WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.632
Volume 11, Issue 8, 21-31 Research Article ISSN 2278 – 4357

SUSCEPTIBILITY PATTERN OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM HEALTHY COMMUNITY INDIVIDUALS TO *BUCHHOLZIA CORIACEA* ENGL. (FAM: CAPPARACEAE) LEAF EXTRACT

Clement Oliseloke Anie[1]* and Olukayode Temitope Egbon[2]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy Delta State University Abraka.

[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy Federal University Oye-Ekiti.

Article Received on
31 May 2022,

Revised on 20 June 2022,
Accepted on 10 July 2022

DOI: 10.20959/wjpps20228-22765

***Corresponding Author**
Clement Oliseloke Anie
Department of Pharmaceutical Microbiology, Faculty of Pharmacy Delta State University Abraka.

ABSTRACT

Objective: The *in vitro* antimicrobial activity of crude extract of leaf extracts of *Buchholzia coriacea* against Methicillin-resistant *Staphylococcus aureus*. **Methods**: The aqueous and methanolic extracts *Buchholzia coriacea* were investigated for their phytochemical constituents; while the MRSA was identified using oxacilin sensitivity disk. Antimicrobial activity was evaluated using seeded plate method. Phytochemical screening revealed the presence of tannin, anthroquinone, glycosides, steroids, terpenoid, alkaloids. The minimum inhibitory concentration of methanol and aqueous leaf extracts of *B. coriacea* on selected MRSA isolates. **Results**: The results show that every isolates had growth at 12.5 mg/ml concentration but none of them had at 200 mg/ml – 50 mg/ml concentrations. At 25 mg/ml concentration isolate 3 had growth. MIC of the methanol leaf extract of *B. coriacea* against isolates 1, 2, 4 and 5, was 25mg/ml, while that for isolate 3 MIC is 50 mg/ml. MIC of aqueous leaf extract of *B. coriacea* on selected MRSA isolates. MIC of aqueous leaf extract of *B. coriacea* on selected MRSA isolates shows that all the isolates were able to grow at 25 mg/ml and 12.5 mg/ml concentrations. Contrarily, none had growth at 200 mg/ml and 100mg/ml concentrations. However at 50 mg/ml concentration, isolate 3 had growth, while other isolates had no growth 50 mg/ml. Therefore, the aqueous extract had minimum

inhibitory concentration for isolate 3 at 100 mg/ml as against 50 mg/ml for isolates 1, 2, 4 and 5. The aqueous extract had higher MIC concentrations than the methanol leaf extract in agreement with earlier reported. **Conclusion**: Further study to be carried out to fractionate the leaf extracts which could enhance the bacterial activity.

KEYWORDS: Methanol, *Buchholzia coriacea*, Aqueous extract.

INTRODUCTION

Staphylococcus aureus is a species of bacteria commonly found on the skin and or in the noses of healthy people The nose is one of the few openings that bacteria have direct access to get inside the body. Thus the nose and nasal passages can be perfect environments for some bacteria. *S. aureus* has been recognized as one of the most important bacteria pathogen significantly contributing to hospital and community-acquired infections all over the world. Methicillin resistant staphylococcus aureus (MRSA) is known to be associated with several severe infections Anie, 2020. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major causes of hospital-acquired infections worldwide. It is widespread in communities and prevalent in hospital and even amongst the livestock. The mortality and morbidity amongst patients with infections associated with MRSA are usually high owing to increasing resistance to several antibiotics Anie *etal* (2017). Ever since the introduction of antimicrobial drugs that are β-lactamase–stable into medical use, methicillin-resistant *Staphylococcus aureus* (MRSA) have surfaced universally as significant nosocomial pathogens; even their prevalence in the community is rising considerably. Though *S. aureus* has been discovered to be one of the commonest aetiology of mastitis in bovine and even some other serious diseases of animal such as septicaemia, wound, joint infections, bone, and MRSA strains infrequently have been isolated from animals. Strains of MRSA have now been isolated from cows with mastitis, dogs and horses with lesions, and cats and dogs that were carriers. The transmission of MRSA between animals and the human beings (e.g., dogs, pigs, horses,) has been reported nevertheless the transmission between humans and cows has not, to our knowledge. We explain a first recognized case of MRSA transmission between a person and cows Anie *etal* (2020). In Nigeria, *B. Coricea* is known to be a perennial plant that grows as a tree and its locally called “Uwuro” in Yoruba, and “Uke” in Ibo. In Bini it is called “Ovu”. The parts of the plant that is generally eaten include the seeds usually eaten raw or cooked. It is known to be food of the brain that improves brain memory; it is used for hypertension treatment and prevention of premature aging capability Nweze *etal.*, (2006).

This study is aim at determining the antimicrobial properties of the leaf extract of *B. coriacea* on Methicillin-resistant *Staphylococcus aureus.*

MATERIALS AND METHODS

Ethical Consideration

Samples were collected, after proper explanation of information regarding the study to the participants and approval from the Delsu health centre was sought. Oral consent was obtained for participation in the study.

Sample collection

The nasal samples from subjects in the designated study areas were obtained with sterile swab sticks, which were carefully and gently inserted into the inner area and rubbed over the anterior nares of both nostrils.

Preparation of Mcfarland turbidity equivalent standard

In preparation Mcfarland standard solution, 1% v /v solution of sulphuric acid was prepared by addition of 1 ml of concentrated sulphuric acid to 99 ml of water and 1% w/v solution of barium chloride was prepared by dissolving 0.5 g of barium chloride in 50 ml of water. McFarland equivalent standard was prepared by adding 0.6 ml barium chloride solution to 99.4 ml of 1 % v/v sulphuric acid and mixed properly (Anie *etal.*, 2022).

Isolation and characterization of bacteria

The collected swab samples were immediately taken Pharmaceutical Microbiology Laboratory Delta State University Abraka and then streaked on the surface of sterile freshly prepared nutrient agar plates. The inoculum on the agar plates were streaked out on another agar media in order to obtain discreet colonies. The culture plates were incubated at 37° C for 24 hours and were observed for any growth. The colonies obtained were again inoculated in nutrient agar to achieve discreet colonies. Pure colonies were further cultured on mannitol salt agar. After obtaining pure strains, identification tests were carried out using standard microbiological procedures Cheesbrough (2006).

Phenotypic detection of MRSA using Oxacillin disc

The Susceptibility testing of all S *aureus* isolates, was conducted using the agar diffusion method on the Muller Hinton agar using oxacillin sensitivity disc. The inhibition zones

observed were measured. These were interpreted as susceptible, intermediate and resistant according to standard specifications Anie *etal*., (2022).

Collection and identification of plant material

The plant was authenticated by Odewo, Akinniyi Samuel of Forestry Institute of Nigeria, Jericho, Ibadan, fresh leaves of *Buchholzia coriacea* was collected and used for this study.

Preparation of plant leaf for extraction

The plant leaves were air-dried at room temperature for five days to avoid denaturing of the active constituents, after which they were milled using mortar and pestle into a coarse powder.

Extraction

Fifty grams (50 g) of the powdered sample was weighed and soaked in 300 ml of extracting solvents (water, methanol) for five days while being stirred at intervals. Five millimetres (5ml) of the ethanol was added to the extraction flask containing powdered leaf sample in water to prevent deterioration of the product. At the end of five days, the content was filtered using a filter paper then aqueous filtrate was freeze dried. The dried extract was stored in a sample bottle until required for analysis Okafo *etal* (2019) Enwa *etal.,* (2015)

Phytochemical screening of the plant extract

Five grams (5 g) of the plant aqueous extract was dissolved in 50 ml of water in a beaker as a stock solution of the plant extract from where different volumes of the extract were taken for different phytochemical screenings Anie (2020).

Antimicrobial screening test using Kirby-Bauer Method

The agar well diffusion method was employed in the assay. Nineteen (19) ml of sterilized Mueller-Hinton agar was seeded with 1 ml of the microorganisms and poured into a Petri dish to solidify. A cork borer (sterile) of 5 mm in diameter was carefully used to bore hole in the agar. With the aid of a sterile syringe, two (2) drops of the plant methanol extract, were dispensed into the wells depending on the concentrations of the plant extracts indicated on different wells. Methanol (50 %v/v) and ciprofloxacin (50 µg /ml) were used as negative and positive controls respectively. The plates were left on the work bench undisturbed to allow the antibacterial agents to diffuse for 2 hours at room temperature. Two hours later, plates were incubated at 37°C for 24 hours. The zones of inhibition were observed around the wells

and measured in millimeters (mm) using a metre rule after 24 hrs of incubation and recorded. The mean of the triplicate results were recorded accordingly Anie *etal*., (2011).

Minimum Inhibitory Concentration Determination

The MICs of the methanol and aqueous leaf extracts of *Buchholzia coriacea* against the MRSA isolates were determined using the agar dilution method. Serial doubling dilutions of the leaf extract were carried out using molten Mueller- hinton agar to obtain concentrations of 200 mg/ml,100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml. The mixtures were dispensed into sterilized Petri dishes and allowed to cool and solidify uniformly. Overnight cultures of microorganism (2 drops) were streaked on the well set agar surface. The inoculated agar plates were at room temperature for 2 hours when the inocula have been absorbed completely by the medium. The plates were placed in inverted positions within incubator for 24 hours at 37°C and the plates were observed and recorded. The least concentration of the extract that inhibited the growth of a bacterial isolate (MRSA), that is where no growth was observed, was then taken as the MIC. Anie *etal*., (2022)

RESULTS

According to Table 1, out of the 100 individuals screened for MRSA, 7(7%) were resistant to oxacillin, with 93 (93%) isolates susceptible. Five of these resistant strains occurred in males, while two (2) occurred in females. Phytochemical analysis of methanol leaf extract of *B. coriacea* revealed the presence of tannin, terpenes, flavonoid, steroid, saponin, reducing sugar and alkaloid cardiac glycoside as shown in Table 2.

The antibacterial activity of methanol and aqueous leaf extract of *B. coriacea* on MRSA isolates is presented shown in Table 3.

Table 1: Incidence of MRSA among healthy Community Individuals.

Source	Number of MRSA	Percentage
Male	5	71.4
Female	2	28.6
Total	7	100

Table 2: Phytochemical constituents of leaf extract of *B. coriacea.*

Phytochemicals	Result
Alkaloids	+
Glycosides	++
Resins	+
Flavonoids	+
Steroids	+
Terpenoids	+
Tannins	++
Anthraquinones	+
Reducing sugar	++

+ = present.

- = absent.

Antibacterial activity of methanol and aqueous leaf extracts of *B. coriacea* on selected MRSA isolates from healthy community individuals

The antibacterial activity of methanol and aqueous leaf of *B. coriacea* extract on selected MRSA isolates is presented in Table 3. The inhibition zone diameters (IZD) indicates that methicillin- resistant *S. aureus* (MRSA) isolates 1,2,5 showed the greatest susceptibility to methanol leaf extract (20 mm) at 200 mg/ml concentration; followed by isolate 4, with an IZD of 12.5 mm at 200 mg/ml, and isolate 3, with an IZD of 13 mm also at 200 mg/ml. Generally, the IZDs ranged between 6 mm at 15.5 mg/ml and 20 mm (at 200 mg/ml) concentration. Comparing this result with the control 50µg/ml of ciprofloxacin, it is pertinent to note that the control performed best on isolate 1 with IZD of 35 mm and least on isolate 5with IZD of 28mm.The IZD of the control (ciprofloxacin) was generally higher than those of methanol leaf of the plant.

While the antibacterial activity of aqueous the leaf of *B. coriacea* extract extract on selected MRSA isolates is presented in Table 3. The IZD result evidenced that methicillin- resistant *S. aureus* (MRSA) from Abraka study location isolate 2 showed the greatest susceptibility to aqueous leaf extract (19 mm) at 200 mg/ml concentration; followed by isolates, 1 and 5, with IZD of 18 mm at 200 mg/ml, isolate 3 with an IZD of 13 mm and isolate 4 with IZD of 17mm. According to the results, the IZDs ranged between 5mm at 12.5 mg/ml and 19 mm at 200 mg/ml concentration. The control (50µg/ml of ciprofloxacin), performed best on isolate 1 with IZD 35 mm and lowest on isolate 5 with IZD 28 mm. The IZD of the control (ciprofloxacin) was at all times higher than those of the aqueous leaf extract of the plant.

Table 3: Antibacterial activities of methanol and aqueous leaf extracts of *B. coriacea* on selected MRSA isolates from healthy community individuals

Isolates	Mean zone of inhibition (mm) at different concentration (mg/ml) of methanol leaf extract *Buchholzia coriacea.*						
	200	100	50	25	12.5	Positive control	Negative control
Abk 1	20	18	17	13	11	35	-
Abk2	20	16	14	12	12	32	-
Abk 3	13	11	9	8	6	28	-
Abk 4	15.5	14	12	11	7	30	-
Abk5	20	19	17	15	13	32	-
Isolates	Mean zone of inhibition (mm) at different concentration (mg/ml) of aqueous leaf extract of *B. coriacea.*						
	200	100	50	25	12.5	Positive control	Negative control
Abk 1	18	16	14	13	10	35	-
Abk2	19	14	12	10	10	32	-
Abk 3	13	10	8	7	5	28	-
Abk 4	17	13	11	9	6	30	-
Abk5	18	15	13	10	10	32	-

MIC of methanol and aqueous and leaf extracts of *B. coriacea* on selected MRSA isolates from healthy community individuals

Table 4 shows the MIC of methanol leaf extract of *B. coriacea* on selected MRSA isolates. The results show that every isolates had growth at 12.5 mg/ml concentration but none of them had at 200 mg/ml – 50 mg/ml concentrations. At 25 mg/ml concentration isolate 3 had growth. MIC of the methanol leaf extract of *B. coriacea* against isolates 1, 2, 4 and 5, was 25mg/ml, while that for isolate 3 MIC is 50 mg/ml.

MIC of aqueous leaf extract of *B. coriacea* on selected MRSA isolates from healthy community individuals is presented in Table4. MIC of aqueous leaf extract of *B. coriacea* on selected MRSA isolates from Abraka study location shows that all the isolates were able to grow at 25 mg/ml and 12.5 mg/ml concentrations. Contrarily, none had growth at 200 mg/ml and 100mg/ml concentrations.

However at 50 mg/ml concentration, isolate 3 had growth, while other isolates had no growth 50 mg/ml. Therefore, the aqueous extract had minimum inhibitory concentration for isolate 3 at 100 mg/ml as against 50 mg/ml for isolates 1, 2, 4 and 5. The aqueous extract had higher MIC concentrations than the methanol leaf extract.

Table 3: MIC of methanol and aqueous leaf extracts of *B. coriacea* on selected MRSA isolates from healthy community individuals.

Isolates	Different concentrations methanol leaf extract of *Buchholzia coriacea* (mg/l)					MIC (mg/ml)
	200	100	50	25	12.5	
Abk 1	-	-	-	-	+	25
Abk2	-	-	-	-	+	25
Abk 3	-	-	-	+	+	50
Abk 4	-	-	-	-	+	25
Abk5	-	-	-	-	+	25
Isolates	**Different concentrations aqueous leaf extract of *Buchholzia coriacea* (mg/l)**					
	200	100	50	25	12.5	
Abk 1	-	-	-	+	+	50
Abk2	-	-	-	+	+	50
Abk 3	-	-	+	+	+	100
Abk 4	-	-	-	+	+	50
Abk5	-	-	-	+	+	50

Key: + = positive

- = negative

DISCUSSION

Previous studies have shown that many plants extracts which are known to contain these phytochemicals, have antimicrobial activities and as such, have been helpful as antimicrobial agents. The presence of these phytochemicals in the leaf extract of *B. coriacea* is probably the basis of its antimicrobial activities. This result is in agreement with the result of Ajaiyeoba *etal*.,(2003): Anie *etal*., (2011). Phytochemical analysis carried out on this leaf extracts of *Buchholzia coriacea* revealed the presence of tannin, anthroquinone, glycosides, steroids, terpenoid, alkaloids Onyekaba *etal*., (2011). The antibacterial activity of methanol and aqueous leaf of *B. coriacea* extract on selected MRSA isolates from healthy community individuals is presented in Table 3. The inhibition zone diameters (IZD) indicates that methicillin- resistant *S. aureus* (MRSA) from healthy community individuals isolates 1,2,5 showed the greatest susceptibility to methanol leaf extract (20 mm) at 200 mg/ml concentration; followed by isolate 4, with an IZD of 12.5 mm at 200 mg/ml, and isolate 3, with an IZD of 13 mm also at 200 mg/ml. Generally, the IZDs ranged between 6 mm at 15.5 mg/ml and 20 mm (at 200 mg/ml) concentration. Comparing this result with the control 50µg/ml of ciprofloxacin, it is pertinent to note that the control performed best on isolate 1

with IZD of 35 mm and least on isolate 5with IZD of 28mm.The IZD of the control (ciprofloxacin) was generally higher than those of methanol leaf of the plant.

While the antibacterial activity of aqueous the leaf of *B. coriacea* extract extract on selected MRSA isolates from healthy community individuals is presented in Table 3. The IZD result evidenced that methicillin- resistant *S. aureus* (MRSA) from healthy community individuals isolate 2 showed the greatest susceptibility to aqueous leaf extract (19 mm) at 200 mg/ml concentration; followed by isolates, 1 and 5, with IZD of 18 mm at 200 mg/ml, isolate 3 with an IZD of 13 mm and isolate 4 with IZD of 17mm. According to the results, the IZDs ranged between 5mm at 12.5 mg/ml and 19 mm at 200 mg/ml concentration. The control (50µg/ml of ciprofloxacin), performed best on isolate 1 with IZD 35 mm and lowest on isolate 5 with IZD 28 mm. The IZD of the control (ciprofloxacin) was at all times higher than those of the aqueous leaf extract of the plant.

Table 4 shows the MIC of methanol leaf extract of *B. coriacea* on selected MRSA isolates from healthy community individuals. The results show that every isolates had growth at 12.5 mg/ml concentration but none of them had at 200 mg/ml – 50 mg/ml concentrations. At 25 mg/ml concentration isolate 3 had growth. MIC of the methanol leaf extract of *B. coriacea* against isolates 1, 2, 4 and 5, was 25mg/ml, while that for isolate 3 MIC is 50 mg/ml.

Table 4. MIC of aqueous leaf extract of *B. coriacea* on selected MRSA isolates from Abraka study location shows that all the isolates were able to grow at 25 mg/ml and 12.5 mg/ml concentrations. Contrarily, none had growth at 200 mg/ml and 100mg/ml concentrations.

However at 50 mg/ml concentration, isolate 3 had growth, while other isolates had no growth 50 mg/ml. Therefore, the aqueous extract had minimum inhibitory concentration for isolate 3 at 100 mg/ml as against 50 mg/ml for isolates 1, 2, 4 and 5. The aqueous extract had higher MIC concentrations than the methanol leaf extract in agreement with earlier reported (Mbata).

CONCLUSION

The leaf extracts possessed antibacterial activity against bacterial isolates, with the methanol leaf extract at 25mg/ml had better antibacterial activity than the aqueous leaf extract at the same concentration. The minimum inhibitory concentration of the methanol leaf extract was lower than the MIC of the aqueous extract. Overall, our result is in support of traditional

medicinal values of this plant Further study to be carried out to fractionate the leaf extracts which could enhance the bacterial activity.

ACKNOWLEDGMENTS: We thank Mr Michael Oghenejobo for his assistance in the Laboratory

Conflicts of Interest: No conflict of interest among the authors.

REFRENCES

1. Anie C O. Evaluation of Biofilm and Enterotoxin and Producing Capacity of Methicillin resistant Staphylococcus aureus Isolated from Healthy Persons. Journals of applied science and Environmental management, 2018; 22(12): 1881-1884.
2. Anie C O, Ugwu M C, Ibezim E C, Esimone C O. Antibiogram of Methicillin- Resistant *Staphylococcus aureus* Isolates among Healthy Human Subjects in Oleh, South-Southern Nigeria International Journal of Current. Microbiology and Applied Sciences, 2017; 6(9): 3710-3716.
3. Anie CO. Susceptibility Pattern of Staphylococcus aureus isolated from Cows in Obiarukwu Delta State. Sokoto journal medical laboratory Sciences, 2019; 4(2): 35-41.
4. Anie C.O Esimone CO Ibezim Arhewoh MI 2022 Tropical journal of Pharmaceutical research, 21(4): 825-831.
5. Anie CO. The Antimicrobial Activities of Root Extract of *Buchholzia coriacea* Engler (Wonderful Kola) Sokoto journal medical laboratory Sciences, 2020; 5(1): 006 44–51.
6. Ibezim EC, Kenechukwu FC, Odimegwu DC, Builders PF, Kabele-Toge B, Anie C, Igwilo CO, Otuu FC, Onyechukwu C. Antimicrobial efficacy of a syrup formulation from methanol extract of Garcinia kola seed Afr J Pharm Res Dev, 2011; 3(1): 22-27.
7. Anie CO, Matthew I. Arhewoh, Henry A. Okeri. Antimicrobial Activity Of Crude Extracts Of *Diospyros monbuttensis* (Fam: Ebenaceae) Root And Stem Barks. International Journal of Biomedical Research, 2011; 2(1): 18- 24. 27-32.
8. Anie C O, Egbon O T, Okwunwa S, Elue E, Antimicrobial Activity And Potency Of The Methanolic Extract Of *Anona Muricata* (Soursop) Against Isolate from Wound Swab World Journal of Pharmaceutical Research, 2022; 11(4): 246-257.
9. Ajaiyeoba EO, Onocha P A, Nwozo SO, Sam W. Antimicrobial and cytotoxic evaluation of Buchholzia coriacea stem Bark, *Fitoterapia*, 2003; 74: 706-709.

10. Okafo S O, C O, Anie CO, Nwanua MC. Formulation and evaluation of antimicrobial topical creams from ethanol extract of *Vernonia ambigua* leaves. Nigerian Journal of Pharmaceutical Research, 2019; 15(2): 249-255.
11. Enwa F O, Oghenejobo M, Anie CO,. Ayeh A. Antibacterial Screening of the Ethanol and Aqueous Extract of the Fruit Peel of *Persea Americana* Mill against Selected Enteric Bacteria. Academia Journal of Microbiology Research, 2015; 4(3): 040-046, March 2016 DOI: 10.15413/ajmr..0109.
12. Anie CO, Nwabuokei IG, Oghenejobo M, Enwa FO. The Antibacterial Effect of the Leaf Extract of *Buchholzia coriacae(Capparidaceae)* on Gram-Negative Nasal Isolates Sch. Acad. J. Pharm, 2015; 4(4): 226-231.
13. Onyekaba T U, Arute JE, Anie, C.O. Antimicrobial and phytochemical screening of leaf extract of *Buchholzia coriacea*,(Wonder ful Cola).*Journal of Pharmaceutical and Allied Sciences*, 2011; 8(1): 1283-1288.
14. Onwuliri F C, Mabitine N. Antimcrobial activities of the leaf extract of acacia ataxacantia de. Afican J.Nat. Sc, 2005;p10.
15. Mbata TI, Duru CM, Onwumelu H A. Antibacterial activity of crude seed extracts of *Buchholzia coriacea* E. On some pathogenic bacteria. *Journal of Developmental Biology and Tissue Engineering*, 2009; 1: 001–005.