Biochemistry and Molecular Biology
2016; 1(3): 27-33
http://www.sciencepublishinggroup.com/j/bmb
doi: 10.11648/j.bmb.20160103.11

Antimicrobial Potentials of *Parkia clappertoniana* Jacq, *Boswellia dalzielli* Hutch and *Carica papaya* L. Ethanolic Extract on Multi- drug Resistant Diarrheal *salmonallae* and *shigellae* Bacteria

Maryam Sani Lawal[1, *], Abdulsalami Mohammed Sani[2], Denwe Samuel Dangmwan[2], Usman Yahaya[3]

[1]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria

[2]Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria

[3]Trial Afforestation Research Station, Forestry Research Institute of Nigeria (FRIN), Kaduna, Nigeria

Email address:

mynetmyn@yahoo.com (M. S. Lawal) msabdulsalami@nda.edu.ng (A. M. Sani), dangmwansamuel@gmail.com (D. S. Dangmwan), usmanyahayaks@yahoo.com (U. Yahaya)

[*]Corresponding author

To cite this article:

Maryam Sani Lawal, Abdulsalami Mohammed Sani, Denwe Samuel Dangmwan, Usman Yahaya. Antimicrobial Potentials of *Parkia clappertoniana* Jacq, *Boswellia dalzielli* Hutch and *Carica papaya* L. Ethanolic Extract on Multi- drug Resistant Diarrheal *salmonallae* and *shigellae* Bacteria. *Biochemistry and Molecular Biology.* Vol. 1, No. 3, 2016, pp. 27-33. doi: 10.11648/j.bmb.20160103.11

Received: September 9, 2016; **Accepted**: September 22, 2016; **Published**: October 14, 2016

Abstract: Medicinal plants constitute the major component of traditional medicine practiced worldwide due to the economic viability, accessibility, and ancestral experience. Three medicinal plants (*Boswelllia dalzielli* Hutch, *Carica papaya* L. and *Parkia clappertoniana* Jacq) used locally in diarrheal treatment were investigated. This study was carried on isolated and confirmed strains of multi-drug resistant isolates of *salmonallae* and *shigellae* bacteria isolated from diarrheal patients in kaduna metropolis, Nigeria. The antidiarrheal activity of the three plant extracts against multidrug resistant strains of *Shigella* and *Salmonella* was performed by agar well diffusion method using standard guidelines. The findings indicated that *Boswelllia dalzielli*, *Carica papaya* and *Parkia clappertoniana* had growth inhibitory effect against the tested bacteria. Ethanolic extract of *Boswelllia dalzielli* exhibited the highest inhibitory effect at 100mg/ml concentration on both *Salmonella* and *Shigella* isolates. When the results were statistically analyzed using Duncan's multiple range test ($P<0.05$), the highest efficacy was recorded with the ethanol extracts of *Boswelllia dalzielli* (26.00 ± 1.00). On the basis of these findings, it can be assumed that ethanol extracts of *Boswelllia dalzielli*, *Carica papaya* and *Parkia clappertoniana* could be potential sources for antidiarrheal drugs.

Keywords: Diarrhea, Multidrug Resistance, Medicinal Plants

1. Introduction

Diarrheal diseases are leading cause of mortality and morbidity, especially in children in developing countries, including Nigeria and other Sub-saharan countries resulting in a major health care problem [1]. Infections due to a variety of bacterial etiologic agents, such as pathogenic *Escherichia coli, Vibrio cholerae, Areomonas spp., Shigella spp., Salmonella spp., Pseudomonas spp., Klebsiella spp., Campylobacter spp., and Staphylococcus aureus* are most common. Infectious diseases are the world's major danger to human health and account for approximately 550,000 deaths/day [2]. Most of the severe conditions are claimed due to pathogenic bacteria.

Antibacterial drugs are one of the most important tools in combating bacterial infections and saving human life from severe invasion of many infectious diseases. However, from the past few decades, these health opportunities are under risk as many frequently used antibiotics have become less effective against certain illnesses. This is not only due to the toxic reactions, but also for the emergence of multi-drug-

resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics [3].

Multiple drug resistance (MDR), or multi-resistance is a condition enabling disease-causing microorganisms (bacteria, viruses, fungi or parasites) to resist distinct antimicrobials, first and foremost antibiotics, but also antifungal drugs, antiviral medications, anti-parasitic drugs, chemicals of a wide variety of structure and function targeted at eradicating the organism. Therefore, alternative antimicrobial sources are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic relevance of ancient remedies, such as plants [4].

Medicinal plants have been acknowledged as potential sources of new compounds of therapeutic value and as sources of lead compounds for drug design and development [5]. Medicinal plants generally exhibit multiple non-specific effects which are usually complementary or synergistic. In most cases, a plant is often used for a variety of ailments which may then suggest a concerted or wholesome therapy. These therapeutic agents can have antispasmodic effects, delay intestinal transit, suppress gut motility, inhibit intestinal motility, stimulate water adsorption or reduce electrolyte secretion [6] and some of these effects have been attributed to the presence of numerous phytochemicals (such as alkaloids, tannins, flavonoids and terpenes). Considering the vast potentiality of plants as sources for antimicrobial drugs, this study aimed at detecting the antibacterial activities of some natural plant extracts and investigating their effects on some multi-drug resistant human clinical bacterial isolates to common commercial antibiotics.

2. Materials and Methods

2.1. Collection, Identification and Treatment of Plant Materials

Fresh plant parts were collected at Trial Afforestation Research Station, Forestry Research Institute of Nigeria, Afaka Kaduna. The plants were authenticated by a taxonomist in the Department of biological sciences NDA Afaka Kaduna to confirm their identities with voucher numbers NDA BIO 1529, 1530 and 1531 for *Carica papaya, Parkia clappertoniana* and *Boswellia dalzielli spp* respectively. The samples were then dried at room temperature after which they were milled into coarse powder with an electric blender; the milled samples were kept in tight bottles.

2.2. Extraction Procedure

Ethanolic extracts of the plants were prepared using the method described by [7]. One hundred grams of the blended samples each were separately measured into a conical flask and 1000 ml of ethanol was added; covered with a cork, and left on a shaker at 100 r.p.m. for 24 hours after which the extract was filtered and squeezed through four layers of muslin cloth. The filtrate was concentrated under reduced pressure in a rotavapor at 40°C to recover the solvent. The extracts obtained were stored in sterile McCartney bottles and kept in the refrigerator at 4°C and later used for antimicrobial tests.

2.3. Phytochemical Screening of the Plant Extracts

Chemical constituents of the extracts were analysed to detect the presence of particular compounds using standard procedures according to the methods of [8, 9]

2.4. Collection and Maintenance of Test Organisms

Pure cultures of bacteria *(Salmonella spp.* and *Shigella spp*.) isolated from clinical specimens were obtained from the microbiology department of Ahmadu Bello University Teaching Hospital Zaria ,Yusuf Dan-tsoho memorial hospital, Kaduna National Institute For Trypanosomiasis Reasearch (NITR), Kaduna and Gold Bond Medical Laboratories, Kaduna. The organisms were maintained on Nutrient agar slants at 4°C and were routinely sub-cultured during storage.

2.5. Morphological and Biochemical Characterization of Isolates

Isolates were characterized and identified based on morphological and biochemical characterization using standard techniques described by [10].

2.6. Antibiotic Susceptibility Screening

The sensitivity/resistance of the bacterial strains to antibiotics was carried out using Kirby-Bauer disc diffusion method. A multi disc containing Augmentin (30 µg), Ciprofloxacin (10 micrograms), Septrin (30 micrgramms), Chloramphenicol (30 microgramms), Sparfloxacin (10 microgramms), Amoxacillin (30 microgramms), Gentamycin (10 microgramms), Pefloxacin (30 microgramms), Tarivid (10 micrgramms) and Streptomycin (30 microgramms) was employed.

2.7. Antibacterial Properties of Plant Extracts

The antibacterial properties of ethanolic plant extracts were determined using the agar well diffusion method of [11]. Twenty-four (24) hour old broth cultures of each test organism (standardized inoculum) were swabbed onto sterile Mueller Hinton agar in Petri dishes using sterile cotton swab. A sterile stainless steel cork borer of size 5mm in diameter was used to make wells on the plates. The holes were filled with 10mg/ml, 20mg/ml, 30mg/ml 50mg/ml and 100 mg/ml of the extracts. Each was labelled appropriately. Control experiment was also set up where the holes were filled with sterile distilled water by the use of sterile 2 ml syringes.

The plates were incubated at 37°C for 24 hours after which the result were read by measuring the diameter of zones of inhibition around the wells with the aid of a ruler and recorded. The antimicrobial readings were done in triplicates and diameters of zones of inhibition (mm) were expressed as means.

2.8. Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the

extracts was determined for each of the test organisms in triplicates in test tubes. To 0.5 ml of varying concentrations of the extracts (5, 25, 50, 75, 100, 125, 150, 175 and 200 mg/ml) in test tubes, Nutrient broth (2 ml) was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. A tube containing nutrient broth only was seeded with the test organisms, as described above, to serve as controls.

The culture tubes were then incubated at 37°C for 24 h. After incubation the tubes were then examined for microbial growth by observing the turbidity.

2.9. Minimum Bactericidal Concentration (MBC)

Visual observation of growth inhibition on solid medium was used to determine MBC as described by [12]. To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile Nutrient agar by streaking. Nutrient agar plates only were streaked with the respective test organisms to serve as controls. All the plates were then incubated at for 37°C 24 h. After incubation the concetration at which no visible growth was seen was noted as the Minimum Bactericidal Concentration (MBC).

3. Result

3.1. Identification of Organisms

In this research, two enteric bacteria namely *Salmonella* and *Shigella* were studied. Isolates collected from the different healthcare institutions were subjected to biochemical test (their ability to utilise or oxidise certain chemical substances to produce a certain colour). They were identified based on their morphological and biochemical reactions (table 1)

Table 1. *Characterization and Biochemical reaction of Salmonella spp and Shigella spp isolates from diarrhoea.*

Phenotypic Traits and Tests	Appearances and responses of Bacterial Isolates	
	Salmonella spp.	***Shigella spp***
Morpology	**smooth, colourless, colonies. 2-4mm**	**smooth, colourless colonies. 2-3mm**
Shape	Rod	Rod
Gram reaction	-ve	-ve
Glucose	+ve	+ve
Sucrose	+ve	+ve
Lactose	-ve	-ve
Oxidase	-ve	-ve
Indole	-ve	-ve
Hydrogen Sulphide	+ve	-ve
Gas production	+ve	+ve
Ornithine	+ve	+ve
Urea	-ve	-ve
Citrate	+ve	-ve
Motility	Motile	non-motile
Triple Sugar iron (butt)	A	A
Triple Sugar iron (slant)	K	K

Key: +ve Positive, -ve Negative, A:Acid,K: AlkalineA/A; utilize glucose, lactose and sucrose.

3.2. Phytochemical Screening

Phytochemical screening of the plant extracts showed presence of some phyto compounds (Table 2).

Table 2. *Qualitative analysis of the phytochemicals of the ethanolic extracts of Boswellia dalzielli, Parkia clappertoniana and Carica papaya.*

Plant	Alkaloids	Glycoside	Flavonoid	Saponin	Tannin	Steroids	Phenols	Protein
BDL	+	_	+	+	+	+	+	_
BDB	+	+	+	+	+	+	_	_
CPL	+	+	_	+	+	+	+	_
CPB	+	+	+	+	+	+	_	_
PCL	+	+	+	+	+	_	_	_
PCB	+	+	_	+	+	+	+	

KEY: BDL= *Boswellia dalzielli* leave, BDB= *Boswellia dalzielli* bark, CPL= *Carica papaya* leave, CPB = *Carica papaya* bark, PCL= *Parkia clappertoniana* leave PCB= *Parkia clappertoniana* bark.

3.3. Susceptibility Testing of Conventional Antibiotics against Salmonella and Shigella Species

Susceptibility testing of conventional antibiotics against *Salmonella* and *Shigella* species shows that Augmentin, Amoxacillin and Streptomycin were the most resisted antibiotics, having no effect on any of the isolates exposed to them (Table 3), Chloramphenicol was the most effective with 25±1.00 and 23±1.00 against *Salmonella* and *Shigella* respectively. Followed by Sparfloxacin (15.33±0.58) for *Shigella* and *Salmonella* with 21.00±1.00. Drug resistance was also observed for Gentamycin, perfloxacin and Tarivid.

The result of the susceptibility of antibiotics presented a varying degree of resistance to the different drugs used against the different organisms but in all, from the result of antibiotics susceptibility tests, all isolates were sensitive to at least one of the Ten antibiotics and resistant to more than three they were tested against. The result were labeled Resistant (R), Intermediate(I) and Susceptible(S) after being compared to the approved performance standards for antimicrobial susceptibility testing.

Table 3. *Zones of inhibition of Antibiotics with Salmonella spp. and Shigella spp.*

	Zones of inhibitions	
Antibiotics	***Shigella***	***Salmonella***
Chloramphenicol	23.00 ± 1.00(S)	25.00 ± 1.00(S)
Sparfloxacin	15.33 ± 0.58(I)	21.00 ± 1.00(S)
Septrin	7.17 ± 0.29(R)	14.67 ± 0.58(I)
Ciproflaxacin	20.50 ± 3.04(S)	21.00 ± 1.00(S)
Amoxicillin	0.00 ± 0.00(R)	0.00 ± 0.00(R)
Augmentin	0.00 ± 0.00(R)	0.00 ± 0.00(R)
Gentamycin	0.00 ± 0.00(R)	8.33 ± 0.58(R)
Pefloxacin	0.33 ± 0.58(R)	0.00 ± 0.00(R)
Tarivid	4.83 ±0.29(R)	7.00 ± 1.00(R)
Streptomycin	0.00 ± 0.00(R)	0.00 ± 0.00(R)

KEY: R= Resistant: I=Intermediate :S= Susceptible
Values are means ± standard deviation of three replicates

3.4. Susceptibility Testing of Boswellia Dalzielli, Parkia Clappertoniana and Carica papaya Extracts on Salmonella and Shigella Species

The results showed that the plant extracts had activity on all the test organisms at varying degree (Tables 4 and 5). The absence of zones of inhibition around each well signified resistance. The zone of inhibition against the isolates increased with increase in concentration of the plant extracts. Against *Salmonella* isolate the highest zone of inhibition was observed at 100mg /ml with an inhibition of 26±1.00 (Table 4) by ethanol extract of *Boswellia dalzielli* stem bark followed by the leave extract with 25.50±0.50 (Table 4). No zone of inhibition was observed at 10mg/ml by ethanol extracts of *Carica papaya* and *Parkia clappertoniana* (Tables 4). Table 5 showed that at 100mg/ml the highest inhibition was observed against *Shigella* at 26 ±1.00 by *Boswellia dalzielli* ethanol leave extract.

Table 4. *Zones of Inhibition of Ethanolic Extracts of Boswellia dalzielli, Parkia clappertoniana and Carica papaya with Salmonella spp.*

Plant extract	**concentration (mg/ml)**				
	10	**20**	**30**	**50**	**100**
BDL	$8.00^{c} \pm 1.00$	$14.60^{d} \pm 0.85$	$15.00^{c} \pm 1.00$	$21.00^{d} \pm 1.00$	$25.50^{d} \pm 0.50$
BDB	$4.17^{b} \pm 1.04$	$6.30^{c} \pm 0.92$	$11.20^{b} \pm 1.57$	$17.40^{c} \pm 2.42$	$26.00^{d} \pm 1.00$
CPL	$0.00^{a} \pm 0.00$	$2.50^{ab} \pm 0.50$	$8.33^{ab} \pm 0.58$	$13.03^{b} \pm 0.58$	$16.00^{b} \pm 2.00$
CPB	$0.00^{a} \pm 0.00$	$1.67^{a} \pm 2.87$	$4.90^{a} \pm 4.25$	$7.93^{a} \pm 2.50$	$12.33^{a} \pm 4.16$
PCL	$0.00^{a} \pm 0.00$	$1.00^{a} \pm 1.00$	$11.13^{b} \pm 1.79$	$12.73^{b} \pm 0.64$	$17.00^{bc} \pm 1.00$
PCB	$0.00^{a} \pm 0.00$	$4.33^{bc} \pm 0.58$	$10.50^{b} \pm 0.50$	$15.23^{bc} \pm$ c2.08	$19.77^{c} \pm 0.56$

a,b,c,d means within a column in each plant part with different superscripts are significantly different ($P<0.05$). Values are means ± standard deviation of three replicates.
BDL= *Boswellia dalzielli* leave, BDB= *Boswellia dalzielli* bark, CPL= *Carica papaya* leave, CPB = *Carica papaya* bark, PCL= *Parkia clappertoniana* leave PCB= *Parkia clappertoniana* bark.

Table 5. *Zones of Inhibition of Ethanolic Extracts of Boswellia dalzielli, Parkia clappertoniana and Carica papaya with Shigella spp.*

Plant extract	**concentration (mg/ml)**				
	10	**20**	**30**	**50**	**100**
BDL	$8.83^{d} \pm 0.29$	$10.83^{c} \pm 0.29$	$19.40^{e} \pm 0.53$	$23.33^{e} \pm 0.58$	$26.00^{d} \pm 1.00$
BDB	$0.00^{a} \pm 0.00$	$5.00^{b} \pm 1.00$	$8.50^{c} \pm 0.50$	$12.00^{c} \pm 1.00$	$15.67^{b} \pm 0.58$
CPL	$0.00^{a} \pm 0.00$	$4.67^{b} \pm 0.58$	$6.67^{b} \pm 0.58$	$10.33^{b} \pm 0.58$	$14.67^{b} \pm 0.58$
CPB	$0.00^{a} \pm 0.00$	$0.67^{a} \pm 0.58$	$3.33^{a} \pm 0.58$	$7.00^{a} \pm 1.00$	$11.00^{a} \pm 1.00$
PCL	$1.33^{b} \pm 0.58$	$5.67^{b} \pm 0.58$	$8.83^{c} \pm 0.29$	$11.67^{bc} \pm 0.58$	$15.33^{b} \pm 0.58$
PCB	$2.33^{c} \pm 0.58$	$5.33^{b} \pm 0.58$	$10.00^{d} \pm 0.00$	$15.33^{d} \pm 1.15$	$20.00^{c} \pm 1.00$

a,b,c,d means within a column in each plant part with different superscripts are significantly different ($P<0.05$). Values are means ± standard deviation of three replicates.
BDL= *Boswellia dalzielli* leave, BDB= *Boswellia dalzielli* bark, CPL= *Carica papaya* leave, CPB = *Carica papaya* bark, PCL= *Parkia clappertoniana* leave PCB= *Parkia clappertoniana* bark.

3.5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) was observed by lack of turbidity in each test tube used for the minimum inhibitory concentration test after 24 hours at 37C (Figure 1 and 2) of which the while the appearance of turbidity indicated the growth of test organism. The effects of the extracts were also observed.

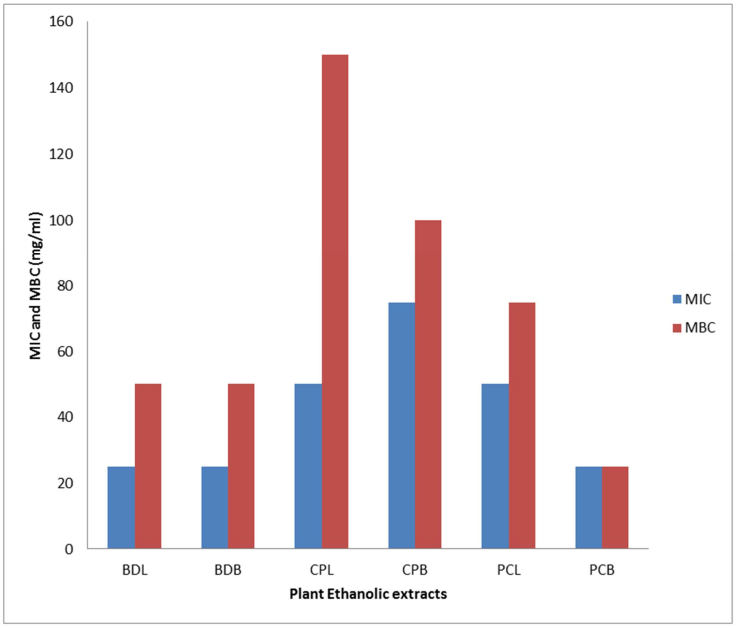

Figure 1. *Minimum Inhibitory Concentration MIC (mg/ml) and Minumum Bactericidal Concentration MBC(mg/ml) of Boswellia dalzielli, Parkia clappertoniana and Carica papaya ethanolic extract for Salmonella spp.*

BDL= *Boswellia dalzielli* leave, BDB= *Boswellia dalzielli* bark, CPL= *Carica papaya* leave, CPB = *Carica papaya* bark, PCL= *Parkia clappertoniana* leave PCB= *Parkia clappertoniana* bark.

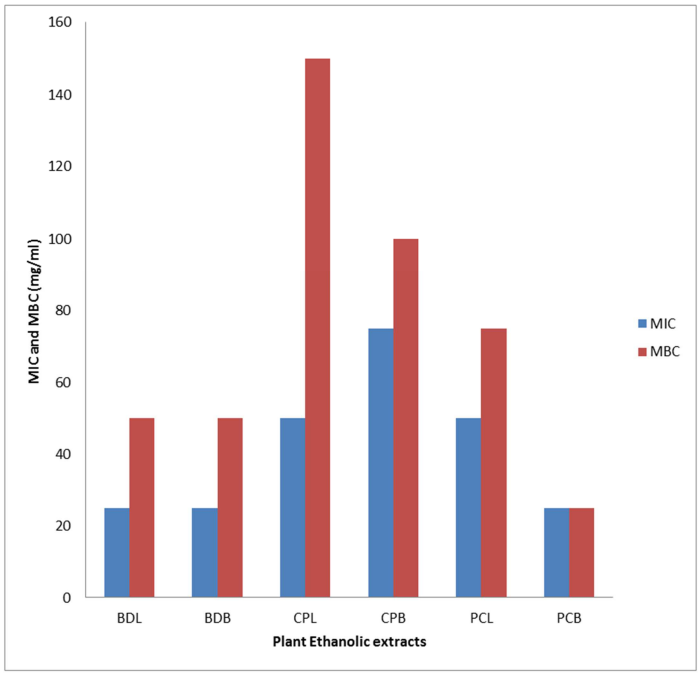

Figure 2. *Minimum Inhibitory Concentration MIC (mg/ml) and Minumum Bactericidal Concentration MBC(mg/ml) of Boswellia dalzielli, Parkia clappertoniana and Carica papaya ethanolic extract for Shigella spp.*

KEY: BDL= *Boswellia dalzielli* leave, BDB= *Boswellia dalzielli* bark, CPL= *Carica papaya* leave, CPB = *Carica papaya* bark, PCL= *Parkia clappertoniana* leave PCB= *Parkia clappertoniana* bark.

4. Discussion

Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. The study was conducted with a view to investigate the antimicrobial properties of some medicinal plant extracts against some human clinical bacterial isolates. In this study, the isolated *sallmonallae* and *shigallae* bacteria were resistant to most (70%) of the commonly used antibiotics (Table 3). Drug resistance of bacteria to antibiotics has been attributed to the misuse and overuse of antibiotics as well as the possession of drug resistance plasmids [13]. There seemed to be complete resistance to Augmentin, Pefloxacin, Tarivid and Amoxicillin by both *Salmonella* and *Shigella* isolates (Table 3) in this study, which is in disagreement with reports from [14, 15]. Resistance is a natural biological response of microbes to antimicrobials and is currently a worrisome scenario affecting many parts of the world [16, 17]. Apart from intrinsic resistance, gene transfer and mutation are among the underlying mechanisms involved in the development of antimicrobial resistance by microbes [16]. Ethanolic extract of the bark and leaves of *Boswellia dalzielli* showed high potency in their bactericidal action against the test bacteria. This might be due to the ability of the solvent to extract active ingredients (bioactive compounds) from the plant materials. This research suggest that ethanolic extracts of screened plants would be helpful in treating diseases in man caused by enterotoxin producing bacteria like *Salmonella Shigella*. The antibacterial activities of the extracts increased as the concentration increased as found out in this work (Table 4 and 5). This does not differ from the research findings of [18] who reported that the tannins isolated from medicinal plants possess remarkable toxic activity against bacteria and fungi and may assume pharmacological importance in future.

The demonstration of activity against the test bacteria provides scientific bases for the local usage of these plants in the treatment of various bacterial related ailments. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant bacteria. The low MIC value observed in our study (Figure 1 and 2) using some of the extracts is a good indication of high efficacy against these bacteria as was observed in a study by [19]. This outcome is good considering that diarrhoea is on the rise and also becoming recalcitrant to first-line antibiotics for its treatment in developing countries, including Nigeria. Other extracts notably *Carica papaya* had high Minimum Inhibitory Concentration (MIC) may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds [19].

5. Conclusion

In conclusion, except for Chloramphenicol, ciproflaxacin and sparfloxacin for which both *Salmonella* and *Shigella* isolates were susceptible, a high level of multi-drug resistance was detected. Notably, the bacteria seemed to have developed complete resistance to Augmentin, Streptomycin, Pefloxacin and Amoxicillin. Therefore, traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries.

References

[1] Bangar, R., Mamatha, B. and Indira B. (2011). A novel treatment approach towards Emerging Multidrug Resistant Enteroaggregative *Escherichia coli* (EAEC) causing acute/persistent diarrhea using medicinal plant extracts. *Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2 (1)*: 1-9.

[2] Ahmad, I. and Beg, A. Z (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacol 74 (2):* 113-23.

[3] Hancock R. E., Nijnik A. and Philpott DJ (2012). Modulating immunity as a therapy for bacterial infections. *Nat Rev Microbiol; 10(4)*: 243-54.

[4] Mandal, S., Deb Mandal, M., and Pal, N. K. (2010). Synergistic anti-*taphylococcus aureus* activity of amoxicillin in combination with *Emblica officinalis* and *Nymphae odorata* extracts. *Asian Pacific Journal of Tropical Medicine, 3*: 711-714.

[5] Newman DJ, Cragg GM, Snader KM. (2003). Natural Products as sources of new drugs over the period 1981-2002. *Journal of Natural Products., 66*: 1022-1037.

[6] Palombo EA. (2006). Phytochemicalsfrom traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. *Phytotherapy, 20*(9): 717–724.

[7] Barry A. L. and Thornsberry C. (1991). Susceptibility tests: Manual of Clinical Microbiology. 5th ed. Washinghton: America Society for Microbiology.

[8] Odebiyi A. and Sofowora A. E. (1990). Phytochemical screening of Nigerian medicinal plants. *Part III. Lloydia,* 41: 234-246.

[9] Trease, G. E, and Evans, M. C. (1983). *Textbook of Pharmacognosy* (12th ed.) London: *Bailiere, Tindal*. Pp. 343-383.

[10] Aneja K. R. (2007). *Experiments in Microbiology, Plant Pathology and Biotechnology.* Pp 547-554.

[11] Bauer A, Kirby W. M. J., Sherris C. and Truck M. (1966). Antibiotic susceptibility testing by a standard single disc method. Am. *Journal of Clinical. Pathology*., 44: 493.

[12] Mitscher L. A, Leu R., Bathala M. S., Wu W. and Beal J. L. (1972). Antimicrobial agents from higher plants. Introduction, rational and methodology. Uoydia 35. 157-166.

[13] DubMandal, M. (2005). Experiments on exploration of environmental bacteria degrading a pesticide used in agriculture. *Thesis*, University of Jadavpur.

[14] Mache A., Mengistu Y. and Cowley S. (1997). *Shigella* serogroups identified from adult diarrhoeal out-patients in Addis Ababa, Ethiopia: antibiotic resistance and plasmid profile analysis." *East Africa Medical Journal*, 74 (3): 179-182.

[15] Asrat D. (2008). "*Shigella* and *Salmonella* serogroups and their antibiotic susceptibility patterns in Ethiopia." *East Mediterranean Health Journal*., 14 (4): 760-767

[16] Sharma R, Sharma CL. and Kapoor B. (2005). "Antibacterial resistance: Current problems and possible solutions." *Indian. Journal of Medical Science*, (59): 120-129.

[17] Khatun F., Faruque A. S., Koeck J. L., Olliaro P., Millet P., Paris N., Malek M. A., Salam M. A. and Luby S. (2011). "Changing species distribution and antimicrobial susceptibility pattern of Shigella over a 29-year period (1980-2008)." *Epidemiology and Infections*, 139 (3): 446-452.

[18] Banso, A and Adeyemo, S. O. (2007). Evaluation of antimicrobial properties of tannins isolated from Dichrostachys cinerea. *African Journal of Biotechnology*, 6 (15): 1785.

[19] Doughari, J. H., Elmahmood, A. M. and Manzara, (2007). S. *African Journal of Microbiology Research* pp. 037-041.