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Evaluation of Antibacterial and Phytochemical Analysis of Root Bark Extracts of *Guiera senegalensis* against Methicillin Resistant *Staphylococcus aureus* (MRSA)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MG, NIT and MA designed the research, writing of the manuscript was done by all the authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The study was to evaluate the antibacterial activity and phytochemical screening of *Guiera senegalensis* extracts against methicillin resistant *Staphylococcus aureus* (MRSA).
Place and Duration of Study: A total of 70 samples of wound, High Vaginal Swab (HVS) and Skin were collected from Muhammad Abdullahi Wase specialist Hospital, Kano from September 2015 to December 2015.
Methodology: The aqueous and ethanol extracts from *Guiera senegalensis* root bark was tested using well diffusion method for their antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) isolated from wound, skin and HVS samples of patients attending Muhammad Abdullah Wase specialist Hospital, Kano.
Results: The results showed that the ethanolic extracts of the root bark produces impressive antibacterial activity against some of the test organisms with zone of inhibition ranging between 24mm and 20mm, the aqueous root extracts had low inhibitory effect than the ethanolic extract with zone of inhibition of 21mm and 15mm. This is therefore indicates that the root bark extracts

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partitioned in solvent of different polarities were effective against some MRSA. Phytochemical screening of the extracts revealed the presence of phyto-compounds such as alkaloids and tannins which are known to inhibit bacterial growth by different mechanisms from those of synthetic drugs.
Conclusion: The phyto-constituents present may be responsible for the *Guiera senegalensis* antibacterial activity hence, *Guiera senegalensis* can be used as antibacterial agent against Methicillin resistant *Staphylococcus aureus*.

Keywords: Guiera senegalensis; phytochemicals; antibacterial activity; methicillin resistant Staphylococcus aureus; Well diffusion.

1. INTRODUCTION

Guiera senegalensis Gmel (Combretaceae) (i.e. sabara in Hausa) is a shrub of savannah region of West and Central Africa [1]. The literature reports several recorded uses for *G. senegalensis* in traditional medicine to treat various illnesses [2]. It is recognized as being active against cough, respiratory congestion and fever [3], and is prescribed as an antitussive [4,5], to ease breathing and to treat lung and bronchial disorders. It is also used against malaria [6]. *S. aureus* is widely considered a major factor of nosocomial infections ranging from minor skin infections, osteomyelitis and endocarditis, to more serious infections including fatal necrotizing pneumonia [7]. Although these infections were historically treatable, beginning in the 1980s, methicillin resistant *S. aureus* (MRSA) strains have spread rapidly in susceptible hospitalized patients, dramatically changing the therapy available for preventing and treating staphylococcal infections [8]. Although MRSA infections were relatively uncommon among healthy individuals in the community, a dramatic change was observed in 2003 when new strains of MRSA were reported to be responsible for outbreaks of community-acquired cutaneous infections and severe pneumonia [9]. MRSA infections present a challenge to infection control and treatment strategies, resulting in increased morbidity, mortality, and length of hospitalization and health care costs [10].

Therefore, the present study was to determine the phytochemical and antibacterial activity of aqueous and ethanolic extracts of *Guiera senegalensis* as an alternate therapy against MRSA.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant materials (root bark) of *Guiera senegalensis* plant was collected from Danhassan town in Kura Local Government Area of Kano State, Nigeria. Botanical Identification and Authentification of the plant materials was done by a staff of the department of plant Biology, Herbarium unit, Bayero University, Kano. Voucher specimen number: Bayero University, Kano Herbarium Accession Number BUKHAN 0032 and deposited there for future reference. Fresh root bark of *Guiera senegalensis* was dried in the open air and ground to powder form and kept in cellophane bag at 4ºC before extraction.

2.2 Preparation of Extracts

The plant parts were thoroughly washed and air dried and ground to powder. Briefly twenty five gram (25g) of root bark was soaked separately in 250ml of distilled water and 250ml of (95%) ethanol in each case were allowed to stand for one weeks with regular shaking. The obtained extract was filtered by using whatman No.1 filter paper each filtrate was concentrated under reduced pressure on a rotary evaporator till golden viscous mass was obtained for ethanol extracts and for aqueous extracts steam bath. Finally, the prepared extracts were stored at 4^{o}c for further use. 1g of each aqueous extract and ethanol prepared (each separately) was taken and the aqueous extract was dissolved in 5ml sterile distilled water, while ethanolic extracts were dissolved in 5ml of 5% DimethylSulphoxide (DMSO). Thus 200mg/ml of stock was obtained as a standard concentration of aqueous and ethanolic extracts.

2.3 Phytochemical Screening

The phytochemical screening of the crude extracts was carried out according to the method of Tiwari et al. [11], for possible detection of some secondary metabolites such as alkaloids, tannins, saponins, Flavonoids, glycosides, steroids and phlobatannins.

2.4 Evaluation of Antibacterial Activity

Staphylococcal isolates were obtained from Muhammad Abdullahi Wase Specialist Hospital, pathology department, Microbiology unit. They were re-isolated and the pure cultures subcultured on nutrient agar slants. They were stored at 4ºC until required. An *in vitro* test using the agar diffusion method as describe by Esimone et al. [12] was adopted for the study. All the test bacteria used were incubated and introduce into nutrient agar broth. About 15ml of sterile molten nutrient agar in a Petri dish was seeded with 1.0ml of standardized broth cultures of the bacteria (5-9 × 10^7cfu/ml) and swirled gently to ensure uniform distribution of the microorganisms and then solidify on a flat surface. After 6mm diameter well were bored in the agar with sterile cork borer and filled with 0.1ml of different concentration of the extracts. The Petri dish were allowed to stand for 1hr at room temperature for pre-diffusion to occur and then incubated at 37ºC for 24hrs. At the end of incubation zone of inhibition that developed were measured with transparent ruler in millimeter (mm) and average zone of inhibition was calculated. Gentamicin 125 mg/ml was used as positive control. Diameters of zones of inhibition ≥10mm exhibited by plant extracts were considered active [13].

2.5 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration, (MIC) of the extracts was determined using the tube dilution method [14]. Dilution of the plants extracts was incorporated in nutrient broth in 1:1 ratio Initial rough estimates of the MIC values of the plant extracts against the test organisms were estimated to determine the range of MIC values. Consequently, the following concentrations were prepared for each extract, using the dilution formula: 400, 200, 100, 50, 25, 12.5, 6.25 mg/ml. In addition, 0.1ml of standard suspension of the test organisms was added to each tube. The tubes were incubated at 37ºC for 24 hours. A tube containing extract and growth medium without inoculum would be included to serve as control. The presence of growth (turbid solution) or absence of growth (clear solution) at the end of incubation period was recorded. The lowest concentration of the extract showing no growth was regarded as the minimal inhibitory concentration (MIC). The minimum bactericidal concentration, (MBC) was determined by sub culturing the last test dilution that showed visible growth (turbidity) and all others in which there was no growth on a fresh Mueller Hilton agar and incubated for further 24 hours at 37ºC. The dilution that shows no single bacterial colony was taken as the minimum bactericidal concentration (MBC) as reported by Forbes et al. [14].

2.6 Statistical Analysis

The data was analyzed using One-Way Anova and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the mean ± standard deviation. Significance level for the differences was set at $p<0.05$.

3. RESULTS

3.1 Phytochemicals Analysis of the Root Bark of *Guiera senegalensis*

Phytochemicals analyses of the root bark of *Guiera senegalensis* is presented in Table 1. From the results, alkaloids, anthraquinones, flavonoids, glycosides, steroids and tannins were present root of *Guiera senegalensis.*

Table 1. Phytochemicals analysis of the root bark extract of *Guiera senegalensis*

Phytochemical component	Root bark
Alkaloid	+
Anthraquinone	+
Flavonoids	+
Glycoside	+
Saponins	+
Steroid	+
Tannin	+
Terpenoid	+

3.2 Anti-MRSA Activity

3.2.1 Ethanol root bark extract

Anti-MRSA activities of the different concentration of ethanolic extract of the root bark of *Guiera senegalensis* is presented in Table 2. From the results, diameter of zone of inhibition (mm) at 100, 200, 300, 400, 500 and 1000 against the control for the bacterial isolates 09, 036, 063,217, 266 and 319 were observed to be 8.25±1.86, 9.75±2.16, 10.12 ± 2.27, 11.25 ± 2.5, 12.37 ± 2.79, `14.12 ± 3.21 and control 15.75 ± 3.43; respectively. No significant differences ($p > 0.05$) were observed at various concentrations of the *Guiera senegalensis* root bark extract and the control.

3.2.2 Aqueous root back extract

The mean zones of inhibition of aqueous extract of the root bark of *Guiera senegalensis* at concentration such as 100, 200, 300, 400, 500 and 1000 mg/ml against the control for the bacterial isolates 09, 036, 063,217, 266 and 319 were observed to be 1.75 ± 1.16, 3.25 ± 1.35, 6.62 ± 1.26, 8.12 ± 1.31, 10 ± 1.64, 13.37 ± 2.08 and 21 ± 0.00 respectively. There is significance differences ($p < 0.05$) were observed at various concentrations of the *Guiera senegalensis* root bark extract and the control.

3.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration and minimum bactericidal concentration of the extract is represented in Table 4. The result showed that the extract can inhibit the growth at 25-50mg/ml. the MBC of the extracts against the MRSA isolates is found to be 200mg/ml.

4. DISCUSSION

In this study, the phytochemical screening of aqueous and ethanolic extract of root bark of *Guiera senegalensis* revealed the presence of alkaloid, Flavonoids, glycosides, saponins, steroid, tannins, Anthraquinones and terpenoids was found to be absent in the leaf. This result correlates with the finding of Williams et al. [15].

The anti-MRSA activity of the crude extracts of *Guiera senegalensis* were determined in comparison with the standard antibiotic (Gentamicin) against the test organisms. There was a significance differences between the zones of inhibition by the crude extracts and the antibiotic (control). The inhibitory effects of the crude extracts could be attributed to the phytochemical components of the crude extracts as reported in previous study by Kubmarawa et al. [16].

The results obtained indicates that the root bark of *Guiera senegalensis* partitioned in solvent of different polarities were effective against some of the test organisms. The ethanolic extracts of the root bark produces impressive anti-MRSA activity against some of the test organisms with zone of inhibition ranging between 24mm and 20mm, the aqueous root extracts had low inhibitory effect than the ethanolic extract with zone of inhibition of 21mm and 15mm. this is therefore indicates

Table 2. Anti-MRSA activity of the different concentration of ethanolic extract of the root bark of *Guiera senegalensis*

Isolate (MRSA)	Concentration of the extract (mg/ml)						
	Diameter of zone of inhibition formed (mm)						
	100	200	300	400	500	1000	Control
IS1	12	14	16	18	20	24	21
IS2	00	00	00	00	00	00	00
IS3	00	00	00	00	00	00	00
IS4	10	13	14	14	15	17	21
IS5	14	15	15	16	19	21	21
IS6	10	12	12	14	15	17	00
IS7	10	12	12	14	15	17	00
IS8	10	12	12	14	15	17	21

IS = Isolate

Table 3. Anti-MRSA activity of the different concentration of aqueous extract of the root bark of *Guiera senegalensis*

Isolate (MRSA)	Concentration of the extract (mg/ml)						
	Diameter of zone of inhibition formed (mm)						
	100	200	300	400	500	1000	Control
IS1	00	00	06	08	10	12	21
IS2	00	00	00	08	10	15	00
IS3	00	00	00	00	00	00	00
IS4	08	10	12	13	15	20	21
IS5	00	08	08	10	10	14	21
IS6	08	08	08	08	12	14	00
IS7	00	08	08	10	15	17	00
IS8	00	00	09	08	10	15	21

IS = Isolate

Table 4. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the extracts

Isolates (MRSA)	Extracts/MIC/MBC (mg/ml)	
	Ethanol extract	Aqueous extract
IS1	25/50	200/200
IS4	25/25	200/200
IS5	50/50	200/200
IS6	25/50	200/200
IS7	50/50	200/200
IS8	25/25	200/200

IS = Isolates

that the root bark extracts partitioned in solvent of different polarities were effective against some of the test organisms (MRSA). Other workers have also shown that root bark methanolic and aqueous extracts of *Guiera senegalensis* inhibited the growth of various microorganisms at different concentration [15].

The extracts (ethanolic and aqueous) of *Guiera senegalensis* were active against MRSA strains with ethanol extract demonstrating higher anti-MRSA activity than water extracts. Previous studies by Mamman and Isa [18] indicated that the crude extracts of these plants were effective against *S. aureus*. The present study was slightly conformed to their findings but the only area of concern is that while their studies only dealt with the effect of crude ethanolic extracts on *S. aureus*, this study focused on the effect of crude extract on the MRSA, and determination of both MIC & MBC values of the extracts. The MIC values were range between 50 to 25 mg/ml obtained for ethanol extracts and aqueous extracts was between the ranges of 200mg/ml to 400mg/ml. Similarly, the MBC values for ethanol extract 25 mg/ml and aqueous extract are 200mg/ml.

Literature reports several recorded use for *G. senegalensis* in traditional medicinal to treat various illness [2]. It is used externally as an antiseptic healing preparation for wounds, stomatitis, gingivitis and syphilitic cankers [3].

The observed antibacterial effect on the isolates is believe to be due to the presence of tannins, flavonoids and saponins which have been shown to possess antibacterial properties [16]. Some workers have also attributed the observed antimicrobial effects of plant extracts due to the presence of secondary metabolites [17].

The zone of inhibition exhibited by the extracts against some of the methicillin resistant *S. aureus* have justified their used by traditional medical practitioners in the treatment of microbial infections such as sore, open wounds and boils, MRSA or *S. aureus* have been implicated in cases of boils, open wounds and sores and extracts of *Guiera senegalensis* were effective against these microbes. The MIC and MBC exhibited by the extracts against MRSA are of great significance in the health care delivery system, since it could be used as an alternative to orthodox antibiotics in the treatment of infection cause by these microbes, especially as the developed resistance to known antibiotics [16]. So their use will reduce cost of obtaining health care.

The inability of the extracts to inhibit two strains of MRSA by the aqueous extract and ethanol extract may be that the possess a higher mechanism for detoxifying the active principles in these extracts. Although some bacterial strain are known to possessed mechanism which convert substances that inhibit their growth to non toxic compound. The *S. aureus* produces an enzyme called penicillinase which converts the antibiotic penicillin to penicillinoic acid which is no longer inhibitory to its growth [19].

5. CONCLUSION

The results of this study showed that aqueous and ethanol root bark extract from *Guiera senegalensis* is effective antibacterial agent against some strains of MRSA, due to the presence of the phytochemical compounds. There is a need of plants phytochemical compounds isolation because they can serve as templates for production of new antibacterial factors.

ETHICAL APPROVAL

Ethical approval (HMB/GEN./488/VOL.1) was obtained from Kano State Hospital Management Board based on the consent of Murtala Muhammad Specialist Hospital ethical committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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