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Antibacterial potential of the flavonoids from the fruits of *Voacanga africana* Stapf and *Tabernaemontana contorta* Stapf on diarrheagenic bacteria: A Comparative study between the crude extracts and their fractions

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Abstract
Diarrhea, ranked second cause of death in children under five years globally, is one of the main causes of death in these children in Cameroon. The development phenomena of resistant strains, the side effects, and the costs of often restrictive drugs have motivated populations to look towards phytotherapy. The objective of this study was to carry out a comparative of the antibacterial activity of flavonoids of crude extracts and fractions of the fruit of *Voacanga africana* Stapf and *Tabernaemontana contorta* Stapf on the germs responsible for diarrhea. The fruits have been macerated in a hydro-ethanolic solution followed by the extraction of flavonoids by differential fractionation with solvents of increasing polarity. The polyphenols and flavonoids content of the crude extracts and the fractions have been determined by colorimetric methods and the antibacterial activity by the macro-dilution using *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* strains. Finally, a comparison of the results was obtained. Phytochemical screening highlighted classes of compounds common to the two species, such as flavonoids and alkaloids and differences; the assay showed that the crude extract of *Voacanga africana* contains many more polyphenols with a value of 143.10 ± 1.01 mg EAT / g of dry extract, but *Tabernaemontana contorta* contains more flavonoids, including its extract ethyl acetate has the highest value, 40.65 ± 0.02 mg EQ / g of dry extract. For the antibacterial activity, the flavonoids of the ethyl acetate fraction of *T. contorta* have greater antibacterial activity against the strains tested with MICs ranging from 6.25 to 12.5 mg / mL followed by the raw extract of *V. africana*. This contribution could offer applications in the medical field for the fight against diarrhea.

Keywords: *Voacanga africana*, *Tabernaemontana contorta*, hydro-ethanolic extract, fractionation, antibacterial activity, diarrhea

Introduction
In developing countries, infectious diseases (IDs) represent a real public health issue due to their frequency and severity [1]. One of the most common problems of these IDs is the development of bacterial diarrhea [2]. Diarrhea has been commonly referred to as the discharge of more than three losses of liquid stools per day [2]. The ranked second cause of mortality in children under five years, diarrhea is the cause of 525,000 child deaths per year, and there are around 1.7 billion cases of diarrhea each year in the world [3]. In developing countries, it is one of the most fatal conditions [4], generally observed in children. In Cameroon, childhood diarrhea is one of the leading causes of death in children under five after malaria, measles, and respiratory tract diseases [5]. The prevalence of this pathology is around 18.9 %, with a high incidence in rural areas (19.7 %) compared to the main cities like Yaoundé (14.4 %) [2] several factors contribute to the high prevalence and severity of diarrhea. These include non-compliance with hygiene rules, difficult access to safe drinking water, population overcrowding, immunodeficiency, and chronic diseases [6]. Antibiotics have been often used for the treatment of acute bacterial diarrhea [7], but the misuse of antibiotics increases antibiotics resistance of the bacterial strains the population [8]. The demographic growth and the poverty of the populations of the developing countries limit access to health care facilities, constituting a brake on good hygiene. This contributes to the increase in demand for traditional medicines [9], further jeopardizing caretaking attempts with antibacterial agents. The improved traditional drugs obtained from medicinal plant extracts would then be an alternative to antibiotic resistance, the reason why populations are turning to herbal medicine [10].

Our study is part of the strategy developed by the Cameroonian authorities to make the production of traditionally improved medicines (MTA) accessible to the general public and thus enhance the local flora while contributing to the search for alternative solutions to the management of acute infectious diarrhea especially those of bacterial origin.

It is in this context that we were interested in the antibacterial powers of polyphenols, more precisely in the flavonoids from two plants: *Tabernaemontana contorta* staff and *Voacanga africana*, which have been widely used in traditional medicine. They are used as anticancer, for skin conditions, in malaria patients [11, 12] as well as other condition in the gastrointestinal tract [13] .The present work consisted in examining the activity of the various extracts and fractions of the fruits *Tabernaemontana contorta* staff and *Voacanga africana* on bacterial strains, in order to determine the plant with the best antibacterial activity to make a drug in the near future. To carry out this work, a hydro-ethanolic extraction of our two plants has been carried out followed by fractionation in which the content of polyphenols and flavonoids have been determined then the antibacterial activity was evaluated and compared.

Material and Methods

Plant Material

The plant material used consisted of the ripe fruits of *Tabernaemontada contorta* and *Voacanga africana* (Figure 1) who have been collected at Mount Eloudem (Nkolbisson – Yaounde) and identified in the National Herbarium of Cameroon under reference numbers 26138/SFR/Cam and 9227/SFR/Cam respectively between January and February 2020. The fruits of these harvested plants have been washed, cut and, then dried at room temperature in the dark to preserve as much as possible the integrity of the molecules. They were then reduced to powder (800 µm to 1600 µm), and packed in poly bags and stored at 4°C for laboratory works. The extract preparation, fractionation, and chemical screening were performed in the Laboratory of Chemistry of Université des Montagnes (UdM), and the antibacterial potential tests conducted in the Laboratory of Microbiology of the Université des Montagnes (UdM) teaching hospital in Banekane (West-Cameroon).

Fig 1: Fruits of *T. contorta* (left) and *V. africana* (right)

Microorganisms

The three bacterial strains tested for the antibacterial activity of crude extracts and flavonoid fractions of *Tabernaemontana contorta* and *Voacanga africana* were: *Staphylococcus aureus* (ATCC 25923)*:* Gram + bacteria; *Echerichia coli* (ATCC 25922): Gram – bacteria; *Klebsiella pneumoniae* (ATCC 13883): Gram – bacteria. They were all provided by the microbiology laboratory of the University des Montagnes.

Extraction and fractionation

Preparation of extracts

The fruit of *Voacanga africana* (320 g) and *Tabernamontana contorta* (320 g) have been extracted by maceration with an ethanol-water mixture (70 :30) in constant agitation (800 trs/min) on a magnetic agitator PRO HPS-7 Lab Plus Series with the renewal of solvent every 24 hours for three times. The hydroethanolic solutions were filtered on Whatman N°3 paper then evaporated to dryness on a rotavapor (Rotavapor® BUCHI R-201) under reduced pressure at 40 ˚C to obtain the dry extracts. The extraction yield was calculated using the following formula:

$$R = \frac{(mass\ of\ raw\ extract)}{mass\ of\ powder} \times 100$$

Preparation of partitions

The partitioning has been done following the method of Xuan Li et al. (2020) [14]. A portion of the residue (15 g) was suspended in 100 mL deionized water and then portioned successively with ethyl acetate (200 mL) and n-butanol (200 mL) three times at room temperature. After filtration, the filtrate of each part has been concentrated at reduced pressure to obtain ethyl acetate soluble part, n-butanol soluble part and water-soluble part, respectively. The dry residues were then recovered and then stored at 4°C before carrying out the antibacterial tests.

Phytochemical screening

The presence of the main chemical groups in the extracts has been investigated using the tests described by Bassène (Bassène, 2012) [15] and Bruneton [16]: flavonoids (Shinoda test), tannins (Stiasny reaction followed by that of ferric chloride), alkaloids (Dragendorff and Mayer reagent) , sterols and terpenes (Liebermann-Buchard reaction) and saponosides (foam index).

Test for Polyphenols

Perchloride test

To about 3 mL of the extract, 2 to 3 drops of $FeCl_3$ have been added. The presence of polyphenols was put to evidence by the development of a greenish color.

Test for flavonoids

In 1 ml of extract, 1 ml of ethanol has been added. The mixture was then treated with a few drops of iso-amyl alcohol, and 0.05 g of magnesium shavings, and three drops of concentrated H_2SO_4. We obtained a pink red color, thus reflecting the presence of flavonoids in our extract.

Alkaloids test

The alkaloids have been characterized using Dragendorff's reagent (Potassium iodobismuthate) and Mayer's (Iodomuric reagent).

Mayer's reagent test

In 15 mL of extract, 4 mL of 10 % sulfuric acid was added. 5 mL of the obtained solution was subsequently transferred into two tubes. In the first tube, 3 drops of Mayer's reagent were added and let to rest for 15 minutes. We had the appearance of a white yellow or light-yellow precipitate in both extracts.

Dragendorff reagent test
In the second tube three drops of Dragendorff's reagent were added, we had the appearance of a yellow-orange precipitate in both extracts. These two tests indicate the presence of alkaloids.

Test for saponins
5 mL of the extract have been boiled for 5 minutes. After cooling, the contents of the tubes were stirred vertically for 15 seconds, then let to stand. We had the appearance of a persistent foam with a height of more than one centimeter, indicating the presence of saponins for the extract of *Tabernaemontana contorta* and no persistent foam for the extract of *Voacanga africana*

Test for steroids and terpenoids
In 1 mL of extract, 1 mL of methanol has been introduced and dissolved. 0.2 mL of each of the following reagents was added: chloroform, glacial acetic anhydride, concentrated H_2SO_4. We obtained a violet then greenish coloration for the extract of *Tabernaemontana contorta* indicating the respective presence of terpenoids and steroids but no coloration for extract of *Voacanga africana*

Test for tannins
In 1 mL of extract, was added 1 mL of methanol. To the resulting solution, five drops of 0.5 % sulfuric acid and five drops of 1 % ferric chloride were added. We obtained blue-blackish staining for *Voacanga africana* extract, indicating the presence of tannins and no staining for *Tabernaemontana contorta* extract.

Polyphenols and flavonoids content
Polyphenol content
The polyphenol contents of the crude extracts and the fractions have been determined by the Folin-Ciocalteu method described by Mbopi et al. (2021) [17]. In 100 µL of the solution of each extract and fraction, 500 µL of the Folin-Ciocalteu reagent have been added, then 2 minutes after 2 mL of carbonate of 20 % sodium have been added. After 30 min of incubation, the absorbances were then read on a spectrophotometer at 760 nm. Three tests have been carried out for each concentration of product tested. A standard curve established from a dilution series of tannic acid (25-50-75-100-125mg / L) has been processed in the same way as the extracts. The results have been expressed in milligrams equivalent of tannic acid per gram of dry extract (mg EAT/g).

Flavonoid content
The method for determining flavonoids in crude extracts and fractions had been based on the principle of direct determination by aluminum chloride (Mahdi, 2012) [18]. In 500 µL of solution of each extract and fraction was added 1.5 mL of 95 % ethanol followed by 100 mL of 10 % aluminum chloride and 100 µL of 1M sodium acetate and finally 2.8 mL of distilled water. After 30 min of incubation, the absorbances were then read on a spectrophotometer at 415 nm. Three tests have been carried out for each concentration of product tested. A calibration curve has been established from a dilution series of quercetin (60-120-180-240-300 mg / L). The results have been expressed in milligram equivalent of quercetin per gram of dry extract (mg EQ / g).

Antibacterial Activity
Bacterial inoculums preparation
An aseptic condition for each bacterial strain, the bacteria Were subculture on Muller-Hinton-Agar agars in kneading dishes, by the streak method, then incubated at 37 °C for 24 hours to obtain young colonies used for the preparation of the bacterial inoculum.
From the 24 hours colonies, 1 to 3 colonies were picked using a platinum loop and introduced into 2 to 3 mL of sterile physiological water, to obtain turbidity similar to that of point 0.5 on the McFarland scale, corresponding to a variant concentration between 10^6 and 10^8 Colonial Forming Units / mL (CFU / mL)

Sensitivity test
Bacterial sensitivity to our samples has been assessed using the antibiogram principle [19]. 20 µL of each solution at 100 mg / mL of extract and fraction was inoculated by the disc method on Petri dishes previously containing bacterial strains containing Mueller-Hinton medium, then incubated in an oven at 37 °C for 18 to 24 hours after being left at room temperature for 15 min. The presence of an inhibition zone obtained corresponds to an absence of bacterial growth and thus reflects an antibacterial activity of our fractions.

Determination of the minimum inhibitory concentration (MIC)
The determination of the MIC has been based on evaluating of the susceptibility of a microorganism to an antimicrobial substance. It has been defined as the smallest concentration that will inhibit any visible growth of a microorganism after incubation at 37 °C for 18 to 24 hours. The technique used is that of macro-dilution [20]. In the different test tubes of the dilution range were introduced 1 mL of Mueller-Hinton broth, then in the first tube of the range 1 mL at 50 mg / mL (for the F1), 100 mg / mL (for the E2, F2 and E1) and 200 mg / mL (for F5, F4 and F3) was introduced into the first tube of the dilution range following which cascade dilutions of ratio 2 were performed in Mueller Hinton's broth of so as to obtain a concentration range of 25 mg / mL to 0.156 mg / mL; 50 mg / mL to 0.156 mg / mL; 100 mg / mL to 0.156mg / mL for the 50mg / mL (F1), 100mg / mL (E2, F2 and E1) and 200 mg / mL (F5, F4 and F3) fractions respectively. Then 15 µL of bacterial inoculum was added to each tube, then incubated at 37 °C. After 24 h, the MIC of each extract and fraction have been deduced from the first tube of the range within which growth did not occur. The experiment has been repeated three times.

Determination of the minimum bactericidal concentration (MBC)
The minimum bactericidal concentration (MBC) corresponds to the lowest concentration of substance capable of killing more than 99.9 % of the initial bacterial inoculum (i.e., less than 0.11 % of survivors) after 18 to 24 hours of incubation at a temperature of 37 ° C. The tubes of the range in which the bacterial growth have been not visualized were streaked on Petrie dishes containing Mueller-Hinton-Agar medium and then incubated for 24 hours at 37 °C. The CMB of each extract and fraction has been deduced from the smallest concentration at which no culture has been observed. The experiment has been repeated three times. The MBC/MIC ratios has been then assessed for the bacteriostatic or the bactericidal potential on the test organisms. When it was higher than 4, the extract was said to have a bacteriostatic action while equal to or lower than 4, it was regarded as bactericidal. The extract was said to have an absolute

bactericidal action when the MIC was equal to the MBC (MBC/MIC = 1).

Result and Discussion

Extraction and fractionation

The results of extraction and fractionation are shown in Table 1 (extraction) and 2 (fractionation)

Table 1: Result of the extraction of powder from fruits of *Voacanga africana* and *Tabernaemontana contorta*

Plant material	Mass of dry powder (g)	Mass of raw extract (g)	Yield (%)
Voacanga africana	320	51.68	16.15
Tabernaemontana contorta	320	18.94	5.92

Table 1 shows the results of the extraction carried out on our two plants. The hydro ethanolic extraction of 320g of *Voacanga africana* and *Tabernaemontana contorta* gave a mass of the crude extract of 51.68 g and 18.94 g, thus giving a yield of 16.15% and 5.92 %, respectively.

The extraction yield from the hydroethanolic mixture maceration method of the two species showed that the fruits of *Voacanga africana* have a better yield (16.15 %) than those of the fruits of *Tabernaemontana contorta* (5.92 %). This difference could be due to the chemical composition of the fruits of *Voacanga africana*, which is probably richer in polar compounds, especially polyphenols, than that of the fruits of *Tabernaemontana contorta*.

This result is different from the work of Olaleye [21], who found a yield of 9.75 % for the species *Voacanga africana*. This difference can be explained by the nature of the solvent and the part of the plant used. Indeed, in the latter's work, maceration was carried out on the leaves with methanol as the extraction solvent. The fruits being one of the sources of polyphenols [22], the hydroethanolic mixture being polar will better extract the polyphenols this justifying our higher yield.

Table 2: Result of partitioning of raw extracts

Plant materials	Solvents	Mass of partitions(g)	Yields (%)
T. contorta	Ethyl acetate	1.74	11.62
	n-butanol	2.28	15.22
	water	10.98	73.20
V. africana	Ethyl acetate	5.06	33.73
	n-butanol	1.18	7.87
	water	8.76	58.40

Table 2 shows the result of the fractionation by the different solvents of increasing polarity on our two crude extracts. The aqueous fractions showed higher yields (73.20 % and 58.40 %). This shows that in our study, extracts there is a predominance of polar compounds, in particular flavonoids of the di-glycoside and tri-glycoside type [23].

Phytochemical screening

Table 3 shows the result of the phytochemical screening carried out on our extracts.

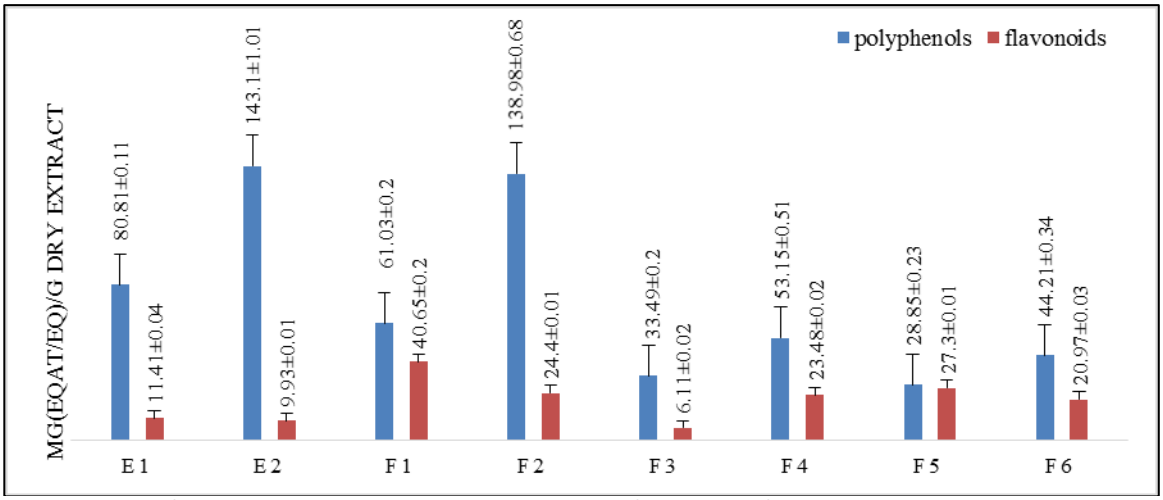
Table 3: Phytochemical screening results

Secondary metabolites		HAv	HAt
Alkaloids	**Ma**	+	+
	Dr	+	+
polyphenols		+	+
flavonoids		+	+
Tannins		+	+
Quinones		+	-
Terpenoids		-	+
Steroids		-	+
saponins		-	+

HAv: hydro-alcoholic extract of *Voacanga Africana*; HAt: hydro-alcoholic extract of *Tabernaemontana contorta*; Ma: Mayer; Dr: Dragendorff; (+): present; (-): absent

The table 3 of phytochemical screening results show the presence of classes of compounds such as alkaloids, polyphenols, flavonoids, tannins and quinones for the extract of *Voacanga africana* and alkaloids, polyphenols, flavonoids, terpenoids, steroids and saponins for *Tabernaemontana contorta.*, The difference in our two extracts resides in the presence of tannins, and quinones for the extract of *Voacanga africana* and saponosides, terpenoids, and steroids for the extract of *Tabernaemontana contorta*. This would be in agreement with the results of Joshua [24]. This result also made it possible to justify the extraction yield because *Voacanga africana* contains more polar compounds, especially more phenolic compounds. This difference could justify the result of the antibacterial activity.

Polyphenols and flavonoids content



E1: Raw extract of *Taberneamontana contorta*; E2: Raw extract of *Voacanga africana*
F1: Ethyl acetate fraction of *Taberneamontana contorta*; F2: Ethyl acetate fraction of *Voacanga africana;* F3: Aqueous fraction of *Taberneamontana contorta;* F4: Aqueous fraction of *Voacanga africana;* F5: 1-butanol fraction of *Taberneamontana contorta;* F6: 1-butanol fraction of *Voacanga africana*

Fig 2: Polyphenols and flavonoids content of crude extracts and the different fractions of the fruits of *Voacanga africana* et *Tabernaemontana contorta*

This result shows us that the raw extract of *Voacanga africana* has the highest polyphenol content (143.10 ± 1.01 mg EAT / g of dry extract) but has one of the lowest values in terms of flavonoids content (9.93 ± 0.02 mg EQ / g of dry extract). The ethyl acetate fraction of *Taberneamontana contorta* has a polyphenol content value (61.03 ± 0.2 mg EAT / g of dry extract) close to its flavonoid content (40.65 ± 0.02 mg EQ / g of dry extract) as well as the polyphenol content of the 1-butanol fraction of *Taberneamontana contorta* (28.44 ± 0, 23 mg EAT / g of dry extract) and its flavonoid content (27.13 ± 0.01 mg EQ / g of dry extract). We also note that the fraction of *Voacanga africana* with ethyl acetate has a value quite close (138.98 ± 0.68 mg EAT / g of dry extract) to the value of the crude extract of *Voacanga africana* but with a fairly low value for the flavonoid content (24.40 ± 0.68 mg EQ / g of dry extract). These results were obtained from the tannic acid calibration curve for polyphenols (y = 0.9867x - 0.0004 with an R^2 = 0.9979) and the quercetin calibration curve for flavonoids (y = 7.0767x - 0.0118 with an R^2 = 0.9988)

Concerning the quantification of polyphenols and flavonoids, the results obtained show that *Voacanga africana* contains many more polyphenols with the highest value on the raw extract (143.10 ± 1.01 mg EAT / g of dry extract), which is in agreement with the results of the phytochemical screening (flavonoids, tannins) and the fact that the hydro-ethanolic extraction preferentially extracts the polar compounds from which the polyphenols originate [23]. This is also observed in *Taberneamontana contorta* which despite, having a lower polyphenol content, its raw extract still has the highest value (80.81 ± 0.11 mg EAT / g) concerning its fractions. On the other hand, *Taberneamontana contorta* has a much higher content of flavonoids observed in its ethyl acetate fraction (40.65 ± 0.02mg EQ / g) than that of *Voacanga africana* which has the highest content in its fraction. With ethyl acetate and water (24.40 ± 0.68 and 23.48 ± 0.02 mg EQ / g respectively). This shows that *Taberneamontana contorta* contains much more flavonoids of the aglycone, mono, and diglycosidic type and that *Voacanga africana* contains in equal parts flavonoids of the aglycone, mono and diglycosidic type but also flavonoids of the di, tri and tetraglycosidic type. The solvents used during the fractionation of the crude extracts extract, for ethyl acetate, flavonoids of the aglycones, mono and diglycosidic type and for water, flavonoids of the di, tri and tetraglycosidic type [23].

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Table 4 shows the result of the determination of the minimum inhibitory concentration and minimum bactericidal concentration of the crude extracts on the strains of bacterial

Table 4: MIC and MBC values and MIC/MBC ratios of crude extracts and partions of *Voacanga africana and Tabernaemontana contorta*

Fractions	*Klebsiella pneumonia*			*Escherichia coli*			*Staphylococcus aureus*		
	MIC (mg/ml)	MBC (mg/ml)	Rapport CMB/CMI	MIC (mg/ml)	MBC (mg/ml)	Rapport CMI/CMB	MIC (mg/ml)	MBC (mg/ml)	Rapport CMI/CMB
E1	25	-	Nd	50	100	2	25	100	4
E2	25	-	Nd	12.5	--	Nd	3.125	6.25	2
F1	6.25	-	Nd	12.5	25	2	12.5	25	2
F2	50	-	Nd	25	-	Nd	50	50	1
F3	100	100	1	25	100	4	50	-	Nd
F4	100	-	Nd	50	-	Nd	50	100	2
F5	25	-	Nd	12.5	50	4	25	50	2

E1: Raw extract of *Taberneamontana contorta*; E2: Raw extract of *Voacanga africana*
F1: Ethyl acetate fraction of *Taberneamontana contorta*; F2: Ethyl acetate fraction of *Voacanga africana;* F3: Aqueous fraction of *Taberneamontana contorta;* F4: Aqueous fraction of *Voacanga africana;* F5: 1-butanol fraction of *Taberneamontana contorta;* F6: 1-butanol fraction of *Voacanga africana*; Nd: Not defined

The results in Table 4 shows that the crude extract of *Voacanga africana* exhibited greater activity with MICs ranging from 25 to 3.125 mg / mL on the three bacterial strains tested. This result can be attributed in addition to the metabolites in common to the two species to the tannins present in the fruit extract of *Voacanga africana* which has proven an antibacterial activity on the bacterial strains responsible for infections in humans such as *Clostridium sp* , *Escherichia coli*, *Staphylococcus aureus* [25]. Their properties to bind to calcium ions involved in the metabolism of bacteria affects the permeability of the bacterial wall, thus disrupting the absorption of trace elements essential for bacterial growth [26]. The antibacterial activity of the flavonoid fractions with ethyl acetate (MIC ranging from 6.25 to 12.5 mg / mL), 1-butanol (MIC ranging from 12.5 to 25 mg / mL), and aqueous (MIC ranging from 50 to 100 mg / mL) of *Tabernaemontana contorta* was superior to that of the flavonoid fractions *of Voacanga africana*. This could probably be due to the high content of flavonoids in the fruit fractions of *Tabernaemontana contorta*. In fact, flavonoids exert their antibacterial activity mainly by inhibiting the synthesis of DNA and certain enzymes in the bacterial membrane [27].

The flavonoid fractions of the fruits of *Taberneamontana contorta* showed superior antibacterial activity than the crude extract of *Taberneamontana contorta*. This result illustrates that the antibacterial activity of the fruits of *Taberneamontana contorta* is attributed to the flavonoids.

On the other hand, the raw extract of *Voacanga africana* presented an antibacterial activity superior to its flavonoid fractions. The antibacterial activity of the fruits of *Voacanga africana* would therefore be linked to a synergistic effect between the different classes of metabolites [28].

The aqueous fraction of *Taberneamontana contorta* showed a high activity on the *Klebsiella pneumoniae* strain, encapsulated, invasive bacterium, reputed to produce beta-lactamase [29]. This result could be due to the action of flavonoids such as tri glycosides which bind to glucuronic acids or sugars in the *Klebsiella pneumoniae* capsule by O-glycoside bonds causing capsule fragility [30].

For the *Escherichia coli* and *Klebsiella pneumoniae* strains, the CMBs of the extract and fractions of *Voacanga africana* (at concentrations of 50 and 100 mg / mL) were not obtained, unlike *Staphylococcus aureus*, showing that the latter would have a predominant action on gram + bacteria.

The activity of the extract and fractions of the fruits of *Taberneamontana contorta* on *Escherichia coli* and *Staphylococcus aureus* indicates that they act on bacteria independent of gram.

The lowest MICs and CMBs of extracts and fractions of the fruits of our two species were obtained on *Staphylococcus aureus*, revealing that they had a greater activity on this gram + bacterium. Gram + bacteria have a very permeable membrane. They have many layers of peptidoglycan representing up to 90 % of the constituents of the bacterial wall rich in metal ions which could be the substrate for secondary metabolites [30].

The CMB / CMI ratio revealed that the extracts and fractions of *Voacanga africana* have a bactericidal effect on *Staphylococcus aureus*. In contrast, the extracts and fractions of the fruits of *Taberneamontana contorta* have a bactericidal effect on *Escherichia coli* and *Staphylococcus aureus*. This bactericidal effect of the extract and fractions of the fruits of *Taberneamontana contorta* would be attributed to the presence of terpenoids and saponins only in the latter, which are capable of infiltrating the lipid bilayer to cause the rupture of the cell membrane causing the leakage of the bacterial content. The lowest MICs and CMBs of extracts and fractions of the fruits of our two species have been obtained on *Staphylococcus aureus*

According to table 4, analysis of the results shows that the extracts and fractions of the fruits of these two plants have antibacterial activity on the strains tested. The ethyl acetate fraction of *Taberneamontana contorta* has greater antibacterial activity (MIC flanging from 6.25 to 12.5 mg / ml) on bacterial strains compared to all other fractions tested; which shows that the aglycone, mono and diglycosidic flavonoids contained in this fraction have a very great antibacterial power on the germs responsible for diarrhea. On the other hand, the ethyl acetate fraction and even the *Voacanga africana* aqueous fraction does not exhibit an activity comparable to that of the ethyl acetate fraction of *Taberneamontana contorta* but yet the crude extract of *Voacanga africana* shows a quite similar antibacterial activity (MIC ranging from 3.125 to 25 mg / mL) to that of the ethyl acetate fraction of *Tabernaemontana contorta*. This would suppose that the flavonoids of the aglycone, mono and diglycosidic type and of the tri and tetraglycosidic type contained in the ethyl acetate fractions of and aqueous *Voacanga africana* would not have a very great antibacterial power on their own but in synergy with other compounds contained in the fruits of *Voacanga africana*; especially since compounds such as tannins and alkaloids have an antibacterial activity [31, 32]

During this work, many CMBs could not be determined at the concentrations tested, especially for the extracts and fractions of *Voacanga africana* on two bacterial strains; but values obtained on the *Staphylococcus aureus* strain showed greater activity (CMB = 6.25 mg / ml) on the crude extract. This could suggest a more accentuated spectrum of action on Gram +. On the other hand, the MBC values of the extract and fractions of *Taberneamontana contorta* with the ethyl acetate fraction, which showed high activity (MBC = 25 mg / mL) would rather suggest a spectrum of action as much on Gram + as the Gram-. It should also be noted that the ethyl acetate fraction *Taberneamontana contorta* has a bactericidal effect on the strains *Escherichia coli* and *Staphylococcus aureus* (CMI / CMB ratio = 2). *Voacanga africana* crude extract has a bactericidal effect on *Staphylococcus aureus.*

According to a classification of the activity of plant extracts according to the value of their MIC revealed by Kuete, the extracts used in this study, despite showing activity are weakly active on all bacteria. Indeed, for this author, the activity, of an extract is significant if the MIC <100 µg / mL It is said to be moderate when the MIC is between 100 and 625 µg / mL (100 <MIC < 625 µg / ml), and is low if the MIC> 625 µg / mL [33].

Conclusion

Through the comparative study of the antibacterial activity of the flavonoids of crude extracts and fractions of the fruit of *Voacanga africana* and *Tabernaemontana contorta* on the germs responsible for diarrhea, it appears that the two plants contain common and specific classes of compounds. *Voacanga africana* contains many more polyphenols where as *Tabernaemontana contorta* contains many more flavonoids, which gave the ethyl acetate fraction greater antibacterial activity compared to the antibacterial activity *Voacanga africana*. This could be due to a synergy of action of all the compounds present in the plant. Our results confirm that the extracts and fractions of the two plants could possibly

compete with the antibiotics used in the treatment of bacterial diarrhea. These tests contribute to the scientific validation of the massive traditional use of these species by populations. In perspective, it would be important to further research on a wide range of microbial strains and to identify the active constituents responsible for the antibacterial activity.

References

1. Bendahou M, Muselli A, Grignon-Dubois M, Benyoucef M, Desjobert JM, Bernardini AF et *al*. Antimicrobial activity and chemical composition of *Origanum glandulosum* Desf. Essential oil and extract obtained by microwave extraction: comparison with hydrodistillation. Food Chem 2008;106(1):132-139.
2. Nguendo JB, Salem G. Épidémiologie géographique des maladies diarrhéiques à Yaoundé (Cameroun). *M@ppemonde* 2008;89:1-17.
3. Organisation Mondiale de la santé. Maladies diarrhéiques. https://www.who.int/fr/news-room/fact-sheets/detail/diarrhoeal-diseases. Mai 2017. 20 Mai, 2020
4. Coulibaly MD, Kadio A. Place des diarrhées bactériennes dans les pays en développement. Pan Afr Med J 1998;4:1-4.
5. Dupeyron C. Les diarrhées aiguës bactériennes. http://devante.org/articles/les-diarrhees-aigues-bacteriennes. Avril 1997. 26 Mai 2020.
6. Dosso K, N'guessan B, Bidie A, Gnangoran B, Méite S, Yapo A, et *al*. Antidiarrhoeal Activity of an Ethanol Extract of the Stem Bark of *Piliostigma Reticulatum* (Caesalpiniaceae) in Rats. African J Tradit Complement Altern Med 2011;9:242-9.
7. Ngo T. Antidiarrhoeal, antimicrobial and antioxidant properties of two Cameroonian medicinal plants: *Trilepisium madagascariens* DC. Leuwen Berg (Moraceae) and *Entada abyssinica* Steud. EXA. Rich. (Mimosaceae). [Thèse]. Physiologie animale : Université de Dschang Cameroun 2011.
8. Monnet DL. Consommation d'antibiotiques et résistances bactériennes. Ann Fr Anesth Reanim 2000;19:409-17
9. Collège des Universitaires de Maladies Infectieuses et Tropicales. e-Pilly TROP Maladies infectieuses tropicales. 25eme édition Alinéa Plus 2016.
10. Organisation Mondiale de la santé. Stratégies de l'OMS pour la médecine traditionnelle 2002-2005. Points marquants de l'an 2000 en matière de médicaments essentiels et politiques pharmaceutiques. Genève : OMS 2002
11. Nagib C. Policlinique médicale universitaire, Lausanne - Médecin moderne, médecin connecté. Swiss Sport Exerc Med 2017;66(4):49-52.
12. Zocoler MA, De Oliveira AJB, Sarragioto VL, Vidotti GJ. Qualitative determination of indole alkaloids of *Tabernarmontana fuchsiaefolia* (Apocynaceae). J Braz chem 2005;16(6b):1372-1377
13. Van Beek TA, Kuijlaars FLC, Thomassen PHAM, Verpoorte R, Swendsen AB. Antimicrobially active alkaloids from *Tabernaemontana pachysiphon*. Phytochem 1984;23(8):1771-1778
14. Li X, Deng Y, Kang L, Chen L, Zheng Z, Huang W, *et al*. Cytotoxic active ingredients from the seeds of *Voacanga africana*. S Afri J Bot 2020;137:1-9
15. Bassène E. Initiation à la Recherche sur les Substances Naturelles: Extraction- Analyse-Essais Biologiques. Presse Universitaire de Dakar : Dakar 2012;17(94-96):140.
16. Bruneton J. Pharmacognosie, phytochimie, plantes médicinales. 3ème Ed, Edition Technique et Documentation-Lavoisier, Paris 1999, 421-499.
17. Mbopi YP, Fozeng DH, Nguekeu YM, Bitchagno TM., Ngantchouko NB, Awouafack MD, *et al*. Chemical constituents, antioxidant activity and bactericidal effect of *Dicliptera verticillata* (Acanthaceae) along with total phenolic content. S Afr J Bot 2021;142:216-221.
18. Mahdi B. une approche phytochimique pour différencier deux espèces du genre Zygophyllum [Mémoire de master]. Chimie appliquée : Université Kasdi Merbah Ouargla 2012.
19. Krief S. Métabolites secondaires des plantes et comportement animal : surveillance sanitaire et observations de l'alimentation des chimpanzés (*Pan troglodytes schweinfurthii*) en Ouganda : Activités biologiques et étude chimique de plantes consommées. [Thèse]. Ecologie et Chimie des substances naturelles : Ouganda 2005.
20. Kuate Tokam CR, Fotsing Kwetche PR, Ngoupayo J, Ndelo J, Kouamouo J, Gamwo Dongmo S, *et al*. Phytochemical screening, antibacterial and antifungal activity of crude aqueous and methanol extracts of stem bark of *Garcinia brevipedicellata*. Int J Pharm Biomed Res 2013;4:221-226
21. Olaleye SB, Oke JM, Etu AK, Omotosho IO, Elegbe RA. Antioxidant and anti-inflammatory properties of flavonoid fraction from the leaves of *Voacanga africana*. Niger J Physiol Sci 2004;19 (1-2):69-76.
22. Benguerba A. Etude phytochimique et de la phase butanolique de l'espèce *Inula crithmoides L* [Mémoire de master]. Chimie organique option Phytochimie : Université Mentouri Constantine 2008.
23. Ferhat M. Recherche des substances bioactives de *Centaurea microcarpa* coss et dur. [En ligne]. Mémoire de Master. Algérie : Université de M'sila 2009. Disponible sur https://www.memoireonline.com/04/10/3329/m_Recherche-de-substances-bio-actives-de-centaurea-microcarpa-coss-et-dur11.html. Consulté le 27 Mai 2020
24. Joshua PE, Oka SA, Eze CS. Estimation of phytochemical, total phenolic and total flavonoid contents of methanol extract of *Voacanga africana* root bark and its fractions. Bio-Research 2017;15:988-993.
25. Leitao DP, Polizello AC, Ito IY, Spadaro AC. Antibacterial screening of anthocyanic and proanthocyanic fractions from *Cramberry juice*. J. Med. Food 2005;8(1):36-40
26. Pahane MM, Mouafo HT, Noumo TN, Djuikoue IC, Nyondo C, Tchamba MN, *et al*. Assessment of the microbial quality of braised products from Bangangte city and antibiotic susceptibility of selected pathogens isolated therein. Asian J Biotechnol Bioresour Technol 2021;7(2):30-50
27. Ulanowska K. Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA and protein synthesis in some bacterial strains. Arch. Microbial 2006;184(5):271-278.
28. Ganfon H, Houvohessou JP, Assanhou AG, Bankole HS, Gbemou J. Activité antibactérienne de l'extrait éthanolique et des fractions d'*Anogeissus leiocarpa* (DC) Guill. Et Perr.(Combretaceae).Int. J. Biol Chem Sci 2019;13(2):643-51

29. Boughachiche R, Sebais S. Caractérisation morphologique, biochimique et mutagénèse des *Klebsiella pneumoniae* au CHU de Constantine [Mémoire]. Université de Mentouri Constantine 2016.
30. Flandrois J, Courcol R, Lemeland J, Ramuz M, Sirot J, Soussy C, *et al*. Bactériologie Médicale. On : Presse Universitaire de Lyon 2011;45(18):40540-8.
31. Djahra AB, Bordjila O, Benkherara S. Extraction, séparation et activité antibactérienne des tanins de marrube blanc (*Marrubium vulgare* L.*).* Phytothérapie 2013;11:348-352
32. Muñoz V, Moretti C, Sauvain M, Caron C, Porzel A, Massiot G, et *al*. Isolation of Bis-indole Alkaloids with Antileishmanial and Antibacterial Activities from *Peschiera van heurkii* (Syn. *Tabernaemontana van heurkii).* Planta Med 1994;60:455-459.
33. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Med 2010;76:1479-1491.