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Full Length Research Paper

Preliminary phytochemical and antimicrobial screening of the leaf extract of *Cassia singueana* Del.

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Ethanolic and aqueous extracts of *Cassia singueana* Del. leaves were screened for their antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogen, Escherichia coli, Salmonella typii* and *Shigella dysentery*. The result indicated that the extracts inhibited the growth of one or more test pathogens. The ethanolic extract showed a broad spectrum of antimicrobial activity. Phytochemical investigation revealed the presence of tannins, saponins, alkaloids, glycosides, flavonoids, carbohydrate and terpenes. The minimum inhibitory concentration (MIC) ranges from 1.0 x 10^4 to 4x10^3 µg/ml.

Key words: *Cassia singueana*, *Caesalpiniaceae*, leaf extracts, antimicrobial activity, phytoconstituents.

INTRODUCTION

Man has earlier discovered within his environment the wealth of importance of plants as therapeutic agents. This knowledge together with their toxic potentials had passed down from generation to generation. Of the 300,000 plant species acclaimed world wide only about 5% have been investigated scientifically for their medicinal purposes (Sanusi and Rabo, 2004). Researchers have reported that developing countries rely mainly on plants for the treatment of their prevailing ailments especially in areas where hospitals are not accessible (Lambo, 1979). In industrialized countries it is known that over 30% of all prescription drugs are from plant origin (Iwu et al., 1999).

Cassia singueana Del. *(*family Caesalpiniceae*)* is a woody annual herb or under shrubs between 1.2 and 1.5 m high with small yellow lowers. It is wide spread in India and tropical Africa including northern Nigeria, especially in cultivated or old clearings by the road side and open grassy areas (Dalziel, 1956; Irvine, 1961).

The active principles of many drugs found in plants are secondary metabolites (Ghani, 1990; Doelis, 1993). Therefore, basic phytochemical investigation of this extracts for major phytoconstituents is also vital. In the present study, the water and ethanolic extracts of *C. singueana* Del. leaves were screened for phytochemical constituents and antimicrobial activity against *Staphylococcus aureus, Streptococcus pyogen, Escherichia coli, Salmonella typhi* and *Shigella dysentery*.

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MATERIALS AND METHODS

The leaves of *C. singueana* Del. were collected from Maiduguri metropolis, Borno State, Nigeria. The plant materials were identified by Professor S. S. Sanusi of the Biological Science Department, University of Maiduguri and Voucher specimen No. 46BA was deposited in the research laboratory of chemistry Department University of Maiduguri. The solvents used for the preparation of the leaf extracts were water and ethanol.

Preparation of plant extracts

The plant materials were dried at room temperature and then powdered using a grinder. The powdered sample (100 g) was subjected to soxhlet extraction using 300 ml of each of the solvents (water and ethanol) respectively. The resulting extracts were concentrated on a hot water bath and kept for further investigation.

Phytochemical screening

Phytochemical screening for major constituents was undertaken

Table 1. Inhibition zone of *Cassia singueana del.* (water extract/drug) against the tested microorganisms.

	Zones of inhibition (mm)				
Extract/drug (mglml)	***Staphylococcus aureus***	***Streptococcus pyogene***	***E. coli***	***Shigella dysentery***	***Salmonella typhi***
200	12	6	0	0	0
300	15	9	0	0	2
400	15	10	0	3	2
500	18	10	4	5	5
250 (GTC)	25	15	10	12	10

GTC = Gentamicin.

Table 2. Inhibition zones of C*assia singueana* Del. (ethanolic extract/drug) against tested microorganisms.

	Zones of inhibition (mm)				
Extract/drug (mglml)	***Staphylococcus aureus***	***Staphylococcus pyogen***	***E. coli***	***Shigella dysentery***	***Salmonella typhi***
200	18	9	2	2	2
300	20	10	6	4	5
400	24	12	12	10	7
500	24	13	12	10	9
250 (GTC)	30	18	20	25	17

GTC = Gentamicin.

using standard qualitative methods as described by Odebiyi and sofowora (1990) and Fadeyi (1983) the extract were screened for the presence of glycosides, alkaloids, tannins, flavonoid, saponins and terpenes.

Test organisms

Standard strains of *S. aureus, S. pyogen*, *E. coli, S. typhi* and *S. dysentery* were obtained from the department of medical microbiology, university of Maiduguri teaching hospital, Maiduguri, Nigeria.

Antimicrobial screening test

The paper disc diffusion method was used to determine the antimicrobial activity of the extract from *C. singueana* Del. using standard procedures (Erickson et al., 1960; Bauer et al., 1996). Solutions of the extract of varying concentrations, ranging from 200 to 500 mg/ml were prepared. Nutrient agar was prepared, sterilized and used as the growth medium for the microorganisms. 20 ml of sterilized medium was poured into each sterilized petridish, covered and allowed to solidify. The Mueller-Hinton sensitivity agar plate was then seeded with the test microorganisms by the spread plate technique, and was left for about 30 min to dry. The sterilized paper discs were soaked in the prepared solution of the extracts with varying concentration and were dried at 50°C. The dried paper discs were then planted on the nutrient agar seeded with the test microorganisms. The plates were incubated at 37°C for 24 h after which they were inspected for zones of inhibition of growth. The zones of inhibition were measured and recorded in millimeters. A control experiment was also set up using pure DMSO for each of the test organisms.

Determination of minimum inhibitory concentration (MIC)

MIC of the ethanolic and aqueous extract of *C. singueana* Del. which showed the highest antibacterial activity in the disc diffusion assay were determined based on broth dilution technique with a standard method (Krivoshan et al., 1989). The inocula of micro organisms were prepared from 12 h broth cultures. Stock solutions of extracts (200 mg/ml) were diluted with nutrient broth cultures. Stock solution of extracts (200 mg/ml) were diluted with nutrient broth in serial tenfold dilutions using nutrient broth to make dilution ranging from 200 mg/ml (2×10^5 µg/ml) to 0.2 mg/ml (2×10^2 µg/ml) and inoculated with 0.2ml of the test microorganisms. The inoculated tubes were then incubated at 37^0C for 24 h and were inspected for non-turbidity. The least concentration of the extract which prevented visible growth was noted and recorded as the minimum inhibitory concentration (MIC).

RESULTS

The results of the antimicrobial tests and minimum inhibitory concentrations for the water and ethanol extracts are presented in Tables 1 to 4.

DISCUSSION

The phytochemical screening revealed the presence of

Table 3. Minimum inhibitory concentration (MIC) of *Cassia singueana Del.* water extract against the tested microorganisms.

	Concentration µglml				
Test organisms	**1 x 10^4**	**3 x 10^4**	**5 x 10^4**	**1 x 10^5**	**2 x 10^5**
Staphylococcus aureus	-	0+	+	+	+
Streptococcus pyogen	-	-	-	-	0+
E. coli	-	-	-	-	-
Shigella dysentery	-	-	-	-	-
Salmonella typhi	-	-	-	-	-

+ = Inhibition; 0+ = minimum inhibition; - = no inhibition (Turbidity).

Table 4. Minimum Inhibitory concentration (MIC) of *Cassia singueana* Del. ethanolic extract.

	Concentration µglml				
Test Organisms	**3x10^3**	**6x10^3**	**1x10^4**	**3x10^4**	**5x10^4**
Staphylococcus aureus	-	0+	+	+	+
Streptococcus pyogen	-	-	0+	+	+
E. coli	-	-	-	0+	+
Shigella dysentery	-	-	0+	+	+
Salmonella typhi	-	0+	+	+	+

+= inhibition; 0+ = minimum inhibition ; -= no inhibition (Turbidity).

tannins, alkaloids, glycosides and terpenes in both aqueous and ethanolic extract while flavonoids and saponnins were only detected in the ethanolic extract only. The ethanolic extract had high concentration of the secondary metabolites. This may be as a result of the solubility of extract in ethanol solvent that the aqueous solvents. The results of zone of inhibition (Tables 1 and 2) demonstrated that ethanolic extract had high inhibitory effect on all the microorganisms than water extract. The minimum inhibitory concentration (MIC) values obtained in the study from the ethanolic extract ranged from 3x10^3 to 5 x 10^4 µg/ml (Table 4).

The lager zones of inhibition exhibited by the ethanolic extract of *C. singueana* Del. may be due to presence of variety of active compounds in the plant such as tannins, alkaloid, and saponins as described by Abo et al. (2000). The minimum inhibitory concentration of 3 x 10^4 µglml was observed with crude extract of *C. singueana* Del. (Table 4) against *S. aureus.* The bacteria were most inhibited by ethanolic extract with MIC of 6x10^3 µglml against *S. aureus* and *S. typhi*, 1 x 10^4 µglml against S. *pyogen* and *S. dysentery*.

The findings were consistent with those of singh (1982), who observed that *Cassia* species contain flavonoids, alkaloid saponins and polysaccharides and showed considerable activity against Gram-positive microorganisms. They also agreed with the findings of Abo et al. (1999) that extracts from the leaves and pods of *Cassia fistula, Cassia pedocarpa* and *Cassia spectabilis* showed significant antibacterial activity. Abo et al. (2000) also found out that the methanol extracts of the leaves and pod of *Cassia alata* and *Cassia sieberiena.* Possess antimicrobial property.

Conclusion

The result of the experiment showed that the leaf of *C. singueana Del.* may have some valuable anti-microbial activities against Gram positive and Gram negative microorganisms. This property tends to support the traditional medicinal stage in the treatment of bacteria infections. The result of the study justified the use of the plant in the treatment of diseases of microbial origin in herbal medicine.

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