*Asian Journal of Applied Sciences (ISSN: 2321 – 0893)*
*Volume 06 – Issue 04, August 2018*

# *In vitro* Assessment of *Euclea crispa* (Thunb.) Leaf Extracts against *Campylobacter* spp. and *Escherichia coli* - Common Diarrhoeal Agents

Kazeem Adekunle Alayande[1, 2,*], Carolina (H) Pohl [1] and Anofi Omotayo Tom Ashafa[2]

[1] Department of Microbial, Biochemical & Food Biotechnology, University of the Free State, P.O. Box 339, Nelson Mandela Drive, 9301 Bloemfontein, South Africa

[2] Phytomedicine and Phytopharmacology Research Group, Department of Plant Sciences, University of the Free State, Qwaqwa Campus, P/Bag X13, Phuthaditjhaba 9866, South Africa

**Corresponding author's email: jkadekunle2 [AT] gmail.com*

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**ABSTRACT—** ***Diarrhoea is a common childhood disease with high mortality rate. This study thus aimed at assessing effect of Euclea crispa leaf extract and its fractions against diarrhoea causing bacterial isolates and determining time-kill dynamics by each of the potent fractions. Susceptibility of each isolates was determined by agar well diffusion while the minimum bacteriostatic and bactericidal concentrations were determined by agar dilution method. Time-Kill dynamics was evaluated over a period of 120 min against Escherichia coli (1323) representing Gram negative isolates. The zones of inhibition exhibited by the leaf extract at 20 mg/ml range between 17±0.28 and 22±0.00 mm while that of the partitioned fractions at 10 mg/ml are between 14±0.00 and 22±0.00 mm. MICs of the leaf extract range between 0.31 and 2.50 mg/ml. The lowest MIC (0.08 mg/ml) is exhibited by the fractions partitioned into ethyl acetate, n-butanol and water while that of n-hexane and chloroform is 0.16 mg/ml. The lowest MBC exhibited by all the fractions is 0.31 mg/ml except that of the chloroform (1.25 mg/ml). Total mortality was achieved by the ethyl acetate fraction at a concentration of 2 × MIC after 120 min of contact time, meanwhile the mortality rate achieved by n-butanol, n-hexane, aqueous and chloroform fractions were 98, 94.6, 91.8 and 83.7% respectively under similar condition. This study showcase significant antidiarrhoeal potential of Euclea crispa leaf extracts and equally indicates a source of readily available therapeutic agent against diarrhoeal infection in South Africa and environs.***

**Keywords—** Diarrhoea, Bacteriostatic, Bactericidal, Time-kill dynamics, FT-IR

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## 1. INTRODUCTION

Diarrhoea infection remains a major public health problem across developing nations. It is a common cause of childhood diseases with high mortality rate among children under the age of five [1]. Diarrhoea is the second leading killer of children, nearly one fifth of the children under the age of five die due to dehydration, compromised immunity or malnutrition associated with diarrhoea infection [2]. It was estimated in 2011 that diarrhoea accounted for 10% of the 6.9 million deaths among children [3]. Among the major bacterial pathogens implicated in diarrhoeal infection across the globe are *Escherichia coli*, *Campylobacter*, *Salmonella*, *Shigella*, *Vibrio*, and *Yersinia* species. And data from United States indicate that *Campylobacter* and *Salmonella* spp. are the most frequent, followed by *Shigella* spp. and Shiga toxin-producing *E.* coli [4]. Although most diarrhoeal episodes are self limiting and the resulting dehydration can be controlled with mere oral rehydration therapy [5]. However, it would be appropriate to respond to diarrhoeal infection promptly as it symptomizes, most importantly the more severe conditions which may degenerate into complications or eventual death.

Medicinal plants provide cheaper and readily available therapeutic source for most infectious diseases due to some valuable bioactive compounds that are evidently reside in them. Historically, plants have provided source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and wellbeing [6]. About 80% of the people living in developing countries rely directly on infusion or poultices of plants for their medical remedies [7]. Flavonoids which is one of the chemical groups found in medicinal plants has been reported to have antidiarrhoeal activity by Schuier *et al*. [8].

*Euclea crispa* (Thunb.) commonly referred to as blue guarri is a small evergreen tree with average height of between 2 and 6 m. It is from the family Ebenaceae [9]. It is also known as one of the most common trees in South Africa and is extensively utilized in traditional medicine for a wide range of diseases [10]. Alfred [11] reported the traditional use of decoction from the root of *E. crispa* as antitussive by the people of Zimbabwe and it is also in use in South African

healing tradition as psychoactive agent [12]. The antimicrobial activity of the leaf extract against various human pathogens had also been reported [10, 13]. This study is therefore designed to carry out intensive *in vitro* assessment of antidiarrhoeal property of the leaf extracts of *E. crispa*.

# 2. METHODS

## 2.1 Collection of plant sample

Fresh leaf sample of *Euclea crispa* was harvested from Puthaditjhaba, South Africa. This was authenticated at the University of the Free State herbarium with collection of Taylor and Van Wyk (1994, reference number: 6404000-400). The leaves were thereafter air dried at room temperature to a constant weight and then powdered. The powdered plant sample was then extracted and partitioned as previously described [13]

## 2.2 Microorganisms

Microorganisms used in this study include typed cultures and locally isolated organisms. This include; *Campylobacter coli* (ATCC 33559), *C. coli* (ATCC 43478), *C. jejuni* (ATCC 49943), *C. jejuni* (ATCC 29428), *C. jejuni* (NCTC 11322), *C. jejuni* (NCTC 11351), *Escherichia coli* (ATCC839), *E. coli* 1304, *E. coli* 1080, *E. coli* B1634, *E. coli* B 98, *E. coli* B 841, *E. coli* B771, *E. coli* 1323, *E. coli* WCD1.

## 2.3 Chemical groups profiling of the fractions

This was assessed through fourier transform infrared spectrometry (FT-IR) analysis. FT-IR spectra of each of the potent fractions were obtained on a FT-IR spectrometer (PerkinElmer, precisely) with ATR attachment (PIKE, miRonle).

## 2.4 Susceptibility test

Agar-well diffusion method as described by and EUCAST [14] was employed to determine which of the selected isolates was sensitive to the plant extracts using Mueller-Hinton agar, a lose agar designed to allow free and proper diffusion of antimicrobial agents into the agar matrix. Nutrient broth (Oxoid, UK) cultures of the bacterial isolates (18 to 24 h) were standardized using McFarland scale (0.5 McFarland standard). The standard inoculums (0.1 ml) was subcultured into Mueller-Hinton Agar (Oxoid, UK) and poured into a Petri dish. This was allowed to set before wells were bored into the agar medium using a sterile cork borer (6 mm). The wells were filled with prepared solution of the extract and the plates were incubated at 37 $^0$C for 24 h. The *campylobacter* spp. were inoculated on MHA with 5% sheep blood and incubated under micro-aerophilic condition [15]. After which the plates were observed for zones of inhibition. The effects of the extract on bacterial isolates were compared with that of streptomycin (1 mg/ml) and tetracycline (0.1 mg/ml). Sterile distilled water and 10% methanol were used as control and the experiment was carried out in replicates of three.

## 2.5 Determination of minimum inhibitory concentrations (MICs)

This was determined against susceptible isolates via agar dilution methods as described by EUCAST [16] and CLSI M07A10 [17]. Two-fold dilutions of the extract were prepared and 2 ml of different concentrations of the aliquot was added to 18 ml of sterile molten Nutrient Agar to give final concentrations range of 0.313 to 20.0 mg/ml (leaf extract) and 0.078 to 10 mg/ml (fractions). This was aseptically poured into a sterile Petri dish and allowed to set. The surface of the medium was allowed to dry before streaking with 18 h old standard inoculums (0.5 McFalland standard) and then incubated at 37 $^0$C for 48 h. After which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that inhibited the growth of the bacteria cells.

## 2.6 Determination of minimum bactericidal concentrations (MBCs)

This was determined as described by Akinpelu *et al*. [18]. Samples were picked from the line of streaks with no visible growth on the MICs result plates and sub-cultured onto freshly prepared Nutrient Agar plates. The plates were incubated at 37 $^0$C for 48 h. The MBCs were taken as the lowest concentration of the extracts that did not show any visible growth on the new set of Nutrient Agar plates.

## 2.7 Determination of killing rate by the potent fractions

The killing rate by the potent fractions was determined following the method described by Odenholt *et al.* [19] and Akinpelu *et al*. [20] with little modification. This was carried out against *Escherichia coli* (1323) as a representative of Gram negative isolates. Nutrient Broth culture (18-24 h) was standardized (0.5 McFalland standard) and viable count was determined. Thereafter, 5 ml of the known cell density of the bacterial suspension was added to 45 ml of different concentrations of the fractions relative to the MIC. The resulting suspension was mixed properly and held at room temperature while the killing rate was determined over a period of 120 min. Exactly 0.5 ml of the suspension was withdrawn at appropriate time intervals and transferred into 4.5 ml Nutrient Broth recovery medium containing 3%

Tween80. This was serially diluted in sterile physiological saline and 0.1 ml of the final dilution was plated with Nutrient Agar. The plate was incubated at 37 $^{0}$C for 48 h. The control experiment was set up without inclusion of extract. Viable counts were made in triplicates for each sample and reduction in the colony counts indicate killing by the antimicrobial agents.

## 3. RESULTS

The FT-IR analysis of the potent fractions indicated the presence of certain functional groups *viz;* alkanes, alkenes, alkynes, alcohol, phenol, aldehyde, aromatics, ether, sulfoxides, amides and amines (figure 1 & table 1).

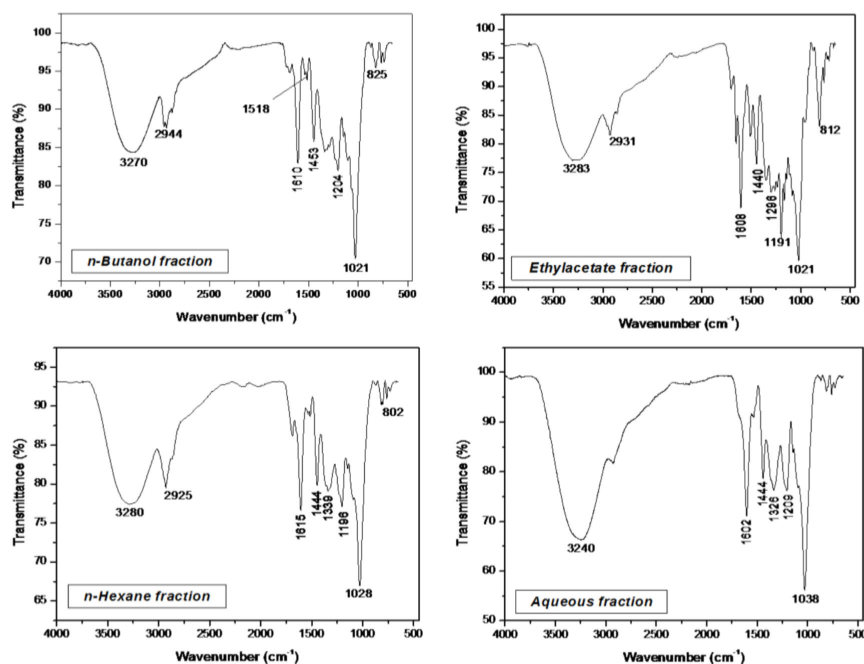


**Figure 1:** FT-IR Spectra of the n-butanol, ethyl acetate, n-hexane and aqueous fractions of *E. crispa* leaf extract

**Table 1:** Chemical group profiling of the potent fractions

| Functional groups | Functional groups description | BUT | ETY | nHEX | AQU |
|---|---|---|---|---|---|
| | | Absorption frequency (cm$^{-1}$) | | | |
| | C–O–H bending | 1453 | 1440 | 1444 | 1444 |
| Alcohol, Phenol | O–H stretch | 3270 | 3283 | 3280 | 3240 |
| Alkanes | C–H stretch | 3270 | 3283 | 3280 | 3240 |
| Alkenes | =C–H out of plane bend | 825 | 812 | 802 | – |
| | C=C stretch | 1610 | 1600 | 1615 | 1602 |
| Alkynes | ≡C-H stretch | 3270 | 3283 | 3280 | 3240 |
| Sulfoxides | S=O stretch | 1021 | 1021 | 1028 | 1038 |
| Aromatics | C=C stretch | 1610 | 1608 | 1615 | 1602 |
| | C=C stretch | 1518 | – | – | – |
| Aldehyde | –CHO stretch | 2944 | 2931 | 2925 | – |
| | N–H bending | 1610 | 1600 | 1615 | 1602 |
| Amide ($1^{0}$, $2^{0}$) | N–H stretch | 3270 | 3283 | 3280 | 3240 |
| Amines | N–H ($1^{0}$) bending | 1610 | 1608 | 1615 | 1602 |
| | C–N stretch | 1204 | – | 1196 | 1209 |
| Ethers | C–O stretch | 1204 | 1296 | 1196 | 1209 |

Keys: BUT = n-Butanol fraction, ETY = Ethyl acetate fraction, nHEX = n-Hexane fraction and AQU = Aqueous fraction

The susceptibility test indicates that all the test isolates were sensitive to the plant extract and its fractions with different degrees of inhibition zones. The widest zone of inhibition (22±00 mm) was exhibited by n-butanol and ethyl

acetate fractions against a number of *Campylobacter* and *E. coli* strains at a concentration of 10 mg/ml, while the minimum (14±00 mm) was exhibited by aqueous fraction against *Campylobacter* spp. under the same condition and none of the *Campylobacter* spp. was sensitive to the chloroform fraction (table 2 and 3). The lowest minimum inhibitory concentration (MIC) by the leaf extract was 0.31 mg/ml against a few strains of *E. coli*, while the minimum of 0.08 mg/ml was exhibited by n-butanol, ethyl acetate and aqueous fractions against different strains of *E. coli* (table 4). On the other hand, the lowest minimum bactericidal concentration (MBC) exhibited by the leaf extract was 1.25 mg/ml while the lowest MBC exhibited by the n-butanol, ethyl acetate, n-hexane and aqueous fractions was 0.31 mg/ml and that of the chloroform fraction was 0.63 mg/ml (table 5).

**Table 2:** The sensitivity patterns of *Euclea crispa* leaf extract against test diarrhoeal agents

| **Bacterial isolates** | **Zones of inhibition (mm) **** | | | |
|---|---|---|---|---|
| | ***Euclea crispa* (20 mg/ml)** | **Streptomycin (1 mg/ml)** | **Tetracycline (0.1 mg/ml)** | **SDW** |
| *Campylobacter coli* (ATCC 33559) | 18±0.29 | 26±0.58 | 25±0.58 | 0 |
| *C. coli* (ATCC 43478) | 20±0.50 | 27±1.00 | 26±0.00 | 0 |
| *C. jejuni* (ATCC 49943) | 22±0.00 | 26±0.00 | 28±1.00 | 0 |
| *C. jejuni* (ATCC 29428) | 21±0.58 | 26±0.76 | 29±0.00 | 0 |
| *C. jejuni* (NCTC 11322) | 20±0.00 | 27±0.58 | 25±1.00 | 0 |
| *C. jejuni* (NCTC 11351) | 18±0.58 | 26±0.00 | 28±1.15 | 0 |
| *Escherichia coli* (ATCC839) | 20±0.00 | 27±0.00 | 26±0.58 | 0 |
| *E. coli* 1304 | 18±0.00 | 25±1.15 | 25±0.50 | 0 |
| *E. coli* 1080 | 19±0.58 | 25±0.00 | 27±0.00 | 0 |
| *E. coli* B1634 | 20±0.00 | 27±0.58 | 25±0.58 | 0 |
| *E. coli* B 98 | 18±1.00 | 28±0.00 | 29±1.15 | 0 |
| *E. coli* B 841 | 20±1.00 | 27±1.00 | 28±0.00 | 0 |
| *E. coli* B771 | 20±1.15 | 26±0.58 | 25±1.00 | 0 |
| *E. coli* 1323 | 18±1.15 | 27±0.00 | 27±0.50 | 0 |
| *E. coli* WCD1 | 21±0.58 | 28±0.28 | 27±0.58 | 0 |

KEY: ATCC = American type culture collection, NCTC = National collection of type cultures, SDW = Sterile distilled water, (mm) ** = As Mean of three replicates.

**Table 3:** Sensitivity patterns of fractions obtained from *Euclea crispa* extract against test diarrhoeal agents

| **Bacterial isolates** | **Zones of inhibition (mm) **** | | | | | |
|---|---|---|---|---|---|---|
| | **BUT (10 mg/ml)** | **ETH (10 mg/ml)** | **nHEX (10 g/ml)** | **AQU (10 mg/ml)** | **CHL (10 mg/ml)** | **MET (10%)** |
| *Campylobacter coli* (ATCC 33559) | 20±0.28 | 20±0.00 | 20±0.00 | 14±0.00 | 0 | 0 |
| *C. coli* (ATCC 43478) | 20±0.58 | 20±1.00 | 18±0.58 | 14±0.50 | 0 | 0 |
| *C. jejuni* (ATCC 49943) | 22±0.00 | 20±0.58 | 18±0.00 | 16±0.00 | 0 | 0 |
| *C. jejuni* (ATCC 29428) | 22±0.58 | 22±0.00 | 19±1.04 | 16±0.58 | 0 | 0 |
| *C. jejuni* (NCTC 11322) | 22±0.50 | 22±0.00 | 18±0.00 | 16±0.00 | 0 | 0 |
| *C. jejuni* (NCTC 11351) | 20±0.00 | 20±0.58 | 18±0.58 | 16±1.00 | 0 | 0 |
| *Escherichia coli* (ATCC839) | 20±0.00 | 20±1.15 | 18±0.28 | 20±0.00 | 18±1.00 | 0 |
| *E. coli* 1304 | 22±1.00 | 22±0.00 | 16±0.50 | 16±1.15 | 18±0.00 | 0 |
| *E. coli* 1080 | 20±1.15 | 20±1.00 | 18±0.00 | 20±0.58 | 18±0.00 | 0 |
| *E. coli* B1634 | 18±0.50 | 22±0.58 | 18±1.15 | 22±0.00 | 16±0.50 | 0 |
| *E. coli* B 98 | 18±0.00 | 20±0.58 | 17±1.00 | 16±1.00 | 18±0.00 | 0 |
| *E. coli* B 841 | 20±0.00 | 20±0.00 | 18±1.00 | 21±0.00 | 16±0.58 | 0 |
| *E. coli* B771 | 18±1.00 | 21±1.15 | 18±0.50 | 16±0.00 | 17±0.28 | 0 |
| *E. coli* 1323 | 20±0.00 | 22±0.00 | 18±1.00 | 18±0.58 | 16±0.00 | 0 |
| *E. coli* WCD1 | 22±0.50 | 20±1.00 | 20±0.00 | 19±1.15 | 18±0.58 | 0 |

KEY: ATCC = American type culture collection, NCTC = National collection of type cultures, BUT = n-Butanol fraction, ETH = Ethyl acetate fraction, nHEX = n-Hexane fraction, CHL = Chloroform fraction, AQU = Aqueous fraction, MET = methanol, 0 = Not sensitive, (mm) ** = As Mean of three replicates.

**Table 4:** The minimum inhibitory concentrations (MICs) of the leaf extract of *E. crispa,* fractions and standard antibiotics exhibited against test diarrhoeal agents

| **Bacterial isolates** | **ECE (mg/ml)** | **BUT (mg/ml)** | **ETH (mg/ml)** | **nHEX (mg/ml)** | **AQU (mg/ml)** | **CHL (mg/ml)** | **STREP (mg/ml)** | **TET (mg/ml)** |
|---|---|---|---|---|---|---|---|---|
| *Campylobacter coli* (ATCC 33559) | 1.25 | 0.63 | 0.63 | 0.31 | 2.50 | ND | 0.063 | 0.025 |
| *C. coli* (ATCC 43478) | 1.25 | 0.63 | 0.31 | 0.31 | 1.25 | ND | 0.063 | 0.013 |
| *C. jejuni* (ATCC 49943) | 0.63 | 0.31 | 0.63 | 0.31 | 2.50 | ND | 0.031 | 0.013 |
| *C. jejuni* (ATCC 29428) | 0.63 | 0.31 | 0.31 | 0.31 | 1.25 | ND | 0.016 | 0.006 |
| *C. jejuni* (NCTC 11322) | 1.25 | 0.31 | 0.63 | 0.63 | 1.25 | ND | 0.016 | 0.025 |
| *C. jejuni* (NCTC 11351) | 1.25 | 0.63 | 0.63 | 0.63 | 2.50 | ND | 0.008 | 0.013 |
| *Escherichia coli* (ATCC839) | 1.25 | 1.25 | 0.63 | 1.25 | 2.50 | 1.25 | 0.016 | 0.013 |
| *E. coli* 1304 | 1.25 | 0.63 | 0.08 | 1.25 | 1.25 | 0.16 | 0.125 | 0.100 |
| *E. coli* 1080 | 0.63 | 0.63 | 0.31 | 1.25 | 0.63 | 0.63 | 0.031 | 0.050 |
| *E. coli* B1634 | 0.63 | 0.31 | 0.16 | 0.31 | 0.08 | 0.31 | 0.031 | 0.050 |
| *E. coli* B 98 | 0.63 | 0.63 | 0.31 | 0.63 | 1.25 | 0.63 | 0.008 | 0.006 |
| *E. coli* B 841 | 0.31 | 0.16 | 0.16 | 0.63 | 0.08 | 0.63 | 0.004 | 0.100 |
| *E. coli* B771 | 0.31 | 0.31 | 0.16 | 0.31 | 2.5 | 0.31 | 0.250 | 0.100 |
| *E. coli* 1323 | 0.63 | 0.16 | 0.08 | 0.31 | 0.16 | 0.63 | 0.004 | 0.050 |
| *E. coli* WCD1 | 0.31 | 0.08 | 0.16 | 0.16 | 0.16 | 0.63 | 0.004 | 0.006 |

KEY: ATCC = American type culture collection, NCTC = National collection of type cultures, ECE = *Euclea crispa* leaf extract, BUT = n-Butanol fraction, ETH = Ethyl acetate fraction, nHEX = n-Hexane fraction, CHL = Chloroform fraction, AQU = Aqueous fraction, STREP = Streptomycin, TET = Tetracycline, ND = Not determined.

**Table 5:** The minimum bactericidal concentrations (MBCs) of the leaf extract of *E. crispa*, fractions and standard antibiotics exhibited against test diarrhoeal agents

| Bacterial isolates | ECE (mg/ml) | BUT (mg/ml) | ETH (mg/ml) | nHEX (mg/ml) | AQU (mg/ml) | CHL (mg/ml) | STREP (mg/ml) | TET (mg/ml) |
|---|---|---|---|---|---|---|---|---|
| *Campylobacter coli* (ATCC 33559) | 5.00 | 1.25 | 1.25 | 1.25 | 5.00 | ND | 0.125 | 0.050 |
| *C. coli* (ATCC 43478) | 2.50 | 1.25 | 0.63 | 1.25 | 2.50 | ND | 0.125 | 0.025 |
| *C. jejuni* (ATCC 49943) | 1.25 | 0.63 | 1.25 | 0.63 | 5.00 | ND | 0.063 | 0.025 |
| *C. jejuni* (ATCC 29428) | 1.25 | 0.63 | 0.63 | 0.63 | 2.50 | ND | 0.063 | 0.025 |
| *C. jejuni* (NCTC 11322) | 2.50 | 0.63 | 1.25 | 1.25 | 2.50 | ND | 0.031 | 0.050 |
| *C. jejuni* (NCTC 11351) | 5.00 | 0.63 | 0.63 | 1.25 | 2.50 | ND | 0.031 | 0.025 |
| *Escherichia coli* (ATCC839) | 2.50 | 1.25 | 1.25 | 2.50 | 2.50 | 2.50 | 0.031 | 0.025 |
| *E. coli* 1304 | 2.50 | 1.25 | 0.31 | 2.50 | 2.50 | 0.63 | 0.125 | 0.100 |
| *E. coli* 1080 | 1.25 | 1.25 | 0.63 | 2.50 | 1.25 | 1.25 | 0.063 | 0.100 |
| *E. coli* B1634 | 1.25 | 0.63 | 0.31 | 0.63 | 0.63 | 1.25 | 0.063 | 0.013 |
| *E. coli* B 98 | 1.25 | 1.25 | 0.63 | 1.25 | 1.25 | 1.25 | 0.031 | 0.100 |
| *E. coli* B 841 | 1.25 | 0.31 | 0.31 | 0.63 | 0.31 | 1.25 | 0.016 | 0.100 |
| *E. coli* B771 | 1.25 | 0.63 | 0.31 | 0.63 | 2.50 | 0.63 | 0.250 | 0.100 |
| *E. coli* 1323 | 1.25 | 0.31 | 0.31 | 0.63 | 0.63 | 0.63 | 0.016 | 0.050 |
| *E. coli* WCD1 | 1.25 | 0.31 | 0.31 | 0.31 | 0.31 | 1.25 | 0.016 | 0.013 |

KEY: ATCC = American type culture collection, NCTC = National collection of type cultures, ECE = *Euclea crispa* leaf extract, BUT = n-Butanol fraction, ETH = Ethyl acetate fraction, NHEX= n-Hexane fraction, CHL = Chloroform fraction, AQU = Aqueous fraction, STREP = Streptomycin, TET = Tetracycline, ND = Not determined.

At a concentration of 1 × MIC after 15 min of contact time, the mortality rate by the fractions partitioned into n-butanol, ethyl acetate, n-hexane, chloroform and water are 58.6, 51.4, 44.9, 49.4 and 39.7% respectively. Under similar condition after 90 min of contact time, increase in the mortality rate was also observed for all the fractions and finally after 120 min of contact time the rate achieved by each of the fractions is 85.7, 92.9, 7 9.4, 80.6 and 75.4% respectively (fig. 2). When the concentration of the fractions was increased to 2 × MIC, a general increase in the killing rate was observed. At this concentration, total elimination of the bacterial cells was achieved by ethyl acetate fraction after 120 min of contact time while 98, 94.6, 83.7 and 91.8% mortality were achieved by n-butanol, n-hexane, chloroform and aqueous fractions respectively (fig. 3). At a concentration of 3 × MIC, similar trend was observed. Total elimination of the bacterial cells was achieved by ethyl acetate fraction after 60 min of contact time and after 120 min by the fraction partitioned into n-butanol (fig. 4).

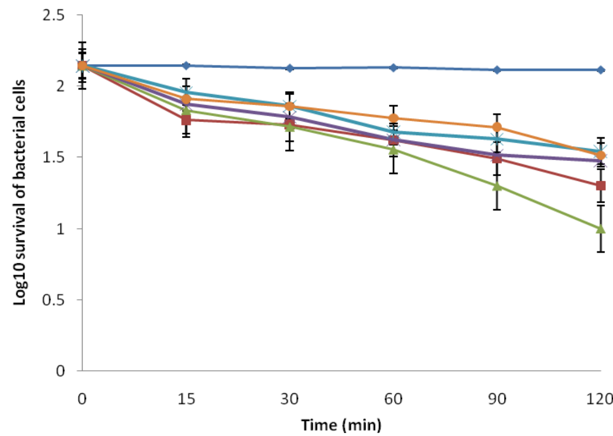


**Figure 2:** The rate of killing of *E. coli* (1323) by the fraction partitioned into water (), n-Butanol (), Chloroform (), Ethyl acetate (), n- Hexane fraction () at 1× MIC. Control ()

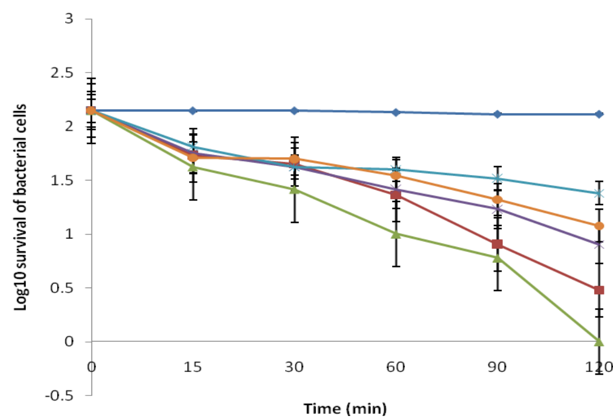


**Figure 3:** The rate of killing of *E. coli* (1323) by the fraction partitioned into water (), n-Butanol (), Chloroform (), Ethyl acetate (), n- Hexane fraction () at 2 × MIC. Control ()

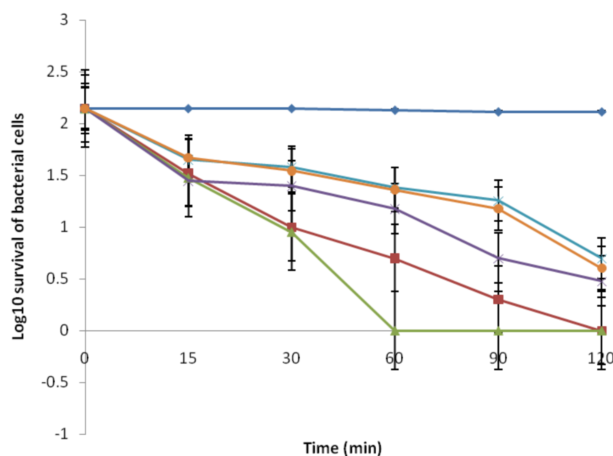


**Figure 4:** The rate of killing of *E. coli* (1323) by the fraction partitioned into water (), n-Butanol (), Chloroform (), Ethyl acetate (), n- Hexane fraction () at 3 × MIC. Control ()

## 4. DISCUSSION

Susceptibility test reveals that all the test isolates were sensitive to the leaf extracts except for the fraction partitioned into chloroform which is not active against test *Campylobacter* spp. Mean while, agar dilution assay used to determine MICs/MBCs clearly indicates various degree of potency by different fractions. The lowest MIC value of 0.08 mg/ml was exhibited by the fractions partitioned into n-butanol, ethyl acetate and water against different strains of *E. coli*. This

organism is best-known among human intestine microbial flora and as well as versatile gastrointestinal pathogen. While different strains of *E. coli* that cause diarrhoea are known to have distinct set of virulence-associated features [21], this finding thus indicates effectiveness of the leaf extract against diarrhoea caused by *E. coli*. n-Hexane fraction appeared the most potent against *Campylobacter* spp. with the lowest MIC value of 0.31 mg/ml against 67% of the test isolates. This organism is the etiological agent of Campylobacteriosis commonly associated with eating raw or undercooked poultry. It is one the most common types of bacteria causing diarrhoea with estimated 2.4 million people each year in the United States [22]. Following a report from world health organization [23], *Campylobacter* is one of the most frequently occurring bacterial agents of gastroenteritis and it is ranked fourth among identified pathogens after rotavirus, *Salmonella* and *Cryptosporidium*. [24-25]. Biocidal effect of these extracts against *Salmonella* Typhimurium and *Shigella flexneri* was demonstrated in our previous study [13] which further buttress effectiveness of our candidate plant against a wide range of bacterial caused diarrhoea infection. Considering the relative degree of potency exhibited by individual fractions, bioactive principles from the leaf of *E. crispa* can be extracted both by polar and fairly polar solvents. Therefore suggest the plant as a potent and veritable source of antidiarrhoeal agents for pharmaceutical industries.

## 5. CONCLUSION

This study demonstrates significant antidiarrhoeal potential against prominent diarrhoea causing bacterial isolates which compared favourably with standard antibiotics. *E. crispa* therefore provides a source of readily available and affordable therapy for diarrhoeal victims in South Africa and its environs. It equally indicates a source antimicrobial drugs template for pharmaceutical industries. Work is ongoing in our laboratory to further purify and isolate the bioactive principles from each of the potent fractions.

## 6. ACKNOWLEDGEMENT

The authors acknowledge research committee of the University of the Free State.

## 7. REFERENCES

[1] S. Karambu, V. Matiru, M. Kiptoo, J. Oundo, "Characterization and factors associated with diarrhoeal diseases caused by enteric bacterial pathogens among children aged five years and below attending Igembe District Hospital, Kenya", Pan. Afr. Med. J. vol. 16, pp. 37, 2013.

[2] L. Liu, H.L. Johnson, S. Cousens, J. Perin, S. Scott, J.E. Lawn, I. Rudan, H. Campbell, R. Cibulskis, M. Li, C. Mathers, R.E. Black, For the Child Health Epidemiology Reference Group of WHO and UNICEF. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000, Lancet. vol. 379 pp. 2151–2161, 2012.

[3] C.F. Lanata, C.L. Fischer-Walker, A.C. Olascoaga, C.X. Torres, M.J. Aryee, R.E. Black, Global Causes of Diarrheal Disease Mortality in Children <5 Years of Age: A Systematic Review. PLoS ONE vol. 8, no. 9, e72788, 2013.

[4] J. Liu, J. Gratz, A. Maro, H. Kumburu, G. Kibiki, M. Taniuchi, A.M. Howlader, S.U. Sobuz, R. Haque, K.A. Talukder, S. Qureshi, A. Zaidi, D.M. Haverstick, E.R. Houpt, Simultaneous Detection of Six Diarrhea-Causing Bacterial Pathogens with an In-House PCR-Luminex Assay, J. Clin. Microbiol. vol. 50, no. 1, pp. 98–103, 2012.

[5] C.L. Fischer, W.D. Sack, R.E. Black, Etiology of Diarrhea in Older Children, Adolescents and Adults: A Systematic Review. PLoS Negl. Trop. Dis. vol. 4, no. 8, e768, 2010.

[6] O.O. Igbinosa, E.O. Igbinosa, O.A. Aiyegoro. Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn). Afr. J. Pharm. Pharmacol. vol. 3, no. 2, pp. 058–062, 2009.

[7] J.H. Doughari. Evaluation of antimicrobial potentials of stem bark extract of *Erythrina senegalensis* DC. Afr. J. Microbiol. vol. 4, no. 17, pp. 1836–1841, 2010.

[8] M. Schuier, H. Sies, B. Illek, H. Fischer. Cocoa-related flavonoids inhibit CFTR-mediated chloride transport across T84 human colon epithelia. J. Nutr. vol. 135, no.10, 2320–2325, 2005.

[9] S. Nicolette, Millennium seed bank project. Kirstenbosch gardens, Available at: http://www.plantzafrica.com. (accessed 10.06.15), 2010.

[10] S. Magama, J.C. Pretorius, P.C. Zietsman. Antimicrobial properties of extracts from Euclea crispa subsp. crispa (Ebenaceae) towards human pathogens. S. Afr. J. Bot. vol. 69, no 2, pp. 193–198, 2003.

[11] M. Alfred. An ethnobotanical survey of medicinal plants used by the people in Nhema communal area, Zimbabwe J. Ethnopharmacol. vol. 136 pp. 347–354, 2011.

[12] J.F. Sobiecki. A preliminary inventory of plants used for psychoactive purposes in southern African healing traditions. Trans. Roy. Soc. S. Afr. vol. 57, no. (1&2) pp. 1–24, 2006.

[13] K.A Alayande, C.H. Pohl, A.O.T. Ashafa. "Time-kill kinetics and biocidal effect of Euclea crispa leaf extracts against microbial membrane", Asian Pac. J Trop. Med. vol. 10 no. 4, 390–399, 2017.

[14] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. 2016, Version 6.0, http://www.eucast.org

[15] Clinical and laboratory standards institute (CLSI). “Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria” Approved Guideline-Third Edition (M45, 3rd ed.), Wayne, PA. 2016, ISBN 1-56238-918-1.
[16] European committee for antimicrobial susceptibility testing (EUCAST). Determination of minimum inhibitory concentrations (MICs) of antibacterial agent by agar dilution. Clin. Microbiol Infect. vol. 6, pp. 509–515, 2000.
[17] Clinical and laboratory standard institute (CLSI), Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard- tenth edition. CLSI document M07 A10 (ISBN 1-56238-988-2). 950 west valley road suit 2500, Wayne, PA 19087 USA vol. 35 no. 2, 2015. ISBN 1-56238-988-2.
[18] D.A. Akinpelu, E.O. Abioye, O.A. Aiyegoro, F.O. Akinpelu, A.I. Okoh. Evaluation of antibacterial and antifungal properties of Alchornea laxiflora (Benth.) Pax. & Hoffman. Evid. Based Complement. Alternat. Med. 2015; doi.org/10.1155/2015/684839
[19] I. Odenholt, O. Cars, E. Löwdin. “Pharmacodynamic studies of amoxicillin against *Streptococcus pneumoniae*: comparison of a new pharmacokinetically enhanced formulation (2000 mg twice daily) with standard dosage regimens”, J. Antimicrob. Chemother. vol. 54 pp. 1062–1066, 2004.
[20] D.A. Akinpelu, K.A. Alayande, O.A. Aiyegoro, F.O. Akinpelu, A.I. Okoh. “Probable mechanisms of biocidal action of *Cocos nucifera* Husk extract and fractions on bacteria Isolates”, BMC Complement. Altern. Med. vol. 15, pp. 116, 2015a.
[21] R.M. Robins-Browne, E.L. Hartland. “Advances in pediatric gastroenterology and hepatology: *Escherichia coli* as a cause of diarrhea”, J Gastroenterol Hepatol. vol. 17, pp. 467–475, 2002.
[22] California department of public health (CDPH). Division of Communicable Disease Control. Campylobacteriosis fact sheet. State of California-health and human services agency, 2013 (accessed 09.08.15) https://www.cdph.ca.gov/HealthInfo/discond/Documents/Campylobacter.pdf
[23] World health organization (WHO). “The global view of Campylobacteriosis: report of an expert consultation Utrecht, Netherlands, 9-11 July 2012. Estimating the true incidence of Campylobacteriosis”, 2013 (accessed 09.08.15), www.who.int/iris/bitstream/10665/80751/1/9789241564601_eng.pdf
[24] A. Saeed, D. Johansson, G. Sandstr¨om, H. Abd. “Temperature Depended Role of Shigella flexneri Invasion Plasmid on the Interaction with Acanthamoeba castellanii”, Int. J. Microbiol., Article ID 917031, 8 pages. doi: 10.1155/2012/917031. 2012.
[25] S. Ashkenazi, D. Cohen. “An update on vaccines against *Shigella*”, Ther. Adv. Vaccines. vol. 1, no. 3, pp. 113–123, 2013.