Asian Journal of Research and Reports in Gastroenterology

3(4): 23-32, 2020; Article no.AJRRGA.60090

Phytochemical Screening, Antibacterial and Anti-Diarrheal Activity of *Combretum dolichopetalum*

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

Editor(s):
(1) Dr. John K. Triantafillidis, IASO General Hospital, Greece.
Reviewers:
(1) Sheila Mgola Maregesi, Muhimbili University of Health and Allied Sciences, Tanzania.
(2) M. Sakthi Priya, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), India.
(3) Shweta Rajawat, Maulana Azad National Institute of Technology (MANIT), India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60090

Original Research Article

Received 06 June 2020
Accepted 13 August 2020
Published 21 August 2020

ABSTRACT

Background and Study Aim: In Nigeria *Combretum dolichopetalum* is used to treat various diseases such as gastrointestinal disorders, blood in stool and diarrhoea. The study aimed at investigating on the phytochemical contents, antibacterial activity and antidiarrhoeal activity of *C. dolichopetalum* leaves.
Methodology: Collected leaves of *C. dolichopetalum* leaves were sun dried and powdered. The extract (MECD) was prepared by cold maceration using methanol. MECD was subjected fractionation by open column chromatography eluted with *n*-hexane, 10% MeOH-DCM. Ethyl acetate, *n*-butanol and water five fractions (HxF, MMCF, EtOAcF, BuF and AqF). These were subjected to phytochemical screening by standard chemical tests and antibacterial testing on standard microorganisms and clinical isolates and, antidiarrhoeal testing against castor oil induced diarrhea in rats was performed using MECD.
Results: Detected phytochemicals included; Alkaloids, Flavonoids, Glycosides, Resins, Saponins, Steroids, Tannins, Terpenoids etc. MECD demonstrated antidiarrhoeal activity in all tested concentration in a dose dependent manner. Varying zones of inhibition was observed among tested microorganisms showing sensitivity to at least four test samples except for *S. kintambo* was sensitive to MECD and MMCF. None the tested microbes showed sensitivity towards AqF. The lowest MIC value of 15.63 mg/ml of test samples was observed against clinical isolated and

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P. aeruginosa. S. kintambo showed resistance to most tested samples except to MMCF and MECD with the MIC value of 62.5 mg/ml.
Conclusion: The plant *C. dolichopetalum* (leaves) is rich in several phytochemicals and possess antidiarrhoeal and antibacterial activity. It can provide standardized herbal formulation if proven to be safe.

Keywords: Combretum dolichopetalum; anti-bacterial; anti-diarrhoea; phytochemicals.

1. INTRODUCTION

Diarrhoea (from the Greek word διappoia; literally meaning "through-flowing") is an abnormal faecal discharge characterized by frequent and fluid stool and/or the presence of blood and mucus with increased neutrophil polymorphs in the stool; usually due to disease of the small intestine and involving fluid and electrolyte loss. Epidemiologically, it is the passage of three or more loose or watery stool. This increase is mainly due to excess water, which makes up 60-85% of faecal matter. However, no universally accepted definition of clinical values as guidelines for the more commonly used determinants of clinically meaning diarrhoea elevated stool output (>200 – 250 g/day) [1]; this measure varies greatly in dietary fibre intake; watery, difficult to control bowel movements (qualitative assessment); and bowel movement frequency exceeding three bowel movements *per* day [1].

Therefore, diarrhoea is a symptom of the secretory response of the gastrointestinal mucosa to a wide variety of stimuli. The increased secretion increases the watery content and fluidity of the stool. If the primary disease causes inflammation or ulceration of the intestinal mucosa, particularly of the large intestine and rectum, blood and mucus may be present in the stool. A wide variety of conditions including enteric infections, alteration in digestion and absorption of food, a variety of hormonal factors or even a response to a parenteral infection can result in the symptom of diarrhoea. Nevertheless, the mechanism leading to this increased fluid content of the stool may be acute or chronic and may represent a symptom of gastrointestinal infection that will respond differently to pharmacological therapy. Acute infective diarrhoea is the major clinical problem in tropical countries [2]. Diarrhoea is an important cause of under nutrition as patients eat less with reduced absorption of nutrients; nutrient requirements also increase as result of infection. It is often the underlying reason for malnutrition changing from marginal to severe in a child. It is also an important cause of growth retardation because children with diarrhoea suffer from loss of appetite, restriction and malabsorption [1].

Ethnopharmacological studies has reported medicinal uses of some species of the genus Combretum against various bacterial infections such as gonorrhea, syphilis and symptoms like diarrhoea, hypertension and even cancers. Combrestatin A_4 phosphate (CA4P) derived from African bush willow, *Combretum caffrum* represent the lead compound in a group of novel tubulin depolymerizing agents being developed as vascular targeting agents (VTAs). VTAs are drugs that induce rapid and selective vascular dysfunction in tumours [3]. It induces mitotic arrest by inhibiting microtubule assembly and has high affinity for tubulin binding [4]. Similarly, it has selective toxicity to proliferating endothelial cells and induces vascular shutdown in tumour models *in vivo* [5,6].

In Nigeria, the aqueous extract of *Combretum dolichopetalum,* is used in folklore medicine for the relief or cure of stomach ache, blood in stool, diarrhoea and related gastrointestinal disorders. The extract contains contained tannins, alkaloids, glycosides, flavonoids and saponins, and was able to protect rats from gastric and duodenal ulcer [7,8]. In pyloric ligation with histamine administration in rats, oral administration of the extract inhibited gastric ulcer incidence and volume of acid produced [7], later shown result from inhibition of gastric emptying Gastric emptying [7].

The present study however evaluated the antibacterial and anti-diarrhoea potentials of methanol extract of *Combretum dolichopetalum* leaves.

2. MATERIALS AND METHODS

2.1 Plant Material Sampling

The leaves of *Combretum dolichopetalum* were collected between the month of February, 2005 from Orba, Udenu Local Government Area of Enugu State. The botanical identification was confirmed by Mr. A. Ozioko of Bioresources

Development and Conservation Programme (BDCP). The voucher specimen was deposited in the BDCP herbarium (BDCP 0094).

2.2 Experimental Animals

Wistar albino rats of either sex bred in the animal house of Faculty of Biological Sciences and Veterinary Medicine were used for the study. They were acclimatized in the animal house for five days with free access to water and food before the start of the experiment. The rats were fed with standard pellets (Guinea Feeds, Plc, Nigeria). The animals were maintained under standard 12-hour light-dark cycle throughout the duration of the study. The rats were randomly assigned to different control and treatment groups in the course of the experiments.

2.3 Microbial Organism

Sensitivity screening, minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) used standard microorganism namely; *Pseudomonas aeruginosa* (ATCC 10145); *Staphylococcus aureus* (ATCC 12600) and *Escherichia coli* (ATCC 11775) obtained from Bioresources Development and Conservation Programme (BDCP), *Salmonella kintambo* was donated by Veterinary Microbiology and Pathology, University of Nigeria, Nsukka and four isolates of *Escherichia coli* collected from Public Health Unit, Department of Microbiology, University of Nigeria, Nsukka.

2.4 Chemicals and Reagents

All chemical used were of analytical grade and supplied by Sigma Aldrich, Germany.

2.5 Extraction of the Plant Material

The leaves were shade dried for 5-7 days. The leaves were milled to fine powder and 70 grams of the powder was extracted by macerating in methanol (1:10 w/v). The filtrate was dried *in vacuo* to obtain the methanolic extract of *Combretum dolichopetalum* (MECD). The MECD was fractionated using solvents of increasing polarity in column chromatography to obtain the fractions used in the study. The various yields of the extract and the fractions were calculated using the formula:

$$\frac{Ye}{We} \times \frac{100}{1} = \text{Percentage yield of the extract or fractions}$$

Where

Ye is the weight (*g*) of Methanolic Extract and the fractions respectively; and

We is the weight (*g*) of the plant material or the methanolic extract.

2.6 Column Chromatography

Exactly 551 *g* of MECD was mixed thoroughly with adequate quantity of silica gel (particle size 0.063-0.200 *i.e* 70 – 200 Mesh) to produce free flowing powder. The powder was placed on top of the dry packed column (4 x 80 *cm* diameter by length) and eluted first with n-hexane, until the eluents became clear. 10% methanol-methylene chloride (MMCE) was further used to elute the constituents. This was followed by the elution with ethyl acetate (EToAcE), butanol (BuE) and water (AqE).

2.7 Phytochemical Analyses

The phytochemical analyses (flavonoids, saponins, terpenes, tannins, carbohydrates, reducing sugars, glycosides, proteins, vitamin A, vitamin E and alkaloids) were conducted on the Methanolic Extract (MECD), *n*-Hexane (HxE), 10% Methanol-Methylene Chloride (MMCE), ethyl acetate (EToAcE), *n*-Butanol (BuE),Water (AqE) [9].

2.8 Microbiological Assays

2.8.1 Sensitivity test

The agar-well diffusion technique was used to pre-screen the antimicrobial activity of the extracts according to Lovian [10]. The standard type cultures: *Pseudomonas aeruginosa* (ATCC 10145); *Staphylococcus aureus* (ATCC 12600); *Escherichia coli* (ATCC 11775); *Salmonella kintambo* (SCRL 113*)* and four *E. Coli* isolates (*E.coli-1, E.coli-2, E.coli-3* and *E.coli-4*) were used for the investigation. The standard and clinical isolate were maintained on Mueller Hinton agar slants at 4^0C; purified and serially sub-cultured in the Mueller Hinton agar plates at 37^0C for 24 hours prior to use. Disc dose, 100 *µl* of 250 *mg/ml*, of the test sample was applied on appropriately labelled wells made in gelled agar containing 1 x 10^6 organisms *per ml*. After an hour of pre-diffusion time, the plates were incubated at 37^0C for 24 *hours.* The activity of the extracts and fractions were assessed by measuring the zones of inhibition, the values

were compared with those of the standard antibiotics, Ciprofloxacin (20 *µg/ml*). The experiment was carried out in triplicates and the average inhibition zone diameter recorded.

2.8.2 Minimum Inhibitory Concentration (MIC)

The MIC screening for each extract (MECD, HxE, EToAcE, MMCE, BuE) and test organisms was done using modified agar-well diffusion technique [11]. The sterile Mueller Hinton agar was inoculated with the relevant microorganisms. A two-fold serial dilution of the extract (250 *mg/ml*) was prepared to achieve a concentration range of 125 – 7.8125 *mg/ml*.100 *µl* of each dilution was introduced into the aseptically bored cups in the standardised inoculums of the test bacterial culture. The plates were incubated at 37 ^{0}C for 24 hours and the inhibition diameter read after incubation. The assay was carried out in duplicate and MICs of the test agents were taken as the lowest concentration of the extract showing clear zone of inhibition.

2.8.3 Minimum Bacteriocidal Concentration (MBC)

The agar well diffusion technique was employed in the study [11]. A 2 mm-diameter agar disc cut out from the inhibition zones of last three consecutive wells in each dilution showing inhibition in above MIC results. The discs were incubated into a fresh sterile nutrient broth medium. The broth cultures were incubated for 18 *hrs* at 37^{o}C, after which 0.1 *ml* was spread over a fresh sterile Mueller Hinton agar plate. In turn, this was also incubated at 37^{o}C for 18 *hrs* and the MBC was read as the least concentration showing no visible growth.

2.9 Antidiarrhoeal Testing

The anti-diarrhoeal activity of the MECD in castor oil- induced diarrhoea in rat model was carried out according to Venkatesan et al. [12]. Using Three-dose regimen (100, 200, and 400 *mg/kg* body weight *p.o*). Before animal study commenced, The MECD were administered orally to the other three groups of rats at the dose levels of 100, 200 and 400 *mg/kg* respectively. After one hour of treatment, all the animals were challenged with 1 *ml* of castor oil orally, and observed for consistency of faecal material [12]. The frequency of defaecation was observed on hourly basis for 4 *h* [13]. The percentage inhibition of the extract was calculated as follows:

2.10 Statistical Analysis

Data obtained was statistically analyzed using SPSS ver.22. One-way analysis of variance was employed to test for significant differences at 95% confidence level. Data was expressed as mean±S.D.

3. RESULTS

3.1 Extractive Yields

Yield of MECD and its corresponding fractions obtained in column chromatography is presented in Table 1.

Table 1. Percentage yield for the various extracts

Agent	Percentage Yield [%(*w/w*)]
MECD	6.99
HXE	8.01
MMCE	32.18
EToACE	12.70
BUE	10.57
AqE	4.32

3.2 Phytochemical Analysis

The phytochemical constituents of the extracts in Table 2 indicate that the MMCE and EToAcE have similar phytochemical constituents. They both showed the presence of alkaloids, terpenoids, steroids, glycosides, saponins, tannins and carbohydrates. The MECD contained all the phytochemicals in other extracts. The alkaloidal extract however contained a few constituents but had more of the alkaloidal tannates. The hexane extract contained more of flavonoids. They all contained various quantities of the principal constituents as indicated below:

3.3 Bioassay Guided Screening Studies

3.3.1 Anti-diarrhoeal effect of the MECD

The Table 3 indicates preliminary anti-diarrhoeal study of the MECD against castor oil induced diarrhoea. The extract inhibited the castor oil-induced diarrhoea in a dose-dependent manner. The dose-dependent (100, 200 and 400 *mg/kg p.o.*) post absorptive faecal weight indicated that the extract offered its best protection at 400 *mg/kg*. Although the different doses of the extract exhibited various levels of inhibition, only the

Table 2. The phytochemical constituents of the various extracts

Phytoconstituents	HxE	EToAcE	MECD	MMCE	BuE	AqE
Alkaloids	+	++	++	++	+	+
Terpenoids	+	+++	+	+++	++	-
Steroids	+	+++	+	+++	+	-
Acidity	-	-	+	+	-	+
Glycosides	-	+	+	+++	+	+
Resins	+	++	+	+	+	+
Carbohydrates	+	++	++	+	+	++
Saponins	+	++	+	++	+	-
Tannins	-	+++	++++	++	+	-
Flavonoids	+++	++	+	+	-	-
Fats and oil	+	-	+	-	-	-
Reducing sugar	-	+	-	-	-	-
Protein	-	-	+	+	-	-
Vitamin E	+	++	++	+	-	+
Vitamin A	+	+	+	+	+	_

- implies absent; + implies present in small amount; ++ implies present in moderately high concentration; +++ implies present in very high concentration; ++++ implies excessively present

atropine were able to produce significant ($p<0.05$) anti-diarrhoea protective potency.

3.4 Antimicrobial Studies

3.4.1 Sensitivity screening

The Table 4 summaries the microbial susceptibility screening of the pure and clinical isolates of the various fractions of the MECD. The AqE had no activity on the pure and clinical isolates used for the study. The activity of the various fractions on the organism showed varied activity with *S. kintambo*. The MECD and MMCE showed antibacterial activity to all the organisms when compared with the standard antibiotics [Ciprofloxacin (20 *µg/ml*)]. They also showed antibacterial activity against the Gram +ve and Gram –ve bacterial organisms used in the studies. The overall activity levels of the fractions are as follows:

MECD>MMCE>HXE>EToAcE>BUE>AqE. The BUE showed no activity on the Gram +ve organisms used in the study.

3.5 Antimicrobial Screening – MIC and MBC Assays

Based on the observed no activity of AqE on the microbial susceptibility screening, it was eliminated from the MIC and subsequent antidiarrhoeal predictive guided bio-assays. Table 5 shows the Minimum Inhibitory Concentration (MIC) of the pure and clinical isolates using the agar well diffusion. The results of the inhibitory effect showed similar pattern of antibacterial activity with the susceptibility screening. It shows no and little bacteriostatic activities at the nominated lower concentrations, 7.81 and 31.25 mg/ml respectively. The *S. kintambo* indicated high resistance to the antibacterial activity of all the extracts except at high concentrations (125 and 62.5 mg/ml) of MMCE and MECD. Fig. 1 indicates varied trend of bacteriocidal activity of all the extracts with MMCE & MECD showing consistent activity in all the tested organisms.

Table 3. Effect of Methanolic Extract of *Combretum dolichopetalum* (MECD) on mean defecation in 4 h in castor oil-induced diarrhoea in rats

Groups	Mean defecation in 4 hr. (Mean ± S.D)	% Inhibition of defecation
ET_{10} 2 *ml/kg*	1.52 ± 0.29	-
Atropine 3 *mg/kg*	0.65 ± 0.44	57.24*
$MECD_{100\ mg/kg}$	1.20 ± 0.21	20.92*
$MECD_{200\ mg/kg}$	0.52 ± 0.01	39.47*
$MECD_{400\ mg/kg}$	0.75 ± 0.48	50.66*

** P < 0.05 compared to negative control (ANOVA; LSD post hoc); MECD=Methanol extract of Combretum dolichopetalum; Values of mean defecation are shown as Mean ± SD (n = 5)*

Table 4. Antimicrobial activity of the extract – Sensitivity Screening

Extract	Concentration (*mg/ml*)	Inhibition Zone Diameter (*mm*)							
		S. aureus	***P. aeruginosa***	***S. kintambo***	***E. coli***	***E. coli-1***	***E. coli-2***	***E. coli-3***	***E. coli-4***
Control	250	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
EToAcE	250	15.66±1.73	20.33±1.16	0.00±0.00	17.66±1.16	15.67±1.16	0.00±0.00	17.67±0.58	16±1.00
AqE	250	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
MMCE	250	17.33±0.58	20.67±2.52	19.67±0.58	17.67±1.16	18.67±0.58	15.00±2.00	18.33±0.58	15.67±0.58
HxE	250	22.67±1.53	17.67±0.58	0.00±0.00	27.67±1.16	23.67±0.58	16.67±0.58	21.00±0.00	17.67±0.58
MECD	250	20.00±2.65	19.33±1.53	19.00±1.00	19.33±0.58	17.33±0.58	18.00±1.00	19.00±1.73	16.33±1.16
BuE	250	0.00±0.00	19.33±1.53	0.00±0.00	17.33±0.58	17.00±1.73	15.00±2.00	18.00±1.73	17.33±0.58
Ciprofloxacin	20 *µg/ml*	23.33±0.58	28.33±2.52	18.33±1.15	25.67±0.58	21.00±2.00	15.00±0.00	26.33±0.58	17.67±0.58

Control = DMSO (Dimethylsulphoxide)

Table 5. The Minimum Inhibitory Concentration (MIC) of the various extracts for the pure and clinical isolates using Agar- well diffusion techniques

Extract	Concentration (*mg/ml*)	Inhibition zone diameter (*mm*)							
		S. aureus	***P. aeruginosa***	***S. kintambo***	***E. coli***	***E. coli-1***	***E. coli-2***	***E. coli-3***	***E. coli-4***
Control	125	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
EToAcE	125	12.00±1.41	18.00±1.41	0.00±0.00	15.00±0.00	12.00±0.71	0.00±0.00	15.5±0.71	13.5±0.71
	62.5	11.00±0.00	13.5±0.71	0.00±0.00	12.5±0.71	10.50±0.71	0.00±0.00	13.00±0.00	10.50±0.00
	31.25	9.50±0.71	10.50±0.71	0.00±0.00	10.50±0.71	8.50±0.71	0.00±0.00	11.00±0.00	9.00±0.00
	15.63	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	8.50±0.71	0.00±0.00
	7.81	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
MMCE	125	15.00±0.00	19.00±0.00	16.00±1.41	15.45±0.71	15.50±2.12	13.50±0.71	15.00±1.41	14.00±1.41
	62.5	9.50±0.71	15.00±0.00	13.00±0.00	12.50±0.71	13.50±0.71	10.00±1.41	12.50±0.71	11.00±0.00
	31.25	0.00±0.00	12.00±0.00	0.00±0.00	9.00±0.00	10.50±0.71	8.00±0.00	10.00±0.00	8.00±0.00
	15.63	0.00±0.00	10.00±0.00	0.00±0.00	0.00±0.00	9.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	7.81	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
HxE	125	22.5±2.12	15.00±1.41	0.00±0.00	26.00±0.71	21.00±0.00	14.50±0.71	16.00±1.41	16.00±1.41
	62.5	19.50±0.71	12.50±0.71	0.00±0.00	19.50±0.71	18.50±0.71	11.50±0.71	13.50±0.71	13.00±1.41
	31.25	16.00±1.41	10.00±0.00	0.00±0.00	11.50±0.71	12.50±0.71	10.00±0.00	9.00±1.41	11.00±0.00
	15.63	13.00±1.41	0.00±0.00	0.00±0.00	0.00±0.00	9.00±1.41	0.00±0.00	0.00±0.00	8.50±0.71
	7.81	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
MECD	125	17.50±0.71	18.00±1.41	16.50±0.71	17.50±0.71	15.50±0.71	16.50±0.71	18.50±0.71	14.50±0.71
	62.5	15.00±0.00	13.00±0.00	13.00±0.00	14.00±0.00	10.00±0.00	13.50±0.71	14.50±0.71	11.50±0.71
	31.25	10.50±0.71	10.00±0.00	0.00±0.00	10.00±0.00	0.00±0.00	10.50±0.71	10.00±0.00	9.00±0.00
	15.63	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	8.50±0.71	0.00±0.00	0.00±0.00
	7.81	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
BuE	125	0.00±0.00	17.00±0.00	0.00±0.00	14.50±0.71	13.00±0.00	13.50±0.71	17.00±0.00	15.50±0.71
	62.5	0.00±0.00	12.50±0.71	0.00±0.00	12.00±0.00	11.00±0.00	10.50±0.71	15.00±0.00	11.50±0.71
	31.25	0.00±0.00	9.50±0.00	0.00±0.00	0.00±0.00	9.00±0.00	8.00±0.00	10.00±0.00	9.00±0.00
	15.63	0.00±0.00	9.00±0.72	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	8.00±0.00
	7.81	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

n = 2; Diameter of cork borer = 6 mm; Control = DMSO (Dimethylsulphoxide)

4. DISCUSSION

The use of natural medicines for the treatment of diseases and ailments is increasing globally. In developing countries, a quarter of infant and childhood mortality is related to diarrhoea. Use of conventional pharmaceutical antidiarrhoeal drugs reduces the symptoms of diarrhoea (loose stool consistentency, frequency of defeaction, and excessive stool weight) by effect on intestinal transit, mucosal transport or luminal content. However oral rehydration therapy has reduced acute diarrhoeal disease mortality whereas chronic diarrhoea remains a life threatening problem, in which malnutrition is a common coexisting and complicated factor. Number of factors, such as infective, immunological and nutritional are involved in the perpetuation of the diarrhoea syndrome [2]. Many plants conveniently available in Nigeria used in traditional folklore medicine for treatment of diarrhoea are known to offer a protective effect on the intestinal tract.

In spite of the importance of diarrhoea as a public health problem, it is counted with relatively reduced number of drugs for its treatment. The World Health Organization (WHO) encouraged studies for the treatment and prevention of diarrhoeal diseases depending on traditional medical practices [14]. The above consideration stimulated the interest to evaluate the antidiarrhoeal effect of *Combretum dolichopetalum* against castor oil induced-diarrhoea and antimicrobial efficacy of some of its extract in a bioassay-guided manner.

The preliminary screening of anti-diarrohoeal effect of the Methanolic Extract of *Combretum dolichopetalum* (MECD) indicates its dose-dependent reduction of the number of times of defecating. The extract showed more potency than the standard anti-diarrhoea drug (atropine) used. Thus, it was selected for futher bioassay-guided separation studies. Chromatography differential solvent separation of the MECD extract with 10 % Methylene chloride-methanol (MMCE), Ethylacetate (EtoAcE), *n*-Butanol (BUE), *n*-Hexane (HxE) and Water (AqE) respectively yielded the various extracts used subsequently in bioassay-guided manner to rationalise the antidiarrhoea effect of MECD. The phytochemical analyses of the extracts indicated the presence of several bioactive components in the extract and fractions. The MECD contained all the phytochemical constituents in other extracts. The MMCE and EToAcE has similar phytochemical constituents (alkaloids, tannins, terpenoids, steroids, glycosides, saponins and carbohydrates). Among the fractions, AqE contained the least number of phytochemical constituents while EToAcE had the highest. The phytochemical constituents may be responsible for the activity noted with the MECD. Previous report have demonstrated that antidiarrhoeal activity with tannin and alkaloids [15], saponins,

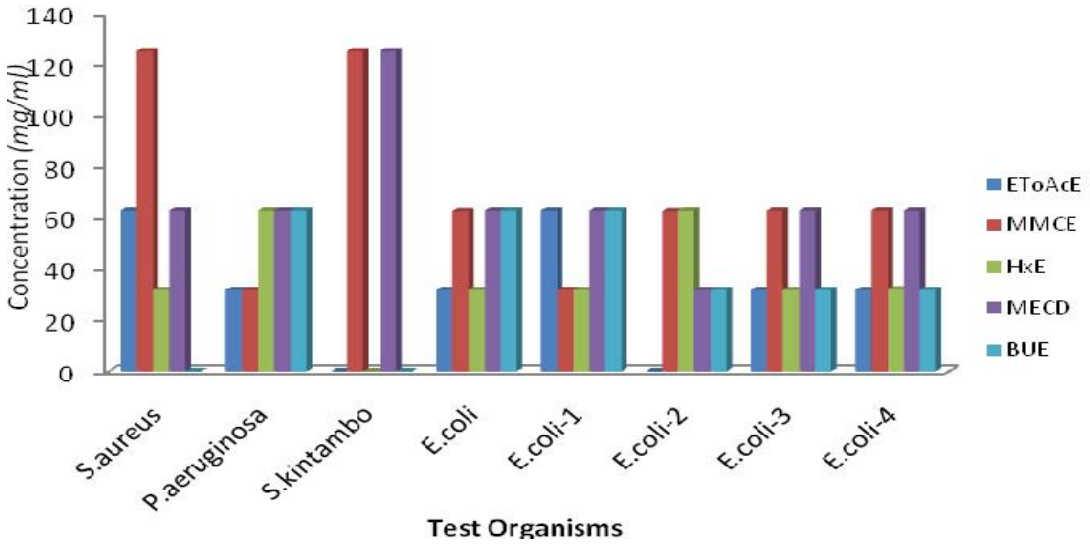

Fig. 1. Minimum Bactericidal Concentration (MBC) of the various extracts for the pure and clinical Isolates

reducing sugars and sterols and/or terpenes containing extracts. One or more of these phytochemials may be responsible for the antidiarrhoeal activity of methanol extract. HxE indicates much presence of flavonoids compared to all the extracts - tanins, alkaloids, steroids, terpenoids and saponins were evident in the MMCE and EToAcE compared to MECD. The extract EToAcE, MMCE and BuE indicated positive reactions to tannin and glycosides, suggesting a possible presence of flavone glycoside (glucotannin).

The link between bacteria in causing disease have been well established in literature [16]. Also, plant extract has been used for purification of water [17], considering that water can serve as a means of transmitting bacteria that causes diarrhoea into the body. Thus, the preliminary antibacterial sensitvity screening of extracts is important and showed that they exhibited antibacterial activity against pure and clinical isolates (Gram positives and Gram negatives) compared to the standared antibiotics, ciprofloxacine. According to Suffredini et al. [18], gram negative bacteria are hardly susceptible to plant extract in doses as low as 200 *mg/ml*, thus concentrations of 250 *mg/ml* were used in testing the antibacterial sensitivity screening activity. The AqE was the least active plant extract against all the tested bacterial species showing no activity. The AqE was eliminated subsequently for the Minimum inhibitory and Bacteriocidal Concentration test (MIC and MBC).

A growing body of evidence indicates a relationship between colonization by micro organism and a variety of gastrointestinal diseases. The implication of enteropathogenic *E.coli*, *Vibrio cholerae* and non-typhoid Salmonella to mention but a few, in pathogenesis of diarrhoea and the use of antibiotics in its management stimulated the study of antimicrobial activity of the extracts. However, all extract showed an *in vitro* antibacterial activity (MIC) aganist at least two referenced bacterial strains and three of the clinical isolates of *E.coli (1-3)*. *E.coil* 4 and *P.aerugenosa* were the most sensitive bacteria to the extract with 85.71 % of plant extracts showing activity against *E.coli 4* at concentrations in range 15.63- 31.25 *mg/ml*. *Salmonella kintambo* was least sensitive with only 74.14% of extract having inhibitory activity. The most active extracts against *S.kintambo* are MMCE and MECD. The bacterial susceptibility trend for the various extract are in the order *P.aeruginosa* > *E.coil* 4 > *E.coil* 3 > *E.coil* 1 > *E.coil* > *E.coil* 2 >*S.aureus* > *S.kintambo.*

Antimicrobial therapy is indicated effective in reducing the duration of infectious diarrhoea for moderate and severe episodes and ciprofloxacin is considered the effective drug of choice for treatment of traveller's diarrhoea in adult in most regions of the world. Also a large body of evidence show that antimicrobial agents can reduce the severity and duration of some intestinal infections, especially in those bacteria and infections that produce acute watery diarrhoea. Antimicrobials are also useful in bacterial intestinal infections that cause systemic involvement. The MIC trend have similar bacteriocidal activity (MBC) pattern indicating the presence of common phytochemical principle for the consistent antibacterial activity of MMCE and MECD in all the test organisms. Nonetheless, the broad spectrum activity against all the bacterial strains and at tested concentrations 15.63 – 125 *mg/ml* of MMCE and MECD suggest a possible common compound phytochemical constituents activity on the bacterial strains. Though low sensitivity of some organisms could be attributed to the fact that bacteria is naturally resistant to many antibiotics due to the permeability barrier offered by its outer membrane.

5. CONCLUSION

In conclusion, the extract possessed antidiarrhoea potentials and it fractions showed antimicrobial potentials against the test organism. This can be labeled to the rich phytocompounds, present in the plant extract such as tannins, steroids and terpenes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the Ethical Committee of the University of Nigeria, Nsukka, Enugu State, Nigeria, with approval number UNN/FOS/BCH/23/5243.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/60090