

## Evaluation of antimicrobial efficacy of hydrazide-hydrazone derivatives against food borne pathogens”.

### **Abstract:**

Bacteria are a real public health problem because of their involvement in many diseases. Their resistance to antibiotics has become one of the most important problems in the fight against infectious diseases worldwide. The objective of this work was to evaluate the antibacterial activity of hydrazide-hydrazone derivatives against pathogenic strains isolated from food using the agar diffusion method in cups and the macro-dilution method in liquid medium.

In this work, a survey was conducted among food vendors on the four security corridors of the city of Daloa. A structured questionnaire was administered to 127 vendors and a total of 60 food samples were collected from the four corridors and analysed.

The results indicate that the majority of food sales at these security corridors are carried out by women (88.12%). The vendors were mostly adults (59.84%) aged between 15 and 35, with no formal education (50.39%). Microbiological analysis of the 2 types of food showed the presence of enterobacteria above the recommended thresholds. Concerning the antimicrobial effectiveness explored, four substances (AEV5, AB2, AB3 and AB5) revealed antibacterial activity. The MBC/MIC ratio of all these substances being less than 4 indicates that these synthesised substances all have bactericidal activity. As a result, substances derived from S-alkylation and Mannich bases can be used to combat bacterial infections.

**Keywords:** biological properties, hydrazide-hydrazone derivatives, food poisoning, bacterial strains, enterobacteria, food, Daloa

### **Introduction**

Food is the first consumer good used to satisfy the primary needs of every human being. The quality of this food is vital to the health of consumers (Fung *et al.*, 2018). Food is the main source of micro-organisms when it is not eaten in a healthy way. Every year, several million people suffer from food-borne illnesses (Kirk *et al.*, 2015 ;Fung *et al.*, 2018). These food-borne illnesses are generally known as food poisoning. They are diseases that are often infectious and accidental, contracted as a result of ingesting food contaminated with pathogenic bacteria. Such contamination generally results from inadequate food handling, preservation or cooking methods (Ferreira *et al.*, 2013). These infectious diseases are a global concern. The number of cases of food poisoning is constantly rising around the

world. Every year, there are more than 600 million cases of food poisoning in the world (OMS, 1996; Fleckenstein *et al.*, 2021). There have been numerous cases of food poisoning in Côte d'Ivoire. In May 2022, in the town of Bondoukou, in the east of the country, around a hundred people aged between 2 and 32 suffered from food poisoning after eating a dairy product sold in an ice cream parlour (Ministère de la santé, 2022). Food-borne diseases are therefore a major concern for governments (Bernard, 2014). Most are caused by bacteria such as *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp*, *Clostridium spp* and *Campylobacter spp* (Antunes *et al.*, 2020; Vieira *et al.*, 2021). Antibiotics are most commonly used to treat this condition. Unfortunately, the misuse of antibiotics and the horizontal transfer of resistance genes have led to the emergence of bacteria resistant to certain antibiotics. Among the concerns raised by this issue, bacterial resistance to antibiotics has been recognised by the World Health Organisation as one of the most serious threats to global health, food security and development. Its spread could change medicine in the coming years. Antibiotic resistance is all the more worrying given that the use of antibiotics is often uncontrolled (Pons *et al.*, 2014). Faced with the constant threat of infectious agents, it is essential to find new antimicrobial molecules. Hydrazide-hydrazone derivatives could offer an alternative for better management of cases of food poisoning (Popiolek, 2017). Indeed, these substances are a potential source for strengthening the fight against bacteria that are increasingly resistant to antibiotics. The main objective of this work is to evaluate the antibacterial activity of synthesised hydrazide-hydrazone derivatives on strains isolated from street foods sold in the commune of Daloa.

The specific objectives set were: research the presence of germs responsible for food poisoning in foods sold on the four security corridors of the city of Daloa, highlight the antibacterial effect of hydrazide-hydrazone derivatives on pathogenic strains and determine the antibacterial parameters (MIC, CMB) of these substances.

## **Materials and methods**

### **Materials**

#### **Presentation of the study area**

Daloa is the capital of the Haut Sassandra region. Its position at the crossroads of the roads linking respectively the West and the North-West to the rest of the country, on the one hand, to neighboring Guinea and Liberia to the West, to Mali and Burkina-Faso in the North, on the other hand, gives it the status of a crossroads city (Bolou, 2021).

### **Biological material**

#### **Bacterial strains**

The microbial support consists of:

-Bacterial strains isolated and identified from foodstuffs sold on the various corridors. A total of six (6) strains were selected. These were four (4) strains of *E.coli* coded *E.coli A1*, *E.coli A2*, *E.coli A3*, *E.coli A4* and two (2) strains of Enterobacteriaceae coded *Ent P1*, *Ent P2*.

-A strain of *E. coli* resistant to third generation cephalosporins (C3G ®) from the bio-collection of the National Reference Center for Antibiotics of the Institute Pasteur (Abidjan, Côte d'Ivoire) used for quality control of the tests.

### **Other materials**

-**Hydrazide-hydrazone derivatives and mannich bases:** 4 codified substances (AEV3, AEV5, AEV6, AEV8) derived from benzimidazole S-alkylation and 5 codified mannich base substances (AB2, AB3, AB4, AB5 and AB7) synthesised and described as new bioactive molecules were provided by the Laboratory of Constitution and Reaction of Matter, Department of Sciences of Structures of Matter and Technology, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire for this study.

### **Methods**

A socio-demographic survey of 127 sellers on the different security corridors (Man, Issia, Bouaflé and Vavoua) was carried out over a period of one month (from March 31 to April 30, 2022). The survey sought information on the age, gender and level of education of the vendors. It also looked at the hygienic state of food preparation, conditions of sale, conditions of food preparation and storage, and the level of knowledge about hygiene practices among male and female vendors.

### **Sample collection and transport**

Food samples were collected from four security corridors in the city of Daloa, namely the Issia corridor (South), the Vavoua corridor (North-West), the Bouaflé corridor (East) and the Man corridor (West). A total of 30 solid food samples and 30 liquid food samples were collected from vendors. The samples were packaged in labelled sterile jars, then placed in a cooler containing an ice accumulator and transported to the laboratory.

**Isolation and identification of bacteria from the food samples** The preparation of the mother suspension of samples and decimal dilutions was carried out in accordance with standard NF EN ISO 6887-1, 2017, which defines the general rules for the preparation of the mother suspension and dilutions. Culture media were prepared and used in accordance with the manufacturers' instructions. Two types of inoculation were carried out. Surface

inoculation for the detection of *E.coli* on Rapid*E.coli* medium (Bio-Rad, France) and inoculation in the mass with VRBL (Biokar, France) and VRBG (Biokar, France) media for the detection of Enterobacteriaceae. Cultures were incubated at 37°C for 24 hours. Colonies were observed and counted, identified and preserved in 10% glycerol.

#### **Preparation of solutions of hydrazide-hydrazone derivatives and mannich bases**

Stock solutions of the chemical substances were prepared at a concentration of 16 µg/mL. The substances in powder form were first diluted in ethanol at 96°C.

#### **Sensitivity testing and determination of inhibition diameters**

The Mueller-Hinton agar diffusion technique described by Bssaibis *et al.* in 2009 was used in this study. This technique involved obtaining log phase colonies transplanted onto nutrient agar using the streak method. A bacterial inoculum estimated at 10<sup>6</sup> CFU/mL adjusted by opacity to 0.5 McFarland was prepared in physiological water (NaCl 0.9%). of this inoculum was then swabbed onto Muller-Hinton agar (GMH, Bio-Rad, France). Cups were made by sinking the large end of a sterile Pasteur pipette into the inoculated Muller-Hinton agar and left to dry. These plates were then filled with 50 µL of the chemical preparation and incubated at 37°C for 24 hours. The inhibition diameters were then determined by caliper measurement. The effectiveness of the substances was assessed according to the criteria of Ponce *et al.* (2003). Thus, a substance is said to be ineffective if the inhibition diameter is less than 8 mm, effective if the diameter is between 9 and 14 mm, very effective for a diameter between 15 and 19 mm and extremely effective if the diameter is greater than 20 mm.

#### **Determination of antibacterial parameters Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

The determination of the antibacterial parameters was made for substances with an inhibition diameter greater than 9 mm according to the macro-dilution method in a liquid medium described by (Koné *et al.*, 2004).

#### **Preparation of the bacterial inoculum**

A bacterial inoculum was prepared from a fresh colony in 10 mL of sterile MH broth and incubated at 37°C for 3 hours. Then 0.3 mL of this pre-culture was diluted in 10 mL of sterile MH broth and homogenised.

#### **Preparation of test concentration ranges**

A concentration range for the test substances was obtained using the double dilution method. A stock solution with a concentration of 1000 µg/mL (2mg in 2ml of 70° ethanol) was prepared. A series of 2-fold dilutions were made from this solution to obtain

concentration ranges from 500 µg/mL to 7.81 µg/mL.) Then, in sterile haemolysis tubes, 1 mL of each concentration range of the substance was brought into contact with 1 mL of bacterial inoculum. In a growth control tube, 1 mL of sterile distilled water was added to the inoculum, while 2 mL of sterile Muller-Hinton broth (MHB) was added to the sterility control. The preparations were incubated for 24 hours at 37°C. Afterwards, observation with the naked eye was carried out and the lowest concentration for which no bacterial growth was observed with the naked eye corresponded to the MIC.

The MBC is determined by transferring a sample of the tubes showing no growth to a fresh medium devoid of the molecules to be tested. This involved diluting the starting inoculum from  $10^{-1}$  to  $10^{-4}$  and inoculating these different dilutions using a calibrated 2 µL loop in 5 cm long streaks on Muller-Hinton agar, then incubating at 37°C for 24 hours (preparation A). After reading the MICs, the contents of tubes in which there was no visible growth were used to inoculate the GMH on 5 cm long streaks (preparation B). The MIC was determined by comparing the bacterial growth of preparations A and B. The smallest concentration in the tube with less than 0.01% viable bacteria compared to the initial inoculum is the MBC.

The CMB/CMI report made it possible to clarify the mode of action of the chemical substance.

According to Nayaka *et al*, (2021), the substance is bactericidal when its CMB is equal to its MIC or if the CMB/MIC ratio is less than or equal to 4. It is said to be bacteriostatic when its CMB is greater than its MIC or if the CMB/MIC ratio is higher than 4. When this ratio is equal to 32, the strain is said to be tolerant.

### **Statistical analysis**

All tests were carried out in triplicate and the numerical values obtained are expressed as the arithmetic mean plus the corresponding statistical standard deviation. STATISTICA 7.1 software was used for these analyses. Analyses of variance (ANOVA) based on multiple DUNCAN tests with a significance level of 5% were performed in order to compare the variables measured on different strains with the substances tested. Means, standard deviations and graphs were produced using EXCEL 2016.

## Results and discussion

### Results

#### Socio-demographic profile of the people surveyed and their level of knowledge about hygiene

The results of the survey of food vendors from the four corridors are shown in table I. In terms of gender, the results show that 11.82% of vendors are men and 88.12% are women. In terms of level of education, vendors are poorly educated. Only 7.08% had completed higher education, 27.55% had attended secondary school, 14.96% had completed primary school, and 50.39% had no education at all. In terms of age, 15.74% of sellers are under 14, 59.84% are between 15 and 35, and 24.42% are over 36. In terms of knowledge of hygiene, the results show that 54.5% of respondents have some knowledge of hygiene and 45.5% have no knowledge of hygiene.

**Table I: Socio-demographic profiles of vendors on the various corridors**

Socio-demographic profiles		Staff	Frequency (%)
Sex	Male	15	11.82
	Female	112	88.12
Age	0-14 years	20	15.74
	15-35 years	76	59.84
	35 years and over	31	24.42
Level of study	No level of education	64	50.39
	Primary	19	14.96
	Secondary	35	27.55
	Superior	9	7.08
Level of hygiene knowledge	Yes	69	54.5
	No	58	45.5

## Antibacterial effect of chemicals derived from benzimidazole S-alkylation on bacterial strains

Table II below shows the inhibition diameters of the four benzimidazole S-alkylation substances (AEV3, AEV5, AEV6 and AEV8) on strains isolated from food. It should be noted that the four substances were ineffective on the 4 *E.coli* strains isolated because they had an inhibition diameter of less than 9 mm. The substances AEV5 and AEV8 were highly effective and effective respectively only on the *Ent P2* strain with diameters of 17.3 mm and 10 mm. On the *Ent P2* strain, all the substances in this chemical group were found to have no antibacterial effect.

**Table II: Diameters (mean  $\pm$  SD) of the zones of inhibition of chemical substances derived from benzimidazole S-alkylation on bacterial strains**

Bacterial strains	Inhibition diameters (mm)			
	S-alkylation benzimidazole			
	AEV3	AEV5	AEV6	AEV8
<i>E. coli</i> A1	0,8 $\pm$ 0,1 <sup>b</sup>	5,3 $\pm$ 0,6 <sup>d</sup>	0,6 $\pm$ 0,2 <sup>b</sup>	0,5 $\pm$ 0,0 <sup>b</sup>
<i>E. coli</i> A2	0,7 $\pm$ 0,2 <sup>a</sup>	0,8 $\pm$ 0,1 <sup>a</sup>	0,7 $\pm$ 0,2 <sup>a</sup>	0,7 $\pm$ 0,0 <sup>a</sup>
<i>E. coli</i> A3	0,7 $\pm$ 0,1 <sup>b</sup>	7,2 $\pm$ 0,6 <sup>a</sup>	0,7 $\pm$ 0,2 <sup>bs</sup>	0,5 $\pm$ 0,0 <sup>b</sup>
<i>E. coli</i> A4	0,7 $\pm$ 0,2 <sup>a</sup>	0,7 $\pm$ 0,1 <sup>a</sup>	0,6 $\pm$ 0,2 <sup>a</sup>	0,9 $\pm$ 0,0 <sup>a</sup>
<i>Ent P1</i>	0,6 $\pm$ 0,2 <sup>c</sup>	17,3 $\pm$ 0,6 <sup>a</sup>	0,8 $\pm$ 0,1 <sup>c</sup>	10 $\pm$ 0,0 <sup>b</sup>
<i>Ent P2</i>	0,8 $\pm$ 0,1 <sup>a</sup>	0,6 $\pm$ 0,2 <sup>a</sup>	0,7 $\pm$ 0,2 <sup>a</sup>	0,5 $\pm$ 0,1 <sup>a</sup>

NB: Values with the same letter in superscript are statistically identical, at the 5% threshold.

## Effect of various Mannich-based chemicals on the *in vitro* growth of bacterial strains

The results of the sensitivity of bacterial strains to Mannich-based substances are presented in Table III. All the substances were ineffective on the *Ent P2* strain. No zone of inhibition was observed. The results also show that the substances AB2, AB3, AB4 and AB7 are extremely effective on the *E. coli* A2 strain with diameters between 21 and 27 mm and similarly for AB2 and AB3 on the *E. coli* A1 strain with diameters of 21.0  $\pm$  0.0 and 23.0  $\pm$  0.0 respectively. The largest inhibition zone diameters were observed on *E. coli* A2 (27.0 $\pm$ 0 mm) for AB2 (Table III).

**Table III: Diameters (mean  $\pm$  SD) of the zones of inhibition of Mannich-based substances on bacterial strains**

Bacterial strains	Inhibition diameters (mm)				
	Mannich-based				
	AB <sub>2</sub>	AB <sub>3</sub>	AB <sub>4</sub>	AB <sub>5</sub>	AB <sub>7</sub>
<i>E. coli</i> A1	21,2 $\pm$ 0,8 <sup>b</sup>	23,0 $\pm$ 0,0 <sup>a</sup>	—	18,3 $\pm$ 0,5 <sup>c</sup>	13,0 $\pm$ 1,6 <sup>d</sup>
<i>E. coli</i> A2	27,9 $\pm$ 1,4 <sup>a</sup>	24,5 $\pm$ 2,5 <sup>b</sup>	21,2 $\pm$ 0,8 <sup>c</sup>	16,3 $\pm$ 0,2 <sup>d</sup>	22,6 $\pm$ 1,4 <sup>bc</sup>
<i>E. coli</i> A3	17,2 $\pm$ 0,7 <sup>b</sup>	17,0 $\pm$ 0,2 <sup>b</sup>	12,3 $\pm$ 0,7 <sup>c</sup>	19,0 $\pm$ 0,0 <sup>a</sup>	—
<i>Ent P1</i>	17,0 $\pm$ 1,3 <sup>a</sup>	15,3 $\pm$ 0,7 <sup>a</sup>	14,0 $\pm$ 0,8 <sup>a</sup>	19,0 $\pm$ 1,2 <sup>a</sup>	10,0 $\pm$ 0,8 <sup>a</sup>
<i>Ent P2</i>	—	—	—	—	—

NB: Values with the same letter in superscript are statistically identical, at the 5% threshold.

#### Antibacterial parameters of synthesised chemicals

Table IV presents the results of the determination of antibacterial parameters (MIC, MBC and MBC/MIC) of the substances that showed an inhibitory action on the bacterial strains by their inhibition diameter. These were AEV5 and AEV8 on the *Ent P1* strain, AB2 on *E. coli* A2, AB3 on *E.coli* A1 and AB5 on *Ent P1*. The MBC/MIC ratio for all the substances was less than 4. The substances all have bactericidal power on the bacterial strains used.

**Table IV: Antibacterial parameters of benzimidazole S-alkylation derivatives and Mannich bases**

	S-alkylation benzimidazole		Mannich-based		
	AEV5	AEV8	AB2	AB3	AB5
	<b>Bacterial strains</b>				
	<i>Ent P1</i>	<i>Ent P1</i>	<i>E.coli</i> A2	<i>E.coli</i> A1	<i>Ent P1</i>
MIC ( $\mu$ g/ mL)	31,25	31,25	125	62,5	62,5
MBC ( $\mu$ g/ mL)	62,5	62,5	250	62,5	125
MBC/MIC	2	2	2	1	2
Effect	Bactericidal	Bactericidal	Bactericidal	Bactericidal	Bactericidal

## Discussion

The aim of this work was to evaluate the antibacterial activity of the chemical substances synthesised on bacterial strains responsible for food poisoning. The strains were isolated from food sold on the various security corridors in the town of Daloa. The socio-demographic survey on the sale of food at these various corridors revealed that this activity is mainly carried out by women (88.12%), compared with 11.82% for men. Vendors are generally very young, with 59% aged between 15 and 35, and the vast majority have no formal education. These same observations have been made by Khomotso and Chelule (2020) and also by Attien *et al* (2021), in fact the commercial activity of street food in Africa is mostly carried out by women and especially that are linked to catering. Actually, according to Rahman *et al* (2016), the predominance of women in the street food sector could be due to the fact that women are the main breadwinners in the majority of households. The results of the microbiological analysis of the various foodstuffs revealed the presence of microorganisms such as: enterobacteria, faecal coliforms and *E. coli*. These germs have also been found in street foods in the work of certain authors such as Mosupye and Von Holy (2000), Attien *et al*, (2022) and Zébré *et al*, (2022). The presence of these germs in these foods is thought to be due to handling by the vendors. These germs are indicators of poor hygiene. They are evidence of faecal contamination (Koffi-Nevry *et al.*, 2012). Hygiene conditions and the practices of food handlers in sales outlets are generally inadequate (Attien *et al.*, 2022; Zébré *et al.*, 2022). A lack of understanding of the concept and importance of hygiene is thought to be behind the high levels of contamination in the various samples analysed (Cardinale *et al.*, 2005; Attien *et al.*, 2021). Consumer health is therefore becoming a global concern. In order to combat food-borne illnesses effectively in the face of the growing threat of bacteria resistant to conventional antibiotics, new chemical molecules derived from benzimidazole S-alkylation and Mannich bases have been synthesised and tested on bacteria isolated from food. The results obtained with the benzimidazole S-alkylation substances show that only the Ent P1 strain is sensitive to the AEV5 and AEV8 substances. This observation is less surprising given that enterobacteria are highly resistant to antimicrobials (Iredell *et al.*, 2016; Li *et al.*, 2021). According to Zniber *et al*, (2009), the lack of effect could be explained by taking into account the structure of the molecule tested (the size of the molecules) as well as the physiology and genetic potential of the target cell. But according to Iredell *et al*, (2016), Enterobacteriaceae resistance to antibiotics, particularly  $\beta$ -lactam antibiotics, is increasingly dominated by the mobilisation of single continuously expressed genes that code for effective drug-modifying enzymes. As for Mannich-based substances, they have almost all shown

antibacterial activity on all strains with the exception of the *Ent P2* strain. The antibacterial activity of these substances has been demonstrated in several previous studies. Indeed, this family of substances is effective on both Gram+ and Gram- bacteria (Gheorghe, 2015). Determination of the antibacterial parameters shows that the substances have an antibacterial effect. The MBC/MIC ratio is less than 4. These substances could therefore be used as antibacterial agents.

## Conclusion

This work made it possible to demonstrate the effectiveness and *in vitro* antibacterial properties of derivatives resulting from S-alkylation and Mannich-based substances on six bacterial strains (four (4) strains of *E. coli* and two (2) strains of enterobacteria) isolated from foods. The MICs obtained ranged between 31.25 and 125 µg/mL. As for the CMBs, they were between 31.25 and 250 µg/ML. CMB/MIC ratios indicated chemicals with bactericidal activity. These results made it possible to conclude that some of these synthesized chemical compounds have antibacterial properties. Thus, these molecules could contribute to the fight against bacterial infections.

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