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Sensitivity of *Escherichia Coli* and *Staphylococcus Aureus* to Stem Bark and Root Extracts of *Faidherbia Albida* (Winter Thorn)

[*1]Umar A. T., [1]Liman M. N., [1]Puma H. U.,Salawudeen A.[1],[1]Kawuwa U. A.
[1]*Department of Microbiology, Gombe State University, PMB 127 Gombe State, Nigeria*

Abstract: *Occurrences of drug resistant bacteria have been on the rise globally, thereby presenting a looming threat to efficiency of conventional antibiotic therapy, and hence the need to seek alternatives to antibiotics. The aim of this study was to evaluate the sensitivity of Escherichia coli and Staphylococcus aureus to extracts from root and stem bark of Faidherbia albida(winter thorn). Extraction of phytochemicals from the plant parts was done using cold maceration method with methanol as solvent, resulting in percentage yields of 6.50% and 5.25% for stem bark and root extracts respectively. The qualitative phytochemical analysis of the crude plant extracts revealed the presence of tannins, alkaloids, and saponins in the stem bark extracts, while the root extracts tested positive for only flavonoids and steroids. The test organisms were collected from specialist hospital Gombe, Gombe State, Nigeria, and confirmed to be Staphylococcus aureus and Escherichia coli using Gram's staining, Microscopy, and appropriate standard biochemical tests, which showed S. aureus colonies to be Gram positive, purple, spherical in shape, arranged in clusters, catalase positive and coagulase positive, while E. colicolonies wereGram negative, rod shaped, catalase and indole positive. Susceptibility of these bacteria to theplant extracts were determined using Well diffusion method, which produced zones of inhibition ranging from 10-20mm for S. aureus and 8-10mm for E. coli, for distinct plant extracts, while a combination of the extractsproduced only 8mm zones of inhibition against both of these bacteria. Minimum inhibitory concentration (MIC) of the stem bark extracts was determined to be 0.5mg/ml using tube dilution method while minimum bactericidal concentration (MBC) was determined to be 1.0mg/ml using agar diffusion method. Therefore, F. albida stem bark has the potential to serve as a source of plant-based medicines against infections caused by these bacteria, and thus offering some alternative to antibiotics and a solution to the economic problems and disease complications resulting from antibiotic resistance by these microorganisms.*

Keywords: *Sensitivity, Escherichia coli,Staphylococcusaureus,Faidherbia albida, Root,Stem bark*

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I. Introduction

Globally, drug resistant types of pathogenic microorganisms such as *Staphylococcus aureus* and *Escherichia coli* have been taking over from their antibiotic-susceptible kin as main causes of transmittable illnesses(Christou, 2011), thereby making this issue of antibiotic resistance a global threat to conventional antibacterial therapy (Huttner et al., 2013).One of the popular opportunistic human pathogenic bacteria is this*S. aureus*, which has been shown to be responsible for a notable array of diseases and health conditions such as bacteremia, endocarditis, formite-borne diseases, osteoarticular, pleuropulmonary, skin, and soft tissue infections, etc.,(Tong et al., 2015).

In recent years, the occurrences of infections by antibiotic-non-responsive strains of *S. aureus* have been on the rise in some nationslike The Gambia (Bojang et al., 2017), andin Australia and New Zealand where out of 1,153 Children with *S. aureus* bacteremia (SAB), 50 died within a month(McMullan et al., 2016), and also in USA, where nearly 400,000 hospitalizations occurred due to *S. aureus* infections in 2003 and has been estimated that methicillin resistant *S. aureus* is responsible for 19,000 mortalities in patients on admission yearly in America(Boucher & Corey, 2008).

Another common bacterium which can cause serious infections is *E. coli*, which has been implicated globally in health conditions and illnesses such as diarrhea, septicemia, meningitis, and urinary tract infections in children(Makvana & Krilov, 2015). In 2010, *E. coli* was shown to be responsible for about 323,000,000 illnesses, 464,000 deaths, and more than 40,000,000 disability adjusted life years (DALYs)(Kirk et al., 2015).Other estimates from 2010 indicated that there were over 1.7 billion cases of diarrhea in children younger than 5 years of age globally(Walker et al., 2013).The occurrences of illnesses resulting from infections by drug resistant *E. coli* have also been on the rise worldwide, hence making these antibiotic resistant strains of *E. coli* to become a topic of serious concern (Bryce et al., 2016;Rao et al., 2014).

In some European countries, burden estimates resulting from infections by both methicillin-resistant *S. aureus* (MRSA) and *E. coli* in 2007 revealed 42,894 illnesses, 8,215 excess deaths, and 375,748 excess hospital

days with a total hospital expenditure of 62.1 million Euros(de Kraker et al., 2011).In another study involving 112 patients, 44.5% and 34.4% mortality rates were seen from septic shock and severe sepsis respectively, where *E. coli* and *S. aureus* accounted for 25% and 21.4% of the bloodstream pathogens in the cases(Artero et al., 2010).

Natural extracts of plant origin contain various compounds with noteworthy antimicrobial activities. It has been estimated that almost 80% of the global community utilize plant-based medicines for curing a lot of ailments, which is backed up by some studies that have shown how some plants in Africa possess noteworthy activities against certain antibiotic non-responsive disease causing microorganisms(Tchinda et al., 2017).

Hence, this work was set up with the aim of determining the antibacterial activity of root and stem bark extracts of *Faidherbia albida* against *Staphylococcus aureus* and *Escherichia coli.*

II. Materials And Methods

Collection, Authentication, and Preparation of Plant Materials

The fresh plant parts were collected from Malamsidi town of Gombe State, Nigeria, and authenticated at the Herbarium of Biological Sciences Department, Gombe State University. The plant parts were then dried under a shade for fourteen days, grounded to powder form using aluminum mortar and pestle, and stored in an air-tight container at room temperature before use(Tchinda et al., 2017).

Extraction

Cold maceration technique was used to extract compounds from the plant parts. One hundred and fifty grams (150g) of powdered plant material were extracted in six hundred milliliters (600mls) of methanol for three days with shaking for thirty (30) minutes daily. The mixture was then filtered and evaporated to dryness(Zhang et al., 2018).

Phytochemical Analysis

Screening for Tannins

Five grams of each plant extract were added to 3mls Ferric chloride solution (5%) in separate setups. Development of a deep-green color specified the existence of tannins, and vice-versa(Ezeonu & Ejikeme, 2016).

Screening for Saponins

Frothing, which persevered on moderate heating was accepted as aninitialindication for the existence of saponins, and vice-versa. Limiteddroplets of olive oil were added and development of soluble emulsion established the existence of Saponins(Astuti et al., 2011).

Screening for Flavonoids

About 2g of each plant extract was completely detanned with acetone, 5mls of 20% NaOH was added to an equivalent volume of the water-reconstituted extract. Development of a yellow solution showed the existence of flavonoids, and vice-versa(Ndebia & Kamgang, 2007).

Screening for Steroids

One hundred milligrams of each plant extract wereliquified in 2 mls of chloroform. Sulphuric acid was cautiously added to procedure a lower layer. A reddish brown color at the boundarydesignated the existence of steroids, and vice-versa(Watal et al., 2014).

Screening for Alkaloids

Wagner's reagent was prepared and 2mls were added to 0.5g of each extract. Development of a reddish-brown colored precipitate designated the existence of alkaloids and vice-versa(Santos et al., 2003).

Antibacterial Activity Tests

Test Isolates

Clinical isolates of presumed *S. aureus* and *E. coli* were collected from Microbiology Laboratory of Specialist Hospital Gombe, Gombe State and maintained on nutrient agar slants in a refrigerator prior to use. Appropriate confirmatory tests including Gram's staining, microscopy and biochemical tests were employed for confirmation of the isolates (Cheesbrough, 2006).

Stock Solution of Extracts

A 200mg/2ml concentration of the extracts werere-constituted by dissolving 0.2g in 2mls of 20% v/v dimethyl sulfoxide (DMSO) (Charles et al., 2012).

Inoculum Standardization

Direct colony suspension methodwas employed; 24h old colonies of the confirmed isolates were picked using sterile wire loop and dissolved in 2mls of sterile normal saline. The turbidity was then adjusted with sterile normal saline to match the turbidity of 0.5 McFarland Standard(CLSI, 2015).

Sensitivity

Agar well diffusion methodwas used. Mueller-Hinton Agar was prepared and dispensed in Petri dishes. Set plates were incubated overnight to ensure sterility before use. Suspension of the test isolates were standardized

as stated previously. From the stock of 200mg/2ml extracts, serial dilutions were made to obtain 100, 50, 25, 12.5, and 6.25 mg/ml extract dilutions. Each labeled media plate was uniformly inoculated with the test organism by using a sterile cotton swab rolled in the suspension to swab the plate surface in a form that lawn growth can be formed. A sterile cork borer of 8mm diameter was used to make wells on the media. 0.1ml of the various extract concentrations were dropped into each well using a micropipetteand appropriately labeled. The plates were incubated at 37^{O}C for 20 hours. Antibacterial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation. 0.064mg/ml of gentamicin was used as positive control and DMSO as negative control(Klančnik et al., 2010).

Minimum Inhibitory Concentration (MIC)

Broth dilution techniquewas employed to ascertain the MIC. Nutrient broth was prepared and dispensed into test tubes (10mls each). The test isolates were then inoculated into the different concentrations and incubated at 37°C for 24 h. The test tubes were observed for change in turbidity which indicates growth. The lowest concentration of extracts in the broth which showed no change in turbidity was recorded as the MIC. Two control tubes were maintained. These includeda tubecontaining extract without inoculum, and another tube containing the growth medium and inoculum(Yusha et al., 2011).

Minimum Bactericidal Concentration (MBC)

This was determined using an agarinoculation method. Mueller Hinton Agar (MHA) was prepared, poured into sterile Petri dishes and allowed to cool. The contents of the test tubes within the range of concentrations with no growth from the MIC test were picked (100µl each) and inoculated separately onto different MHA plates, incubated at 37°C for 24 hours after which the plates were observed for any visible growth. The MHA plate with the lowest concentration of extract without any visible colony growth was considered as the MBC(Yusha'u et al., 2010).

III. Results And Discussion

The results of extraction (Table 1) showed percentage yields of 6.5% and 5.25% for *F. albida* stem bark and root extracts respectively. Both extracts were smooth in texture and brown in color.

The phytochemical analysis of the crude plant extracts (Table 2) revealed the presence of tannins, alkaloids, and saponins in the stem bark extract, while the root extract tested positive for only flavonoids and steroids. These phytochemicalshave been shown to have good antimicrobial activity(Silva et al., 2012;Madhumitha & Saral, 2011; Kaur & Arora, 2009).

Sensitivity of the test isolates to the plant extracts revealed zones of inhibition of 8mm to 20mm when *F. albida* stem bark and root extracts were tested on *S. aureus* (Table 3). When both extracts from the same plant were tested on *E. coli*, zones of inhibition of 8mm to 10mm were recorded. When combination of both root and stem bark extracts were tested on both organisms, zones of inhibition of 8mm were recorded for both test isolates. For the MIC (Table 4), the lowest extract concentration which gave absence of turbidity changein the broth media tubes after 24h was taken as evidence of growth inhibition (MIC), and further absence of growth after sub-culturing from MIC tube onto MHA plates and incubating for another 24h was taken as evidence of total cell death and hence the MBC. The activity showed by the stem bark extract against*S. aureus* can be attributed to the presence of alkaloids and tannins in the extract, because these phytochemicals have previously been attributed with good antimicrobial properties because they act against bacteria by binding and precipitating proteins, and by destroying various molecular targets in a cell, respectively(Compean & Ynalvez, 2014; Simões et al., 2009;Acamovic & Brooker, 2005).

Table 1: Extraction of plant parts

Plant Part	Percentage yield	Color	Texture
F. albida stem bark	6.50%	Dark Brown	Semi Smooth
F. albida root	5.25%	Light Brown	Very Smooth

Table 2: Phytochemical analysis of the plant extracts

Plant Part	Alkaloids	Flavonoids	Tannins	Steroids	Saponins
F. albida stem bark	+	-	+++	-	+++
F. albida rook	-	+	-	+	-

KEY: - = absent, + = present (normal response), +++ = present (high response)

Table 3: Sensitivity of the test isolates to the plant extracts

	***F. albida*SBE (mg/ml)**				***F. albida*RE (mg/ml)**				**Control**
	1.0	**0.5**	**0.25**	**0.125**	**1.0**	**0.5**	**0.25**	**0.125**	**G**
S. aureus	20mm	12mm	8mm	8mm	8mm	8mm	8mm	8mm	16mm
E. coli	10mm	8mm	8mm	8mm	8mm	8mm	8mm	8mm	30mm
EC(BO)	8mm	8mm	8mm	8mm	8mm	8mm	8mm	8mm	

KEY: SBE; stem bark extract, RE; root extract, G; gentamicin, EC; extracts combination,BO; both organisms

Table 4: MIC and MBC of the stem bark extract against *S. aureus*

Minimum Inhibitory Concentration (MIC)	0.5mg/ml
Minimum Bactericidal Concentration (MBC)	1.0mg/ml

IV. Conclusion

It can be concluded that *F. albida* stem bark has some phytochemicals with good activity against *S. aureus*, and so can serve as a source of plant-based antibacterial agent which can be further exploited as a viable option for replacement of antibiotics in treating infections caused by this bacterium, thereby reducing oreliminating the economic and health challenges resulting from antibiotic resistance by this noteworthy opportunistic pathogen.

AUTHORS CONTRIBUTIONS

- Umar A. T. carried out the MIC and MBC tests, and drafted the manuscript.
- Liman M. N. collected the plant parts, prepared them, extracted the phytochemicals, and prepared stock solution of the extracts.
- Puma H. U. carried out the initial sensitivity tests and checked the article for grammar errors.
- Kawuwa U. A. collected, confirmed the test bacteria, and standardized inocula of the test isolates.
- Salawudeen A. carried out the phytochemical analysis of the plant extracts.

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