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THE ANTIMICROBIAL ACTIVITY OF THE STEM-BARK AND LEAF OF *PARKIA CLAPPERTONIANA* KEAY FAMILY LEGUMINOSAE AGAINST SELECTED MICROORGANISMS.

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Abstract

In this work, the antimicrobial activity of the stem bark and leaves of *Parkia clappertoniana* Keay was investigated. The activity of the crude extract was tested on both Gram-positive and Gram-negative bacteria. A comparative study of the effects of the methanolic and aqueous extracts revealed that the methanolic extract was more potent. The screening also revealed that both the stem bark and leaves of *P. clappertoniana* were effective against all test organisms. The activity was however more pronounced on Gram-positive organisms with *Staphylococcus aureus* being more susceptible and *Pseudomonas aeruginosa* being most resistant. Since the traditional herbalist claims that the plant cures diarrhoea and dysentery and if the disease condition is caused by bacteria, then it can be concluded that his claims might be true based on the results obtained.

Keywords: *Parkia clappertoniana*; antimicrobial activity; Gram-positive bacteria; Gram-negative bacteria

Introduction

A large proportion of the population in a number of developing countries (including Nigeria) still rely on traditional practitioners, including traditional birth attendants, herbalists and bone-setters and on local medicinal plants to satisfy their primary health care needs. Traditional medicines have maintained its popularity in a number of Asian countries, such as China, India, Japan and Pakistan. The Japanese per capita consumption of herbal medicine appears to be highest in the world (WHO, 1996). Herbalists in the Jos metropolis of Plateau State claim that they use *P. clappertoniana* stem bark and leaf extracts for the treatment of diarrhoea, dysentery and abdominal pain.

Parkia clappertoniana Keay (Leguminosae) is a widespread savanna tree commonly known as the 'Dorowa'. It is easily recognized by its bright red pendulous flowers. Its local (Nigerian) names include: *Dorowa* (Hausa); *Narehi* (Fulani); *Lonchi* (Nupe); *Nune* (Tiv); *Igba* (Yoruba); *Ugba* (Etsako); *Eyiniwan* (Edo) *Ogirilli* (Igbo).

The bark, leaves, flowers and pods have innumerable medicinal and food utilizations, the pods in particular (husk and pulp) are staple food for humans, and are stored in households (Le Houerou 1976).

The bark of the lower part of the tree is often cut away for medicinal uses: an infusion of the bark is regarded as a tonic. A decoction of the husks is taken cold as an astringent for diarrhoea. The young unexpanded flower buds called "*gundatuntu*" in Hausa are sometimes used as a medicine or preventive for leprosy. The leaves and roots beaten up

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with water are remedy for some eye problems in Gambia. The bark is used by the Madingoes in Gambia as a specific toothache antidote. The seeds are said to be capable of rendering foul stinking water potable (Keay and Onoche 1964).

The present study investigated the possible anti-bacterial property of the crude drug extracts of the stem-bark and leaf of *Parkia clappertoniana* with a view to verify the claim.

MATERIALS AND METHODS

Plant Materials. The stem-bark and the leaves of *Parkia clappertoniana* were collected from the Federal School of Forestry, Bauchi Road, Jos in Plateau State, Nigeria and identified by Thomas Yakubu of the Department of Pharmacognosy, University of Jos. The plant materials were dried in the sun for two days (leaves) and five days in the case of the stem-bark. After drying they were pounded separately to fine powders using pestle and mortar. The powders were packed separately, labeled and thereafter used in the extraction procedures.

Preparation of the Crude Plant Extracts.

Aqueous Extracts

a) Hot Decoction

80g of the powdered stem-bark were placed into a 1 litre beaker containing 700ml distilled water. Content of the beaker was heated at constant temperature of 4 hours. The decoction was then filtered using a suction pump. The filtrate was evaporated to dryness at 60^{o}C.

b) Cold extraction

The same amount of powdered plant material was soaked in distilled water in a round bottom flask 24 hours and then transferred to a flask shaker for 3 hours. The menstrum was filtered with help of a suction pump and the filtrate evaporated to dryness also at 60^{o}C. The above procedures were carried out using the powdered leaves sample.

Ethanolic Extract

a) Hot Extraction

50g of the powdered stem-bark was loaded into a soxhlet extractor and exhaustively extracted with ethanol over a period of 4 days. The extract was then evaporated to dryness at 80^{o}C over a hot water bath. The above procedure was repeated using 50g of powdered leaves but extraction was done over 3 days.

b) Cold Extraction

50g of powdered stem-bark was soaked in 70% ethanol in a round bottom flask for 24 hours after which the flask was mechanically shaken for 3 hours. The extracted mix was filtered and evaporated to dryness as usual. The procedure was repeated using the powdered leaves sample.

Assays/ Test Organisms

For the assays, the dry extracts were reconstituted in distilled water and tested at various concentrations against clinical isolates of *Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Proteus mirabilis* and *Staphylococcus aureus* obtained from the Jos University Teaching Hospital (JUTH). A laboratory strain of *Bacillus subtilis* was also used in the screening. Gentamycin B.P was used as the reference drug. The bacterial cultures were maintained on slants of nutrient agar (Oxoid). Prior to testing, they were subcultured in nutrient broth for 24 hours at 37^{o}C then each culture was suitably diluted with sterile distilled water to obtain the suspensions containing approximately 10^{6} cells/ml used in the tests.

Antibacterial screening of the plant extracts was carried out by the Agar cup diffusion method in which plates of nutrient agar (Oxiod) seeded with the various bacterial cultures were used. Extract concentrations ranging from 200mg/ml to 25mg/ml and reference drug were applied to each dish. A pre-diffusion time of 1 hour was allowed before incubation aerobically at 37^{o}C for 24

hours and appropriate controls were also set up. Zone diameters were measured (mm) and the mean diameters recorded. The minimum inhibitory concentrations (MIC) of the extracts were determined using the tube dilution method. The minimum bactericidal concentration (MBC) was also determined from the MIC results.

Killing Time. The effect of ½MIC, MIC and 2MIC concentrations of the extract on the viability of Gram-positive *Bacillus subtilis* and *E. coli* (Gram-negative) were also determined. A known inoculum of test organism was placed in contact with a specified extract concentration and samples of the extract-organism mixture were withdrawn at 20 minutes intervals and viable counts carried out to determine the time it would take to kill all viable cells. The results were graphically displayed.

RESULTS AND DISCUSSION

The results of the sensitivity tests show that both the aqueous and ethanolic extracts of *P. clappertoniana* possess activity against the organisms used. The results suggest that the soxhlet and hot water extracted crude drugs were more active than the drug extracted by non-heat methods. It is possible that the longer duration of extraction with 70% ethanol and the heat applied to the aqueous extract caused more of the active ingredients(s) to be extracted. Since the majority of the constituents of the stem-bark of *P. clappertoniana* are tannins and condensed tannins, ethanol must have been a good solvent for the constituents (Trease, 1989). The extract showed some degree of frothing on agitation, thus the likelihood of the presence of saponins cannot be excluded. Saponins have been known to possess marked antimicrobial activity (Waff, 1968; Wolters, 1966).

The results of the sensitivity tests show that both extracts (ethanolic and aqueous hot and cold extracted) are more active against Gram-positive bacteria especially *Bacillus subtilis,* and then *Staphylococcus aureus, Proteus mirabilis* was the most susceptible of the Gram-negative organisms (Tables 1, 2, 3). On the other hand, *Pseudomonas aeruginosa* seemed resistant to the action of the (hot) aqueous extract of the stem-bark (Table 1) whilst it was fairly susceptible to the ethanolic extracts of both stem-bark and leaves (Table 2 & 4).

It is interesting to note that *P. aeruginosa* (and other pseudomonads) has been reported to be resistant to most antimicrobial agents (especially in the presence of Ca^{++} and Mg^{++} (Jawetz et al 1984), yet it was susceptible to the extracts of *P. clappertoniana.* Similarly the other Gram-negative organism *Shigella dysenteriae* which causes dysentery, is shown to be fairly susceptible to all the extracts apart from the stem-bark, cold ethanolic extract. The results also show that the MIC for the ethanolic stem-bark and leaf extracts is 5mg/ml for all the test organisms except *Staph aureus* which was inhibited at 10mg/ml. But for the aqueous stem-bark, all other test organisms were inhibited at 20mg/ml except *Pr. mirabilis* which had a MIC of 10mg/ml (Table 5). Thus it can be said that *Staph aureus* requires a higher concentration for drug to inhibit its growth. This is in accordance with the report of Jawetz et al. 1984 which states "Staphylococci rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems." On the other hand *Pr. mirabilis* susceptibility to the action of the extracts can be attributed to its reported sensitivity to various antimicrobial agents such as Penicillins, aminoglycosides, cephalosporins and chloramphenicol (Jawetz, et al. 1984).

In the MBC test, growth was observed after subculturing the MIC tubes on an antibacterial agent-free agar. This suggests that the crude extract of *P. clappertoniana* has a bacteriostatic effect on both Gram-positive

and Gram-negative bacteria. However it is noted that a bacteriostatic agent can be made cidal by merely increasing its concentration (Marshal and Hrenoff, 1987). This fact is shown in the results of effect of concentrations of extract on the viable count of chosen test organisms. From figures 1 and 2 it was observed that doubling the concentration of the extract lead to a corresponding decrease in number of viable organisms. At twice MIC (10mg/ml) the population was reduced to less than 1 viable cell in 140 minutes with *B. subtilis* and 160 minutes in the case of *E. coli.* The control which contained no extract continued an exponential growth. Thus the extract can become a bactericidal agent at higher concentrations.

Parkia clappertoniana is used traditionally as an antidiarrhoeal and antiamoebic. *E. coli* has been implicated in diarrhoea of bacterial origin (Sack, 1975; Rowe 1977). Enterotoxigenic *E. coli* is the most common cause of traveler's diarrhoea (Jawetz et. al. 1984). Certain strains of staphylococci produce enterotoxin which causes food poisoning and therefore implicated also in diarrhoea. Ingestion of 25µg of enterotoxin B results in vomiting and diarrhoea in humans and monkeys. Certain strains of *Bacillus,* although rarely caused diseases in humans, can grow in foods and produce an enterotoxin that causes vomiting and bloody diarrhoea (Jawetz et. al. 1984). *Shigella dysenteriae* is a facultative anaerobe which causes bacillary dysentery and abdominal cramps. Therefore, the fact that both the aqueous and ethanolic extracts of *Parkia clappertoniana* were active against these organisms (*E. coli, Staph aureus, B. subtilis, Shigella dysenteriae*) may explain the basis for the use of the preparation from this plant in the said disease states. This study represents a contribution towards the accumulation of data on useful medicinal plants in this sub-region.

Table 1a: Zones of inhibition of hot aqueous extract of stem bark *P. clappertoniana* on the test organisms.

Test Organisms	*Diameter, Zone of Inhibition (mm) at given amounts applied					
	Extracts (mg)					Gentamicin (µg)
	40	20	10	5	2.5	40
Staph aureus	20.0	18.0	14.0	12.0	10.0	15.0
B. subtilis	20.0	18.0	15.0	12.0	11.0	16.0
P. mirabilis	20.0	18.0	16.0	14.0	12.0	25.0
Ps. aeruginosa	12.0	10.0	10.0	10.0	10.0	16.0
E. coli	20.0	17.0	14.0	12.0	10.0	18.0
Sh. dysenteriae	18.0	16.0	14.0	12.0	10.0	16.0

* mean zone diameters from two replicates are recorded in all tables.

Table 1b: Zones of inhibition of cold aqueous extract of stem bark *P. clappertoniana* on the test organisms.

Test Organisms	*Diameter, Zone of Inhibition (mm) at given amounts applied				
	Extracts (mg)				Gentamicin (µg)
	40	20	10	5	40
Staph aureus	15.0	12.0	12	10	16
B. subtilis	15.0	13.0	12	10	16
P. mirabilis	15.0	13.0	12	10	20
Ps. aeruginosa	14.0	13.0	10	10	17
E. coli	15.0	14	12	10	18
Sh. dysenteriae	15.0	13	12	10	18

Table 2a: Zones of Inhibition of hot ethanolic extract of stem bark of *Parkia clappertoniana* on test organisms.

Test Organisms	*Diameter, Zone of Inhibition (mm) at given amounts applied				
	extracts (mg)				extracts (mg)
	40	20	10	5	40
Staph aureus	22.0	20.0	18.0	16.0	16.0
B. subtili	20.0	18.0	15.0	13.0	18.0
P. mirabilis	20.0	18.0	15.0	15.0	24.0
Ps. aeruginosa	15.0	13.0	12.0	10.0	18.0
E. coli	18.0	16.0	14.0	11.0	15.0
Sh. dysenteriae	18.0	15.0	12.0	10.0	18.0

Table 2b: Zones of Inhibition of cold ethanolic extract of stem-bark of *P. clappertoniana* on test organisms.

Test Orgnisms	*Diameter, Zone of Inhibition (mm) at given amounts applied				
	extracts (mg)				Extracts (mg)
	40	20	10	5	40
Staph aureus	16.0	14.0	13.0	11.0	14
B. subtilis	16.0	14.0	12.0	10.0	16
P. mirabilis	16.0	14.0	12.0	10.0	16
Ps. aeruginosa	14.0	12.0	10.0	10.0	14
E. coli	15.0	13.0	11.0	10.0	15
Sh. dysenteriae	13.0	10.0	10.0	10.0	15

Table 3: Zones of Inhibition of cold aqeuous extract of leaves of *P. clappertoniana* organisms.

Test Orgnisms	*Diameter, Zone of Inhibition (mm) at given amounts applied				
	extracts (mg)				extracts (mg)
	40	20	10	5	40µg
Staph aureus	18	15	12	10.0	12
B. subtilis	20	16	13	11.0	17
P. mirabilis	18	16	14	12.0	15
Ps. aeruginosa	16	14	11	10.0	15
E. coli	17	14	12	10.0	16
Sh. dysenteriae	16	13	11	10.0	15

Table 4: Zones Inhibiton of ethanolic extract of leaves (Hot, Cold), of *P. clappertoniana* on test organisms.

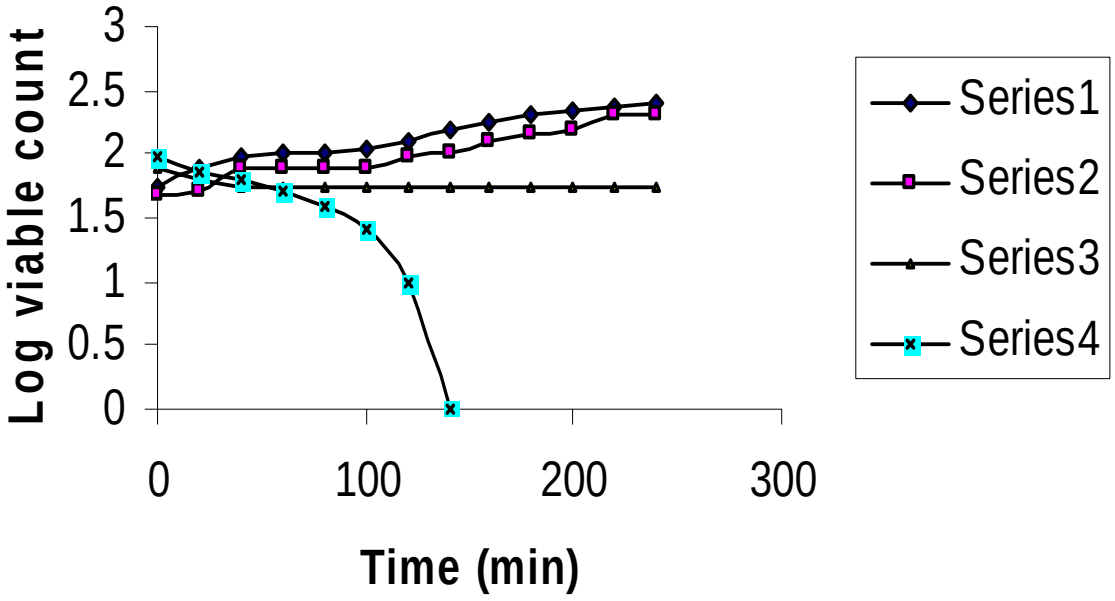
Test Orgnisms	*Diameter, Zone of Inhibition (mm) at given amounts applied				
	extracts (mg)				extracts (mg)
	40	20	10	5	40µg
Staph aureus	20, 18	18, 16	16, 13	15, 10	16, 16
B. subtili	18, 21	15, 18	13, 14	12, 12	16, 15
P. mirabilis	22, 20	20, 18	18, 14	16, 12	18, 20
Ps. aeruginosa	14, 17	12, 14	10, 12	10, 10	16, 17
E. coli	14, 17	12, 15	12, 13	10, 10	16, 18
Sh. dysenteriae	13, 15	11, 14	10, 11	10, 10	15, 17

Table 5a: Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of ethanolic and aqueous extract of the stem bark *P. clappertoniana* on test organisms.

Organisms	Ethanolic		Aqueous	
	MIC	MBC	MIC (mg/ml)	MBC (mg/ml)
Staph aureus	10	>20	20	>40
B. subtilis	5	20	20	>40
P. mirabilis	5	20	10	>40
Ps. aeruginosa	5	20	20	>40
E. coli	5	20	20	>40
Sh. dysenterae	5	20	20	>40

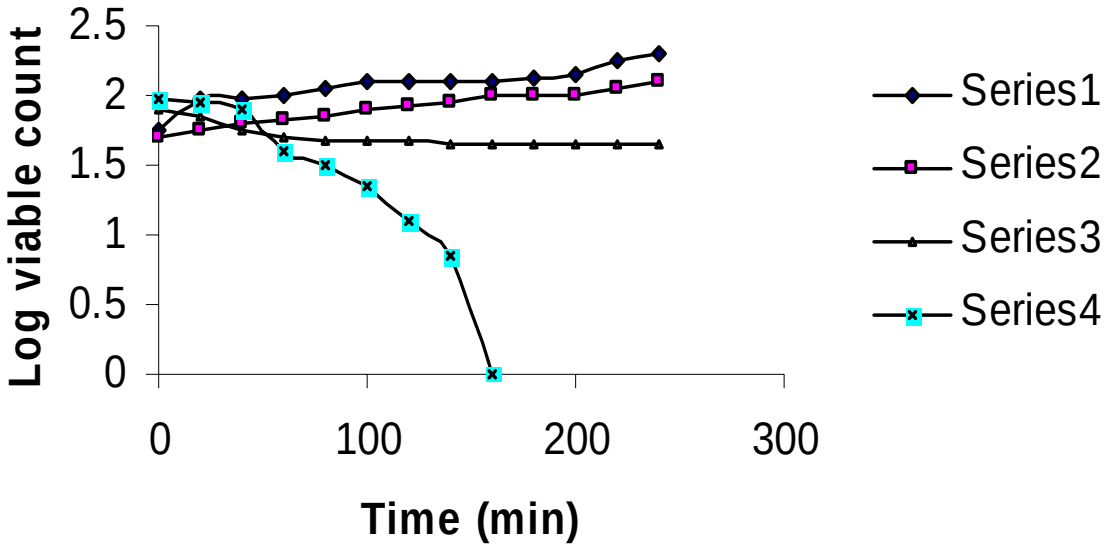
Table 5b: Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of ethanolic and aqueous extract of leaves of *P. clappertoniana* on test organisms.

	Ethanolic		Aqueous	
Organisms	MIC	MBC	MIC (mg/ml)	MBC (mg/ml)
Staph aureus	10	20	10	20
B. subtilis	5	10	2.5	10
P. mirabilis	5	10	5	20
Ps. aeruginosa	5	20	5	20
E. coli	5	20	5	20
Sh. dysenteriae	5	20	5	20



Series 1 – Control; Series 2 – ½ MIC; Series 3 – MIC; Series 4 - 2MIC

Fig 1: Effect of different concentrations of ethanolic stem bark extract of *Parkia clappertoniana* on the viable count of *B. subtilis*



Series 1 – Control; Series 2 – ½ MIC; Series 3 – MIC; Series 4 - 2MIC

Fig 2: Effect of different concentrations of ethanolic stem bark extract of *Parkia clappertoniana* on the viable count of *E. coli*

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