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Evaluation of Antibacterial Effects of *Combretum dolichopetalum* Methanol Leaf Extract on some Pathogenic Bacteria.

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ABSTRACT

Antimicrobial resistance (AMR) is a threat to global public health and a challenge in treating infectious diseases. This has resulted in a shift of research attention to natural substances such as medicinal plants that could be a source of developing novel drugs. Combretum dolichopetalum has been reported to have antidiarrhoeal effects in rats. In this study, the antibacterial potential of different concentrations of methanolic extract of Combretum dolichopetalum was evaluated against seven pathogenic bacteria (Escherichia coli, Bacillus cereus, Salmonella gallinarum, Aeromonas hydrophilia, Pasteurella multocida, Klebsiella pneumoniae and Shigella dysenteriae) at different concentrations using the agar well diffusion method. Antibiogram of these organisms were also carried out by the Kirby Bauer disk diffusion method. Zones of inhibition were calculated as means ± SEM. Result showed that the plant extract had significant concentration dependent antibacterial activity compared to distilled water and some known drugs ($P<0.05$). All concentrations showed activity against all bacteria, unlike the drugs, which had selective activity, with concentration of 400 mg/ml, showing the highest activity. Tested bacteria exhibited a high level of resistance to synthetic drugs ranging from 48% to 100%. Multi-drug resistance was also observed. S. gallinarum, B. cereus and E. coli exhibited 100% resistance to all test antibiotics. All organisms showed 100% resistance to Ampicillin-Clavulanic acid and Ceftriaxone. Results from this study show that C. dolichopetalum possesses antimicrobial activity as it inhibited growth of all test bacteria.

Keywords: AMR, zones of inhibition, *Combretum dolichopetalum,* pathogenic bacteria.

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INTRODUCTION

The discovery of antibiotics was a turning point in the development of medicine, but the emergence and spread of multidrug resistant (MDR) strains of pathogenic organism; resulting from the indiscriminate use of antibiotics, has become a challenge and a major threat to global public health [1,2]. Also challenging, is the consistent rise in the evolution of new mutant resistant strains of various microorganisms, which poses a risk to both human and animal health.

Since the beginning of human history, many plants have been used as source of relief from various ailments such as fevers, gastrointestinal disorders, burns, skin infections [3, 4]. Medicinal plants have been discovered to be rich in a variety of secondary metabolites, which have been found to be responsible for their antimicrobial properties [5]. These metabolites include several phytochemicals such as tannins, alkaloids, flavonoids, terpernoids and other phenolic compounds which possess both antimicrobial and antioxidant properties [6].

According to the World Health Organization, medicinal plants are the best sources for a variety of drugs and 80% of the world's population depend on herbal medicine for respite from various ailments [7]. With the rise in MDR pathogens, the search for an alternative, safe and effective solution is imperative.

Combretum dolichopetalum belonging to the family *Combretaceae* is commonly known as "achicha nza" (food of the sun bird) in Igboland and "okoso" in Edo Nigeria [8]. The leaves and roots are extensively used in ethnomedical practices of many cultures. The antiulcer, anti-hepatotoxic, trypanociddal, anti-inflammatory, anti-diabetic, gastric anti-secretory, anti-diarrheal and anti-spasmolytic activities of *C. dolichopetalum* have been reported by previous workers [8, 9, 10].

This study is therefore aimed at evaluating the antibacterial effects of the extract of *C. dolichopetalum* on some selected pathogenic microorganisms and also comparing its antibacterial effect with that of selected synthetic antibacterial drugs.

MATERIALS AND METHODS

Collection and Identification of Plant

The fresh leaves of *C. dolichopetalum (E&L)* were sourced from Alakwo, Owerri in Imo State Nigeria and identified by Mr. A.O. Ozioko, a taxonomist of Bioresource development and conservation programme, Enugu State Nigeria.

Preparation of Plant Extract.

Fresh leaves of *C. dolichopetalum* were dried at room temperature and pulverized using electric blender. The ground leaves was then extracted in 80% methanol using soxhlet apparatus as described by Shekins *et al.,* [11]. The extract was dried using the hot air oven at the temperature of 35°C to obtain a yield percentage of the extract. The percentage yield of the sample was determined as follows:

$$\text{Percentage yield (\%)} = \frac{\text{weight of the extract}}{\text{Weight of the dried powder}} \times 100$$

The extract was then reconstituted by dilution in distilled water to different concentration (25, 50, 100, 200, and 400) mg/ml as described by Shekins *et al.,* [11], before using for antimicrobial sensitivity.

Evaluation of Antibacterial Activities

Test Organisms

A total of seven microorganisms were investigated; *Bacillus cereus (C), Escherichia coli(E), Pasteurella multocida (DN), Salmonella gallinarum (SA), Aeromonas hydrophila (DL), Klebsiella pneumonia (BL) and Shigella dysenteriae (BN)*. All isolates were obtained and well identified by colonial morphology, gram staining and a series of biochemical tests according to Bergey's Manual of Systematic Bacteriology [12].

Preparation of :
A loopful of the test organisms were inoculated in Nutrient broth and incubated overnight at 37° C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5 × 108 CFU/ml.

Antibacterial Assay
Antibacterial assay of extracts of *C. dolichopetalum* was performed using the agar well diffusion method as described by Srinivasan *et al*., [13].

The Mueller Hinton Agar (MHA) plates were lawn cultured by swabbing small volumes of the microbial broth on the plates and then evenly seeded and streaked onto the agar plate surface by means of sterile cotton swab and incubated for an hour at 37°C. Afterwards, six wells of 6 mm were bored in the inoculated media using a sterile cork-borer (6 mm in diameter). With the aid of a micropipette, five different concentrations of the plant extract solutions (25mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml, 400 mg/ml distilled water was used as control) were carefully placed in the respective wells in the plate. Each sample was tested in triplicates.

Antibiogram
Antimicrobial susceptibility test for all the microbial isolates was also carried out with commercial antibacterial agents: Amoxicillin-Clavulanic acid (0.03mg), Chloramphenicol (0.03mg), Ciprofloxacin (0.005mg), Ceftriaxone (0.03mg), Gentamicin (0.01mg) and Tetracycline (0.03mg) in order to compare their antimicrobial potency with the plant extracts. This test was conducted by the modified Kirby Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guideline [13]. The plant extracts and antibiotic discs were allowed to diffuse for about 30 minutes and then the plates were incubated for 24 hrs. After overnight incubation, the plates were observed for the formation of a clear zone around the wells which corresponds with the diameters of the zones of inhibition. These zones of inhibition (ZOI) were observed and measured in mm.

Statistical Analysis
Data were analysed using the Statistical Package for Social Sciences (SPSS) version 17.0 for Windows. The inhibition zones were calculated as means ± SEM. The statistical difference of the mean zones of inhibition of the extract and synthetic drugs for individual bacterium was carried out by using the one-way analysis of variance (ANOVA) and values were considered significant at $P < 0.05$. Means were separated by least square deviation (LSD).

RESULTS
Percentage yield of *C. dolichopetalum* was 8.12 %. In the parallel study, the antibiogram revealed that all organisms tested were resistant to at least two or more antibiotics used (Table 1). Resistance of organisms to the tested antimicrobial drugs ranged from 28.6% to 100%. All the organisms were resistant to Ceftriaxone and Amoxicillin-Clavulanic acid, while four (57.1%) organisms were susceptible to chloramphenicol. *E.coli, S. gallinarum* and *B. cereus* were resistant to all the drugs, while *P. multocida* and *A. hydrophila* recorded highest sensitivity (57.1%) to the drugs used in this study.

Table 1: Antibiogram of test bacteria to antimicrobial agents

ORGANISM	TE	C	CRO	CN	AMC	CIP
P. multocida	S	S	R	S	R	S
A. hydrophila	S	S	R	S	R	S
K .pneumoniae	S	S	R	R	R	R
S. dysenteriae	R	S	R	S	R	R
B. cereus	R	R	R	R	R	R
E. coli	R	R	R	R	R	R
S. gallinarum	R	R	R	R	R	R

*TE-tetracycline; C- chloramphenicol; CRO- ceftriaxone; CN- gentamicin; AMC- amoxicillin/Clavulanic acid; CIP- Ciprofloxacin.

The methanol extract of *C. dolichopetalum* plant showed antibacterial efficacy that was also greater when compared to distilled water and some of the synthetic antibacterial agents used in this study (Table 2). All the bacteria in this study were susceptible to the extract. The extract had concentration dependent antibacterial effect on all the organisms, seen as significantly different ($P<0.05$) inhibition zones when compared to distilled water (Table 2). The extract at 400 mg/ml had highest inhibition for *P. multocida*, (17.00±1.45); *A. hydrophilia* (20.00±1.15); *K. pneumoniae* (23.00±1.15); *S. dysenteriae* (20.00±2.88) and *B. cereus* (17.00±1.45) respectively. At 100 mg/ml, the extract had highest inhibition for *E. coli* (25.00±1.15) and *S. gallinarum* (22.00±1.15) respectively. Thus, *K. pneumoniae* demonstrated the highest significant ($P< 0.05$) susceptibility to the extract compared to distilled water; followed by *S. dysenteriae* and *A. hydrophilia* and *P multocida*. None of the drugs were effective against *B. cereus,* as they showed a mean zone of inhibition of 0.0 mm to all synthetic drugs, but a zone of inhibition 17.3 mm at the concentration of 400 mg/ml. *A. hydrophila* and *P. multocida* were highly susceptible to Ciprofloxacin (28.33 mm and 29.66 mm respectively), followed by Chloramphenicol (19.5 mm and 19.0 mm respectively) as compared to the mean zones of inhibition (20.66 mm and 18.66mm) at 400 mg/ml respectively. While all the extract concentrations significantly inhibited all the bacterial isolates compared to distilled water, tetracycline did not inhibit *S. dysenteriae*, *B. cereus*, *E. coli* and *S. gallinarum*; Chloramphenicol did not inhibit *B. cereus*, *E. coli* and *S. gallinarum*; Ceftriaxone did not inhibit any of the bacterial isolates; Gentamycin did not inhibit *B. cereus*, *E. coli* and *S. gallinarum*; Amoxicillin-clavulanic acid did not inhibit any of the bacteria and Ciprofloxacin did not inhibit *B. cereus*, *E. coli* and *S. gallinarum*. There were no significant differences between their zones of inhibition and distilled water ($P>0.05$) compared to distilled water.

Table 2 : **Effect of *Combretum dolichopetalum* leaf extract and known drugs on inhibition zones of bacterial isolates (your conc are in mg while the drugs are in microgram. Please explain.**

Treatment	Zones of inhibition (mm)						
	P. multocida	*A.hydrophila*	*K.pneumonia*	*S.dysenteriae*	*B. cereus*	*E. coli*	*S.gallinarum*
DIST.WATER	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CDME 25mg/ml	2.00±0.00*	4.00±0.57*	2.00±0.57*	2.00±0.00*	2.00±0.57*	13.00±1.70	17.00±2.02
CDME 50mg/ml	8.00±0.57*	16.00±1.15*	10.00±1.50*	10.00±0.57*	8.00±0.10*	18.00±0.57*	18.00±1.73*
CDME100mg/ml	11.00±0.57	13.00±1.15*	13.00±0.57*	18.00±0.00*	12.00±1.15*	25.00±1.15*	22.00±1.15*
CDME 200	15.00±1.15*	18.00±1.73*	15.00±0.57*	12.00±1.15*	16.00±1.73*	13.00±0.57*	20.00±1.73*
CDME 400	17.00±1.45*	20.00±1.15*	23.00±1.15*	20.00±2.88*	17.00±1.45*	20.00±1.45*	20.00±2.33*
TE(0.03mg)	17.00±0.57*	15.30±0.33*	16.33±0.88*	0.00	0.00	0.00	0.00
C(300.03mg)	18.66±1.15*	20.66±0.33*	22.66±0.81*	23.33±0.33*	0.00	0.00	0.00
CRO(0.03mg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CN(0.01mg)	14.33±0.66	15.66±0.33	3.66±0.33*	16.00±0.00	0.00	0.00	0.00
AMC(0.03mg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CIP(0.005mgg)	28.33±0.66*	29.66±0.33*	15.00±0.57*	16.33±0.33*	0.00	0.00	0.00

* Means are significantly different at P<0.05; DIST.WATER: distilled water; CDME: *Combretum dolichopetalum* methanol extract (mg/ml); TE: tetracycline; C: chloramphenicol; CRO: ceftriaxone; CN: gentamycin; AMC: amoxicillin-clavulanic acid; CIP: ciprofloxacin

DISCUSSION

Chemotherapeutic success in the use of synthetic antimicrobial drugs has been threatened enormously by the global spread of resistant pathogens. Medicinal plants are presenting an alternative, more available and cost-effective option. This study investigated the effects of *Combretum dolichopetalum* against selected microorganisms. The extracts showed maximum activity against the selected pathogens. This agrees with findings of researchers that state that plant extracts exhibit antimicrobial potency [14, 15]. The mean zones of inhibition observed in this work increased with increasing concentration. This agrees with [5, 16]. Also in this study, the antibacterial findings of *Combretum dolichopetalum* extract at all concentrations were able to inhibit the growth of *S. gallinarum and E.coli*. This disagrees with previous findings [17]; where poor antimicrobial activity of *C. dolichopetalum* against *E.coli* isolates was recorded, but is consistent with the investigations of Asres *et al.,* [18]; that indicated high antibacterial activity against *E. coli* and *S. dysenteriae* by acetone extracts of *Combretum molle*. Antidiarrheal activities of methanolic extracts of *C. dolichopetalum* leaves have been established [10, 19]. This antibacterial activity could be linked to the already established anti-diarrhoeal activity as most cases of diarrhoea are caused by enterobacteriacae, for instance, *E. coli* and *S. gallinarum* are aetiologic agents of colibacillosis and salmonellosis of poultry respectively, with of diarrhoea as a major clinical sign.

The high (100%) resistance exhibited by all tested bacteria to Ceftriaxone and Amoxicillin-Clavulanic acid and multiple antimicrobial resistance recorded is in agreement with previous studies [16, 20]; and may be due to ability of virulent factors possessed

by these organisms to destroy the beta-lactam rings of these antibiotics. Sensitivity of tested bacteria to ciprofloxacin, tetracycline and gentamicin seen in this study has also been previously reported [21]. *E. coli* and *S. gallinarum* were resistant to all tested synthetic drugs and has been previously reported. However, these bacteria that cause colibacillosis and salmonellosis in poultry with high morbidity and mortality were very susceptible to the extract. The high sensitivity of tested bacteria to chloramphenicol may be due to its reduced usage in treatment of food animals, as a result of its ban in food-producing animals [23]. This must have reduced development and spread of resistant genes to the drug.

Conclusion

This study reveals that *Combretum dolichopetalum* has antibacterial activity against all tested bacteria, and so can inhibit growth of bacterial pathogens. This antibacterial activity shown by this plant should be exploited in tackling infectious diseases and multi drug resistance especially in poultry production. Same studies should be carried out on other species of bacteria and fractions of the plant evaluated for their antibacterial potential. Furthermore, investigations to evaluate the antiviral, antifungal and antiparasitic activities of *C. dolichopetalum* are expedient.

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