**Phytoconstituents and Antibacterial Activity of Moringa *Oleifera* Leaf on *Staphylococcus aureus* from Roasted Meat (‘Suya’) in Port Harcourt, Nigeria**

**ABSTRACT**

Antibiotic resistance to conventional antibiotics has continued to be a therapeutic challenge in clinical settings. To this end, alternative therapeutic approaches are being sought after. One such approach is the use of antimicrobial inhibitors from natural sources like medicinal plants. This study aimed to explore the antibacterial potential of aqueous and ethanolic leaf extracts of *Moringa oleifera* plant against *Staphylococcus aureus* isolated from roasted meat ‘Suya’ in Port Harcourt, Nigeria. The antibacterial activity of *Moringa oleifera* leaf extracts against *S. aureus* was determined in vitro in aqueous and ethanolic extracts using the cup plate method at four different extract concentrations (400 mg/ml, 200 mg/ml 100 mg/ml and 50 mg/ml). Analysis of phytoconstituents revealed the presence of tannins, flavonoids, alkaloids, saponins and terpenes. The ethanolic extract revealed a significant degree of antibacterial activity against *S. aureus* isolates, and this inhibition varied at different concentrations while the aqueous extract was ineffective on the test *S. aureus* isolates. The ethanolic extract showed zones of inhibition of 25mm at a concentration of 400 mg/ml, 21mmat 200mg/ml, 13mm at 100mg/ml and 8mm at 50mg/ml. Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) were at 200 mg/ml for the ethanolic plant extract, while no MIC and MBC were observed for the aqueous plant extract. Antibiotic susceptibility testing using the Kirby Bauer disc diffusion method revealed that *Staphylococcus aureus* was susceptible to Ciprofloxacin, Amikacin and Norfloxacin but resistant to Nalidixic acid. The results from this study have further confirmed the promising antibacterial potential of *M. oleifera* in inhibiting the activities of *S. aureus*.

*Keywords: Staphylococcus aureus, Roasted Meat (‘Suya’), Antibacterial Activity, Phytoconstituents, Moringa oleifera.*

**INTRODUCTION**

*Staphylococcus aureus* is part of the normal flora of the surface of the skin as well as mucous membranes especially the anterior nares in healthy persons (Wertheim *et al.,* 2005). The diversity of pathological conditions caused by *S. aureus* evident in humans include osteomyelitis, bacteremia, pneumonia, systemic diseases, urinary tract infections and food poisoning. (Lina *et al.,* 1999). *S. aureus* has been identified as a food borne hazard by food microbiologists rating it as the third major cause of food borne diseases across the world (Bean *et al.,* 1997; Tirado & Schimdt, 2001). *S. aureus* because of its non-fastidious nature can grow on substrates with a wide temperature range of 7–480C °C, reduced water activity (aw) of 0.86 and pH values of 4.2 to 9.3 (Narmanno *et al.,* 2005). Research has shown that *S. aureus* cannot compete favorably with the naturally occurring microbiota in raw foods, as a result, contamination is due to improper handling practices and storage conditions that favor the multiplication of the organism and its associated enterotoxins (Stewart, 2005). Food handlers whose hands and noses have been colonised by the organism have been suggested to be the major source of staphylococcal food contamination (Giami, *et al.,* 2019; Wertheim *et al.,* 2005).This contamination usually occurs through secretions from their respiratory tracts or by direct contact (Kluytmans & Wertheim, 2005).‘Suya’ is a local Nigerian term for barbecued, spiced, smoked or roasted meat. It is a delicacy that originated in Northern Nigeria among the Hausas and has become popular in all parts of Nigeria (Edema *et al.,* 2008). This street food has been reported to harbor enterotoxigenic strains of *S. aureus* strains which mostly occurs through cross contamination from their respective food handlers (Giami *et al.,* 2019).Also, this delicacy is prepared from boneless meat (Abdullahi *et al.,* 2004) whereas meat and meat products have been implicated as one of the commonly incriminated foods that enables the *S. aureus* to thrive (Wieneke *et al.,* 1993). The recent rise in antibiotic resistance has become a public health concern and *S. aureus* has becomea formidable bacteria known for its resistance to a wide array of antibiotics (CDC, 2019; Lowy, 1998). Food can serve as a means of transfer of drug-resistant strains of the bacteria to humans giving rise to infections that become a therapeutic challenge due to the limited chemotherapeutic options available to clinicians as a result of the surge in antibiotic resistance (Hammad *et al.,* 2012). it has therefore become necessary to explore alternative therapeutic options. One of such avenues with great potential is the use of plant extracts with potential in inhibiting bacterial activities (Leone & Garmer, 2019). *Moringa oleifera*, often referred to as the "drumstick tree" or "miracle tree," has emerged as a plant of interest in this context. *Moringa oleifera* often referred to as the "drumstick tree" or "miracle tree," has been celebrated in traditional medicinal systems for centuries because of its nutritional and therapeutic properties. Various components of the Moringa tree, such as its leaves, seeds, and bark, have undergone investigation for their potential therapeutic properties associated with the presence of bioactive compounds, such as alkaloids, flavonoids, polyphenols, terpenes, and terpenoids (Walter *et al*., 2011). It has therefore become imperative to evaluate the in vitro antibacterial effect of *Moringa oleifera* leaf extract on *Staphylococcus aureus,* especially strains isolated from the food chain, to protect public health and perhaps provide therapeutic solutions to infections caused by antibiotic resistant strains. This study was carried out to evaluate the invitro inhibitory effect of *Moringa oleifera* leaf extract on *Staphylococcus aureus* isolated from roasted meat ‘Suya’ in Port Harcourt metropolis, Nigeria.

**MATERIALS AND METHODS**

**Sample Collection**

The roasted meat ‘Suya’ samples were purchased from different selling points in the five major zones that make up Port Harcourt metropolis in the paper wrap used by the sellers to package them. It was then transferred using a sterile spatula into sterile containers with ice packs and sent immediately to the laboratory for analysis.

**Isolation and Identification of *S. aureus***

Ten (10g) of a representative portion of the roasted meat ‘suya’ sample was homogenized in 90ml of sterile peptone water (0.1%). A wire loop, sterilized by flaming in a Bunsen burner, was used to inoculate the food sample from the stock solution onto mannitol salt agar for the presumptive identification of *S. aureus* which was incubated aerobically at 370C for 24hours as described by El-Jakee et al. 2019. Further confirmatory tests such as Gram’s staining and biochemical tests were done according to standard methods by Cheesebrough, 2000.

**Collection of *Moringa oleifera* Plant**

The plant material used was harvested from the Rivers State University farm and transferred into sterile polythene bags, which were labelled appropriately and taken directly to the laboratory for analysis. The fresh leaves were confirmed by a botanist from the Department of Plant Science and Biotechnology at the Rivers State University. The fresh leaves were then dried in shade.

**Preparation of Aqueous and Ethanolic Plant Extract**

The dried plant leaves were processed according to standard methods as described by Alsadig *et al,* 2016, Briefly, one hundred grams of the dried plant leaves were ground to powder with a grinder and extracted as follows: Two amounts of 25g of the powdered leaves were separately extracted in 500ml conical flasks with ethanol (ethanolic extraction) for 3 hours in soxhlet apparatus. Then the extracts were filtered and evaporated under reduced pressure using Rotavapor. Another 25g of the powdered leaves were separately extracted in 500ml conical flasks with 100ml of sterile distilled water (aqueous extraction) by infusion overnight, then it was filtered (through Whatman No.1 filter) and the filtrate was dried and weighted. The following concentrations 400 mg/ml, 200mg/ml, 100mg/ml, and 50mg/ml of these extracts (ethanolic and aqueous respectively) were prepared and used in antimicrobial susceptibility testing.

**Phytoconstituent Screening**

Phytoconstituent screening was carried out for the aqueous and ethanolic extracts of the leaves using the methods as described by Abdulfatai *et al*. 2018.

**Test for Phenols and Tannins**

About 0.5grams of the aqueous and ethanol extracts respectively was mixed separately and 2ml of 2% solution of ferric chloride (FeCl3-) was then added and observed for a change in colour.

**Test for Alkaloids**

This test was intensified by weighing 0.5grams of each of the aqueous and ethanol extracts separately and mixed with 1% hydrochloric acid and shaken for two minutes. The mixture was filtered and drops of Mayers reagent was added to each different mixture and it was observed for colour change.

**Test for Saponins**

This test was intensified by weighing 0.5grams of each of the aqueous and ethanol extracts separately and mixed with 5ml of distilled water in a test tube and was vigorously shaken and observed for any change in colour.

**Test for Flavonoids (Alkali Reagent Test)**

This test was intensified by weighing 0.5grams of each of the aqueous and ethanol extracts and were mixed separately. 2ml of 2% solution of NaOH was then added and observed for colour change after which 1-2 drops of dilute acid were added.

**Test for Terpenes**

About 0.5g of each of the aqueous and ethanol extracts were dissolved separately in 5ml of water, 2-3 drops of 10% of ferric chloride solution was then added and observed for colour change.

**Sterility Test of Leave Extract**

A drop of each of the extracts was placed on a sterile Muller Hinton agar plate and incubated at

37oC for 24hours.

**Evaluation of Antibacterial Activity of *Moringa* *oleifera Leaves* Extract**

The antibacterial activity of the extracts was determined using the agar-well diffusion method (cup plate method). Mueller-Hinton agar media was sterilized by autoclaving, cooled, and 1ml of a 24-hour-old culture of the test organism, whose turbidity was adjusted to a 0.5 McFarland standard, was added to 19 ml of already prepared Mueller-Hinton agar. Using a sterile cock borer, wells of 5mm diameter were bored on the agar surface, and 0.1ml of both the aqueous and ethanol extracts at the following concentrations 400mg/ml, 200mg/ml, 100mg/ml and 50mg/ml were dispensed into each well. The aqueous and ethanolic plant extracts had four bores each, one bore for each concentration. The plates were allowed to stand for one hour for the pre-diffusion of the extracts to occur before incubating for 24 hours at 37 °C and were then observed for the presence of zone of inhibition and their diameters measured in millimeters (mm).

**Evaluation of Antibiotic Sensitivity of *S. aureus* using the Disc Diffusion Method**

The antibiotic susceptibility pattern of the test organism was determined using disc diffusion method according to Bauer *et al.* 1966 on Mueller–Hinton agar plates. A standardized inoculum of 0.5 McFarland turbidity of the test organism was prepared from pure overnight culture and tested against antibiotic discs of Ciprofloxacin (10μg), Norfloxacin (10μg), Amikacin (10μg) and Nalidixic Acid (30μg). The antibiotic discs were placed on the media about two centimeters apart. After overnight incubation at 37°C aerobically, the culture was examined for zone of inhibition of bacterial growth around the respective discs, which was measured in millimeters (CLSI, 2018).

**Minimum inhibitory concentration (MIC) Determination**

The minimum inhibitory concentration (MIC) refers to the lowest concentration of an antimicrobial agent that completely halts the growth of a test organism and can be detected without the need for magnification (CLSI, 2006). Aqueous and ethanolic concentrations were prepared by

dilution using distilled water to obtain different concentrations of 400mg/ml, 200mg/ml, 100mg/ml, and 50mg/ml. one (1) ml of each extract concentrations and that of Mueller Hinton broth was mixed, and 0.1ml of standardized inoculum of the test organism was added to each of the test tubes above. The tubes were incubated at 37 °C for 24 hours. Tubes containing broth and leaf extracts were used as positive control while tubes containing broth and inoculum were used as negative controls. The tubes were observed after 24 hours of incubation (Cheesebrough, 2000).

**Determination of Minimum Bactericidal Concentration (MBC)**

Sterile Mueller Hinton agar plates were separately inoculated with culture from each of the MIC tubes that showed no evidence of turbidity. The plates were incubated at 370C for 24 hours. The MBC was determined as the highest dilution that yielded no single bacterial colony on the agar surface (Cheesebrough, 2000).

**Data Analysis**

Results were presented in tables and interpreted accordingly using descriptive statistics (Ogbe *et al.,* 2011).

**RESULTS**

**Evaluation of Plant Extract Sterility**

The sterility test carried out on the aqueous and ethanol plant extracts revealed the absence of any contaminant (Table 1).

Table 1. Evaluation of Plant Extract Sterility

|  |  |
| --- | --- |
| ***Moringa oleifera* Plant Extract** | **Sterility** |
| **Aqueous** | **-** |
| **Ethanolic** | **-** |

Key: (-) absence of contaminant (An indication of sterility)

**Phytoconstituents of the Plant Extract**

The phytochemical screening of the aqueous and ethanolic extract revealed the presence of various bioactive compounds. The aqueous and ethanolic extracts revealed the presence of tannins, flavonoids, alkaloids and saponins. The ethanolic extract alone revealed the presence of terpenes. Phenols were not found in both aqueous and ethanolic extracts. (Table 2).

A blue-black coloration indicated the presence of Phenols and Tannins following the addition of ferric chloride (Ajayi & Fadeyi 2015; Krishnapriya, 2017). The presence of alkaloids was confirmed by the formation of a yellow cream precipitate following the addition of Mayer’s reagent (Ajayi & Fadeyi 2015; Krishnapriya, 2017). The formation of stable foam was an indication of the presence of Saponins after the addition of distilled water (Mohammed *et al.,* 2017). Flavonoids were evident by the formation of an intense yellow color which turned colorless upon the addition of few drops of dilute acid (Ajayi & Fadeyi 2015; Mohammed, 2018). Terpenes were confirmed by the formation of a violet precipitate following the addition of ferric chloride.

**Table 2: Phytochemical Composition and Antibacterial Activities of *Moringa oleifera* Plant Extract.**

|  |  |  |
| --- | --- | --- |
| Phytoconstituents | Aqueous Extract | Ethanolic Extract |
| Flavonoids | + | + |
| Alkaloids | + | + |
| Terpenes | - | + |
| Saponins | + | + |
| Tannins | + | + |
| Phenols | - | - |

Key: (+) = Presence, (-) = Absence

**Antibacterial Activity of Aqueous and Ethanolic Extract of *Moringa oleifera* on *Staphylococcus aureus.***

Ethanolic extract of *M. oleifera* revealed significant inhibitory activity on the test *S. aureus* isolates and this inhibition varied at different concentrations while the aqueous extract was ineffective on the test *S. aureus* isolates. The ethanolic extract showed zones of inhibition of 25mm at a concentration of 400 mg/ml, 21mmat 200mg/ml, 13mm at 100mg/ml and 8mm at 50mg/ml (Tables 3&4).

**Table 3. Antibacterial Activity of Aqueous Extract of *Moringa Oleifera on S. aureus.***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Concentration (mg/ml) | 400 | 200 | 100 | 50 |
| Zone of inhibition (mm) | **-** | **-** | **-** | **-** |

**Table 4. Antibacterial Activity of Ethanolic Extract of *Moringa Oleifera on S. aureus.***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Concentration (mg/ml) | 400 | 200 | 100 | 50 |
| Zone of inhibition (mm) | 25 | 21 | 13 | 8 |

Key: Resistant: zone diameter = ≤15mm, Intermediate: Zone diameter =16-20mm, Sensitive: Zone diameter = ˃ 21mm. Std disc conc: Standard disc concentration.

**Minimum Inhibitory Concentration of Aqueous and Ethanolic Extract of *Moringa oleifera* on *S. aureus****.*

The MIC for the ethanolic extract was at a concentration of 200mg/ml for *S. aureus*. no MIC was recorded for the aqueous extract (Table 5).

**Table 5. Minimum Inhibitory Concentration (MIC) of Aqueous and Ethanolic Extract of *Moringa oleifera* on *S. aureus****.*

|  |  |  |
| --- | --- | --- |
| Extract concentration (mg/ml) | Aqueous | Ethanolic |
| 400 | + | - |
| 200 | + | - |
| 100 | + | + |
| 50 | + | + |

Key: (+) = growth. (-) = No growth

**Minimum Bactericidal Concentration (MBC) of Aqueous and Methanolic Extract of *Moringa oleifera* on *S. aureus***

The MBC for the ethanolic extract was at a concentration of 200mg/ml for *S. aureus*. No MBC was recorded for the aqueous extract (Table 6).

**Table 6. Minimum Bactericidal Concentration (MBC) of Aqueous and Methanolic extract of *Moringa oleifera* on *S. aureus***

|  |  |  |
| --- | --- | --- |
| Plant Extract concentration (mg/ml) | Aqueous | Ethanolic |
| 400 | + | - |
| 200 | + | - |
| 100 | + | + |
| 50 | + | + |

Key: (+) = growth. (-) = No growth

**Antibiogram of *S. aureus* Isolates using Conventional Antibiotics**

*S. aureus* was susceptible to Ciprofloxacin, Amikacin and Norfloxacin with the following diameters for their zones of inhibition 24mm, 23mm and 21mm respectively but was observed to be resistant to Nalidixic acid with a 4mm zone of inhibition diameter (Table 7.)

**Table 7. Antibiogram of *S. aureus* Isolates using Conventional Antibiotics**

|  |  |  |
| --- | --- | --- |
| Antibiotic  | Std disc conc (µg) | Diameter of inhibition zones of S. *aureus* (mm) |
| Ciprofloxacin | 10 | 24 |
| Norfloxacin | 10 | 23 |
| Amikacin | 10 | 21 |
| Nalidixic Acid  | 30 | 4 |

Key: Resistant: Zone diameter ≤15mm, Intermediate: Zone diameter 16-20mm, Sensitive: Zone diameter greater than 21mm. Std disc conc: Standard disc concentration.

**Antimicrobial Activity of *Moringa Oleifera* Leaf Extracts against *S. aureus* Compared to Conventional Antibiotics.**

The commercial antibiotics Ciprofloxacin, Norfloxacin and Amikacin used on the test organism were more effective than the *Moringa oleifera* plant extract.

**DISCUSSION**

The absence of growth on the sterile Mueller Hinton agar plates confirmed the sterility of the *Moringa oleifera* leaf extracts indicating that there was no contamination from external sources (Ajayi & Fadeyi 2015; Cheesebrough, 2000). The presence of the bioactive phytoconstituents tannins, flavonoids, alkaloids, saponins and terpenes in the plant extracts is a confirmation of the medicinal properties of the plant as suggested by literature and is most likely responsible for the inhibitory activities exhibited by the plant extract against the test organism *S. aureus* (Mohammed, 2018). Saponins, flavonoids and alkaloids have been reported to be responsible for the antibacterial activities recorded in medicinal plants (Anwar *et al.*, 2017). These findings can be compared with the work of Akinyeye et al. 2014 whose study similarly found tannins, saponins and alkaloids in *Moringa oleifera* leaf extract. The ethanolic extract was seen to be more effective than the aqueous extract in inhibiting the growth of *S. aureus*. This difference in activity is likely as a result of the differences in polarity of the two solvents as non-polar solvents tends to be associated with poorextraction of the phytoconstituents which is directly responsible for the plant bioactivity.These findings, agree with those of Kiran & Tafida 2013 who also reported significant activity in the ethanolic extract over the aqueous extract, in contrast, studies carried out by Alsadig et al. 2016 reported that both ethanolic and aqueous leaf extracts exhibited significant activity against *S. aureus* and other bacterial pathogens. These variations could be attributed to the choice of solvent used as well as the method of extraction used which plays a key role in the expression of phytoconstituents (Abdulfatai *et al.,* 2018; Pal *et al.,* 1995).

The MIC for the ethanolic plant extract was at a concentration of 200mg/ml against the test organism *S. aureus* and no MIC was recorded for the aqueous plant extract. The MBC was also recorded at a concentration of 200mg/ml for the ethanolic plant extract with no significant inhibitory activity in the aqueous plant extract. These results are in contrast with report obtained by Sheriffdeen et al. 2021 who recorded lower MIC values of 60mg/ml for both the ethanolic and aqueous extract against clinical isolate of *S. aureus* and MBC values of 80mg/ml for both the aqueous and ethanolic extracts against *S. aureus*. These variations may be due to the slight difference in the phytoconstituents recorded in both studies (Magaji *et al.,* 2020; Patricia *et al.,* 2019). Research has shown that phytoconstituents of medicinal plants vary with the geographical location in which the plant is grown and harvested (Abdulfatai *et al.,* 2018). *S. aureus* isolated from roasted meat “suya’’ in this study when exposed to conventional antibiotics was observed to be susceptible to Ciprofloxacin, Amikacin and Norfloxacin but resistant to Nalidixic acid, this result was in agreement with results obtained by Giami et al. 2019 where *S. aureus* isolated from roasted meat ‘suya’ was also sensitive to Ciprofloxacin, However, slight variations were observed when results from this study was compared to work done by Alsadig et al. 2016 who recorded intermediate sensitivity among *S. aureus* isolates isolated from urinary tract infected patients. Also, Alsadig et al. 2016 recorded that the commercial antibiotics used on the test isolate were observed to be more effective than the *Moringa oleifera* leaf extracts in inhibiting the growth of *S. aureus.*

**CONCLUSION**

The results of this study revealed the presence of the phytoconstituents tannins, flavonoids, alkaloids, saponins and terpenes in the extract of *Moringa oleifera* plant leaves with the ethanolic plant exhibiting a more inhibitory effect on *S. aureus* than the aqueous plant extract*.* The conventional antibiotics were also observed to be more effective than the plant extract in inhibiting the growth of *S. aureus* isolates. Further studies could explore different extraction methods, as this is an important determining factor in the activity of the bioactive compounds. Additionally, it is possible that an even higher concentration of the ethanolic plant extract could exhibit higher inhibitory activity against *S. aureus.*

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