

RESEARCH

MICROBICIDAL AND MICROBISTATIC POTENTIAL OF *BOSWELLIA SACRA* STEM BARK EXTRACTS AGAINST SELECTED CLINICAL ISOLATESMacDonaldIdu^{1*}, Frank C. Okeke² and Benjamin Ogunma Gabriel³¹Phytomedicine Research Group, Department of Plant Biology and Biotechnology, University of Benin, Benin City, PMB 1154, Edo State, Nigeria²Department of Biological Sciences, University of Abuja, Nigeria³Department of Science Laboratory Technology, University of Benin, Benin City, PMB 1154, Edo State, NigeriaEmail: alexthemain076@gmail.com

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Abstract: Objectives: The study showed the antimicrobial properties of the stem bark extracts against selected strains of microbes. The antimicrobial study of the stem bark extracts was established using a standard method. The study investigated the microbicidal and static effect of *Boswellia sacra* stem bark extracts against clinical isolates. The results obtained showed that the sixteen clinical isolates obtained, *Shigella dysenteriae* (10.30%) was most predominant followed by *Escherichiacoli* (9.30%) while *Bacillus subtilis* and *Vibrio cholera* were the least occurring bacteria at 2.10% each. Most of the isolates were gram-negative (53%) and the others (47%) were gram-positive. Sugar utilization test revealed that all the isolates (100%) utilized glucose and sucrose. From the sensitivity test of *Boswellia sacra* stem bark extracts on clinical isolates, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Alcaligenes faecalis*, *Streptococcus pyogenes* showed the highest zones of inhibition while *Vibrio cholerae*, *Streptococcus mutans* and *Enterococcus faecalis* showed the least zones of inhibition of the fractions used. Ethanol fraction had the highest activity on the test isolates while N-butanol and N-hexane recorded the least. Susceptibility of clinical isolates to standard antibiotics sensitivities of the test bacteria against commercially available standard antibiotics. *Staphylococcus epidermidis* was the most susceptible while *Vibrio cholera* was mostly resistant to the drugs. The Zone of inhibition of extracts were compared with that of different standards like ampicillin, ciprofloxacin, norfloxacin, and chloramphenicol for antibacterial activity and nystatin and griseofulvin for antifungal activity. In conclusion, this study adheres to the ethnomedicinal claim that *Boswellia sacra* scientifically validated of its potency as antimicrobial effect against several micro-organisms.

Keywords: Microbicidal, Static, *Boswellia sacra*, Stem bark, Clinical isolates

INTRODUCTION

In the 1960's when many groups of essential antibiotics including tetracyclines, cephalosporins, aminoglycosides, macrolides, etc. were discovered, challenges of chemotherapy initially appeared solved. The excitement and promise of having attained the final death knell of the 'evil' microorganisms was, however, short-lived. Since then till now the microorganisms have had an unwavering upper hand in the battle with bioscientists as their genetic ability for metamorphosis improved with each attack on them. Termed 'antimicrobial resistance (AMR)', this phenomenon has exasperated the efforts of researchers, medical and biomedical scientists due to the increasing cases of treatment failures even with multidrug approach (Mayers et al., 2009). The magnitude of this threat recently prompted an unusual level of attention from world leaders. Antimicrobial resistance (AMR) occurs when

bacteria, viruses, parasites and fungi develop resistance against medicines that were previously able to deter them (Singer et al., 2016). From time, plants have been the main source of restorative and healing agents for mankind. Irrespective of the habitat- terrestrial, marine or arboreal, mankind and most terrestrial organisms have relied on plants for nutrition and medicine (Abreu et al., 2012). This is because plants yield a wide range of complex, structurally diverse and functionally compatible bioactive agents (Cos et al., 2006). The natural plant products in their pure states or as extracts, often serve as a source from which several new drugs could be obtained as a result of their inherent biochemical diversity (Cos et al., 2006). In recent years, biomedical researchers have centered on the investigation of plants, microbial extracts, essential oils, pure secondary metabolites and new synthesized organisms as potential antimicrobial agents (Runyoro et al., 2006; Mabona et al., 2013). AMR is considered by WHO as one of the greatest

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and most urgent global risks requiring international and national attention. This has become a serious concern to public health (Guschin et al., 2015). Some plants are known to exhibit or express some capability to overcome this resistance in certain organisms. This finding led to the investigation of the mechanisms of action and the isolation of the implied active compounds exhibited by these plants. Of particular essence is the elucidation of the effect of the plant extracts with respect to their microstatic and microcidal action and the spectra of microorganisms affected (Panda, 2014). With the current level of global call and attention given to antimicrobial resistance, investigation is still ongoing for plants, microbial extracts, essential oils, pure secondary metabolites which could yield potential antimicrobial agents (Runyoro et al., 2006; Mabona et al., 2013). With the current level of biomedical research and understanding, no easy solution appears to be in sight with respect to developing some irresistible micro biocides because the fundamental premises on which this system is based have not changed. The most popular and safest source of these antibiotics remains natural products obtainable from plants, animals, microorganisms and even the physical environment (Berdy, 2005). *Boswellia species* have been noted to exhibit antimicrobial, antiseptic, antiviral, antifungal properties (Kafuti et al., 2018; Afsar et al., 2012). The objective of this study is to evaluate the antimicrobial activity of *Boswellia sacra* stem bark extracts.

MATERIALS AND METHODS

Collection and identification of plant

The plant sample used in this research was obtained from Kibiya Local Government Area in Kano State in April 2017. The headquarters of the Local Government is Kibiya. The plant was identified and authenticated at the Faculty of Science, University of Abuja, Abuja, Nigeria and further authenticated by Prof. Odaro Timothy, in the Department of Plant Biology and Biotechnology, University of Benin with Voucher number UBH-B152

Preparation of Extracts

The stem bark and leaves of the plant were air-dried under shade for 7 weeks. Thereafter each was pulverised using wooden mortar and pestle. Some quantity (about 200gms) of the stem bark was placed in five separate containers each containing 1.5 litres of the solvents- ethanol, ethyl acetate, n-hexene, n-butanol, chloroform. These were left for 24 hours. Thereafter the contents in each of the containers were filtered using muslin cloth. With the residue in the cloth discarded, the crude extracts were preserved for use in determining the phytochemical contents. For *Boswellia sacra* leaves, extracts of plant sample were prepared

using methanol. Each extract type was prepared by soaking in methanol in air tight containers for 72 hours with occasional stirring. The extracts were then filtered and taken to a rotatory evaporator (RE 300, Bibby Scientific, UK) for concentration at low pressure. The extracts were thereafter stored in a sterile airtight container and kept in a refrigerator till they were required.

Microbiology: Sterilization of Apparatus and Media

All glass ware used were thoroughly washed with detergent and rinsed with tap water. They were air dried and sterilized in the oven at 160°C for 2 hours. All the media used were prepared according to the manufacturer's specifications and autoclaved at 121°C for 15 minutes.

Clinical Isolates

Clinical specimens including stool, urine, blood, high vaginal Swab and wound samples were collected from phlebotomy units of Delta State University Teaching Hospital Abraka. Serial dilutions of all samples were done and 0.2 ml of 10⁻⁴ dilution of each sample was inoculated in duplicate onto Nutrient agar (NA) plates and other media designed for the detection of specific group of bacteria.

Identification of Clinical Isolates

Bacterial colonies were initially characterized by morphology and microscopic appearance, and further identified by biochemical tests. The biochemical tests included catalase, oxidase, urease, hydrogen sulphide production and motility test, glucose utilization test, lactose, sucrose, maltose, citrate utilization tests. The biochemical and physiological characteristics of clinical isolates were ascertained according to Bergey's Manual of Determinative Bacteriology (Bergey and Hplt, 1998).

Sensitivity Testing of *Boswellia sacra* Extract on Clinical isolates

The sensitivity testing of the crude extract of the plant was determined using Agar Well Diffusion method as described by Mbata *et al.* (2006). The test microorganisms were first grown in nutrient broth for 18h and standardized to 10⁸ cfu/ml of 0.5 McFarland standards. A volume of 0.1ml was sub-cultured into sterile molten Mueller-Hinton agar and poured into plates. They were allowed to set and wells were bored with a sterile 6mm cork borer. The wells were then filled with 35mg/ml of the crude extract while streptomycin (1mg/ml) was used as positive control. The cultured plates were allowed to stand for 1-2h for proper diffusion of the antimicrobial agents before incubating overnight at 37°C for 24 hours. Zones of inhibition produced by the activity of the extract were recorded appropriately. The fractions obtained from the crude extract were tested for antimicrobial activity using concentration of 300mg/ml on the test microorganisms.

Antibiotic Susceptibility Test

All the isolates were subjected to antibiotics susceptibility test using modified Kirby-Bauer agar disk diffusion method (Bauer *et al.*, 1966) on Mueller-Hinton agar. The isolates were tested against nine commonly used antibiotics namely Ceftazidime (CAZ); Cefluroxime (CRX); Gentamycin (GEN); Coftriaxone (CTR); Erythromycin (ERY); Cloxicillin (CXC); Ofloxoxin (OFL); Amoxicillin/Clavulinate (AUG); and Ciprofloxacin (CPX) all of Abtek Biological, England and 0.5 McFarland standard suspension was used for standardization of bacterial suspensions. The diameter of the zone of inhibition of 24-hour growth of each isolate was carefully measured.

From the result obtained the isolates could be grouped as susceptible, intermediate or resistant according to interpretative chart of complete growth inhibition zone diameter sizes for bacteria according to the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standard Institution, 2012).

Data Analysis

Results were analysed with Graph pad prism version 6. Data were presented as Mean ± S.E.M, and statistical significance was calculated using one-way ANOVA, followed by Dunnett's test where P<0.05 were considered statistically significant.

RESULTS

Table 1. Occurrence of bacteria isolates in samples

Isolates	PERCENTAGE OF BACTERIAL ISOLATES					
	Urine %	High vaginal swab (n=2) %	Blood (n=2) %	Wound (n=2) %	Stool (n=2) %	Total %
<i>Escherichia coli</i>	1(11.10)	2 (22.20)	2 (22.20)	2 (22.20)	2 (22.20)	9 (9.30)
<i>Pseudomonas aeruginosa</i>	2 (40.00)	0 (0.00)	1 (20.0)	2 (40.00)	0 (0.00)	5 (5.20)
<i>Shigella dysenteriae</i>	2 (20.00)	2 (20.00)	2 (20.00)	2 (20.00)	2 (20.00)	10 (10.30)
<i>Salmonella enterica</i>	2 (28.60)	1 (14.30)	2 (28.60)	1 (14.30)	1 (14.30)	7 (7.20)
<i>Klebsiella pneumonia</i>	2 (33.30)	1 (16.70)	0 (0.00)	1 (16.70)	2 (33.30)	6 (6.20)
<i>Micrococcus flavus</i>	2 (25.00)	2 (25.00)	1 (12.50)	2 (25.00)	1 (12.50)	8 (8.20)
<i>Bacillus subtilis</i>	0 (0.00)	0 (0.00)	0 (0.00)	2 (100.00)	0 (0.00)	2 (2.10)
<i>Streptococcus pyogenes</i>	2 (33.30)	2 (33.30)	0 (0.00)	1 (16.70)	1 (16.70)	6 (6.20)
<i>Streptococcus mutans</i>	2 (25.00)	2 (25.00)	1 (12.50)	2 (25.00)	1 (12.50)	8 (8.20)
<i>Alcaligenes faecalis</i>	2 (40.00)	0 (0.00)	0 (0.00)	1 (20.00)	2 (40.00)	5 (5.20)
<i>Staphylococcus aureus</i>	1 (20.00)	2 (40.00)	0 (0.00)	2 (40.00)	0 (0.00)	5 (5.20)
<i>Vibrio cholera</i>	1 (50.00)	0 (0.00)	0 (0.00)	1 (50.00)	0 (0.00)	2 (2.10)
<i>Corynebacterium rubrum</i>	1 (25.00)	0 (0.00)	0 (0.00)	2 (50.00)	1 (25.00)	4 (4.10)
<i>Proteus vulgaris</i>	1 (25.00)	2 (50.00)	0 (0.00)	1 (25.00)	0 (0.00)	6 (6.20)
<i>Staphylococcus epidermidis</i>	2 (28.60)	2 (28.60)	0 (0.00)	1 (14.30)	2 (28.60)	7 (7.20)
<i>Enterococcus faecalis</i>	2 (66.70)	0 (0.00)	0 (0.00)	1 (33.30)	0 (0.00)	3 (3.10)
TOTAL	27 (27.80)	19 (19.60)	9 (9.30)	26 (26.80)	16 (16.50)	97 (100.00)

$\chi^2 = 39.05$; $df = 64$; $P = 0.99$

A total of sixteen isolates were obtained and shown on Table 1. *Shigelladysenteriae* (10.30%) was most predominant followed by *Escherichiacoli* (9.30%)

while *Bacillussubtilis* and *Vibriocholera* were the least occurring bacteria at 2.10% each.

Identification of clinical isolates

Table 2. Biochemical Test of Clinical Isolates

S/N	Cultural Characteristics	Probable organism										
		Catalase	Citrate	Indole	Motility	Glucose	Lactose	Oxidase	H ₂ S	Gram	Sucrose	
1	Cream, ovoid	+	-	+	+	+	+	-	-	-	+	<i>Escherichia coli</i>
2	Cream, ovoid	+	-	+	+	+	+	+	-	-	+	<i>Pseudomonas aeruginosa</i>
3	Cream, elongate	-	-	+	-	+	+	+	-	-	+	<i>Shigella species</i>
4	Cream, ovoid	+	-	+	+	+	+	+	+	-	+	<i>Salmonella species</i>
5	Cream, ovoid	+	+	-	+	+	+	+	+	-	+	<i>Klebsiella pneumoniae</i>
6	Cream, ovoid	+	-	+	+	+	+	-	-	-	+	<i>Micrococcus flavus</i>
7	Cream, ovoid	+	-	+	+	+	+	+	-	-	+	<i>Bacillus species</i>
8	Cream, elongate	-	-	+	-	+	+	+	-	+	+	<i>Streptococcus pyogenes</i>
9	Cream, ovoid	+	-	+	+	+	+	+	-	+	+	<i>Streptococcus mutans</i>
10	Cream, ovoid	+	+	-	+	+	+	+	+	-	+	<i>Alcaligenes faecalis</i>
11	Cream, ovoid	+	-	+	+	+	+	-	-	+	+	<i>Staphylococcus aureus</i>
12	Cream, ovoid	+	-	+	+	+	+	+	-	+	+	<i>Vibrio cholera</i>
13	Cream, elongate	-	-	+	-	+	+	+	-	-	+	<i>Corynebacterium rubrum</i>
14	Cream, ovoid	+	-	+	+	+	+	+	-	+	+	<i>Proteus vulgaris</i>
15	Cream, ovoid	+	+	-	+	+	+	+	-	+	+	<i>Proteus mirabilis</i>
16	Cream, ovoid	+	-	+	+	+	+	-	-	+	+	<i>Staphylococcus epidermidis</i>
17	Cream, ovoid	+	-	+	+	+	+	+	-	+	+	<i>Enterococcus faecalis</i>

Key: Gram = Gram reaction. + indicates present. – indicates absent.

Biochemical identification and characterizations of bacteria associated with clinical samples. The identification of the isolates was carried out using different morphological and biochemical tests. The results of these tests are shown on Table 2. Most of

the isolates were Gram negative (53%) and the others (47%) were Gram positive. Sugar utilization test revealed that all the isolates (100%) utilized glucose and sucrose.

Table 3. Sensitivity test of *Boswellia sacra* extracts on clinical (bacterial) isolates

S/N	ORGANISM	EIHANOL 300mg/ml	N-HEXENE 300mg/ml	N-BUTANOL 300mg/ml	EIHYL ACETATE 300mg/ml	CHLOROFORM 300mg/ml
1	<i>Escherichia coli</i>	13.0±5.7	11.0±7.4	16.0±4.4	15.0±5.0	15.0±3.5
2	<i>Pseudomonas aeruginosa</i>	17.0±4.4	6.0±4.4	9.0±2.7	10.0±1.7	15.0±1.2
3	<i>Shigella dysenteriae</i>	16.8±2.0	7.0±2.2	8.0±2.2	15.0±0.0	11.0±3.2
4	<i>Salmonella typhi</i>	7.0±4.8	7.0±2.2	14.6±2.8	17.2±1.4	11.8±2.6
5	<i>Klebsiella pneumoniae</i>	18.4±0.8	8.8±2.5	16.2±0.5	1.0±2.5	5.0±0.0
6	<i>Micrococcus flavus</i>	18.2±1.4	12.2±3.8	11.0±2.7	17.4±1.1	13.0±2.9
7	<i>Bacillus subtilis</i>	18.0±4.2	12.5±5.7	17.7±4.4	16.2±4.3	13.5±6.4
8	<i>Streptococcus pyogenes</i>	20.0±0.0	6.6±4.0	13.3±2.0	13.3±2.0	13.6±2.2
9	<i>Streptococcus mutans</i>	0.0±0.0	11.3±1.1	8.3±4.1	17.0±1.8	5.0±2.5
10	<i>Alcaligenes faecalis</i>	19.0±1.4	18.0±2.8	7.5±10.6	10.0±7.0	5.0±0.0
11	<i>Staphylococcus aureus</i>	11.7±9.2	7.5±5.0	10.7±4.3	13.2±2.7	12.0±4.9
12	<i>Vibrio cholera</i>	0.0±0.0	5.0±7.0	2.5±3.5	12.5±3.5	2.5±3.5
13	<i>Corynebacterium rubrum</i>	7.3±6.4	10.6±1.1	11.0±5.2	15.3±0.7	5.0±0.0
14	<i>Proteus vulgaris</i>	5.0±7.0	15.0±7.0	7.5±3.5	10.0±7.0	9.0±12.7

15	<i>Proteus mirabilis</i>	5.0±7.0	13.0±0.0	11.0±1.4	15.0±0.0	10.0±7.0
16	<i>Staphylococcus epidemidis</i>	21.6±2.8	15.6±0.6	7.3±4.7	10.0±8.6	11.6±5.7
17	<i>Enterococcus faecalis</i>	20.0±0.0	20.0±0.0	12.5±3.5	7.5±10.6	2.5±3.5

At lower concentrations of different fractions of the extract, little or no susceptibilities were seen whereas, at higher concentration like 300mg/ml the test organisms became highly susceptible to most of the fractions.

Table 3 shows the sensitivities. The zones of inhibition for susceptible test microorganisms ranged between 0.0±0.0mm and 21.6±2.8mm.

Staphylococcus epidermidis, *Enterococcus faecalis*, *Alcaligenes faecalis*, *Streptococcus pyogenes* showed the highest zones of inhibition while *Vibrio cholerae*, *Streptococcus mutans* and *Enterococcus faecalis* showed the least zones of inhibition of fractions used. Ethanol fraction had the highest activity on the test isolates while N-butanol and N-hexane recorded the least.

Table 4. Susceptibility of various isolates from the different clinical samples to the multiple antibiotic drugs

Isolates\Antibiotics	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	CPX
<i>Escherichia coli</i>	5.2 ± 10.2	11.2 ± 14.2	9.8 ± 12.2	9.6 ± 13.0	10.4 ± 14.9	2.8 ± 12.5	16.2 ± 6.0	13.6 ± 6.7	12.6 ± 11.4
<i>Pseudomonas aeruginosa</i>	5.2 ± 14.5	11.0 ± 15.0	6.2 ± 13.3	12.6 ± 11.4	17.4 ± 5.0	13.2 ± 17.8	16.4 ± 10.7	7.0 ± 15.1	9.2 ± 18.0
<i>Shigella dysenteriae</i>	6.0 ± 17.8	11.4 ± 16.5	10.2 ± 17.3	16.0 ± 3.7	15.4 ± 8.5	15.4 ± 4.6	15.6 ± 9.4	5.0 ± 10.0	10.8 ± 12.7
<i>Salmonella typhi</i>	5.2 ± 10.5	10.4 ± 15.9	8.6 ± 18.2	9.6 ± 15.1	11.4 ± 17.0	8.0 ± 13.2	10.0 ± 15.8	3.2 ± 9.7	7.4 ± 13.9
<i>Klebsiella pneumonia</i>	0.0 ± 0.0	6.6 ± 18.7	2.0 ± 8.9	15.4 ± 20.5	15.0 ± 19.7	5.0 ± 14.1	14.8 ± 18.7	3.0 ± 13.4	13.0 ± 16.7
<i>Micrococcus flavus</i>	2.75 ± 9.9	9.6 ± 11.3	3.6 ± 9.8	7.8 ± 14.8	9.8 ± 11.2	4.6 ± 13.1	9.8 ± 11.8	12.4 ± 2.7	13.8 ± 7.2
<i>Bacillus subtilis</i>	2.7 ± 9.5	12.0 ± 6.1	7.25 ± 15.3	12.5 ± 3.0	13.0 ± 2.4	3.0 ± 10.3	11.2 ± 6.7	13.2 ± 2.2	15.0 ± 4.2
<i>Streptococcus pyogenes</i>	0.0 ± 0.0	11.0 ± 1.7	9.0 ± 11.2	6.7 ± 8.2	13.7 ± 6.6	0.0 ± 0.0	7.3 ± 7.3	13.7 ± 7.8	14.0 ± 6.6
<i>Streptococcus mutans</i>	4.0 ± 9.7	10.7 ± 13.4	5.0 ± 12.2	10.0 ± 12.2	13.7 ± 4.9	5.0 ± 12.2	15.0 ± 6.4	12.3 ± 3.5	16.7 ± 8.6
<i>Alcaligenes faecalis</i>	7.5 ± 3.5	13.0 ± 1.4	13.5 ± 2.1	0.5 ± 0.7	11.5 ± 0.7	7.5 ± 10.6	6.5 ± 9.1	14.0 ± 2.8	14.0 ± 2.8
<i>Proteus vulgaris</i>	2.5 ± 3.5	0.0 ± 0.0	0.0 ± 0.0	16.5 ± 5.6	15.0 ± 5.6	16.5 ± 4.9	21.5 ± 0.7	2.5 ± 3.5	5.0 ± 7.1
<i>Proteus mirabilis</i>	2.5 ± 3.5	22.0 ± 2.8	21.5 ± 4.9	20.5 ± 3.5	20.0 ± 7.1	21.0 ± 1.4	20.0 ± 0.0	2.5 ± 3.5	13.0 ± 4.2
<i>Staphylococcus epidermidis</i>	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.3 ± 7.8	9.3 ± 5.3	6.7 ± 16.3	7.3 ± 17.9	0.0 ± 0.0	0.0 ± 0.0
<i>Enterococcus faecalis</i>	5.0 ± 0.0	10.0 ± 14.1	9.0 ± 12.7	20.0 ± 2.8	17.0 ± 2.8	17.5 ± 6.3	20.5 ± 0.7	5.0 ± 0.0	15.0 ± 0.0

Key: CAZ-Ceflazidime; CRX-Cefluoro xime; GEN-Gentamycin; CTR-Coftriaxone; ERY-Erythromycin; CXC-Cloxicillin; OFL-Oflo xo xin; AUG-Amoxicillin/Clavulinate and CPX- Ciprofloxacin.

Susceptibility of clinical isolates to standard antibiotics

Table 4 shows the sensitivities of the test bacteria against commercially available standard antibiotics used at a concentration of 300mg/ml. Sensitivities

ranged from 0.0±0.0 to 24.5±14.8 of Ceflazidime and Ofloxoxin respectively. *Staphylococcus epidermidis* was the most susceptible while *Vibrio cholera* was mostly resistant to the drugs.

Table 5. Comparing the antimicrobial activities of extracts with antibiotics

Clinical isolates	Extracts	Antibiotics (comparable)
1 <i>Escherichia coli</i>		OFL
2 <i>Pseudomonas aeruginosa</i>		OFL
3 <i>Shigella dysenteriae</i>		CTR,ERY, CXC, OFL
4 <i>Salmonella typhi</i>	100%	
5 <i>Klebsiella pneumonia</i>	100%	
6 <i>Micrococcus flavus</i>	100%	
7 <i>Bacillus subtilis</i>	100%	
8 <i>Streptococcus pyogenes</i>	100%	
9 <i>Streptococcus mutans</i>	100%	
10 <i>Alcaligenes faecalis</i>	100%	
11 <i>Proteus vulgaris</i>		CTR,ERY, CXC, OFL
12 <i>Proteus mirabilis</i>		100%
13 <i>Staphylococcus epidermidis</i>	100%	
14 <i>Enterococcus faecalis</i>		CTR

DISCUSSION

Of the sixteen clinical isolates used in this work, *Shigella dysenteriae* and *Escherichia coli* were most predominant. From the result of the sensitivity test of *Boswellia* stem bark extracts on bacterial isolates, the largest zones of inhibition were seen with the ethanolic fraction at high concentration (300mg/ml). At lower concentrations no significant activities were observed. The five organic extracts employed in this work, the ethanol extract had the highest activity. It is closely followed by the ethyl acetate fraction. The chloroform fraction was the least active. *Escherichia coli* was most sensitive to the n-butanol fraction while *Proteus vulgaris* was most sensitive to n-hexane fraction. *Salmonella enterica*, *Streptococcus mutans*, *Staphylococcus aureus*, *Vibrio cholera*, *Corynebacterium rubrum* and *Proteus mirabilis* were most sensitive to the ethyl acetate extract of *Boswellia sacra*. The ethanol extract had the highest activity against *Staphylococcus epidermidis* (21.6mm), *Enterococcus faecalis* (20.0mm) and *Streptococcus pyogenes* (20.0mm). This result tends to agree with that of Danlami et al. (2015) in which the ethyl acetate and ethanol extracts of leaves and stem bark of *Boswellia dalzielii* showed more activity against the isolates than the hexane extracts. Again they observed that both extracts showed significantly higher antimicrobial activity towards *Escherichia coli* and *Klebsiella pneumonia*. Susceptible microorganisms are those microorganisms which cannot grow if the drug is present and so the drug effectively hinders the growth or multiplication of the organism. In this work the growth inhibition range is from 11mm to 21.6mm. The growth inhibition zone measured ranged from 11 to 20 mm for all the sensitive bacteria and the results showed that the extracts of *Boswellia ovalifoliolata* were found to be effective against all the microbes tested. The N-hexane extract also equally effectively inhibited (20.00mm) the growth of *Enterococcus faecalis* as the ethanolic extract (20.00mm). Weckesser et al. (2007) found that both *Boswellia elongata* and *Boswellia ameero* exhibited a strong antimicrobial effect only against Gram positive bacteria. In this work, *Escherichia coli* was most sensitive to the n-butanol extract (16.00mm) and least sensitive to the n-hexane extract (11.00mm). Again, it was reported that the extract of *Boswellia serrata* exhibited significant antibacterial and antifungal properties. *Boswellia serrata* dry extract was highly effective against selected aerobic and anaerobic bacteria such as *Streptococcae*, *Corynebacteria*, *Clostridium perfringens* and *Propionibacterium acnes* (Danlami et al. 2015). The results were compared with standard antibiotic drugs. In this work, extracts

of *Boswellia sacra* found to be active against clinical isolates are shown on Table 5. *Escherichia coli* (OFL), *Pseudomonas aeruginosa* (OFL), *Shigella dysenteriae* (CTR,ERY,CXC,OFL), *Salmonella typhi*, *Klebsiella pneumonia*, *Micrococcus flavus*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Alcaligenes faecalis*, *Proteus vulgaris* (CTR, ERY, CXC,OFL), *Proteus mirabilis*, *Staphylococcus epidermidis*, *Enterococcus faecalis* (CTR) (Weckesser et al., 2007).

CONCLUSION

Boswellia species certainly have exhibited significant *in vitro* antimicrobial capabilities compared with the common antibiotics. The traditional use of the plant for many diseases is apparently justified. More work will be done to utilize the antibiotic, antifungal and antiviral properties of this plant for the treatment various ills affecting humans.

REFERENCES

- Abreu A.C., McBain A.J. and Simoes M. (2012). Plants as sources of new antimicrobials and resistance-modifying agents. *Natural Products Report*.29:1007. [Google Scholar](#)
- Afsar V., Reddy Y.M. and Saritha R.V. (2012). *In Vitro* Antioxidant Activity and Anti Inflammatory Activity of Methanolic Leaf Extract of *Boswellia serrata*. *International Journal of Life Sciences, Biotechnology and Pharma Research*,1:4. [Google Scholar](#)
- Bergey D.H. and Holt J.G. (1998). *Bergey's Manual of Determinative Bacteriology* 9th edition. Philadelphia: Lipincott Williams and Wilkins p83-88. [Google Scholar](#)
- Bauer A.W., Kirby W.M., Sherris J.C. and Turck M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45 (4): 493-496. [Google Scholar](#)
- Berdy, J. (2005). Bioactive microbial metabolites *Journal of Antibiotics*, 58:1-26. [Google Scholar](#)
- Clinical and Laboratory Standards Institute (2012). *Performance Standards for Antimicrobial Disk Susceptibility Tests*, Approved Standard, 7th edition, Clinical and Laboratory Standards Institute document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA. [Google Scholar](#)
- Cos P., Vlietinck A.J., Berghe V. D. and Maes L. (2006). Anti-infective potential of natural products:

How to develop a stronger in vitro 'proof-of-concept'. *Journal of Ethnopharmacology*, **106**: 3.290-302.

[Google Scholar](#)

Danlami U., Daniel G.J., David B. and Galadanchi K.M. (2015). Phytochemical, Nutritional and Antimicrobial Screening of Hexane, Ethyl Acetate and Ethanolic Extracts of *Boswelliadalzielii* leaves and stem bark. *American Journal of Bioscience and Bioengineering*, **3**(5):76-79.

[Google Scholar](#)

Guschin, A., Ryzhikh P. and Rummyantseva, T. (2015). Treatment efficacy, treatment failures and selection of macrolide resistance in patients with high load of *Mycoplasma genitalium* during treatment of male urethritis with Josamycin. *BioMed Central Infectious Disease*, **15**:1-7.

[Google Scholar](#)

Kafuti, Y., Alemika, T.E., Ojerinde, O.S., Taba, K., Mpiana, P.T., Balogun, O. and Kindombe, N.M. (2018). Phytochemical studies, *in vitro* antioxidant and antiproliferative of stem bark of *Boswelliadalzielli* Hutch *GPH-Journal of Advanced research in Applied Science*, **1**:1.

[Google Scholar](#)

Mabona, U., Viljoen, A. and Shikanga, E. (2013). Antimicrobial activity of Southern African medicinal plants with dermatological relevance: from an ethnopharmacological screening approach, to combination studies and the isolation of a bioactive compound. *Journal of Ethnopharmacology*, **148**:45-55.

[Google Scholar](#)

Mayers, D. L., Lerner, S.A. and Ouelette, M. (2009). *Antimicrobial Drug Resistance C: Clinical and Epidemiological Aspects*, vol. 2, Springer Dordrecht Heidelberg, London pp. 681-1347.

[Google Scholar](#)

Mbata, T.I., Debiao, L. and Saikia, A. (2006). Antibacterial activity of the crude extract of Chinese Green Tea (*Camelliasinensis*) on *Listeria monocytogenes*. *Internet Journal of Microbiology*, **2**(2).

[Google Scholar](#)

Panda, S. (2014). Screening Methods in the Study of Antimicrobial Properties of Medicinal Plants. *International Journal of Biotechnology and Research*, **2**:1:1-35.

[Google Scholar](#)

Runyoro, D.K., Matee, M.I. and Ngassapa, O.D. (2006). Screening of Tanzanian medicinal plants for anti-Candida activity. *BioMed Central Complementary and Alternative Medicine*, **6**:11.

[Google Scholar](#)

Singer, A.C., Shaw, H., Rhodes, V. and Hart, A. (2016). Review of Antimicrobial Resistance in the Environment and its relevance to Environmental regulators, *Frontiers of Microbiology*, 2016.01728.

[Google Scholar](#)

Weckesser, S., Engel, K., Simon-Haarhaus, B., Wittmer, A., Pelz, K. and Schempp, C.M. (2007). Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. *Phytomedicine* **14**:508-516.

[Google Scholar](#)

