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Research Article
Antibacterial Activity of *Abrus precatorius* (Linn.) Leaf Extract Against Multi-resistant Wound Bacterial Isolates

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Abstract

Background and Objectives: There are increasing reports of isolation of multi-drug resistant bacteria from infected wounds. There is, therefore, urgent need to identify these pathogens and find the best ways of improving wound care and management. In the midst of the reported multi-resistance of wound pathogens to conventional antibiotics, this study was carried out to investigate some of the folkloric uses of ethanolic and aqueous extracts of *A. precatorius* against multi-drug resistant wound pathogens recovered from patients from a referral hospital in Nigeria. **Materials and Methods:** One hundred wound samples from patients presenting at a referral hospital in Nigeria were screened in this study using microbiological methods. The susceptibility of the recovered isolates against ethanolic extracts of *Abrus precatorius* was evaluated. **Results:** Sixty-six bacterial organisms were isolated and 3 different microbial species were most predominately isolated: *Staphylococcus aureus* (45%) followed by *Pseudomonas aeruginosa* (24%) and *Escherichia coli* (15%). Susceptibility to ethanol extract of *Abrus precatorius* was high among *S. aureus* (87%) followed by *E. coli* (67%) then *P. aeruginosa* (60%) and for the aqueous extract, 43% of the recovered *S. aureus* isolates were susceptible while there was no effect observed for *E. coli* and *P. aeruginosa* wound isolates. Interestingly, apart from its anti-bacteria activity, it also showed potent activity on multi-drug resistant bacterial wound isolates. **Conclusion:** Findings from the present study substantiate the folkloric use of *A. precatorius* leaf extract for wound treatment.

Key words: *Abrus precatorius,* wound pathogens, wound management, bacteria, susceptibility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wound care constitutes an important part of routine care given by health professionals to the community population[1]. An effective management of wounds, especially chronic wounds in health care setting can have an impact in population health, reducing morbidity and improving function and quality of life. Wounds presented by patients vary from one setting to another, ranging from acute surgical wounds, traumatic wounds such as those that occur following an accident, burn wounds or chronic wound such as; diabetic foot, leg and peptic ulcers. All wounds are contaminated with micro-organisms that are part of the saprophytic micro flora of the skin and the type of and quantity of these micro-organisms varies from one wound to another[2]. Factors such as; origin or source of the wound, body location, size and duration of the wound must be considered in wound management because of their impact on wound colonization and infection[3]. Microbial colonization of wounds are characterized by the presence of multiplying micro-organisms in the surface of a wound but with no immune response from the host and with no associated clinical signs and symptoms.

Emergence of resistant strains of pathogenic micro-organisms has also continued to pose a major health concern about the efficacy of several drugs, most importantly and antibiotics in current use. Causes of the widespread and development of antibiotic resistance are multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics, environmental factors, often inappropriate use of antibacterial agents such as; broad-spectrum drugs and incomplete compliance with basic infection control practices such as hand washing[4].

This increasing rate of development of resistance to commonly used antibiotics has led to the search for newer, more effective, affordable and readily available sources with less side effects, in particular, from local medicinal plants (herbs) as the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs[5].

These antimicrobial substances are of natural origin and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as 'forgotten plants' Taking into account the increasing demand for natural ingredients that might be used as food additives, components of functional foods, preventing plant diseases and nutraceuticals as well as for other applications. It is reasonable to revise the 'forgotten plants' by assessing their applicability and benefits using modern scientific analytical methods[6]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by micro-organisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents[7].

One of such 'forgotten plants' is *Abrus precatorius,* a member of the family Fabaceae, order fabales, genus *Abrus* and species *Precatorius*. It's a slender, perennial climber that twines around trees, shrubs and hedges. It is a legume with long, pinnate-leafleted leaves. It's commonly known as jequirity, Crab's eye, rosary pea, precatory pea or bean, John Crow Bead. The plant is native to India and grows in tropical and subtropical areas of the world where it has been introduced. It has a tendency to become weedy and invasive[8].

Its leaf is effective in wound healings, infections with acne sores or boils and wounds. It also helps in getting rid of itching and other skin related problems. The plant kingdom synthesizes diverse active compounds which are valuable in the treatment and control of many diseases. These compounds are principally secondary metabolites[9,10].

In order to investigate some of the folkloric uses of *A. precatorius*, this study tested the susceptibility of bacterial wound isolates recovered from patients attending a referral hospital in southeast Nigeria.

MATERIALS AND METHODS

Research duration: The study was conducted in the Department Of Microbiology Research Laboratory, University of Nigeria, Nsukka, for duration of 4 months (September-December, 2015). The study made use of microbial techniques of culture of wound samples, isolation of pathogens and identification under the microscope, using of biochemical tests such as Gram-stain technique, oxidase test, Indole test, catalase test as well as the use of selective media such as; MacConkey, Mannitol Salt Agar and Eosin Methylene Blu (All from Sigma-Aldrich, Darmstadt, Germany) were used in the identification of the bacteria isolates. The preparations of agar were used according to the manufacturer's instructions.

Sample collection and processing: Wound samples were collected from ESUTH Teaching Hospital, Parklane, Enugu and transported to microbiology laboratory UNN in an ice bag for culture and isolation. A total of 100 samples from both female and male patients were collected by using sterile swab sticks

with the patient's consent. Samples were screened and bacterial isolates were recovered and identified using standard microbiological procedures.

Ethical approval: Ethical approval with reference number ENU/15/ESUTH/65597 was obtained from Enugu State University, Teaching Hospital Parklane, Enugu, for the isolation of bacteria isolates from infected wounds and informed consent was obtained from all those who participated in the study.

Collection and extraction of plant material: Healthy disease free, mature fresh leaves of *Abrus precatorius* were collected locally from Nsukka, environ and identified by an experienced botanist, Mr. Ozioko. The collected leaves were washed thoroughly 2-3 times with clean tap water and rinsed with sterile distilled water and air dried. After drying, the dried samples were ground into fine powder using an electric grinder. This process breaks the leaves to smaller pieces thus exposing the internal tissues and cells to solvents thus facilitating their easy penetration into the cells to extract the constituents. Water and absolute ethanol were used for the extraction of the plant material using cold maceration method as described by Oyagede *et al.*[10] with slight modifications. One hundred grams of the finely blended dried leaves were extracted in 1 L of absolute ethanol and hot water separately in different bowls with a cover and then allowed to stand for 24 h after which the mixture was separated by sieving, using muslin cloth and concentrated by allowing the solvents to vaporize naturally using a tray and air dried at room temperature such that the aqueous rich extract is obtained.

Phytochemical analysis of *A. precatorius* leaf extract: *Abrus precarious* leaves were screened for the presence of various phytochemicals such as; saponins, alkaloids, tannin, glycosides and flavonoids etc. as previously described by Harborne[11].

Antimicrobial susceptibility test of plant extracts on predominant isolates: Antimicrobial activity of *Abrus precatorius* extracts was determined using the procedures described by Clinical and Laboratory Standard Institute[12]. Five clean test tubes were arranged in a test-tube rack and labeled serially. The dried extract was dissolved in 70% DMSO to dissolve the extract and make a slurry, after which the slurry was dissolved in sterilized distilled water to get a working solution of 500 mg mL^{-1}. Different 2-fold concentration of the extract were used: 500, 250, 125, 62.5 and 31.5 mg mL^{-1} and sterile water as control. Using a sterile wire loop (flamed wire loop), a colony of the test organism was picked and emulsified in a test-tube containing 3-4 mL of sterile normal saline to get a suspension of the test organism. The suspension was standardized to 0.5 McFarland standard (1.5×10^8 CFU mL^{-1}). Then, dipping a sterile swab stick into the standardized suspension and swaved on the test-tube to remove the excess, the test organism was inoculated on to well-labeled nutrient agar medium plates in triplicates and carefully spread by streak plate method on the plate for even distribution over the medium. The agar plates were allowed to stand for about 3-5 min to allow the inoculum to be absorbed by the medium after which wells were bored on the agar using sterile cork borer (6 mm). An aliquot of 300 µL from the different concentrations of the extract were separately introduced using a micropipette into the different labeled wells bored (A-E). An aliquot of about 300 µL of sterile distill water was introduced into the well (CON) bored at the center of the agar plate medium to serve as a negative control. This process was carried out in triplicate for all the bacterial isolates tested and allowed to stand on the bench for 1 h for pre-diffusion and thereafter incubated at 37°C for 24 h. After incubation, the resulting inhibition zones were measured in millimeters (mm) using a transparent meter rule. Antimicrobial activities were expressed in terms of the mean value of inhibition zone diameter (mm) of triplicate experiments.

Determination of minimum inhibitory and bactericidal concentration of plant extract: The minimum inhibitory concentration of the extracts was determined for the test organisms in triplicates at varying concentrations of 500, 250, 125, 62.5 and 31.5 mg mL^{-1}. These concentrations were achieved by diluting serially with Mueller-Hinton broth. Then about 300 µL of the already standardized organism was introduced into each tube using a micropipette. A control experiment was set up which contained Mueller-Hinton broth and test organisms as positive control while the Mueller-Hinton broth and plant extract were used as negative control. The tubes were incubated at 37°C for 24 h and observed for turbidity as an indication of growth. MIC was designated as the lowest sample concentration showing no turbidity which indicated complete inhibition of growth[12,13]. The minimal bactericidal concentration was determined by selecting the tubes that showed no growth during the MIC determination and a loopful from each of the tubes sub-cultured on nutrient agar plates and incubated for 24 h at 37°C. The MBC were determined as the least concentration that showed no visible growth.

RESULTS

Detail of samples used: Of the one hundred wound samples collected from 80 patients, 69 were females and 11 were males. The age range for females was 30-80 years and for males, 15-80 years. Table 1 shows the details of different wound samples screened in the study as well as the type of wounds screened.

Culture and isolation of wound pathogens: Sixty-six organisms were isolated consisting of five different species. The most common wound bacteria recovered was *Staphylococcus aureus* (45%) followed by *Pseudomonas aeruginosa* (24%) and *Escherichia coli* (15%). Most of the wounds were infected by just one organism. However, there were a few polymicrobial wound infections (2 or at most 3 microbial species) with the most common association being *S. aureus* and *P. aeruginosa* in both sexes.

Phytochemical evaluation of *A. precatorius* leaf extract: Table 2 shows the result of the phytochemical screening of *A. precatorius* leaves and the degree at which the phytochemical constituents are present. This analysis showed the presence of alkaloids, saponins, sterols, terpenoids, glycosides, tannins and flavonoids.

Sensitivity patterns of ethanolic extract of *A. precatorius* leaves against predominant wound bacteria isolates: Table 3 shows the sensitivity patterns of ethanolic extract of *A. precatorius* leaves against predominant isolates (*S. aureus, P. aeruginosa and E. coli*) recovered from the screened wound samples against the various concentrations.

All the three most predominant wound bacterial isolates recovered in the study were susceptible to the plant material. However, *S. aureus* appeared to be more susceptible considering that some of the species were sensitive at lower concentration (31.5 mg mL^{-1}). Three of the fifteen isolates of *Pseudomonas aeruginosa* were sensitive at the next lower concentration (62.5 mg mL^{-1}). This is remarkable considering that this organism is known to show multiple resistance to antibiotics as well as the fact that only crude extracts were used in present testing. There is a possibility that refined extracts will do better. This could be an interesting area to focus in future studies. Interestingly, one isolate of *E. coli* was also susceptible at 62.5 mg mL^{-1}.

Minimum inhibitory concentration and minimum bactericidal concentration: Table 4-6 show the minimum inhibitory and minimum bactericidal concentrations of the multi-resistant isolates obtained from the screened wound samples. These data represent the concentration at which *A. precatorius* leaf extract (ethanolic and aqueous) inhibited the screened wound pathogens and the concentration at

Table 1: Details of the different samples screened in the study

Sample source/categories	No. of collected	Sample (%)
Bed sore samples	2	2
Surgical wound samples (includes diabetic sores)	48	48
Burns	20	20
Accident victims	20	20
Cancer sores	10	10
Total	100	100

Table 2: Preliminary phytochemical screening of ethanolic extract of *A. precatorius* leaves

Constituents	Test performed	Indication for positive test	Relative degree
Reducing sugar	Fehling's solution	Brown precipitate	+
Proteins	Biuret	Violet	+++
Carbohydrates	Molisch test	Reddish-brown ring	+
Flavonoids	Alkaline reagent test	Yellow	+++
Saponins	Froth test	Froth formation	++
Terpenoids	Salkowski	Reddish-brown	++
Alkaloids	Mayer's regent test	Creamy precipitate	++
Tannins	Ferric chloride	Blue-red ring	+++

+: Present, ++: Moderately present, +++: Highly present, -: Absent

Table 3: Sensitivity patterns of ethanolic extract of *A. precatorius* leaves against predominant isolates recovered from screened wound samples against various concentrations

	Concentration of extract used/Zones of inhibition (%)					
	A 500	B 250	C 125	D 62.5	E 31.5	Control water
Microbial species isolated (No.)	(mg mL^{-1})					
Staphylococcus aureus (n = 15)	14 (87.5)	14 (87.5)	14 (87.5)	1 (31.3)	2 (12.5)	-
Pseudomonas aeruginosa (n = 15)	3 (60)	3 (60)	3 (60)	3 (60)	-	-
Escherichia coli (n = 10)	2 (66.7)	2 (66.7)	2 (66.7)	1 (33.3)	-	-

-: No Inhibition zone, CON: Control (water), n: Number of isolates tested, A: Extract concentration of 500.0 mg mL^{-1}, B: Extract concentration of 250.0 mg mL^{-1}, C: Extract concentration of 125.0 mg mL^{-1}, D: Extract concentration of 62.5 mg mL^{-1}, E: Extract concentration of 31.3 mg mL^{-1}

Table 4: Minimum inhibitory and minimum bactericidal concentrations (MIC/MBC) of the water extract of *A. precatorius* leaf against selected predominant bacteria wound isolates (*S. aureus*)

Isolate number	MIC (mg mL^{-1})	MBC (mg mL^{-1})
S. aureus **(n = 7)**		
S2	250	500
S4	250	500
S15	250	500
S22	250	500
S32	125	250
S37	250	500
S39	250	250

Table 5: Minimum inhibitory and minimum bactericidal concentrations (MIC/MBC) of the ethanolic extract of *A. precatorius* leaf against *S. aureus*

Isolate number	MIC (mg mL^{-1})	MBC (mg mL^{-1})
S. aureus **(n = 14)**		
S2	250	500
S4	250	250
S15	250	500
S22	250	200
S24	125	250
S25	250	500
S32	125	250
S37	250	500
S39	250	500
S48	250	500
S52	125	250
S53	250	500
S57	125	250
S61	250	500

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, S2, 4, 15, 22, 24, 25, 32, 37, 39, 48, 52, 53, 57 and 61 are multidrug resistant *S. aureus*

Table 6: Minimum inhibitory and bactericidal concentrations (MIC/MBC) of the ethanolic plant extract of *A. precatorius* leaf against multi-resistant *E. coli* and *P. aeruginosa*

Isolate number	MIC (mg mL^{-1})	MBC (mg mL^{-1})
E. coli **(n = 2)**		
S5	250	500
S6	125	500
S9	-	-
P. aeruginosa **(n = 3)**		
S16	125	250
S18	250	250
S20	-	-
S41	-	-
S62	125	250

-: No minimum inhibitory concentration value, MIC: Minimum inhibitory concentration value, S5, S6 and S9 are multidrug resistant *E. coli*, S16, S18, S20, S41 and S62 are multidrug resistant *P. aeruginosa*

which it killed/inhibited the pathogens. It was observed that at concentrations of 125 mg mL^{-1}, most of the screened wound pathogens were inhibited at a concentration of 250-500 mg mL^{-1}.

Currently, there are no reference datasets or endpoints with which to compare the data presented in Table 4-6.

DISCUSSION

Effective management of wounds, especially chronic wounds has an impact on population health by reducing morbidity and improving the quality of life. Hence, the importance of this study in wound care and management. Many drugs have entered the international market through exploration of ethno-pharmacology and traditional medicine.

The phytochemical constituents of a plant often determine its physiological action on the human body. These metabolites are usually responsible for the pharmacological activities of medicinal plants[14]. Saponins and flavonoids have been reported by Nair *et al.*[15] to possess wound-healing activity which is likely to be part of the high bactericidal properties of *A. precatorius* leaf extract at high concentrations against the screened wound pathogens as it was observed in present study. Terpenoids are known to promote wound-healing process, mainly due to their astringent and antimicrobial activities which seem to be responsible for wound contraction and increased rate of epithelialization[16]. Flavonoids and their derivatives are known to decrease lipid peroxidation by improving vascularity leading to slowing down of cell necrosis[17]. Antioxidants are agents that protect cells against damage caused by molecules known as free radicals. The antioxidant activities of extracts are mainly due to the presence of phenolic compounds such as; flavonoids, phenolic acids, tannins and phenolic diterpenes[18]. Hence, the phyto-constituents of *A. precatorius* leaf extract such as; tannins and flavonoids, play a major role in wound healing by preventing and protecting oxidative damage from free radicals as recorded by Ayoola *et al.*[19], that tannins and flavonoids help in preventing and protecting oxidative damage from free radicals. From this study, evaluation of the *A. precatorius* leaf extract on the most prevalent bacteria involved in wound infection showed a dose-dependent (concentration) inhibitory activity against the isolated micro-organisms such as *S. aureus* (MIC of $\geq$125 mg mL^{-1}) and *P. aeruginosa* (MIC of $\geq$250), which are the two organisms mostly implicated in chronic and non-healing wounds as reported by Bjamsholt *et al.*[20]. The plant extracts showed lower activity against the Enterobacteriaceae, *E. coli* and *P. aeruginosa,* this can be due to the possession of outer membrane by Gram- negative bacteria that serves as an effective barrier[16] showed that at a concentration of $\geq$125 mg mL^{-1}, the organisms were inhibited but requires a higher concentration to kill them, this is in conformity with the studies done by Nair *et al.*[15].

The use of *Abrus precatorius* in the traditional setting for treating different kinds of ailments is well known. This study tested the three most common bacterial isolates recovered

from wounds in a Nigeria referral hospital. The extracts showed activity against all the three organisms though their activity varied slightly among these isolates.

Phlobatannins have also been demonstrated to have wound-healing activity. Therefore, the wound-healing potential of *A. precatorius* can be attributed to the contributions of its individual phytoconstituent.

It was noted that the ethanolic extract of *A. precatorius* leaf has greater effect in the inhibition of the predominant wound pathogens isolated than the aqueous extract, which may be due to the fact that ethanol is a better solvent for the extraction of the active compounds from plant materials when compared to the distilled water used in the case of aqueous extracts.

Conventional wound treatment requires more or less, the combined effects of antibiotics, anti-inflammatory agents, astringents and antipyretics, *A. precatorius* has been reported to possess antibacterial, anti-inflammatory, antioxidant and antipyretic effects by Girish and Satish[21]. The extract can, therefore, be effectively used in the treatment of wounds as we have ascertained its bactericidal effect against multidrug resistant pathogens screened in present study. Thus, the antimicrobial activity of the leaf extract on these wound isolates may partly contribute to the wound-healing effect by eliminating infection and thus allowing initiation of natural tissue repair processes. It also suggested that *A. precatorius* leaf extract may play useful role in accelerating the healing of old wounds by eradicating already established infection.

This study also observed that the MIC and MBC were slightly high. However, it is important to note that it was used crude extract. The purified extract might show better activity. In the traditional setting, another thing to note is that extracts are sometimes combined together in treating an ailment. This is because in traditional settings, herbalists most times use other herbs in combination with *A. precatorius* to stimulate a better activity of *A. precatorius* extract thereby making such a combination more potent against micro-organisms. It will be interesting in future research to separately test individual and combined forms of these extracts against wound pathogens to ascertain the exact potency of such combinations.

The present research revealed that *A. precatorius* is a unique source of many potential phytochemicals such as; proteins, flavonoids, tannins, saponins, terpenoids, alkaloids, carbohydrates and reducing sugar. The first three were more abundant. It is difficult to ascribe the activities of these extracts to any of the phytochemical contents without performing further experiments. It is also possible that the activities of the *A. precatorius* extract is as a result of a combination of one or more of the phytochemical constituents as has been previously established in other studies with other plant materials by Akah *et al.*[22]. The presence of these phytochemicals makes this plant very important and versatile for its various medicinal properties i.e., anti-diabetic, neuroprotective, anti-cancer, anti-microbial, analgesic, wound healing and many more. The present research revealed that *A. precatorius* is a unique source of many potential phytochemicals which makes this plant very important and versatile for its large number of medicinal properties i.e. anti-diabetic, neuroprotective, anti-cancer, anti-microbial, analgesic and many more as reported by Nascimento *et al.*[23]. This may indicated that extensive research is yet to be done in this very potent medicinal plant. Hence extensive research should be done to exploit the therapeutic ability to fight against various diseases. Above collected literature conclude that *A. precatorius* is quite promising as a multipurpose medicinal agent as it is having very potential pharmacognostic and pharmacological applications[23].

It is worthwhile to establish from this study that *E. coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* are the three most implicated wound pathogens recovered in the study as established by previous studies by Meaume *et al.*[1]. This information may be relevant to clinicians as it gives them the likely idea of what to expect. In certain circumstances, clinicians sometimes administer antibiotics to wound patients without first obtaining culture and antibiotic sensitivity data. Specifically, the frequency of bacteria isolated from screened infected wound samples has *Staphylococcus aureus* (45%) as the most prevalent followed by *Pseudomonas aeruginosa* (24%) and *Escherichia coli* (15%). This is in conformity with the findings from a previous study by Meaume *et al.*[1] that demonstrated a similar trend though in a different geographical location.

The data showed that the ethanolic extract was most effective on *S. aureus* (87%) followed by *E. coli* (66.7%) and *P. aeruginosa* (60%) while the aqueous extract only showed activity on MDR *S. aureus* (43%) and no effect on MDR *E. coli* and *P. aeruginosa.* As already noted, it would seem that MIC/MBC are somehow elevated at 250-500 mg mL^{-1}. However, it is important to highlight the multi-resistant nature of these isolates against conventional antibiotics. Additionally, the extracts are crude in nature and have not been purified. Since the isolates exhibited multi-resistance, there was no point displaying the MIC/MBC values of conventional antibiotics tested. Future studies may warrant testing more bacteria wound isolates to make a more robust conclusion of these findings.

CONCLUSION

Natural sources of antioxidants have generated a lot of great interest due to their potentials to substitute synthetic ones. This study has identified the most prevalent wound bacteria pathogen in the area under study. It was also able to provide some justification for the folkloric use of *A. precatorius* leaf extract in wound care especially that of the ethanolic extract. Present study findings also showed the potentials of *A. precatorius* leaf extract in wound healing considering the high constituents of its phytonutrients. However, further research is needed to ascertain other important parameters such as the side effects if any, safe dosage and toxicity of the *Abrus precatorius* leaf extract to humans and most importantly, the mechanisms involved.

SIGNIFICANCE STATEMENT

This study was able to justify the folkloric use of *A. precatorius* leaf extract in wound management and treatment of infected wounds. It also established that the extract is effective against wound bacteria especially multidrug resistant ones. The extract concentration would appear to be high in some cases, but this ought to be downplayed considering the fact that only the crude unpurified extract was tested.

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