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Application of Medicinal Plants to Overcome Antibiotic Resistance in Some Selected Multi-Drug Resistant Clinical Isolates.

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ABSTRACT

Antibiotic resistance among bacteria is becoming a major problem in the treatment of many infections. Hence, there is need to find an alternative way by using medicinal plants to overcome the antibiotic resistance and source of novel drug for the treatment of these bacterial diseases. This work aimed at evaluating the possibility of applying hexane extracts of *Allium sativum* bulbs, *Calotropis procera* leaves, *Acacia nilotica* pods, and *Mitracarpus scaber* whole parts to overcome the antibiotic resistance in some selected clinically isolated bacterial strains (*Staphylococcus aureus*, *Klebsilla pneumoniae* and *Streptococcus pneumoniae*). The efficacy of the hexane extracts was tested by using Agar well diffusion assay method and zones of growth inhibition were measured in millimeter (mm). The results indicated that, all the plants extracts exhibited antibacterial activity against one or more tested pathogens. The hexane extract of *A. nilotica* showed stronger and broad spectrum activity against the tested isolates as compared to the other extracts that demonstrated moderate activity. Noticeably, no antibacterial activity was found in the hexane extract of *C. procera* against the tested bacteria except on *Klebsilla pneumoniae* at concentration of 100mg/ml. Therefore, this study ascertained the value of the medicinal plants to be used as alternatives in the treatment of bacterial infections so as to overcome completely or minimize the resistance on the synthetic or commercial antibiotic drugs.

INTRODUCTION

Antibiotic resistance is a form of drug resistant whereby some sub-populations of a microorganism, usually a bacterial species, are able to survive after exposure to one or more antibiotics. In other word, the term "antibiotic resistance" is refers to the ability of a microorganism to withstand the effect of an antibiotic. The use of antibiotics is limited because bacteria have evolved defenses against certain antibiotics. One of the main mechanisms of defense is inactivation of the antibiotic. This is the usual defense against penicillin and chloramphenicol, among others. Another form of defense involves a mutation that changes the bacterial enzyme affected by the drug in such a way that the antibiotic can no longer inhibit it. This is the main mechanism of resistance to the compounds that inhibit protein synthesis, such as the tetracycline [1].

The problem of resistance has been exacerbated by the use of antibiotics as prophylactics, intended to prevent infection before it occurs. Indiscriminate and inappropriate use of antibiotics for the treatment of the common cold and other common viral infections, against which they have no effect, removes antibiotic-sensitive bacteria and allows the development of antibiotic-resistant bacteria [2].

The pathogens resistant to multiple antibiotics are considered as Multi-drug resistant pathogens [3]. Multi-drug resistant organisms are resistant to treatment with several, often conventional antimicrobial agents. Some of the most important types of multiple drug resistant organisms that have been encountered include; *Staphylococcus aureus*, *Klebsilla pneumonia* and *Streptococcus pneumonia*.

In considering the antibiotic resistance as a major problem in the treatment of bacterial infections, there is need to find alternative way of treating infectious diseases using plants which are abundant in our environment and their extracts may overcome the antibiotic resistance so as to serve as a source of novel drugs for the treatment of these diseases.

MATERIALS AND METHODS

Plant Sampling

The healthy plants, *Allium sativum*, *Calotropis procera*, *Acacia nilotica*, and *Mitracarpus scaber* were obtained from Birnin Kebbi, Kebbi State, Nigeria. The plants were identified and authenticated in the Botany Unit, Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero.

Preparation of Plant Sample

The required parts of the plants to be used were cleaned and air dried in a room. The dried samples were pulverized using a mortar and pestle. The ground samples were used for the preparation of the hexane extracts.

Table 1: Parts of the Plants Used

Plant	Parts of the Plant Used
<i>Allium sativum</i>	Bulbs
<i>Calotropis procera</i>	Leaves
<i>Acacia nilotica</i>	Pods
<i>Mitracarpus scaber</i>	Whole plant

Multi-Drug Resistant Clinically Isolated Bacteria

The multi-drug resistant bacteria (*Staphylococcus aureus*, *Klebsilla pneumonia* and *Streptococcus pneumonia*) were obtained from Sir Yahaya Memorial Hospital Birnin Kebbi. The bacterial strains were identified on the basis of cultural and morphological characteristics and the sensitivity test were conducted to test their resistance to so many antibiotics drugs. *Staphylococcus aureus* was resistant to penicillin, amoxicillin, chloramphenicol, cloxacillin, erythromycin, streptomycin, and tetracycline. *Streptococcus pneumonia* was resistant to penicillin, amoxicillin, cloxacillin, and erythromycin. *Klebsilla pneumonia* was resistant to pefloxacin, tarivid, streptomycin, chloranphenicol, sparfloxacin, amoxicillin, augmentin, and septrin.

Culture and Maintenance of the Bacterial Strains

The isolates were maintained by sub-culturing them into a new prepared nutrient agar slant and stored in an incubator at 37°C.

Preparation of Inoculums

The Bacterial inoculums were prepared by sub-culturing of the test organisms from nutrient agar slants on another prepared nutrient agar plates and incubated at 37°C for 24 hours. The pure cultures on the nutrient agar plates were used as the inoculums.

Preparation of Extracts

The hexane extracts of the plants were obtained according to the method described by [4], with slight modifications. 100g of each dried powder of each plant samples was mixed with 400 ml of n-hexane. The mixture was gently stirred, tightly covered with cotton wools and foiled, then allowed to stand for 3 days at room temperature. Each extract was decanted and filtered through muslin cloth. The filtrates obtained were placed in a water bath and allowed to stand to evaporate the solvent. The residues obtained were used for testing the antibacterial activity of the plants extracts.

Tests for Antibacterial Efficacy

The Agar well diffusion method described by [5] was used to determine the antibacterial activity of the plant extracts. The Mueller-Hinton agar media were prepared according to the method described by [6]. The prepared agar plates were inoculated with test organisms (different bacterial inoculums). Five wells (holes) were made into the set agar in Petri-dishes containing the inoculums using a sterile syringe of 10mm diameter. The different concentration (50mg/ml, 75mg/ml, and 100mg/ml) of the extract were prepared. A 0.25ml (5 drops) volume of each prepared concentration (50mg/ml, 75mg/ml, and 100mg/ml) of the extract was dispensed into the different agar wells in the media. Reference drugs; Gentamycin (50mg/ml) was serve as positive control, while the solvent (hexane) was used as negative control. The cultures were incubated at 37°C for 24 hours. The zones of inhibition around the wells were measured as an indication of the antibacterial activity of the plant extract. The experiment was conducted in two replicates.

Measuring of Zone Inhibition

The zone of inhibition is the diameter over the growth of microorganism inhibited due to the presence of an antimicrobial agent [7]. After the incubation period, the plates were observed for zones of inhibition (indicated by clear zones) around the wells. The antibacterial activities of the extracts were assessed by measuring the diameter of the zone of inhibition in (mm) around the wells using a transparent measuring ruler. The actual zone of inhibition was calculated by subtracting the diameter of the well from the measured diameter (including the well diameter).

RESULTS

Table 2: Antibacterial Activity of Pods Hexane Extract of *Acacia nilotica*

Multi-Drug Resistant Bacteria	Zone of Inhibition (mm)			
	50mg/ml	75 mg/ml	100 mg/ml	Gentamycin (50mg/ml)
<i>K. pneumonia</i>	2.5±0.707	4.0±1.414	5.0±1.414	10.5±2.121
<i>S. aureus</i>	3.0±1.414	4.0±1.414	4.5±0.707	6.5±0.707
<i>S. pneumonia</i>	4.0±2.828	5.0±2.828	7.5±0.707	8.0±0.00

Values are presented as mean ± standard deviation of triplicates. Gentamycin was used as positive control.

Table 3: Antibacterial Activity of *Mitracarpus scaber* Hexane Extract

Multi-Drug Resistant Bacteria	Zone of Inhibition (mm)			
	50 mg/ml	75 mg/ml	100 mg/ml	Gentamycin (50mg/ml)
<i>K. pneumonia</i>	-	-	2.5±2.121	23.5±0.707
<i>S. aureus</i>	3.5±0.707	5.0±1.414	5.5±0.707	23.0±2.828
<i>S. pneumonia</i>	-	-	-	9.5±0.707

Values are presented as mean ± standard deviation of triplicates. Gentamycin was used as positive control.

Table 4: Antibacterial Activity of Bulbs Hexane Extract of *Allium sativum*

Multi-Drug Resistant Bacteria	Zone of Inhibition (mm)			
	50 mg/ml	75 mg/ml	100 mg/ml	Gentamycin (50mg/ml)
<i>K. pneumonia</i>	1.0±0.000	1.5±0.707	2.5±0.707	10.0±1.414
<i>S. aureus</i>	1.5±0.707	2.0±0.000	3.5±0.707	19.5±0.707
<i>S. pneumonia</i>	1.0±0.000	1.5±0.707	2.0±1.414	21.0±0.707

Values are presented as mean ± standard deviation of triplicates. Gentamycin was used as positive control.

Table 5: Antibacterial Activity of Leaves Hexane Extract of *Calotropis procera*

Multi-Drug Resistant Bacteria	Zone of Inhibition (mm)			
	50 mg/ml	75 mg/ml	100 mg/ml	Gentamycin (50mg/ml)
<i>K. pneumonia</i>	-	-	1.5±1.414	21.5±2.121
<i>S. aureus</i>	-	-	-	5.5±0.707
<i>S. pneumonia</i>	-	-	-	22.0±1.410

Values are presented as mean ± standard deviation of triplicates. Gentamycin was used as positive control.

DISCUSSION

Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide. An antibiotic resistant bacterium is a threat which is becoming increasingly common. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. The search for antimicrobials from natural sources has received much attention and efforts have been put in to identifying compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism. These compounds have significant therapeutic applications against human pathogens including bacteria, fungi or virus [8]. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds. So, a return to natural substances is an absolute need of our time [8].

Plants remain one of the main sources of natural products for new therapies particularly in poor countries, because most of them are less cost, and can affect a wide range of antibiotic resistant microorganisms, and another interesting reason is that, the herbal medicines have fewer adverse effects compared to the conventional ones. Therefore, plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay. Many reports are available on the antibacterial properties of these plants. Some of these observations would help in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the plants for developing commercial drug for applications to overcome antibiotic resistant in the resistant strains. Hence, in this investigation, the hexane extracts of *A. nilotica*, *Allium sativum*, *M. scaber* and *C. procera* were evaluated for exploration of their antimicrobial activity against certain Gram negative (*Klebsilla*) and Gram positive bacteria (*S. aureus* and *Streptococcus*), which was regarded as human pathogenic bacteria that are resistant to many antibiotic drugs.

Antibacterial activities of the plants extracts were tested using well diffusion method. The hexane extract of the *A. nilotica* pods showed the highest zone of inhibition on *S. pneumoniae* (7.5±0.707mm) at 100mg/ml and the lowest on *K. pneumoniae* (2.5±0.707mm) at 50mg/ml as recorded in the (Table 2). The result obtained revealed that, *A. nilotica* indicated a good antibacterial activity against the tested microorganisms. This result is supported by the result previously reported by [9] who reported that, the different parts of the plant have antibacterial activity against the tested microbes. It was observed that, the Gram positive bacteria among the tested bacteria are more susceptible to the plant extract with the highest zone of inhibition for *Streptococcus pneumonia*. While the Gram negative bacteria (*Klebsilla*) showed less sensitivity to the plant extract when compared to the Gram positive bacteria (*Streptococcus*). The hexane extract of the *M. scaber* showed the highest zone of inhibition on *S. aureus* (5.5±0.707mm) at 100mg/ml and the lowest on *K. pneumoniae* (2.5±2.121mm) at 100mg/ml as seen in (Table 3). The result obtained revealed that, *M. scaber* has good antibacterial activity against the tested microorganisms, except *S. pneumoniae* which has no zone of inhibition at even the large concentration (100mg/ml) of the plant extract. This result is in line with the result previously reported by [7], who reported that, the plant extracts have antibacterial activity.

The hexane extract of the *A. sativum*, showed the highest zone of activity on *S. aureus* (3.5±0.707mm) at 100mg/ml and the lowest on *K. pneumoniae* and *S. pneumonia* (1.0±0.000mm) at 50mg/ml as shown in (Table 4). This result revealed that, the hexane extract of the *A. sativum* has poor antibacterial activity against the tested microorganism when compared to the other tested extract. This result is supported by the result previously obtained by [10, 11, 12], who reported that, the plant extracts have antibacterial activity. But the result in other way contradicted the results reported by [13, 14], who reported that, the hexane extract of the plant did not showed any antibacterial activity on the tested bacteria. The controversy might be because of the concentration of the extract used in their research, which was

50mg/ml compared to the concentration used in this work. The large size of the zones of inhibition indicated the potency of the active principles of the plant. It was recorded that an increase in the concentration of the extract yielded higher activity as shown by the diameter of zone of inhibition.

Table 5 showed the antibacterial activity of *C. procera*. The hexane extract of the *C. procera*, inhibited only *K. pneumonia* (1.5 ± 1.414 mm) at concentration of 100mg/ml and no any other zone of inhibitions observed for the other tested bacteria. This result showed that, the hexane extract of the *C. procera*, has relatively poor antibacterial activity against the tested bacteria. This result is supported by the result previously obtained by [15], who reported that, the hexane extract of the plant has no antibacterial activity on the tested bacteria. According to the studies reported earlier by [4, 16, 17], the latex of the plant and other solvent extract of the plant have some antibacterial activities on some of the tested bacteria.

CONCLUSION

This research concluded that, the n-hexane solvent extraction was suitable for verifying the antibacterial properties of these medicinal plants. These plants have very promising antimicrobial activities and thus can be used traditionally to cure various infectious diseases cause by these resistant bacteria, and could serve as useful source of new antibacterial agents.

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