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Exposure to Plant Extract Causes the Variation of Antibiotic Susceptibility of Two Bacterial Strains (*Salmonella* Serotype Typhi and *Staphylococcus aureus*)

Fabrice Ezo'o Mengo[1], Stéphanie Claire Tchonang[1], Hermann Ludovic Kemaleu[1], Sylvain Leroy Sado Kamdem[2*] and Jean Justin Essia Ngang[2]

[1]Department of Biochemistry, Faculty of Science, The University of Yaounde 1, P.O.Box 812, Yaoundé, Cameroon.
[2]Department of Microbiology, Faculty of Science, The University of Yaounde 1, P.O.Box 812, Yaoundé, Cameroon.

Authors' contributions

This work was carried out in collaboration between all authors. Authors JJEN and SLSK designed, coordinated and supervised the study. Authors FEM, SCT and HLK performed the lab experiments. Authors FEM, SLSK and JJEN wrote the paper. Authors SLSK, FEM, SCT and HLK participated in data analyses. All the authors read and approved the final version of the manuscript.

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ABSTRACT

Introduction: Several authors have associated the development of antibiotic resistance to the use of antibiotics. But this development of resistance could also be associated with plant extracts. This article explores the impact of exposure to different plant extracts of *Salmonella* serotype Typhi and *Staphylococcus aureus* on their sensitivity to antibiotics.
Methods: According to the informations obtained from traditional medicine healers, 13 plants

**Corresponding author: E-mail: sadosylvain@hotmail.com;*

powders from different parts were selected to compose mixtures that were used to produce the extract by decoction. The susceptibility test through inhibition diameter analyses and the minimal inhibition concentration were performed using the decoctions and the two strains. Different microorganisms were exposed to a fresh broth containing the extract at fixed or increased concentration in every 24 h for 14 days. Subsequently, after the 14 days, these strains were grown in the same broth renewed at 24 h without added extract for another 14 days. Antibiogram using three antibiotics was performed at 48 h. Variation of inhibition diameter was used to evaluate the impact of extract exposure to the sensitivity.

Results: The two strains subjected to a molecular pressure of the plant extracts acquired resistance to the antibiotics tested, regardless of the concentration of the plant extract used for the exposure. The sensitivity of *Salmonella* Typhi exposed to two of the decoctions decreased vis-a-vis the three antibiotics tested and this decrease persisted 14 days repeated sowing in new broth without plant extract despite the lack of antimicrobial. In addition, exposure of *Staphylococcus aureus* to the extracts of *Chromoleana odorata* and *Erigeron floribundus* showed a decrease in sensitivity of this strain to Erythromycin. But this sensitivity compared to other antibiotics has decreased after non antimicrobial exposure.

Conclusion: This study shows that continuous exposure of bacteria to some plant extracts reduces the sensitivity of these strains. As a result, the development of antibiotic resistance is not only related to the uncontrolled use of antibiotics.

Keywords: Antibiotics; plant extracts; antimicrobial activity; continuous exposure; sensitivity.

ABBREVIATIONS

SXT/TSU: Cotrimoxazole; C: Chloramphenicol; GM: Gentamicine; ERY: Erythromycine; CIP: Ciprofloxacin; Dec.0: Decoction0; Dec.1: Decoction1; Dec.2: Decoction2; Dec.3: Decoction3; CO: Chromolaena odorata; EF: Erigeron floribundus; LC: Lantana camara; TP: Tridax procumbens; ΔP: Difference in inhibition diameter obtained during exposure period between the beginning and the end of the exposure; ΔA: Difference in inhibition diameter obtained during non exposure period between the beginning and the end of this period; MICs: Minimal inhibitory concentrations.

1. INTRODUCTION

The antibiotic resistance which today represents as a global public health problem is the cause of the search for new molecules, with a potential reserve being the use of medicinal plants. The observations made over the past 20 years showed that the number of new drugs reaching the market has unfortunately fallen [1,2] while the level of resistance has increased among pathogenic bacteria, including *Salmonella* and *Staphylococcus* types. This is the case for the methicillin-resistant *Staphylococcus aureus*, as well as *Salmonella* serotype Typhi and *Salmonella* serotype *Typhimurium* resistant to second generation fluoroquinolones and to ceftriaxone [3]. Several authors have attributed antibiotic resistance to the selective pressure of antibiotic use in clinical, veterinary and agricultural practices [4]. A correlation between the use of antibiotics, the emergence and spread of bacterial resistance is indeed well documented [5].

Infections such as typhoid fever and meningitis caused by resistant bacteria have become difficult to treat and the treatment cost is increasingly high. The use of medicinal plant products or in combination with antibiotics is becoming a serious alternative in developing countries.

In general, combination studies of medicinal substances, particularly antibiotics along with extracts and essential oils obtained from plants are often described in the literature [6,7,8]. This strategy is indeed of great interest for potential clinical applications, since it reduces the possible side effects of current treatments by reducing the dose of the compound used [6], thus also limiting the development of antibiotic based resistance phenomena.

However, recent studies have shown that bacteria with antibiotic resistance traits can be isolated from people who have not been subjected to their significant exposure to

Table 1. List of medical plants used against *Salmonella* Typhi and *Staphylococcus aureus* in Cameroon

Scientific name	Family	Common name	Part used	Associated plants	Method of preparation	How to use	References
Citrus medica	*Rutaceae*	Cedratier	Fruit		Decoction	Oral	[12]
Mangifera indica	*Anacardia-ceae*	Andok (Ewondo)	Bark	Citron	Decoction	Oral	[11]
Carica papaya	*Caricaceae*	Fofo (Ewondo)	Leaves	Datrier, salt germ	Maceration, decoction, trituration	Oral	[14,10]
Bidens pilosa	*Asteraceae*	Colé-colé	Bark	Aloès	Trituration	Oral	[14,10]
Senna alata	*Fabaceae*	Datrier	Leaves		Boil, maceration	Oral	[11]
Cymbopo-gon citratus	*Poaceae*	Citronnelle Ossanga (Ewondo)	Leaves	Citron, cane of the twins	Decoction, Maceration	Oral	[10]
Alstonia boonei	*Apocynaceae*	Ekuk (Ewondo)	Ecorces	Nivaquine leaf	Maceration, fermentation	Oral	[14]
Panax ginseng	*Araliaceae*	Ginseng	Fruit, racine	Kinkeliba in fruit	Infusion, maceration	Oral	[14]
Entandrophragma sp.	*Meliaceae*	Sapeli blanc	Ecorces	Baobab, dattier	Macération, fermentation	Oral	[14]
Voacanga africana	*Apocynaceae*	Voacanga d'Afrique	Seed, Leaves	Cola nuts	Maceration, decoction	Oral	[14]
Enantia chlorantia	*Annonaceae*	Nfol	Ecorces	Citrus limon	Decoction	Oral	[14]
Irvingia gabonensis	*Irvingiaceae*	Andok beti (Ewondo)	Ecorces	Ngongui	Decoction	Oral	[15]
Musanga cecropioides	*Cecropiaceae*	Asseng	Ecorces		Decoction	Oral	[15]
Chromolae-na odorata	*Asteraceae*	Kondengui (Ewondo)	Leaves	Kings of herbs	Trituration	Der-mal, Oral	[13]
Erigeron floribundus	*Asteraceae*	Vien nguim	Leaves		Friction in warm water	Der-mal	[13]

antibiotics, living in remote locations and undergoing very little geographical mobility [9]. This suggests that there are agents other than antibiotics that induce resistance.

Literature presents many plants that are used as a mixture in the form of decoctions to treat people with typhoid fever, caused by *Salmonella* Typhi in Cameroon. *Bidens pilosa, Carica papaya* and *Cymbopogon citratus* [10]; *Senna alata* and *Mangifera indica* [11] and *Citrus médica* [12] are among them. Several macerated plants are also used against skin diseases or gastrointestinal infections, as is the case of *Chromolaena odorata* and *Erigeron floribundus*, whose antibacterial activity against *Staphylococcus aureus* has been demonstrated [13]. Yet in a country where modern medicine and traditional medicine are used, it is not uncommon to see many therapeutic failures of modern medicine after treatment with traditional medicine. This justifies the interest of this work which aimed at elucidating the influence of *Salmonella* Typhi and *Staphylococcus aureus* exposure to plant extracts on the antimicrobial activity of various antibiotics. In particular, those used in the fight against these two pathogens. This study was carried out in the Laboratory of Microbiology of the University of Yaounde 1, Cameroon between August 2013 and April 2015.

2. MATERIALS AND METHODS

2.1 Plants Selection

Several medicinal plant species used in the treatment of typhoid and staphylococcal skin infections were harvested in Cameroon central region from August 2013 to September 2014. Some of these plants and the parts used are presented in Table 2. They were selected based on indications from the literature and information received from traditional healers and vendors of medicinal plants. Botanical identification and authentication were done at the National Herbarium of Cameroon

2.2 Preparing Aqueous Extracts of Plants

The preparation of the decoctions used in this work was prepared based on recipes from traditional healers for the treatment of typhoid fever.

Indeed, the harvested plants were washed, dried and ground. The various powder samples were then weighed and mixed in water in the ratio of (w / v) 1: 2 for the first solution obtained from the plants (1: 9: 10), 2: 3 for the second solution obtained from the plants (1: 7: 8), 1: 2 for the third solution obtained from the plants (4: 8: 11: 12;: 13) and 2: 3 for the last solution obtained from the plants (1: 2: 3: 4: 5: 6). The solutions obtained were boiled for 30 minutes and then cooled to room temperature. The decoctions were filtered using Whatman No. 1 filter paper and the filtrate was dried in an oven at 45°C until a constant weight was achieved. Decoctions thus obtained and codified as :Dec.0; Dec.1; Dec.2 and Dec.3 respectively and stored at 4°C for later use.

Table 2. Medicinal plants selected for assessing the antibacterial activity

N°	Scientific name	Mass/litre	Part used	Reference	Location of collection
1	*Citrus medica*	120 g	Fruit	65106/HNC	Mokolo market
2	*Mangifera indica*	200 g	Bark	5734/HNC	Mount Eloumden
3	*Carica papaya*	180 g	Leaves	18647/SFR/CAM	Mendong
4	*Bidens pilosa*	40 g	Bark	42254/HNC	Mount Eloumden
5	*Senna alata*	80 g	Leaves	1871YA	Mount Eloumden
6	*Cymbopogon citratus*	30 g	Leaves	48536/SFR/CAM	Mendong
7	*Alstonia boonei*	88 g	Ecorces	2151/SRF/CAM	Mount Eloumden
8	*Entandrophragma*	102 g	Ecorces	29933/HNC	Mount Eloumden
9	*Allium cepa*	250 g	Bulbs	034/UDS	Mokolo market
10	*Allium sativum*	200 g	Bulbs	44810/HNC	Mokolo market
11	*Kalanchoe crenata*	100 g	Ecorces	50103/YA	Mokolo market
12	*Annickia chlorantha*	60 g	Ecorces	2949/SFR/CAM	Mokolo market
13	*Picralima nitida*	240 g	Fruit	1942/SRFK	Mokolo market
14	C*hromolaena odorata*	100 g	Leaves	952/SRF/CAM	Campus UY1
15	*Erigeron floribundus*	100 g	Leaves	48832/HNC	Campus UY1

The anti-staphylococcal decoction was made from four plant extracts: *Chromolaena odorata*; *Erigeron floribundus; Enantia chlorantha* and *Irvingia gabonensis*. For this, the different parts of each harvested plant were washed with water, dried and finely ground using an electric grinder. The resulting powder was soaked in water in a 10% ratio (w / v) for 48 h. The solution was filtered under vacuum on Whatman No. 1 filter paper and the filtrate was dried in the oven at 45°C. The dry extract obtained was kept in a refrigerator at 4°C for future use.

2.3 Bacterial Strains

Staphylococcus aureus NCTC 10652 and *Salmonella* Typhi *32* that are involved in skin diseases and typhoid fever respectively, were used in this work and provided by the Microbiology Laboratory of the Food Science Department of the University of Bologna (Italy).

2.4 Antibiotics

The following antibiotics: Gentamicin (GM), Cotrimoxazole (SXT/TSU) (Strides Acrolab Ltd), Erythromycin (ERY) (Alice pharma Pvt Ltd.), Ciprofloxacin (CIP) (Maxheal Pharmaceuticals Ltd.) and Chloramphenicol (C) (Baijingyu Nanjing Pharmaceutical Co. Ltd) were used. The choice of these antibiotics was based on the frequency of use and their family affiliation.

2.5 Antimicrobial Analysis

2.5.1 Inhibition diameter analysis

The antimicrobial activity of antibiotic substances and plant extracts used was determined by the diffusion method on agar medium [16]. Antibiotic solutions and plant extracts were prepared at concentrations of 1 mg/ml and 100 mg/ml respectively. Agar was seeded from a bacterial suspension of 10^6 UFC / ml. 10 µl of each antibiotic solution and 20 µl of each extract test solution and the solvent used as control (sterile water) were deposited on different filter paper disks (6 mm diameter). The impregnated disks were dried for 24 hours at 37°C to complete evaporation of the solvent and then deposited on the surface of the agar. After a 24-hour incubation period at 37°C, the various diameters of the inhibition halos obtained around the disks were measured.

2.5.2 Determination of MICs

MICs were determined according to the method described by Cos et al. in 2006 [16]. A geometric progression of reason 2 of the concentrations of plant extracts ranging from 0.1 mg/ml to 1600 mg/ml was carried out. For antibiotics, the range of concentrations ranged from 0.001 to 0.5 mg/ml. Subsequently, these tubes were seeded with a volume of 100 µl of the inoculum (10^6 UFC / ml) diluted 1/100; and were incubated for 24 hours at 37°C. The experimental controls consisted of (I) a broth without inoculum but the inhibitory substance and (II) broth with inoculum without the inhibitory substance. After incubation, the presence of haze indicated visible growth of the bacteria, while the absence of haze involved the antibacterial effect of the tested substances. MIC was determined to be the lowest concentration that inhibited any visible growth.

2.6 Continuous Exposure of Bacterial Strains to Plant Extracts

Strains of *Salmonella* Typhi and *Staphylococcus aureus* were cultured in every 24 hours for 14 days in nutrient broth containing the plant extracts at a fixed concentration and increasing sub-lethal concentrations. When growth was visible in the tubes, 100 µl of this culture was taken to inoculate two different broths: firstly a new nutrient broth containing the same concentration of the plant extract and secondly a new nutrient broth containing the double concentration the plant extract. This was repeated in every 24 hours for 14 days.

After 14 days of exposure to the extracts « exposure period », the strains were grown again in every 24 hours for 14 additional days in nutrient broth without plant extracts «non-exposure period » to verify a possible change of strain behaviour towards the antimicrobial. The diameters of the inhibition halos of the different antibiotics vis-a-vis the strains studied exposed or unexposed to plants extracts were evaluated in every 48 hours by performing a standard antibiogram using the diffusion method with the disks described above.

The study of the antibiotic sensitivity of strains exposed to plant extracts over time was based on the determination of the indices ΔP and ΔA where, ΔP is the difference in inhibition diameter obtained during exposure period calculated as initial inhibition diameter minus final inhibition diameter. ΔA is the difference in inhibition diameter obtained during the non-exposure period calculated as inhibition diameter at the beginning minus inhibition diameter at the end. Thus: if ΔP<0, the strain became more sensitive

to antibiotics during the exposure period, if ΔP>0, it became more resistance to antibiotics during the exposure period, and if ΔA<0, the strain gained more resistance to antibiotics during the non-exposure period with respect to the exposure period; if ΔA>0, the strain became more sensitive to antibiotics during the non period exposure with respect to the exposure period.

3. RESULTS

3.1 Activity of Antimicrobial Substances

The sensitivity of the bacterial strains (*Salmonella* Typhi and *Staphylococcus aureus*) vis-a-vis different tested antimicrobial is presented in Table 3. The results shows that *Salmonella* Typhi and *Staphylococcus aureus* were sensitive to both the antibiotics and the plant extracts tested in this work. The antimicrobial activities of the extracts tested, however, remained much lower than those of antibiotics. The diameters of the inhibition halo obtained between 25.0 mm and 42.0 mm and between 7.5 mm and 12.00 mm respectively for antibiotics and plant extracts (Fig. 1). Ciprofloxacin (36.00 mm) and Chloramphenicol (29.00 mm) have the best antibacterial activity against *Salmonella* Typhi and for *Staphylococcus aureus* it is Cotrimoxazole (42.00 mm) and Erythromycine (40.00 mm). Regarding the plant extracts, Dec 1 has the highest antibacterial activity against *Salmonella* Typhi with an inhibition diameter of 12.00 mm, followed by the extracts of Dec 0 and Dec 3 whose Inhibitory activities were similar. Furthermore, *Erigeron floribundus* and *Chromolaena odorata* showed the best activities against *Staphylococcus aureus NCTC 10652* with inhibition diameters of 12.00 mm and 11.50 mm respectively.

The results reported in Table 3 shows MIC values Typhi ranging from 0.008 to 0.320 mg / ml for antibiotics, and 6.25 to 1600.00 mg/ml for plant extracts. These results confirm the best activities of antibiotics compared to that of plant extracts. Dec 0, Dec 1 and Dec 3 were the most active decoctions against *Salmonella* and with comparable activities (MICs equal to 800 mg/ml). Moreover, extract of *Chromoleana odorata* was the most active against *Staphylococcus aureus NCTC 10652* with a MIC equal to 6.25 mg/ml.

3.2 Antibiotic Susceptibility of Strains Exposed to Plants Extracts

Continuous exposure of strains to plant extract resulted in a variation in their sensitivity to the antibiotics tested (Tables 4,5,6,7). Three most active antibiotics for each strain was used to monitor their strain behaviour towards them after exposure to plant extracts and the period of none exposure.

According to the present work, the monitoring of the antibacterial activity of the three antibiotics tested on *Salmonella* Typhi before and after its exposure to decoctions 1 and 3 shows that this strain has developed resistance to the three antibiotics (ΔP>0), this regardless of the concentrations used (Figs. 2 and 3). In addition, it was seen that this resistance developed during the increasing concentration exposed period which was maintained over time during the non exposure period (ΔA<0) with all the antibiotics. When the exposure period was at a fixed concentration of extract (Dec.1), only Chloramphenicol induced resistance was lost. In case of exposure to Dec.3, the resistance to antibiotic-induced during the exposure period was maintained only for Ciprofloxacin in the two tests and Cotrimoxazole only during the increasing concentration exposed period.

The results obtained during the exposure period of *Staphylococcus aureus* to *Chromoleana odorata* show that this strain has become more sensitive to all antibiotics (ΔP<0) except Erythromycin at a fixed concentration (ΔP>0). Similar results were obtained during the exposure period of *Staphylococcus aureus* to *Erigeron floribundus* (Figs. 4 and 5). The strain has become more sensitive to all

Table 3. Minimum inhibitory concentrations of antimicrobials (mg/ml)

	C	CIP	SXT/TSU	Dec.0	Dec.1	Dec.2	Dec.3
Salmonella Typhi	0.32	0.008	0.128	800	800	1600	800
	GM	**ERY**	**SXT/TSU**	**CO**	**EF**	**LC**	**TP**
Staphylococcus aureus	0.008	0.008	0.125	6.25	25	25	25

SXT/TSU: Cotrimoxazole; C: Chloramphenicol; GM: Gentamicine; ERY: Erythromycine; CIP: Ciprofloxacin; Dec.0: Decoction0; Dec.1: Decoction1; Dec.2: Decoction2; Dec.3: Decoction3; CO: Chromolaena odorata; EF: Erigeron floribundus; LC: Lantana camara; TP: Tridax procumbens

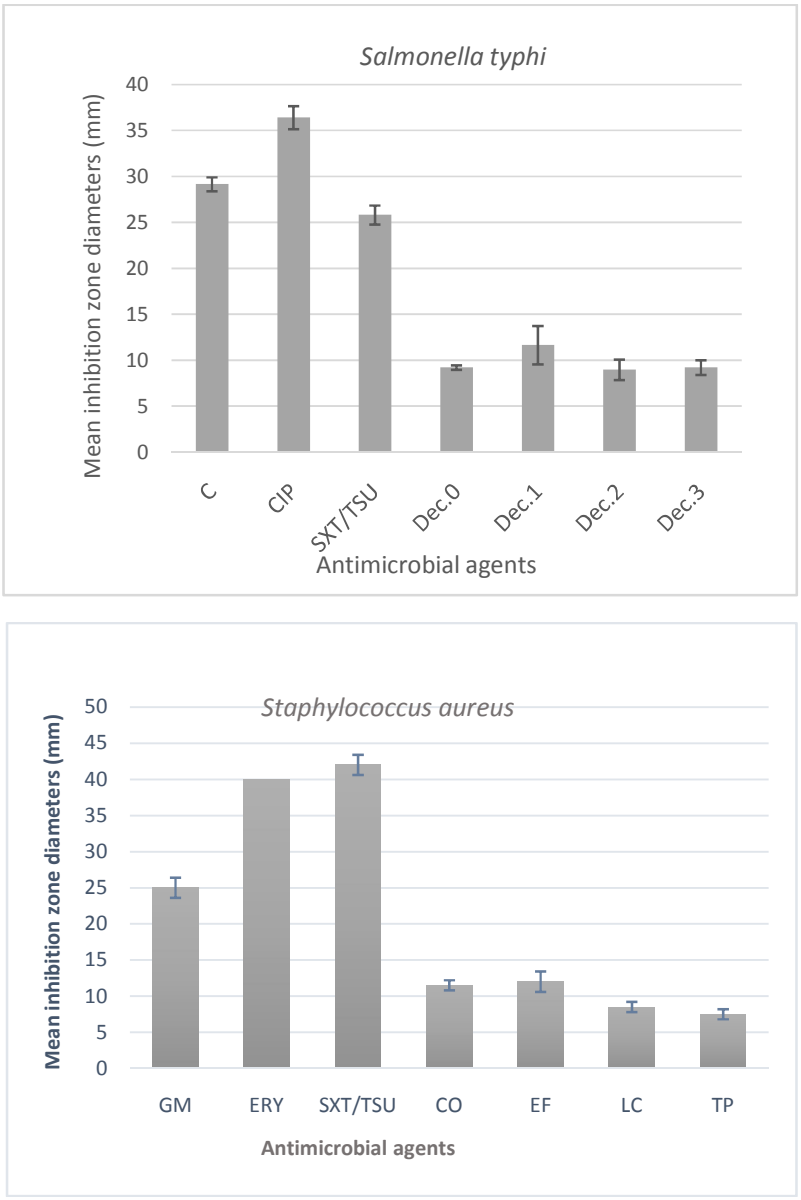

Fig. 1. Values of the mean diameters of the inhibition halos of antimicrobials tested vis-a-vis *Salmonella Typhi* and *Staphylococcus aureus*

SXT/TSU = Cotrimoxazole, C = Chloramphenicol, GM = Gentamicine, ERY= Erythromycin, CIP=Ciprofloxacin, Dec.0 = Decoction0, Dec.1 = Decoction1, Dec.2 = Decoction2, Dec.3 = Decoction3, CO= Chromolaena odorata, EF=Erigeron floribundus, LC= Lantana camara, TP= Tridax procumbens

antibiotics (ΔP<0) except Erythromycin at a fixed and increasing concentration (ΔP>0). Moreover, strains of *Staphylococcus aureus* obtained after exposure to *Chromoleana odorata* became less sensitive to all antibiotics during non exposure period (ΔA<0), and strains obtained after exposure to *Erigeron floribundus* had a lower sensitivity to Gentamicin (only fixed concentration) and Cotrimoxazole during non exposure period (ΔA<0). In addition, we observed a gain of sensitivity of *Staphylococcus aureus* to Gentamicin (at increasing concentration) and Erythromycin during non exposure period.

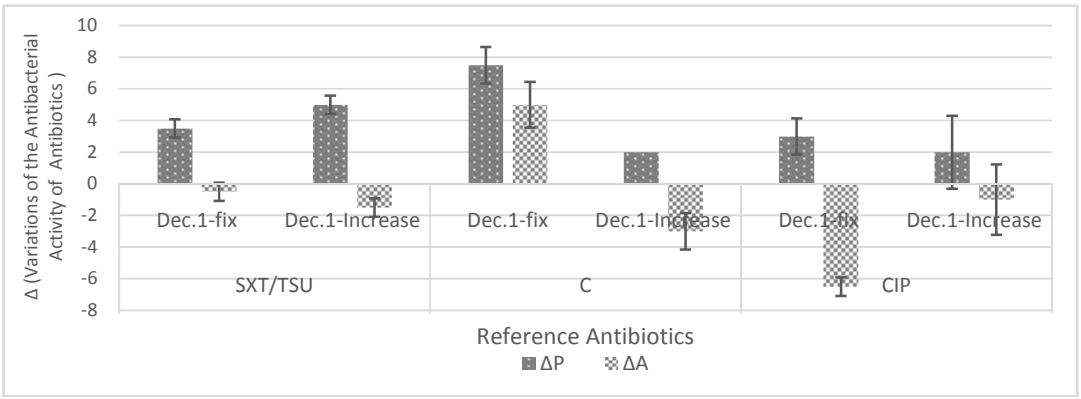

Fig. 2. Variations of the antibacterial activity of reference antibiotics against *Salmonella Typhi* in repeated culture in the presence and absence of fixed and increasing Dec.1

ΔP: difference in inhibition diameter obtained during exposure period calculated as initial inhibition diameter minus final inhibition diameter; ΔA: difference in inhibition diameter obtained during the non-exposure period calculated as inhibition diameter at the beginning minus inhibition diameter at the end. ΔP<0: the strain became more sensitive to antibiotics during the exposure period; ΔP>0: it became more resistance to antibiotics during the exposure period; ΔA<0: the strain gained more resistance to antibiotics during the non period exposure with respect to the exposure period; ΔA>0: it became more sensitive to antibiotics during the non period exposure with respect to the exposure period. SXT/TSU: Cotrimoxazole; C: Chloramphenicol; CIP: Ciprofloxacin; Dec.1-fix: Decoction1 at a fixed concentration; Dec.1-Increase: Decoction1 at increasing concentration

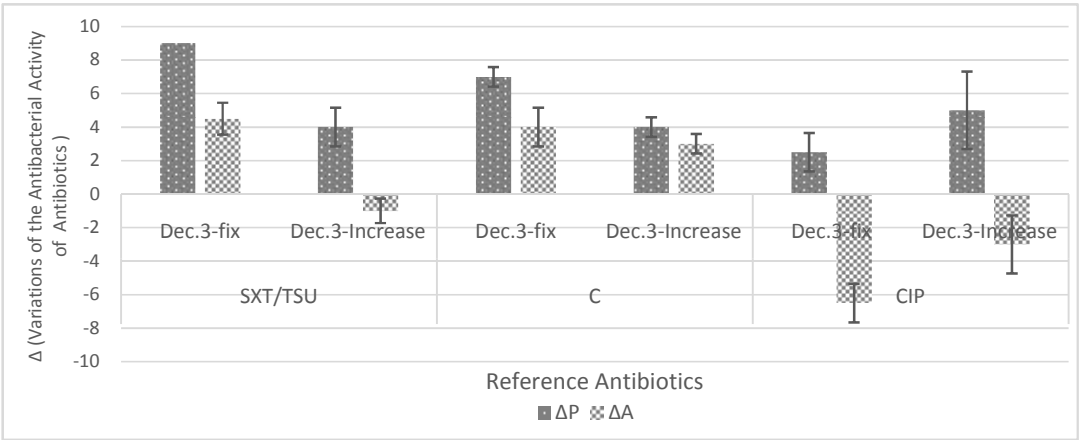

Fig. 3. Variations of the antibacterial activity of the reference antibiotics against *Salmonella Typhi* in repeated crops in the presence and absence of the fixed and increasing Dec.3

ΔP: difference in inhibition diameter obtained during exposure period calculated as initial inhibition diameter minus final inhibition diameter; ΔA: difference in inhibition diameter obtained during the non-exposure period calculated as inhibition diameter at the beginning minus inhibition diameter at the end. ΔP<0: the strain became more sensitive to antibiotics during the exposure period; ΔP>0: it became more resistance to antibiotics during the exposure period; ΔA<0: the strain gained more resistance to antibiotics during the non period exposure with respect to the exposure period; ΔA>0: it became more sensitive to antibiotics during the non period exposure with respect to the exposure period. SXT/TSU: Cotrimoxazole; C: Chloramphenicol; CIP: Ciprofloxacin; Dec.3-fix: Decoction3 at a fixed concentration; Dec.3-Increase: Decoction3 at increasing concentration

4. DISCUSSION

The low activity of plant extracts observed compared to antibiotics can be related to the presence of various molecules present in these plants. In fact, unlike antibiotics which are pure molecules, plant extracts are mixtures of active molecules plus other substances such as polysaccharides, polypeptides capable of binding to the active compounds and hide or decrease their activity [17,18].

Table 4. Mean diameters of *Salmonella* Typhi inhibition rings after repeated cultures in the presence and absence of Dec.1 at a fixed concentration and growing for all antibiotics

Days	Mean diameter of inhibition halo (mm)					
	SXT/TSU		C		CIP	
	Dec.1-fix	Dec.1-increase	Dec.1-fix	Dec.1- increase	Dec.1-fix	Dec.1- increase
1	26,00±1,04f	26,00±1,04g	29,00±0,76d	29,00±0,76f	36,50±1,25g	36,50±1,25g
9	13,00±0,00c	14,00±0,00c,d	25,00±1,00b	26,50±0,29d	26,00±0,58b,c	27,50±0,58e
15	14,00±0,58c	12,50±0,58b,c	22,00±1,73a	28,00±0,58d,e	25,00±1,15a,b	26,00±0,00b,c,d,e
23	10,00±2,00b	9,00±1,00a	22,00±0,00a	23,00±1,15a,b	25,00±1,15a,b	25,00±0,58a,b,c
29	14,00±0,58c	11,50±0,58a,b	24,00±0,29b	22,00±1,73a	23,00±0,00a	29,50±0,50f

The average diameters of the inhibited zones affected by the same letter in the same column are not significantly different at p <0.05. Each value represents the mean ± standard deviation of the mean diameter of the inhibition zones. SXT/TSU: Cotrimoxazole; C: Chloramphenicol; CIP: Ciprofloxacin; Dec.1-fix: Decoction1 at a fixed concentration; Dec.1-Increase: Decoction1 at increasing concentration

Table 5. Mean diameters of *Salmonella* Typhi inhibition rings after repeated cultures in the presence and absence of Dec.3 at fixed and increasing concentration for all antibiotics

Days	Mean diameter of inhibition halo (mm)					
	SXT/TSU		C		CIP	
	Dec.3-fix	Dec.3- increase	Dec.3-fix	Dec.3- increase	Dec.3-fix	Dec.3- increase
1	26,00±1,04j	26,00±1,04h	29,00±0,76f,g	29,00±0,76f,g	36,50±1,25i	36,50±1,25g
9	13,00±0,00e	15,00±3,06f	26,00±2,00d,e	27,50±2,31e,f	28,00±0,00e,f	30,00±1,53e
15	8,00±0,00a	13,50±1,15d,e	23,00±1,15b	25,50±1,15c,d	25,50±1,15c,d	23,00±0,00b
23	11,00±1,15b,c	10,50±0,87b,c	24,50±0,58b,c	21,00±1,15a	23,00±1,15b	24,00±0,58b
29	13,00±1,53d,e	13,00±1,00d,e	24,00±0,00b,c	25,50±0,58c,d	24,00±0,58b,c	25,00±0,00c

The average diameters of the inhibited zones affected by the same letter in the same column are not significantly different at p <0.05. Each value represents the mean ± standard deviation of the mean diameter of the inhibition zones. SXT/TSU: Cotrimoxazole; C: Chloramphenicol; CIP: Ciprofloxacin; Dec.3-fix: Decoction3 at a fixed concentration; Dec.3-Increase: Decoction3 at increasing concentration

Table 6. Mean diameters of *Staphylococcus aureus* inhibition rings after repeated cultures in the presence and absence of fixed and increasing concentration of *Chromolaena odorata* (CO) for all antibiotics

Days	Mean diameters of inhibition halo (mm)					
	SXT/TSU		ERY		GM	
	CO-fix	CO- increase	CO-fix	CO- increase	CO-fix	CO- increase
1	42,00±1,41g	42,00±0,70h	40,00±1,41j	40,00±1,41f	25,00±0,70d	25,00±1,41e
9	29,00±1,41d	32,00±1,41e,f	28,50±2,12b	30,00±0,00a	14,00±1,41a	14,00±0,00a
15	29,00±0,00d	29,50±0,70c,d	33,00±0,00g	34,50±2,12d,e	16,00±0,00b	18,50±0,70b
23	23,50±0,70a	24,50±1,41a	30,00±1,41d	34,50±0,70d,e	17,00±0,00b	21,50±2,12d
29	30,00±1,41d	28,50±1,41b,c	29,50±1,41c	31,50±1,41a,b	16,50±2,12b	19,50±2,12b,c

The average diameters of the inhibited zones affected by the same letter in the same column are not significantly different at p <0.05. Each value represents the mean ± standard deviation of the mean diameter of the inhibition zones. SXT/TSU: Cotrimoxazole; C: Chloramphenicol; Ery: Erythromycin; CO-fix: Chromolaena odorata at a fixed concentration; CO- increase: Chromolaena odorata at increasing concentration

Table 7. Mean diameter of *Staphylococcus aureus* inhibition rings after repeated cultures in the presence and absence of fixed and increasing *Erigeron floribundus* (EF) for all antibiotics

Days	Mean diameters of inhibition halo (mm)					
	SXT/TSU		ERY		GM	
	EF-fix	EF- increase	EF-fix	EF- increase	EF-fix	EF- increase
1	42,00±0,70h	42,00±0,70h	40,00±2,12g	40,00±1,41g	30,00±0,10f	25,00±0,70h
9	32,00±0,00e	24,00±1,41b	35,00±0,70e	34,50±2,12d,e	24,00±2,00c,d	12,50±0,70g
15	25,5,00±2,12a	27,00±1,41c,d	28,50±1,41a	29,00±0,70a,b	24,50±1,53d	15,50±0,70b
23	27,50±0,70b	28,50±0,70d,e	29,00±0,70a	31,50±1,41c	24,00±0,58c,d	22,50±0,70f,g
29	30,00±0,00d	42,00±0,70h	37,00±1,41f	40,00±1,41g	24,50±0,87d	25,00±0,70h

The average diameters of the inhibited zones affected by the same letter in the same column are not significantly different at p <0.05. Each value represents the mean ± standard deviation of the mean diameter of the inhibition zones. SXT/TSU: Cotrimoxazole; C: Chloramphenicol; Ery: Erythromycin; EF-fix: Erigeron floribundus at a fixed concentration; EF- increase: Erigeron floribundus at increasing concentration

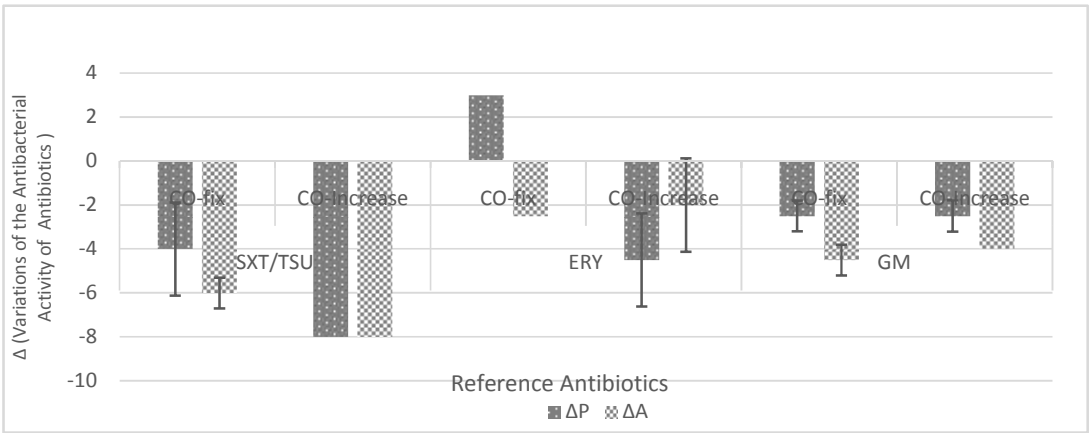

Fig. 4. Variations of the antibacterial activity of reference antibiotics against *Staphylococcus aureus* in the presence and absence of *Chromolaena Odorata* (CO) at a fixed and increasing concentration

ΔP: difference in inhibition diameter obtained during exposure period calculated as initial inhibition diameter minus final inhibition diameter; ΔA: difference in inhibition diameter obtained during the non-exposure period calculated as inhibition diameter at the beginning minus inhibition diameter at the end. ΔP<0: the strain became more sensitive to antibiotics during the exposure period; ΔP>0: it became more resistance to antibiotics during the exposure period; ΔA<0: the strain gained more resistance to antibiotics during the non period exposure with respect to the exposure period; ΔA>0: it became more sensitive to antibiotics during the non period exposure with respect to the exposure period. SXT/TSU: Cotrimoxazole; C: Chloramphenicol; Ery: Erythromycin; CO-fix: Chromolaena odorata at a fixed concentration; CO- increase: Chromolaena odorata at increasing concentration

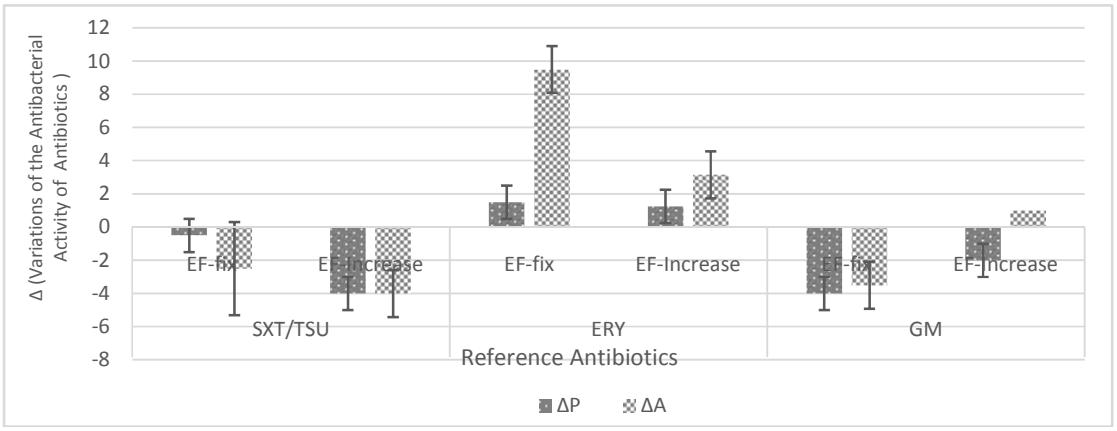

Fig. 5. Variations in antibacterial activity of reference antibiotics against *Staphylococcus aureus* in the presence and absence of *Erigeron floribundus* (EF) with fixed and increasing concentration

ΔP: difference in inhibition diameter obtained during exposure period calculated as initial inhibition diameter minus final inhibition diameter; ΔA: difference in inhibition diameter obtained during the non-exposure period calculated as inhibition diameter at the beginning minus inhibition diameter at the end. ΔP<0: the strain became more sensitive to antibiotics during the exposure period; ΔP>0: it became more resistance to antibiotics during the exposure period; ΔA<0: the strain gained more resistance to antibiotics during the non period exposure with respect to the exposure period; ΔA>0: it became more sensitive to antibiotics during the non period exposure with respect to the exposure period. SXT/TSU: Cotrimoxazole; C: Chloramphenicol; Ery: Erythromycin; EF-fix: Erigeron floribundus at a fixed concentration; EF- increase: Erigeron floribundus at increasing concentration

The resistance developed by *Salmonella* Typhi to the three antibiotics (Chloramphenicol, Ciprofloxacin, Cotrimoxazole) during its exposure to decoctions 1 and 3 and maintained during the non exposure period with most antibiotics may have been caused by a permanent adaptation, which instead of producing a metabolic burden as claimed by some authors [19,20] has

produced specific changes in bacterial metabolism that are beneficial for the growth of the bacteria [21,22,23].

During the exposure period of *Staphylococcus aureus* to *Chromoleana odorata* and *Erigeron floribundus* this strain has become more sensitive to most of the antibiotics (ΔP<0). This gain of sensitivity can be explained by the absence of an external membrane in *Staphylococcus aureus,* exposing its cell wall. Furthermore, Kosanić and Ranković [24] suggested that the cell wall structure and composition of bacteria could account for the different sensitivity to antimicrobial compounds. The lost sensitivity observed during non exposure period after exposure to *Chromoleana odorata* and *Erigeron floribundus* can be provoked by a phenotypic adaptation of microorganisms to environmental conditions [25].

The effect of plant extracts on the acquisition of resistance in *Salmonella* Typhi and *Staphylococcus aureus* could be related to the mechanism of action of each antibiotic and to the antibacterial activity of the phytomolecules contained in these plant extracts. According to Tenover [26] the antimicrobial compound interferes with the cell wall, the membrane, nucleic acid and enzymes. Chloramphenicol, Gentamicin and Erythromycin target ribosomes. But Chloramphenicol works by inhibiting the formation of peptide bonds by inhibition of peptidyl transferase while Gentamicin and Erythromycin act as inducing decoding error and inhibiting elongation of peptide chain respectively [27]. The three antibiotics interfere with protein synthesis, which is not the case with Cotrimoxazole, which interferes with the synthesis of folic acid and Ciprofloxacin, inhibiting nucleic acid synthesis. Furthermore, studies have shown that the plants contained in the decoctions as well as *Chromolaena odorata* and *Erigeron floribundus* contain secondary antibacterial metabolites such as tannins, saponins, flavonoids, quinones, phenols, beta-cyanins, cardioglycosides, coumarins, alkaloids and steroids [13,28]. Some of these metabolites contain aromatic forms in their structures as well as certain functional groups [29] found in the structure of the antibiotics tested. This can cause effects similar to those related to the use of antibiotics, thus explaining the acquisition of resistance of *Staphylococcus aureus* exposed to these plant extracts. Indeed, a study conducted by Mori et al. [30] has shown that metabolites containing aromatic forms inhibit nucleic acid synthesis just like the Ciprofloxacine.

Furthermore, no significant difference was observed between the strains exposed to fixed concentrations of the extracts and those exposed to increasing concentrations vis-a-vis antibiotics tested. This goes against the work of Michael van der Horst et al. [31] whose strains exposed to antibiotics at fixed sub-lethal concentrations exhibited behaviour different from those exposed to antibiotics at increasing concentrations. The acquisition of antibiotic resistance may therefore not be closely related to the increase in the concentration of an antimicrobial substance as long as it remains sub-lethal, but to the nature of the molecule to which the microorganism is exposed.

The results presented here indicate that antibiotics are not the only actors in the emergence and spread of resistant bacteria as claimed by several authors [5,32]. Because all strains exposed to plant extracts exhibited different behaviour from unexposed strains. Many of them became less sensitive to the antibiotics tested. This decrease in the activity of antibiotics translated by the decrease of their diameters of inhibition. However, the behaviour varies according to the plant and the antibiotic tested, thus highlighting the diversity of mechanisms involved in the acquisition of resistance described by several authors [31,33].

5. CONCLUSION

The exposure of *Salmonella* Typhi and *Staphylococcus aureus* to some of the extracts of plants traditionally used in Cameroon in the treatment of typhoid and skin diseases has shown a reduction in the sensitivity of these strains to the antibiotics prescribed in the fight against these infections. These results, therefore, show that the selective pressure use of plants in traditional treatments can, as in the case of the selective pressure use of antibiotics in clinical and agricultural practices, be the cause of emergence and the spread of antibiotic-resistant bacteria, even though some of this resistance is not permanent. These could explain the case of several therapeutic failures of the populations admitted in hospitals after a long uncontrolled use of medicinal plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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