Chemistry Research Journal, 2021, 6(6):148-153

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Antimicrobial Activity and Brine Shrimp Lethality Test of *Cassia Sieberiana* D.C. Leaves Extracts

S. Abubakar[1], O.A. Adoum[2]

[1]Department of Pure and industrial Chemistry Umaru Musa Yar'adua University, P.M.B, 2218, Katsina, Nigeria
[2]Department of Pure and industrial Chemistry, Bayero University, P.M.B, 3011, Kano, Nigeria
abubakar.sani@umyu.edu.ng

Abstract The leaves powered of *Cassia sieberiana* D.C (Fabaceae) was extracted using ethanol and partitioned into various fractions. The crude ethanol extract and solvent fractions were screened for cytotoxicity using Brine Shrimp Test (BST).The ethyl acetate extract was found to be very active on BST at1.175µg/ml. The extracts and solvent fractions were further tested against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. The highest zone of growth inhibition (13.0mm) at 10,000µg/ml was exhibited by the ethylacetate fraction of the leaves of *C. sieberiana* on *Salmonella typhi*. The potency was comparable to the standard antibiotics (i.e ciprofloxacin) used.

Keywords *Cassia sieberiana* D.C, Cytotoxicity, Antimicrobial activity

Introduction

Cassia sieberiana D.C. (Fabaceae) commonly called ''Marga'' in Hausa, is a small tree 15m high, with a short, twisted bole, spreading crown with drooping branches. Leaves alternate, composite, Paripinnate (Sometimes with a terminal leaflets), 20-30cm long, with 5-9 pairs of opposite leaflets [1]. It is a Savanna tree, growing in almost any kind of soil [2]. *Cassia* species were known in folk medicine for their laxative and purgative uses [3]. The prevalence used of the root part of *Cassia sieberiana* by herbalist in sub-Saharan Africa, for the treatment of Oxidative stress related diseases such as diabetes might be attributed to the presence of flavonoids and stilbene [4].

The root and the leaves powder were reported to be used for treating skin diseases such as ringworm, scabies, and eczema [5]. An infusion obtained from the plant was reported to be administered for the treatment of sore throat [6]. The root infusion was used as a purgative and vermifuge [7]. It was reported that an aqueous extract of the leaves is used for the treatment of peptic ulcer [8]. The extract was also used to treat other gastro-intestinal disorders such as stomachache and diarrhea [9].

Moreover, biological activities on extracts of the leaves of *Cassia sieberiana* have been documented. Methanol extract of the leaves and pods of the plant exhibited significant antimicrobial activity against *P. aeruginosa*, *S. aureus*, *Proteus mirabilis*, *C. Albicans*, *A. niger* and *A. flavus* [10].

The ethyl acetate fraction has demonstrated potent α-glucosidase inhibition and strong free radical scavenging (DPPH and $ABTS^{+}$) [4].. It has been reported that several bioactive compounds isolated from roots or bark extracts of *Cassia sieberiana* and these include: β-Sitosterol, Calcium Oxalates, tannins, sterols, epicatecol and leucopelargonical [11]. Emodin **1** have been Isolated from the ethylacelate extract [12]. The plant also contains 1,3,8-tri-hydroxy-2-methyl anthraquinone, aloe-emodin, Sennosides and rheinemodin [7, 13-14]. Other compound

isolated from the root of *Cassia sieberiana* (Figure 1) include, piceatannol **2** as a light brown solid, islandicin**3** as reddish powder, kaempferol **4** as a yellow powder, chrysophanol **5** as yellow powder, and quercetin **6** as a yellow solid [4]. The high number of hydroxyl group coupled with the *ortho*-dihydroxyl groups in compounds **3** and **6** were reported to be responsible for their strong enzyme inhibitory activity [4].

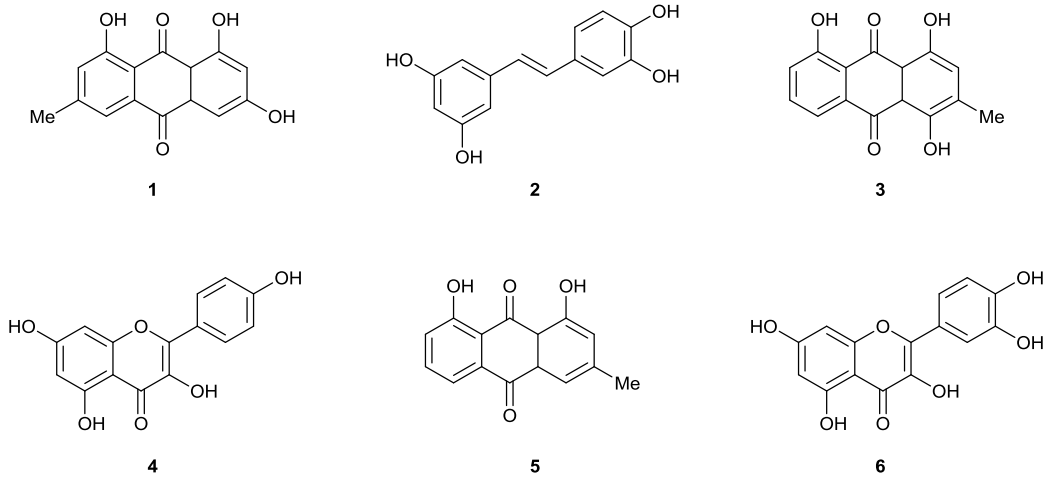

Figure 1: Phytochemical compounds isolated from root part of Cassia sieberiana

This study screened the various extracts obtained from the leaves of *Cassia sieberiana* against some selected organisms and determined their brine shrimp lethality.

Materials and Methods

Collection of Plant Materials

The plant materials used in this study were collected in June, 2007, from Yako village in Kiru Local Government Area of Kano State, Nigeria. The plants were identified by Mallam Baba Ali Garko (Staff of Bayero University, Kano), and authenticated by Mr. Mohammad Musa of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria. A voucher specimen (No1387) has been deposited at Herbarium.

Extraction of Plant Material

The air-dried and grounded plant sample (200g) were extracted percolation with absolute ethanol (700ml) at room temperature for two weeks. The percolates were evaporated to dryness *in vacuo* to afford a residue coded (F001) [15].

Fractionation of Crude Extract

The crude extract (F001) was solvent partitioned to give chloroform (F002), water (F003) soluble fractions and ethylacetate (F004). The chloroform soluble fraction (F002) was further partitioned between n-hexane and methanol to given-hexane (F005) and methanol (F006) soluble fractions (Table 1). All the fractions were concentrated *in vacuo*, weight of the fractions was recorded and stored in a freezer until tested [15-16].

Brine Shrimp Lethality Bioassay

A brine shrimp lethality (BST) bioassay is capable of detecting a broad spectrum of bioactivity present in crude extracts. The technique is easily mastered, costs little and utilizes small amount of test materials. The bioassay provides a front-line screening that can be backed up by more specific and more expensive bioassays once the activity has been detected [17].

The plant extract was screened against brine shrimp larvae of Artemia saline according to the method described [15, 18]. In this test, sea water obtained from Lagos Beach, Nigeria was used to culture the Artemia larvae. To enhance

the solubility of test, dimethylsulphoxide was added to test materials and control vials. The results obtained are depicted in Table 2.

Antimicrobial Bioassay

Pure cultures of *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* were obtained from the Microbiology Laboratory, Aminu Kano Teaching Hospital, Kano, Nigeria. The three bacterial cultures were maintained in nutrient agar slant at 4 °C before use.

Preparation of Inocula

The inoculum was prepared from the stock cultures which were maintained on nutrient agar slant at 37 °C overnight and sub-cultured in nutrient broth using a sterilized wire loop and incubated at 37 °C for 24 hours. The density of suspension to be inoculated was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (1% v/v).

Preparation of Sensitivity Disc

A paper puncher was used to prepare discs of about 6mm diameter from whatman's No 1 filter paper. Batches of 100 discs were transferred into Bijou bottles and sterilized in theoven at 110 °C for 24hours. The stock solution of 10mg/ml of the plant extract for the bioassay was prepared by dissolving 0.01g of each fraction of *C. sieberiana* in 1ml Dimethyl sulfoxide (DMSO) (i.e 10,000µg/ml). Three concentrations of 5000, 2000, and 1000µg/ml were prepared by dissolving 0.5ml. 0.2ml and 0.1ml of the stock solution into 0.5ml, 0.8ml and 0.9ml of DMSO, respectively. One milliliter (1ml) of the extract from 10,000µg/ml, 5000µg/ml, 2000µg/ml and 1000µg/ml concentrations were each transferred into separate bottles containing 100discs. Since each disc can absorb 0.01ml, the four bottles yielded discs of 100µg/disc, 50µg/disc, 20µg/disc, and 10µg/disc, respectively.

Antibacterial Susceptibility Test

Disc agar diffusion method described by (Kirby-Bauer 1966) and demonstrated by Mukhtar and Tukur [19] was employed for antibacterial assay. Four concentrations 100µg/disc, 50µg/disc, 20µg/disc, and 10µg/disc for each fraction of *C. seiberiana* extract were prepared. A sterile wire loop loaded with standard culture was used in streaking agar plates distributed evenly and aseptically in an inoculation chamber. A standard antibiotic disc Ciprofloxacin (30µg, control disc) were aseptically pressed firmly at the center using sterile forceps unto the inoculated plates. The zone diameter of inhibition was measured to the nearest whole number using a transparent meter ruler (Table 3).

Table 1: Some physical parameters of the crude and various fractions of *Cassia sieberiana* D. C. leave extracts

Fraction	Weight(g)	Texture
F001	19.90	Oily brownish substance
F002	10.20	Gummy yellowish substance
F003	2.70	Crystalline brown substance
F004	1.65	Sticky brown substance
F005	2.40	Sticky dark green substance
F006	2.10	Sticky greenish substance

Key: F001 = crude ethanol extract; F002 = Chloroform soluble fraction;F003 = water soluble fraction;F004 = ethylacetate soluble fraction; F005 = n-hexane soluble fraction; F006 = Methanol soluble fraction.

Table 2: The activity (in BST) of various fractions of *Cassia sieberiana* D. C.

Fractions	LC_{50} µg/ml	Remark
F001	73.50	Active
F002	>1000	Inactive
F003	>1000	Inactive
F004	1.175	Active
F005	15.35	Active
F006	21.40	Active

LC_{50} is determined at 95% confidence interval

Table 3: Antimicrobial activity of various fractions of *C. sieberiana* D. C. on the bacterial isolates

Fractions	Concentration (µg/ml)	Test organisms (Zone of inhibition mm)		
		S. aureus	***S.typhi***	***E. Coli***
F001	1000	0	8	8
	2000	9	10	11
	5000	12	11	13
	10000	15	16	15
	Control	16	32	35
F002	1000	8	0	0
	2000	9	0	8
	5000	11	0	10
	10000	13	8	13
	Control	14	12	28
F003	1000	0	0	0
	2000	0	0	0
	5000	0	8	8
	10000	12	11	11
	Control	30	20	18
F004	1000	7	8	0
	2000	8	9	8
	5000	9	10	9
	10000	12	13	13
	Control	15	15	27
F005	1000	0	0	8
	2000	0	8	10
	5000	9	11	12
	10000	12	13	14
	Control	32	35	25
F006	1000	8	8	7
	2000	10	10	9
	5000	13	12	11
	10000	16	14	13
	Control	40	25	27

Key: Zone of inhibition for disc = 6mm

Result and Discussions

Some physical parameters (weight, colour and texture) of the crude extract and various factions of the leaves of *C. sieberiana* D. C. were presented in (Table 1). Result of the preliminary BST screening of solvent partitioned extracts of the leaves of *C. sieberiana* D. C. showed that about 67% of the plant extracts were active in BST (Table 2). The

bioassay showed that ethylacetate soluble extract (F004) of *C. sieberiana*LC_{50}=1.175µg/ml (Table 2) exerted highest lethal activity. The cytotoxicity in BST n-hexane soluble extract (F005) and methanol soluble extract (F006) were moderate. The lowest activity was found in the ethanol extract (F001) LC_{50}=73.05µg/ml. Meanwhile, the chloroform and aqueous soluble extracts have BST at LC_{50} values greater than 1000µg/ml and are therefore not active on brine shrimp. This may partly be the reason why some shrimps prefer to associate with the plants in their sea environment [20]. These results suggest potency in the plants extracts under investigation.

The antibacterial activities were carried out on all the fractions obtained as shown in the table 3. It was reported that susceptibility of bacterial culture to extract was determined by measurement in the following ranges; 0-7 mm indicates inactive; 8-7 mm indicates weak activity while 12 mm and above indicates strong activity [21].

However, results from the antimicrobial screening test are shown in Table3.The zone of growth inhibition (13.0) at 10,000µg/ml was exhibited by the ethylacetate fraction of the leaves of *Cassia sieberiana* on *S. typhi*. The potency of the ethylacetate fraction was comparable to the standard antibiotics (i.e ciprofloxin) used. The ciprofloxin have a universal activity against the three test organisms *S. aureus, S. typhi* and *E. coli* with zone of inhibition ranging from 12.0 to 40.0mm respectively. Ethanol and Chloroform fractions of *Cassia sieberiana* exhibited zone of growth inhibition 15mm and 13mm against *S.aureus*, at 10,000µg/ml.

Conclusion and Recommendation

The ethylacetate fraction of the leaves of *C. sieberiana* provided a scientific basis for the ethnomedicinal uses of plant in the Northern region of Nigeria to cure typhoid fever. The zones of inhibition exhibited by the ethanol and chloroform fractions of *C. sieberiana* on *staphylococcus aureus* justified their uses by traditional medicinal practitioners in the treatment of sores, bores, and open wounds.

The cytotoxic activity observed on the ethylacetate, n-hexane, methanol and ethanol extracts of *C.sieberiana* may lead to the discovery of new cytotoxic compounds. The extracts should also be evaluated for the pesticide activity. Further research to detect and characterize bioactive compounds of the leaves of *Cassia sieberiana* needs to be carried out.

Acknowledgement

Our gratitude to Prof. M. D. Mukhtar of Biological Science Department, Bayero University Kano.

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