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Research Paper

Antibacterial activity of the aqueous leaf extract of *cassia occidentalis* (sanga-sanga) on *salmonella typhimurium*

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The *Cassia occidentalis* leaves was obtained from Usmanu Danfodiyo University Sokoto, Fish farm and it was authenticated at Usmanu Danfodiyo University herbarium after it was taken to Microbiology Laboratory Usmanu Danfodiyo University, Sokoto for aqueous extraction Fifty grams of grounded leaves was dissolved in 500 ml of distilled water and the extract was evaporated in hot air oven at 40°C for 9 days. The concentrations were tested against *S. typhi* that was obtained from specialist Hospital Sokoto (SHS) after the test isolate was tested for viability and confirmed at the Microbiology Laboratory Usmanu Danfodiyo University, Sokoto. The leaf extract was tested at different concentrations of 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml. But there was no activity recorded at 20 mg/ml. And the minimum inhibitory concentration (MIC) was recorded at 30 mg/ml and minimum bactericidal concentration (MBC) was recorded at 60 mg/ml. The phytochemical screening reveals the presence of Flavonoid (++), Tannin (+), Saponin (+++), Glycoside (+), Cardiac glycoside (+), Steroid (+), Saponin glycoside (+), Anthraquinones (+), and Volatile oil (+). The presence of these photochemical may be attributed to the antibacterial activity of the extracts.

Key words: Antibacterial activity, bacteria, photochemical, *salmonella typhimurium*, *cassia occidentalis*

INTRODUCTION

Plants are important source of drugs; especially in traditional medicine (Bako *et al*., 2005). It is a common practice in Nigeria and other apart of the world to use plant in the form crude extracts, decoction, infusion or tincture to treat common infection chronic conditions (Odeja, 2005). According to WHO, over 70% of the world population rely on medicinal plants of primary health care and there are reports from various researchers on natural substance of plant origin which are biologically active with desirable antimicrobial and antioxidant properties (WHO, 2008).*Cassia occidentalis* is an unarmed slender upright short lived (annual or biennial) shrub, 0.5-2.5 m tall distinguished leaves consist of 3-7 pairs of leaflets (2-10 cm long and 2-3 cm wide) that have pointed tip; amounted gland at base of leaf stalk no glands between leaflets.

There is a conspicuous dark colored gland near the base of the stalk of each leaf (*Lazzaride et al*., 1997). *Cassia occidentalis* called as “ewe oriesi” in Yoruba and coffee *Senna* English belongs to the family *caesalpiniacea*, subfamily *caesalpinioideae*. It is an ayurvedic plant with huge medicinal importance (Arya *et al*., 2010) leaves of *Cassia occidentalis* plants have ethno medicinal importance like paste of leaves is externally applied on healing wounds, sore, itch, cutaneous disease, bone fracture, ringworm skin diseases, throat

and fever (Arya *et al*., 2010).

Salmonella typhimurium is facultative intracellular pathogens (Jantsch *et al.,* 2011). Many infections are due to ingestion of contaminated food. Salmonella serovars can be divided into two main groups typhoidal and non typhoidal Salmonella. Non typhoidal serovars are more common, and usually cause self- limiting gastrointestinal disease.

They can infect a range of animal, and are zoonotic, meaning they can be transferred between humans and other animal (Jantsch *et al.,* 2011). The clinical presentation of typhoid fever varies from a mild illness with low-grade fever, malaise, and slight dry cough to a severe clinical picture with abdominal discomfort and multiple complications.

Many factors influence the severity and overall clinical outcome of the infection. They include the duration of illness before the initiation of appropriate therapy, the choice of antimicrobial treatment, age, the previous exposure or vaccination history, the virulence of the bacterial strain, the quantity of inoculums ingested, host factors (Wain *et al*., 1994).

The definitive diagnosis of typhoid fever depends on the isolation of *S. typhi* from blood, bone marrow or a specific anatomical lesion.

Evidence suggests that the fluoroquinolones are the optimal choice for the treatment of typhoid fever in adults and that they may also be used in children.

The recent emergence of resistance to fluoroquinolones, however, suggests that their widespread and indiscriminate use in primary care settings should be restricted (Papoff and minor*,* 1997) previous pharmacological investigation showed that *S. occidentalis* leaves extracts have antibacterial (Saganuwan and Gulumbe, 2006), antimalaria (Tona *et al*., 2001) antimutagenic (Jafri *et al*., 1999; Sharma *et al.,* 2000), antiplasmodial (Tona *et al*., 2001) anticarcinagenic (Sharma and Jumar, 2003) and hepato- protective (Yadav *et al*., 2009) activity.

However, studies on this showed that the nature and amount of the photochemical varies according to the season and geographical, location (Yadav *et al*., 2009).

Thus this study is aimed in determining the antibacterial activity of aqueous leaf extracts of *Cassia occidentalis* on *Salmonella typhimurium* since, *Salmonella typhimurium*, is a bacterium of medical significance and its emergence is a worry to the populace.

Thus, previous researches have shown that the susceptibility and resistance profile of the bacterium to different antibiotics most of which might be due to environmental conditions or the ability of the organism to withstand the surge of chemical pressure caused either by the host immune system or the drugs.

It then becomes necessary to determine the antibacterial activity of the aqueous extracts of this *Cassia occidentalis* leaves on the bacterium *Salmonella typhimurium*.

MATERIALS AND METHODS

Sample collection and identification

The *cassia occidentalis* leaves were obtained from the garden of Usmanu Danfodiyo University fish farm. The fresh leaves were taken to Usmanu Danfodiyo University herbarium and identified and authenticated and the Voucher No. is (UDUH/ANS/0110). Pure culture of the test isolate i.e. *Salmonella typhimurium* was obtained from the Microbiology Laboratory, Specialist hospital Sokoto, (SHS) Sokoto state Nigeria. It was transported to Microbiology Laboratory Usmanu Danfidiyo University, Sokoto where it was tested for viability and confirmed by subjecting it to different biochemical test.

Sample processing

The leave was allowed to dry at room temperature for 6 days. The dried leaves was grounded using mortar and pestle and the grounded leaves was sieved with a mesh of size 0.5 mm. the powdered sample was stored in aluminum foil at room temperature (28°C). The test organism (*salmonella typimurium*) was subculture in nutrient agar and it was incubated for 24 h where it was later stored in nutrient agar slant at 4°C (Cheesbrough, 2000).

Preparation of the aqueous extract

The preparation of aqueous extract was carried out as described by (E-ole *et al*., 1994). Fifty Grams of grounded *Cassia occidentalis* leaves was dispensed in 500 ml of distilled water in 1000 ml capacity conical flask the mixture was stirred vigorously and it was allowed to stand for 24 h. It was stirred again and it was filtered through a whattman filter paper No.1 lined funnel into a conical flask, the filtrate was evaporated with hot air oven at 40°C and the solid crude extract was obtained (E-ole *et al*., 1994).

Gram staining

A smear was prepared on a clean grease free slide and heat fixed, the smear was placed on a staining rack and flooded with crystal violet for 60 s; then rinsed with water and excess water drained off to avoid diluting the mordant. Then the smear was flooded with iodine solution (mordant) for 30 s and washed with water. The smear was decolorized with alcohol and was added drop-wisely on the tilled slide and washed with water. The slide was then flooded with safranin for 60 s and finally washed with water. The slide was allowed to air dry and heat fixed before being examined under oil immersion

with microscope using X100 objective lens. Gram positive bacteria appeared blue to purple while Gram negative bacteria appeared pink to red (Cheesbrough, 2000).

Biochemical test

Oxidase test

This test was done as described by Cheesbrough (2000). A loopful amount of the test organism was emulsified on the oxidase strip. The development of an intense purple color within 30 s indicates the oxidase positive while failure to develop an intense color within 30 s indicates a negative test.

Indole test

This test was done as described by Oyeleke and Manga, (2008). A little portion of the bacteria was inoculated onto 5ml of sterile peptone-water enriched with 1% tryptophan and was incubated at 37°C for 48 h. To the culture, 0.5 ml of Kovac's reagent was added and gently shaken. In the positive test, indole (present in the culture) dissolved in the reagent which then became red, ad formed a layer at the surface of the medium while a yellow layer at the surface of the medium denoted a negative result.

Citrate utilization test

A little portion of the isolate was inoculated into Koser's citrate medium and incubated at 37°C for 72 h. A positive citrate test was confirmed by formation of bright blue color while the initial green color of the medium denoted a negative test (Oyeleke and Manga, 2008)

Urease test

A sterile straight wire was used to inoculate the test organism in a medium which contains urea and the indicator phenol red by stabbing through the centre of the medium. It was incubated at 37°C for 24 h. A change in color of the indicator to pink-red indicated a urease positive test while the initial yellow color indicates a negative test (Cheesbrough, 2000).

Methyl Red (MR) and Voges-Proskaeur (VP) test

MR test was carried out as described by Ochei and Kolhatkar, (2000). A little portion of each isolate was inoculated into the glucose phosphate peptone water medium ad was incubated at 37°C for 48 h. Few drops of methyl red were added to the culture. MP positive was indicated by red color formation at the surface of the medium while no change denoted negative. MR test is usually done in conjunction with VP test. Retaining the initial color in the medium during MR test indicated a positive VP test; therefore an organism was either MR or VP positive.

Triple sugar iron (TSI)

Triple sugar iron was prepared and with a sterile needle the culture from solid medium was streaked on the surface of the slant and the butt was subbed three times. Incubation was at 35°C for 24 h. Gas formation was determined by the appearance of one or several bubbles. In the butt, vigorous gas formations can result in cracks or it may be pushed from the bottom. TSI glucose fermenters were indicated by the butt becoming yellow. If both the butt and slope were yellow, it indicated fermentation of lactose and sucrose. Fermentation of lactose and sucrose but not glucose was deleted with red butt and yellow slope (Oyeleke and Manga, 2008). A little portion of each isolate was stabbed into triple sugar iron agar and incubated at 37°C for 24 h. Motility was observed by the spread of the organisms outwards from the stabbed areas (Ochei and Kolhatkar, 2000). A little portion of each isolate was inoculated by streaking and stabbing for 24 h into triple sugar iron and incubated at 37°C. Evolution of blackening on the medium indicated a positive test while no blackening indicated negative (Cheesbrough, 2000).

Antibacterial activity assay

The antibacterial activity assay of the extract was done using the agar diffusion method (Cheesbrough, 2000). The organism to be tested was inoculated into sterile nutrient agar and incubated at 37°C for 24 h. Then loop of inoculums were transferred into 5 ml of nutrient broth and incubated at 37°C for 24 h. Wells of 5 mm in diameter were made with a sterile cork borer in another plate containing sterile nutrient Agar and the inoculums of the test organism was spread on the solid plates with the aid of sterile swab moisten with the bacterial suspension. The different concentration of the aqueous extract of the *Cassia occidentalis* was placed in the wells made in inoculated plates. The plates were incubated at 37°C for 24 h to observe presence or absence of zone of inhibition in millimeters (mm), (Girish and Satish, 2008).

Determination of minimum inhibitory concentration (MIC)

MIC of the extracts was carried out using the tube dilution

techniques described by (Cheesbrough, 2000). A double fold serial dilution was made using Muller-Hinton broth. Equal volume of Muller-Hinton broth and extracts was dispensed into sterile tubes. A quantity of 0.1 ml of standardize inoculums were added to each test tube which was incubated aerobically at 37°C for 2 h. A tube with broth and inoculums served as organic control. A tube with broth and extract served as extract control. The lowest concentration of the extract which inhibited microbial growth was recorded as the minimum inhibitory concentration (MIC)

Determination of Minimum Bactericidal Concentration (MBC)

Sterile Muller-Hinton agar plates were inoculated with sample from each of the test tube that showed visible growth from the MIC test. The plates were incubated at 37°C for 24 h. The concentrations used were 120mg/ml, 60mg/ml and 30mg/ml, and the lowest concentration of the extracts that yielded no growth was recorded as the minimum bactericidal concentration (MBC) (Cheesbrough, 2000).

Phytochemical screening of extract

Qualitative test

Test for flavonoids

Three milligrams (3 mg) aliquot of the filtrate and one milligram of ten percent (10%) NaOH (Sodium Hydroxide Solution) were added, when yellow colour is developed then it indicates the possible presence of Flavonoids (Oyekele and Manga, 2008).

Test for tannins

Ferric chloride solution 5% ferric chloride solution was added drop by drop into the 2ml of the extract and the color produced was noted. The presence of dark green color was indicative of the presence of tannins (Harbone *et al*., 1973).

Test for saponin

Ten (10) ml of distilled water was added to 0.5cm³ of the extracts, it was shacked vigorously with the test tube for 2minutes. The presence of frothing indicates saponins presence, (Oyekele and Manga, 2008).

Test for glycosides

2.5 ml of 50% H_2SO_4 was added to 5 ml of extract in a test tube. The mixture was heated in boiling bath for 15 min. It was allowed to cool and neutralized with 10% NaOH and 5 ml of fehling's solution was added and the mixture was boiled. A brick-red precipitate was observed which indicate the presence of glycoside (Harbone *et al*., 1973).

Test for alkaloids

2 ml of extract was stirred with 2ml of 10% aquences hydrochloric acid. 1 ml was treated with few drops of Wangers' reagent and second 1 ml portion was treated similarly with Mayer reagent. Precipitation was observed for the presence of alkaloids (Harbone *et al*., 1973).

Test for cardiac glycosides (Keillerkilliani's test)

2 ml of 3.5% ferric chloride solution were added and allowed to stand for one minute. One ml of concentrate H_2SO_4 was carefully pour dawn the wall of the tube so as to form lower layer. A reddish brawn ring when formed at the interface indicates the presence of cardiac glycoside (Oyeleke and Manga, 2008).

Test for saponin glycosides

2.5 ml of extract was added to 2.5 ml of fehiling's solution A and B. a bluish green precipitate was observed for the presence of saponin glycosides (El-ole *et al*., 1994)

Test for balsam

2.5 ml of extract was mixed with equal volume of 90% ethanol. 2 drop of alcoholic ferric chloride solution was added to the mixture. A dark green color was observed for three presence of balsam (El-Ole *et al.,* 1994)

Test for anthraquinones

2 ml of the plant extract was shaken with 10 ml of benzene and 5 ml of 10 ammonia solution was added. The mixture was shaken and the presence of pink color in the ammonical (lower) phase was observed for the presence of anthraquines (Harbone *et al*., 1973).

Test for volatile oil

1 ml of the fraction was missed with dilute HCl. A white precipitate was formed which indicated the presence of volatile oil (Evans and Gupta, 1980).

Test for steroid

2 ml of the extract was dissolved in 2 ml of chloroform and 2 ml of Sulphuric acid was carefully added and the lower layer was formed. A reddish brown color was

Table 1. Antibacterial activity of the extracts on *S. typhi* at different concentrations.

Mg/ml	*S. typhi*
20	0.00 mm
40	12.00 mm
60	15.00 mm
80	16.00 mm
100	18.00 mm

Table 2. Minimum inhibitory concentration (MIC) on *S. typhi.*

Plant	Concentrations (mg/ml)								MIC Mg/ml
	120	60	30	15	7.5	3.75	1.87	0.94	
Cassia occidentalis	-	-	-	+	+	+	+	+	30

Key - = No growth+ = Growth found

Table 3. Minimum Bactericidal Concentration (MBC) on *S. typhi.*

Plant	Concentrations			MBC
	120	60	30	Mg/ml
Cassia Occidentalis	-	-	+	60

Key - = No growth, + = Growth found

observed for the presence of Steroid (Harbone *et al*., 1973).

RESULTS AND DISCUSSION

Table 1 showed the antibacterial activity of the extracts at different concentrations on the *S. typhi*. At 20 mg/ml no activity was recorded. However at concentration of 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml an activity was recorded. These activity increases as the concentration was increased. In this study, the antibacterial activity of the aqueous leaf extract of *Cassia occidentalis* was determined at concentration of 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml. The antibacterial activity of the aqueous leaf extract was recorded at concentration of 40 mg/ml (12 mm), 60 mg/ml (15 mm), 80 mg/ml (16 mm), and 100 mg/ml (18 mm). This shows that from 40mg/ml as the concentration was increased the rate of antibacterial activity increases. This is in conformity with Odeja*,* (2005), Egharevba *et al.,* (2010) and Sadiq *et al.,* (2012), whom all proved that the *Cassia occidentalis* leaf extract is active against Microorganism at different concentration. Also, it is noted that the rate of antibacterial activity increases (Singh and Singh, 2007).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined respectively as to determine the minimum concentration which the extract is active on the test isolate (Tables 2 and 3). And it was discovered that at concentration of 30 mg/ml MIC was noted while at 60 mg/ml MBC was noted. This shows that the leaf extract will be able to kill the organism at 60 mg/ml but at concentration lesser than that, it may only hinder the growth but not kill it.

The ability of such activity on the bacteria may be due to the possession of lipopolysaccharide layer which allows the direct exposure of inner membrane layer to the natural activities of the antibacterial (Munyendo *et al.,* 2011).The phytochemical analysis of the aqueous was presented in (Tables 4 and 5). Saponin was present high amount, Flavonoids was present in moderate amount, Tannins, Glycoside, Cardiac glycosides, Steroids, Saponin glycoside, Anthraquinones and Volatile oil were present in trace amount. However Balsam and Alkaloid are not detected.

The antibacterial activity of the leaf extract may be related to the presence of phytocompounds fond in the extract. Even though some phytocompounds were found in trace amount. The phytocompounds include: Flavonoid, Tannin, Saponin, Glycoside, Cardiac glycoside, Steroid, Saponin glycoside, Anthraquinones and Volatile oil.

The presence of these metabolites suggests great potential for the plant as a source of phytomedicines. The antibacterial activity is greatly influenced by the presence of Flavonoid, Anthraquinones and Saponin.

Table 4. Phytochemical analysis.

Phytochemical	Result
Flavonoid	++
Tannins	+
Saponins	+++
Glycoside	+
Alkaloid	N.D
Cardiac glycoside	+
Steroids	+
Saponin glycosides	+
Balsam	N.D
Anthraquines	+
Volatile oil	+

Key: + = Present in trace amount, ++ = Present in moderate amount, +++ = Present in high amount, N.D = Not detected

Table 5. Biochemical analysis of *Salmonella typhi*

Biochemical parameters	Oxidase	Indole	Citrate	Urease	VP	Glucose	Sucrose	Lactose	H_2S	Gas	Motility
Salmonella typhi	-	-	-	-	-	+	-	-	+	-	+

Conclusion and recommendation

The antibacterial activity of *Cassia occidentalis* leaf extract was recorded at a concentration of 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml, having a minimum inhibitory concentration (MIC) of 30 mg/ml and minimum bactericidal concentration (MBC) of 60 mg/ml. This activity is due to presence of phytochemical compounds even though some of these compounds were found in trace amount. Thus more research should be conducted on the activities of this extracts on other bacteria of medical significance as well as increasing in its concentration.

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