Research Article

Cytotoxicity and antibacterial activities of methanol extract of *Cochlospermum tinctorium* roots and its fractions

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Abstract

Background: It is well-known fact that medicinal plants are the resources of promising drugs for many ailments. Although, a number of research have been conducted on these plants, however, study related to their cytotoxic and antibacterial activity is of paramount important due to worldwide increase microbial resistance to antibiotics. **Objective:** The objective of present study was to determine the cytotoxicity and antibacterial activities of *Cochlospermum tinctorium* roots extract against some clinical isolates of bacteria. **Material and Methods:** Water and methanol were used in extraction of *Cochlospermum tinctorium* roots by percolation. The antibacterial activity was carried out using agar well diffusion technique against *E. coli*, *Proteus specie*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*, the minimum inhibitory concentration and the minimum bactericidal concentration were also determined using standard procedure The extracts were also subjected to Phytochemical Screening, Brine-shrimp lethality assay, the Lc 50 values were determined by linear regression analysis method. **Results:** The result of phytochemical screening revealed the presence of secondary metabolites, the result of antibacterial activity ranged from 7.23±0.20mm to 32.67±0.58mm inhibition zones, N-hexane extract have the highest zone of inhibition (32.67±0.58mm) against *K. pneumoniae* at 2000µg/ml concentrations, the extract was active on all the tested microorganisms at different concentrations, the MIC was lowest for *K. pneumoniae* at (62.5µg/ml) and highest for *S. aureus* and *E. coli* at (125µg/ml), the MBC result showed that, the extract was bactericidal to *K. pneumoniae* at (62.5 µg/ml) followed by *S. aureus* at (125µg/ml) and *E. coli* at (250µg/ml), the cytotoxicity of the plant extract (LC50) showed that, the plant was weakly toxic. **Conclusion:** These findings therefore support the local use of *C. tinctorium* root extracts for the treatment of bacterial infections in Northern Nigeria.

Keywords: Brine shrimp lethality assay, *Cochlospermum tinctorium*, cytotoxic, linear regression analysis, antibacterial

Introduction

Microbial infections are major public health problems in the developed and developing countries. Antibiotics are used to treat these infections. Due to indiscriminate use of commercial antibiotics, the incidence of multiple antibiotic resistance in

human pathogens is increasing (Jeyachandran and Mahesh, 2007). Infectious diseases caused by bacteria and fungi affect millions of people worldwide, throughout the history of mankind, infectious diseases have remained a major cause of death and disability. The discovery of antibiotics was an essential part in combating bacterial infections that once ravaged humankind (Usha et al., 2010). The use of plants and herb extract in the treatment of human ailments is a very ancient art, a practice that has been passed on for generations and scientists in Africa and other developing countries and other are conducting research into local

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plants abundant in the continent for their possible use in traditional medicine (Nneamaka, 1991).

Medicinal plants are the resources of promising drugs for many diseases. The biological and pharmacological properties of many plants are still unknown (Ayyanar and Ignacimuthu, 2008; Agbafor et al., 2011; Roy et al., 2011; Vinoth et al., 2011; Mishra and Mishra, 2011). World-over, the scientists are exploring the possibilities of utilizing or finding out pharmacologically active compounds from medicinal plants (Agbafor et al., 2011). For example, screening of medicinal plants for their phytochemicals, antioxidant, anticancer and antimicrobial activities is the prime concern for finding out an effective phytochemically active principle (Ayyanar and Ignacimuthu, 2008; Agbafor et al., 2011; Roy et al., 2011; Vinoth et al., 2011; Mishra and Mishra, 2011). Majority of these kind of works are concerned with the study of aqueous or solvent extracts of plant parts and testing them individually for selective pharmacological activities, such as antibacterial (Mishra and Mishra, 2011), hepatoprotective (Dhanasekaran and Ganapathy, 2011), hypoglycemic and hypolipidemic activities (Sharma et al., 2007).

Cochlospermum tinctorium is a savannah plant found in fallow farms across Northern Nigeria. It is a shrub that grows up to 10m 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 in Hausa (Adam et al., 2017), Yarudi in Fulani (Musa et al., 2012), Ichachafolo in Igala (Adam et al., 2017) and Sewutu in Yoruba (Adam et al., 2017).

Cochlospermum tinctorium is a familiar herb in the traditional medicinal preparation in Northern Nigeria, where decoctions of the whole roots are used as remedy for gonorrhea, 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).

Materials and Methods

Sample Collection

The roots of *Cochlospermum tinctorium* was collected from Birnin Kudu Local Government, Jigawa State, Nigeria between July to August, 2019. The plant was authenticated at the Herbarium unit of the department of Biological sciences, Bayero University Kano and the specimen voucher number (BUKHAN 24) was obtained there.

Preparation of plant material and extraction

The fresh roots of the plant were air dried, pulverized to fine

powders using porcelain mortar and pestle. The pulverized material weighing 300g was soaked into 1.2L of methanol solvent at a ratio of 1:4 for two (2) weeks according to standard procedure described by Veeramuthu et al, (2006). The plant extracts were filtered and the methanol solvent were evaporated using rotary evaporating machine (Recirculating chiller) at 74°C.

Fractionating of the plant extract

After extraction/ evaporation, the plant extract was fractionated using various solvents i.e polar, semi-polar and non-polar by adding 150ml of each solvent into the crude extract (methanol). The fractions are as N-Hexane (Non-polar), Crude (Polar), Ethyl acetate (Semi/moderately polar), Residual aqueous fraction (Polar)

Phytochemical screening

The extract was subjected to various phytochemical tests to identify the constituent's secondary metabolites such as flavonoids, alkaloids, tannis, steroids and volatile oil using standard methods as described by Harborne (1984), Sofowara (1993) and Trease and Evans (1989).

Test organisms

Bacterial isolates: *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus specie*, *Staphylococcus aureus*, *Klebsiella pneumonia* isolated from patients attending Microbiology Laboratory of Murtala Muhammad Specialist Hospital (MMSH) Kano were obtained. 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 of the plant extracts were determine using hole-in bioassay plate procedure as reported by Karou et al., (2006) using Nutrient agar was incubated for 24 hours at 37°C. About five (5) discrete colonies were aseptically transferred using sterile wire loop into a tube containing sterile normal saline (0.85% NaCl) and it was adjusted to a turbidity of 0.5 MacFarland standards. The suspensions were streaked on the surface of Nutrient 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 500µg/ml, 1000µg/ml and 2000µg/ml concentrations of the extract. Standard antibiotics Amoxicillin was used as positive control. The plates were incubated for 24 hours 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-tube containing 9 mL of double strength broth. The test tubes were inoculated using standard inocula and incubated at 37°C for 24 hours. MICS was recorded as lowest concentration of the extract showed no visible growth of the broth.

Determination of Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration was aseptically determined by inoculating aliquots of culture from MIC tubes that showed no growth, on sterile nutrient agar plates incubated at 37°C for 48 hours. MBC was recorded on the lowest concentration of the extract that showed no bacterial growth (National Committee for Clinical Laboratory Standard, 2006).

Determination of Cytotoxicity (Brine Shrimp Assay)

Cytotoxic activity of the root extracts were tested against brine shrimps hatched in saline solution (Known as naupili) (Meyer, 1982). Brine shrimp eggs were hatched in a plastic rectangular

container that was one-quarter filled with saline solution with general aeration. A plastic separator (with holes) for unequal compartment was placed in a container. Eggs were separated into the larger and darker compartment. After 48 h, mature naupili were collected from the smaller and illuminated side. Ten shrimps were transferred to a sterile test tubes containing 10µg/ml, 100µg/ml, and 1000µg/ml concentrations of the root extract of *Cochlospermum tinctorium*, which was then incubated at 25°C for 24 h, after which the survivors were counted with the aid of a 3X magnifying glass. DMSO and saline solution were used as negative controls and potassium dichromate as the reference standard. Lethal dose were calculated by linear regression analysis (Patil and Magdum, 2012).

Results and discussion

The phytochemical screening of the crude, hexane, ethyl acetate and methanol extract of *C. tinctorium* showed the presence of alkaloids, flavonoids, phenol, tannis, steroids, triterpens, glycosoids and saponin (Table 1). 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

Table 1. Phytochemical Screening of root extract of *Cochlospermum tinctorium* (Cochlospermaceae)

S. No.	Plant Constituents	Fractions			
		CRD	HEX	E. A	MEOH
1.	Alkaloids	+	+	+	-
2.	Flavonoids	+	-	-	+
3.	Phenol	+	+	+	+
4.	Tannis	-	+	+	-
5.	Steroids	-	+	+	-
6.	Triterpens	+	+	+	+
7.	Glycosoid	-	+	+	-
8.	Saponin	-	-	-	+

+: Present, -: Absent, CRD: Crude, HEX: Hexane, E.A: Ethyl Acetate, MEOH: Methanol

Table 2. Antibacterial activity of crude extract of *Cochlospermum tinctorium* (Cochlospermaceae)

S. N.	Test Organisms	Concentrations (µg/ml) Zone of inhibition (mm)			
		2000	1000	500	Control (Amxl)
1.	<i>E. coli</i>	20.33±0.88 ^a	16.33±0.88 ^b	13.00±0.58 ^c	6.0±0.0 ^d
2.	<i>Proteus spp</i>	16.33±0.58 ^a	6.0±0.0 ^b	6.0±0.0 ^b	24.78±0.70 ^d
3.	<i>P. aeruginosa</i>	8.72±0.26 ^a	6.0±0.0 ^b	6.0±0.0 ^b	21.09±1.01 ^d
4.	<i>K. pneumonia</i>	19.00±1.00 ^a	19.28±0.78 ^a	13.33±0.58 ^c	22.78±0.70 ^d
5.	<i>S. aureus</i>	15.67±0.58 ^a	11.53±0.41 ^b	12.99±0.32 ^c	14.33±0.58 ^d

Values are expressed as Mean ± S.D of 3 replicates, values with different superscripts within the same row are considered significant (p<0.05), well =6mm

Table 3. Antibacterial activity of hexane extract of *Cochlospermum tinctorium* (Cochlospermaceae)

S. No.	Test Organisms	Concentrations ($\mu\text{g/ml}$) /Zone of inhibition (mm)			
		2000	1000	500	Control (Amxl)
1.	<i>E. coli</i>	11.23 $\pm$ 0.20 ^a	15.45 $\pm$ 0.51 ^b	8.72 $\pm$ 0.26 ^c	20.00 $\pm$ 1.00 ^d
2.	<i>Proteus spp</i>	6.00 $\pm$ 0.00 ^a	6.00 $\pm$ 0.00 ^a	6.00 $\pm$ 0.00 ^a	16.72 $\pm$ 0.25 ^d
3.	<i>P. aeruginosa</i>	6.00 $\pm$ 0.00 ^a	6.00 $\pm$ 0.00 ^a	6.00 $\pm$ 0.00 ^a	19.45 $\pm$ 0.51 ^d
4.	<i>K. pneumonia</i>	32.67 $\pm$ 0.58 ^a	31.45 $\pm$ 0.51 ^b	30.55 $\pm$ 0.51 ^b	30.55 $\pm$ 0.51 ^b
5.	<i>S. aureus</i>	6.00 $\pm$ 0.00 ^a	6.00 $\pm$ 0.00 ^a	6.00 $\pm$ 0.00 ^a	14.89 $\pm$ 0.20 ^d

Values are expressed as Mean $\pm$ S.D of 3 replicates, values with different superscripts within the same row are considered significant ($p < 0.05$), well = 6mm

Table 4. Antibacterial activity of ethyl acetate extract of *Cochlospermum tinctorium* (Cochlospermaceae)

S/N	Test Organisms	Concentrations ($\mu\text{g/ml}$) /Zone of inhibition (mm)			
		2000	1000	500	Control (Amxl)
1.	<i>E. coli</i>	16.17 $\pm$ 0.29 ^a	6.00 $\pm$ 0.00 ^b	6.00 $\pm$ 0.00 ^b	33.45 $\pm$ 0.51 ^d
2.	<i>Proteus spp</i>	9.48 $\pm$ 0.13 ^a	8.72 $\pm$ 0.26 ^b	6.00 $\pm$ 0.00 ^c	15.95 $\pm$ 0.09 ^d
3.	<i>P. aeruginosa</i>	6.00 $\pm$ 0.00 ^a	6.00 $\pm$ 0.00 ^a	6.00 $\pm$ 0.00 ^a	21.33 $\pm$ 0.67 ^d
4.	<i>K. pneumonia</i>	19.55 $\pm$ 0.51 ^a	11.30 $\pm$ 0.28 ^b	9.78 $\pm$ 0.38 ^c	22.72 $\pm$ 0.26 ^d
5.	<i>S. aureus</i>	6.00 $\pm$ 0.00 ^a	6.00 $\pm$ 0.00 ^a	6.00 $\pm$ 0.00 ^a	14.55 $\pm$ 0.51 ^d

Values are expressed as Mean $\pm$ S.D of 3 replicates, values with different superscripts within the same row are considered significant ($p < 0.05$), well = 6mm

Table 5. Antibacterial activity of Aquous methanolic extract of *Cochlospermum tinctorium* (Cochlospermaceae)

S. No.	Test Organisms	Concentrations ($\mu\text{g/ml}$) /Zone of inhibition (mm)			
		2000	1000	500	Control (Amxl)
1.	<i>E. coli</i>	9.45 $\pm$ 0.51 ^a	8.55 $\pm$ 0.51 ^a	6.00 $\pm$ 0.00 ^c	12.89 $\pm$ 0.19 ^d
2.	<i>Proteus spp</i>	12.41 $\pm$ 0.36 ^a	6.00 $\pm$ 0.00 ^b	6.00 $\pm$ 0.00 ^b	22.55 $\pm$ 0.51 ^d
3.	<i>P. aeruginosa</i>	19.34 $\pm$ 0.00 ^a	19.34 $\pm$ 0.00 ^a	19.34 $\pm$ 0.00 ^a	19.34 $\pm$ 0.00 ^a
4.	<i>K. pneumonia</i>	14.87 $\pm$ 0.23 ^a	11.45 $\pm$ 0.51 ^b	7.23 $\pm$ 0.20 ^c	21.22 $\pm$ 0.70 ^d
5.	<i>S. aureus</i>	19.33 $\pm$ 0.58 ^a	11.45 $\pm$ 0.51 ^b	7.23 $\pm$ 0.20 ^c	19.45 $\pm$ 0.51 ^a

Values are expressed as Mean $\pm$ S.D of 3 replicates, values with different superscripts within the same row are considered significant ($p < 0.05$), well = 6mm

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 current findings agrees with the work of (Tijjani et al., 2009) who works on Phytochemical screening of methanolic root extract of *Cochlospermum tinctorium*. The result of the phytochemical screening is in agreement with other studies such as analysis of the ethanol leaf extract of *Nymphaea lotus* by Akinjogunla et al. (2010) and Afolayan et al. (2013).

The results of antibacterial activity of the root extract of *Cochlospermum tinctorium* against *E. coli*, *Proteus specie*, *P. aeruginosa*, *K. pneumomiae* and *S. aureus* ranged from 7.23 $\pm$ 0.20mm to 32.67 $\pm$ 0.58mm inhibition zones, N-hexane

extract have the highest zone of inhibition (32.67 $\pm$ 0.58mm) against *K. pneumomiae* at 2000 $\mu\text{g/ml}$ concentrations, the extract was active on all the tested microorganisms at different concentrations. The results showed that, the antibacterial activity against the tested organisms were in the following order: Methanol aqueous, crude, ethyl acetate, N-hexane root extract (MEOH>CRD>E.ACT>HEX)(Table 2, 3, 4 &5). Furthermore, the susceptibility profile of *E. coli*, *Proteus specie*, *P. aeruginosa*, *K. pneumomiae* and *S. aureus* to the plant extract is an indication that, the extract can be harnessed as alternative treatment of remedy for bacterial infections (Marano et al., 2000; Clemente et al., 2013; Liebana et al., 2013). These findings are in agreement with the work of (Holetz et al., 2002) who work on the screening of some

Table 6. Minimum inhibitory concentration (MIC) of crude extract of *Cochlospermum tinctorium* (Cochlospermaceae)

S. No.	Test Organisms	Concentrations (µg/ml)					+ve Control	-ve Control	Inference (MIC)
		250	125	62.5	31.25	15.625			
1.	<i>E. coli</i>	-	-	+	+	+	+	-	125
2.	<i>Proteus spp</i>	+	+	+	+	+	+	-	N.I
3.	<i>P. aeruginosa</i>	+	+	+	+	+	+	-	N.I
4.	<i>K. pneumonia</i>	-	-	-	+	+	+	-	62.5
5.	<i>S. aureus</i>	-	-	+	+	+	+	-	125

+: Turbid, -: No growth, N.I= No Inhibition

Table 7. Minimum bactericidal concentration (MBC) of crude extract of *Cochlospermum tinctorium* (Cochlospermaceae)

S. No.	Test Organisms	Concentrations (µg/ml)					+ve Control	-ve Control	Inference (MBC)
		250	125	62.5	31.25	15.625			
1.	<i>E. coli</i>	-	+	+	+	+	+	-	250
2.	<i>Proteus spp</i>	+	+	+	+	+	+	-	N.A
3.	<i>P. aeruginosa</i>	+	+	+	+	+	+	-	N.A
4.	<i>K. pneumonia</i>	-	-	-	+	+	+	-	62.5
5.	<i>S. aureus</i>	-	-	+	+	+	+	-	125

+: Positive, -: Negative, N.A= Non-Applicable

Table 8. Brine shrimp lethality assay of the methanolic crude and fractionated extracts of *Cochlospermum tinctorium* (Cochlospermaceae)

Concentrations (µg/ml)	No. of shrimps	Crude	Fraction		
		Mortality at 24hrs	Immortality at 24hrs	Mortality (%)	Lc50 Value
1000	10	10.00±0.00	0.0±0.0	100	3.165
100	10	10.00±0.00	0.0±0.0	100	
10	10	8.33±0.33	1.67±0.33	83.3	
		N-Hexane	Fraction		
1000	10	10.00±0.00	0.0±0.0	100	1.175
100	10	10.00±0.00	0.0±0.0	100	
10	10	9.67±0.33	0.33±0.33	96.7	
		E. Acetate	Fraction		
1000	10	10.00±0.00	0.0±0.0	100	1.175
100	10	10.00±0.00	0.0±0.0	100	
10	10	9.67±0.33	0.33±0.33	96.7	
		Methanol	Fraction		
1000	10	10.00±0.00	0.0±0.0	100	15.019
100	10	7.67±0.33	2.33±0.33	76.7	
10	10	4.67±0.33	5.33±0.33	46.7	

The mortality and immortality rates were taken in Triplicates and Standard Error of Mean (Mean ± SEM) was used. LC 50/≤1000µg/ ml is toxic (Meyer et al., 1982)

plants used in the Brazilian folk medicines for the treatment of infectious diseases, however at variance with that of (Rahman et al., 2011) who work on partial substitution of cumim seed meal by *Jatropha* meal as potential protein source for meal and

reported that, the plant extracts does not have activity on *P. aeruginosa* and *E. coli*. The result obtained in this study have affirmed the hypothesis that, there is significant relationship between the root extract of *Cochlospermum tinctorium* and antibacterial activity against *E. coli*, *Proteus specie*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus* ($P < 0.05$).

The minimum inhibitory concentration (MIC) was lowest for *K. pneumoniae* (62.5µg/ml) and highest for *S. aureus* and *E. coli* (125µg/ml). The minimum bactericidal concentration (MBC) result showed that, the extract was bactericidal to *K. pneumoniae* at (62.5µg/ml) followed by *S. aureus* at (125µg/ml) and *E. coli* at (250µg/ml).

The result of Cytotoxicity (Lc 50) revealed that, after 24 hours of interaction between the crude extract and the shrimps, all the ten (10) average number of shrimps are not viable (died) at 1000µg/ml and 100µg/ml concentrations of the crude extract representing (100%) of the average mortality rate, while at 10µg/ml concentration of the extract, the average number of 1.67 are viable (alive) with the average mortality rate of 8.33 representing (83.3%) all with Lc50 value of 3.165, the result of cytotoxicity revealed that, n-hexane and ethyl acetate fractions are more potents with Lc50 value of 1.175 followed by the crude fraction with Lc50 value of 3.165 and methanol aqueous fraction was least potent with Lc50 value of 15.019, the result showed that, the plant extract is weakly toxic which ascertain its safety as alternative antibacterial agent, the current finding is in agreement with (Meyer, 1982) who work on a convenient general bioassay for active plant constituents and also reported that, the cytotoxicity range $\leq 1000\mu\text{g/ml}$ is toxic.

Conclusion

Due to the presence of some secondary metabolites obtained from the result of phytochemical screening. These compounds may be responsible for the antibacterial activity of the plant extract. The activity was found to be concentration and time dependent. The results of cytotoxicity showed that, the plant was weakly toxic. These findings therefore support the local use of *C. tinctorium* root extracts for the treatment of bacterial infections such as gastrointestinal, urinary tract etc in Northern Nigeria.

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Conflicts of interest

We declared that we have no conflict interests

Ethical Clearance

An approval of the study was obtained from the research ethics

committee of Kano State Ministry of Health, Nigeria.

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