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Phytochemical Screening and Antibacterial Evaluation of *Randia acuminata* Root Extract against Wound Pathogens

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Authors' contributions

This work was carried out in collaboration between all authors. Author OUMJ designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors ANU and SIE managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the present study was to determine common pathogens associated with infected wounds and the phytochemical composition and antibacterial activities of *Randia acuminata* root extract against isolates associated with infected wounds.
Study Design: The ethanolic extract was used for the phytochemical screening of the root extract. The disc diffusion method was used to assess the antibacterial activities of the alkaloid fraction of the root extract against isolated pathogens.
Place and Duration of Study: The study was carried out at the University of Uyo Teaching Hospital (Microbiology Laboratory) and Department of Microbiology (Microbiology Laboratory), University of Uyo, Uyo, Akwa Ibom State, Nigeria between the months of April, 2017 – August, 2017.
Methodology: The isolation of infective agents from wound specimens and the phytochemical screening of the root extract were performed using standard methods. The disc diffusion method was used to evaluate the antibacterial activities of the alkaloidal fraction of the root extract on

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Staphylococcus aureus and species of *Streptococcus, Pseudomonas, Klebsiella* and *Clostridium*.
Results: Alkaloid, saponins, phlabotanin, anthraquinone deoxysugar, cardiac glycoside and terpenes constitute the bioactive components of the root extract. The pathogens associated with infected wounds and their frequency of occurrence were *Staphylococcus aureus* (35%) and species of *Streptococcus* (15%)*, Pseudomonas* (20%) *Klebsiella* (20%) and *Clostridium* (10%). Susceptibility of the organisms to the root extract varied with concentration 30 < 40 < 50mg/ml The antibacterial activity of the extract suggests concentration-dependent inhibitory response by the test organisms.
Conclusion: The results of this study indicate that the alkaloid fractions of *R. acuminata* root extract has potent antibacterial effect and can be incorporated into antiseptics for wound cleaning/dressing.

Keywords: Antibacterial; alkaloid fraction; concentration-dependent; inhibitory response; wound pathogens.

1. INTRODUCTION

Wound contaminants are likely to originate from three main sources: the environment, surrounding skin and endogenous sources involving mucous membranes [1]. Infection occurs when virulence factors expressed by one or more microorganisms in a wound out-competes the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provokes a series of local and systemic host responses [2]. The progression of a wound to an infected state involve a multitude of microbial and host factors including the type, site, size, and depth of the wound, the extent of nonviable exogenous contamination, the level of blood perfusion to the wound, the general health and immune status of the host, the microbial load, and the combined level of virulence expressed by the types of microorganisms involved. Most acute and chronic wound infections involve mixed populations of both aerobic and anaerobic microorganisms [1,3]. The increasing incidence of multidrug resistance of human pathogenic microorganisms in recent years and the side effect of antibiotics on the host, have raised concern on the need for new antimicrobial compounds with novel mechanism of action to combat re-emerging infectious diseases [4,5]. This globally developing scenario has given rise to a constant and innovative search for new and effective antimicrobial agents from various sources including plants with medicinal properties [6,7,8,9,10]. *Randia acuminata*, a tropical plant found usually in moist forests, distributed in Sierra Leone, Zaire, Nigeria, other parts of Africa and the world at large, is one among many plants with varying medicinal values. In Nigeria, the plant is known by different names such as 'Atu ubie' (Igbo), 'Pako ijebu' (Yoruba), and 'Okok edi' (Ibibio) [11]. Notably, parts of the plant such as the stems are used as chewing sticks in Southern and western Nigeria, whereas the root extract is employed as an enema for dysentery and as an aphrodisiac. The pulped leaves are used externally as ligaments for lumbago and muscular pains, the juice from the incised fruits are used as an eye drop in Sierra Leone while the stem is believed to be an aphrodisiac [12]. As a result of its ethno-medical applications and foaming properties, we evaluated the effect of the alkaloid fraction from *Randia acuminata* root extract against bacteria associated with wound infections.

2. METHODOLOGY

2.1 Collection and Identification of Plant

Fresh *Randia acuminata* plant of ten years and four months (10.4 yrs.) whose root each weighed an average of 22.4 g was collected during the onset of wet season from Uyo local government area of Akwa Ibom state, identified at the Department of Botany and Ecological studies and transported to the Pharmacognosy Laboratory, University of Uyo, Uyo, for phytochemical extraction and fractionation.

2.2 Processing, Extraction and Phytochemical Screening of *Randia acuminata* Root

The root of *Randia acuminata* was cut into tiny bits and air-dried at room temperature and milled into powdery form to increase the surface area.

150 g of the plant powdered part was exhaustively cold extracted using 50% ethanol for 72 hours. The liquid extract was filtered and the filtrate concentrated at 40°C using a rotatory evaporator as described and adopted by Tedwins et al. [13]. The concentrated extract was transferred into empty beakers and weighed to determine the weight of the individual extracts.

The phytochemical screening of the extract was performed according to the methods described in Trease and Evans Pharmacognosy [14].

2.3 Fractionation

Fractionation was carried out following the procedures described in Trease and Evans Pharmacognosy [14]

Acid-base partitioning was carried out by dissolving the concentrated ethanol extract in 5% Hydrochloric acid (HCl) and shaken with Trichloromethane ($CHCl_3$). The aqueous fraction was neutralized with Ammonium Hydroxide (NH_4OH) and shaken with $CHCl_3$ to obtain crude alkaloidal fraction. The alkaloidal fraction was purified by chromatographing it on silica gel column (60-120 mesh size, 23 x 75 cm) and eluted with a gradient of n-Hexane with ethyl acetate (9:1), ethyl acetate with methanol (9:1). The fractions collected in 25 ml test tubes were pooled together on the basis of their Thin Layer Chromatography (TLC) characteristics (silica gel G254, ethyl acetate) under the UV and the Dragendroff‟s spray reagent. [14].

2.4 Source and Identification of Clinical Isolates

The clinical isolates were obtained by culturing wound specimens obtained from patients with established infected wound cases at the University of Uyo Teaching Hospital, Uyo. The wound samples were collected using sterile swabs and inoculated by direct streaking on blood agar and Reinforced Clostridial agar [15]. Inoculated plates were incubated at 37°C for 24 hours aerobically (blood agar) and anaerobically (Clostridial agar) using an anaerobic jar with gaspak. Discrete colonies were subcultured by streaking on freshly prepared Nutrient agar plates. The inoculated plates were incubated at 37°C for 24 hours. The bacterial isolates were characterized based on their colonial morphology, microscope appearance and biochemical characteristics using standard procedures [15,16]. Pure isolates were preserved on agar slants in MacCartney bottles as stock cultures in the refrigerator at 4 ± 0.2°C until needed for use.

2.5 Susceptibility Test Using Standard Antibiotics

The susceptibility test was performed using the National Committee for Clinical Laboratory Standards (NCCLS) modified Kirby- Bauer disc diffusion technique as described by Cheesbrough [15]. The antibiotic test was carried out to detect organisms that were sensitive or resistant to standard antibiotics (positive control). Mueller Hinton agar was prepared, poured into sterile petri dishes and allowed to set. Test organisms from an overnight culture were inoculated into 0.5% of normal saline and incubated for 2 to- 4 hours at 37°C. The turbidity of the bacterial suspension was compared and adjusted to the turbidity of the McFarland standard as described by Cheesbrough [15] (bacterial suspension was diluted to an optical density of 0.1 at optical activity of 625nm). The bacterial suspension (10^6 cfu/ml) was inoculated on the prepared culture medium using a sterile swab (equivalent of spread plating with 0.1ml of bacterial suspension using calibrated syringe, and spread using a sterile spreader) Antimicrobial disc(Abtek Biologicals) containing antibiotics(antibiotics with standard zones of inhibition in the order; 0-8mm = Resistant,9 – 14mm = Intermediate,15mm and above = Sensitive) was placed on the surface of the plates containing the test organisms. Each round end of the disc was pressed down to touch the surface of the media. The plates were incubated at 37°C for 24 hours. After incubation, the plates were observed and zones of inhibition around the discs measured [15].

2.6 Antimicrobial Assay of the Extract

The NCCLS modified Kirby- Bauer disc diffusion technique as applicable to the susceptibility test using standard antibiotics was used to test the antimicrobial activity of the extract [15]. Nutrient broth was prepared and sterilized using standard microbiological procedures. The test organisms were inoculated into the nutrient broth and incubated for 18 hours at 37°C. No. 1 Whatman's filter paper was cut into small discs using a perforator as described and adopted by Tedwins et al. [13] and the discs were sterilized at 180°C for 15 minutes in the oven. Mueller Hinton agar was prepared and sterilized using autoclave at 121°C for 15 minutes, poured into sterile petri dishes, allowed to set and labeled. The test organisms from the 18 hour broth culture prepared according to Cheesbrough [15] same as used for testing with standard antibiotics were also inoculated using a similar method as applicable to testing with standard antibiotics [15]. The root extract was measured into 30, 40 and 50 mg/mL concentrations using 50% ethanol as diluent. Using sterile forceps, the discs were

dipped into the extract at various concentrations and placed on the surface of the Mueller Hinton agar plate. This was labeled properly and incubated at 37°C for 24 hours. Negative control was also set up using the sterile small discs made from the filter paper soaked only in ethanol of same concentrations as extracts. The results obtained for experiments using test extract and negative control (disc soaked only in ethanol) were normalized to combat any likely effect ethanol as a diluent may have on the test organisms.

2.7 Statistical Analysis

The results were computed statistically using statistical package for social sciences (SPSS) software package version 17. The frequency distribution values of the isolates were expressed as percentage while other values are expressed as mean ± standard deviation. All experiments were carried out in triplicates.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Phytochemical composition of the root extract

Table 1. shows the phytochemical analysis of *R. acuminata* root extract. The phytochemical screening of *Randia acuminata* root extract revealed the presence of bioactive components such as Alkaloid, Saponins, Phlabotannin, Anthraquinone, Deoxysugar, Cardiac glycoside and Terpenes.

3.2 Microorganisms associated with infected wounds

The microorganisms associated with the assessed infected wounds and their occurrence include *Staphylococcus aureus* 49 (35%), *Streptococcus pyogenes* 21 (15%), *Pseudomonas aeruginosa* 28 (20%), *Klebsiella pneumonia* 28 (20%) and *Clostridium difficile* 14 (10%). This is presented in Fig. 1.

3.3 Antimicrobial assay of root extract

Susceptibility of the test organisms to the plant root extract varied and was concentration-dependent (Figs. 2 and 3). However, the 50mg/mL concentration of the extract exerted zone clearing effect on *Streptococcus pyogenes* and *Clostridium difficile* similar to the Levofloxacin.

3.4 Antimicrobial assay using standard antibiotics

Susceptibility test using standard antibiotics showed Levofloxacin and Ciprofloxacin with the highest potency against the Gram positive and Gram negative bacteria respectively (Tables 2 and 3)

3.2 Discussion

The phytochemical screening of *R. acuminata* root extract indicates the strong presence of alkaloids as one of the bioactive component. Alkaloids are pharmacologically active compounds used as local anesthetics, stimulants and analgesics. This bioactive ingredient from other plants is known to act also as bactericidal, anti-cancer, anti-hypertensive, cholinomimerics and spasmolysis, vasodilators, arrhythmic, anti-asthma and anti-malarial agent [7,9,17]. Studies reported by Garba and Okeniyi [18] showed that total alkaloids of plants such as *Jatropha curcas*, *Carica papaya*, *Magnifera indica*, *Calotropis procera* and *Psidium guajava*, showed appreciable level of antimicrobial (broad spectrum) activities against *Staphylococcus aureus* and species of *Streptococcus, Lactobacillus, Actinomycetes* and *Candida albicans* at extract concentration of 6×10^2 µcm^3. The presence of this compound in the root extract therefore indicates potential therapeutic value.

In this study, a total of one hundred and forty bacterial isolates were recovered from ninety wound specimens collected from different patients. The organisms from the wound specimens and the percentage occurrence include *Staphylococcus aureus* (35%), *Streptococcus pyogenes* (15%), *Pseudomonas aeruginosa* (20%), *Klebsiella pneumoniae* (20%) and *Clostridium difficile* (10%). The results of this study indicate that microorganisms associated with the assessed wounds were mixed population of bacteria with the Gram positive bacteria outnumbering the Gram negative (Fig. 1). These organisms have been implicated in bite, burn, traumatic and surgical wound infections [2,19,20]. This results also indicate *Staphylococcus aureus* as the dominant organism (35%) while *Clostridium difficile* revealed the least occurrence (10%). The isolation of *Clostridium difficile* among the recovered microbes, however, indicate this organism as an uncommon bacteria associated with wound infections.

Table 1. Phytochemical properties of *Randia acuminata* root extract (ethanolic extract)

Bioactive components	
Flavonoids	-
Anthraquinone	++
Saponin	++
Terpenes	++
Alkaloid	++
Deoxysugar	+
Tannins	-
Phlabotannin	+
Cardiac glycoside	++

Key: (-) Not detected; (+) Low, (++) High

The results of this study indicate *Staphylococcus aureus* as the most frequently isolated bacterial pathogen and corroborate with studies revealing the profile of automobile accident wound infections [19,20,21,22,23] and surgical site infections [24]. Several reports have also implicated species of *Pseudomonas, Klebsiella* and *Streptococcus* in wound infections [19,21,25]. The dominance of *Staphylococcus aureus* among the pathogens is attributed to their existence as a normal flora of the human body and can thus easily colonize and infect wounds on the body as an opportunistic pathogen. The isolation of *Clostridium difficile* is indicative of wound contamination with soil probably during injury and unhealthy personal health habit of the patients.

The susceptibility profile of the test organisms suggests a wide range of antimicrobial response (Tables 2 and 3). For instance, *Staphylococcus aureus* was resistant to most of the antimicrobial agents (There was no zone of inhibition for Ciprofloxacin, Norflox, Gentamicin, Amoxil, Streptomycin, Ampiclox and Rifampicin except for Erythromycin (21 mm) and Chloramphenicol (22 mm). This level of resistance poses great challenge in the treatment of the assessed wounds and can lead to fatal outcomes. However, the test organisms showed the highest susceptibility to Levofloxacin (22 ± 1 mm) and Ciprofloxacin (20 mm) for Gram positive and Gram negative bacteria respectively. High sensitivity of wound pathogens to ciprofloxacin has been reported [19,21,24,26] as well as high resistance to erythromycin, streptomycin and gentamicin [24].

Alkaloids are pharmacologically active compounds and certain plant alkaloids are medicinally significant [9,17,18]. The Alkaloidal fraction of *Randia acuminata* root extract at 30, 40 and 50 mg/mL concentrations showed significant antibacterial activities against the test organisms (Figs. 2 and 3) The antibacterial activities generally indicated concentration-dependent inhibitory response by the wound pathogens. Least (12 mm) and highest (23 mm) zones of inhibitions were observed at 30 and 50 mg/mL concentrations of the root extract respectively. The antibacterial effect of the alkaloid fraction of the root extract at 50 mg/mL concentration compared favorably with the antimicrobial activities of the standard antibiotics (Levofloxacin and Ciprofloxacin) which served as control (positive control). The results of this study indicate that alkaloidal fractions of

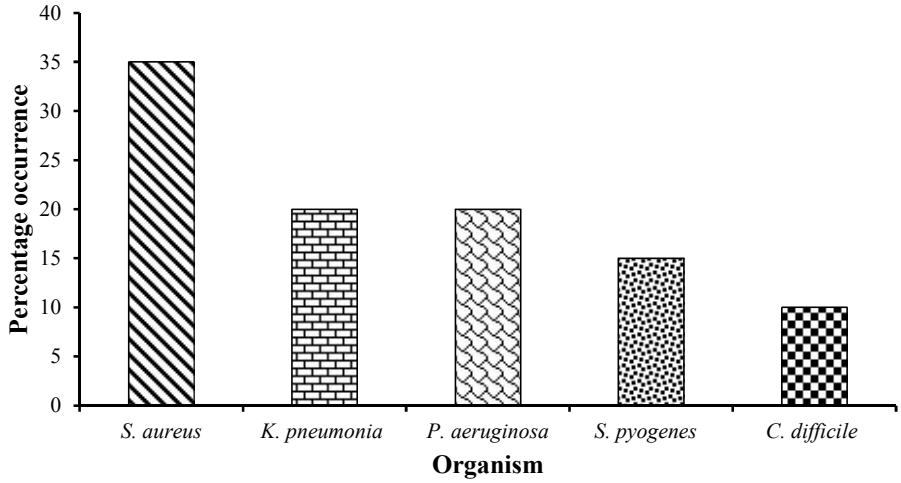

Fig. 1. Percentage occurrence of microorganisms associated with wounds on human subjects (n = 60; human subjects where 90 wound specimens (swabs) were obtained)

Table 2. Susceptibility of the Gram positive microorganisms to different antimicrobial agents

Organisms	Inhibition zone (mm)									
	CPX	**NB**	**CN**	**AMX**	**S**	**RD**	**E**	**CH**	**APX**	**LEV**
Streptococcus pyogenes	21 ± 0.7	0	15 ± 0.7	13 ± 0.5	15 ± 0.2	9 ± 1.4	0	0	0	22 ±0.2
S. aureus	0	0	0	0	0	0	21 ± 1.2	22 ± 0.5	0	23 ± 0.5
Clostridium difficile	0	0	0	0	0	0	20 ± 0.5	16 ± 0.7	0	22 ± 0.7

Values are the mean of triplicate determinations

0 - 8mm = resistant; 9 – 14 mm = Intermediate; 15 mm - above = Sensitive

Key: *CPX-Ciprofloxacin, NB-Norflox, CN - Gentamycin, AMX - Amoxil, S- Streptomycin, RD- Rifampcin, E- Erythromycin, CH - Chloramphenicol,, APX - Ampiclox, LEV- Levofloxacin*

Table 3. Antimicrobial susceptibility of the Gram negative bacteria (Triplicate values)

Organism	Inhibition zone (mm)									
	OFX	**PEF**	**CPX**	**AU**	**CN**	**S**	**CEP**	**NA**	**SXT**	**PN**
Klebsiella pneumoniae	20±0.5	13.5± 0.5	20.5±0.7	0	21± 0.5	17.5± 0.4	0	0	15± 0.5	13±0.5
Pseudomonas aeruginosa	19±0.5	21.5± 0.3	20.5± 0.1	0	0	14±0.6	0	0	16.5±0.9	20.5±0.7

Values are the mean of triplicate deteminations

Key: *0-8 mm = Resistant, 9 - 14 mm = Intermediate, 15 mm - above = Sensitive*

OFX- Tarivid, PEF-Perflacin, CPX - Ciprofloxacin, AU –Augmentin, CN – Gentamycin, S- Streptomycin, CEP – Ceporex, NA – Nalidixic acid, SXT –Septrin, PN – Ampicillin

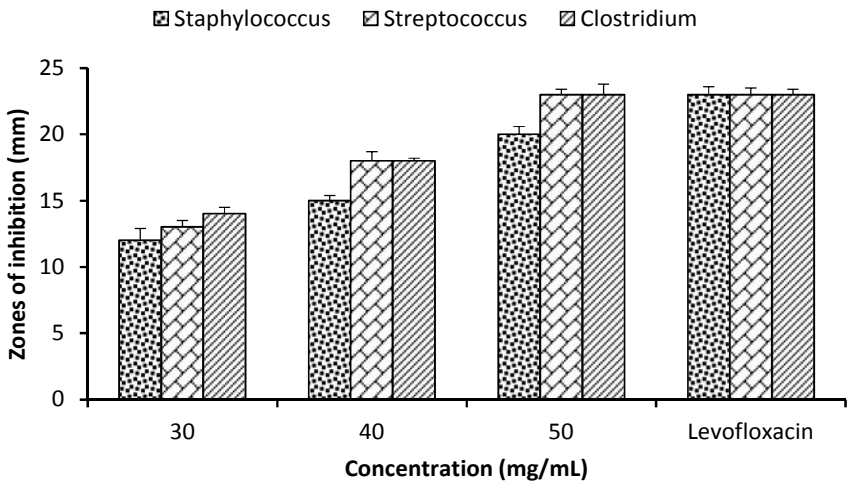

Fig. 2. Response of Gram positive microorganisms to root extract (alkaloid fraction) and standard antimicrobial agent

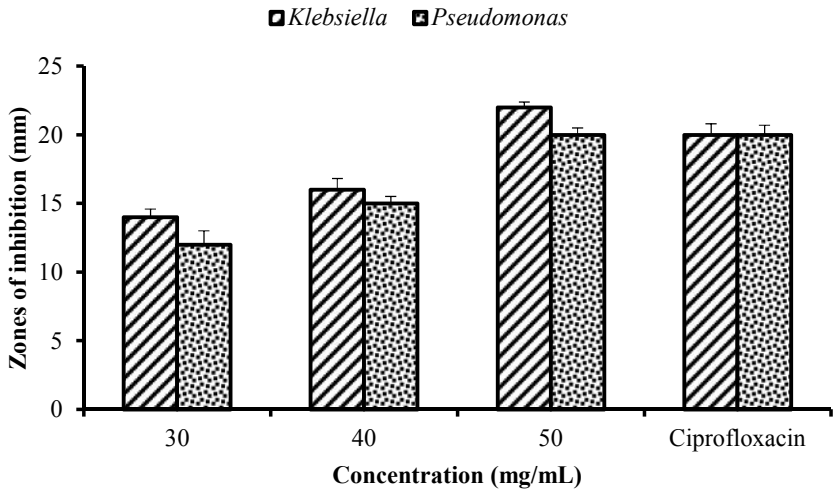

Fig. 3. Response of Gram negative microorganisms to alkaloid fraction of *R. acuminata* root extract

R. acuminata root extract have significant antibacterial activities against wound pathogens. This results corroborates with studies indicating the antibacterial activities of some plants used as local chewing sticks such as *Salvadora persica* (arak tree), *Azadirachta indica* (Neem), *Garcivna kola*, *Anogeissus leiocarpus, Vitex donana, Terminalia glaucescens* and *Sorindela warnackei* against oral pathogens [11,12,13,27].

4. CONCLUSION

The study assessed the common infective agent associated with wounds, the bioactive components of *R. acuminata* root extract and the antibacterial efficacy of the alkaloidal fraction of the extract on wound pathogens. *Staphylococcus aureus* and *Clostridium difficile* showed highest and least occurrence among the bacterial isolates associated with the assessed wounds. The phytochemical screening of the extract revealed high alkaloid content while it's antibacterial activities against the wound pathogens revealed potency at 50 mg/mL which compared favourably with the standard antibiotics (Levofloxacin). The results of this study indicate additional therapeutic values of the plant extract. Therefore, alkaloid fractions of *R.*

acuminata root extract can be incorporated in the production of antiseptics for wound cleaning/dressing.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards. Permission was obtained from Akwa Ibom state Ministry of Health, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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