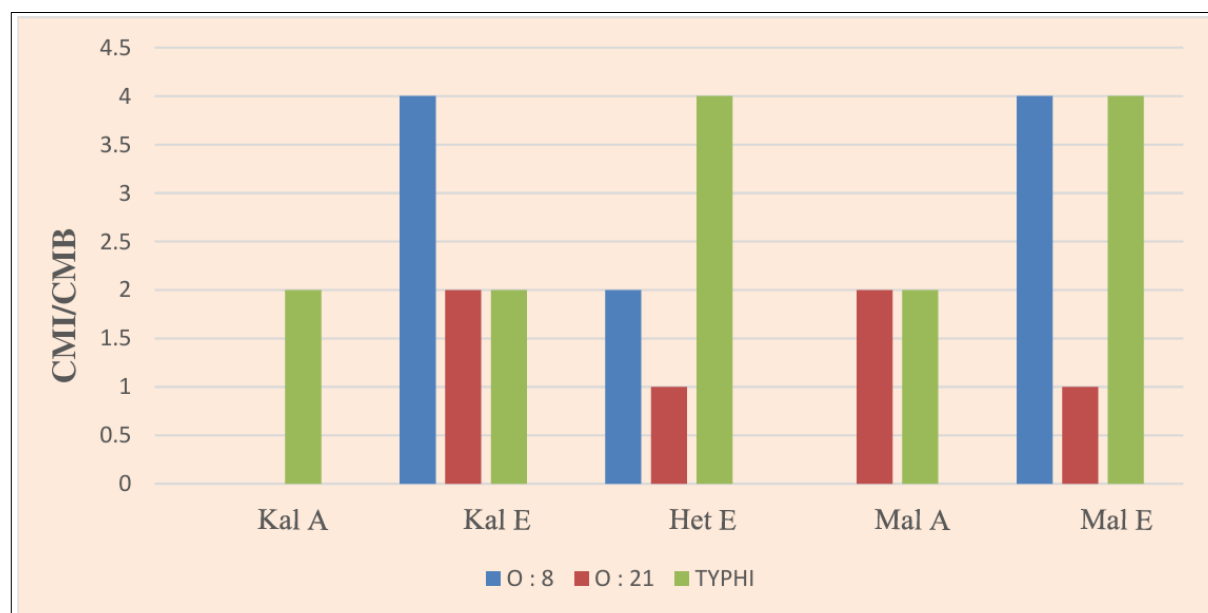


**Fig 1:** Effects of plant extracts on the strains tested

$CMB / CMI \leq 2$ , the substance is said to be bactericidal;  $CMB / CMI > 2$ , the substance is said to be bacteriostatic. Kal A: aqueous extract of *Kalanchoe crenata*; Kal E: ethanolic extract of *Kalanchoe crenata*; Mal A: aqueous extract of *Mallotus oppositifolius*; Mal E: ethanolic extract of *Mallotus oppositifolius*; Het E: ethanolic extract of *Heterotis rotundifolia*

## Conclusion

The results of this work reveal that the selected plants possess dose-dependent antibacterial activity on *Salmonella* strains of various origins. Among these plants, the leaves of *Kalanchoe crenata*, of *Mallotus oppositifolius* and *Heterotis rotundifolia* are those which offer greater activity on all the plants studied. These strong antibacterial activities observed at the level of these enteropathogenic strains make plausible a possible use of these various plant extracts as an alternative to the use of antibiotic molecules. Substituting these plant extracts for the classic antibiotics used in breeding could therefore solve on the one hand the problem of the appearance and emergence of multiresistant bacteria, of the overuse of antibiotic molecules.

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