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Research Article

Efficacy of *Plukenetia conophora* against Multidrug Resistant Staphylococcus aureus isolated from Wound.

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ABSTRACT

Due to the increasing resistance of the superbug, *Staphylococcus aureus*, and many other pathogenic microorganisms to conventional antibiotics, there is a need to search for new and better antimicrobial substances. This study was carried out to evaluate the antimicrobial efficacy of the African walnut plant, *Plukenetia conophora* against antibiotic-resistant *Staphylococcus aureus*. 350 wound swab samples were obtained. Isolation, characterization, identification and antibiotic susceptibility testing of *Staphylococcus aureus* isolates were done using standard microbiological procedures. Antimicrobial activity screening of the different extracts on the antibiotic-resistant isolates was performed with the agar well diffusion technique. Varying concentrations (100mg/ml, 50mg/ml, 25mg/ml,12.5mg/ml) of both the aqueous and ethanol extracts of the leaves of the plant were assayed for antimicrobial potential and their antimicrobial activity was compared with the antibiotic, Nitrofurantoin 300µg (NIT) for each of the antibiotic-resistant Staphylococcus aureus isolates. Only the leaf extracts possessed antimicrobial potential against antibiotic-resistant *Staphylococcus aureus*. Also, ethanol extracts of the leaves displayed the higher antimicrobial potential of the leaf extracts is dose-dependent and is higher than that of NIT upon comparison. The MIC and the MBC were found to be 25mg/ml and 100 mg/ml respectively for both ethanol and aqueous leaf extracts. Flavonoids, alkaloids, tannins, and phenols were observed to be responsible for the antimicrobial potential of the leaves of *Plukenetia conophora*. This study reveals that the leaves of *Plukenetia conophora* possess antimicrobial property and as such can be used as an alternative to conventional antibiotics for the treatment of infections caused by multidrug-resistant *Staphylococcus aureus.*

Keywords: *Resistance, Multidrug, Staphylococcus, Walnut, Extract*

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INTRODUCTION

1n the past decade, the number of antibiotic-resistant bacteria has increased drastically (Ogbolu and Alli, 2012). In recent time, different agents employed in the treatment of infectious diseases cause by antibiotic-resistant bacteria are no longer potent. This called for concern because a wide species of bacteria is becoming resistant to more than one antibiotic (Piddock, 2006; Ogbolu and Alli, 2012). The development of antimicrobial resistance in clinical settings is a growing problem worldwide and it requires international approaches. Antimicrobial resistance (AMR) has been on increase and it has been a very serious challenge in preventing and treatment of infections caused by antibiotic-resistant bacteria (Andy and Okpo, 2018; Prestinaci *et al*., 2015). The problem of AMR is especially urgent to tackle due to the fact that resistance bacteria are on the increase of which *Staphylococcus aureus* is one (Andy and Okpo, 2019).

S. aureus is one of the major pathogens that are found on human skin and mucous membrane. Studies has revealed that 30% of the world's population is colonized by this organism (Salgado *et al*., 2003). S. aureus as a well-known human pathogen have reached epidemic proportions globally (Grundmann *et al*., 2006; Stefani and Goglio, 2010). Despite the fact that it is a normal flora of human, its implicated in various diseases such as mild skin infections (boils and rashes), and life-threatening diseases (Persistent bacteremia, sepsis, and pneumonia) (Tong *et al*., 2015; Kresiel *et al*., 2010). This implies that *S. aureus* silently stays as natural flora and yet sometimes threatens our life as an opportunistic pathogen. The pathogenicity of S. aureus is attributed to virulence factors possessed by this organism (Adhesins, coagulase, catalase, etc), and toxins. Its ability to form biofilm and evasion of immune strategies also contributes to its virulence (Foster *et al*., 2014; Otto, 2014). Its multidrug resistance strains have made it to be one of the most prevalence pathogenic bacteria (Hiramatsu *et al*., 2014).

S. aureus is resistant to an array of antibiotics including Cephalosporins, Penicillins, Carbapenems (making the Beta-lactam armamentarium clinically ineffective) (Turner *et al*.,

2019), Linezolid (Flamms *et al*., 2014), Daptomycin (Cafisco *et al*., 2014; Cavalcante *et al*., 2014) and Vancomycin (Mc Guinness *et al*., 2017; Foster, 2017; CDC, 2002; WHO, 2014). Antibiotic-resistant strains of S. aureus are often more implicated with high morbidity rate and mortality rate compared to its susceptible strains (Fowler *et al*., 2006; Hanberger *et al*., 2011). Studies has revealed that Methicillin-Resistant-Staphylococcus aureus (MRSA) causes more deaths in the US hospitals than HIV/AIDS, viral hepatitis, and tuberculosis in combination (Boucher and Corey, 2008; Klevens *et al*., 2006). MRSA strains has accounted for about 75% infections in some parts of the world (Chen and Huang, 2014; Schaumburg *et al*., 2014; Tokajian, 2014). In addition to the health challenge post by antibiotic-resistant strains of this organism, S. aureus, also imposes an economic burden. (Le *et al*., 2013).

In addition to the multidrug-resistant problem, antibiotics has some kind of effects on the host. (Parekh *et al*., 2005; Ogbolu and Alli, 2012). These triggers the need to find an alternative antimicrobial drug for the treatment of infectious diseases. As a result, there has been growing interest among researchers to develop natural plant products that will be use as new antimicrobial and antioxidants agents (Takon *et al*., 2013; Takon *et al*., 2015).

Of recent, focus has been on plant extracts and biological active ingredient from plant (Parekh *et al*., 2015), and more studies are being carried out on medicinal plants (Enitan *et al*., 2014). This interest in medicinal plants is due to the fact that they are cheap, readily available and accessible by the local populace and due to the fact that synthetic medications have numerous side effects, plant extracts are being considered (Mahesh and Satish, 2008). The efficacies and little or no known side effects and low cost of these plants products as well as their availability and ability make them a drug of choice to succeed where most synthetic and conventional agents have made little progress (Udobi and 0naolopa, 2009; Takon *et al*., 2015). In Nigeria, many medicinal plants are being used to treat diseases and *Plukenetia conophora* is one of those plants (Mahesh and Satish, 2008).

The plant *Plukenetia conophora*, also refers to as Tetracarpidium conophorum or the tropical African walnut (Oyekale *et al*., 2015; Ayodeji and Aliyu, 2018) belongs to the family *Euphorbiaceae* (Enitan *et al*., 2014; Malu *et al*., 2009; Amaeze *et al*., 2011). It is a perennial woody climbing shrub of about 10 – 20 feet long, most commonly found in some parts of Nigeria and in other West African countries like Gabon and Cameroun (Enitan *et al*., 2014) and also in America, Europe, and Asia (Malu *et al*., 2009). The leaves, bark, root, and fruit of Plukenetia conophora have some medicinal properties. (Enitan *et al*., 2014; Amaeze *et al*., 2011). The leaves are used to treat several medical conditions such as constipation, indigestion, dysentery, intestinal worm infection etc (Enitan *et al*., 2014). In most cases, the bark is used as laxative in tea and also studies have revealed that it can be used to control blood pressure, while the root is used for hemorrhoids, frostbite and varicose ulcer (Enitan *et al*., 2014). It has been reported that the African walnut is also used to treat male fertility issues in Southern Nigeria (Amaeze *et al*., 2011; Obianime and Uche, 2010; Ikpeme *et al*., 2014). In Africa, especially in Nigeria, the water extracts of the root provide a soothing beverage for fever and malaria infection and its antibacterial efficacy (Malu *et al*., 2009).

The extracts of *Plukenetia conopora* has shown potential antimicrobial activities against a wide spectrum of bacteria and fungi as demonstrated by Ajaiyeoba and Fadare (2006) (Enitan *et al*., 2014). Also, the leaves extracts have been demonstrated to have a good free radical scavenging activity. Very little is known about the effect of the African walnut on antibiotic-resistant S. aureus. Thus, this research was initiated to know whether African walnut would exert any antimicrobial effect on antibiotic-resistant S. aureus.

MATERIALS AND METHODS

Preparation of extracts: The plant samples (leaves, stem, and root) were dried at room temperature for 2 weeks. The dried samples were crushed with pestle and mortar and then reduced to a fine powder using a grinder. 10g of the dried fine-powdered samples of leaves, running stem, and roots were weighed out separately and placed in conical flasks to which 100 ml of distilled water was then added. The extraction was allowed for 4 days with intermittent shaking at room temperature to obtain the aqueous extract. The same procedure was repeated with ethanol as the solvent to obtain the ethanol extract. Following extraction, filtration was done using sterile Whatman filter paper (No. 1). The filtrates obtained from the filtration procedure were then evaporated in a water bath to remove excess distilled water and ethanol until a solid mass of both the aqueous and ethanol extracts was obtained respectively. These extracts were placed in sterile sample bottles and preserved at -10º C.

Phytochemical screening: The different parts of *Plukenetia conophora* (leaves, running stem, and roots) were subjected to qualitative and quantitative phytochemical screening using methods described by Kebede *et al*., 2021; Takon *et al*., 2015; Malu *et al*., 2009. Alkaloids, tanins, steroids, saponins, flavonoids, phenols, phlobatanins, terpenoids, anthraquinones and glycosides were assayed.

Sterility test: About 2ml of the extracts was introduced into 10 ml of Muller Hinton broth separately and it was incubated for 24 hours at 37ºC. Sterility of the extracts was observed by the absence of turbidity or clearness of the broth after the incubation period. To further confirm the sterility of the extracts, a loopful of tube's content was plated. Absence of growth confirmed extracts' sterility.

Preparation of various concentrations of aqueous and ethanol extracts: Aqueous and ethanolic extracts of the running stem, roots, and leaves were reconstituted in distilled water (for aqueous extracts) and dimethyl sulphoxide (for ethanol extracts) respectively, to obtain various concentrations of the extracts. Thus, 2g of the aqueous extracts (extracts of the running stem, roots, and leaves) were reconstituted in 10ml of distilled water to obtain a 200mg/mL extract solution for the various extracts obtained from different parts of the plant. Lower concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, and 6.25mg/ml of the extracts was obtained using double dilution method. The same procedure was repeated for

ethanol extracts of the various plant parts with dimethyl sulphoxide as the solvent.

Isolation and purification: Three hundred (350) wound swabs samples were obtained from the diagnostic laboratory of Medical Microbiology and Parasitology Unit of the University of Calabar Teaching Hospital were dipped in sterile peptone water in test tubes to dislodge and activate the organisms collected on them.0.1ml of the peptone water in to which the different wound swabs were separately dipped was measured out and inoculated on sterile mannitol salt agar media in labeled Petri dishes using the spread plate technique. Distinct yellow colonies observed on the inoculated media after the period of incubation (24 hours) were subcultured in mannitol salt media to obtain distinct, pure colonies of the bacteria of interest and for easy identification of the isolates morphologically.

Characterization and identification of isolates: The subcultured colonies were observed and their morphological characteristics such as colony form (shape and size), colour of colonies, edge of the colonies, colony texture and consistency, elevation of the colony, and biological characteristics such as a change in colour of the media were studied. The purified isolates were characterized by Gram staining and various biochemical tests.

Antibiotic susceptibility test: Following the standardization of the inoculum in nutrient broth and adjustment of the turbidity to 0.5 McFarland standard, the antibiotic susceptibility testing was done using the Kirby Bauer's disc diffusion method (Bauer *et al*., 1966)

Antimicrobial activity screening test: Different strains of antibiotic resistant Staphylococcus aureus were subjected to antimicrobial activity testing with the plant extracts using agar well diffusion method as described by Ayodeji and Aliyu, 2018; Enitan *et al*., 2014; Udobi and Onaolopa, 2009.

Comparison of the antimicrobial activity of various concentrations of the leaf extracts: Concentration of 100mg/ml of the leaf extract was prepared by dissolving 0.1g of the aqueous and ethanol extracts in 1ml of sterile distilled water and dimethyl sulphoxide respectively. Another 100mg/ml concentration of the aqueous and ethanol leaf extracts was made and 1ml of Mueller Hinton broth was added to it to give a concentration of 50mg/ml. Same procedure was carried out obtain concentrations of 25mg/ml, 12.5mg/ml, and 6.125mg/ml of the aqueous and ethanol extracts. The surfaces of sterile Mueller Hinton agar in Petri dishes were uniformly seeded with a standardized suspension of the bacterial inoculums. 6mm holes were bored in the seeded agar using a standardized stem borer. The Petri dishes were appropriately labeled. 0.1ml of the various concentrations of the aqueous and ethanol extracts of the leaves were introduced separately into the bored holes using sterile syringes. Equal volumes of the solvents (sterile water and dimethyl sulphoxide) were also measured into the holes to serve as negative controls. The entire procedure was repeated for 9 more isolates and the plates were incubated for 24 hours at 37ºC.

Determination of MIC and MBC: Plant extracts that showed potent antibacterial effect on the test isolate during the susceptibility testing were further subjected to MIC and MBC determination using standard protocol and procedure obtained from CLSI guideline with slight modification according to method of Weiegand *et al*., 2008; Kebede *et al*., 2021; Wikler *et al*., 2011. Test was carried out in triplicates with Nitofurantoin (30µg) as a positive control**.**

RESULTS

The cultural, morphological, and biochemical characteristics presented by the test isolates are summarized in Table 1. The test isolates were found to grow on mannitol salt agar producing yellow colonies with a change in colour of the media from red to yellow due to fermentation of the sugar (mannitol) resulting in acid production which reduces the pH of the media.

The Antibiotic Sensitivity of the different Staphylococcus aureus isolates, isolated from clinical samples were assayed using the Kirby Bauer disc diffusion method. A total of eight antibiotics were used against the different Staphylococcus aureus isolates. Their effects shown by zones of clearance were measured in millimeters and diameters of these zones were compared with the Clinical Laboratory Standard Institute (CLSI) standard so as to determine whether the isolates were resistant, intermediate or susceptible to the antibiotics used. The result of the antibiotic susceptibility test is presented in Table 2.

Table 1
Cultural, morphological and biochemical characterization of isolates

Isolate code	Colony shape	Margin	Surface	Elevation	Colony pigment	Cell shape	Cells arrangement	Gram reaction	Catalase	Coagulase	Citrate	Oxidase	Sugar utilization (M, G, S, L)	H_2S production	Motility	Indole	Methyl red	Voges Proskauer
WS-1 T0 WS-2	Circular	Entire	Smooth	Convex	Yellow	Cocci	Grape-like clusters	+	+	+	+	-	+	-	-	-	+	+

KEY: M - Mannitol, G - Glucose, L - Lactose , S - Sucrose, + = Positive , - = Negative

Table 2:
Antibiotic susceptibility test result of identified isolates

Antibiotics	Conc. (µg)	CLSI standard			Zones of inhibition for ten *Staphylococcus aureus* isolates (mm)									
					WS1	WS2	WS3	WS4	WS5	WS6	WS7	WS8	WS9	WS10
		R	I	S										
CAZ	30	≤14	15-17	≥18	0	0	0	0	0	0	0	0	0	0
CRX	30	≤14	15-17	≥18	0	0	0	0	0	0	0	0	0	0
GEN	10	≤12	13-14	≥15	0	0	11	0	0	0	0	15	0	0
CXM	5	≤15	16-18	≥19	0	0	0	0	0	0	0	0	0	0
OFL	5	≤14	15-17	≥18	0	0	12	0	0	0	0	11	0	0
AUG	30	≤19	-	≥20	0	0	0	0	0	0	0	0	0	0
NIT	300	≤14	15-16	≥17	12	9	20	15	8	8	9	10	11	12
CPR	5	≤15	16-20	≥21	0	0	10	0	0	0	0	0	0	0

KEY: **CAZ – Ceftazidime, CRX – Cefuroxime, GEN – Gentamicin, CXM – Cefixime, OFL – Ofloxacin, AUG – Augmentin, NIT – Nitrofurantoin, CPR – Ciprofloxacin, 0 –** No inhibition zone, **WS –** Wound swab, **Conc. –** Concentration**, CLSI –** Clinical Laboratory Standard Institute, **R –** Resistant, **S –** Sensitive, **I –** Intermediate, **mm –** Millimete

Table 3:
Antimicrobial activity screening of the various extracts of the different parts of the plant

S. aureus Isolates	Ethanol Extracts (100mg/ml)(mm)			Aqueous Extracts (100mg/ml)(mm)		
	Leaves	Stem	Roots	Leaves	Stem	Roots
WS1	19	0	0	16	0	0
WS2	18	0	0	16	0	0
WS3	22	0	0	20	0	0
WS4	20	0	0	15	0	0
WS5	16	0	0	16	0	0
WS6	17	0	0	15	0	0
WS7	15	0	0	12	0	0
WS8	20	0	0	19	0	0
WS9	20	0	0	18	0	0
WS10	18	0	0	18	0	0

KEYS: 0 – No inhibition Zone, WS - Wound Swab

The result of the antimicrobial activity screening of the various extracts of the different parts of Plukenetia conophora (stem, root and leaves) is presented in Table 3. Both the aqueous and ethanol extracts of the different parts (stem, roots and leaves) of the plant, Plukenetia conophora were assayed for antimicrobial properties on separate antibiotic resistant Staphylococcus aureus isolates. No zones of inhibition were observed for the aqueous and ethanol extracts of both the stem and roots. However, zones of inhibition were observed for both the aqueous and ethanol extracts of the leaves of the plant. The various zones of inhibition observed were measured. The result of the assay is shown in Table 3.

The antimicrobial activity of various concentrations of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml of both aqueous and ethanol extracts of the leaves were compared. The various concentrations of ethanol extract were observed to have higher antimicrobial activity when compared with the various concentrations of the aqueous extract as showed by wider zones of inhibition. This is presented in two tables; Table 4 and Table 5.

Table 4:
Effects of different concentrations of ethanol leaf extract

Isolates	Different Concentrations of the Extract (mg/ml)			
	100	50	25	12.5
WS1	19	16	16	15
WS2	18	17	16	10
WS3	22	18	18	14
WS4	20	20	16	15
WS5	16	16	14	14
WS6	17	13	13	11
WS7	15	13	10	11
WS8	20	18	17	15
WS9	20	19	18	15
WS10	18	15	13	13

KEY: WS - Wound Swab

Table 5
Effects of different concentrations of aqueous leaf extract

Isolates	Different Concentrations of the Extract (mg/ml)			
	100	50	25	12.5
WS1	16	15	15	13
WS2	16	16	14	12
WS3	15	15	14	14
WS4	15	15	12	11
WS5	16	14	14	14
WS6	15	13	13	12
WS7	12	10	10	9
WS8	17	15	14	12
WS9	18	15	15	13
WS10	18	16	14	14

KEY: WS- Wound Swab

Table 6:
Qualitative phytochemical analysis of the different parts (stem, roots, and leaves) of *Plukenetia conophora*

PHYTOCHEMICALS	ROOTS	STEM	LEAVES
Alkaloids	+	+	+
Phenols	-	+	+
Saponins	-	-	-
Steroids	+	-	+
Glycosides	-	-	-
Anthraquinones	-	-	-
Tannins	-	+	+
Phlobatannins	-	-	-
Flavonoids	-	+	+
Terpenoids	+	+	+

KEY: + = Detected, - = Not detected

Different parts of the plant, Plukenetia conophora were analysed to determine the presence of some phytochemicals. The leaves were observed to possess some phytochemicals that are absent in stem and roots. Result of the qualitative phytochemical analysis of the stem, roots and leaves is presented in Table 6.

The phytochemicals found in the different parts (running stem, roots and leaves) of the plant, Plukenetia conophora were quantified using Ultraviolet-Visible absorption spectroscopy. The result of the quantitative analysis is presented in Table 7. The antimicrobial assay of the various phytochemicals extracted from the leaves of the plant, Plukenetia conorphoa showed that steroids and terpenoids exhibited no antimicrobial activity while alkaloids, tannins, phenols, flavonoids exhibited antimicrobial activity as shown by the zones of clearance around the agar well into which they were introduced. The result is presented in table 8.

Table 7:
Quantitative phytochemical analysis of the different parts (stem, roots, and leaves) of *Plukenetia conophora*

PHYTOCHEMICALS	LEAVES (%)	STEM (%)	ROOTS (%)
Alkaloids	30	10	20
Flavonoids	20	40	-
Terpenoids	3	11	9
Tennins	4.3	7.6	-
Steroids	8.1	-	14.2
Phenols	8	6	-

KEY: - = Not detected

The antimicrobial activity of various concentrations of the aqueous and ethanol extracts were compared with Nitrofurantoin- an antibiotic. The comparison was done with measured diameters of the zones of inhibitions produced by the various concentrations of the aqueous and ethanol extracts and those produced by the antibiotic (Nitrofurantoin) when used separately on the test isolates. The results of this comparison are presented in Figures 1, 2, 3, 4, 5, 6, 7 and 8.

DISCUSSION

The efficacy *Plukenetia conophora* as an antimicrobial agent was evaluated against multidrug-resistant Staphylococcus aureus. The different parts of the plant tested for antimicrobial activity were the running stem, roots, and leaves. The nuts were not assayed for antimicrobial properties because of the seasonality of the nut and its resultant unavailability during the period of this study.

Table 8
Antimicrobial activity of the different phytochemicals in the leaves of *Plukenetia conophora*

Phytochemicals	Zone of inhibition (mm) of antibiotic-resistant *Staphylococcus aureus* isolates									
	WS-1	WS-2	WS-3	WS-4	WS-5	WS-6	WS-7	WS-8	WS-9	WS-10
ALKALOIDS	19	11	15	15	17	21	15	16	17	20
FLAVONOIDS	15	9	14	14	14	15	14	15	13	19
PHENOLS	12	15	11	13	15	13	12	11	13	13
STEROIDS	0	0	0	0	0	0		0	0	0
TANNINS	14	19	16	15	17	16	14	14	14	19
TERPENOIDS	0	0	0	0	0	0	0	0	0	0

WS - Wound Swab ; 0 – No inhibition Zone

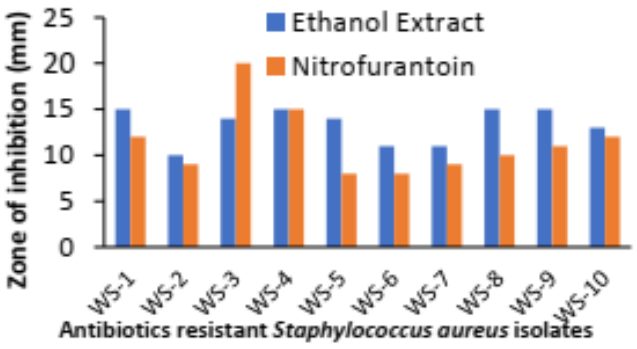

Figure 1:
Comparison of 12.5mg/ml concentration of ethanol leaf extract with Nitrofurantoin 300µg

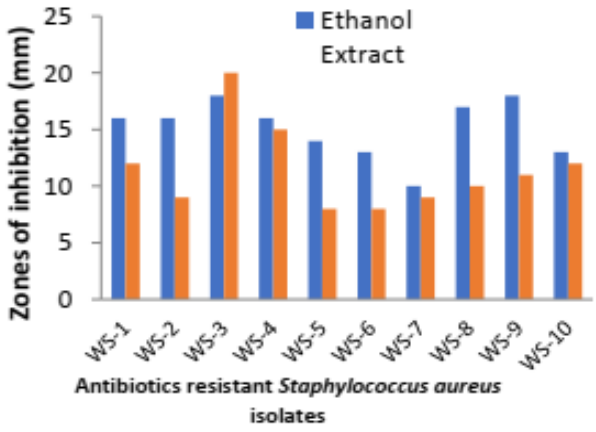

Figure 2:
Comparison of 25 mg/ml concentration of ethanol leaf extract with Nitrofurantoin 300µg

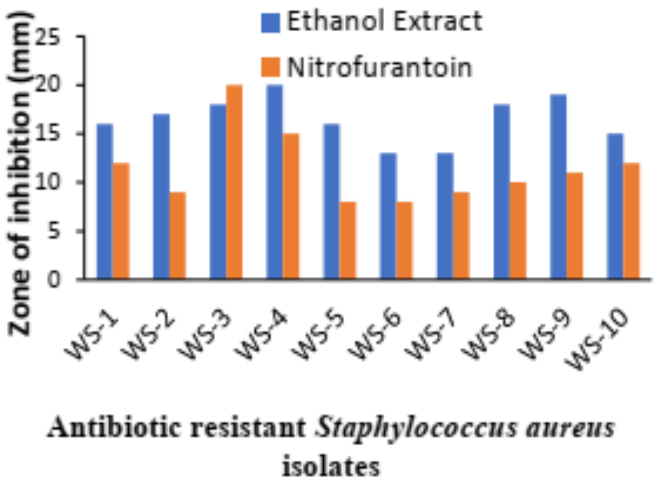

Figure 3:
Comparison of 50 mg/ml concentration of ethanol leaf extract with Nitrofurantoin 300µg

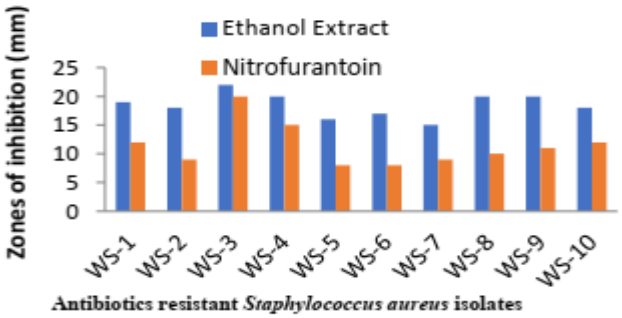

Figure 4:
Comparison of 100 mg/ml concentration of ethanol leaf extract with Nitrofurantoin 300µg

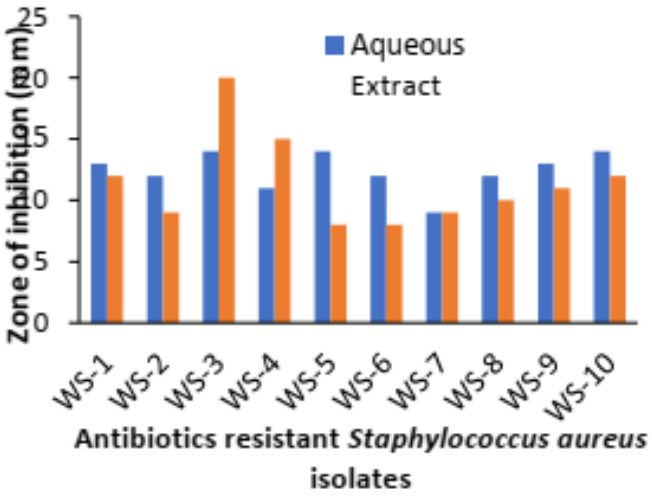

Figure 5:
Comparison of 12.5mg/ml concentration of aqueous leaf extract with Nitrofurantoin 300µg

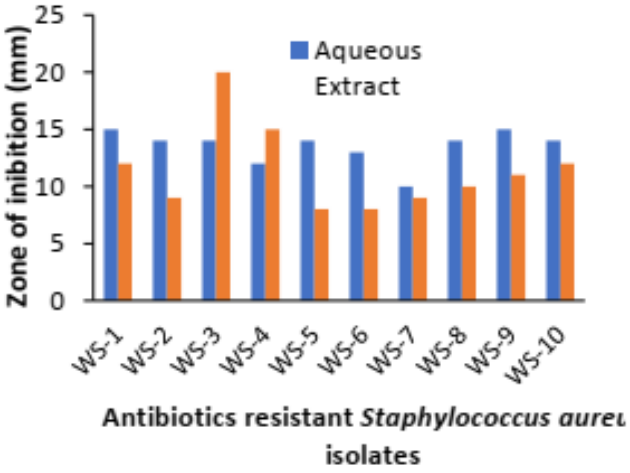

Figure 6:
Comparison of 25 mg/ml concentration of aqueous leaf extract with Nitrofurantoin 300µg

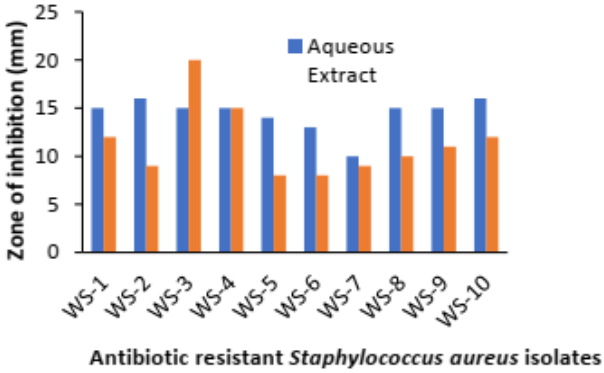

Figure 7:
Comparison of 50 mg/ml concentration of aqueous leaf extract with Nitrofurantoin 300µg

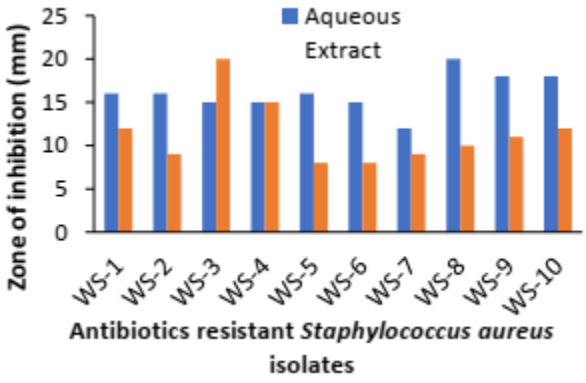

Figure 8:
Comparison of 100 mg/ml concentration of aqueous leaf extract with Nitrofurantoin 300µg

A total of sixteen clinical specimens (wound swabs and eye swabs) were obtained from the Microbiology and Parasitology unit of the University of Calabar Teaching hospital. Ten Staphylococcus aureus pure colonies were isolated from ten clinical samples out of the sixteen clinical samples collected. The test isolates were found to grow on mannitol salt agar producing yellow colonies with a change in

colour of the media from red to yellow due to fermentation of the sugar (mannitol) resulting in acid production which reduces the pH of the media.

From the antibiotic susceptibility test result obtained in this study, only one of the isolates of *Staphylococcus aureus* (WS-3) was found to be susceptible to Nitrofurantoin (NIT) but resistant to the rest of the antibiotics used. Also, Nitrofurantoin was observed to have an intermediary effect on isolate WS- 4. The isolate (WS- 4) however was observed to be resistant to the rest of the antibiotics used. This reveals the widespread occurrence and distribution of antibiotic-resistant strains of *Staphylococcus aureus* in medical settings (Chambers and Deleo, 2009; Boucher and Corey, 2008). This observation corroborates with that of Garoy *et al*., (2019) who reported the increase in the occurrence of antibiotic-resistant Staphylococcus aureus in two referral hospitals in Asmara. The antibiotic-resistant profile of these isolates also suggests the indiscriminate use of the antibiotics tested and more. In this study, the aqueous and ethanol extracts of the running stem, roots, and leaves were extracted by maceration. Both the aqueous and ethanol extracts of the different parts (stem, roots, and leaves) of the plant, *Plukenetia conophora*, were assayed for antimicrobial properties on separate antibiotic-resistant *Staphylococcus aureus* isolates. No zone of inhibition was observed for the aqueous and ethanol extracts of both the stem and roots.

The various zones of inhibition observed in the aqueous and ethanolic extract were measured. The result of the assay is presented in Table 3. This result is however similar to reports made by a study conducted by Ogbolu and Alli (2012). Also, this observation is contradictory to the findings made by Ajayeoba and Fadare (2006) as reports from their research recorded antimicrobial properties against Staphylococcus aureus in the roots and stem of the plant, *Plukenetia conophora.* However, the strain of the Staphylococcus aureus on which the ethanol extracts of the roots and stem had been tested may not have been antibiotic-resistant; as it was not stated in the report. On the other hand, both the aqueous and ethanol extracts of the leaves of the plant, *Plukenetia conophora* were observed to possess antimicrobial properties on the test isolates as shown by the zones of clearance observed around the agar wells, to which a particular concentration (100mg/ml) was introduced. This conforms to the study carried out by Ajayeoba and Fadare (2006) and is also in accordance with the study carried out by Bello *et al*., (2013) where the leaves were reported to possess antimicrobial activity. This result is however contradictory to the study carried out by Ogbolu and Alli (2012) where the methanol and ethanol extracts of the leaves were reported to be void of antimicrobial properties.

When assayed, the antimicrobial potential of the aqueous and ethanol extracts of the leaves was found to increase with the increase in concentration. In other words, the aqueous and ethanol extracts of the leaves displayed concentration-dependent antimicrobial activity. This observation is in agreement with the study carried out by Enitan *et al*., (2014). This was demonstrated by an increase in zone diameter with an increase in concentration. Apart from the increase of antimicrobial activity of the extracts with an increase in concentration, worthy of note is the greater antimicrobial activity expressed by the ethanol extracts when compared with the aqueous extract. The antimicrobial activity of various concentrations of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml of both aqueous and ethanol extracts of the leaves were compared. The various concentrations of ethanol extract were observed to have higher antimicrobial activity when compared with the various concentrations of the aqueous extract as showed by wider zones of inhibition as presented in two tables; Table 4 and Table 5. This observation is harmonious with findings made by Uthayarasa *et al*., (2010) and Gberikon *et al*., (2015) that ethanol extracts show higher antimicrobial activity than aqueous extracts because ethanol can extract more of the phytochemicals that will inhibit the growth of bacteria as opposed to aqueous solutions.

The antimicrobial activity of various concentrations of the aqueous and ethanol extracts were compared with Nitrofurantoin- an antibiotic. The comparison was done with measured diameters of the zones of inhibitions produced by the various concentrations and those produced by the antibiotic (Nitrofurantoin) when used separately on the test isolates. The results of this comparison are presented in Figures 1, 2, 3, 4, 5, 6, 7, and 8.

Comparison of antibacterial activity of the various concentrations of aqueous and ethanol extracts of the leaves revealed that the ethanol extracts had a greater antimicrobial effect than the aqueous extract as demonstrated by the zones of inhibitions exhibited by various concentrations of both extracts. The various concentrations of the ethanol extract had larger inhibition zones when compared with their aqueous counterpart.

Qualitative phytochemical analysis of the running stem, roots, and leaves of *Plukenetia conophora* was carried out to test for the presence of flavonoids, alkaloids, phenols, tannins, terpenoids, steroids, anthraquinones, saponins, glycosides, and phlobatannins. Different parts of the plant, *Plukenetia conophora* were analysed to determine the presence of some phytochemicals. The leaves were observed to possess some phytochemicals that are absent in stem and roots. The result of the qualitative phytochemical analysis of the stem, roots, and leaves is presented in Table 6. The leaves were found to contain the highest number of phytochemicals tested for (six); closely followed by the stem (five) and then the roots (three). The qualitative phytochemical analysis revealed the presence of alkaloids, phenols, steroids, terpenoids, tannins, and flavonoids in the leaves. Alkaloids, phenols, tannins, flavonoids, and terpenoids were the phytochemicals found present in the stem of the plant, and alkaloids, steroids, and terpenoids were found in the roots. The analysis further revealed that only two (alkaloids and terpenoids), out of the ten phytochemicals qualitatively analyzed, were present in all three parts of the plant (root, stem, and leaves). Though it is difficult to determine the mechanism of actions of the bioactivity of the leaf extract of the study plant (*Plukenetia conophora*), it is possible to say that the leaves have antibacterial activity based on phytochemicals found during the phytochemical screening of the leaves. Although some of these phytochemicals associated with antimicrobial activity are present in the stem of *Plukenetia conorphora*, the absence of antimicrobial activity may be due to lesser amounts of the phytochemicals in the stem.

The phytochemicals found in the different parts (running stem, roots, and leaves) of the plant, *Plukenetia conophora* were quantified using Ultraviolet-Visible Absorption Spectroscopy. The result of the quantitative analysis is presented in Table 7. This was carried out to determine the percentage composition of the various phytochemicals found in them (stem, roots, and leaves). For alkaloids and terpenoids (the phytochemicals found in all the plant parts analyzed), the percentage composition of alkaloids was found to be highest in the leaves and smallest in the stem while the percentage composition of terpenoids was found to be highest in the stem and smallest in the leaves. The stem was found to have a higher percentage composition of flavonoids (40%) when compared with the percentage composition of flavonoids found in the leaves (20%). The percentage composition of tannins was slightly higher in the stem (7.6%) than in the leaves (4.3%) of the plant. The antimicrobial assay of the various phytochemicals extracted from the leaves of the plant, *Plukenetia conorphoa* showed that steroids and terpenoids exhibited no antimicrobial activity while alkaloids, tannins, phenols, flavonoids exhibited antimicrobial activity as shown by the zones of clearance around the agar well into which they were introduced. The result is presented in Table 8.

The different concentrations of the aqueous and ethanol extracts of the leaves were prepared and inoculated with appropriate volume of inoculums to determine the minimum inhibitory concentration (MIC) of the extracts. Growth of inoculum was observed by a change in colour of the dissolved extracts. The growth of microorganisms changed the colour of the dissolved extracts as compared with the control. The lowest dilution of the extracts that inhibited the growth but did not kill the organism was defined as MIC. The minimum inhibitory concentration of both the aqueous and ethanol extracts of the leaves was found to range between 25mg/ml and 50mg/ml. The broth dilution tubes containing 25mg/ml and 50mg/ml concentration of the extracts showed no visible turbidity or change in colour of the dissolved extract when inoculated with the test isolates. Thus, 25mg/ml concentration of both the aqueous and ethanol leaf extracts were found to be the MIC. However, when loopfuls from the tubes containing these concentrations of the extract and the inoculum were plated on sterile nutrient agar media, growth was observed to develop on the media.

The minimum bactericidal concentration (MBC) of both the aqueous and ethanol extracts was found to be 100mg/ml. No turbidity or change in colour of the dissolved extract of 100mg/ml concentration was observed upon inoculation with the test isolates and incubation. Loopfuls from tubes containing this concentration of the extracts (100mg/ml) gave no growth on sterile nutrient agar after the period of incubation for both the aqueous and ethanol extracts. Thus, the aqueous and ethanol extracts of the leaves of *Plukenetia conophora* were found to be bactericidal on antibiotic-resistant Staphylococcus aureus at 100mg/ml concentration.

In conclusion, the evaluation of the antimicrobial efficacy of the different parts of the African walnut plant (*Plukenetia conophora*) on antibiotic-resistant Staphylococcus aureus revealed that only the leaves of the plant produced in vitro antimicrobial activity against the test organism. The stem and the roots of the plant, however, showed no in vitro antimicrobial effect on antibiotic-resistant Staphylococcus aureus from the results obtained. Results of the comparative antimicrobial efficacy of the extracts of the leaves further revealed that the ethanol extract of the leaves of the plant has higher antimicrobial potential than the aqueous extract; thus, showing that the solvent employed in the extraction process has a significant role in antimicrobial efficacy of the extracts. Flavonoids, alkaloids, phenols, and tannins are phytochemicals present in the leaf of the plant, suggested to give it its antimicrobial property. Increased antimicrobial activity with an increase in the concentration of the extracts shows concentration-dependency of the leaf extracts. Comparison of the antimicrobial efficacy of varying concentrations of the leaf extracts with Nitrofurantoin 300µg (an antibiotic) portrayed the leaf extracts as better antimicrobial agents. The data on the distribution and occurrence of antibiotic-resistant S. aureus suggests that there has been an indiscriminate use of the antibiotics tested in the study area.

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