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**Phytochemical and Antibacterial Studies of Root Extract of *Cochlospermum tinctorium* A. Rich. (Cochlospermaceae)**

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**Abstract:** Methanol extract of the root of *Cochlospermum tinctorium* was evaluated for antibacterial activities using hole-in-plate bioassay technique against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Corynebacterium ulcerans*, *Proteus mirabilis* and *Shigella dysenteriae* using ciprofloxacin (10 µg mL<sup>-1</sup>) and gentamicin (10 µg mL<sup>-1</sup>) as reference standards. The extract was active on all the test organisms at concentration of 2000 µg mL<sup>-1</sup>. The activity of the extract against *S. dysenteriae* was found to be more potent with MIC 100 and MBC 500 µg mL<sup>-1</sup>. Time kill studies showed that the antibacterial activities were time dependent. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins and cardiac glycosides. These phytochemicals could be responsible for the antimicrobial activities exhibited by the extract and hence justify the ethnomedicinal uses of *C. tinctorium*.

**Key words:** *Cochlospermum tinctorium*, phytochemical, antibacterial, time kill studies

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## INTRODUCTION

For centuries, medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health care facilities are grossly inadequate. In Africa, traditional medicine is of great value and more than 70% of the people refer to traditional healers concerning health issues (Kamanzy *et al.*, 2002). In Nigeria, as in other African countries, several roots, leaves, fruits and barks of plants are used for different medicinal purposes; some of which have been discovered in many researches to be rich in secondary metabolites like tannins, alkaloids, flavonoids, phenols, steroids and volatile oils which are responsible for their therapeutic activities (Cowan, 1999; Rabe and Vanstoden, 2000). The continued investigation of the secondary plant metabolites has led to important breakthroughs in pharmacology and has helped tremendously in the development of modern pharmacotherapeutics in Africa and other parts of the world (Nwaogu *et al.*, 2007).

*Cochlospermum tinctorium* is a savannah plant found in fallow farms across Northern Nigeria. It is a shrub that grows up to 10 m high. The leaves are alternate, palmate lobed with stipules. The inflorescence consists of bright yellow flowers that are regular or slightly irregular and borne in racemes or panicles. Fruits are elongated 3-5 valve capsule containing seeds that are embedded in cotton foam (Hutchinson and Dalziel, 1963). The plant is commonly known as Rawaya (Hausa) and is a familiar herb in the traditional medicinal preparations in Northern Nigeria, where decoctions of the whole roots

are used as remedy for gonorrhoea, jaundice and gastrointestinal diseases (Mann *et al.*, 2003). Elsewhere, the roots are used as remedy for malaria and schistosomiasis (Traore *et al.*, 2006) and are also found to have hepatoprotective effects (Diallo *et al.*, 1992).

Earlier studies have indicated that triacylbenzenes and long chain volatile ketones (arjunolic acid), apocarotenoids, tannins (ellagitannins) (Diallo *et al.*, 1991) and 3-O-E-p-coumaroylaliphitic acid (Ballin *et al.*, 2002) were all isolated from the roots of *C. tinctorium*. Few pharmacological studies have been conducted on the plant. In this study, we report the phytochemical, antibacterial and time kill studies of methanol extract of *C. tinctorium*, with a view to gaining insight into its therapeutic potentials in its local use for the treatment of gastrointestinal ailments in Northern Nigeria.

## MATERIALS AND METHODS

### Sample Collection

The roots of *Cochlospermum tinctorium* were collected from Dakace village along Jos road, Zaria, Nigeria in July, 2006. The plant was authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria and voucher specimen (No. 2314) was deposited there.

### Preparation of Plant Material and Extraction

The fresh roots of the plant were air dried, pulverized to fine powder using a porcelain mortar and pestle. The pulverized material weighing 150 g was extracted exhaustively with methanol by simple percolation (cold extraction) for one week. The extract was filtered and concentrated in vacuo at 40°C using a rota vapor after which about 7.05 g of crude methanol extract was obtained.

### Phytochemical Screening

The extract was subjected to various phytochemical tests to identify the constituent secondary metabolites using standard methods as described by Harborne (1984), Sofowora (1993) and Trease and Evans (1989).

### Test Organisms

#### Six Clinical Bacterial Strains

*Escherichia coli*, *Klebsiella pneumoniae*, *Corynebacterium ulcerans*, *Staphylococcus aureus*, *Proteus mirabilis* and *Shigella dysenteriae* were obtained from the Department of Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Shika. The isolates were purified on nutrient agar (OXOID) plates and characterized using standard microbiological and biochemical procedures as described by Cowan and Steel (1974).

### Determination of Antibacterial Activity

The antibacterial activity was carried out by utilizing the hole-in-plate bioassay procedure as reported by Karou *et al.* (2006). Pure culture of the organisms was inoculated on to Mueller-Hinton Agar (MERCK) incubated for 24 h at 37°C. About 5 discrete colonies were aseptically transferred with a sterile wire loop into a tube containing sterile normal saline (0.85% NaCl) and were adjusted to a turbidity of 0.5 MacFarland standards. The suspension was used to streak on the surface of Muller Hinton agar plates with sterile swab. A sterile 6 mm diameter cork borer was used to make holes into the set agar in petri dishes containing the bacterial culture. The wells were filled with 500, 1000 and 2000  $\mu\text{g mL}^{-1}$  concentrations of the extract. Standard antibiotics ciprofloxacin (10  $\mu\text{g mL}^{-1}$ ) and gentamicin (10  $\mu\text{g mL}^{-1}$ ) were used as reference or positive control. The plates were incubated for 24 h at 37°C. All the tests were performed in triplicate and the antibacterial activities were expressed as mean diameter of inhibition zones (mm) produced by the plant extract.

#### Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration was carried out using micro broth dilution in accordance with National Committee for Clinical Laboratory Standard (2006). Serial dilution of the least concentration of the extract that showed activity was prepared using test tubes containing 9 mL of double strength broth. The tests tubes were inoculated with the suspension of the standardized inocula and incubated at 37°C for 24 h. MICs were recorded as the lowest concentration of extract showing no visible growth of the broth.

#### Determination of Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration was determined by aseptically inoculating aliquots of culture from MIC tubes that showed no growth, on sterile nutrient agar plates and incubating at 37°C for 48 h. MBC was recorded as the lowest concentration of extract showing no bacterial growth.

#### Determination of Rate of Kill

The rate of kill was determined only for bacterial isolates most susceptible to the extract. Inocula were grown over night at 37°C in Mueller Hinton Agar (MHA). The over night broth was adjusted to a 0.5 McFarland standard in nutrient broth and a further dilution was made by inoculating 200 µL in glass flask containing 20 mL of sterile nutrient broth. The flask was shaken and incubated for 90 min at 37°C to ensure that the organisms were out of their lag phases and into their logarithmic phases.

After the 90 min initial incubation period, 20 µL of the culture of each organism was inoculated into test tubes of nutrient broth containing 10 mL of MIC of the extract for each organism. Control was set up consisting of two duplicate test tubes as negative control (without extract) and positive control with 0.5 µg mL<sup>-1</sup> of ciprofloxacin. All test tubes were incubated at 37°C and samples sub cultured at 2, 4, 6, 8, 10, 12 and 24 h. Colony counts were performed by making serial dilutions in physiological saline, plating 100 µL of each dilution on to MHA and incubated for 48 h at 37°C. Viable counts were calculated to give cfu mL<sup>-1</sup> and kill curve were plotted with time against logarithm of the viable count. Each experiment was performed twice on separate occasions.

### RESULTS AND DISCUSSION

Results of the phytochemical screening revealed the presence of alkaloids, flavonoids, tannins and cardiac glycosides, but saponin was found to be absent (Table 1). Antibacterial activity of the extract at 2000 µg mL<sup>-1</sup> showed that *S. aureus* and *S. dysenteriae* exhibited the highest zone of inhibition (19.0 mm) whereas *K. pneumoniae* and *P. mirabilis* had the lowest (11.0 mm) (Table 2). The extract exhibited MIC at 100 µg mL<sup>-1</sup> against *S. dysenteriae*, 500 µg mL<sup>-1</sup> against *S. aureus* and *C. ulcerans*, 1000 µg mL<sup>-1</sup> against *E. coli* and *P. mirabilis* and 1500 µg mL<sup>-1</sup> against *K. pneumoniae* (Table 3). The MBC showed that the extract was bactericidal against *S. dysenteriae* at 500 µg mL<sup>-1</sup>, *S. aureus* and *C. ulcerans* at 1000 µg mL<sup>-1</sup>, *E. coli* and *P. mirabilis* at 1500 µg mL<sup>-1</sup> and *K. pneumoniae* at 2000 µg mL<sup>-1</sup> (Table 3). Time kill studies showed that there was no viable *S. dysenteriae* after 6 h exposure (Fig. 1). However, *S. aureus* was completely killed after 10 h exposure to the extract (Fig. 2). The killing of *S. dysenteriae* was more rapid than that of *S. aureus* (Fig. 3).

Table 1: Results of phytochemical screening

Plant constituents	Results
Alkaloids	+
Tannins	+
Cardiac glycosides	+
Saponins	-
Flavonoids	+
Cyanogenic glycosides	-

+: Present, -: Absent

Table 2: Zone of inhibition diameter (mm) produced by extract of *C. tinctorium*

Test organisms	Concentration of extracts ( $\times 10^3 \mu\text{g mL}^{-1}$ )			Ciprofloxacin ( $10 \mu\text{g mL}^{-1}$ )	Gentamicin ( $10 \mu\text{g mL}^{-1}$ )
	0.5	1.0	2.0		
<i>S. aureus</i>	-	15.3	19.0	12	14
<i>C. ulcerans</i>	-	11.0	17.2	20	10
<i>K. pneumoniae</i>	-	-	11.0	15	12
<i>E. coli</i>	-	9.2	14.3	20	0
<i>P. mirabilis</i>	-	5.2	11.0	21	14
<i>S. dysenteriae</i>	4.2	9.0	19.0	19	9

-: No activity at that concentration

Table 3: MIC and MBC of *C. tinctorium* extract ( $\mu\text{g mL}^{-1}$ )

Test organisms	MIC ( $\times 10^{-3} \mu\text{g mL}^{-1}$ )					MBC ( $\times 10^{-3} \mu\text{g mL}^{-1}$ )				
	2.0	1.5	1.0	0.5	0.1	2.0	1.5	1.0	0.5	1.0
<i>S. aureus</i>	-	-	-	*	+	-	-	∅		
<i>C. ulcerans</i>	-	-	-	*	+	-	-	∅		
<i>K. pneumoniae</i>	-	*	+	++	++	∅	-			
<i>E. coli</i>	-	-	*	+	++	-	∅			
<i>P. mirabilis</i>	-	-	*	+	++	-	∅			
<i>S. dysenteriae</i>	-	-	-	-	*	-	-	-	∅	

\*: MIC, ∅: MBC, -: No growth, +: Turbid, ++: Very turbid

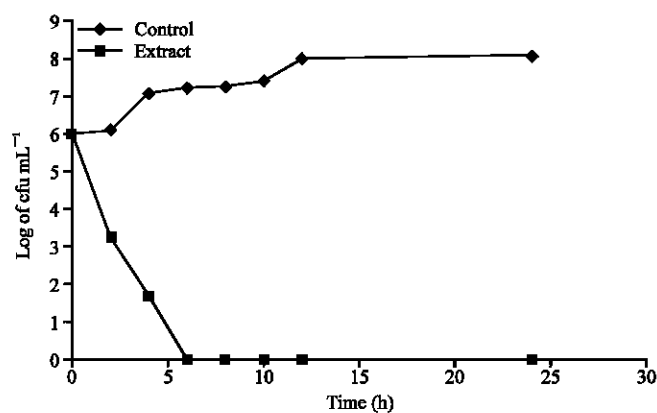


Fig. 1: Killing of *S. aureus* by *C. tinctorium* extract

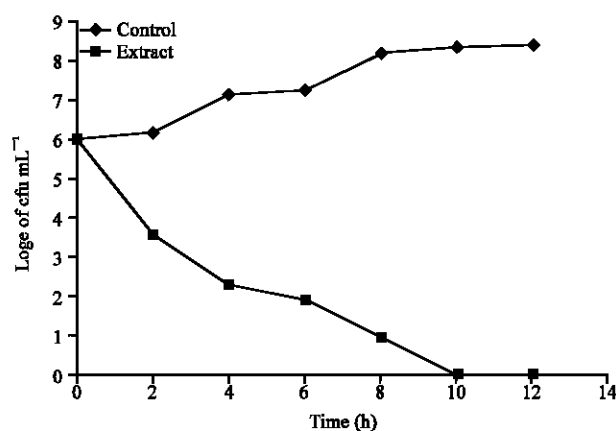


Fig. 2: Killing of *S. dysenteriae* by *C. tinctorium* extract

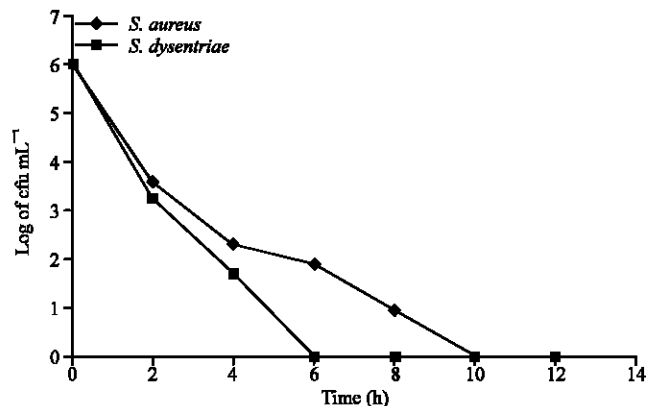


Fig. 3: Comparison of killing of *S. aureus* and *S. dysenteriae*

The phytochemical screening of methanol extract of *C. tinctorium* showed the presence of flavonoids, alkaloids, tannins and cardiac glycosides. These metabolites have been shown to be responsible for various therapeutic activities of medicinal plants (Trease and Evans, 1989). Flavonoids especially are known to be effective antimicrobial agents against a wide array of microorganisms; the activity is attributed to their ability to complex with extra cellular and soluble proteins and with bacterial cell wall (Cowan, 1999).

The result of antibacterial activity showed that the extract was active on all the tested microorganisms at different concentrations. The zones of inhibitions were highest at 2000  $\mu\text{g mL}^{-1}$  for all the organisms. These zones were found similar to the zones produced by the standard antibiotics ciprofloxacin and gentamicin at 10  $\mu\text{g mL}^{-1}$  each (Table 2). The MIC was lowest for *S. dysenteriae* (100  $\mu\text{g mL}^{-1}$ ) followed by *S. aureus* and *C. ulcerans* (500  $\mu\text{g mL}^{-1}$ ) and highest for *K. pneumoniae* (1500  $\mu\text{g mL}^{-1}$ ) (Table 3). These findings are in agreement with the findings of Holetz *et al.* (2002), however contradicts the findings of Suffredini *et al.* (2006), which reported that their experience of screening 1200 plant extracts against four bacterial species showed that gram-negative bacteria are hardly susceptible to plant extracts in doses as low as 200  $\text{mg mL}^{-1}$ . The MBC result showed that the extract was bactericidal to *S. dysenteriae* at 500  $\mu\text{g mL}^{-1}$ , followed by *S. aureus* and *C. ulcerans* (1000  $\mu\text{g mL}^{-1}$ ) (Table 3). Time kill studies on *S. dysenteriae* (Fig. 1) and *S. aureus* (Fig. 2) showed that the killings were time dependent. It was also found that the killing of *S. dysenteriae* was more rapid (after 6 h) than that of *S. aureus* (after 10 h) (Fig. 3). Okemo *et al.* (2001) reported that MIC (4  $\text{mg mL}^{-1}$ ) of *Azadirachta indica* extract completely wiped out *S. aureus* in 6 h and noted that the killing was both dosage and time dependent. This suggests that at higher concentration the organism would be killed at a faster rate.

## CONCLUSION

The result obtained from the phytochemical screening has shown the presence of some secondary metabolites. These compounds may be responsible for the antibacterial activity of the plant extract. The activity was found to be concentration and time dependent. These findings therefore support the local use of *C. tinctorium* root extracts for treatment of gastrointestinal, urinary tract and other infectious diseases in Northern Nigeria. Further investigation using bioactivity guided fractionation is currently going on to determine the active constituent(s) in our laboratory.

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