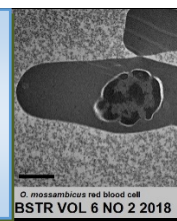


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Antibacterial Activity and Phytochemical Analysis of *Cassia occidentalis* Leaf Extract on *Salmonella Typhimurium*

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ABSTRACT

Different concentration extracts of *Cassia occidentalis* (Caesalpiniaceae) leaves were screened for their antimicrobial activity against *Salmonella Typhimurium*. The pattern of inhibition was varied with the concentration used. Antibacterial activity of the extracts at different concentrations on the *S. Typhimurium* was recorded with no activity at 20 mg/mL. Nevertheless, at concentrations of 40, 60, 80 and 100 mg/mL, activity was observed. This activity was seen increased as the concentration increases. At the minimum bactericidal concentration (MBC) of 60 mg/mL, the bacteria were killed with no tidal activity at 30 mg/mL. The antibacterial activity of *Cassia occidentalis* leaf extract was recorded at concentrations of 40 mg/mL (12 mm), 60 mg/mL (15 mm), 80 mg/mL (16 mm) and 100 mg/mL (18 mm) with a minimum inhibitory concentration (MIC) of 30 mg/mL and MBC of 60 mg/mL. This was due to the presence of phytochemical compounds. Some of these compounds were found in a trace amount, whereas saponin was present high amount, flavonoids were present in a moderate amount, while tannins, glycoside, cardiac glycosides, steroids, saponin glycoside, anthraquinones and volatile oil were present in trace amount. However, balsam and alkaloid are not detected.

INTRODUCTION

Plants are an important source of drugs especially in traditional medicine [1]. It is a common practice in Nigeria and other parts of the world to use the plant in the forms of crude extracts, decoction, infusion or tincture to treat common infection chronic conditions [2]. According to the WHO, over 70% of the world population rely on medicinal plants for primary health care, and there are reports from various researchers on natural substances of plant origin that are biologically active with desirable antimicrobial and antioxidant properties [3].

Cassia occidentalis is an unarmed slender upright short-lived (annual or biennial) shrub with 0.5-2.5 m tall and distinguished fetid odour. It is once a compound leave consisting of 3-7 pairs of leaflets (2-10 cm long and 2-3 cm wide) with pointed tip; amounted gland at the base of leaf stalks no glands between leaflets. There is a conspicuous dark coloured gland near the base of the stalk of each leaf [4]. *Cassia occidentalis* is known as "ewe ori esi" in Yoruba and coffee *Senna* English belongs to the family of *caesalpiniaceae*, subfamily *caesalpinoideae*. It is an ayurvedic plant with huge medicinal importance [5]. The leaves of *Cassia occidentalis* plants have ethno medicinal importance like the paste of leaves can be externally applied to heal wounds,

sore, itch, cutaneous disease, bone fracture, ringworm skin diseases, throat and fever [5]. Previous pharmacological investigations showed that *S. occidentalis* leaves extracts have antibacterial [6], antimalarial [7], antimutagenic [8], antiplasmodial [7] and anticarcinogenic [9] properties. Studies on hepato protective activity however showed that the nature and amount of the phytochemicals vary according to seasons and geographical locations [10].

Salmonella, a bacterium of medical significance, is of high worry to the populace. Previous studies demonstrated that the susceptibility and resistance profile of the bacterium to different antibiotics most of which may 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 *Cassia occidentalis* leaves on *Salmonella Typhimurium*.

MATERIALS AND METHODS

Sample collection

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. Pure culture of the test isolate, which *Salmonella* Typhimurium, was obtained from the Microbiology Laboratory, Specialist hospital Sokoto, (SHS) Sokoto state Nigeria. It was transported to Microbiology Laboratory Usmanu Danfodiyo University, Sokoto where it was tested for viability and confirmed by subjecting it to different biochemical tests as described by [11,12,13]. The isolates were maintained on freshly-prepared nutrient agar for further use.

Sample processing

The leaves were allowed to dry at room temperature for six days. The dried leaves were grounded using mortar and pestle, which were then sieved with a 0.5 mm mesh. The powdered sample was stored in aluminium foil at room temperature (28°C). The test organism (*Salmonella* Typhimurium) was subcultured in nutrient agar and incubated for 24 hours where it was later stored in nutrient agar slant at 40°C [11].

Preparation of aqueous extract

The preparation of aqueous extract was carried out as described by [14]. Whatman filter paper No.1 was lined funnel into a conical flask, while the filtrate was evaporated with hot air oven at 40°C to obtain the solid crude extract.

Determination of minimum inhibitory concentration (MIC)

The MIC of the extracts was carried out using the tube dilution technique described by [11]. A double fold serial dilution was made using Muller-Hinton broth. An equal volume of Muller-Hinton broth and extracts was dispensed into sterile tubes. A quantity of 0.1 mL of standardised inoculum was added to each test tube, which was incubated aerobically at 37°C for 2 hours. A tube with broth and inoculum served as organic control. Meanwhile, the tube with broth and extract served as the extracted control. The lowest concentration of the extract inhibiting microbial growth was recorded as the minimum inhibitory concentration (MIC)

Determination of minimum bactericidal concentration (MBC)

Sterile Muller-Hinton agar plates were inoculated with samples from each test tube showing visible growth from the MIC test. The plates were incubated at 37°C for 24 hours. The concentrations used were 120, 60 and 30 mg/mL, whereas the lowest concentration of the extracts that yielded no growth was recorded as the minimum bactericidal concentration (MBC) [11].

Phytochemical screening of extract

Qualitative test

Test for flavonoids

Three milligrams (3 mg) aliquot of the filtrate and 1 mg of 10% NaOH were added. If yellow colour is developed, it indicates the possible presence of flavonoids [13].

Test for tannins

The of ferric chloride solution (5%) was added drop wise into the 2 mL extract and the colour produced was noted. The presence of dark green colour indicates the presence of tannins [15].

Test for saponin

Exactly 10 mL of distilled water was added to 0.5 cm³ of the extract, which was vigorously shaken with test tube for 2 min. The presence of frothing indicates the presence of saponins [13].

Test for glycosides

Exactly 2.5 mL of 50% H₂SO₄ was added to 5 mL of extract in a test tube. The mixture was heated in a boiling bath for 15 min. It was allowed to cool and neutralised with 10% NaOH, and 5 mL of Fehling's solution was added, and the mixture was boiled. A brick-red precipitate was observed, which indicates the presence of glycoside [15].

Test for alkaloids

Exactly 2 mL of extract was stirred with 2 mL of 10% aqueous hydrochloric acid. 1 mL was then treated with few drops of Wanger's reagent and the second 1 mL portion was treated similarly with Mayer's reagent. Precipitation was observed for the presence of alkaloids [15].

Test for cardiac glycosides (Keiller killiani's test)

Exactly 2 mL of 3.5% ferric chloride solution was added and allowed to stand for 1 min. 1 mL of concentrated H₂SO₄ was carefully poured on the wall of the tube to form a lower layer. A reddish brown ring, when formed at the interface, indicates the presence of cardiac glycoside [13].

Test for saponin glycosides

Exactly 2.5 mL of extract was added to 2.5 mL of Fehling's solution A and B. A bluish-green precipitate was observed for the presence of saponin glycosides [16].

Test for balsam

Exactly 2.5 mL of extract was mixed with an equal volume of 90% ethanol. Two drops of alcoholic ferric chloride solution were added to the mixture. Dark green colour was observed for the presence of balsam [16].

Test for anthraquinones

2 mL of the plant extract was shaken with 10 mL benzene and 5 mL of 10 ammonia solution was added. The mixture was shaken and the presence of pink colour in the ammoniacal (lower) phase was observed for the presence of anthraquinones [15].

Test for volatile oil

Exactly 1 mL of the fraction was missed with dilute HCl. A white precipitate was formed, which indicates the presence of volatile oil [17].

Test for steroid

Exactly 2 mL of the extract was dissolved in 2 mL of chloroform, and 2 mL of sulphuric acid was carefully added and forming the lower layer. A reddish-brown colour was observed indicating the presence of steroid [15].

RESULTS AND DISCUSSION

The result for the antibacterial activity of the aqueous extract of *Cassia occidentalis* on *Salmonella* Typhimurium was observed. **Table 1** displays the antibacterial activity of the extracts at different concentrations on the *S. Typhimurium*. At 20 mg/mL no activity was recorded. However, at concentrations of 40, 60, 80 and 100 mg/mL, activity was recorded. This activity increases as the concentration were increased

Table 1. The antibacterial activity of the extracts at different concentrations on the *S. Typhimurium*.

Extract concentrations (mg/mL)	Antibacterial activity (MM)
20	0.00
40	12.00
60	15.00
80	16.00
100	18.00

The MIC is presented in **Table 2**. It was demonstrated that the concentration of 30 mg/mL inhibited the growth while growth was observed as from 15 mg/mL. While **Table 3** depicts the MBC at the concentration of 60 mg/mL, where the bacteria were killed with no bacteriicidal activity at 30 mg/mL.

Table 2. The growth of *Cassia occidentalis* on 30 mg/mL of MIC.

S/n	Plant	Concentrations (mg/ml)								Mic (mg/ml)
		120	60	30	15	7.5	3.75	1.87	0.94	
1	<i>Cassia occidentalis</i>	-	-	-	+	+	+	+	+	30

Key:
- = No growth
+ = Growth found

Table 3. The growth of *Cassia occidentalis* on 60 mg/mL of MIC.

S/n	Plant	Concentrations			Mbc (mg/ml)
		120	60	30	
1	<i>Cassia occidentalis</i>	-	-	+	60

Key:
- = No growth
+ = Growth found

The phytochemical analysis of the aqueous is presented in **Table 4**. Saponin was present high amount, flavonoids were present in a moderate amount, while tannins, glycoside, cardiac glycosides, steroids, saponin glycoside, anthraquinones and volatile oil were present in trace amount. However, Balsam and Alkaloid were not detected

Table 4. The phytochemical analysis of the aqueous.

S/n	Phytochemical	Result
1	Flavonoid	++
2	Tannins	+
3	Saponins	+++
4	Glycoside	+
5	Alkaloid	N.d
6	Cardiac glycoside	+
7	Steroids	+
8	Saponin glycosides	+
9	Balsam	N.d
10	Anthraquinones	+
11	Volatile oil	+

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

DISCUSSION

In this study, the antibacterial activity of the aqueous leaf extract of *Cassia occidentalis* was determined at concentrations of 20, 40, 60, 80 and 100 mg/mL. The antibacterial activity of the aqueous leaf extract was recorded at concentrations 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 40 mg/mL as the concentration increases the rate of antibacterial activity increased. This conforms with [18] and [19], whom all proved that the *Cassia occidentalis* leaf extract is active against microorganism at different concentrations. Besides, it was noted

that the rate of antibacterial activity increased [20]. The MIC and MBC were also determined to determine the minimum concentration where the extract was active on the test isolate. In addition, 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 is able to kill the organism at 60 mg/mL but may only hinder the growth at concentration lesser than that. The ability of such activity on the bacteria might be due to the possession of the lipopolysaccharide layer that allows the direct exposure of inner membrane layer to the natural activities of the antibacterial [21].

The antibacterial activity of the leaf extract may be related to the presence of phytochemicals found in the extract even though some phytochemicals were found in trace amount. The phytochemicals include flavonoid, tannin, saponin, glycoside, cardiac glycoside, steroid, saponin glycoside, anthraquinones and volatile oil. The presence of these metabolites suggests a great potential for the plant as a source of phytomedicines. The antibacterial activity is greatly influenced by the presence of flavonoid, anthraquinones and saponin.

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