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The antimicrobial activities of some medicinal plants on *Escherichia coli* as an agent of diarrhea in livestock

[*1]**Chukwuka, K.S**; [2]**Ikheloa, J.O**; [3]**Okonko, I.O**; [4]**Moody, J.O** and [2]**Mankinde, T.A**

[1]*Department of Botany, University of Ibadan, Ibadan, Nigeria*
[2]*Department of Veterinary Microbiology & Parasitology, University of Ibadan, Ibadan, Nigeria*
[3]*Department of Biochemistry & Microbiology, Lead City University, Ibadan, Nigeria*
[4]*Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria*

ABSTRACT

This study was carried out to isolate and identify Escherichia coli from diarrheic fecal samples of calves and piglets in Ibadan, Nigeria and to determine the antimicrobial activities of some medicinal plants on these Escherichia coli isolates as an agent of diarrhea in livestock. It also aimed at extracting the therapeutic component of the ethnomedicinal plants using different solvents and determining the efficacy of the plants extracts on E.coli by disc diffusion and minimal inhibitory concentration (MIC) methods. E. coli strains showed a high frequency of single or multiple drug resistance (MDR) to ≥3 of the antimicrobial agents tested (3 to 6 antibiotics). The bacteria were found to be resistant to ampicillin, nalidixic acid, nitrofurantoin, co-trimoxazole, streptomycin and tetracycline. Chloroform, methanol and warm water extracts of the leaves of Ageratum conyzoides, Adansonia digitata, Annona muricata, Bryophyllum pinnatum Kuiz, Cassia sieberiana, and Ocimum gratissimum traditionally employed for the treatment of gastrointestinal infections were prepared and evaluated for its antimicrobial activities. Extracts of Adansonia digitata, Bryophyllum pinnatum, Cassia sieberiana and Ricinus communis did not inhibit E. coli at the various concentrations. Only methanol extracts of Ageratum conyzoides, Annona muricata and Ocimum gratissimum showed some degree of inhibition which varies with the E. coli strain tested. The methanol extracts of the plant parts were more potent than the chloroform and water extracts which showed no activity against the test organisms. Neat (100%) concentration of methanol preparation inhibited the growth of Escherichia coli. The minimum inhibitory concentration (MIC) exhibited by Ocimum gratissimum against the E. coli strains was 20,000µg/ml. The presence of these MDR- E. coli strains in these diarrhoeic piglets indicates public health hazard and calls for particular attention and warning signal for the possible occurrence of food borne intoxication. The result suggests that the preparation of these three plants exhibited significant in vitro antimicrobial activity against common gastrointestinal isolates and may be employed for the routine treatment of gastrointestinal infection as an alternative to antibiotics chemotherapy.

Keywords: *Ageratum conyzoides*, *Annona muricata*, disc diffusion methods, *Escherichia coli*, methanol extracts, Minimal inhibitory concentration (MIC), *Ocimum gratissimum*, Piglets, Public health concerns

INTRODUCTION

There are several diseases of the gastrointestinal tract of farm animals in which diarrhea is a major clinical finding. Diarrhea is the increased frequency of defecation of watery faeces. Commonly incriminated bacterial agents of diarrhea include *Escherichia coli*, *Salmonella spp*, *Clostridium perfringens* types B & C, and *Mycobacterium paratuberculosis* [1]. Bacterial agents induce diarrhea in newborn animals particularly calves and piglets in the first week of life. It is one of the most common disease complexes which the large animal clinicians have to contend with. It causes a significant economic loss in herbs of cattle and swine. It may assume even greater importance in the future as livestock production becomes more intensified.

Escherichia coli serovars are the causative agents of colibacillosis. The utilization of antimicrobial drugs has played important role in animal husbandry, since they are used in prophylaxis, treatment and growth promotion [2]. In the developed world, the extensive use of antibiotics in agriculture, especially for prophylactic and growth promoting purposes, has generated much debate as to whether this practice contributes significantly to increased frequencies and dissemination of resistance genes into other ecosystems. In developing countries like Nigeria, antibiotics are used only when necessary, especially if the animals fall sick, and only the sick ones are treated in such cases [3].

There is currently a world trend to reduce the use of antibiotics in animal food due to the contamination of meat products with antibiotic residues, as well as the concern that some therapeutic treatments for human diseases might be jeopardized due to the appearance of resistant bacteria [4]. During the last decade, the use of antimicrobial drugs for growth promotion and therapeutic treatment in food animals has received much attention. The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections [5]. The reservoir of resistant bacteria in food animals implies a potential risk for transfer of resistant bacteria, or resistance genes, from food animals to humans [6]. Subsequent emergence of infections in humans, caused by resistant bacteria originating from the animal reservoir, is of great concern. These unintended consequences of antimicrobial drug use in animals led to termination of antimicrobial growth promoters in food animals in countries in the European Union, including Denmark, where the consumption of antimicrobial drugs by production animals was reduced by 50% from 1994 to 2003 [6]. The effective treatment and control of diarrhea in cattle and piglets can be unreliable. Considerable progress had been made in the treatment of the effects of diarrhea, such as dehydration and acidosis but less so in the control of these disease complexes [7].

There have been several approaches to combat diarrhea in livestock among these are the resurgence of interest in the use of medicinal plants and this subject has received extensive coverage in many publications [7]. This has a wide range of benefits to man. From trees and shrubs amongst others, man obtains medicine and foods [8-10]. A good number of trees and shrubs have been claimed by ethnoveterinary practitioners, ethnomedical practitioners and other local people to have medicinal benefits against infectious and/or non infectious animal and/or human diseases [11]. Throughout history plants have been used by human beings for medicinal purposes and even in modern times have formed the basis of many pharmaceuticals in use [12].

A recent review has shown that approximately 25% of modern medications are plant derivatives, while 75% of new drugs against infectious diseases that have arrived between 1981 and 2002 originated from natural sources [13]. Medicinal plants are those plant from which one or more of it's tissues contain substances that can used for the therapeutic purposes or which are precursors for the synthesis of useful drugs. It is possible to distinguish between medicinal plant whose constituents have well established medicinal properties and plants whose constituents have not been thoroughly evaluated for therapeutic purposes [7].

Despite the progresses in modern medicine, it has been reported that more than 70% of the developing world's population still depends on complementary and alternative systems of medicine, otherwise known as traditional medicine [14]. Many of these medicines are plant based, and include curcumin, resveratrol, baicalein, boswellic acid, betulinic acid, ursolic acid, and oleanolic acid are now studied as possible drugs for the future against inflammatory diseases [15]. Edible plant extracts have shown promising anti-tumorigenic activity [16]. A large number of traditional medicinal preparations used in various countries of the world have shown promise as treatment for herpes virus infections, which cannot be completely cured by the available anti-herpes drugs like nucleoside analogs [17]. The various ailments for which searches are going on in plants for newer drugs are too numerous to mention; to cite just a few examples, new plant-derived compounds are being searched for treatment of malaria because of the increasing resistance of *Plasmodium falciparum* against currently available anti-malarial drugs [18-19], searches for anti-fungal, molluscicidal and larvicidal compounds in African medicinal plants have been reviewed [14], anti-leishmanial activity in Israeli plants has been reviewed by El-On *et al.* [20], and anti-trypanosomal and cytotoxic activities of pyrrolizidine alkaloid-producing plants of Ethiopia has been reported by Nibret *et al.* [21]. The various ailments treated by the plants included respiratory tract infections, gastrointestinal disorders, sprains and fractures, skin ailments, malaria, mental disorders, hepatic disorders, diabetes, influenza, urinary tract infections, hypertension, debility, measles, chicken pox, toothache, gynecological problems, sexual disorders, and helminthiasis. Some plants were also used as antidote to poison, diuretic, and abortifacient [14].

Efforts are now being made in different countries to carry out research into traditional medicine and to integrate it with orthodox medicine. However, not much has been done to salvage traditional medicine as it applies to veterinary practice. Although, there are no professional traditional veterinary medical practitioners in Nigeria, herd men, village elders and people who kept animals for domestic purposes were experienced in the diagnosis and treatment of specific animal diseases. Some of these herbs are used mainly for the treatment of diseases found in animal only. Nigerians have developed a stock of empirical information concerning the therapeutic values of our local plants, and it is widely believed that remedies of natural origin are harmless, and carry no risk. However, there is a possibility of inadvertently applying extracts of toxic plants in a few cases.

It is therefore important that the knowledge of traditional treatments of animal diseases be documented and detailed studies be carried out to establish the efficacy of these herbs. Also many of the medicinal plants acclaimed to be of therapeutic value are yet to be completely exploited hence a need for further investigation in order to determine the chemical composition and the efficacy of extracts of the plants in combating diseases. Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins [8, 11, 22-24].

This study aimed at isolating and identifying *Escherichia coli* from diarrhoeic fecal samples of calves and piglets in Ibadan, Nigeria. It also aimed at extracting the therapeutic component of the medicinal plants using different solvents and determining the efficacy of the plants extracts on *E.coli* by disc diffusion and minimal inhibitory concentration (MIC) methods.

MATERIALS AND METHODS

Collection of samples

The various medicinal plants were obtained at the campus of the University of Ibadan. They include: *Ageratum conyzoides L; Adansonia digitata L; Annona muricata (PERS); Bryophyllum pinnatum Kuiz; Cassia sieberiana D. C.; Ocimum gratissimum L.*

Extraction of the medicinal plants

Three (3) extracts were obtained from each of the medicinal plants namely; chloroform, methanol and water extracts. Chloroform and methanol base extraction involved three major processes, using the continuous alcohol extraction method with slight modification. The plants were subjected first to chloroform base extraction and then to methanol base extraction.

Standardization of *E. coli* Used for Disc

Diffusion Susceptibility Test

A discrete colony was inoculated into tryptone soya broth and incubated aerobically at 37^0C for 18 to 20hrs. The broth was diluted by adding a loopful of the overnight culture to 5.0ml of physiological saline contained in bijou bottle. This gave a concentration of approximately 10^5 - 10^6 CFU per ml. With a sterile Pasteur pipette, about 2.0ml of the diluted suspension was transferred to sensitivity test Agar. The plate was hand rocked back and forth and side ways to allow the culture to spread over the whole surface. The excess fluid was removed with a Pasteur pipette and discarded into a strong disinfectant. The plate was left to dry in an inverted position on the working bench.

Determination of the effects of the Extracts

Discs of about 5.0mm in diameter were prepared from Whatmann filter paper. About 0.05ml of each of the plant extracts was then impregnated into the disc by a single channel micro pipette. The discs were then applied onto the surface of the already inoculated agar. The plate was left on the working bench to allow for the diffusion of the plant extract into the agar before incubating at 37^0C for 20hrs. The test was carried out for chloroform, methanol and water extracts of the plants. Zones of inhibition was then observed and measured in millimeter after aerobic incubation at 37^0C for 18 – 20hrs.

Determination of the MIC of the Plant Extract on *E. coli* Using Tube Dilution Method

The standard inoculums used were determined by Miles and Mistra method. The minimum inhibitory concentration (MIC) test was carried out only for *Ocimum gratissimum*. There *E. coli* strains were used for the test. The strains were designated ‘A’, ‘4’ and ‘5’. ‘A’ was isolated from calf faecal sample, ‘4’ is *E. coli* 021, strain, while, sample ‘5’ is *E. coli* 022 strain. Trypticase soy broth was delivered into a row of 9 test tubes for each of the strains. About 0.9ml of the broth was delivered into the first test tubes and about 0.5ml of the broth was delivered into the rest of the test tubes. Also, 0.1ml of known concentration of methanol extract of *Ocimum gratissimum* was then delivered into the first test tube. The concentration of the *Ocimum gratissimum* used was 2.0 x 10^4µg/ml. A doubling dilution was then carried out down the row of the test tubes except the 9^{th}. This was done by transferring 0.5ml of the mixture in the first tube into the second tube. This dilution was repeated in the third test tube as well as in the other tubes until the last

was reached. The 0.5ml of the mixture in 8th tube was discarded. The 9th test tube which served as a control contained 0.5ml of broth without the extract. This procedure gave antibiotic concentration of 2.0 x 10^4µg/ml in the first test, 1.0 x 10^4µg/ml in the second and so forth. Equal volume, that is, 0.4ml of the prepared diluents of the organism was then added to the row of the test tubes. The preparation was incubated at 37^0C for 24hrs after which the content of the tubes were inoculated onto blood agar and then incubated aerobically at 37^0C for 24 hrs.

Chloroform extract, ethanol extract, and water extract of all the medicinal plants were tested on three (3) strains of the *E. coli*. Growth was determined by visual observation for turbidity and by planting a loopful of the broth containing the *E. coli* and the extract of *Ocimum gratissimum* on blood Agar and incubated aerobically at 37^0C for 24hrs.

RESULTS

Susceptibility of *E. coli* Strains to Plants Extracts by Diffusion Test

Extracts from four of the plants did not inhibit *E. coli* when used in their undiluted forms. Extracts that did not inhibit *E. coli* were from *Adansonia digitata, Bryophyllum pinnatum, Cassia sieberiana* and *Ricinus communis*. Three of the plants namely *Ageratum conyzoides, Annona muricata* and *Ocimum gratissimum* however were observed to show some degree of inhibition which varies with the *E. coli* strain tested. The results of the susceptibility of *E. coli* strains to plants extracts are as shown in Table 1. The activities of the extracts also varied between solvents with the ethanol extracts demonstrating the highest activity against all the test bacteria. The methanol extracts of the plant parts were more potent than the chloroform and water extracts which showed no activity against the test organisms (Table 1).

Table 1: Antimicrobial effects of plant extracts on *E. coli* strains

Plants	Strain of *E. coli*	Chloroform	Methanol	Water
Ageratum conyzoides	A	-	10mm	-
	4	-	-	-
	5	-	12mm	-
Annona muricata	A	-	-	-
	4	-	15mm	-
	5	-	11mm	-
Ocimum gratissimum	A	-	13mm	-
	4	-	12mm	-
	5	-	13mm	-

Legend: A = isolated strain from diarrhoeic pig; 4 = E. coli 021; 4 = E. coli 022; - = no inhibition

Table 2: Minimum Inhibitory Concentration of *Ocimum gratissimum* on *E. coli* Strain A

E. coli strain A load (Cfu/ml)	Concentration of *Ocimum gratissimum* µg/ml	Growth Observed
7.75 x 10^3	20,000	No growth
7.75 x 10^3	10,000	Presence of growth
7.75 x 10^3	5,000	Presence of growth
7.75 x 10^3	2,500	Presence of growth
7.75 x 10^3	1,250	Presence of growth
7.75 x 10^3	625	Presence of growth
7.75 x 10^3	312.5	Presence of growth
7.75 x 10^3	156.25	Presence of growth
7.75 x 10^3	0.0	Presence of growth

Minimum Inhibitory Concentration (MIC) Test

Table 2 shows the MIC of the extract of *Ocimum gratissimum* on *E. coli* strain A. It showed that the extract of *Ocimum gratissimum* inhibited *E. coli* strain A at MIC value of 20,000µg/ml.

Table 3 shows the MIC of the extract of *Ocimum gratissimum* on *E. coli* strain 4. It showed that the extract of *Ocimum gratissimum* also inhibited *E. coli* strain 4 at MIC value of 20,000µg/ml.

Table 3: Minimum Inhibitory Concentration of *Ocimum gratissimum* on *E. coli* Strain 4

***E. coli* strain 4 load (Cfu/ml)**	**Concentration of *Ocimum gratissimum* µg/ml**	**Growth Observed**
1.13×10^4	20,000	No growth
1.13×10^4	10,000	Presence of growth
1.13×10^4	5,000	Presence of growth
1.13×10^4	2,500	Presence of growth
1.13×10^4	1,250	Presence of growth
1.13×10^4	625.0	Presence of growth
1.13×10^4	312.5	Presence of growth
1.13×10^4	156.25	Presence of growth
1.13×10^4	0.0	Presence of growth

Table 4 shows the MIC of the the extract of *Ocimum gratissimum* on *E. coli* strain 5. This also showed that the extract of *Ocimum gratissimum* on inhibited *E. coli* strain 5 at MIC value of 20,000µg/ml.

Table 4: Minimum Inhibitory Concentration of *Ocimum gratissimum* on *E. coli* Strain 5

E. coli strain 5 load (Cfu/ml)	Concentration of *Ocimum gratissimum* µg/ml	Growth Observed
1.18×10^4	20,000	No growth
1.18×10^4	10,000	Presence of growth
1.18×10^4	5,000	Presence of growth
1.18×10^4	2,500	Presence of growth
1.18×10^4	1,250	Presence of growth
1.18×10^4	625	Presence of growth
1.18×10^4	312.5	Presence of growth
1.18×10^4	156.25	Presence of growth
1.18×10^4	0.0	Presence of growth

DICUSSION

In the tropical environment, diarrhea is responsible for mortality in young animals especially piglets. Survivors lose weight and may not be able to reach weaning weight due to atrophy of the intestinal mucusa. The Entero-pathogenic *E. coli* remains an important cause of neonatal diarrhea. This has been confirmed by the findings of other workers [2, 25-28]. *E. coli* coliforms make-up approximately 10% of microorganism of humans and is used as indicator organisms of faecal contamination and is associated with poor environmental sanitation [25]. However, many drug-resistant human fecal *E. coli* isolates may originate from poultry, whereas drug-resistant poultry-source *E. coli* isolates likely originate from susceptible poultry-source precursors [26]. It is estimated that nearly 90% of all antibiotic agents use is in food animals, are given at subtherapeutic concentrations prophylactically or to promote growth [2]. Antimicrobial drug resistance in *E. coli* isolated from wild birds has been described [27]. *E. coli* and the *E. coli* 0157: H7 strain has previously been isolated from meat samples [28].

E. coli strain showed a high frequency of single or multiple drug resistance (MDR). Multidrug resistance was defined as resistance to ≥3 of the antimicrobial agents tested [29], which revealed the multi-drug resistant pattern and ability of these organisms to many antimicrobials (3 to 6 antibiotics). The bacteria were found to be resistant to Ampicillin, Nalidixic acid, Nitrofurantoin, Co-trimoxazole, Streptomycin and Tetracycline. The MDR pattern reported on *E. coli* in this study is comparable to previous studies [27, 30]. One suspected source of drug-resistant *E. coli* in humans is use of antimicrobial drugs in agriculture [26]. This use presumably selects for drug-resistant *E. coli*, which may be transmitted to humans through food supply [26]. Supporting this hypothesis is the high prevalence of antimicrobial drug–resistant *E. coli* in retail meat products, especially poultry reported by Schroeder et al. [31] and Johnson et al. [26], and the similar molecular characteristics of fluoroquinolone-resistant *E. coli* from chicken carcasses and from colonized and infected persons in Barcelona, Spain, in contrast to the marked differences between drug-susceptible and drug-resistant source isolates from humans [26]. Such multidrug resistance has important implications for the empiric therapy of infections caused by *E. coli* and for the possible co-selection of antimicrobial resistance mediated by multidrug resistance plasmids [32].

Resistance to tetracycline was the most frequent in their study. A prominent reason for concern with regard to these MDR organisms is the recognized emergence of antimicrobial resistance among key species. However, a number of studies in the literature indicated a gradual increase in the emergence of antibiotic-resistant microorganisms especially in hospitals [33]. Many factors apart from antibiotic exposure can contribute to the development of antibiotic resistance in bacterial isolates [32]. Overcrowding in holding pens also increases the percentage of antimicrobial resistant enteric bacteria shed into the environment by pigs [32]. Other factors that may disturb gastrointestinal microflora are starvation or other dietary changes, fear and other conditions like cold [3].

Surveillance of *Campylobacter* antimicrobial drug resistance was implemented in France in 1999 for broilers in conventional and free-range broiler farms and in 2000 for pigs as part of a surveillance program on resistance in sentinel bacteria (*Escherichia coli* and *Enterococcus* spp.) and zoonotic bacteria (*Salmonella* spp. and *Campylobacter* spp.) in animal products for human consumption [34]. In the overall, the most common multidrug resistance (>3 drugs) patterns included resistance to Ampicillin, Nalidixic acid, Nitrofurantoin, Co-trimoxazole, Streptomycin and Tetracycline. Resistance to Nalidixic acid and Nitrofurantoin has been previously reported in Ibadan by Okonko et al. [35]. Resistance to co-trimoxazole and streptomycin remained high. Resistance to ampicillin is of clinical interest because this drug may be used for the treatment of severe infections [32]. Cotrimoxazole resistance in line with other studies in recent time remained stable and it is consistent with Oteo et al. [29] and Okonko et al. [32, 36].

E. coli contamination is usually associated with contaminated water and food (animal feeds) and their presence reflects the degree of purity or signals faecal contamination of both human and animal origin [32]. The presence of *E. coli* in these diarrheic piglets should receive particular attention, because their presence indicate public health hazard and give warning signal for the possible occurrence of food borne intoxication [4, 32]. Although the number of isolates was relatively small, the level of multidrug resistance was high. The high rates of resistance found in the present study can be explained by the spread of use of antibiotics agents given to farm animals in Nigeria as prophylaxis, growth promoters or treatment. According to Abdellah et al. [2] and Okonko et al. [32], the multiple resistances observed were also to those antimicrobials frequently employed in veterinary practices. Therefore, in line with the assertions of Okonko et al. [32], we recommend that more restrictions on the irrational use of antibiotics and public

awareness activities should be undertaken to alert the public of the risks of the unnecessary use of antibiotics.

Ethnoveterinary medical practice has been reported to be widespread among herdsmen and village livestock producers in northern Nigeria [37]. This study also investigated the efficacy of some medicinal plants that are believed to have anti-diarrhoeic effect. Extracts of seven plants were tested for antibacterial effect on E. coli. Four of the plants namely: *Adansonia digitata, Bryophyllum pinnatum, Cassia sieberiana,* and *Ricinus communis* showed no inhibition for *E. coli* strains irrespective of the solvent in which the extract was contained. This is consistent with previous studies. The less susceptibility of these four plants namely (*Adansonia digitata, Bryophyllum pinnatum, Cassia sieberiana,* and *Ricinus communis*) might be due to environmental mutation acquired by the organisms [38]. The survey by Harun-or-Rashid et al. [37] indicated that the traditional cattle healers of Birishiri use quite different variety of plant species to treat cattle ailments.

Adansonia digitata (Baobab) is a traditional African medicinal plant with numerous applications, including treatment of symptoms of infectious diseases [39]. Many parts of the plant (*Adansonia digitata*), especially leaves, fruit pulp, seeds and bark fibres, have been used traditionally for medicinal and nutritional purposes [40] and some commercial enterprises produce standardized preparations derived from seeds, fruit pulp and leaves of this plant [39]. The medicinal applications include treatments for intestinal and skin problems and various uses as anti-inflammatory, anti-pyretic and analgesic agents [39]. Recent research in animals has confirmed the presence of such activities in specific extracts [38-39, 41-43]. In addition antibacterial, antiviral and anti-trypanosome activities have been reported [11, 38]. Plant extracts can be evaluated for inflammatory properties in such a system and direct antiviral effects can also be tested against the same viruses [39, 44-45].

Yagoub [38] also reported that *Adansonia digitata* showed no antimicrobial activity against *E. coli* irrespective of the solvent in which the extract was contained. However, this also differs from the findings of other authors. The extracts of *Adansonia digitata* was reported to have had the most potent antiviral properties, especially the DMSO extracts on influenza virus, herpes simplex virus and respiratory syncytial virus and influenza virus was the most susceptible virus [39]. The leaves of *Adansonia digitata* has been used to treat diarrhea, and as anti-dysentery as well as for the treatment of smallpox and measles as an eye instillation [46-47]. The bark has also been used as antidote to strophanthus poisoning [47]. The extracts were also reported to be active against herpes simplex, Sindbis and poliovirus. The fractions of pericarp were also reported to be active against *Staphylococcus aureus, S. epidermidis, Streptococcus mutans,* and *Pseudomonas aeruginosa* and strongly antitrypanocidal [47].

In a study by El-Mahmood and Doughari [48], *S. aureus, S. pyogenes* and *P. mirabilis* were more susceptible to *Cassia alata,* while *E. coli* and *P. aeruginosa* were less sensitive. In another study, *Cassia occidentalis* and *C. zambensicus* exhibited active antimicrobial activity against the enterobacteria tested, that is, *S. typhi* and *E. coli* than *Newbouldia leavis* and the combination of the three plant sources [49]. *Ricinus communis* is used orally for body ache, maintain good health and for treating loss of hair [37]. Bessong and Obi [50] and Bessong et al. [51-52] reported the inhibitory activities of *Ricinus communis* against HIV-1.

Three of the plants (*Ageratum conyzoides, Annona muricata* and *Ocimum gratissimum*) showed some degree of inhibitions with ethanol extract of the plant. This is consistent with previous studies by various authors. Paste of the whole plant of *Ageratum conyzoides* is used as an insect

repellent and for treatment of wounds and itches. Certainly, the available studies indicate that plants like *Ageratum conyzoides*, present excellent potential for development of drugs leading to treatment of both widespread but common ailments like gastrointestinal disorders, as well as complicated ailments like diabetes and heart diseases, which are prevalent throughout the world population and which cannot be treated satisfactorily with modern allopathic medicine [14].

Ocimum gratissimum showed a distinguished antibacterial activity against *E. coli* and this is line with the observations of earlier workers [53]. This study however, showed the minimum inhibitory concentration of *O. gratissimum* to be rather high. With improved technology, better active principle could be extracted from the plant. This could be of value in the treatment of diarrhea due to *E. coli* in livestock. In the study by Adebayo-Tayo and Adegoke [53], *O. gratissimum* and *H. floribunda* exhibited the highest degree of activity and activity index. In their study, the extract of *O. graissimum* ("Nton") was reported to have had the highest mean inhibitory zone of 23 mm against *S. flexineri* [53]. In the same study by Adebayo-Tayo and Adegoke [53], the MIC results of *S. flexineri* in 26.24 mg/ml of *O. gratissimum* excite them to analyze for its MBC which was 47.24 mg/ml. However, the extracts showed the highest degree of inhibition against *S. flexineri* at concentration of 80 mg/ml, followed by *S. anceps* and the least was observed from *K. senegalensis* while at 40 mg/ml, *O. gratissimum* had the highest inhibitory concentration against *S. flexineri* [53].

In this study, *Ocimum gratissimum* has proven antibacterial properties useful in controlling diarrhea. Farmers should be encouraged to plant it on their farms. It can be fed to weaners. It is of value in controlling post-weaning diarrhea. Cattle farmers do not always avoid modern veterinary drugs because of their higher prices or lack of accessibility; but because in their actual observations, they found out that the use of medicinal plants for treatment of certain cattle ailments can provide better efficacy [54]. Modern research has also started to validate at least some of the veterinary uses of medicinal plants [37, 55].

The activities of the plants extracts also varied between solvents with the methanol extracts demonstrating the highest activity against all the test bacteria. This is consistent with other studies [48, 56-57]. In this study, the methanol extracts of the plant parts were more potent than the chloroform and water extracts, which showed no inhibitory activity. This is similar to the reports of Elmahmood and Amey [57] but contrary to observations of Roy et al. [56]. It has been reported that different phytoconstituents have different degrees of solubility in different types of solvents depending on their polarity [48]. In a traditional setting, water is the solvent largely used to prepare these concoctions [57]. The higher activity demonstrated by organic solvents in this study is therefore an indication that less of the bioactive principles are extracted when water is used as a solvent [48].

In conclusion, the development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents [58]. The 3 plants (*Ageratum conyzoides, Annona muricata* and *Ocimum gratissimum*) that showed some degree of inhibitions with ethanol extract may be indicative of the presence of antibiotics compounds in them. In line with Yagoub [38], this may help to discover new phytochemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. More investigation on the subject especially the effect of these 3 plants (*Ageratum conyzoides, Annona muricata* and *Ocimum gratissimum*) on other agents of diarrhea such as *Salmonella spp* and *Yersinia spp* will be rewarding. This investigation might aid the possibility of the use of plants in drug development for human consumption possibly for the treatment of gastrointestinal, urinary tract and wound infection as well as typhoid fever. The noted medicinal plants with no antibacterial

activity on *E. coli* but which have been empirically proven to have anti-diarrhoeic effects may be due to astringent properties possessed by extract of the plants. This act by preventing loss of electrolytes and water to the intestinal lumen. The extracts may show antibacterial activities against other agents of diarrhea that have not been tested in this investigation. There is the need to carry out further test on other agents of diarrhea in order to know the spectrum of micro-organisms that are susceptible to the different fractions of *Ageratum conyzoides, Annona muricata* and *Ocimum gratissimum*. According to Yagoub [38], the effect of these plants on more pathogenic organisms and toxicological investigation and purification however, needs to be carried out. Thus, in line with the assertions of Harun-or-Rashid et al. [37], our findings present considerable potential for further scientific research on these plant species, which can lead to development of cheaper and more efficacious drugs. However, toxicological analysis of the crude extracts is recommended in order to assess their safety in the body of a patient when administered.

REFERENCES

[1] Blood DC, Handerson JA, Radostits OM. **1994**. Diseases of the Newborn. In: Veterinary Medicine, 8th Edition, Bailliere Tindal Publishers.
[2] Abdellah C, Fouzia RF, Abdelkader C, Rachida SB, Mouloud Z. **2009**. *African Journal of Microbiology Research; 3(5)*: 215-219
[3] Chikwendu CI, Nwabueze RN, Anyanwu BN. **2008**. *African Journal of Microbiology Research; 2*: 012-017
[4] Kabir SML. **2009**. *African Journal of Biotechnology; 8 (15*): 3623-3627
[5] Nawaz SK, Riaz S, Riaz S, Hasnain S. **2009**. *African Journal of Biotechnology; 8 (3)*: 365-368
[6] Heuer OE, Jensen VF, Hammerum AM. **2005**. *Emerg. Infect. Dis.; 11(2)*: 344-345
[7] Akerele O. **1991**. Medicinal plants: Policies and priorities conservation of medicinal plants. Great Britain Cambridge University Press. Cambridge.
[8] Edeoga HO, Okwu DE, Mbaebie BO. **2005**. *Afr. J. Biotechnol. 4(7)*: 685-688.
[9] Kala CP. **2007**. *Curr. Sci. 93(12)*: 1828-1834.
[10] Sharief MU, Rao RR. **2007**. *Curr. Sci. 93(11):* 1623-1628.
[11] Masola SN, Mosha RD, Wambura PN. **2009**. *African Journal of Biotechnology, 8 (19)*: 5076-5083
[12] Schmidt B, Ribnicky DM, Poulev A, Logendra S, Cefalu WT, Raskin I. **2008**. *Metabolism, 57 (Suppl 1)*: S3-9.
[13] Bedoya LM, Bermejo P, Abad MJ. **2009**. *Mini Reviews in Medicinal Chemistry, 9(5)*: 519-525.
[14] Mohammed R, Israt JM, Fahmidul-Haque AKM, Md. Ariful-Haque M, Parvin K, Jahan R, Chowdhury MH, Rahman T. **2009**. *Adv. in Nat. Appl. Sci., 3(3):* 402-418.
[15] Gautam R, Jachak SM. **2009**. *Medicinal Research Reviews, 29(5)*: 767-820.
[16] Amara AA, El-Masry MH, Bogdady HH. **2008**. *Pakistan Journal of Pharmaceutical Sciences, 21(2)*: 159-171.
[17] Chattopadhyay D, Khan MT. **2008**. *Biotechnology Annual Review, 14*: 297-348.
[18] Batista R, Ade Silva Jr. J, de Oliveira AB. **2009**. *Molecules, 14(8)*: 3037-3072.
[19] Kihampa C, Joseph CC, Nkunya MH, Magesa SM, Hassanali A, Heydenreich M, Kleinpeter E. **2009**. *Journal of Vector Borne Diseases, 46(2)*: 145-152.
[20] El-On, J., L. Ozer, J. Gopas, R. Sneir, H. Enav, N. Luft, G. Davidov and A. Golan-Goldhirsh, **2009**. *Annals of Tropical Medicine and Parasitology, 103(4)*: 297-306.
[21] Nibret E, Sporer F, Asres K, Wink M. **2009**. *Journal of Pharmacy and Pharmacology, 61(6)*: 801-808.

[22] Latha PS, Kannabiran K. **2006**. *Afr. J. Biotech. 5(23)*: 2402-2404.
[23] Awoyinka OA, Balogun IO, Ogunnowo AA. **2007**. *J. Med. Plants Res. 1(3)*: 63-65.
[24] Biradar YS, Jagatap S, Khandelwal KR, Singhania SS. **2007**. Exploring of Antimicrobial Activity of Triphala Mashi-an Ayurvedic Formulation, Oxford J. [http://ecam.oxfordjournals.org/cgi/content/full/nem002v1] Cited **2010** October 6.
[25] Prescott LM, Harley JP, Klein DA. **2005**. Microbiology. 6th ed. McGraw- Hill, New York; pp. 833-842.
[26] Johnson JR, Sannes MR, Croy C, Johnston B, Clabots C, Kuskowski MA, Bender J, Smith KE, Winokur PL, Belongia EA. **2007**. *Emerg. Infect. Dis.; 13(6)*:838-846
[27] Dolejska M, Cizek A, Literak I. **2007**. *Journal of Applied Microbiology; 103*:11–19.
[28] Oyeleke SB. **2009**. *African J. Microbiology Research; 3(5)*: 245-248
[29] Oteo J, Lázaro E, de Abajo FJ, Baquero F, Campos J, *Emerg. Infect. Dis., 11(4)*: 546-553
[30] Sjölund M, Bonnedahl J, Hernandez J, Bengtsson S, Cederbrant G, Pinhassi J, et al. **2008**. *Emerg. Infect. Dis.; 14(1)*:70-75
[31] Schroeder CM, Naugle AL, Schlosser WD, Hogue AT, Angulo FJ, Rose JS, et al. **2005**. **2000**. *Emerging Infectious Diseases, 11(01)*:113-115
[32] Okonko IO, Nkang AO, Fajobi EA, Mejeha OK, Udeze AO, Motayo BO, Ogun AA, Ogunnusi TA, Babalola TA. **2010**. *EJEAFChe 9 (3)*: 514-532
[33] Suchitra JB, Lakshmidevi N. **2009**. *African J. Microbio. Res.; 3 (4)*:175-179
[34] Gallay A, Prouzet-Mauléon V, Kempf I, Lehours P, Labadi L, Camou C, Denis M, de Valk H, Desenclos J-C, Megraud F. **2007**. *Emerg. Infect. Dis.; 13(2)*: 259-266
[35] Okonko IO, Donbraye-Emmanuel OB, Ijandipe LA, Ogun AA, Adedeji AO, Udeze AO. **2009a**. *Middle-East J. Scientific Research; 4 (2)*: 105-109
[36] Okonko IO, Soleye FA, Amusan TA, Ogun AA, Ogunnusi TA, Ejembi J. **2009b**. *Global J. Pharmacology; 3(2)*:69-80
[37] Harun-or-Rashid MD, Tanzin R, Ghosh KC, Jahan R, Khatun MA, Rahmatullah M. **2010**. *Adv. in Nat. Appl. Sci., 4(1)*: 10-13.
[38] Yagoub SO. **2008**. *Research Journal of Microbiology*; 3(3): 193-197
[39] Vimalanathan S, Hudson JB. **2009**. *Journal of Medicinal Plants Research; 3(8)*: 576-582
[40] Chadare FJ, Linnemann AR, Hounhouigan JD, Nout, MJR, Van Boekel MAJ. **2009**. *Crit. Rev. Food Sci. Nutr. 49*: 254-274.
[41] Palombo EA. **2006**. *Phytother. Res. 20*: 717-724.
[42] Ajose FOA. **2007**. *Int. J. Derm. 46 (suppl 1*): 48-55.
[43] Karumi Y, Augustine AI, Umar IA. **2008**. *J. Biol. Sci. 8*: 225-228.
[44] Sharma M, Schoop R, Hudson JB. **2008**. *Phytother. Res. 23*: 863-867.
[45] Sharma M, Anderson SA, Schoop R, Hudson JB. **2009**. *Antiv. Res. 83*: 165-170.
[46] Shahat AA. **2006**. *Pharmaceutical Biology; 44*:445-450
[47] Doughari JH. **2006**. *Tropical Journal of Pharmacy Research*; 5: 597-603
[48] El-Mahmood AM, Doughari JH. **2008**. *African Journal of Pharmacy and Pharmacology; 2(7)*: 124-129
[49] Ajayi O, Akintola TA. **2010**. *African Journal of Microbiology Research; 4 (4)*: 314-316
[50] Bessong PO, Obi CL. **2006**. *African Journal of Biotechnology; 5 (19)*: 1693-1699
[51] Bessong PO, Obi CL, Andreolar M-L, Rojas LB, Pouysegu L, Igumbor E, Meyer JJM, Guideau S, Litvak S. **2005**. *J. Ethnopharmacol. 99(1)*: 83-91.
[52] Bessong PO, Rojas LB, Obi CL, Tshisikawe PM, Igumbor EO. **2006**. *Afri J. Biotechnol. 5(6)*: 526-528.
[53] Adebayo-Tayo BC, Adegoke AA. **2008**. *Journal of Medicinal Plants Research; 2(11)*: 306-310
[54] Luseba D, Van der Merwe D. **2006**. *Onderstepoort Journal of Veterinary Research, 73*: 115-122.

[55] Farooq Z, Iqbal Z, Mushtaq S, Muhammad G, Iqbal MZ, Arshad M. **2008**. *Journal of Ethnopharmacology, 118*: 213-219.
[56] Roy J, Shakaya DM, Callery PS, Thomas JG. **2006**. *Afr. J. Trad. Compl. Alt Med. 3(20)*: 1-7.
[57] Elmahmood AM, Amey JM. **2007**. *Afr. J. Biotechnol. 6(11)*: 1272-1275.
[58] Alim A, Goze I, Cetin A, Atas AD, Vural N, Donmez E. **2009**. *African Journal of Microbiology Research; 3(8)*: 422-425