The Pharmaceutical and Chemical Journal, 2021, 8(6):18-24

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Bioassay Guided Isolation of Antimicrobial and Cytotoxic Components from Ethyl Acetate Extracts of *Cassia sieberiana* D.*C.* (Fabaceae)

S. Abubakar

Department of Pure and industrial Chemistry Umaru Musa Yar'adua University, P.M.B, 2218, Katsina, Nigeria
abubakar.Sani@umyu.edu.ng

Abstract The leaves extracts of *Cassia sieberiana* D.C. were screened for cytotoxicity using Brine Shrimp Test (BST) and antimicrobial bioassay against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* using crude ethanol extract, Chloroform soluble fraction, aqueous soluble fraction, ethyl acetate soluble fraction, methanol soluble fraction and n-hexane soluble fraction. The Ethyl acetate fraction obtained proved to be most active in inducing complete lethality at minimum doses in BST and also active on *Salmonella typhi*. The Bioactivity result was used to guide the column chromatography, which led to the isolation of pure compound CSB-8, which was found active in the BST with LC_{50} value of 34(722-182) µg/ml and showed remarkable activity on *Salmonella typhi* (zone of inhibition 25mm) at 10,000 µg/ml. The ^{1}H-NMR, ^{13}C NMR, FTIR and GC-MS spectra of the compound suggested the proposed structure to be 2-pentadecanone.

Keywords Brine shrimp, *Cassia sieberiana* D.C., Antimicrobial bioassay

Introduction

Plant materials have been an essential part of human society since the civilization started. Medicinal plants are boon of nature to cure a number of ailments of human beings [1]. The medicinal uses of plants in the Savanna belt of Nigeria have been reported, but investigations providing scientific support for their medicinal uses are grossly inadequate [2]. However, pharmaceutical company's utilized plant extracts to produce relatively crude therapeutic formulations, but with advancement of antibiotics in the mid-twentieth century, drug formulations of fairly-purified compounds have become more typical [3]. Natural products have been the major sources of chemical diversity as starting materials for driving pharmaceutical discovery over the past century [1]. *Cassia* species were known in folk medicine for their laxative and purgative uses [4].

Cassia sieberiana D.C. (Fabaceae) grow as a small trees in Savanna region in West Africa, 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 [5], It has been reported that *C. sieberiana* grown in almost any kind of soil [6]. 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 [7].

The root and the leaves powder were reported to be used for treating skin diseases such as ringworm, scabies, and eczema [8]. An infusion obtained from the plant was reported to be administered for the treatment of sore throat [9]. The root infusion was used as a purgative and vermifuge [10]. It was reported that an aqueous extract of the leaves is

used for the treatment of peptic ulcer [11]. The extract was also used to treat other gastro-intestinal disorders such as stomachache and diarrhea [12].

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* [4]

The ethyl acetate fraction has demonstrated potent α-glucosidase inhibition and strong free radical scavenging (DPPH and $ABTS^{+}$) [7]. 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 [13]. Emodin have been Isolated from the ethylacelate extract [14]. The high number of hydroxyl group coupled with the *ortho*-dihydroxyl groups in compounds and were reported to be responsible for their strong enzyme inhibitory activity [7]. Other compound isolated from the root of *Cassia sieberiana* include, piceatannol as a light brown solid, islandicin as reddish powder, kaempferol as a yellow powder, chrysophanol as yellow powder, and quercetin as a yellow solid [7]. The plant also contains 1,3,8-tri-hydroxy-2-methyl anthraquinone, aloe-emodin, Sennosides and rheinemodin [10, 15-16]. 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 and Identification of Plant Materials

The fresh leaves of *Cassia sieberiana* used in this study were collected from Yako village in Kiru Local Government Area of Kano State, Nigeria on June, 2007. The plants was 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 (No. 1387) 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) [17].

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 [2, 17].

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 [18].

The plant extract was screened against brine shrimp larvae of Artemia saline according to the method described [17, 19]. 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 1.

Antimicrobial Bioassay

Sources of Microorganisms

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 100 discs. 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 [20] and demonstrated by Mukhtar and Tukur [21] 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 2).

Column Chromatography

Silica gel (50-200 mesh) about 250g was mixed with about 500mL of petroleum ether (60-80) to form the slurry. The column outlet was blocked using a clean cotton wool. The slurry was poured into the column (86cm length, 2.0 cm diameter) through a funnel from the top, with addition of some petroleum ether till all the silica gel was poured into the column. A beaker was placed at the bottom of the column for collecting the drained solvent. When the silica gel settled, the column was washed with 500 mL of Chloroform and fresh petroleum ether each. The extract (13.1g) was mixed properly with 20g silica gel until homogeneous mixture was formed; this was then carefully loaded into the column through a funnel. An additional portion of sand was packed to form a protective layer on top of the adsorbent.

The column was run by eluting solvents in order of increasing polarity as followed; Petroleum ether (60-80) 1000mL, a mixture of petroleum ether-Chloroform in a ratio (1:1 1000mL), (1.3, 1000mL) each, chloroform 1000mL, mixture of chloroform -ethyl acetate in a ratio (4:1, 1000mL), (3:2, 1000mL), (1:1, 1000mL), (2:3, 1000mL), (1:4, 1000mL) each, ethyl acetate (1000mL), mixture of ethyl acetate-methanol in a ratio (4:1, 1000mL), (3:2, 1000mL), (1:1, 1000mL), and each methanol (1000mL). The eluents collected in fractions of 100mL receiving bottles were evaporated to dryness and analyzed on TLC plates (Table 6) to determine purity and identical ones with equal R_f values were pooled together [22].

The pooled fraction which afforded pure compound CSB-8 was tested in BST assay (table 1) and against *S.aureus*, *S. typhi* and *E. coli* using agar disk diffusion bioassay (table 2) and finally subjected to spectroscopic analysis.

Table 1: Column chromatography of ethyl acetate fraction *C. sieberiana*

Column Eluents	**Pooled Fractions**	**Pool No**	**Weight (g)**	**LC_{50} Value (µg/ml)**
Petroleum –ether 100%	1,2,3 & 4	CSB-1	0.4391	NT
"	5,6 & 7	CSB-2	0.2390	NT
"	8 & 9	CSB -3	0.1078	NT
"	10,11,12,13 14,15 & 16	CSB-4	0.0683	NT
Petroleum -ether/ chloroform (9:1)	17,18,19 & 20	CSB-5	0.1041	NT
"	21	CSB-6	0.0659	NT
"	22,23,24 25,26,27,& 28	CSB-7	0.0880	-
Petroleum -ether/ chloroform (8:2)	29 & 30	CSB-8	1.01288	34 (722-182)
"	31 & 32	CSB-9	0.1982	NT
"	33 & 34	CSB-10	0.2260	NT
"	35 & 36	CSB-11	0.3543	NT
"	37 & 38	CSB-12	0.9842	NT
"	39,40,41,42,43,44,45&46	CSB-13	0.4863	NT
Petroleum -ether/ chloroform (6:4)	47,48,49,50, 51,52,53&54	CSB-14	0.8068	NT
"	55,	CSB-15	0.1062	NT
"	56,57,58,59,60,61,62,&63	CSB-16	0.6167	NT
"	64,65,66 &67	CSB-17	0.7079	>1000
"	68,69,70,71,72,73,74, 75 &76	CSB-18	0.9419	NT
Petroleum -ether/ Chloroform (5:5)	77,78,79,80,81,82, 83 & 84	CSB-19	2.1782	>1000
"	85,86,87 &88	CSB-20	0.2591	NT
Chloroform 100%	89	CSB-21	0.1656	NT
"	90	CSB-22	0.5367	NT
"	91,92, & 93	CSB-23	1.2619	NT
"	94	CSB-24	0.6221	NT
"	95 & 96	CSB-25	0.9294	NT
"	97 & 98	CSB-26	0.8214	NT
"	99,100,101 & 102	CSB-27	0.2105	NT
Ethyl acetate 100%	103,104 &105	CSB-28	1.4249	NT
Ethyl acetate/ methanol (50:50)	106,107,108,109,110, 111,112,113,114,115, 116,117,118 &119	CSB-29	0.4147	NT
"	120	CSB-30	0.1048	NT
"	121,122,123,124 % 125	CSB-31	1.8602	NT
Methanol (100%)	126	CSB-32	0.0770	NT
"	127,128,129,130,131, 132,133,134,135 & 136	CSB-33	0.3480	NT

Key: LC_{50} is determined at 95% confidence inter, NT = not tested

Table 2: Antimicrobial bioassay of some fractions obtained from column chromatography of ethyl acetate extract of *C. sieberiana*

Fraction	Concentration (µg/ml)	Zone of inhibition (mm)		
		S. aureus	***S. typhi***	***E. coli***
CSB-1	1000	12	15	0
	2000	16	18	0
	5000	19	19	0
	10,000	24	25	0
CSB-3	1000	0	0	0
	2000	0	0	0
	5000	0	0	0
	10,000	0	0	16
CSB-8	1000	0	16	0
	2000	0	18	0
	5000	0	20	0
	10,000	0	26	0

Result and Discussions

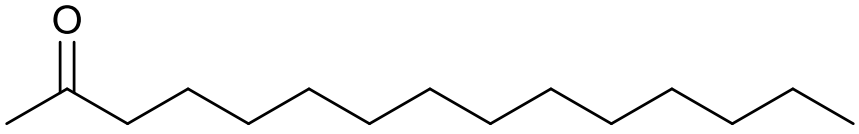
The Brine shrimp test, physical properties and antibacterial activity results of CSB-8 were presented in tables 1 and 2. The ^{1}H-NMR,^{13}C-NMR, FTIR and GCMS spectral data were combine in proposing the structure of the compound. Finally, the structure of compound CBS-8 waspropose to be 2-pentadecanone ($C_{15}H_{30}O$).

Fraction CSB-8 was tested against *S. aureus, S. typhi* and *E. coli* using agar disk diffusion bioassay. Fraction CSB-8 was found to be very active against *Salmonella typhi* (25mm) at 10,000µg/ml while it was inactive against *E. coli* and *Staphylococcus aureus* at all the concentrations tested (Table 2).

The ^{1}H-NMR spectrum of the compound CSB-8 indicated the presence of the following peaks: The triplet observed at δ0.9 ppm indicates the presence of methyl groups at C-15. In addition, the sharp singlet resonance at δ1.3 ppm was assigned tomethylene groups C5-C-14. The presence of signals at δ 1.6 was assigned to secondary alkane proton and 2.2 ppm attributed to methyl group at C-1. However, the ^{13}C NMR spectrum of the compound CSB-8 showed 15 signals or peaks. At δ14.3 [one of (CH_3) at C-15], δ22.9(CH_2), δ25.2(CH_2) δ26.1(CH_2) δ28.8(CH_2) δ29.4(CH_2), δ29.5(CH_2), δ29.6(CH_2), δ29.9(CH_2), δ32.1[(CH_2) at C-14], δ34.6[(CH_3) at C-1], δ64 [one of the (CH_3) at C-3], δ76.6, δ77.2, δ77.8 are assigned to $CDCl_3$.

Analysis of the FTIR spectrum of Compound CSB-8 displayed strong absorption band centred around 2920cm^{-1} represented C-H stretching of methyl and methylene group. The band at 1466cm^{-1}, 1366cm^{-1} are due to C-H bending motion. The band at 724cm^{-1}, (CH_2 rocking mode) indicates (CH_2) n>4. The band at 1177cm^{-1} showed stretching [$CH(CH_3)_2$]. The band at 1734 cm^{-1} indicates the presence of carbonyl (C=O) stretching frequency of a saturated aliphatic ketone.

GC-MS spectrum of Compound CSB-8 showed a characteristic fragmentation pattern of saturated aliphatic ketone. The summary of the ^{1}H-NMR, ^{13}C-NMR, FTIR and GC-MS spectral data guides in proposing the structure of the isolated pure compound CSB-8) as 2-pentadecanone [$C_{15}H_{30}O$].



2-pentadecanone

Conclusion

Based on the research carried out, it clearly shows that *Cassia 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 extracts of *C. sieberiana* may lead to the discovery of new cytotoxic compounds. The bioactivity of compound CSB-8 can ascertain the traditional medicinal claims to the *C. sieberiana* for treating typhoid fever in Hausa land. On the other hand, the bioactivity recorded on the column fractions, CSB-1 and CSB-8 may qualify *C. sieberiana* for further research work.

Recommendation

It is recommended that further work should also be carried out on all the soluble fractions of the plant in order to isolate and characterized more active secondary metabolites and evaluated for the pesticide activity.

Acknowledgement

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

Reference

[1]. Dwight D. B., Min Chu, Uma O., and Vineet R., (2007). The value of natural products to future pharmaceutical discovery. *Journal of the Royal Society of Chemistry* (2007). *Nat Prod. Rep*., 24; 1225-1244.

[2]. Adoum, O.A., Dabo N.T. and Fatope M.O. (1997). Bioactivities of some Savanna Plants in the Brine Shrimp Lethality Test and *In-vitro* Anti-microbial assay. *Inter. J. of Pharmacognosy*, 35 (5): 334 –337.

[3]. Davies J. (1999). ASM News, 65, 304.

[4]. Abo, K.A., Adeyemi A.A. and Jegede I.A. (2000). Spectrophotometric estimation of anthraquinone content and antimicrobial potential of extracts of some *Cassia species* growing in Ibadan, *Nigeria. J. Nat. Prod. Med.* 3:47-50.

[5]. Arbonnier, M. (2004). Trees, shrubs, and Linas of West Africa dry zones. Pp214-463.

[6]. Keay, R.W. (1964). Nigerian Trees. Vol II, Oxford; Clarendon Press pp 69.

[7]. Jibril, S., Hasnah, M. S. and Norazah, B. (2017). Bioassay-Guided Isolation of Antioxidants and α-Glucosidase inhibitors from the Root of *Cassia sieberiana* D.C. (Fabaceae), Rec. Nat. Prod., 11(4): 406-410.

[8]. Benjamin, T.V. (1980). Investigation of *Cassia alata*, a plant used in Nigeria in the treatment of skin diseases. *J. Afri. Med. plants* 3:135-136.

[9]. Dalziel, J.M. and Keay R.W.J. (1954). Flora of west tropical Africa Vol. 1 part 1 pp 724.

[10]. Abegas, B.M., Alemayehu G., Kebede T., Mahajan D. and Nindi M. M. (1996). Quinones and other Phenolic Compound from Marketed African plants. *Chemistry, Biological and pharmacological Properties of African Medicinal Plants* (eds. Hostettman, K.) University of Zimbabwe Publications. pp.63-169).

[11]. Akah, P.A., Orisakwe O.E., Gamaniel K.S. and Shittu A. (1998). Evaluation of Nigerian traditional medicines: Effects of some Nigerian folk remedies on peptic ulcer. *J. Ethnopharmacol*, 62(2):123-127.

[12]. Nwafor, P.A., Okwuasaba F.K. (2001): Effect of Mathanolic extract of *Cassia nigricans* leaves on rat gastrointestinal tract. *Fitoterapia* 72: 206 –214.

[13]. Duquenios, P. and Auton, R. (1968). Contribution to the Chemical study of the leaves of *Cassia Sieberiana* D.C. *Planta Med*. 16(2): 184-190.

[14]. Demirezer, L.O., Kuruuzum-uz A., Bergere I., Schiewe H.J., Zeeck A. (2001). The structures of antioxidant and cytotoxic agents from natural source: anthraquinones and tannis from roots of Rumex patientia, *J. Phytochemistry*, 50; 1213-1217.

[15]. Kinjo, J., Ikeda, T. Watanabe, K. and Nohara, T. (1994): An anthraquinone glycoside from *Cassia angustifolia* leaves. *Phytochem.* 37, 1685-1687.

[16]. Yang, Y. Lim, M. and Lee, H. Emodin isolated from *Cassia obstusifolia* (Leguminosae) seed shows larvacidal activity against mosquito species. *J. Agric, Food. Chem.*, 51: 7629-7631.
[17]. Fatope, M.O., Ibrahim H. and Takeda Y. (1993): Screening of higher plants reputed as pesticides using the brineshrimp lethality assay: *Inter*. *J of Pharmacognosy*, 31 (4): 250-256.
[18]. Pisutthanan, S., Plianbangchang, P., Pisutthanna, N., Ruanruay, S. and Muanrit, O. (2004): Brine Shrimp lethality activity of Thai Medicinal plant in the family Meliaceae. *Naresuam Univ. J*. 12 (2): 13-18.
[19]. Meyer B.N., Ferrigni N.R., Putnam J.E., Jacobson L.B., Nichols, D.E. and McLaughlin, J.L. (1982): Brine Shrimp; a convenient general bioassay for active plant constituents. *Planta Me*. 45:31-34.
[20]. Kirby-Bauer (1966): Antimicrobial sensitivity testing by agar diffusion method African Journal of clinical pathology, 44:493.
[21]. Mukhtar, M. D. and Tukur, A. (2000): Antibacterial activity of *Pistia stratiotes L*. Nigerian *J. of Exp. Bio.* 1(1):51-60.
[22]. Sharma Y. R. and Achaya R. C. (1988): Analytical Methods in Chemistry. *Kalyani Publisher New Delhi*, Ludhiana India. Pp 18-25.