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Evaluation of the Antibacterial Potential of *Bauhinia rufescens* L. and *Euphorbia hirta* L. on Pathogenic Enterobacteria Involved in Gastroenteritis

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A B S T R A C T

This study allowed us to discover the antibacterial potential of hydroethanolic extracts of *Bauhinia rufescens* L. and *Euphorbia hirta* L. on pathogenic enterobacteria. These two plants from the traditional Chadian pharmacopoeia are used in the treatment of gastroenteritis in children and adults. The results obtained justify their use.

Introduction

Diarrhoeal diseases are the third most deadly infection in the world, causing the death of about 2.5 million people a year, including nearly 2 million children under the age of five (3). In the least developed countries, they are the second leading cause of death. Among the agents involved in these diseases, bacteria of the genera *Salmonella* and *Shigella* often occupy a prominent place both in terms of their frequency and the severity of the diseases they cause (1). Thus, significant differences in mortality are observed between developed and developing countries, especially in the first year of life, with an average of 3.9 deaths per 100,000 in the United States compared to 800 to 5,000 per 100,000 (4). Indeed, the WHO estimates that 1.3 billion episodes of diarrhea occur each year in children under five years of age. Mortality associated with gastroenteritis has been estimated at 3 to 5 million cases per year, the majority of which occur in developing countries (5).

In Chad, approximately 19,000 people, including 15,900 children under age 5, die each year from diarrhea (22). Today, the management of infectious gastroenteritis is becoming more complicated due to the emergence of multi-resistant strains. The increase in the resistance of microorganisms to

the antimicrobial agents used is due to the abusive and inappropriate use of antibiotics. Diseases caused by microorganisms are increasingly difficult to treat with existing drugs (20). Thus, the search for new avenues, including the use of plants, is essential, but in order for this traditional medicine to be effective, it must be scientifically validated by further research.

Through this study, we want to understand whether the use of these medicinal plants in Chad for gastroenteritis is justified. Indeed, the evaluation of hydroethanolic extracts of *Euphorbia hirta* L. and *Bauhinia rufescens* L. was done on the pathogenic germs involved and those of reference (*Escherichia coli*, *Salmonella enteritidis*, *Shigella dysenteriae Salmonella typhi* ATCC 14028, *Escherichia coli* ATCC 25922). The general objective is to evaluate the antibacterial activity of hydroethanol extracts of *Euphorbia hirta* L. and *Bauhinia rufescens* L.de on pathogenic bacteria involved in infectious gastroenteritis.

Materials and Methods

Plant materials

The leaves of *Bauhinia rufescens* and the whole plant of *Euphorbia hirta* were harvested in September 2020 25 km from the city of Ndjamena (Chad) in a village called Marra. The harvested plant material was authenticated at the herbarium of the Botany Department of the Faculty of Exact and Applied Sciences of Farcha of the University of Ndjamena (Chad).

Preparation of extracts

The leaves of *Bauhinia rufescens* and the whole plant of *Euphorbia hirta* were dried at room temperature away from the sun and dust, then crushed in a clean mortar before being reduced to a fine powder using an electric mill. 500 g of *Bauhinia rufescens* powder and 300 g of *Euphorbia hirta* powder were macerated in an ethanol/water mixture (70/30). The resulting mixture was incubated for 48 hours at laboratory temperature and frequently agitated. The macerate was then successively filtered with absorbent cotton before being filtered on Whatman filter paper N° 1 under vacuum pump. The solvent was evaporated with the Rotavapor and the total hydroethanol extracts obtained were used to prepare solutions with a concentration of 100 mg/ml which were sterilized by vacuum filtration on 0.45 µm millipore membrane. The recovered extracts were stored at 4°C in the refrigerator prior to testing.

Microbial strains

The microbial strains used consist of pathogenic bacteria isolated and identified in the medical environment at the Bacteriology Laboratory of the Polyclinelle Wossinu-Gbogbode Lomé and reference strains from the Microbiology and Food Quality Control Laboratory (LAMICODA).

Preparation of the microbial suspension

The strains were transplanted on nutrient agar to have young 24-hour bacterial colonies.

Microdilution technique

Microdilution in liquid medium is the reference method for the determination of MIC (Minimum Inhibitory Concentration). It consists in inoculating with bacterial strains a range of wells containing hydroethanol extracts to be tested at increasing concentrations. The MIC corresponds to the first dilution for which no bacterial growth is visible to the naked eye after 18 hours of incubation. The handling is done in a microtiter plate. The culture medium is a Müller Hinton broth. Different concentrations

are prepared with dilutions (of gradient 2) starting from a concentration of 100 mg / ml of each extract. 100 µl of the bacterial suspension are distributed in test wells of the microplate containing the extracts. The test wells consist of: Broth alone, Broth + bacterial suspension and Broth + gentamycin. The plates are then incubated at 37 °C for 24 hours. After incubation, possible growth is revealed by the presence of a cloudiness at the bottom of the well. The MIC is defined as the minimum concentration of extract for which no growth visible to the naked eye is observed. Cups that have shown no visible microbial growth from the MIC and the next well diluted at ½ are re-isolated on nutrient agar. Seeding is done by spreading on the surface of the agar. After 24 h incubation in an oven at 37 °C, the culture media are evaluated for (BMC). Thus, the action of an extract will be considered as bactericidal if the ratio CMB/CMI is equal to 1. The action is said to be bacteriostatic if the CMB/CMI ratio is greater than 1 (12).

Data processing and analysis

The statistical analysis was performed on Excel

Results and Discussion

Inhibitory activities of the various hydroethanol extracts at 50 mg/ml

Table 1 and 2 show respectively the effects of hydroethenolic extracts of *Bauhinia rufescens* and *Euphorbia hirta* on microbial growth. It was found that at a concentration of 50 mg/ml, the hydroethanol extracts from the leaves of *Bauhinia rufescens* L. and the whole plant of *Euphorbia hirta* L. totally inhibited the in vitro growth of all bacterial strains tested (see Tables 1 and 2). At a concentration of 25 mg/ml, it was observed that the hydroethanol extract of *Bauhina rufescens* totally inhibited the growth of E. coli ATCC 25922 and *Escherichia coli* (Table 1) while the hydroalcoholic extract of *Euphorbia hirta* at 25 mg/ml inhibited 100% the growth of *E. coli* ATCC 25922, *Salmonella typhi* ATCC 14028 and *Shigella dysenteriae* (Table 2).

It should be noted that evaluations of the antimicrobial activities of these plants have already been carried out on hydroethanol extracts. The plant powders were macerated in a mixture (ethanol and water in a 70/30 ratio). This extraction method has the advantage of extracting a large quantity of secondary metabolites. The results of our work indicate that the hydroethanol extracts from the leaves of *Bauhinia rufescens* L. and the whole plant of *Euphorbia hirta* L. inhibited the growth of pathogenic Enterobacteriaceae 100% at a concentration of 50 mg/ml.

Sensitivity of *Salmonella typhi* ATCC 14028 and *Salmonella enteritidis*

Salmonella typhi is responsible for most gastroenteritis and especially still in low income countries. This bacterium was sensitive with a MIC of 50 mg/ ml with hydroethenolic extract of *Bauhinia rufescens* but with *Euphorbia hirta* extract, the bacterium was even more sensitive with a MIC of 12.5 mg/ml. These results are in line with studies carried out in India, Thailand and Northern Sudan on the sensitivity of *Salmonella typhi* to ethanolic and aqueous extract of *Euphorbia hirta* with MIC and MBCs that vary according to the part of the plant used, the nature of the solvent and also the technique used *Salmonella typhi* versus the ethanolic and aqueous extract of *Euphorbia hirta* with MIC and MBCs that vary according to the part of the plant used, the nature of the solvent and also the technique used (2, 7 ; 18; 17 ; 6;16) (Table 3 and 4).

Table.1 Effect of hydroethanol extract of *Bauhinia rufescens* on microbial growth

Bacterial strains	Concentration of hydroethanol extract of *Bauhinia rufescens*	
	50 mg/ml	25 mg/ml
***E . coli* ATCC 25922.**	-	-
Escherichia coli	-	-
***Salmonella typhi* ATCC 14028**	-	+
Salmonella enteritis	-	+
Shigella dysenteriae	-	+

+ = microbial growth
-= absence of microbial growth

Table.2 Effect of hydroethanol extract of *Euphorbia hirta* on microbial growth

Bacterial strains	Concentration of hydroethanol extract of *Euphorbia hirta*	
	50 mg/ml	25 mg/ml
***E . coli* ATCC 25922.**	-	-
Escherichia coli	-	+
***Salmonella typhi* ATCC 14028**	-	-
Salmonella enteritis	-	+
Shigella dysenteriae	-	-

+ = microbial growth
-= absence of microbial growth

Table.3 Evaluation of Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentrations (MBC) of the different extracts and their possible effects of *Bauhinia rufescens*

Bacterial strains	Concentration of hydroethanol extract of *Bauhinia rufescens*			
	CMI (mg/ml)	CMB (mg/ml)	CMB/CMI	Effet
***E . coli* ATCC 25922.**	12,5	25	2	Bacteriostatic
Echerichia coli	12,5	25	2	Bacteriostatic
***Salmonella typhi* ATCC 14028**	50	50	1	Bactericidal
Salmonella enteritis	50	50	1	Bactericidal
Shigella dysenteriae	50	50	1	Bactericidal

Table.4 Evaluation of Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentrations (MBC) of the different extracts and their possible effects of *Euphorbia hirta*

Bacterial strains	Concentration of hydroethanol extract of *Euphorbia hirta*			
	CMI (mg/ml)	CMB (mg/ml)	CMB/CMI	Effet
***E . coli* ATCC 25922.**	12,5	25	2	Bacteriostatic
Echerichia coli	50	50	1	Bactericidal
***Salmonella typhi* ATCC 14028**	12,5	25	2	Bacteriostatic
Salmonella enteritis	50	50	1	Bactericidal
Shigella dysenteriae	12,5	25	2	Bacteriostatic

Sensitivity of *Shigella dysenteriae*

Shigella dysenteriae was susceptible to *Bauhinia rufescens* with a MIC of 50 mg/ ml. The observed MIF result of 50 mg/ ml is different from those of Husain *et al.,* (8) in Nigeria who found MIFs of 12.5 mg/ml with the hexane extract and 25 mg/l with the aqueous extract. The efficacy of an extract depends on the solvent used and the extraction method. The hydroethanolic extract of *Euphorbia hirta* allowed us to have even more interesting results with a MIC of 25 mg/ml. Similar results were obtained in a study carried out in Nigeria (7). However, these results are contrary to those of Perumala *et al.*, (2012) in Thailand which were obtained with MIC of 0.5 mg/ ml with ethanolic extract in Thailand (21). (21) The discrepancies recorded with respect to the results of some researchers are partly related to the methods used and the isolates tested. Moreover, the origin of the germs tested (isolation site) can determine their behavior towards the extracts as observed with classical antibiotics.

Sensitivity of *Escherichia coli*

The reference strain (*E. coli* ATCC 25922) was sensitive with a MIC of 12.5 mg/ml both with the hydroethanolic extract of *Bauhina rufescens* and also with that of *Euphorbia hirta*. This finding was made in India (2). For hospital strains the MIC varies from 25 mg/ml to 50 mg/ml depending on the plant and the part used. Many studies carried out throughout the world have confirmed the sensitivity of *Escherichia coli* to extracts of *Bauhinia rufensis* and *Euphorbia hirta* (7, 21, 18, 19, 14, 9).

This work on the evaluation of the antibacterial activities of the hydroethenolic extracts of *Bauhina rufescens* and *Euphorbia hirta*, allowed to highlight the antibacterial potential of the total extracts of these two plants of the Chadian pharmacopoeia used in the traditional treatment of infectious gastroenteritis. These results can be exploited for the purification of the active ingredient(s) of the plants used and the preparation of improved forms of effective remedies based on these two plants whose use in traditional therapy is justified.

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