



Comparative Analysis of Antibacteria Activities of *Parkia biglobosa* Leaves and Husk Oil Extracts Against *Staphylococcus epidermis* and *Pseudomonas aeruginosa* Isolated from African Catfish (*Clarias gariepinus*)

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Abstract

The study sought to determine the antimicrobial effect of methanol oil extract of *Parkia biglobosa* leaves and husk on selected pathogenic bacteria isolated from the oral of African catfish (*Clarias gariepinus*). The experiment was carried out at teaching and Research Laboratory of Oyo State College of Agriculture and Technology. Three catfishes were purchased at Obasanjo farm Nigeria Limited at Igboora. Biochemical test, sensitivity test, minimum Inhibitory Concentration and zone of inhibition were determined on each treatment of *Pseudomonas aeruginosa* and *Staphylococcus epidermis* treated with both extracts of *Parkia biglobosa* leaves and husk oil respectively. The data were analyzed statistically by analysis of variance (ANOVA) probability completely randomized design with least significant difference (LSD) at 5% level. The result revealed that *Pseudomonas aeruginosa* had the highest susceptibility activity (9.00, 12.00, 14.00, 18.00 and 18.00) at all levels of concentration (20%, 40%, 60%, 80% and 100%) respectively, which was also similar for Minimum Inhibition Concentration of *Parkia biglobosa* leaves extract. However, the zone of inhibition showed that at 20% and 40%, *Pseudomonas aeruginosa* reported highest value of 15 and 14 respectively, while *Staphylococcus epidermis* reported higher value at 20% (14) and 40% (13). Conclusively, the results of this study supports the traditional application of *Parkia biglobosa* leaves and husk oil extract and suggests that it can be used as antibacterial agents in novel drugs for the treatment of oral diseases of *Clarias gariepinus*.

Keywords: Antibacterial, Methanol Extract, *Parkia biglobosa* leaves, Husk, *Clarias gariepinus*

Introduction

Parkia biglobosa belongs to the family Leguminosae and the subfamily Mimosoideae (Hopkins, 1983). The tree has a wide distribution ranging across the Sudan and Guinea savanna ecological zones. It is found in 19 African countries (Hall *et al.*, 1997). Investigations into the local perceptions on trees in West Africa revealed *P. biglobosa* as one of the species preferred among conserved fruit-bearing trees (Teklehaimanot, 2004). A survey on families in Burkina Faso on vegetable consumption and seasonality in two communities found that dawadawa (fermented seed of *P. biglobosa*) was consumed in 78 and 85% of all meals (Mertz *et al.*, 2001). About 201,000 tons of *P. biglobosa* fruit is produced annually in Northern Nigeria alone (Sina and Traore, 2002). Sina and Traore (2002) reported that the bark and husk contain tannin. Secondary metabolites are widely applied in medicine, food and the dye industry. Some of them have biological activities (Sparg *et al.*,

2004), others have pharmacological effects (Ansel *et al.*, 1995) and others have multiple uses. Anthraquinones are of fundamental importance both in dye industry and medicine. Anthraquinone glycosides, flavones, and tannin form important ingredients of several laxative medicines and in dyes. In the pharmaceutical industry, natural and synthetic derivatives of anthraquinones and flavonoids are beneficial to mammals and humans with the display of antibacterial, antitrypanosomal and antineoplastic activities (Heyman *et al.*, 2009; Tarus *et al.*, 2002; VelezCruz and Osherooff, 2004). The African cat fish (*Clarias gariepinus*) is recognized as one of the most suitable aquaculture fish species in Africa. Aquaculture research development methods for the production of catfish fingerlings has been regarded as one of the top priorities in Africa, African catfish hormone induced using deoxycorticosterone acetate, human chorionic gonadotropin and common carp pituitaries, has



been carried out successfully (Tarus *et al.*, 2002).

Aquaculture is the controlled production and growing of aquatic organisms for food or ornament (Ozigbo *et al.*, 2014). Current trends in human population growth, habitat and natural stocks destruction versus advances in world agricultural, technology and implementing aqua cultural technology. Aquaculture can contribute substantially to the production of animal protein for human nutrition and other nutritional needs especially in the tropical countries. The successful cultivation of any organism for human consumption demand that resources be easily renewable. It is clearly disadvantageous to cultivate any organism when supply of the young cannot be easily replenished.

Mud catfish (*Clarias gariepinus*) is the most sought after fish species among fish farmers and consumers because it commands a very good commercial value in Nigeria markets (Onyekuru *et al.*, 2019). In recent years, the culture of species of the catfish belonging to the Clariidae family is fast gaining global attention. In Nigeria, the species most cultured are *Clarias gariepinus*, *Heterobranchius* species and their hybrid (Adewolu *et al.*, 2008). They are widely cultured owing to high market price, fast growth rate and ability to withstand adverse pond condition especially low oxygen content (Adewolu *et al.*, 2010)

Bacteria found on fish majorly belong to genera of *Pseudomonas*, *Staphylococcus*, *Flavobacterium*, *Vibrio*, *Micrococcus*, *Bacillus* and *Aeromonas*, some exist as microflora in pond water and large water bodies and they can be found on the gills, skin, fin or in the intestinal tracts of fish under normal conditions but due to environmental stress they may produce epizootics diseases while some are also pathogenic organisms (Oladosu-Ajayi, George, Obasa, Ajayi, & Bankole, 2011). This study

aimed to determine the comparative analysis and antibacterial activity of methanol extract of *Parkia biglobosa* leaves on oral pathogen isolated from African catfish (*Clarias gariepinus*)

Materials and Methods

Sample collection

The leaves and husk of *Parkia biglobosa* were collected from *Parkia biglobosa* plant in the botanical garden of Oyo State College of Agriculture and technology, Igboora.

Preparation of extracts

Fresh leaves and husk of *Parkia biglobosa* was rinsed with distilled water. The leaves and husk were air dried separately at room temperature until properly dried. It was grinded into fine powder with the aid of a binatone blender and sieved. Organic solvent (Methanol) was used for the preparation of the extract. The extract was prepared by weighing 65g of *Parkia biglobosa* leave and 65g of husk powder and into a beaker and 100ml of hot methanol was poured and stirred vigorously until it's properly mixed. The juice was extracted with the use of whatman filter paper.

Collection of African Catfish

Three catfishes were purchased from Obasanjo farm Nigeria Limited in Igboora. Sterile swab was immediately used to collect oral bacteria by swabbing its mouth and inoculated in nutrient broth.

Isolation, Identification and Biochemical test

Organism grown from nutrient broth of the swabbed oral mouth of *Clarias gariepinus* was inoculated in a nutrient agar, isolated and identified. Selected pathogenic from the oral swab were *Staphylococcus epidermis* and *Pseudomonas aeruginosa*.

Table 1: Biochemical test on tested microorganismms

Biochemical Test	<i>Staphylococcus aureus</i>	<i>Psuedomonas aureginosa</i>
Gram stain	+	-
Oxidase test	-	+
Catalase test	+	+
Indole test	-	-
Nitrate test	+	+
Urease test	+	-
Gas	-	+



Sensitivity testing of *Parkia biglobosa* leaves and husk

The sensitivity test of *Parkia biglobosa* leaves and husk was determined by making four ditch (hole) aseptically bored into the nutrient agar plates already sealed with 24hours broth culture of each tested organism i.e (*Staphylococcus epidermis* and *Pseudomonas aeruginosa*) with sterile cork borer (5mm) size. Each hole was carefully filled with 100mg/ml of *Parkia biglobosa* leaves extract with methanol (hot and cold). The experiment was repeated in triplicate.

Each plate was then incubated at 28°C for 24hours and examined for zones of inhibition considered to be sensitive (+) while the hole without inhibition was considered to be insensitive (-) (Sparg *et al.*, 2004).

Minimum inhibition concentration (MIC)

The MIC estimation of the *Parkia biglobosa* leave and husk using the method of Heyman *et al.* (2009). Sterile paper disk sterilized by drying in an oven at 150°C for 1hour was dipped into ranges of *Parkia biglobosa* leave and husk concentration through serial dilution in test tubes. Each soaked disk was carefully and aseptically layered on the nutrient agar plate sealed within 24hours broth culture of each organism (*Staphylococcus epidermis* and *Pseudomonas aeruginosa*) spread on each plate in duplicate with the aid of sterile forceps.

Each plate was incubated inversely at 28°C for 24hours and then examined for the zone of inhibition. The lowest concentration of the extract that inhibited growth was taken as the MIC of the extract on the test organism.

Results

Table 1 shows the biochemical test which differentiate the Gram positive bacteria (*Staphylococcus epidermis*) and the Gram negative bacteria (*Pseudomonas aeruginosa*). The table 2 shows the antibacterial susceptibility test of *Staphylococcus epidermis* and *Pseudomonas aeruginosa* with both leaf and husk extracts used against the isolates, in which 20% concentration leaf extract exhibited 9% from *Pseudomonas aeruginosa* and 7% for *Staphylococcus epidermis*, in 40% concentration of the extract, *Pseudomonas aeruginosa* had 12% while *Staphylococcus epidermis* had 10%. In 60% concentration of the extract, *Pseudomonas aeruginosa* had 14% while *Staphylococcus epidermis* had 12%. In 80% concentration of the extract,

Pseudomonas aeruginosa had 18% while *Staphylococcus epidermis* had 15%, in 100% concentration of the extract, *Pseudomonas aeruginosa* had 18% while *Staphylococcus epidermis* had 16%. In husk extract, 20% concentration of the extract exhibited 10% from *Pseudomonas aeruginosa* and 9% for *Staphylococcus epidermis*, in 40% concentration of the extract, *Pseudomonas aeruginosa* had 13% while *Staphylococcus epidermis* had 11%. In 60% concentration of the extract, *Pseudomonas aeruginosa* had 15% while *Staphylococcus epidermis* had 14%. In 80% concentration of the extract, *Pseudomonas aeruginosa* had 18% while *Staphylococcus epidermis* had 16%, in 100% concentration of the extract, *Pseudomonas aeruginosa* had 19% while *Staphylococcus epidermis* had 18%.

Table 3 shows the minimum inhibition concentration of leaf extracts for both isolates obtained from *Clarias gariepinus* in which 20% concentration extract exhibited 17% from *Pseudomonas aeruginosa* and 17% for *Staphylococcus epidermis*, in 30 concentration of the extract, *Pseudomonas aeruginosa* had 16% while *Staphylococcus epidermis* had 14%. In 40% concentration of the extract, *Pseudomonas aeruginosa* had 14% while *Staphylococcus epidermis* had 12%. In 50% concentration of the extract, *Pseudomonas aeruginosa* had 12% while *Staphylococcus epidermis* had 10%, in 60% concentration of the extract, *Pseudomonas aeruginosa* had 10% while *Staphylococcus epidermis* had 9%. Values obtained from husk extract concentration showed that 20% concentration extract exhibited 19% from *Pseudomonas aeruginosa* and 18% for *Staphylococcus epidermis*, in 30 concentration of the extract, *Pseudomonas aeruginosa* had 17% while *Staphylococcus epidermis* had 16%. In 40% concentration of the extract, *Pseudomonas aeruginosa* had 13% while *Staphylococcus epidermis* had 15%. In 50% concentration of the extract, *Pseudomonas aeruginosa* had 12% while *Staphylococcus epidermis* also had 12%, in 60% concentration of the extract, *Pseudomonas aeruginosa* had 11% while *Staphylococcus epidermis* had 10%.

Therefore, the best MIC has been exhibited by serial dilution of 20% concentration with husk extract, 19% and 18% recorded for *Pseudomonas aeruginosa* and *Staphylococcus epidermis* respectively.



Table 2: Antibacterial Susceptibility of *Parkia biglobosa* leaves extract against *Staphylococcus epidermis* and *Pseudomonas aureginosa* isolated from the oral of *Clarias gariepinus*

	<i>Staphylococcus epidermis</i>	<i>Pseudomonas aeruginosa</i>
20	07.00	09.00
40	10.00	12.00
60	12.00	14.00
80	15.00	18.00
100	16.00	18.00

Unit: mm

Table 3: Antibacterial Susceptibility of *Parkia biglobosa* husk extract against *Staphylococcus epidermis* and *Pseudomonas aureginosa* isolated from the oral of *Clarias gariepinus*

Extract Conc. (%)	<i>Staphylococcus epidermis</i>	<i>Pseudomonas aeruginosa</i>
20	09.00	10.00
40	11.00	13.00
60	14.00	15.00
80	16.00	18.00
100	18.00	19.00

Unit: mm

Table 4: Minimum Inhibition Concentration of *Parkia biglobosa* leaves extract against *Staphylococcus epidermis* and *Pseudomonas aureginosa* isolated from the oral of *Clarias gariepinus*.

Serial dilution (80%) Conc.	<i>Staphylococcus epidermis</i>	<i>Psuedomonas aurigenosa</i>
20	17	17
30	16	14
40	14	12
50	12	10
60	10	09

Unit: mm

Table 5: Minimum Inhibition Concentration of *Parkia biglobosa* husk extract against *Staphylococcus epidermis* and *Pseudomonas aureginosa* isolated from the oral of *Clarias gariepinus*.

Serial dilution (80%) Conc.	<i>Staphylococcus epidermis</i>	<i>Psuedomonas aurigenosa</i>
20	18	19
30	16	17
40	15	13
50	12	12
60	10	11

Unit: mm



Table 6: Determination of zone of inhibition of *Parkia biglobosa* leaves extract against *Staphylococcus epidermis* and *Pseudomonas aureginosa* isolated from the oral of *Clarias gariepinus*.

Concentration (%)	<i>Staphylococcus aureus</i>	<i>Pseudomonas aureginosa</i>
20	15	14
40	14	13
60	11	11
80	09	10
100	09	08

Unit: mm

Table 7: Determination of zone of inhibition of *Parkia biglobosa* husk extract against *Staphylococcus epidermis* and *Pseudomonas aureginosa* isolated from the oral of *Clarias gariepinus*.

Concentration (%)	<i>Staphylococcus aureus</i>	<i>Pseudomonas aureginosa</i>
20	13	16
40	12	13
60	10	12
80	09	11
100	08	09

Unit: mm

Discussion

From the result, the following properties were revealed; anti bacteria (antibiotics), antifungal (antimycotic), anticancerous is inherent (Ademola *et al.*, 2011). The present study showed that the leaf and husk of *Parkia biglobosa* possesses antibacterial potential against *Staphylococcus epidermis* and *Pseudomonas aeruginosa* having much effect on *Staphylococcus epidermis* than *Pseudomonas aeruginosa*. Although when both extracts were compared, it was observed that the husk of *Parkia biglobosa* is more effective and has more antibacterial potential than the leave extract. In line with the present finding, several other studies have repored *Parkia biglobosa* leaf (Ademola *et al.*, 2011), seeds (Alabi, 1996) and bark to have antimicrobial potentials. Several other reports indicated that *Parkia biglobosa* had significant antibacterial activity

in various extracts from different tree parts (Omafuvbe, 2004). The observed result is similar to the work on plant extracts and antimicrobial efficacies of the quinolones and cephalosporins reported by Momoh *et al.* (2011).

Also, the potency of the activity of *Parkia biglobosa* against microbes depends on the extraction solvent used. *Parkia biglobosa* in

organic extracts such as methanol and ethanol were more effective than *Parkia biglobosa* extract in water. This may be due to the fact that there is better solubility of the active components in organic solvent than water (Boer *et al.*, 2005).

The therapeutic value of medicinal plants had been reported to lie in the various chemical constituents. In fact, active principles singly or in combination inhibit greatly the life process of microbes by binding with their protein molecules acting as chelating agents (selective binding polyvalent metal ion so that the latter loses its biological activities), altering their biochemical systems, preventing utilization of available nutrients to the microorganisms (Ansel *et al.*, 1995). In addition, bioactive substances have been reported to confer resistance to plants against bacteria, fungi and pests and therefore explained the demonstration of antibacterial activities by the plant extracts used in this study (Omafuvbe, 2004). With regards to this reported many benefits of *Parkia* due to the high content of vitamins A, B and C, proteolytic enzymes like papin and chymopapin, that had antiviral, antifungal and antibacterial properties embedded in it.

The result of different studies had previously provided evidence about some medicinal plants



and has suggested to be potential source of new antibacterial agents (Babayemi *et al.*, 2004). This work has been able to highlight the possible antibacterial potentials of *Parkia biglobosa* leaf and husk respectively. Extraction of the active component of the plant resulting minimum inhibitory concentrations recorded against well-known Gram positive and Gram negative bacteria, a highly resistant bacterium, provides opportunity to pharmaceutical industries willing to undertake and develop potent antibiotics from *Parkia biglobosa*. Hence, there is the need for government and grant agencies to commit resources to this novel area as it is potential to deliver potent antimicrobial against multi-drug resistant (MDR) strains from collapsing cannot be overemphasized.

Conclusion

The response of antibacterial activities against both *Pseudomonas aeruginosa* and *Staphylococcus epidermis* is an indication that both the leaves and husk of *Parkia biglobosa* are potential source for the production of drugs with a broad spectrum activity. The results of this study affirmed the traditional application of *Parkia biglobosa* leaves and husk and suggested that they can be use as antibacterial agents in novel drugs for the treatment of oral diseases of *Clarias gariepinus*. This situation provides the impetus search for new antimicrobial substances from various sources like medicinal plants.

Recommendation

From the result, the plant oil extracts have traditionally provided a source of hope for novel drugs compounds, as a plant herbal mixture for treatment of human health and wellbeing. Hence, can serve a great significant for therapeutic treatment.

References

- Adewolu., M.A., Ogunsanmi, A.O. and Yunusa, A. (2008). Studies on the Growth Performance and Feed utilization of two clariid catfish and their hybrid reared under different culture systems. *European Journal of Scientific Research*. 23(2): 252-260.
- Ademola I.T, Baiyewu R.A, Adekunle E.A, Omidiran M.B, and Adebawo F.G. An assessment into physical and proximate Analysis of Processed Locust Bean (*Parkia biglobosa*) preserved with common salt. *Journal of Nutrition*, 10:405-408
- Alabi, R.A. (1996). Archaeological reconnaissance of the Badagry coastal area, Southwestern Nigeria. *West African Journal of Archaeology* 26(1): 88-102.
- Ansel HC, Popovich NG, Allen LV (1995). Pharmaceutical dosage forms and drug delivery systems, pp. 120-125.
- Babayemi, O.J., Demeyer, D. and Fievez, V. (2004). Nutritional value of qualitative assessment of secondary compound in seeds of eight tropical browse, shrub and pulse legumes. *Community Applied Biological Science*. Ghent University, 69/1: 103-110.
- de Boer W, Folman L.B, Summerbell R.C, Boddy L. (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiology Rev* 29: 795-811.
- Hall JB, Tomlinson HF, Oni PI, Buchy M, Aebischer DP (1997). *Parkia biglobosa*: A monograph. School of Agricultural and Forest Sciences Publication Bangor, University of Wales, UK, 9:107.
- Heyman HM, Hussein AA, Meyer JJM, Lall N (2009). Antibacterial activity of South African medicinal plants against methicillin resistant *Staphylococcus aureus*. *Pharm. Biol.* 47: 67-71.
- Hopkins, 1983. *Botanical Journal* 87 (2): 135-167.
- Mertz O, Lykke AM, Reenberg A (2001). Importance and Seasonality of Vegetable Consumption and Marketing in Burkina Faso. *Ecol. Bot.* 55(2):276-289.
- Momoh J.E, Udobi C.E. and A.A. Orukotan, (2011). Improving the microbial keeping quality of homemade soymilk using a combination of preservatives, pasteurization and refrigeration. *British Journal of Dairy Science*, 9(5): 29-36.
- Oladosu GA., Ayinla OA, Ajiboye MO. Isolation and pathogenicity of *Bacillus* species. Associated with a septicemic Condition in some Tropical Freshwater Fish Species. *Journal of Applied Ichthyology*, 2011;10:69-72.
- Omafuvbe, B.O., Olumuyiwa S.E., Bolanle A.O. and Steve R.A. (2004). Chemical and Biochemical Changes in African locust Bean (*Parkia biglobosa*) and melon (*Citrullus vulgaris*) seeds during fermentation of condiments. *Pakistan Journal of Nutrition*, 3, 140-145.
- Onyekuru, N.A., Ihemezie, E.J and Chima, C.C (2019). Socio-economic and Profitability Analysis of Catfish Production: A Case Study of Nsukka Local Government Area of Enugu State, Nigeria. *Journal of Tropical*



Agriculture, Food, Economic and Extension.
18(2):51-58.

Ozigbo E, Anyadike C, Adegbite O, Kolawole P (2014). Review of Aquaculture production and management in Nigeria. *Amplified Journal experimental Agriculture*. 4(10):1137-1151.

Sina S, Traore SA (2002). In: Adama AY and Jimoh YA (2011). Production and Classification of Locust Bean Pod Ash (LBPA) as a Pozzolan. Ministry of Works and Infrastructural Development, Minna, Nigeria and Department of Civil Engineering, University of Ilorin, Nigeria. Project Reports.

Sparg SG, Light ME, van Staden J (2004). Biological activities and distribution of plant saponins. *J. Ethnopharmacol.* 94:219-243.

Tarus PK, Machocho AK, Langat TCC, Chhabra SC (2002). Flavonoids from *Tephrosia aequilata*. *Phytochemistry* 60:375-379.

Teklehaimanot Z (2004). Exploiting the Potential of Indigenous Agroforestry Trees: *Parkia biglobosa* and *Vitellaria paradoxa* in subSaharan Africa. *Agrofor. Syst.* 61(1-3):207-220.

Velez CR, Osheroff N (2004). DNA topoisomerases: type II. In: *Encyclopedia of Biological Chemistry*, Elsevier Inc., pp. 806-811.