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ANTIMICROBIAL ACTIVITY OF AQUEOUS EXTRACTS OF *BOSWELLIA DALZIELLI* HUTCH, *CARICA PAPAYA* L. AND *PARKIA BIGLOBOSA* JACQ ON MULTI-DRUG RESISTANT DIARRHEA *SALMONALLAE* AND *SHIGELLAE*

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ABSTRACT

Diarrhea is a disease which remains a problem worldwide despite advances in health interventions. Alternative medicine have been practiced for centuries and remained as an integral part of civilization around the globe. One important aspect of alternative medicine is medicinal plants in which locally available plants or its parts are used in treating diarrhea . Three medicinal plants (*Boswellia dalzielli* Hutch, *Carica papaya* L. and *Parkia biglobosa* Jacq) used locally in diarrhea treatment were investigated. This study was carried on isolated and confirmed strains of multi-drug resistant isolates of *Salmonallae* and *shigellae* bacteria. The experimental treatments consist of different concentrations (10, 20, 30, 50 and 100 mg/ml) of aqueous extracts of bark and leaves of the three medicinal plants in which the concentrations were individually used to screen *Salmonella* and *Shigellae* for susceptibility. Distilled water was used as control. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined in this investigation. The experiment consists of three replicates arranged in a complete randomised block design. The data collected were subjected to analysis of variance (ANOVA). Means separation was carried out using Duncan Multiple Range Test. The findings indicated that *Boswellia dalzielli*, *Carica papaya* and *Parkia biglobosa* had growth inhibitory effect against the tested bacteria. Aqueous extract of *Boswellia dalzielli* exhibited the highest inhibitory effect at 100mg/ml concentration on both *Salmonella* and *Shigella* isolates. Highest efficacy of (23.00 ± 1.00) was recorded with the aqueous extracts of *Boswellia dalzielli*. On the basis of these findings, it can be assumed that aqueous extracts of *Boswellia dalzielli* (23.00), *Carica papaya* (13.77) and *Parkia biglobosa* (17.73) could be potential sources for antidiarrheal drugs.

Keywords: Diarrhea, Multidrug Resistance, Medicinal Plants.

Introduction

Antibiotics have saved the lives of millions of people and have contributed to the major gains in life expectancy over the last century. Antibiotics treatment of common bacterial infections plays an important role in reducing prevalence and death rates of the disease (Reza and Abbas, 2019). However, incorrect usage of antibiotics or misuse in management of diarrhea increases antibiotic resistance. *Shigella* and *Salmonella spp.* are resistant to most antibiotics, and drug treatment related to these bacteria are costly, time consuming and sometimes problematic, particularly in areas with inadequate medical care (Teneja and Mewara, 2016).

The clinical efficacy of many existing antibiotics is being threatened by the

emergence of multi-drug resistance (MDR) pathogens. Diarrhea is caused by host bacterial, viral or parasitic organism most of which are spread by feaces-contaminated water. The increase in antibiotic resistance by microbes to almost all available synthetic antibiotics is a serious public health issue (Vadhana *et al.,* 2015). Infectious diseases caused by resistant micro-organisms are associated with prolonged hospitalizations, increased cost and greater risk for morbidity and mortality. The resistance problem demands that a renewed effort be made to screen various medicinal plants for their potentials anti-microbial traits (Dahiya and Purkayasha, 2012).

Generally, there are proof based studies to confirm the efficiency of medicinal plants and some of these strips of evidence have provided visions into the synthesis of plant based compounds with therapeutics applications (Dhama *et al.,* 2014). Medicinal plants are vital therapeutic resources (Motamedi *et al.,* 2010). They play vital roles in disease prevention and their promotion and use fit into all existing prevention strategies (Sofowara *et al.,* 2013). All plants containing active compounds are important.

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. Thus, plants persist the most abundant natural primary source of active drugs and are priceless in the ethno medicinal treatment of diverse ailments. Medicinal plants are sources of various phytochemicals some of which are usually responsible for their biological activities (Harriet *et al.,* 2020). Interest in plants with antimicrobial properties has been revived as a result of current resistance profiles associated with over and inappropriate use of antibiotics (Chattopadhyay *et al.,* 2009). This research was designed to study the antimicrobial potentiality of different parts of three medicinal plants (*Parkia biglobosa, Boswellia dalzielli* and *Carica papaya*) aqueous extract against multi-drug resistant diarrhea *Salmonellae* and *Shigellae* bacteria isolated from clinical isolates.

Materials and Methods

Experimental Design

Different concentrations (10, 20, 30, 50 and 100 mg/ml) of aqueous extracts of *Boswellia dalzeilli* leaves, *Boswellia dalzeilli* bark, *Carica papaya* leaves, *Carica papaya* bark, *Parkia biglobosa* leaves and *Parkia biglobosa* bark were individually used to screen *Salmonella* and *Shigellae* for susceptibility. Distilled water was used as control treatment. The experiment was arranged in complete randomized block design. Pure culture of bacteria (*Salmonella* and *Shigellae*) were collected and maintained on nutrient agar slants at 4 ^{o}C. Zones of inhibition of the treatments with *Salmonella* and *Shigellae* spp were observed and recorded. The experiment was conducted in triplicates.

Collection and Treatment of Plant Materials

Fresh plant parts were collected at Trial Afforestation Research Station, Forestry Research Institute of Nigeria, Afaka Kaduna. Trial Afforestation Research Station lies on latitudes 10^{o} 33'N and 10^{o} 42'N; Longitudes 7^{o} 13'E and 7^{o} 24'E. The samples were dried at room temperature after which they were milled into coarse powder with an electric blender; the milled samples were kept in tight bottles.

Extraction procedure

Aqueous extracts of the plants were prepared using the method described by Barry and Thernsberry (1991). One hundred grams of the blended samples were each measured into a conical flask and 1000 ml of sterile distilled water was added, covered with a cork, and was mixed together properly and left in a shaker at 100 revolution per minute for 24 hours. The samples were filtered and squeezed through four layers of muslin cloth. The extracts were then sterilized and concentrated at 40°C, the final extracts obtained were stored in sterile McCartney bottles and kept in the refrigerator at 4°C and were used later for antimicrobial tests.

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, Ahmadu Bello University Teaching Hospital Zaria The organisms were maintained on Nutrient agar slants at 4°C and were routinely sub-cultured during storage.

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 (10micrograms),Septrin(30micrgramms),Chloramphenicol(30microgramms),Sparfloxacin(10microgramms), Amoxacillin (30microgramms), Gentamycin (10microgramms), Pefloxacin (30microgramms), Tarivid (10micrgramms) and Streptomycin (30microgramms) was employed.

Antibacterial properties of plant extracts

The antibacterial properties of aqueous plant extracts were determined using the agar well diffusion method of Bauer *et al., (*1966). 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.

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.

Minimum bactericidal concentration (MBC)

Visual observation of growth inhibition on solid medium was used to determine MBC. To determine the MBC, for each set of test tubes in the MIC determination, a loop full 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 37°C for 24 h. After incubation the concentration at

which no visible growth was seen was noted as the Minimum Bactericidal Concentration (MBC).

Statistical Analysis

The data collected were subjected to analysis of variance (AVONA). Comparison of means were done by the New Duncan's multiple range test (DMRT) at 95% confidence level (P=0.05).

Result

Susceptibility Testing of Conventional Antibiotics against *Salmonella* and *Shigella* species

Results from the susceptibility testing using ten (10) antibiotics are shown in table 1. The result were labeled Resistant (R), Intermediate (I) and Susceptible (S) after being compared to the approved performance standards for antimicrobial susceptibility testing. Most of the drugs used are resistance to both *Salmonella* and *Shigelle*, but chloramphenicol was susceptible to *both Shigella* (23.00) and *Salmonella* (25.000).

Table 1: 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)
Amoxycillin	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

Susceptibility Testing of *Boswellia dalzielli, Parkia biglobosa* and *Carica papaya* aqueous extracts on *Salmonella* and *Shigella* species.

The result of antibacterial susceptibility test at 10 mg/ml of the extracts is presented in table 2. It was observed that bark and leaves of *Boswellia dalzielli* exhibited significantly higher ($p< 0.05$) antibacterial activity against *Salmonella* and *Shigella* when compared to extracts of *Parkia biglobosa* (bark and leaves), *Carica papaya* (bark and leaves) and control (distilled water). The result of antibacterial susceptibility test at 20 mg/ml of the extracts is presented in table 3. It was observed that all the plant extracts exhibited significantly higher ($p<0.05$) antibacterial activity against *Salmonella* and *Shigella* when compared to control (distilled water). The result of antibacterial susceptibility test at 30 mg/ml of the extracts is presented in table 4. It was observed that all the plant extracts exhibited significantly higher ($p<0.05$) antibacterial activity against *Salmonella* and

Shigella when compared to control (distilled water). The result of antibacterial susceptibility test at 50 mg/ml of the extracts is presented in table 5. It was observed that all the plant extracts exhibited significantly higher ($p<0.05$) antibacterial activity against *Salmonella* and *Shigella* when compared to control (distilled water). The result of antibacterial susceptibility test at 100 mg/ml of the extracts is presented in table 6. It was observed that all the plant extracts exhibited significantly higher ($p<0.05$) antibacterial activity against *Salmonella* and *Shigella* when compared to control (distilled water).

Table 2: Antibacterial activities of aqueous extract of *Boswellia dalzielli, Parkia biglobosa* and *Carica papaya* (10 mg/ml)

	Zone of inhibition (mm)	
Plants	*Shigella*	*Salmonella*
BDL	$1.00^{c} \pm 0.03$	$1.00^{b} \pm 0.02$
BDB	$0.33^{b} \pm 0.01$	$4.01^{c} \pm 0.03$
PBB	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
PBL	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
CPB	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
CPL	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
Distilled water	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$

Means followed by the same letter are not significantly different ($P<0.05$) using Duncan Multiple Range Test.

BDL= *Bowellia dalzielli* leave, *BDB= Boswellia dalzielli* bark, *CPL= Carica papaya* leave, CPB = *Carica papaya* bark, PBL= *Parkia biglobosa* leave, PBB= *Parkia biglobosa* bark.

Table 3: Antibacterial activities of aqueous extract of *Boswellia dalzielli, Parkia biglobosa* and *Carica papaya* (20 mg/ml)

	Zone of inhibition (mm)	
Plants	*Shigella*	*Salmonella*
BDL	$8.67^{g} \pm 0.02$	$8.00^{e} \pm 0.03$
BDB	$2.67^{e} \pm 0.03$	$5.63^{d} \pm 0.01$
PBB	$0.67^{b} \pm 0.01$	$3.67^{b} \pm 0.07$
PBL	$1.00^{c} \pm 0.00$	$0.00^{a} \pm 0.00$
CPB	$1.83^{d} \pm 0.03$	$5.50^{c} \pm 0.02$
CPL	$4.50^{f} \pm 0.02$	$8.67^{f} \pm 0.03$
Distilled water	$0.00^{a} \pm 0.02$	$0.00^{a} \pm 0.02$

Means followed by the same letter are not significantly different ($P<0.05$) using Duncan Multiple Range Test

Table 4: Antibacterial activities of aqueous extract of *Boswellia dalzielli, Parkia biglobosa* and *Carica papaya* (30 mg/ml)

Plants	Zone of inhibition (mm) *Shigella*	*Salmonella*
BDL	$11.33^{g} \pm 0.03$	$12.63^{g} \pm 0.03$
BDB	$8.00^{f} \pm 0.01$	$9.07^{d} \pm 0.04$
PBB	$3.33^{c} \pm 0.02$	$8.67^{c} \pm 0.05$
PBL	$2.67^{b} \pm 0.03$	$2.51^{b} \pm 0.02$
CPB	$6.00^{d} \pm 0.01$	$10.67^{f} \pm 0.01$
CPL	$7.17^{e} \pm 0.02$	$10.33^{e} \pm 0.01$
Distilled water	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$

Means followed by the same letter are not significantly different (P<0.05) using Duncan Multiple Range Test.

Table 5: Antibacterial activities of aqueous extract of *Boswellia dalzielli, Parkia biglobosa* and *Carica papaya* (50 mg/ml)

Plants	Zone of inhibition (mm) *Shigella*	*Salmonella*
BDL	$20.33^{g} \pm 0.03$	$19.70^{g} \pm 0.03$
BDB	$11.00^{e} \pm 0.00$	$15.87^{f} \pm 0.01$
PBB	$8.67^{c} \pm 0.01$	$11.50^{c} \pm 0.04$
PBL	$5.33^{b} \pm 0.03$	$6.34^{b} \pm 0.03$
CPB	$10.33^{d} \pm 0.01$	$12.33^{e} \pm 0.01$
CPL	$12.67^{f} \pm 0.02$	$11.67^{d} \pm 0.02$
Distilled water	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$

Means followed by the same letter are not significantly different (P<0.05) using Duncan Multiple Range Test.

Table 6: Antibacterial activities of aqueous extract of *Boswellia dalzielli, Parkia biglobosa* and *Carica papaya* (100 mg/ml)

Plants	Zone of inhibition (mm) *Shigella*	*Salmonella*
BDL	$23.00^{g} \pm 0.00$	$22.00^{g} \pm 0.03$
BDB	$12.43^{c} \pm 0.01$	$18.97^{f} \pm 0.01$
PBB	$13.77^{d} \pm 0.02$	$13.33^{c} \pm 0.03$
PBL	$8.00^{b} \pm 0.01$	$8.75^{b} \pm 0.06$
CPB	$15.00^{e} \pm 0.02$	$17.73^{e} \pm 0.03$
CPL	$16.00^{f} \pm 0.04$	$14.50^{d} \pm 0.03$
Distilled water	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$

Means followed by the same letter are not significantly different (P<0.05) using Duncan Multiple Range Test.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against *Shigella spp.* and *Salmonella spp.*

The result of MIC and MBC were presented in (table 7 and 8). The MIC were in the range of 12.5 mg/ml - 150 mg/ml while MBC were in the range of 50 mg/ml - 175 mg/ml.

Table 7: Minimum Inhibitory Concentration MIC (mg/ml) and Minimum Bactericidal Concentration MBC(m/g/ml) of Extracts of *Boswellia dalzielli, Parkia biglobosa* and *Carica papaya* Against *Shigella spp*

Plant Extract	MIC	MBC
BDL	25	50
BDB	75	100
CPL	25	50
CPB	150	175
PBL	25	50
PBB	12.5	50

BDL= *Bowellia dalzielli* leave, *BDB= Boswellia dalzielli* bark, *CPL= Carica papaya* leave, CPB = *Carica papaya* bark, PBL= *Parkia biglobosa* leave, PBB= *Parkia biglobosa* bark.

Table 8: Minimum Inhibitory Concentration MIC (mg/ml) and Minimum Bactericidal Concentration MBC (m/g/ml) of Extracts of *Boswellia dalzielli, Parkia biglobosa* and *Carica papaya* Against *Salmonella spp.*

Plant Extract	MIC	MBC
BDL	25	75
BDB	50	100
CPL	50	75
CPB	150	175
PBL	50	100
PBB	12.5	50

BDL= *Bowellia dalzielli* leave, *BDB= Boswellia dalzielli* bark, *CPL= Carica papaya* leave, CPB = *Carica papaya* bark, PBL= *Parkia biglobosa* leave, PBB= *Parkia biglobosa* bark.

Discussion

In this study, the isolated *salmonallae* and *shigallae* bacteria were resistant to most of the commonly used antibiotics (Table 1). 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 (DubMendal, 2005). There seems to be complete resistance to Augmentin, Pefloxacin, Tarivid and Amoxicillin by both *Salmonella* and *Shigella* in this study, which is in disagreement with reports from (Mache *et al*., 1997; Asrat, 2008). This is a sharp increase from earlier reports indicating the aggravating problem of drug resistance by these microbes over the years. This finding agrees with the finding of Asrat (2008) who reported a high level of resistance to gentamicin and absence of resistance to ciproflaxin. Brooks *et al*. (2006) found a lower level of resistance to the antibiotics used in this study. The only exception was gentamicin to which complete resistance was showed by *shigella* (Brooks *et al*., 2006). Compared to studies reported in other parts of the country (Assefa *et al*., 1997; Roma *et al*., 2000; Yismaw *et al*., 2006; Asrat, 2008), *Shigella* isolates had a lower level of resistance to chloramphenicol while this research detected a high level of susceptibility to chloramphenicol, sparfloxacin and ciproflaxacin. However, there seems to be a similar pattern of high resistance to these drugs in the studies in the rest of the country, even though lower in extent than our findings. This could be due to the fact that streptomycin, augmentin and amoxycillin have been used in the country for a long time and because of their easy availability and potential for misuse.

Aqueous extracts of the bark and leaves showed potency in their bactericidal action against all the test bacteria. This research suggest that aqueous extracts of screened plants would be helpful in treating diseases in man caused by enterotoxin producing bacteria like *Salmonella* and *Shigella*. The antibacterial activities of the extracts increased as the concentration increased as found out in this work (Table 2 and 3). Aqueous extracts of the bark and leaves showed potency in their bactericidal action against all the test bacteria. It was observed that the control, showed no effect on the test bacteria.(Table 4) This does not differ from the findings of (Banso and Adeyemo 2007) 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 observed low MIC and MBC values against these bacteria means that the plant has the potential to treat any ailments associated with these bacterial pathogens effectively.

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. The findings indicated that *Boswelllia dalzielli*, *Carica papaya* and *Parkia biglobosa* had growth inhibitory effect against the tested bacteria. In this manner, plants continue to be a rich source of therapeutic agent.

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