

ANTIBACTERIAL POTENTIALS AND TOXICITY STUDY OF CASSIA OCCIDENTALIS LEAF EXTRACTS AGAINST CLINICAL ISOLATES OF SALMONELLA SP

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ABSTRACT

Cassia occidentalis is an important medicinal herb in traditional healthcare practice and has been reported for various biological activities. The objective of the present study was to analyze the antibacterial potentials and toxicity study of *cassia occidentalis* leaf extracts against clinical isolates of salmonella species. *C. occidentalis* leaves were extracted successively with ethanol, water and methanol as solvent using soxhlet apparatus. The extracts were obtained through rotary evaporator. The extracts were tested in vitro for activity against clinical isolates of *Salmonella typhi* and *Salmonella paratyphi A* and *Salmonella paratyphi B* using agar well diffusion and broth dilution methods. The zones of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The extracts toxicity was investigated using brine shrimp lethality bioassay. The experiments were performed in triplicate and data was analyzed statistically. The in vitro antimicrobial screening revealed that the extracts exhibited varying activities against the different isolates of *Salmonella typhi*, *S. paratyphi A* and *S. paratyphi B*. with zones of inhibition ranging from 7 mm-23 mm, MIC ranging from 62.5 µg/ml – 125 µg/ml and MBC of 125 µg/ml -500 µg/ml, The highest activity observed in *Cassia occidentalis* extracts was 23 mm with methanolic extract against *S. paratyphi A* and *S. paratyphi B*. MIC of 62.5 µg/ml and MBC of 125 µg/ml against *Salmonella typhi*, *S. paratyphi A* and *S. paratyphi B*. The activities observed might be due to the presence of the secondary metabolites like, alkaloids, anthraquinones, sterols, glycosides, saponins, terpenes and flavonoids detected in the plant. The toxicity study carried out revealed that the highest value for LD₅₀ shows nontoxic property in extract was 191.639 µg/ml in the aqueous extract while the lowest was LD₅₀ of 30.765 µg/ml which shows high toxic property observed in methanolic extract. The study also showed that *C. occidentalis* leave is a potential source of antimicrobial compound and has potential use for health benefits.

Key words: *Cassia occidentalis*, *Salmonella sp*, Antibacterial activity, Toxicity

INTRODUCTION

Cassia occidentalis Linn belongs to *Caesalpinaceae* family. It is an erect herb commonly found growing by the roadside ditches and dumpsite in the northern part of Nigeria it is locally known as, *Akidi agbara* (Igbo,) *Abo rere* (Yoruba,) *mazam fari* or *rai-rai* in Hausa and Coffee Senna in English. The roots, leaves and seeds are the parts of the plant used. The leaf-sap is used in eye troubles

in young and old as well as a febrifuge and laxative in The Gambia and Ijo area of Nigeria (Burkill 1995). The leaf is recognized as anti- neuralgic, purgative (in treatment of diarrhea and dysentery) and vermifuge. In Yoruba land, the preparation with palm oil is used to cure convulsion in children. It is an erect herb, commonly found by road sides, ditches and waste dumping sites. *Cassia occidentalis* has been widely used as traditional medicine. Entire parts of the plant have medicinal values (Mohammed *et al.*, 2012). *Cassia* species has been well known for laxative and purgative properties and for the treatment of skin diseases in traditional medicine. *Cassia occidentalis* Linn.has been used as a folklore medicine for hepatotoxicity treatment (Sheebarani *et al.*, 2010). There is now an increasing body of scientific evidence demonstrating that the plant possesses many other beneficial properties. *Cassia occidentalis* leaf powder was tested for anti-inflammatory activity and observed that the transudative, exudative and proliferative components of chronic inflammation were suppressed by these drugs.

Cassia occidentalis Linn was screened for analgesic and antipyretic activity (Sini *et al.*,2011). They found that the ethanol and water extracts of *Cassia occidentalis* possess antinociceptive and antipyretic properties. The aqueous extract of *C. occidentalis* was tested for antidiabetic activity and the study (Laxmi *et al.*, 2010) proved that there was a significant reduction in fasting blood glucose levels in the normal and alloxan-induced diabetic rats. *Cassia occidentalis* Linn belongs to *Caesalpinaceae* family. It is an erect herb commonly found growing by the roadside ditches and dumpsite in the northern part of Nigeria it is locally known as, *Akidi agbara* (Igbo,) *Abo rere* (Yoruba,) *mazam fari* or *rai-rai* in Hausa and Coffee Senna in English. The roots, leaves and seeds are the parts of the plant used. The leaf-sap is used in eye troubles in young and old as well as a febrifuge and laxative in The Gambia and Ijo area of Nigeria (Burkill 1995). The leaf is recognized as anti- neuralgic, purgative (in treatment of diarrhea and dysentery) and vermifuge. In Yoruba land, the preparation with palm oil is used to cure convulsion in children. It is an erect herb, commonly found by road sides, ditches and waste dumping sites. *Cassia occidentalis* has been widely used as traditional medicine. Entire parts of the plant have medicinal values (Mohammed *et al.*, 2012). *Cassia* species has been well known for laxative and purgative properties and for the treatment of skin diseases in traditional medicine. *Cassia occidentalis* Linn.has been used as a folklore medicine for hepatotoxicity treatment (Sheebarani *et al.*, 2010). There is now an increasing body of scientific evidence demonstrating that the plant possesses many other beneficial properties. *Cassia occidentalis* leaf powder was tested for anti-

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MATERIALS AND METHODS

Sample Collection

The plant was collected from Hadejia roadside and authenticated at the Herbarium of the Department of Plant Biology, Bayero University, Kano where a voucher specimen numbered; BUKHAN 0115 was deposited at the herbarium of the Department. The leaf was rinsed with clean water and air-dried for six (6) days under shade, and then pulverized and homogenized using a mechanical grinder. The pulverized leaves were kept in an air-tight cellophane bag until required for used.

Extraction from Pulverized Leave

The powdered samples of the *Cassia occidentalis* leave were extracted following the method of Gupta *et al.* (2009). One hundred (100) g each of the powder leaves of the plant was weighed into 3 different glass containers and sequentially extracted with 500ml each of methanol, ethanol and distilled water by percolation method for three days during which the sealed bottles were shaken at regular intervals. The extracts were filtered through Whatman's filter paper No. 1. and concentrated by using rotary evaporator to yield the crude extracts. The aqueous extract was concentrated on the water bath at 45°C (Fatope *et al.*, 1993).

The percentage yield of each extract was calculated from the respective weights of the extracts using the formula below:

$$\text{Percentage yield} = \frac{\text{Weight of the extract} \times 100\%}{\text{Total weight of sample}}$$

(Harborne 1984)

Preparation of Extract Stock Concentration for Antimicrobial Screening

A test concentration of 30mg/ml, 60mg/ml, 90mg/ml and 120mg/ml for aqueous, methanol and ethanol extracts were prepared by dissolving 0.3g, 0.6g, 0.9g and 1.2g respectively of each extract in 10mls of distilled water in separate test tubes. The same concentration was made for amoxicillin (a commonly used antibiotic against *Salmonella sp*) which serves as the control.

Phytochemical screening

The presence of secondary metabolites in the pulverized plant material was determined using standard methods (Evans 2002; Sofowora 2008).

Antimicrobial Screening of *Cassia occidentalis* Leaf Extracts

Organism Source

The clinical isolates were obtained from the Department of Medical Microbiology Aminu Kano Teaching Hospital (AKTH) in

April 2016. *Salmonella typhi*, *S. paratyphi A* and *S. paratyphi B* identity were confirmed using biochemical tests for identification of catalase, oxidase, indole, motility, citrate utilization, urease production, hydrogen sulfide production as well as acid and gas production using Klinger Iron Agar (Cheesebrough, (2002).

Preparation of the Inoculum

A loopful of the test organism was taken from their respective agar slants and sub-cultured into test tubes containing nutrient broth for the test-tubes were incubated for 24hrs at 37°C. The obtained microorganisms in the broth were standardized using normal saline to obtain a population density, equivalent to a 0.5 McFarland standard as described by National Committee for Clinical Laboratory Standard (NCCLS 2000).

Preparation of Media

The media were prepared according to manufacturer's (AVONCHEM limited, Wellington House waterloo, west Macclesfield Cheshire, England) instruction.

Antibacterial Activity of Extracts using Agar Well Diffusion Method

A sterile cork borer of 5mm in diameter was used to cut well. 10µl of the test solution (extract) was then introduced into the well. The plates were incubated at 37°C for 24hrs, and observed for the zone of inhibition of growth. The zones were measured with a transparent ruler and the result recorded in millimeters. The screening was done in triplicates. Equal concentration of amoxicillin was used as control (Dahiru *et al.*, 2013).

Minimum Inhibitory Concentration Using Broth Dilution Method

The MIC of the extracts was also carried out using broth dilution method as described in Ibekwe *et al.*, 2001. Two-fold serial dilution of the extract in the broth were made from the stock concentration of the extract to obtain 10, 5, 2.5, 1.25, 0.625mg/ml (1000µl, 500 µl, 250 µl, 125 µl, 62.5µl) 0.1ml each of the standardized inocula of the *Salmonella typhi*, *S. paratyphi A* and *S. paratyphi B* were then inoculated respectively into the different concentrations of the extracts in the broth. The tubes containing the test solution were then incubated at 37°C for 24hrs and observed for turbidity of growth. The lowest concentration which showed no turbidity in the test tube was recorded as the MIC (NCCLS 2000).

Minimum Bactericidal Concentration Using Broth Dilution Method

Blood agar was prepared, sterilized at 121°C for 15minutes and was poured into sterile Petri-dishes and left to cool and solidify. The contents of the tubes without growth were then sub-cultured onto the blood agar plates and incubated at 37°C, and observed for colony growth. The MBC was the plate with the lowest concentration of extract and without colony growth (NCCLS 2000).

Determination of Activity Index

The activity index of the crude plant extract was determined using the relation;

$$\text{Activity index (A.I.)} = \frac{\text{Mean of zone of inhibition of the extract}}{\text{Zone of inhibition obtained for standard antibiotic drug}}$$

Determination of Proportion Index

The proportion index was determined using;

$$\text{Proportion index (P.I.)} = \frac{\text{Number of positive results obtained for individual extract}}{\text{Zone of inhibition obtained for standard antibiotic drug}}$$

(Yavad *et al* 2010)

Brine Shrimps Lethality Assay

Test Sample Preparation for Brine Shrimp Bioassay

Cassia occidentalis leaves extracts were dissolved in DMSO (Dimethyl sulfoxide) to obtain stock solution from which various concentrations of 10, 100, and 1000 µg/ml were made by serial dilution after dissolving 1g of the extract in 100ml of the DMSO. Pure DMSO and artificial seawater were used as negative control (Ramachandran *et al* 2011).

Hatching of Brine Shrimp Eggs

Brine shrimps eggs were obtained from Chemistry Department Bayero University Kano. The cysts were hatched in a tank containing artificial seawater made through dissolving a commercial marine salt 38g/L in distilled water (mineral water). The tank was well aerated and the proper light from touch light source was also provided. The nauplii were hatched within 24-36 hours.

Brine Shrimp Lethality Test

The toxicity of extracts was tested at various concentrations viz. 10, 100, and 1000 µg/ml in seawater. Exactly 0.5 ml of diluted test solution was added to the pre marked test tubes containing 4.5ml of artificial sea water. Finally 10 active shrimps were added into each test tube. A vial containing 50µl DMSO diluted to 5 ml was used as control After 24 hours; survivors were counted using a 10x magnification dissection microscope (hand lens) and the percentage of the mortality (%M) of each dose calculated (Ramachandran *et al.*, 2011).

Statistical Analysis of Data

Using probit analysis procedures, the lethality concentration (LC₅₀) was assessed at 95% confidence intervals. LC₅₀ of less than 100 ppm was considered as potent (active) Gupta *et al.*, 1996). (Meyer *et al.*, 1982), LC₅₀ value of less than 100µg/mL is toxic while LC₅₀ value of greater than 1000 µg/mL is non-toxic. The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number, and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the bioactive compounds present in the plant extracts.

RESULTS

Physical Characteristics and the Percentage Yield of the Extracts

The physical characteristics and the percentage yield of the aqueous, ethanolic and methanolic extracts are shown in table 1. *Cassia occidentalis* extracts appeared deep green, in colour with soft textures. The highest percentage yield of the extract was observed in aqueous extract of *C. occidentalis* which was 15.1% of the total sample extracted, followed by the methanolic extract

which had a percentage yield of 7.9% and least percentage yield was ethanolic extract of *C. occidentalis* with 7.7% as shown in table 1.

Preliminary Qualitative Phytochemical Screening

Phytochemical screening for the bioactive components present in the aqueous extract, ethanolic extract and methanolic extract of *Cassia occidentalis* leaves revealed that the extracts were very rich in secondary metabolites which include alkaloids, saponin, terpenoid, flavonoid, anthraquinone, tannins, glycosides and steroids as shown in table 2. The methanolic extract has the highest number of phytochemicals followed by the ethanolic extract while the least phytochemicals was recorded for the aqueous extract. Tannin was detected in all the leave extracts, while flavonoid was not detected in the aqueous and ethanolic extract of *C. occidentalis*. Table 2 showed the distributions of the bioactive phytochemicals in each of the plants extracts.

Antimicrobial Activity of the Plants Extracts against Clinical Isolates

The antimicrobial activities of the methanolic, ethanolic, and aqueous extract and that of Amoxycillin antibiotic at four different concentrations (120mg/ml, 90mg/ml, 60mg/ml, and 30mg/ml for each extract) against the test organisms are indicated in Table 3. No resistance was observed from the culture as zones of inhibition were determined. Methanolic extract gave higher zones of inhibition of 23 mm at 120mg/concentration *S. paratyphi A* and *S. paratyphi B*. this was followed by ethanolic extract in which also no resistance was observed even at the lowest concentration used. The susceptibility pattern of *Salmonella typhi*, *S. paratyphi A* and *S. paratyphi B* against the various extract is generally very high as resistance was only observed at lower concentration 30mg/ml of some of the extract.

(MIC) and (MBC) of the plants Extracts.

The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the extracts of *C. occidentalis* leaves against *Salmonella typhi*, *S. paratyphi A* and *S. paratyphi B* are presented in Table 4. The MIC of the extracts against all the test isolates had range of values of (6.25 – 125µg/ml). Similarly, the minimal bactericidal concentration (MBC), generally do not exceed the minimal inhibitory concentration (MIC) by more than a factor of 2 (Table 4.)

Table 1: Physical Characteristics of the Various Extracts of *Cassia occidentalis* Leaves.

S/N	Parameter	Extracts		
		Methanol	Ethanol	Water
1	Colour	Deep green	Deep green	Green
2	Texture	Soft	Soft	Soft
3	Yield, %	7.90	7.70	15.10

Table 2: Phytochemical Constituents of *Cassia occidentalis* Leave Extracts.

EXTRACT	PHYTOCHEMICALS								
	Alkaloids	Saponins	Tannins	Flavonoids	Steroids	Glycosides	Terpenoids	Anthraquinones	
Aqueous	+	+	+	-	-	+	-	+	
Ethanolic	+	+	+	-	+	+	+	-	
Methanolic	+	+	+	+	+	+	+	+	

KEYS: += Present -= Absent

Table 3: Antibacterial Activity of *Cassia occidentalis* Leaves Extracts against the Test Organisms by Agar Well Diffusion Method.

S / N	Test organisms	Mean zone of inhibition in (mm) produced by various concentration of each extract against the test organism												ACTIVITY INDEX						
		Methanolic extract (mg/ml)			Ethanol extract (mg/ml)			Aqueous extract (mg/ml)			Amoxicillin (mg/ml)			Met	Eth	Aqu				
		120	90	60	30	120	90	60	30	120	90	60	30	120	90	60	30			
1	<i>Salmonella typhi</i>	20	18	1	1	18	1	1	09	17	1	09	0	20	17	15	10	1.0	0.8	0.7
2	<i>Salmonella paratyphi A</i>	23	19	1	1	21	1	1	9	20	1	15	1	21	18	15	13	1.0	1.0	1.0
3	<i>Salmonella paratyphi B</i>	23	18	1	0	20	1	1	9	23	1	15	1	21	17	15	15	0.9	0.9	1.0

P.I: MET=1, ETH=1, AQU=1

Table 4: Minimum Inhibitory (MIC) and Minimum Bactericidal Concentrations (MBC) of *Cassia occidentalis* Leaves Extract

S/ N	Test organisms	Methanolic extract		Ethanol extract		Aqueous extract		Amoxicillin	
		MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
1	<i>Salmonella typhi</i>	62.5	250	125	500	125	500	62.5	250
2	<i>Salmonella paratyphi A</i>	125	500	125	250	125	500	125	500
3	<i>Salmonella paratyphi B</i>	62.5	125	62.5	500	62.5	1000	125	500

MIC = minimum inhibitory concentration,
MBC = minimum bactericidal concentration

Table 5: Brine Shrimp Cytotoxicity Assay of the Individual Extracts

Plant Extract	Organic Solvent	Concentration (ppm or µg/ml)	No. of Shrimps	No. of Survivors	% Mortality	LC ₅₀ (µg/mL) Brine Shrimp Lethality	
<i>C. occidentalis</i> leaves extract	Aqueous	1000	10	3	70	191.639	
		100	10	6	40		
		10	10	8	20		
	Methanol	1000	10	2	80		
		100	10	4	60		30.765
		10	10	6	40		
	Ethanol	1000	10	2	80		
		100	10	5	50		71.427
		10	10	7	30		

Table 6: Result of Isolate Confirmation

Organism	Klinger's iron agar				Motility, indole, urea			citrate
	Slant	Butt	H ₂ S	Gas	Motility	Indole	Urea	
<i>S. typhi</i>	Alk	Acid	Weak+	-	+	-	-	-
<i>S. paratyphi A</i>	Alk	Acid	-	+	+	-	-	-
<i>S. paratyphi B</i>	Alk	Acid	-	+	+	-	-	-

KEYS += present -= absent Alk=Alkaline

DISCUSSION

Water is the best solvent of extraction according to the study of these leaves extract as it gives higher yield of the extract, however methanol gives more yield of the phytochemical compounds, Tannins was found in all the extract and, all the phytochemical tested were found in *Cassia occidentalis*. Babayi *et al.*, 2004, report the presence of the phytochemicals identified in this study. Yadav *et al.*, (2010) studied the antimicrobial potential of *C. occidentalis* leaves with similar result. *Cassia occidentalis* extract have proportional index of 1. *Cassia occidentalis* have activity index more than 1 against *Salmonella typhi*. In brine shrimp lethality bioassay, % mortality increased gradually with increase in concentration of the extracts. An LC₅₀ (concentration killing fifty per cent of the brine shrimp larvae), value greater than 100µg/ml is considered to present a non-toxic compound or extract as reported by Moshi *et al.*, (2010). The brine shrimp toxicity assay showed that methanolic and ethanolic extracts have values less than 100µg/ml therefore considered toxic. Aqueous extract showed LC₅₀ greater than 100µg/ml thus, non-toxic. The high toxicity of *C. occidentalis* leaf extract on brine shrimp larvae may be due to the effect of saponins. Acute toxicity test was conducted in a report with *Cassia occidentalis* and found that this plant did not show any hazardous symptoms or death (Tanimu, 2012). Muhammed *et al.*, (2012) reported that the leaves of *C. occidentalis* are rich in triterpenoidal saponins and these compounds were reported to have high antilipemic, hemolytic and capacity to lower the serum chlesterol level.

Conclusion

Methanol, ethanol and water have the ability of extracting phytochemical from *C. occidentalis* leaves. All extract showed activity against the test organisms in accordance with the extract concentration. The result from MIC and MBC indicates the bacteriostatic property against the test organisms, in accordance with the extract concentration. *Cassia occidentalis* (methanolic, ethanolic and aqueous extract) showed high proportion index similar to control antibiotic Amoxicillin. Toxicity study carried out on the plants extract revealed that aqueous extracts of *C. occidentalis* leave is non- toxic.

Recommendations

From the result obtained

- Brine shrimp lethality assay conducted showed greater percentage of the extract tested were toxic, contrary to the claims by traditional medicine practitioners. Prolong administration of such plants should be avoided especially by community that patronize such plants as herbal cure

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