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Research Article

Role of *Lannea microcarpa* Stem-Bark Acetone Extract in Resolving Antibiotic Resistance of Some Clinically Isolated Bacteria

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Abstract: Currently, situation of bacterial antibiotic resistance is dingy and resistance mechanisms are pandemic, this creates a huge clinical and financial burden on the healthcare system worldwide. If it continues, it may result in pathogens that evade all existing therapeutic agents. This should provide sufficient caution for implementation of new antibiotic alternatives especially from plant resources. Hence, this research was aimed at evaluating the role of *Lannea microcarpa* stem-bark acetone extract in resolving antibiotic resistance of some clinically isolated bacteria. *Lannea microcarpa* stem-bark was obtained within Aliero, Nigeria, while the multidrug-resistant bacterial strains were obtained from Sir Yahaya memorial hospital B/Kebbi, Nigeria. Acetone extract was used, and test organisms were taken from nutrient agar slants and sub-cultured on nutrient agar plates. The antibacterial activities were conducted using agar-well-diffusion method. The phytochemical composition of the stem-bark extract shows the presence of alkaloids, flavonoids, saponins, tannins, anthraquinones, and phenols. Terpenoids and steroids were not detected using the methods used. The result for the antibacterial activity reveals that, the extract exhibited dose dependent activity, however, the extract shown to have high antibacterial activities even at the lower concentration of 75mg/ml compared to the control drug (cloxacin). The higher the concentration of the plant extract the higher the activity and at lower concentration, the activity has not significantly reduced. The results obtained revealed that, *Lannea microcarpa* stem-bark has antibacterial activity against the tested multidrug-resistant bacteria, and this can serve as lead to researchers for the development of effective, safe, readily available and affordable antibacterial drug.

Keywords: Bacteria, Antibiotic resistance, Multidrug-resistance, *Lannea microcarpa*, Stem-bark, Acetone extract, Clinical isolates.

INTRODUCTION

Bacterial antibiotic resistance is a type of drug resistance whereby some sub-populations of bacterial species are able to survive after exposure to one or more antibiotics (Sani, 2014). In other words, "antibiotic resistance means the ability of a microorganism to withstand the effect of an antibiotic" (Witte, 2004).

Several mechanisms have evolved in bacteria which confer them with antibiotic resistance to ensure their survival. These mechanisms can chemically modify the antibiotic, render it inactive through physical removal from the cell, or modify target site so that it is not recognized by the antibiotic (Sani *et al*., 2018). The most common mode is enzymatic inactivation. An existing cellular enzyme is modified to react with the antibiotic in such a way that it no longer affects the microorganism. An alternative strategy utilized by many bacteria is the alteration of the antibiotic target site (Alekshun and Levy, 2007).

All these forms of resistance are transmitted genetically by the bacterium to its progeny. Genes that carry resistance can also be transmitted from one bacterium to another by means of plasmids, chromosomal fragments that contain only a few genes, including the resistance gene. Some bacteria conjugate with others of the same species, forming temporary links during which the plasmids are passed from one to another (Abdulhamid *et al* 2014). If two plasmids carrying resistance genes to different antibiotics are transferred to the same bacterium, their resistance genes

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can be assembled onto a single plasmid (Witte, 2004). The combined resistance can then be transmitted to another bacterium, where they may be combined with yet another type of resistance. In this way, plasmids are generated that carry resistance to several different classes of antibiotic. In addition, plasmids have evolved that can be transmitted from one species of bacteria to another, and these can transfer multiple antibiotic resistance between very dissimilar species of bacteria (Alekshun and Levy, 2007).

Multidrug resistance among many organisms has become a big challenge to infectious disease management. For antibiotic uses that target a primary bacterial infection, there are numerous approaches to either reduce the selection for antibiotic resistance or to replace the antibiotic altogether. Each approach has its own benefits and costs. The most useful replacement for antibiotics is the application of medicinal plants. According to WHO, medicinal plants would be the best source for obtaining variety of drugs. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multi-drug resistant (MDR) strains. A huge number of medicinal plants have been recognized as valuable resources of natural antibacterial compounds (Sani and Abdulhamid, 2016). Considerable numbers of studies have been conducted on the antibacterial activity of medicinal plants which showed promising effectiveness against MDR microorganisms after antibiotics failed to eliminate them (Sani *et al.*, 2014).

MATERIALS AND METHODS

Plant Sampling and Authentication

The *Lannea microcarpa* stem-bark was obtained from Aliero town, Kebbi State, Nigeria. The sample was identified and authenticated in the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero, Nigeria, and was found to belong to the family *Anacardiaceae* with a voucher number 146.

Multidrug-Resistant Clinically Isolated Bacteria

The multidrug-resistant bacteria (*Staphylococcus aureus, Klebsilla pneumoniae, Streptococcus pneumoniae and Salmonella typhi*) were obtained from Sir Yahaya memorial hospital, Birnin Kebbi, Nigeria. Sensitivity tests were conducted on the test organisms, to confirm their multidrug-resistance.

Preparation of Plant Sample

The plant sample was cleaned and air dried at room temperature. The completely dried sample was crushed to coarse powder by grinding with wooden mortar and pestle. The ground sample was use for the preparation of acetone extract.

Preparation of Extract

The acetone extract of the plant was obtained according to the method described by Leonard *et al.*, (2013), with slight modifications. A 200g of the dried powder of the plant sample was dissolved in 500ml acetone. The mixture was gently stirred, tightly covered with cotton wool and aluminum foil, and was allowed to stand for 72 hours at room temperature. The extract was decanted and filtered through muslin cloth. The filtrate obtained was allowed to evaporate at room temperature with continuous weighing until a constant weight was obtained.

Culture and Maintenance of Test Organisms

The isolates were taken from nutrient agar slants and sub-cultured on nutrient agar plates. The plates were incubated in an incubator at 37^{o}C for 24 hours to get sub-cultures of the isolates.

Preparation of Inoculums

After the sub-culturing, to prepare the bacterial inoculums, the sub-cultures were then inoculated on fresh nutrient agar plates using sterile cotton swabs at 37^{o}C for 24 hours. The pure cultures on the nutrient agar plates were used as the inoculums.

Qualitative Phytochemical Screening

Five grams (5g) of the plant extract was dissolved in 40 ml of distilled water and then subjected to phytochemical screening using the method of Mbatchou and Kossono, (2012). The presence of flavonoids, phenols, tannins, saponins, alkaloids, terpenoids, steroids and anthraquinones were tested.

Determination of Antibacterial Activity

The Agar well diffusion method (Pelczar *et al.*, 1993) was used. The Mueller-Hinton agar media were prepared according to the method described by Wolfgang and Hilda, (1976). The prepared agar plates were inoculated with the test organisms. Four wells (holes) were made into the set agar in Petri-dishes containing the inoculums using a sterile syringe of 10mm diameter. The different concentration (75mg/ml, 100mg/ml, and 120mg/ml) of the extract were prepared. A 0.25ml (5 drops) volume of each prepared concentrations of the extract was dispensed into the different agar wells in the media, followed by incubation at 37^{o}C for 24 hours. Cloxacin was used as the positive control while the solvent (acetone) was used as negative control.

Measuring Zones of Inhibition

After the incubation period, the plates were observed for zones of inhibition (indicated by clear zones) around the wells. The antibacterial activities of the extract were accessed by measuring the diameter of the zone of inhibition in millimeter 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.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations of the plant extract were determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Six (6) test tubes were labelled 1-6, to test tubes 2-6 1ml of sterile nutrient broth were added, 1ml of the extract were added to test tube 1 and test tube 2. Doubling dilution was done from tube 2-5 to have extract concentrations (in mg/ml) of 120, 60, 30, 15 and 7.5 respectively. Test tube 6 contains 1ml of sterilized nutrient broth (serving as control for the sterility of the medium). Afterwards, 0.1 ml of a 0.5 McFarland standard of the test organisms in normal saline (0.85% NaCl (w/v)) were inoculated into the test tubes 1-5, shaken and incubated at 37°C for 24 hours. Minimum Inhibitory Concentration is defined as the lowest concentration (in µg/ml) of an antibiotic that inhibits the growth of a given strain of bacteria (Witte, 2004).

Data Analysis

The data collected for the antibacterial activities were subjected to one way analysis of variance (ANOVA) and statistical difference between the means were separated using New Duncan's Multiple Range Test at $P<0.05$ with the aid of a statistical package (IBM SPSS Statistics 20).

RESULTS

The phytochemical composition of the *Lannea microcarpa* stem-bark extract shows that flavonoids, alkaloids, tannins, saponins, phenols and anthraquinones were present, while, terpenoids and steroids were not detected (Table -1).

Table -1: Phytochemical Composition of *Lannea microcarpa* Stem-bark Extract

PHYTOCHEMICAL	OBSERVATION
Flavonoids	+
Alkaloids	+
Tannins	+
Saponins	+
Terpenoids	-
Steroids	-
Phenols	+
Anthraquinones	+

+ = Present, - = Not detected

Table 2 presents the antibiotic susceptibility of the test organisms. These results confirmed their multidrug resistance. It was shown that all the test organisms were susceptible to cloxacin.

Table -2: Antibiotic Susceptibility of the Test Organisms

TEST ORGANISM	ANTIBIOTIC (50MG/ML)								
	Au	**Pe**	**Ch**	**Cl**	**Er**	**Si**	**Ge**	**Te**	**Am**
Streptococcus pneumoniae	-	-	-	+	+	-	-	-	-
Klebsiella pneumonia	-	-	-	+	+	-	-	+	-
Salmonella typhi	-	-	+	+	-	+	+	+	-
Staphlococcus aureus	-	-	+	+	-	+	+	+	+

+ = Effective, - = resistant, Au = Augmentin, Pe = Penicillin, Ch = Chloramphenicol, Cl = Cloxacin, Er = Erythromycin, Si = Sivooflaxacin, Ge = Gentamycin, Te = Tetracycline, Am = Amoxicillin.

The results for the antibacterial activity of *Lannea microcarpa* stem-bark extract (Table 3) reveals that, activity against all the test organisms was exhibited at each extract concentration. It was observed that the higher the concentration of the extract the higher the activity. The extract exhibited significant growth inhibition on all the test organisms compared to the control drug (Cloxacin)

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Table-3: Antibacterial Activity of *Lannea microcarpa* Stem-Bark Extract

Test Organism	Zone of Inhibition (mm)			
	Cloxacin (75mg/ml)	Extract (75mg/ml)	Extract (100mg/ml)	Extract (120mg/ml)
Klebsiella pneumoniae	23.77 ± 4.48^{a}	24.20 ± 2.76^{a}	27.20 ± 2.71^{b}	28.60 ± 2.48^{b}
Salmonella typhi	22.55 ± 2.91^{a}	27.40 ± 2.88^{b}	28.30 ± 0.30^{bc}	28.76 ± 1.97^{c}
Staphylococcus aureus	21.11 ± 0.69^{a}	23.43 ± 2.21^{b}	22.63 ± 2.97^{b}	24.97 ± 1.79^{c}
Streptococcus pneumoniae	22.97 ± 1.79^{a}	27.30 ± 2.17^{b}	27.53 ± 0.92^{b}	26.30 ± 1.48^{b}

Values are presented as mean ± standard deviation of triplicates. Values carrying different superscripts from the control (Cloxacin) for each row are significantly different ($P<0.05$) using ANOVA and Duncan multiple range test.

Table 4 presents the minimum concentrations of the extract at which antibacterial activity on the test organisms is observed. The concentration of 15 mg/ml is the minimum at which the growth of *Streptococcus pneumoniae* was inhibited. But, 30 mg/ml was the minimum extract concentration for the inhibition of the growth of *Staphylococcus aureus*, while 60 mg/ml was the minimum concentration for the growth inhibition of *Klebsiella pneumoniae* and *Salmonella typi*.

Table -4: Minimum Inhibitory Concentrations (MIC) of *Lannea microcarpa* Stem-Bark Extract on the Test Organisms.

TEST ORGANISM	MIC (MG/ML)
Staphylococcus aureus	30
Streptococcus pneumoniae	15
Klebsiella pneumoniae	60
Salmonella typhi	60

DISCUSSION

The antibacterial activity observed with the stem-bark extract of *Lannea microcarpa* can justify its use in traditional medicine for treating ailments, such as diarrhea, gastroenteritis, bacterial infections, toothaches and wound (Tapsoba and Deschamps, 2006). The activity might be due to the presence of polyphenol compounds, such as tannins, phenolic acids and flavonoids (Oleivera *et al*., 2008). Numerous studies on polyphenol compounds indicate that they have antibacterial activity (Adulhamid *et al*., 2016; Berahou *et al*., 2007). Tannins have been found to form irreversible complexes with proline rich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). These observations therefore support the use of *Lannea microcarpa* in herbal cure remedies and this might be the reason for the growth inhibition of the test organisms in this research work. Flavonoids, another constituent of *Lannea microcarpa* stem-bark exhibited a wide range of biological activities like antibacterial, anti-inflammatory, analgesic, cytostatic and antioxidant properties (Hodek *et al*., 2002).

There are high proportions of antibiotic resistance in bacteria that cause common infections (e.g. urinary tract infections, pneumonia, bloodstream infections) in all regions of the world. A high percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterobacteriaceae* (mostly *Klebsiella pneumoniae*) or multidrug-resistant Gram-negative bacteria (Sani *et al*., 2018). Hence, the growth inhibitory effect of *Lannea microcarpa* stem-bark extract on the *Staphylococcus aureus* and *Klebsiella pneumoniae* in this research signifies that the plant can be used for the treatment of infectious diseases caused by the MRSA and the *Klebsiella pneumoniae*.

According to WHO, (2002), medicinal plants would be the best source for obtaining variety of drugs. For this reason; researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multidrug resistant strains. A huge number of medicinal plants have been recognized as valuable resources of natural antibacterial compounds (Abdulhamid and Sani, 2016). Considerable numbers of studies have been conducted on the antibacterial activity of medicinal plants which showed promising effectiveness against multidrug resistant microorganisms after antibiotics failed to eliminate them (Sani *et al*., 2018). The primary benefit of using plant medicines is that they are relatively safer and cheaper than their synthetic counterparts. In addition, plant medicine is a complex combination of different phytochemicals acting by different mechanisms, which

makes it difficult for pathogens to develop resistance (Aiyegoro and Okoh, 2009).

Sani, (2014), studied the effect of hexane extracts of *Allium sativum* bulbs, *Calotropis procera* leaves, *Acacia nilotica* pods, and *Mitracarpus scaber* whole parts to resolve the antibiotic resistance in some selected clinically isolated bacterial strains; *Staphylococcus aureus, Klebsiella pneumoniae and Streptococcus pneumoniae*. 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. When compared, the antibacterial activity of *Lannea microcarpa* stem-bark extract on these resistant bacterial strains in this research work is five (5) fold higher than the activities of all the plant extracts tested by Sani, (2014).

CONCLUSION

It has been revealed that *Lannea microcarpa* stem-bark has the ability to resolve the antibiotic resistance of the multidrug resistant clinically isolated bacterial strains used in this research. Additionally, the plant has very promising antibacterial activity and thus, can be beneficial for the treatment of various infectious diseases cause by these resistant bacteria. This finding can also serve as lead to researchers for the development of effective, safe, readily available and affordable antibacterial drug from plant resources.

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