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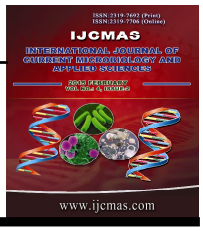

Original Research Article

The Sensitivity of *Escherichia coli* to Extracts of *Combretum fragrans*, *Combretum micranthum* and *Combretum molle* Locally Used in the Treatment of Diarrheal Diseases in the Far-North Region of Cameroon

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Keywords

Combretum, Plant extracts, Sensitivity, *Escherichia coli*, Inhibition zone, Minimum Inhibitory Concentration

A B S T R A C T

This study was undertaken to determine the sensitivity of *Escherichia coli* culture to extracts of three locally used antibacterial plants (*Combretum molle*, *Combretum fragrans* and *Combretum micranthum*) and to identify the phytochemicals present in these plants. Acetone was used as the solvent for the extraction of the leaves. The sensitivity of *Escherichia coli* culture to the plants extracts was determined on Mueller Hinton Agar, following the agar well diffusion technique. Acetone was used as a negative control and Ciprofloxacin as positive control. The Minimum Inhibitory Concentration of the plant extracts was determined by a modified broth dilution method. Phytochemical test was done by detecting major colour changes. Results indicated that all tested plants extracts, as well as Ciprofloxacin, demonstrated antibacterial activity, with diameters of inhibition zones ranging from 8 ± 0.35 to 36 ± 1.80 mm, while acetone had no activity. *Combretum molle* and *C. fragrans* had the greatest inhibitory activity against *E. coli. The* found MIC varied from 0.625 to 2.50 mg/ml. Phytochemical tests showed that the plants contained tannins, proteins, flavonoids, phenols, coumarines and glycosides. These results justify the use of the tested plants as antibacterial by the population. Further studies, involving the plants extracts antimicrobial activities against other micro-organisms and their *in vivo* toxicity and mechanism of action are needed.

Introduction

Diarrhoeal diseases are one of the foremost public health problems worldwide. In the 21st century, diarrhoeal diseases continue to be a major cause of morbidity and mortality worldwide (O'Ryan *et al.,* 2005). Recurrent diarrhoea is prevalent in developing countries, particularly in tropical regions (Pickering, 2004). Enteric pathogens are the most frequent causes of diarrhoea illness, which account for an annual mortality rate

of 3 million and an estimated 4 billion infections worldwide (Talaro, 2005). Almost half of the world's population suffer from diseases associated with insufficient or contaminated water and is at risk from waterborne and foodborne diseases, of which diarrheal diseases are the most deadly (Sindiga *et al.,* 1995).

The pathogenic bacteria most commonly associated with endemic forms of diarrhoea are diarrheagenic *Escherichia coli*, *Salmonella* spp, *Shigella, Vibrio cholerae*, *Aeromonas* and *Pleisomonas* spp (Mamatha, 2006). Diarrhoea is also caused by other agents like viruses and parasites (Palombo, 2006).

Antibiotic resistance is a major clinical cause of concern in treating infections caused by microorganisms (Acharyya *et al.,* 2009). For instance, *E. coli* has showed resistance to the common cheap antibiotics, notably Trimethoprim-Sulphamethoxazole, Kanamycin and Gentamycin (Bebora *et al.,* 1994). As resistance to old antibiotics spreads, the development of new antimicrobial agents has to be expedited if the problem is to be contained. However, the past record of rapid, wide spread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates *et al.,* 2002).

Medicinal plants are effective in the treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic drugs (Koné *et al.,* 2004). Multiple drug resistance has developed due to the indiscriminate use of synthetic antimicrobial drugs (Davis, 1994). In addition, bacteria have evolved numerous defences against the antimicrobials (Ahmad and Aqil, 2006). A combination of these factors is the more reason why scientific research in the field of traditional medicinal plants has to be intensified in order to find alternative antimicrobial drugs. Numerous studies have identified active compounds within herbal plants that are effective antibiotics (Basile *et al.,* 2000) and some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Koné *et al.,* 2004).

The genus *Combretum* includes almost 400 species found all across Africa, many of which are widely used in African traditional medicine (Van Wyk and Gericke, 2000). Several species of the genus have been reported for their biological activities. Different extracts (ethanol, chloroform, methanol or water) of *C. micranthum* antibacterial activity against a number of microorganisms such as *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella* species, *Streptococcus* species, *Proteus vulgaris, Klebsiella* species, *Sarcina lutea, Micrococcus luteus* and *Bacillus subtilis* was noted (Neuwinger, 2000). Antifungal activity against *Candida albicans*, antiviral activity against *Herpes simplex* 1 and 2, antimalarial activity against *Plasmodium falciparum* and antidiabetic activity was also reported (Masoko *et al.,* 2007). Extracts of *C. erythrophyllum* have shown antibacterial activity at different doses against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* (Martini and Eloff, 1998).

Phytochemical studies carried out in the genus *Combretum* have demonstrated the occurrence of many classes of constituents, primarily terpenoids (mainly triterpenes) and phenolic compounds (flavonoids, stilbenoids, phenanthrenes), among others (de Morais Lima *et al.,* 2012).

As the plants of *Combretum* genus are locally and widely used in the treatment of diarrheal diseases in the Far-North region of Cameroon, it becomes necessary to verify the scientific basis of this use and to have an idea on the phytochemical composition of the plants.

The general objective of this study is to evaluate the sensitivity of *E. coli* to extracts of the leaves of *Combretum molle, Combretum fragrans* and *Combretum micranthum.* To attempt this, the following specific objectives are focused: investigation of *E. coli* susceptibility to extracts of the plants leaves, finding of the minimum inhibitory concentration of the leaves extracts and screening of the leaves extracts, in order to identify the phytochemical compounds present in these extracts.

Materials and methods

Description of the study area

The Far-North region is the northern most constituent region of the Republic of Cameroon. It borders the North region to the south, Chad to the east and Nigeria to the west. It is located on latitude 11°00N and longitude 14°30E and has a Sudanno-sahalian climate, with steppes and savannah vegetation. The annual rainfall is about 400-1500 mm and the annual average temperature is 28°C.

Collection of plants materials

Fresh plant leaves of *Combretum molle* R. Br. ex G. Don, *Combretum fragrans* F. Hoffm. and *Combretum micranthum* G. Don were harvested in Ngassa, a small village about a few kilometres from Mindif, in the Far-North Region of Cameroon. The plant leaves were washed and dried at ambient temperature under shade after collection until they were dry. For laboratory analysis, they were ground into powder form using a grinder (MK 10-525-B).

Extraction

All chemicals used in this study were purchased from Sigma-Aldrich chemicals Co (St. Louis, MO, USA) and were of analytic reagent grade. The powdered plant materials were soaked in acetone for 48 hours with intermittent shaking to allow the active phytochemicals to dislodge in the solvent. The acetone soaked plant extracts were then filtered by the use of a Whatman No.1 filter paper and the filtrates evaporated by using a rotary evaporator (W.2000 Heidolph, Germany) set at 72°C to remove the excess solvent. The extracts were stored in air tight containers in the laboratory until when they were used.

Antimicrobial assays

Test cultures

Bacteria strain of *E. coli* was isolated from human faecal matter from patients who presented diarrhoea at the Garoua Annex of the Pasteur Centre of Cameroon. This bacteria isolate was maintained at 4°C on nutrient agar slants until when it was used.

Preparation of the Mueller Hinton Agar (MHA) culture Medium

The preparation was done following the manufacturer instructions. Generally, 7.6 g of the MHA was added to 200 ml of distilled water and was brought to boil using a Bunsen burner. The medium was, later on, sterilized by autoclaving at 121°C for 15 minutes. It was then removed and placed in Petri dishes in a homogenous manner and allowed to solidify. The Petri dishes containing agar were then stored in a refrigerator to be used the following day.

Determination of diameters of growth inhibition zones

The Agar Diffusion Method, according to Collins *et al.* (1995) was used. 0.05 g of each plant extract was reconstituted in 2.5 ml of acetone, leading to a test concentration of 20 mg/ml. One colony of an 18 h conserved bacteria (*E. coli*) culture was dissolved in 2 ml of distilled water in a test tube and adjusted to a density of 0.5 McFarland (approximately 10^6 CFU/ml) and inoculated in prepared MHA plates.

Wells of 6 mm in diameter were made in the agar with a sterile stainless steel cork borer and 0.05 ml of the extracts was added to each well. Controls were comprised of extraction solvent acetone (Negative control) and Ciprofloxacin 5 µg standard disk (Positive control). The agar plates were incubated at 37° C for 48 h. The different zones of inhibition around each plant extract and the controls were recorded. The diameters of these zones of inhibition, around the wells containing the extract, were taken to indicate the antibacterial activity of the plant extracts against the test organism (CASFM, 2014).

Determination of Minimum Inhibitory Concentration (MIC)

The estimation of the MIC of the crude extracts was carried out using a modified Broth Dilution Technique (Akinpelu and Kolawole, 2004). Two-fold dilutions of each of the plants extracts were made, giving concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 mg/l. Then, two aliquots (2 ml) of different concentrations of the two-fold dilutions of each crude extract prepared was added to 18 ml of pre-sterilized molten MHA in test tubes, at temperature of 40°C and mixed to give final concentrations of 10, 5, 2.50, 1.25, 0.625, 0.313, 0.156, and 0.078 mg/ml.

The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry under laminar air flow before inoculating with 48 h bacterial cultures adjusted to a density of 0.5 McFarland. Three series of other Petri dishes were used as controls. One for sterility control containing only 20 ml of sterilized molten MHA, one containing only 20 ml of contaminated MHA for growth control and one containing only 18 ml of MHA and 2 ml of acetone, as negative control, The Petri dishes were later incubated at 37° C for up to 48 h after which they were examined for the presence or absence of growth (Adesokan *et al.*, 2007). The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

Qualitative methods of phytochemical screening

The leaf extracts of each plant were analyzed for alkaloids, flavonoids, glycosides, phenols, saponins, tannins, quinones, cardiac glycosides, coumarines, aromatic amino acids, phytosterols (triterpenoides), proteins and carbohydrates according to Harborne (1998), with slight modifications.

Detection of alkaloids

About 50 mg of each plant extract were stirred with 3 ml of dilute hydrochloric acid and filtered thoroughly. The Dragendorff test was used. To a 1 ml of filtrate, 2 ml of Dragendorff reagent are added. A prominent yellow precipitate confirms the test as positive.

Detection of carbohydrates

The Benedict's test was used to point out the presence of carbohydrates. To 0.5 ml of

each plant extract, 0.5 ml of Benedict reagent was added. The mixture was heated on a boiling water bath for 2 min. A red precipitates indicated presence of sugar.

Detection of glycosides

To test for the presence of glycosides, Legal's test was used. To 2 ml of each plant extract, 3 ml of chloroform and ammonium solution (10%) were added. Formation of pink colour indicated the presence of glycosides.

Detection of proteins

The plant extracts were, each, dissolved in 10 ml of distilled water and filtered through Whatman No.1 filter paper. Millon test was used to test for proteins. To 2 ml of filtrate, few drops of Millon reagent were added. A white precipitates indicated presence of proteins. The Biuret test was also used to detect the presence of proteins as follows. An aliquot of 2 ml of filtrate was treated with two drops of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by 1 ml of sodium hydroxide (40%). The pink colour in ethanol layer indicated presence of proteins.

Detection of amino acids

For each plant extract, 1 ml of NaOH (20%) was added to 1 ml of the filtrate. An orange colouration indicated the presence of amino acids.

Detection of phytosterols (triterpenoides)

The Liberdmann-Burchard test was used to test for the presence of phytosterols. Five mg of each plant extract were each dissolved in 2 ml of acetic anhydride and one or two drops of concentrated sulphuric acid was added slowly along the sides of the test tube. The formation of blue-green colour indicated the presence of triterpenoides and phytosteroids.

Detection of tannins

The Ferric chloride test was used to test for the presence of tannins. Each extract (5 mg) was dissolved in 5 ml of distilled water and few drops of 5% ferric chloride solution were added. The formation of blue-green colour indicated the presence of tannins.

Detection of phenols

Phenols were tested using the Lead acetate test. Each plant extract (5 mg) was dissolved in distilled water and 3 ml of 5 % lead acetate solution was added. A bulky white precipitates indicated the presence of phenols.

Detection of flavonoids

An aqueous solution of the extracts was treated with ammonium hydroxide solution. The yellow fluorescence indicated the presence of flavonoids.

Detection of coumarines

10% NaOH (1 ml) was added to 1 ml of the plant extracts. The formation of yellow colour indicated presence of coumarines.

Detection of saponins

Distilled water (2 ml) was added to each plant extracts and shaken. Formation of 1 cm foam indicated the presence of saponins.

Detection of quinones

Concentrated sulphuric acid (1 ml) was added to 1 ml of each of the plant extract.

Formation of red colour indicated the presence of quinones.

Detection of cardiac glycosides

Glacial acetic acid (2 ml) and few drops of 5% ferric chloride were added to 0.5 ml of each extract. Then, 1 ml of concentrated sulphuric acid was added. Formation of brown ring at the interface indicated presence of cardiac glycosides.

Detection of terpenoids

Chloroform (2 ml) and 1 ml of concentrated sulphuric acid were added carefully to 0.5 ml of extract. Formation of red brown colour at the interface indicated the presence of terpenoids.

Data analysis

All the experiments were run in triplicate. Data were recorded and analyzed, using SPSS 19.0 software. Comparison between the main parameters was made by variances analysis (One-way ANOVA), at the 0.05 level.

Results and Discussion

Antimicrobial assay of plant extracts

The results of antibacterial sensitivity of acetone extracts of *C. molle, C. fragrans* and *C. micranthum* by well diffusion method are depicted in table 1. These results revealed that all extracts are potent antimicrobials against studied *E. coli*. Varying levels of antibacterial activity were observed with zones of inhibition diameters ranging from 08 ± 0.35 mm, for acetone extracts of *C. micranthum* to 15 ± 1 mm, for extracts of *C. molle*. Ciprofloxacin, which was used as a positive control, had a zone of inhibition diameter of 36 ± 1.80 mm and acetone, which was used as the negative control, showed no activity. Hence, any inhibitions observed in the plant extracts were not due to the solvent. An inhibition zone diameter of ≥ 11 mm was chosen as a break-point of bacterial susceptibility of the extracts and the antibiotic (CLSI, 2014). The sensitivity of *E. coli* to the various plant extracts was classified as being resistant, intermediate or sensitive (Table 1).

Minimum Inhibitory Concentration (MIC) of plants extracts against *E. coli*

The MIC for each plant extract was determined. The results show that *C. molle* and *C. fragrans* extracts did not inhibit the growth of the bacteria at a concentration of 0.313 mg/ml, while *Combretum micranthum* extract did not inhibit the growth of the bacteria at a concentration of 1.25 mg/ml (Table 2). *C. molle* and *C. fragrans* extracts inhibited growth at a concentration of 0.625 mg/ml, while *C. micranthum* extract inhibited growth at a concentration of 2.50 mg/ml. These values were taken as the MIC of the extracts. Acetone, on the other hand, did not inhibit the growth of *E. coli*.

Qualitative phytochemical analysis of plants

Preliminary phytochemical analysis of the plant extracts indicated the presence of different phytochemicals (Table 3). The extracts of *C. micranthum* and *C. fragrans* contained high amounts of alkaloids which were completely absent in *C. molle* extracts. Proteins and tannins were present in trace amounts in *C. molle* extracts. Terpenoides were totally absent in extracts of *C. fragrans.* Carbohydrates and saponins were completely absent in all the three plant extracts, while quinones were present only in extracts of *C. micranthum.* All the plants in this study contained tannins.

According to the growth inhibition zone diameters, all the used plants extracts showed different levels of antibacterial activity against tested *E. coli*. The obtained growth inhibition zone diameter of Ciprofloxacin is in accordance with the 30-40 mm CASFM (2014) values, validating, thus, our test. The variation ranged in the order of *Combretum molle* > *Combretum fragrans* > *Combretum micranthum* ($p < 0.05$). Previous studies, on plants extracts antimicrobial activities show that diameters of growth zone inhibition vary with the plants species, the polarity of extraction solvent, the test concentrations of the plants extracts, the extracted part of the plant and the bacterial strains (de Morais Lima *et al.*, 2012; Kokora *et al.,* 2013; Banfi *et al.*, 2014). The varied antibacterial activity, observed in this study, may be due to the presence of different types of phytochemicals in varying concentrations. Tannins, flavonoids, alkaloids, saponins, reducing sugars, sterols and triterpenes have been reported to have antidiarrhoeal as well as antibacterial activity (Lewis, 2003; Palombo, 2006). For instance, flavonoids have an ability to inhibit intestinal motility and hydro-electrolytic secretion, inhibit the intestinal secretory response induced by prostaglandin E2 and have antioxidant properties responsible for the inhibitory effects exerted upon several enzymes (Venkat *et al.*, 2006). Elsewhere, the antidiarrhoeal activity of the phytochemical compounds has been attributed to their antimicrobial activity (Mamatha, 2006). Terpenoides were totally absent in *C. fragrans* extracts but found in extracts of *C. molle* and *C. micranthum*. Earlier studies on the effects of the terpenoides on isolated bacterial membranes reveal their site of action to be at the phospholipids bilayer. They affect bacterial processes that include the inhibition of electron transport, protein translocation, phosphorylation steps and other enzyme-dependent reactions (Seenivasan *et al.*, 2006). These phytochemicals also have some strong antimicrobial significance against some potential enteric pathogens (Edeoga *et al.*, 2005).

Many of the traditionally used medicinal plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit growth of pathogenic bacteria (Palombo *et al.*, 2006). The results in the present study show that the test isolate of *E. coli* is susceptible to extracts of *C. molle* and *C. fragrans* while *C. micranthum* showed mitigated bioactivity against *E. coli*. Many food borne pathogens have developed resistance to antimicrobial agents, including *E. coli* strains (White *et al.*, 2002). This bacterium is known to be drug-resistant (O'Ryan *et al.*, 2005). It has been suggested that this resistance to plant extracts may be due to the presence of the outer membrane of the bacterial cell wall, which acts as a barrier to various environmental factors such as antibiotics or due to the differences in the cell wall composition of various bacteria (Tiwari *et al.*, 2005).

The small zone of inhibition shown by *C. micranthum* indicates that although some plants are used in the management of diarrhoea, not all prescribed antidiarrhoeal medicinal plants may be very effective against enteric pathogens. However, this intermediate position does not rule out the potential of the plant as antibacterial agents for treatment of enterobacterial infections. The weak activity demonstrated by extracts of *C. micranthum in vitro*, does not necessarily mean that the plant would demonstrate weak activity *in vivo*. As, with some drugs, some of these plant extracts may be more potent *in vivo* due to metabolic

transformation of their components into highly active intermediates and to immune adjustment (Ndip *et al.,* 2009).

Results of the minimum inhibitory concentration revealed that *E. coli* is resistant to *C. molle*, and *C. fragrans* extracts at 0.313 mg/ml, while it is resistant to *C. micranthum* extract at 1.25 mg/ml. The MIC for *C. molle* and *C. fragrans* is 0.625 mg/ml, confirming their strong antibacterial properties, while that of *C. micranthum* is 2.5 mg/ml. Varying extracts of the studied *Combretum* species have been found to inhibit *E. coli* growth at different MIC values. For instance, acetone extract of the bark of *C. molle*, ethanol leaves extracts of *C. micranthum*, ethanolic extract of the stem bark of *C. micranthum* and methanol extracts of *C. molle* inhibit *E. coli* growth, respectively, at 50 mg/ml (Asres *et al.,* 2006), 1.25 mg/ml (Banfi *et al.,* 2014), 0.23 mg/ml (Agboke *et al.,* 2012) and 2.5 mg/ml (Saidu, and Abdullahi, 2011). Moreover, the MIC of acetone extract of the leaves of *C. nioroense*, acetone extract of the leaves of *C. acutum*, acetone leaves extract of *C. paniculatum and* methanol leaves extract of *C. calobotrys*, for *E. Coli*, have been found to be, respectively, 20 mg/ml (Coulidiati *et al.,* 2011), 0.625 mg/ml (Coulidiati *et al.,* 2009), 12.5 mg/l (Mbajiuka *et al.,* 2014) and 4.30 mg/l (Eziké *et al.,* 2011). Besides, Elegami *et al.,* (2007) find that the MIC of methanol extracts of leaves of *C. adenogonium*, *C. glutinosum* and *C. aculeatum* are of 4.69, 9.38 and 2.35 mg/l for *E. coli*. The discrepancy between these results can be related to the plants species, their extracted part, and their geographical localization, which acts on their chemical contents, the extraction solvent and the tested bacteria species. Many other plants of the *Combretaceae* family have been found to have antibacterial activity. Ibrahim *et al.,* 2011 have demonstrated that the extracts of *Ocimum gratissimum* inhibit the growth of *E. coli, Klebsiella pneumoniae, Bacillus subtilis and Salmonella typhimurium* at 100 mg/ml, while *Proteus vulgaris*, *Pseudomonas aeruginosa and Staphylococcus aureus* are inhibited at 50 mg /ml. In the same study it is borne out that extract of *Myristica fragrans* has the MIC of 100 mg/ml for *Bacillus subtilis, Salmonella typhimurium and Pseudomonas aeruginosa,* 50 mg/ml for *E. coli, Klebsiella pneumoniae*, *Proteus vulgaris* and 25 mg/ml for *Staphylococcus aureus*. The Antibacterial activity (MIC) of the acetone extracts of the stem bark of *C. molle* against *Helicobacter pylori* 252C (clinical isolate) is shown to be 2.5 mg/ml (Nyenje, 2011). Also, MIC of 2.5 mg/ml has been obtained on the clinical strain of *E. coli*, with aqueous plant extracts of *Terminalia mantaly* (Kokora *et al.,* 2013). These results are comprehensive, because most plants of the *Combretaceae* family are known to contain antimicrobial compounds (Baba-Moussa *et al.,* 1999).

Phytochemical screening is one of the necessary steps to find out the chemical constituents which lead to the isolation of compounds. Many of these compounds are used as the active ingredients of the modern medicine or as the lead compounds for new drug discovery. Phytochemical analysis of the acetone plant extracts of *C. molle, C. fragrans* and *C. micranthum* revealed the presence of varied chemical components. They are known to show medicinal activity as well as exhibiting physiological activity and exhibit anti-inflammatory, anti-oxidant and membrane stabilizing property (El-Mahmood, 2009). Many phytochemical studies of the stem bark of *C. molle* lead to the isolation of triterpenoides, glycosides, tannins, alkaloids, saponins, stilbenes, triterpenes saponin-oleanones trypetitepenes, arjunolic and mollic acids and glycosides which demonstrate cytotoxic,

antifungal, antimicrobial and anti-inflammatory activity, as well as anti-HIV type 1 reverse transcriptase (Asres *et al.*, 2001).

Table.1 Diameters of growth inhibition zones of plants extracts, acetone (solvent) and Ciprofloxacin against *E. coli*

Plant extract	Diameter of inhibition zone (mm)	Susceptibility
Combretum fragrans	12 ± 0.85	Sensitive
Combretum micranthum	08 ± 0.35	Intermediate
Combretum molle	15 ± 1.00	Sensitive
Ciprofloxacin (positive control)	36 ± 1.80	Sensitive
Acetone (negative control)	0	Resistant

<7 = Resistant, 8-10 = Intermediate, ≥11 = Sensitive

Table.2 Minimum Inhibitory Concentration of plants extracts against *E. coli*

Plant extracts	Concentrations (mg/ml)								MIC (mg/ml)
	0.078	0.156	0.313	0.625	1.25	2.50	5	10	
Combretum fragrans	G	G	G	NG	NG	NG	NG	NG	0.625
Combretum micranthum	G	G	G	G	G	NG	NG	NG	2.50
Combretum molle	G	G	G	NG	NG	NG	NG	NG	0.625
Acetone	G	G	G	G	G	G	G	G	

Key: G = Growth, NG = No Growth.

Table.3 Profile of phytochemicals present in plants extracts

Tested chemical group	Plants extracts		
	Combretum fragrans	***Combretum micranthum***	***Combretum molle***
Alkaloids	++	++	_
Aromatic amino acids	++	++	++
Carbohydrates	_	_	_
Cardiac glycosides	++	++	++
Coumarines	++	++	++
Flavonoids	++	++	++
Glycosides	++	++	++
Phenols	++	++	+
Proteins	++	++	+
Quinones	_	++	_
Saponins	_	_	_
Tannins	++	++	+
Terpenoides	_	++	++

Key: - : Absent; +: Trace; ++: Present in appreciable quantity

Extracts of *C. fragrans* and *C. micranthum* had the highest amount of tannins. Tannins have been known to be effective in the prevention of colonization of enteric pathogens and consequently control diarrhoea (Palombo, 2006). These substances also precipitate proteins of the erythrocytes, reduce peristaltic movement and intestinal secretion (Venkat *et al.*, 2006). Tannins are found in large quantities in the bark of trees where they act as a barrier for micro-organisms like bacteria and fungi. They have been found to form irreversible complexes with proline rich protein, resulting in the inhibition of cell protein synthesis (Shimada, 2006). Herbs that have tannins as their main components are astringent in nature and are used for treating inflamed or ulcerated tissues, intestinal disorders such as diarrhoea and dysentery (Parekh and Chanda, 2007). Similar mechanisms of action could be responsible for the antimicrobial actions of the plant extracts under study. In another study, Asres *et al.* (2001) demonstrates good activity of the acetone extract of *C. molle* stem bark against *Mycobacterium tuberculosis* and *Plasmodium falciparum* 3D7. The activity has been attributed to high amount of hydrolysable tannins present in the stem bark of the plant. It is generally believed that tannins are non-selective enzyme inhibitors due to their polyphenolic groups. However, it has been shown that some hydrolysable tannins display selective cytotoxicity (Asres *et al.*, 2001).

In conclusion, plants are studied as potential disease controlling agents in humans as they are relatively safer, affordable and are easily accessible at a local level, such that they can offer an alternative treatment option to the conventional antibiotics. This study has allowed us to determine the antibacterial properties of the acetone leaves extracts of *C. molle*, *C. fragrans* and *C. micranthum* against *E. coli*. The results from these investigations shows that *C. molle* was the most active plant, among the three plant extracts, against *E. coli*, with the highest zone of inhibition diameter. This study also revealed the presence of various phytochemical compounds with antibacterial properties in the three plants extracts. Varying levels of activity were observed within the medicinal plants under study. It may be due to the quality and quantity of the active compound present in each plant extract.

E. coli is developing resistance to commonly employed antibiotics and is a common cause of diarrhoea. Therefore, the plant extracts in this study can be used in the treatment of *E. coli* generated diarrhoeal diseases.

Although this study has provided useful data concerning the antimicrobial activities of leaves extracts of *C. molle*, *C. fragrans* and *C. micranthum* further works are necessary to provide more data. For instance, studies involving a large number of resistant pathogens that cause diarrhoeal diseases are necessary to draw meaningful conclusions; *in vivo* studies in order to confirm the antibacterial activity results obtained must be carried out; deep phytochemical analysis of the plants extracts should be performed to isolate and characterize the specific compounds responsible for the antibacterial activity; the plant extracts should be screened for toxicity *in vivo*, in order to be able to determine the safest dosage for the treatment of diarrhoea in humans.

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