

Comparative Antibacterial Studies On The Root, Stem Bark And Leaf Extracts Of *Parkia Clappertoniana*.

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Citation

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Abstract

The research on medicinal plants is gradually gaining popularity due to millions of people depending on the use of different parts of these materials for various ailments. The antibacterial activity of hot and cold water and ethanolic extracts of the root, stem and leaf of *Parkia clappertoniana* against *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC10145, *Staphylococcus aureus* ATCC 12600 was evaluated using agar-well diffusion and agar dilution methods. All the organisms were susceptible to all the extracts with the diameter of zones of inhibition ranging between 14 mm – 27 mm for hot water extracts, 12 mm – 22 mm for cold water extracts and 12 mm – 25 mm for ethanolic extracts. The Minimum Inhibitory Concentrations ranged between 2.5 mg/ml – 20.0 mg/ml. The rate of kill of the test bacterial species by hot water extract was concentration dependent. Saponin, flavonoids, tannin, glycoside, alkaloid and anthraquinone were the phytochemical constituents detected from the *Parkia clappertoniana* root, stem and leaf extracts. The implications of these findings in the medicinal use of *Parkia clappertoniana* are discussed.

INTRODUCTION

Parkia clappertoniana (Keay) which is commonly known as West African Locust beans belongs to family Leguminosae-Mimosaceae which has about 236 genera¹. The common species of the *Parkia* genus are *Parkia biglobosa*, *P. filicoidea* and *P. clappertoniana*. *Parkia clappertoniana* is a tree of about 18meters high and 3.6meters in width with spreading branches. The plant tends to occur in the savanna country and has been recorded from Gold Coast (Ghana), Togoland (Ghana and Togo), Dahomey (Benin) and Northern Nigeria¹. *Parkia* species have been reportedly used in folk medicine for the treatment of various diseases especially infections^{2, 3}. The roots and leaves of *Parkia clappertoniana* are pounded with water and used as an eye wash; the roots and the leaves were also reported to be active against dental caries, conjunctivitis⁴. It was also reported that an infusion of the stem bark was successfully used for the treatment of many infectious diseases such as diarrhoea, orchitis, dental caries, pneumonia, bronchitis, violent stomachaches, severe cough, infected wounds, otitis, dermatosis, amoebiasis, bilharziosis, leprosis, ankylosis, tracheitis, and conjunctivitis⁴.

Many phytochemical constituents have isolated from *Parkia* species. Lemmich et al.,⁵ isolated 5- deoxyflavones from *Parkia clappertoniana* and phenolics were also isolated from

*Parkia biglobosa*².

Bacterial genera such as *Staphylococci*, *Escherichia* and *Pseudomonads* have been implicated in the above mentioned infectious diseases⁶. *Staphylococcus aureus* occur harmlessly as a normal flora of the skin and mucous membrane and it is one of the commonest bacterial pathogens encountered in the community causing severe food poisoning or minor skin infections to severe life threatening infections⁶.

Escherichia coli are known as part of normal flora but incidentally may cause diseases: urinary tract infection, diarrhoea and hemorrhagic colitis; blood stream sepsis when the normal host defenses are inadequate⁷.

Pseudomonas aeruginosa are wide spread in soil, water and sewage and this can be considered as an indication of their involvement in the natural process of mineralization of organic matter. It has long been a troublesome cause of secondary infections of wound, especially burns, giving rise to blue-green pus. It produces meningitis, when introduced by lumbar puncture and urinary tract infection when introduced by catheters and instruments or irrigating solutions⁷.

Bacterial resistance to antibacterial drugs used in the treatment of some of the earlier mentioned infections has

become a menace, therefore causing untold health challenges to patients. The aim of our study is to check the antibacterial effects of the root, stem and leaf of *Parkia clappertoniana* against the above mentioned pathogens in order to determine which of the plant parts would be the most active.

MATERIALS AND METHODS

Collection of plant materials: The plant *Parkia clappertoniana* root, stem bark and leaf were collected from the Ahmadu Bello University premises and it was carefully authenticated in the Department of Biological Sciences of the University.

Extraction of plant materials: The root, stem bark and leaves were size reduced in order to facilitate drying. The plant parts were dried at room temperature in order to prevent loss of active constituents which may be thermo-labile and drying was continued until constant weight was obtained. Mortar and pestle were finally used to reduce the size of the dried plant parts to powder.

One hundred gram (100 gm) of powdered plant materials was weighed using an analytical balance and kept in the dessicator as to maintain a constant weight. This was transferred into a conical flask placed on a mechanical shaker and 250 ml of 99% ethanol was added and allowed to macerate for 6 hours after which it was decanted into a separating funnel and the filtrate collected. Another 250 ml of 99% ethanol was added to the residue, shaken and allowed to macerate for 6 hours. The total filtrate collected was evaporated to dryness by placing it on water bath at 50°C.

The hot and cold water extracts were obtained by the above procedure using 300 ml each of hot and cold distilled water. The dried extracts were kept inside dessicator before use.

PHYTOCHEMICAL ANALYSIS:

Phytochemical analysis was carried out on the plant extracts for the presence of chemical components such as, saponins, tannins, alkaloids, glycosides, flavonoids, triterpenoids, steroids and anthroquinones⁸.

SUSCEPTIBILITY TESTING:

Twenty milliliter (20 ml) of sterile nutrient agar was poured into the sterile petri-dish and allowed to set. The surface was flooded with 2 ml of 18 hour-broth culture of the test organism which has been standardized according to National Committee for Clinical Laboratory Standards⁹ by gradually adding normal saline to compare its turbidity to McFarland

standard of 0.5 which is approximately 1.0×10^6 cfu/ml. The surface was allowed to dry and sterile No. 4 cork borer was used to bore six holes of about 2.5cm equal size on the surface. 0.1ml of the extract at different concentrations of 5% w/v, 10% w/v and 15% w/v was dropped into each hole and the plate was kept for about one hour at room temperature and incubated at 37°C for 18 hours. The diameter of zones of inhibition was measured after incubation to the nearest millimetre. The experiment was repeated three times and the mean diameter was taken.

Minimum Inhibitory Concentrations (M.I.C.) and Minimum Bactericidal Concentrations (M.B.C.) Determination.

The M.I.C. was determined by agar dilution method. Ten milliliter (10 ml) volume of double strength melted Mueller-Hinton agar at 45°C was diluted with equal volume of the test extract in graded concentrations. These were poured aseptically into sterile Petri-dishes and dried at 37°C for 1 hour with the lid slightly raised. Twenty microlitre (20 µl) of standardized test bacteria (10^6 cfu/ml) were aseptically inoculated on the sterilized paper discs placed on the agar surface at equidistance in triplicates for each concentration of the test plant extract¹⁰.

These were incubated at 37°C for 18 hours. The M.I.C. value was taken as the least concentration of the extract showing no detectable growth. Gentamicin was used as standard antibiotic.

The M.B.C was determined by transferring inoculated discs into a sterile 10ml recovery nutrient broth (Nutrient broth containing 3% v/v Tween 80) from the concentrations that showed no visible growth from the M.I.C. determination. These were incubated at 37°C for 72 hours. The least concentration of the extract that showed no bacterial growth in the recovery liquid medium was taken as the M.B.C.

DETERMINATION OF RATE OF KILL:

The rate at which hot water extract, which proved to be the most active of the tested extracts killed *Staph. aureus* ATCC 021001, *Ps. aeruginosa* ATCC 10145 and *E.coli* ATCC 11775 was tested.

A reaction mixture was made inside a conical flask containing 29 ml of sterile nutrient broth and 1.0 ml of the extract (15% w/v), with 0.1 ml of standardized overnight culture of the test organism. The mixture was shaken at 37°C and at various time intervals of 0, 30, 60 and 90 minutes, 0.1 ml of the mixture was taken using a micropipette, properly

diluted and then plated on the surface of solidified sterile nutrient agar containing 3% Tween 80. It was then allowed to dry and plates were incubated at 37°C for 18 hours and the number of colonies were counted and recorded. This was repeated for 5% w/v and 10% w/v and gentamicin. A control was set containing nutrient broth and the test organism but without the extract. The test results were compared with that of the control.

STATISTICAL ANALYSIS:

Results were expressed as mean ± standard deviation. The data was analyzed using Student’s t-test. P< 0.05 was considered significant and P>0.05 not significant.

RESULTS

PHYTOCHEMICAL ANALYSIS:

The phytochemical analysis of the plant extracts revealed the presence of saponin, tannin, alkaloid, glycoside, flavonoids, triterpenoids, steroids and anthraquinones (Table 1).

Figure 1

Table 1: Phytochemical constituents of *Parkia clappertoniana* root, stem bark and leaf extracts.

Chemical constituents	Root	Stem bark	Leaf
Saponin	+	+	+
Tannin	+	+	+
Alkaloids	+	+	+
Glycosides	+	+	+
Flavonoids	+	+	+
Triterpenoids	+	+	-
Steroids	+	+	-
Anthraquinones	+	+	+

Key: + = present, - = absent

ANTIBACTERIAL SUSCEPTIBILITY TESTING:

The result of the susceptibility test of the organisms to the extracts showed that the extracts had antibacterial activity against all the test bacterial isolates. The comparative study generally showed that the root, stem bark and leaf hot water extracts were more active than their ethanol and cold water extracts respectively. There was a statistical significant difference at P<0.05 between the antibacterial activities of the root hot water extract and the other root extracts, as the hot water extract showed a higher activity than them. The antibacterial activity of the stem bark hot water extract was higher against *Ps. aeruginosa* and *Staph. aureus* than the stem bark ethanol and cold water extract with a statistical significant difference at P< 0.05 but there was no statistical significant difference at P> 0.05 between the antibacterial activity of the stem bark hot water extract and other extracts against *E. coli*. The antibacterial activity of the leaf hot water extract showed no statistical significant difference at P> 0.05

when compared with the activity of the leaf ethanol extract against *Staph. aureus* but there was a statistical significant difference at P< 0.05 between the activities of the leaf hot water extract and the ethanol and cold water extracts against the other test organisms. Generally, the stem bark extracts showed more antibacterial activity than the root and leaf extracts against all the test organisms except *Staph. aureus* where the root hot water extract was more active.

Escherichia coli was more susceptible to the extracts than the other test organisms with statistical significant difference at P< 0.05 (Tables 2 – 4).

The M.I.C. values are lower in the hot water extracts than ethanol and cold water extracts respectively (Table 5).

The rate by which the extracts kill the test organisms was progressive (Figures 1 - 3).

Figure 2

Table 2: Antibacterial susceptibility of the test bacteria species to the hot water extracts.

Test organisms	Conc. of extracts (%w/v)	Zones of Inhibition (mm)		
		Root	Stem bark	Leaf
<i>E. coli</i>	15	26 ± 0.1	27 ± 0.0	26 ± 0.0
	10	25 ± 0.0	25 ± 0.2	22 ± 0.2
	5	18 ± 0.2	20 ± 0.3	20 ± 0.1
<i>Ps. aeruginosa</i>	15	22 ± 0.3	21 ± 0.1	23 ± 0.0
	10	20 ± 0.0	20 ± 0.0	20 ± 0.1
	5	15 ± 0.1	15 ± 0.1	17 ± 0.4
<i>Staph. aureus</i>	15	25 ± 0.5	23 ± 0.0	19 ± 0.0
	10	23 ± 0.2	20 ± 0.2	17 ± 0.3
	5	15 ± 0.2	14 ± 0.0	15 ± 0.1

The results are expressed as mean ± standard deviation

Figure 3

Table 3: Antibacterial susceptibility of the test bacteria species to the cold water extracts.

Test organisms	Conc. of extracts (%w/v)	Zones of Inhibition (mm)		
		Root	Stem bark	Leaf
<i>E. coli</i>	15	21 ± 0.0	22 ± 0.1	20 ± 0.0
	10	19 ± 0.2	20 ± 0.3	18 ± 0.2
	5	15 ± 0.1	17 ± 0.2	14 ± 0.3
<i>Ps. aeruginosa</i>	15	20 ± 0.3	19 ± 0.1	19 ± 0.0
	10	17 ± 0.1	16 ± 0.0	17 ± 0.0
	5	12 ± 0.4	13 ± 0.1	14 ± 0.1
<i>Staph. aureus</i>	15	20 ± 0.0	20 ± 0.5	17 ± 0.0
	10	18 ± 0.2	16 ± 0.2	16 ± 0.0
	5	13 ± 0.1	13 ± 0.2	13 ± 0.0

The results are expressed as mean ± standard deviation

Figure 4

Table 4: Antibacterial susceptibility of the test bacteria species to the ethanolic extracts.

Test organisms	Conc. of extracts (%w/v)	Zones of Inhibition (mm)		
		Root	Stem bark	Leaf
<i>E. coli</i>	15	20 ± 0.1	25 ± 0.0	18 ± 0.1
	10	15 ± 0.3	22 ± 0.2	16 ± 0.2
	5	12 ± 0.2	13 ± 0.1	13 ± 0.0
<i>Ps. aeruginosa</i>	15	20 ± 0.1	18 ± 0.3	18 ± 0.0
	10	16 ± 0.0	15 ± 0.1	16 ± 0.1
	5	12 ± 0.1	12 ± 0.4	13 ± 0.2
<i>Staph. aureus</i>	15	19 ± 0.5	20 ± 0.0	17 ± 0.3
	10	15 ± 0.2	18 ± 0.2	16 ± 0.5
	5	12 ± 0.2	15 ± 0.1	12 ± 0.0

The results are expressed as mean ± standard deviation

Figure 5

Table 5: The Minimum Inhibitory Concentrations (M. I. C) and Minimum Bactericidal Concentrations (M. B. C) of the extracts.

Test organisms	M. I. C. (mg/ml) [M. B. C (mg/ml)]									Gentamicin
	HWE			CWE			ETE			
	RT	SB	LF	RT	SB	LF	RT	SB	LF	
<i>E. coli</i>	2.5 [5]	2.5 [5]	2.5 [5]	5 [10]	5 [5]	5 [10]	5 [10]	2.5 [5]	10 [10]	2.2µg/ml
<i>Ps. aeruginosa</i>	5 [10]	5 [10]	5 [10]	5 [10]	5 [10]	5 [10]	5 [10]	10 [10]	5 [10]	3.2µg/ml
<i>Staph. aureus</i>	2.5 [5]	5 [10]	5 [10]	5 [10]	5 [10]	10 [10]	10 [10]	10 [10]	5 [20]	15.63µg/ml

Key : HWE – Hot Water Extract, CWE - Cold Water Extract, ETE - Ethanolic Extract, RT – Root, SB – Stem Bark, LF – Leaf

Figure 6

Figure 1: Log of death/survival rate of ATCC 11775 on exposure to different concentrations of hot water extract of . and gentamicin.

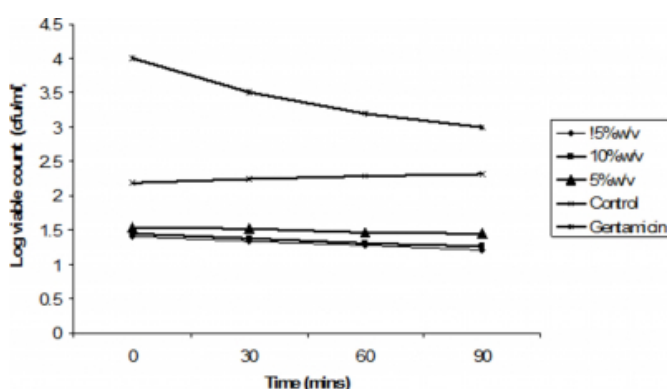


Figure 7

Figure 2: Log of death/survival rate of ATCC 10145 on exposure to different concentrations of hot water extract of . and gentamicin.

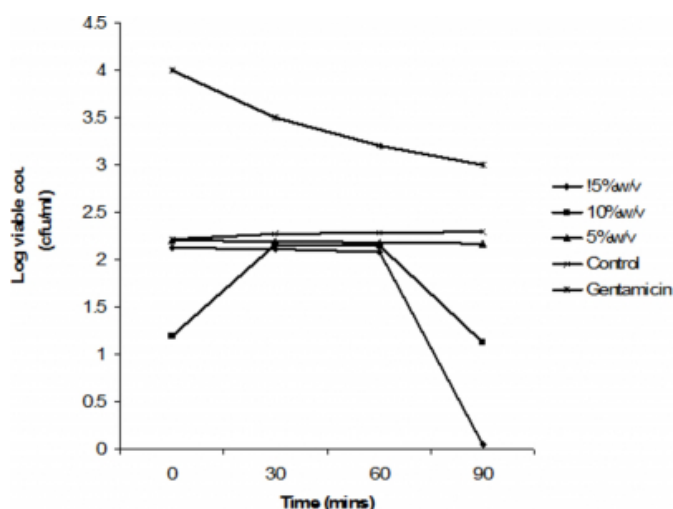
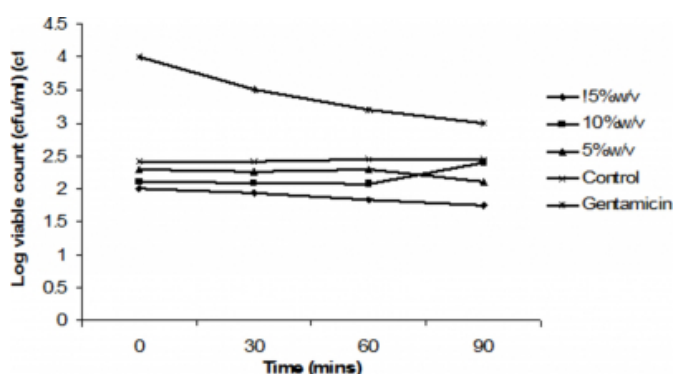


Figure 8

Figure 3: Log of death/survival rate of ATCC 021001 on exposure to different concentrations of hot water extract of . and gentamicin.



DISCUSSION

The phytochemical screening of the plant parts extracts revealed the presence of saponin, tannin, flavonoid, anthraquinones, glycosides, triterpenoids, steroids and alkaloids. The presence of plant secondary metabolites in *Parkia* species, which are known to have broad spectrum of antibacterial activity have been reported^{11, 4, 2}. El-Mahmood and Ameh² reported the presence of phenolics in the root bark of *Parkia biglobosa* which is synonymous to *Parkia clappertoniana*, while it is not revealed in this study but this work revealed the presence of triterpenoids and steroids in the root and stem bark extract. This report is also in agreement with that of Millogo-Kone et al.,⁴ who reported the presence of triterpenoids and steroids in the stem bark of *P. biglobosa*. *Parkia biglobosa* has been ranked among the

richest plants in tannins¹². These plant secondary metabolites have been isolated from various plants by many workers^{13,14},¹⁵ who have also attributed the antibacterial activities of plants to their presence.

All the extracts showed antibacterial activity against all the tested bacteria species. This report disagrees with that of Ajaiyeoba¹¹, who reported that ethanolic and water extracts of *P. biglobosa* had no effect against *Staph. aureus*. *Ps. aeruginosa* was reported to be insensitive to the water and ethanolic extracts of the plant¹⁶. The hot water extracts seem to have more antibacterial activity than ethanol and cold water extracts. Hot water has been reported to be effective in extraction of plant constituents¹⁷. This can also explain the traditional use of hot water to extract plants materials (decoction) by herbal medicine practitioners. The ethanol extracts also were active against the test bacteria species. The results of this work confirm the recent use of the local beer, very rich in ethanol, for the maceration of stem bark and leaf as a prescription by some traditional healers. Patients seem to have more rapid satisfactory effects when used this way⁴.

The stem bark extracts showed more antibacterial activity than the other tested parts of the plant as revealed by this study. This result agrees with that of Millogo-Kone et al.,⁴ who reported that the stem bark of *P. biglobosa* was very active against all the tested organisms. The antibacterial activity of the hot water extract of the stem was confirmed by the rate of kill experiment. The extract killed *E. coli* faster followed by *Staph. aureus* and then *Ps. aeruginosa*.

Escherichia coli ATCC11775 appeared to be the most susceptible to all the extracts. This result confirms those of Olutimayin et al.,¹⁷ Millogo-Kone et al.,⁴ Udobi and Onaolapo³ who reported the antibacterial activity of *P. biglobosa* against *E. coli*.

The conventional antibiotic gentamicin, consistently showed superior antibacterial activity than the extracts similar to the results presented by other workers^{18, 2}. This may be attributed to the fact that herbal medicinal products are prepared from plant and animal origins, most of the time subjected to contamination and deterioration while antibiotics are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures¹⁹.

In conclusion, *Parkia clappertoniana* (Keay) contains chemical constituents which possess antibacterial activity against *E. coli*, the causative agent of diarrhoea, fatal

dehydration, urinary tract and bladder infections; *Ps. aeruginosa*, an opportunistic pathogen in wounds especially burns and other diseases such as HIV/AIDS and *Staph. aureus* causative agent of skin lesions such as boils, pneumonia and gastroenteritis. The eye infection, diarrhea, healing of wounds and inflammation of testicles.

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