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Full Length Research Paper

Cell kill pattern and acute toxicity studies of the aqueous fraction of the methanolic extract of parts of *Parkia biglobosa*

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The pattern and rate of kill of the aqueous fraction of the methanolic extract of the stem bark of *Parkia biglobosa* (WS) against three standard organisms of medical and pharmaceutical importance; *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, as well as the acute toxic effects of the same extract along with those of the leaf (WL) and root (WR) were studied. Results obtained showed the fractions as bactericidal to the test organisms and that the *S. aureus* and *P. aeruginosa* were completely killed by WS within 120 min at 12.5 mg/ml and within 90 min at higher concentrations. LD_{50} results fell within the range of 500 – 5000 mg/kg body weight confirming them to be only slightly toxic and hence not potentially dangerous. These results are discussed in the context of the fact that *P. biglobosa* parts have been reported to be used extensively in the treatment of a wide variety of infections.

Key words: Methanolic, aqueous fraction, *Parkia biglobosa*, bactericidal, slightly toxic.

INTRODUCTION

Plants have traditionally provided a source of hope for novel drug compounds as plant herbal mixtures have made large contributions to human health and well being (Iwu et al., 1999). Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent (Betoni et al., 2006; Shibata et al., 2005) and the African biosphere which is endowed with the highest plant species, continues to be a potential source of these therapeutically useful compounds that can be used to meet immediate needs. These compounds after thorough studies can then be developed into acceptable antibacterial agents.

It is known that plants produce compounds that can be effective antimicrobials and resistance modifying agents. It is also known that the problem of antibiotic resistance has limited the use of most known antibiotics and has made the continual search for new antimicrobials compounds inevitable. Literature is now filled with so much information on compounds that have been isolated from a variety of medicinal plants (Sibanda and Okoh, 2007). Only very little of this (may be 1%) however, have successfully been exploited for clinical use as antibiotics (Gibbons, 2004). Reasons adduced for this include that a number of these compounds have MIC ranges greater than 1000 µg/ml and are believed to really be of no relevance from a clinical point of view (Gibbons, 2004). Another reason is the suggestion that compounds derived from plants are generally weak compared to bacterial or fungal produced antibiotics. May be the most pronounced reason is the fact that a lot of these compounds are so toxic that they cannot be used by humans. There has been no known report of *Parkia biglobosa* poisoning in humans but Fafioye et al. (2004) has reported an LC50 value of 2.4 ppm for the ethanolic extract of *P. biglobosa* against fish (*Clarias gariapinus*) juveniles in an acute toxicity test.

P. biglobosa, also called the African locust bean is a popular tree found scattered around many countries of West Africa. It is a popular food plant whose seeds are fermented to make dawadawa (Daddawa) -a strong smelling tasty food rich in protein by the Hausa people of Northern Nigeria as well as a coffee substitute while the

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pulp is made into a refreshing drink or eaten raw.

Parkia species is used extensively for medicinal purposes by many groups of people; the stem bark is used as a mouth wash as well as for skin infection, bronchitis, pneumonia, sores and diarrhea, violent colic and vomiting especially among the Hausa people of Northern Nigeria.

This paper presents the report of the studies of the pattern and rate of kill of parts of *P. biglobosa* as well as the acute toxicity of these parts.

EXPERIMENTAL

Plant collection and authentication

The plant materials were collected from Samaru village in Kaduna state Nigeria. They were authenticated by the staff of the herbarium section of the Biological Science Department of the Ahmadu Bello University Zaria, Nigeria where a voucher with specimen number 2846 is in place.

Extractions

Each plant material was air dried for five days and then ground into powder in a mortar. 250 g of each was extracted to exhaustion with methanol using a Soxhlet apparatus. Afterwards, the solvents were removed and the extracts obtained stored in the desiccator until needed.

Each of the crude extracts (leaf, stem bark and root) was further fractionated using Petroleum ether, Chloroform and water thus: 20 g of each dried extract was ground in a mortar and dissolved in 200 ml of water before shaking vigorously in a separating flask. The mixture obtained was filtered using a filter paper to remove debris. 200 ml of petroleum ether was then added to the mixture, shaken vigorously and allowed to settle. The petroleum ether layer (on top) was removed and concentrated while a further 200 ml of chloroform was added to the aqueous layer and also vigorously shaken and allowed to settle. The aqueous and the chloroform layers were further separated and while the chloroform portion was concentrated to dryness by allowing to stand on the laboratory bench until all the solvent evaporated (for some other purpose), the aqueous layer was concentrated to dryness using (mild) heat. The resulting fractions were appropriately stored in a desiccator until needed. .

Determination of rate of kill

To four bottles labeled 1, 2, 3 and 4 were added 9 ml each of nutrient broth. Bottle 1 was used as the control while to bottles 2, 3 and 4, were added standardized culture and then 1 ml of 31.25, 62.5 and 125 mg/ml solutions of the aqueous fraction of the stem bark of *P. biglobosa* respectively to give effective concentrations of 3.125, 6.25 and 12.5 mg/ml. The bottles were then incubated at 37^0C and viable counts taken at 30 min intervals by withdrawing 0.1 ml of the mixture in the bottle and diluting in normal saline containing 3% Tween 80. The diluted mixtures were plated on nutrient agar plates and incubated at 37^0C for 24 h. Developed colonies were then counted and the number of colony forming units (Cfu/ml) was calculated.

Acute toxicity studies

The method of Lorke (1983) was used. For each fraction [aqueous fraction of the methanolic extract of leaf (WL), aqueous fraction of the methanolic extract of stem bark (WS) and aqueous fraction of the methanolic extract of root (WR)], nine (9) mice of average weight of 26 g were divided into 3 groups. To each group, one of 3 doses of the fraction was administered through the intraperitoneal route namely 10, 100 and 1000 mg/kg body weight. The animals were then observed over a period of 24 h for mortality. The response of the animals was noted. Doses lower than those where mortality was observed or higher than doses where no mortality was observed were chosen and one experimental animal of average weight of 25 g was administered with a dose each (as directed in the Lorke, 1983 table) and the LD_{50} was calculated using the formular:

$$LD_{50} = \sqrt{C \times D}$$

Where C = lethal dose and D = survival dose

RESULTS

Determination of rate of kill

The general pattern of kill of the test organisms by WS is shown in Figures 1-3. A prominent reduction in cell population on exposure to different concentrations of WS was observed for *S. aureus* and *P. aeruginosa* but not for *E. coli.* Figure 1 shows the cell reduction pattern for these test organisms when exposed to a concentration of 12.5 mg/ml. An initial log cycle reduction for *P. aeruginosa* took about 100 min while for *S. aureus*, it took only 90 min. Generally, it took only about 120 min for the cell population of *S. aureus* and *P. aeruginosa* to be completely killed. The cell population of *E. coli* began to reduce initially but after about 60 min, the population stabilized.

Figures 1 to 3 shows the reduction pattern of the test organisms when exposed to other concentrations of WS (higher and lower than 12.5 mg/ml). It confirmed that higher concentrations of the agent (WS) reduced the population of the test organisms (*S. aureus* and *P. aeruginosa*) to zero within a shorter time than lower concentrations and their susceptibility can then be said to be concentration dependent. The same was not the case for *E. coli* (Figure 1) as no appreciable reduction in the number was observed at the concentration of 12.5 mg/ml.

Acute toxicity studies

Results of toxicity studies showed that all the aqueous fractions (WL, WS and WR) are safe. The stem showed to be more toxic than the root, which showed to be more toxic than the leaf. (Stem > Root > Leaf).

DISCUSSION

After exposure to different concentrations of WS, a reduction in cell population of the test organisms over

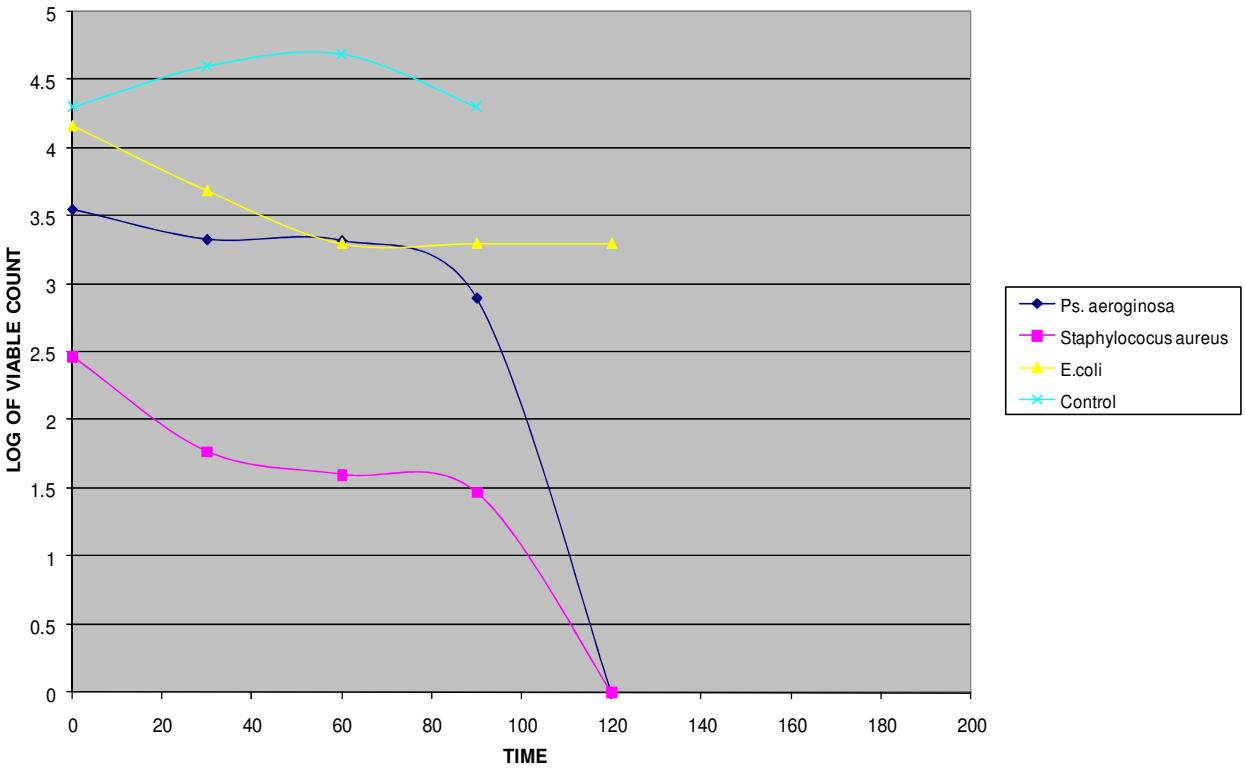

Figure 1. Graph of log variable count of *Staphylococcus aureus, P. aeruginosa* and *Escherichia coli* against time after exposure to 12.5 mg/ml of WS.

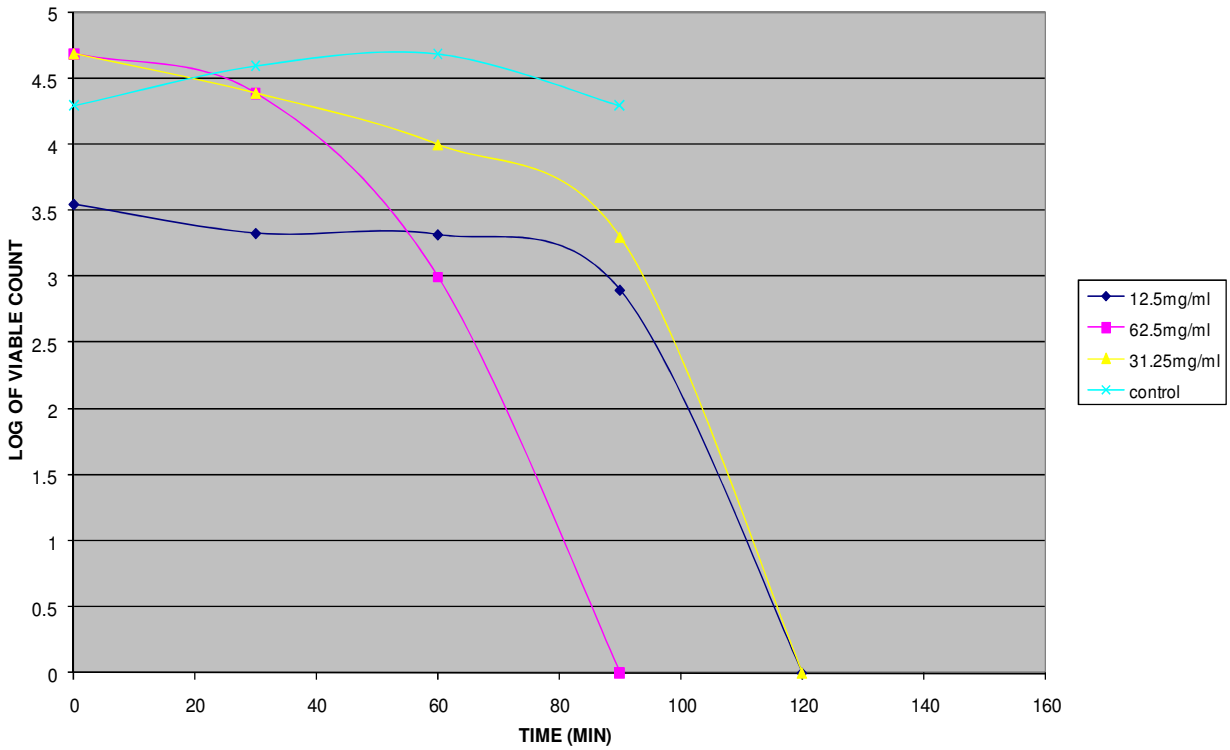

Figure 2. Graph of log variable count of *P. aeruginosa* against time after exposure to different concentrations of WS.

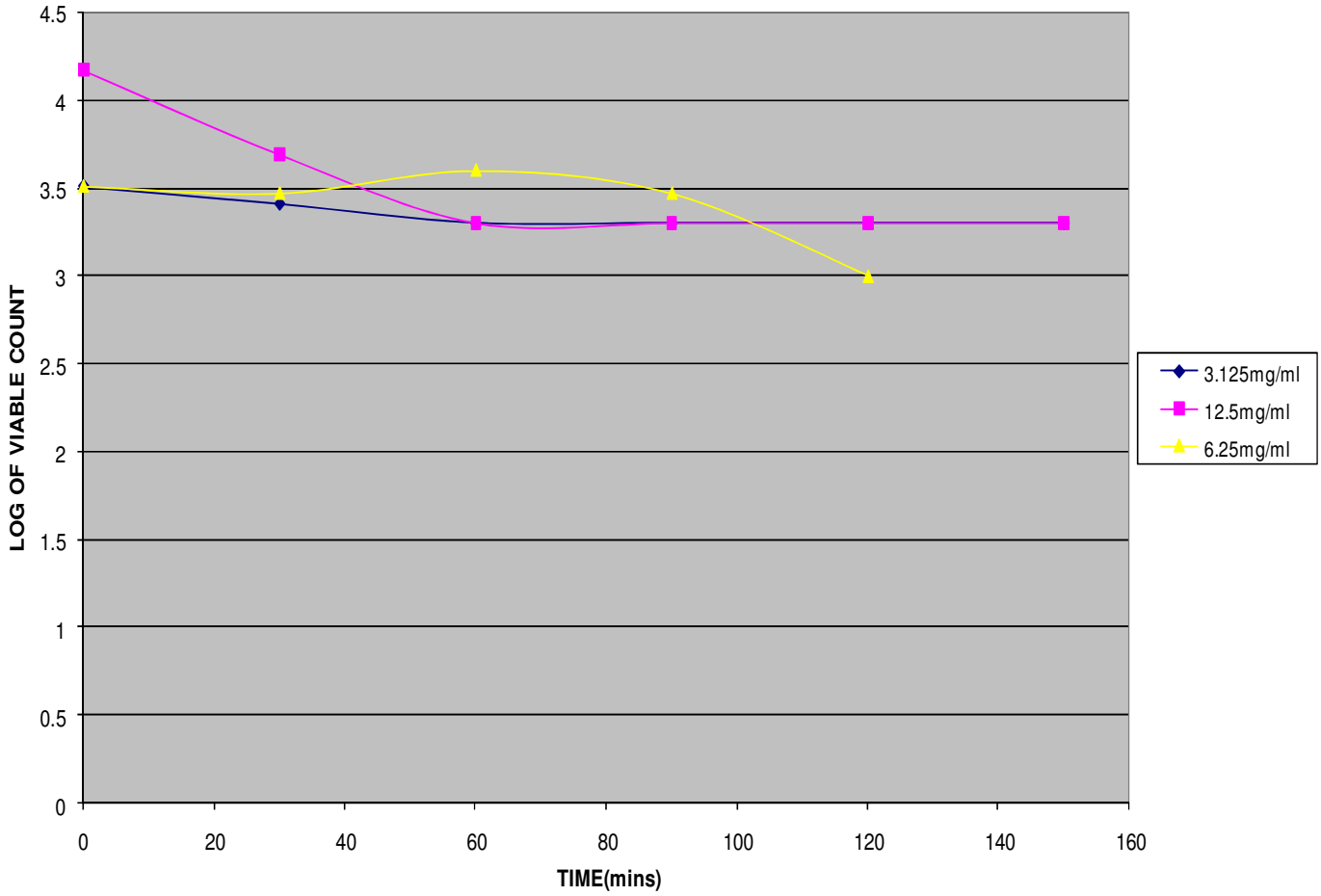

Figure 3. Graph of log variable count of *Escherichia coli* against time after exposure to different concentrations of WS.

time was observed (Figures 1 - 3). *S. aureus* and *P. aeruginosa* which had consistently shown good susceptibility to WS were completely killed by the fraction at a concentration of 12.5 mg/ml within 120 min. At higher concentrations of about 31.25 and 62.5 mg/ml, it took only about 90 min to achieve a total kill of the two organisms. This kill pattern confirms the fraction as bactericidal against the test organisms. It is however not the case with *E. coli*. At a concentration of 12.5mg/ml and lower, no kill was observed for up to 150 min. Although the mode of action of the fractions is yet to be determined, it is known that Gram positive organisms are more sensitive to some antibacterial agents than Gram negative organisms because of their cell wall composition (Oyi, 2001). The composition of the cell wall of bacteria differs between the Gram positive and Gram negative organisms but they both contain a basal structure which is made of N-acetylmuramic acid and N-acetylglucoseamine molecules. The envelope of the Gram negative organisms are however more complex. Besides its cell wall basal structure, there is a second membrane to the exterior which is made up of lipids and protein bilayers. This outer layer confers some characteristics on Gram negative bacteria one of which is its restrictive permeability which accounts for the resistance of Gram negative organisms to antibiotics by preventing the accumulation of these agents to high concentrations at their target sites. The susceptibility of *P. aeruginosa* even though a Gram negative organism, is explained by the fact that the outer membrane of *Pseudomonads* differ significantly from that of other Gram negative bacteria (Hammond and Lambert, 1984).

Acute toxicity results obtained confirmed that the fractions (WS, WR and WL) were only slightly toxic for mice (Tables 1-3). The leaf fraction is less toxic than those of the root and stem bark. Fafioye et al. (2004) reported an LC50 value of 2.4 ppm for the ethanolic extract of the stem bark of *P. biglobosa* against fish (*Clarias gariepinus*) juveniles in an acute toxicity test. The relatively higher toxic level of the stem bark fraction may be explained by the presence in the stem bark and pods of *P. biglobosa*, of a piscicide (an alkaloid parkine) which is a known poison against fish and has also been reported by other researchers (Duker-Eshun et al., 2001).

Table 1. Toxicity studies of the aqueous fraction of the leaf of *P. biglobosa.*

Fraction		Wt of mice (g)	Concentration of fraction injected (mg/kg body weight)	Deaths	LD_{50} mg//kg body weight
WL	Day 1	24.2	1000	$^0/_3$	$\sqrt{CxD}$ = 5000x2900 = 3807
		23.8	1000		
		29.5	1000		
		23.5	100	$^0/_3$	
		31.3	100		
		25.4	100		
		22.0	10	$^0/_3$	
		19.3	10		
		33.4	10		
	Day 2	18.8	1600	$^0/_1$	
		16.7	2900	$^0/_1$	
		24.2	5000	$^1/_1$	

LD_{50} < 1 mg/kg body weight = extremely toxic; LD_{50}1 – 50 mg/kg body weight = highly toxic; LD_{50} 50 – 500 mg/kg body weight = moderately toxic; LD_{50} 500 – 5000 mg/kg body weight = slightly toxic; LD_{50} > 5000 mg/kg body weight = practically non toxic (harmless).

Table 2. Toxicity studies of the aqueous fraction of the root of *P. biglobosa* (WR).

Fraction		Wt of mice (g)	Concentration of fraction injected (mg/kg body weight)	Deaths	LD_{50} mg//kg Body weight
WR	Day 1	30.0	1000	$^2/_3$	$\sqrt{CxD}$ 1600x800 = 1131
		26.6	1000		
		27.8	1000		
		20.8	100	$^0/_3$	
		21.3	100		
		27.3	100		
		34.7	10	$^0/_3$	
		36.4	10		
		30.6	10		
	Day 2	26.1	200	$^0/_1$	
		26.0	400	$^0/_1$	
		22.0	800	$^0/_1$	
		20.0	1600	$^1/_1$	

LD_{50} < 1 mg/kg body weight = extremely toxic; LD_{50}1 – 50 mg/kg body weight = highly toxic; LD_{50} 50 – 500 mg/kg body weight = moderately toxic; LD_{50} 500 – 5000 mg/kg body weight = slightly toxic; LD_{50} > 5000 mg/kg body weight = practically non toxic (harmless).

There is no known reports of *Parkia biglobosa* poisoning in humans. This must explain why it is extensively used in the preparation of medicinal recipes in most parts of West Africa. The results obtained in this work, is therefore, of tremendous interest. This is because most plant products which have been found to have good antibacterial activity cannot be developed into the much desired products to be used against pathogenic microorganisms since they are toxic to humans. It must also be because of this reason that less than 1% of isolated plant compounds known to have good antibacterial activity cannot be used for this purpose (Sibanda and Okoh, 2007)

Since the mode of action of the fractions is known to be concentration dependent, quite a reasonable concentration of the fraction can be used to eliminate the organisms over a short time without the fear of its toxic effect. Further work on chronic toxicity, is however necessary to confirm if the reported toxic constituents of the stem bark will have any effect on humans over time.

Table 3. Toxicity studies of the aqueous fraction of the stem bark of *P. biglobosa* (WS).

Fraction		Wt of mice (g)	Concentration of fraction injected (mg/kg body weight)	Deaths	LD_{50} mg//kg body weight
WS	Day 1	24.0	1000	$^{2}/_{3}$	$\sqrt{CxD}$ 800x400 = 565.7
		22.6	1000		
		27.8	1000		
		27.7	100	$^{0}/_{3}$	
		29.2	100		
		28.4	100		
		27.0	10	$^{0}/_{3}$	
		31.8	10		
		29.6	10		
	Day 2	24.9	200	$^{0}/_{1}$	
		24.0	400	$^{0}/_{1}$	
		23.0	800	$^{1}/_{1}$	
		25.0	1600	$^{1}/_{1}$	

LD_{50} < 1 mg/kg body weight = extremely toxic; LD_{50}1 – 50 mg/kg body weight = highly toxic; LD_{50} 50 – 500 mg/kg body weight = moderately toxic; LD_{50} 500 – 5000 mg/kg body weight = slightly toxic; LD_{50} > 5000 mg/kg body weight = practically non toxic (harmless).

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