**ANTIBACTERIAL AND ANTIBIOFILM ACTIVITIES OF AQUEOUS FRACTION FROM THE STEM-BARK METHANOL EXTRACT OF *PARKIA BIGLOBOSA* AGAINST PATHOGENS ISOLATED FROM CHRONIC WOUNDS IN KADUNA, NIGERIA.**

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Abstract

Biofilm is a 3D structured community of microorganisms usually enclosed in an extracellular polysaccharide matrix and act as a microbial battlefront. They are often polymicrobial and when the associated microorganisms are pathogenic, biofilms become a significant virulence factor and contribute to wound chronicity. The antibiofilm effect of aqueous fraction from stem-bark methanol extract of *Parkia biglobosa* (Jacq.) R.Br.ex G.Don, also known as the African Locust Bean tree, was determined against biofilm-producing bacteria isolated from chronic wounds of patients in Kaduna, Nigeria. One hundred and sixty-eight bacterial isolates were obtained from chronic wounds samples of patients in four selected health facilities between December 2020 to November 2021. The isolates were identified using standard microbiological methods. Biofilm formation was determined and biofilm inhibitory and antibiofilm activities of the aqueous fraction from the stem-bark methanol extract of *Parkia biglobosa* were investigated using microtitre plate method as described by Stepanovic  *et al.,* 2007, Tang  *et al.,* 2011. 115 (68.5%) isolates were multidrug-resistant (MDR) to the antibiotics tested while 60 (52.2%) of the MDR isolates were biofilm-formers. The fraction was found to possess strong biofilm-inhibiting activity of 76.4%±6.3 against *Staphylococcus* spp. at half minimum inhibitory concentration (sub-MIC) and 96.8%±0.2 against *Proteus* spp. at half minimum bactericidal concentration (sub-MBC) and antibiofilm activity against pre-formed biofilms ranged from 74.2%±10.7 against *Staphylococcus* species, 80.0%±4.4 against *Proteus* spp. at sub-MIC and 96.7%±0.1 against *Pseudomonas*  spp. at sub-MBC. Aqueous fraction from stem-bark methanol extract of *Parkia biglobosa* possesses potent biofilm inhibitory and antibiofilm activities thereby providing a potential alternative treatment for bacterial infections.

**Keywords: Biofilm, polymicrobial, chronic wounds, aqueous fraction, stem-bark**

INTRODUCTION

Chronic wounds are hard-to-heal wounds which are often colonized by a host of microorganisms including bacteria, fungi, viruses and protozoa which co-exist in communities enclosed by extracellular matrix known as biofilms (Wu *et al.,* 2018, Durand *et al.,* 2022). Among all microbial and chronic infections, including chronic wounds, 65% and 85% respectively are associated with biofilm formation (Jamal *et al.,* 2018). Biofilms are structured communities of bacteria, fungi and other microorganisms that may be found attached to a surface or in aggregates without being adhered to a surface and are usually enclosed in a self-produced extracellular polysaccharide matrix (Percival *et al.,* 2017). Biofilm is the preferred state of 90% of bacteria (Flemming and Wuertz, 2019) and it serves as a survival mechanism against environmental stress with resulting increase in resistance to antibiotics and disinfectants (Chen *et al.,* 2018). Polymicrobial nature of biofilms in wounds favours the emergence of antibiotic-resistant strains (Anju *et al.,* 2022). Resistance to antibiotics has been shown to be 1000 times more in attached than in planktonic cells either resulting from increased mutation rates, upregulation of efflux pumps or reduced metabolic activity in biofilms (Buch *et al.,* 2019). Antimicrobial resistance (AMR) can increase complications and associated costs of treatment of chronic wound infections (Littmann *et al.,* 2020).

The management of chronic wound include the use of both topical and systemic antimicrobial agents among other treatment protocols (Iyun *et al.,* 2016). This has provided a selective pressure on antibiotic-resistant strains leading to emergence of antibiotic resistance (Sharma *et al.,* 2019). Caputo *et al.,* (2022) reported that 50-71% of chronic wound patients are prescribed at least one antibiotic per wound in the course of treatment. Excessive usage of antibiotics has been associated with patients with chronic wound infections particularly in developing countries where antibiotics are readily accessible over-the-counter (Iqbal *et al.,* 2017, Mohammed *et al.,* 2017).

Overuse of antibiotics have contributed immensely to AMR while at the same time new antibiotics are not be found unlike in the earlier antibiotic era. Alternative natural therapy is being sought to combat the menace of resistance and this search mainly of plants origin may provide a remedy to AMR in the coming decades. Due to the duration of chronic wounds and failure to heal, patients so affected may resort to various alternative particularly traditional and herbal medicines. Plants have been known through the ages of folk medicines as a great source of phytochemicals which have been employed to take care of various ailments, and *Parkia biglobosa* is one of such plants (Builders, 2014). *Parkia biglobosa* (Jacq.) R. Br. Ex. G. Don., also known as the African locust bean tree, is a perennial deciduous tree of the sub-family Mimosoideae and family Fabaceae (Amusa *et al.*, 2014). There are 35 known species of the genus Parkia (tribe Parkieae). Commonly recognized species are *Parkia filicoides, Parkia bicolor, Parkia roxburghii, Parkia biglandulosa* and *Parkia madagascariesis*. Only 3 are found in Africa and 2 of these species are in Nigeria namely *Parkia biglobosa* and *Parkia bicolor* (Adaramola *et al*., 2013). *Parkia biglobosa* is found widely growing in diverse environmental conditions; from the tropical rain forest to arid zones, lower savannah to lowland forest. It is reported to be originally from South America and it is distributed from the Atlantic Coast in Senegal to southern Sudan, northern Uganda and has its greatest belt in West Africa (Amusa *et al.*, 2014). *Parkia biglobosa* occurs naturally in Africa and may be found as the only tree species in a location. It is most conspicuous in anthropogenic landscapes but occurs in rocky hills, e.g. sandstone hills and stony ridges. It is a tree of savannahs and natural forests. It endures very harsh weather conditions such as drought and it is fire-resistant and nitrogen-fixing. It is able to withstand drought because of its deep tap root and its wide crown provides a suitable environment to grow other plants, such as maize, cassava, yams, sorghum and millet (Builders, 2014).

*Parkia biglobosa* is a valuable plant of immense resources for the locals in sub-Saharan Africa due to its multi-purpose function as a source of food, nutrition, medicine and income (Akin-Idowu *et al*., 2018). The plant is of wide economic importance and it has been used for a range of purposes in West Africa as fodder, food, medicine, manure, fuel, timber. The fruit pulp and seeds are both suitable for human consumption. The pulp which is mealy, serves as a major source of energy and nutrients consisting of carbohydrates, proteins, lipids, carotenoids, vitamins A, B, C and oligo-nutrients (Nyadanu *et al*., 2017). It is traditionally consumed by many local Africa populations. The leaves can be boiled, mixed with cereal flour and eaten as vegetable. The young flower buds are also edible and added to salads (Musara *et al*., 2020). The seeds are important sources of plant proteins to rural communities (Akin-Idowu *et al*., 2018) and are also rich in energy, sugars, Vitamin C and lipids and bioactive compounds such as phenolic compounds which have health-promoting properties (Dedehou *et al*., 2016). Parkia seeds are fermented to make a food condiment which is rich in dietary protein and fat-rich flavour; and ingredient of the traditional soups and stews eaten in West Africa and in Nigeria, it is known as dawadawa (Hausa), Iru (Yoruba) and Ogiri (Igbo). It contributes useful amounts of lysine and riboflavin, which are deficient in the diets in this region.

The leaves, bark, roots and flowers have been used in folklore medicine in the treatment of many diseases such hypertension, malaria, respiratory conditions, leprosy, rheumatism, jaundice, wounds, diarrhoeal diseases, toothaches and so on (Dedehou *et al*., 2016, Adaramola *et al*., 2013, Udobi and Onaolapo, 2010).

*Phytochemical composition of Parkia biglobosa*

The stem-bark of *Parkia biglobosa* contains flavonoids, tannins, terpenes, saponins, sterols, phenols, reducing sugars and long-chain esters and catechins of various kinds (Builders *et al.*, 2012, Udobi and Onaolapo, 2009). The use of medicinal plants as treatments against microbial invasion and remedies for diseases dates back to early civilizations and there has been a global resurgence of herbal preparations and integration of phytomedicines in orthodox health care systems (Abioye *et al*., 2013). Emergence of accessible medicinal plants and discovery of new active substances will go a long way to tackle antimicrobial resistance and revolutionize the world of pharmaceuticals (Osemwegie & Dahunsi, 2015).

METHODOLOGY

*Collection of plant material*

The stem-bark of the plant, *Parkia biglobosa* was collected from ABU Dam area of Samaru village in Zaria, Kaduna State. The plant sample was deposited and authenticated by Mr Namadi Sunusi in the Herbarium section of the Department of Botany, Ahmadu Bello University, Zaria with a voucher number (VN) ABU07064.

*Preparation of the plant material*

Upon collection, the stem-bark was washed under clean running tap water, air-dried at room temperature in the laboratory for 7 days and then grounded into coarse powder in a mortar (Harbonne, 1998). The grounded powder was extracted with distilled water and methanol separately using a Soxhlet apparatus. Subsequently, the solvents were removed by gentle heating and evaporation and the extract obtained was stored in a desiccator until needed (Udobi and Onaolapo, 2012). The methanol extract showed better antibacterial activity in a preliminary susceptibility test and was chosen for further tests. The methanol extract of the stem-bark was fractionated sequentially using petroleum ether, chloroform and water.

*Fractionation of the plant extract*

The dried plant extract was first grounded in a mortar, hydrated with distilled water, and then shaken vigorously in a separating flask. The mixture was filtered with the aid of filter paper to remove solid remains or mass. Petroleum ether was added to the filtrate and shaken gently and allowed to stand on the laboratory bench. Upon settling the upper layer of petroleum ether layer was removed gently and concentrated. Chloroform was added to the remaining mixture, shaken gently and left to settle. The chloroform layer was separated from the aqueous layer and concentrated to dryness until all solvent content had evaporated. The aqueous layer was dried using mild heat and the fraction obtained was stored in a dry place until needed (Udobi and Onaolapo, 2012).

*Antibacterial susceptibility testing using the aqueous fraction from stem-bark methanol extract of Parkia biglobosa*

Agar well diffusion method was used to determine the antibacterial susceptibility of the isolates to the aqueous fraction from the stem-bark methanol extract of *Parkia biglobosa*. Sterile Mueller Hinton agar was inoculated aseptically with 100µl of ~108 CFU/ml (0.5 MacFarland standard) concentration of inoculum spread over the agar surface. Wells were bored into the agar plates using sterile 6mm cork-borer and each of the bored wells were sealed with a drop of sterile molten agar. One hundred microlitre of different concentrations 6.25mg/ml, 12.5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml of the fraction was dispensed into the previously labelled wells. The plates were left on the work bench for one hour at room temperature to allow for diffusion of the fraction and then incubated at 37OC for 24 hours. Zones of inhibition were measured to the nearest millimetre (Udobi and Onaolapo, 2009; Jauro *et al*., 2018) and the susceptibility results of the fraction against the isolates was compared to the CLSI interpretative chart for Gentamicin and Ciprofloxacin (CLSI, 2018).

*Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)*

Standardized inoculum of each isolate (~108Cfu/ml) was prepared. Two millilitre (2ml) was mixed in sterile Mueller Hinton broth in test tubes with 2ml of extract to obtain final concentrations ranging from 3.125mg/ml to 100mg/ml. Two test tubes were used as controls; positive control contained only the growth organisms without extract while negative control had only sterile media. The negative control was to confirm the sterility of the broth and the positive control, the viability of the organisms. The test tubes were incubated at 37OC for 24 hours, after which the tubes were removed gently and observed for turbidity at the different concentrations. The lowest concentration of the fraction that showed no turbidity or growth of organism was considered as the minimum inhibitory concentration (MIC).

Subsequently, the contents of the tubes with concentrations of extracts which inhibited growth were plated on to sterile Mueller Hinton agar plates and incubated at 37OC for 24 hours. Afterwards, the plates were observed for growth or no growth. The lowest concentration in which there was no growth of organisms was considered the minimum bactericidal concentration (MBC) (Udobi and Onaolapo, 2009; Jauro *et al*., 2018).

*Determination of biofilm inhibition and antibiofilm activity of aqueous fraction from stem-bark methanol extract of Parkia biglobosa*

Two stages of biofilm development were considered; prevention of biofilm attachment and destruction of 24-hour preformed biofilms.

*Determination of biofilm inhibition by aqueous fraction from stem-bark methanol extract of Parkia biglobosa*

Biofilm inhibition by the aqueous fraction from stem-bark methanol extract of *Parkia biglobosa* was evaluated using 96-well polystyrene flat-bottom microtitre plates with the method described by Antunes *et al*., (2010). Two hundred microlitre of fresh trypticase soy yeast broth (TSB) in 1% glucose inoculated with 20μl of standardized isolates (final concentration 106 CFU/mL) was aliquoted into each well of microtitre plate and cultured in the presence of 20μl sublethal concentrations of the fraction thus; sub-MIC, MIC, and sub-MBC. Wells containing sterile media was used as control. The plates were incubated at aerobically 37°C for 48h.

After incubation, supernatant was pipetted and each well washed thoroughly with sterile physiologic saline to remove free-floating cells and the plates dried in the oven at 60oC for 45 minutes. The biofilm formed was stained with 0.1% aqueous crystal violet solution and incubated at room temperature for 15 min. Following incubation, the excess stain was removed by washing the plate three times with sterile physiologic saline. The plates were allowed to dry completely. Subsequently, the dye bound to the cells was solubilized by adding 150𝜇l of 95% ethanol to each well to distain the wells and incubated at room temperature for 15min. The absorbance was measured using microplate reader at a wavelength of 570nm after gentle shaking. The mean absorbance of the sample was determined and the results expressed as percentage biofilm inhibition.

Percentage biofilm inhibition was calculated using equation described by Olawuwo *et al*, (2022):

Percentage inhibition (%) = Absorbance of control - Absorbance of experimental x 100 (%)

Absorbance of control

*Determination of antibiofilm activity of aqueous fraction from stem-bark methanol extract of Parkia biglobosa*

Antibiofilm activity of the aqueous fraction from stem-bark methanol extract of Parkia biglobosa was determined by a modification of the biofilm inhibition method. Biofilms were allowed to form as previously described in biofilm formation assay. Two hundred microlitres of TSB in 1% glucose was inoculated with 20μl of standardized isolates in 96-well microtitre plates. The plates were incubated at 37OC for 24 hours to allow for formation of biofilms. After 24 hours, the plates were removed from the incubator and 20μl of the extract containing sub-MIC, MIC and sub-MBC were introduced into each of the wells to determine the anti-adherent effect of the fraction on preformed biofilms. The plates were returned into the incubator for another 24 hours after which they were washed as previously described, dried and stained. Absorbance was read using the ELISA plate reader. Results were interpreted by comparing absorbance of test samples with that of TSB (control) and percentage antibiofilm activity determined (Olawuwo *et al*, 2022).

**RESULTS**

The fraction demonstrated concentration-dependent antibacterial activity as activity increased at higher concentration. Whereas *Pseudomonas* spp. was the least susceptibility at the highest concentration used, *E. coli* was least susceptible at the lowest concentration. *Staphylococcus*, *Klebsiella* and *Proteus* species showed similar susceptibilities as concentration increased (**Table 1**).

The minimum inhibitory concentration of the fraction against all the isolates tested indicated higher susceptibility of *Proteus* and *Klebsiella* species (3.125-6.25mg/ml), and least for *Pseudomonas* and *E. coli* (12.5-25mg/ml and 6.25-12.5mg/ml respectively). Minimum bactericidal concentration was lowest for *Staphylococcus* and *Klebsiella* species (6.25-25mg/ml and 6.25-12.5mg/ml), and highest for *Pseudomonas* species and *E. coli* (12.5-50mg/ml) [**Table 2**].

The fraction was observed to possess a strong biofilm inhibitory activity (76.4% ±6.3 for *Staphylococcus* spp.) at concentrations below MIC (6.25-12.5mg/ml) and up to 80.4±3.1 to 81.3±3.0 for *Proteus* *and Klebsiella* species. at 3.125-6.25mg/ml (**Table 3**). The observed activity of the fraction against pre-formed biofilms is presented in **Table 4**  indicating potent antibiofilm activity ranging from 73.6%±13.9 to 80.0%±4.4 against *Klebsiella and Proteus* species at concentrations below the MIC (1.56mg/ml) and 94.9%±2.3 to 96.7%±0.1 against *E.coli*, *Proteus* and *Pseudomonas* at sub-MBC concentration of 6.25mg/ml. Biofilm inhibition above 50% was regarded as good antibiofilm activity while 0-50% was considered as poor and less than 0% was no inhibition or enhancement of biofilm formation (Olawuwo *et al*., 2022).

Table 1. Antibacterial susceptibility patterns of aqueous fraction of *Parkia biglobosa* methanol stem bark extract against isolates from chronic wounds from patients attending selected hospitals in Kaduna

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Zones of inhibition (mm) (mean± standard deviation) | | | | | |
| Concentration of extract (mg/ml) | *Staphylococcus*. spp. (n=102) | *E. coli* (n=24) | *Klebsiella* spp. (n=10) | *Proteus* spp. (n=22) | *Pseudomonas* spp. (n=10) |
| **6.25** | 7.9±2.7 | 6.2±1.1 | 8.0±2.9 | 7.6±2.5 | 6.3±0.7 |
| **12.5** | 9.4±3.5 | 6.8±2.1 | 9.7±2.6 | 8.9±2.9 | 6.5±1.3 |
| **25.0** | 11.0±4.1 | 7.3±2.4 | 11.4±2.6 | 10.7±2.9 | 7.8±1.9 |
| **50.0** | 13.7±4.4 | 9.5±2.6 | 13.6±1.8 | 12.7±2.5 | 9.2±2.5 |
| **100.0** | 16.2±4.4 | 12.7±2.5 | 16.6±2.3 | 15.8±3.4 | 12.9±2.0 |
| **200.0** | 19.3±5.3 | 17.4±3.4 | 19.2±2.6 | 19.3±3.7 | 15.7±2.8 |
| **CN (10μg)**  **CIP (5μg)** | 9.7±2.2  19.0±5.5 | 11.1±3.6  13.9±3.3 | 6.7±1.4  11.3±2.7 | 13.2±2.3  14.0±3.6 | 13.0±2.5  15.3±4.5 |

CN: Gentamicin CIP: Ciprofloxacin

Table 2. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the aqueous fraction from stem-bark methanol extract of *Parkia biglobosa* against chronic wound pathogens in Kaduna, Nigeria

|  |  |  |
| --- | --- | --- |
| **Isolates** | | |
|  | **MIC (mg/ml)** | **MBC (mg/ml)** |
| *Staphylococcus*. spp. | 6.25-12.5 | 6.25-25.0 |
| *Escherichia coli* | 12.5-25.0 | 12.5-50.0 |
| *Proteus* spp. | 3.125-6.25 | 12.5-50.0 |
| *Klebsiella* spp. | 3.125-6.25 | 6.25-12.5 |
| *Pseudomonas* spp. | 6.25-12.5 | 12.5-50.0 |

Table 3. Biofilm inhibitory activity of aqueous fraction from stem-bark methanol extract of *Parkia biglobosa* against isolates from chronic wounds of patients attending selected health facilities in Kaduna, Nigeria

|  |  |  |  |
| --- | --- | --- | --- |
| **Isolates** | **Inhibition (%**±**Standard deviation)** | | |
|  | A | B | C |
| *E.coli* | 80.6±4.3 | 92.8±0.6 | 96.4±1.0 |
| *Pseudomonas* spp. | 83.5±1.3 | 93.2±0.4 | 96.5±0.6 |
| *Proteus* spp. | 81.3±3.0 | 92.6±0.6 | 96.8±0.2 |
| *Klebsiella* spp. | 80.4±3.1 | 92.8±0.4 | 95.7±1.8 |
| *Staphylococcus*. Spp. | 76.4±6.3 | 91.5±1.4 | 96.4±0.5 |

Key:

A: sub-MIC [*Proteus*  and *Klebsiella spp.* (1.56mg/ml), *Staphylococcus* and *Pseudomonas* (3.125mg/ml), *E.coli* (6.25mg/ml)]

B: MIC [*Proteus*  and *Klebsiella spp.* (3.125mg/ml), *Staphylococcus* and *Pseudomonas* spp. (6.25mg/ml), *E.coli* (12.5mg/ml)]

C: sub-MBC [*Staphylococcus*  and *Klebsiella spp.* (3.125mg/ml), *E. coli, Proteus* and *Pseudomonas* (6.25mg/ml)]

Table 4. Antibiofilm activity of aqueous fraction from stem-bark methanol extract of *Parkia biglobosa* against isolates of chronic wounds of patients attending selected health facilities in Kaduna, Nigeria

|  |  |  |  |
| --- | --- | --- | --- |
| **Isolates** | **Antibiofilm activity (%**±**Standard deviation)** | | |
|  | A | B | C |
| *E.coli* | 70.8±11.8 | 88.2±4.6 | 94.9±2.3 |
| *Pseudomonas* spp. | 79.5±2.6 | 92.4±1.3 | 96.7±0.1 |
| *Proteus* spp. | 80.0±4.4 | 91.5±2.2 | 96.2±0.8 |
| *Klebsiella* spp. | 73.6±13.9 | 88.8±6.2 | 96.2±0.5 |
| *Staphylococcus*. Spp. | 74.2±10.7 | 89.2±4.6 | 95.5±1.7 |

Key:

A: sub-MIC [*Proteus* and *Klebsiella* spp. (1.56mg/ml), *Staphylococcus* and *Pseudomonas* (3.125mg/ml), *E.coli* (6.25mg/ml)]

B: MIC [*Proteus* and *Klebsiella* spp. (3.125mg/ml), *Staphylococcus* and *Pseudomonas* spp. (6.25mg/ml), *E.coli* (12.5mg/ml)]

C: sub-MBC [*Staphylococcus* and *Klebsiella* spp. (3.125mg/ml), *E.coli*, *Proteus* and *Pseudomonas* (6.25mg/ml)]

**DISCUSSION**

*Parkia biglobosa* (Jacq.) Benth is a multipurpose plant with wide scale economic, health and ornamental uses in many parts of West Africa. Its documented use for treatment of various diseases across the region is enormous.

The antibacterial activity of the aqueous fraction from stem-bark methanol extract of *Parkia biglobosa* against the various isolates of chronic wounds is concentration-dependent as activity increased with increasing concentration of the fraction. This result is similar to previous studies reported by Udobi and Onaolapo, (2010) against a range of Gram-positive and Gram-negative bacterial isolates using stem-bark methanol extract, Jauro *et al.,* (2018) against methicillin-resistant *Staphylococcus aureus* (MRSA) using methanol extract of the leaf of *Parkia biglobosa.* This may be attributable to the numerous secondary metabolites such as flavonoids, saponins, phenols, terpenoids, etc which reportedly possess antibacterial and antioxidant properties. This effect was comparable to those of conventional antibiotics such as Gentamicin and Ciprofloxacin which were used as reference standards. Similar effect was observed by Osuntokun *et al.,* (2018) in a comparative study of *Parkia biglobosa* and conventional antibiotics against multidrug resistant uropathogenic bacteria in which they reported an ‘outstanding and overwhelming’ activity attributed to the presence of phytochemicals present in the plant. This plant has also been used in traditional medicine to treat wounds (Builders, 2014; Musara *et al*., 2020; Saleh *et al*., 2021). Antibacterial activity against all the isolates tested was concentration-dependent as higher concentration produced greater activity with a minimum inhibitory concentration against respective pathogens ranging between 3.125-6.25mg/ml against *Proteus* and *Klebsiella* species, and 6.25-12.5mg/ml against *Staphylococcus* and *Pseudomonas* species, and 12.5-25.0mg/ml against *E. coli*. The observed zones of inhibition were highest for *Proteus, Klebsiella* and *Staphylococcus* species as concentration increased and often higher or comparable to those of conventional antibiotics; gentamicin and ciprofloxacin. Thus, indicating that the fraction is both bacteriostatic and bactericidal at different concentrations and the MBC not more than four times MIC for all the isolates tested. This result is similar to Udobi and Onaolapo (2010) in which they tested the aqueous fraction from stem-bark methanol of *Parkia biglobosa* against four reference organisms and obtained an MIC ranging from 1.562-25.0mg/ml with the highest MIC against *Escherichia coli* and highest zone of inhibition against *Staphylococcus aureus.* Osemwegie and Dahunsi, (2015) equally reported zones of inhibition were concentration-dependent. The antibacterial activity observed in this study further confirms the traditional use of the plant in the treatment of infections in many parts of Africa where the plant is domiciled and also shows it has a wide spectrum of activities against both Gram-positive and Gram-negative bacteria (Ologundudu *et al*., 2018) thereby confirming previous studies by Udobi and Onaolapo, (2009); Abioye *et al*., (2013); Obajuluwa *et al*., (2013); Osuntokun *et al*., (2018); Ihuma *et al*., (2022).

Aqueous fraction from stem-bark methanol extractof *Parkia biglobosa* showed a high biofilm inhibition and antibiofilm activity above 50% against the test isolates as well as the control, *Pseudomonas aeruginosa* ATCC 27853 and at all concentrations used (half-MIC, MIC and half-MBC). There was a significant association between biofilm inhibition and concentrations used for all bacterial species tested (p<0.05) except *Pseudomonas* spp. Builders *et al*., (2021) reported the effectiveness of stem bark extract in the treatment of burn wounds. Adetutu *et al.,* (2011) in an ethnopharmacological survey and *in-vitro* evaluation of wound-healing plants in south-west Nigeria, reported the stem-bark of *Parkia biglobosa* as one of the five most cited plants for treatment of wounds. In their report, ethanol extract of *Parkia biglobosa* influenced dermal fibroblasts significantly at 15-30µg/ml against the tested isolates (Adetutu *et al.,* 2011). Our results further confirm the traditional use of the plant as medicine. This to the best of our knowledge is the first investigation on the antibiofilm activity of any part of *Parkia biglobosa.* The plant’s secondary metabolites possibly employ the same or similar mechanisms against bacterial virulence in biofilm inhibition to produce good antibiofilm activity.

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