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Abstract

Diarrheal and infectious diseases are the leading causes of morbidity and mortality worldwide. With the emergence of multidrug resistant bacteria, the treatment of these diseases is problematic. This situation stresses the need to search for alternative antibacterial sources notably medicinal plants. The present study aimed to assess the antibacterial activity of three leafy vegetables commonly used to treat diarrheal diseases. Therefore, aqueous and hydro-ethanolic extracts of the leaves of Crateva adansonii, Vernonia amygdalina and Sesamum radiatum were prepared and tested against 12 clinical isolates and 4 reference strains. The antibacterial activities were measured using a microdilution method to determine the Minimal Inhibitory Concentration, Minimal Bactericidal Concentration and the antibiotic power. Susceptibility tests of the extracts were carried out using well diffusion method.

The hydro-ethanolic extracts of the leaves of S. radiatum and C. adansonii and the aqueous extract of S. radiatum had an effective antibacterial effect on the clinical and reference strains isolates. This was supported by Minimal Inhibitory Concentration values ranging between 0.3125 and 5 mg/ml, Minimal Bactericidal Concentration between 0.3125 and 10 mg/ml, a bactericidal power on S. aureus ATCC 25923, Pseudomonas mirabilis A 24974 (reference strains); Staphylococcus aureus, Vibrio cholera and Salmonella Typhi (clinical isolates). For the active extracts, the L Q K L E L W L R Q] R Q H G L D P H W H U V Z H U H V L J Q L ¿ F D Q W O \ G L I I H U H Q W S S. D Q G J U H D radiatum showed the best antibacterial effects on the clinical and reference strains isolates, although reference strains and most of the clinical isolates still more sensitive to antibiotics.

Keywords: Antibacterial activity; Well di usion; S. radiatum; C. adansonii; V. amygdalina

Introduction

In developing countries, infectious diarrhoeas represent a serious public health challenge because of their frequency and gravity. ey are responsible for over 17 million deaths every year across the world with more than half of this burden occurring in Africa [1]. is problem a ects all age groups but parti* [(a)- but gior tpent 4 (b)7-3 (y)7 (b)-7(er 17 mf i)11.nsipen11.uap(e)d g-6 (hsf i)11. r (d g)-5

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ey were constituted of 12 clinical strains isolated from diarrheal faeces samples:Escherichia coli, Staphylococcus aureus, Salmonella Typhi, Salmonella choleraesius, Shigella spp, Shigella exneri, Vibrio cholerae, Citrobacter spp, Proteus mirabilis, Proteus vulgaris, Klebsiella pneumoniae, Klebsiella rhinoscleromatis and 4 reference strains (Escherichia coli ATCC 25922,Staphylococcus aureus ATCC 25923,Pseudomonas aeruginosa ATCC 27853 and Proteus mirabilis A 24974).

Methods

a) Preparation of the extracts:100 g of the vegetables' powder was boiled for 30 minutes in 1000 ml of distilled water. e brew was cooled and ltered once using absorbent cotton and once with Whatman N°1 lters paper. e obtained ltrate was then dried at 50°C in an incubator and served as the aqueous extract (AE).

To make the hydro-ethanolic extract (HE), 100 g of powder was agitated for 72 hours in 1000 ml of 70% diluted ethanol. It was then ltered once on absorbent cotton and once on Whatman N°1 lters paper. e hydro-ethanolic phase was lastly dried at 50°C.

e aqueous and hydro-ethanolic extracts of the vegetables were reconstituted in distilled water at a concentration of 20 mg/ml. e prepared solutions were sterilized by ltration using lter-syringes on 0.22 µm Millipore membrane. e sterility of the stock solutions was veri ed by culturing aliquots of each solution on Mueller Hinton II media and incubated at 37°C for 24 to 48 hours.

b) Preparation of bacterial suspensions: A pure 24 hours colony of each bacteria strain was

emulsi ed in 5 ml of physiological water and adjusted to McFarland 0.5 standard.

c) Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC): is was performed using 96 well plates method described by Houngbeme et al. [14]. 100 µl of the stock solution of each extract was added to 100 µl of the di erent bacterial suspensions in a liquid media containing 100 µl of Mueller-Hinton broth. Positive and negative controls were prepared and respectively made of, 100 µl of MH broth + 100 µl of bacterial suspension and 100 µl of MH broth + 100 µl of the stock solutions of the extracts being tested. e micro-plates were covered with para lm paper and incubated at 37°C for 24 hours. e MIC was estimated with naked eyes compared to the controls and each well was cultured on MH II agar and incubated at 37°C for 24 hours for the determination of the MBC.

e MBC is the smallest concentration of extract at which no bacteria colony can be observed. e antibiotic power (ap) of each extract was therea er calculated with the formula CMB/CMI.

d) Susceptibility tests: A swab of each inoculum was cultured onto Mueller-Hinton II agar plates [15]. Using the tip of sterile pastor pipette, wells of 6 mm of diameter were dug in the agar and 50 µl of each extract was transferred to the wells. A well containing sterile distilled water served as negative control. e petri dishes were kept at ambient temperature for 30 min to 1h for a pre-di usion of the substances before being incubated at 37°C for 24 h [16]. Meanwhile, swabs of each inoculum were cultured onto MH II plates and reference antibiotic disks were used as positive controls. e tests were repeated three times and the antibacterial activity of the extracts was determined by measuring the inhibition zone diameters around each well (Table 1).

e) Statistical analyses: Susceptibility tests were repeated thrice and the results analysed using Stata 11.0 so ware. e data were presented as Mean ± standard error. An analysis of variance (ANOVA single factor) was used to compare the means of the inhibition zone diameters between the two extracts of the same plant, between the aqueous extracts of the

and P. mirabilis A24974 and a bacteriostatic activity on E. coli ATCC 25922 and P. aeruginosa ATCC 27853. S. aureus ATCC 25923 was sensitive to the aqueous extract of S. radiatum and the hydro-ethanolic extract of C. adansonii with the same MIC of 0.625 mg/ml. None of the reference strains was susceptible to the extracts of V. amygdalina. However, a MIC of 2.5 mg/ml was recorded for these extracts on S. aureus ATCC 25923 (Table 2).

S. aureus, E. coli, V. cholerae, S. Typhi and P. vulgaris were the only clinical isolates susceptible to the hydro-ethanolic extract of S. radiatum with MIC values from 0.3125 to 1.25 mg/ml and a bactericidal effect on S. aureus, V. cholerae and S. Typhi. At the same MIC of 0.625 mg/ml, S. aureus, V. cholerae, S. Typhi and P. vulgaris were susceptible to the aqueous extract of S. radiatum with a bactericidal power on S. aureus. Similarly, V. cholerae and P. vulgaris were susceptible to the hydro-ethanolic extract of C. adansonii (MIC = 0.625 mg/ml). No MBC was recorded for the extracts of the leaves of V. amygdalina. Nevertheless, some MIC values were obtained on S. aureus (V. amygdalina AE = 5 mg/ml; V. amygdalina HE = 1.25 mg/ml) (Table 3).

Susceptibility tests

As shown in Table 4 and Figure 1a, S. aureus ATCC 25923 is susceptible to the hydro-ethanolic extract of the leaves of C. adansonii and S. radiatum and the aqueous extracts of S. radiatum.

The inhibition zone diameters obtained with the hydro-ethanolic extract of the leaves of S. radiatum were significantly higher than ($p < 0.05$) those recorded using the aqueous extract of the same plant but also higher than those obtained with the hydro-ethanolic extracts of C. adansonii and V. amygdalina. Apart from S. aureus ATCC 25923, the other reference strains were only sensitive to the hydro-ethanolic extract of the leaves of S. radiatum with inhibition zone diameters v(a)-2(l4 >>

Discussion

e pres ent study aimed to assess the antibacterial activity of the aqueous and hydro-ethanolic extracts of three leafy vegetables commonly used in traditional medicine to treat infectious diarrhoeas. To this end, the extracts were tested on 12 clinical bacteria isolates obtained from diarrheal faeces samples and on 4 reference strains.

S. aureus ATCC 25923 was the most susceptible reference strain to the extracts of C. adansonii (aqueous extract: MIC=1.25 mg/ml; hydro-ethanolic extract: MIC=0.625 mg/ml). However, a total absence of MBC was recorded with the aqueous extract of C. adansonii at all the considered concentrations. Nevertheless, its hydro-ethanolic extract showed a bacteriostatic e ect on S. aureus ATCC 25923 with a MBC of 2.5 mg/ml. Moreover, no MBC was induced by the aqueous extracts of C. adansonii on the clinical isolates. However, V. cholerae and P. vulgaris were susceptible to its hydro-ethanolic extract with a bacteriostatic power on these two isolates. In addition, S. aureus (CMI = 1.25 mg/ml) and Shigella spp (1.25 mg/ml) were also susceptible to this extract though without a MBC. ese results are better than those reported by Lagnika et al. [19] on S. aureus isolated from ryonomys swinderianus (aqueous extract of the leaves of Crateva religiosa MIC = 10 mg/ml; ethanolic extract of the leaves of Crateva religiosa MIC=2.5 mg/ml). is discrepancy could be explained by the fact that the tested bacteria strains were not from the same origin and the two studies were not conducted in the same area. Furthermore, the ndings of the current study are better than those obtained by Ayodeji et al. [20] on E. coli (12.5 mg/ml), S. Typhi (12.5 mg/ml), S. aureus (12.5 mg/ml) and K. pneumoniae (25 mg/ml). Agboke Ayodeji et al. actually carried out their study on the methanolic extract of the leaves of C. adansonii and the reported MIC

against a wide range of bacterial species responsible for diarrheal infections or diseases, especially those belonging to the family of Enterobacteriacea. Probably, they can be used to treat diarrhoeas and food poisoning cases, as well as or enteric bacterial infections.

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