



Phytochemical Characterisation, Compound Isolation, and Anticonvulsant Evaluation of Methanol Stem Bark Extract of *Bombax Costatum* (Bombacaceae)

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Abstract

Bombax Costatum is a deciduous plant widely distributed in West Africa and the Indian subcontinent. It is used in ethnomedicine for the treatment of oedema, snake bites, malaria, epilepsy, amoebiasis, and birth-related complications. Despite its extensive ethnomedicinal applications, its neuropharmacological properties remain underexplored. This work is aimed at the isolation of compounds and anticonvulsant studies on the methanol stem bark extract of *B. costatum* evaluate its anticonvulsant potential using established animal models of seizure. The powdered stem bark of *B. costatum* was extracted by cold maceration in methanol. The resulting crude methanol extract (CME) was suspended in water and fractionated into n-hexane (HF), chloroform (CF), ethyl acetate (EF), and n-butanol (BF) portions. Phytochemical screening and median lethal dose (LD₅₀) were carried out using standard methods. Silica gel Column chromatography was carried out on the chloroform fraction (CF), which yielded 60 sub-fractions, pooled together to give ten main fractions coded as B1 – B10 based on their TLC profile. Fraction B003 and B005 were further purified on an open capillary column in an isocratic manner, leading to the isolation of stigmasterol (compound B5), whose structure was elucidated using IR, 1D, and 2D NMR spectroscopy, respectively. Anticonvulsant activity of the CME was assessed using the maximal electroshock test (MEST) and the pentylenetetrazole test (PTZ) models. Phytochemical screening of the CME revealed the presence of steroids, flavonoids, anthraquinones, terpenoids, glycosides, tannins and carbohydrates, while the chloroform fraction revealed the presence of steroids and triterpenes predominantly. The extract showed no signs of acute toxicity up to 5000 mg/kg. In both MEST and PTZ models, the CME (300 and 600 mg/kg) significantly delayed seizure onset and reduced seizure duration compared to the control group ($p < 0.05$). Although full protection was not observed, the extract demonstrated dose-dependent anticonvulsant effects. These findings suggest that *B. costatum* possesses bioactive phytochemicals with significant anticonvulsant activity, supporting its traditional use in the treatment of epilepsy. The isolation of stigmasterol further highlights the plant's therapeutic potential. Further studies are warranted to elucidate the mechanisms of action and explore its clinical applicability.

Keywords: *Bombax Costatum*, Anticonvulsant Activity, Stigmasterol, Phytochemicals, MES, PTZ, Traditional Medicine

Introduction

A number of African medicinal plants have been reported to possess bioactive constituents capable of exerting anticonvulsant action, hence making them relevant in the management of seizure disorders. These plants include: *Glycyrrhiza glabra* (Ahmed et al., 2019). · *Dalbergia saxatilis* (Ambawade et al., 2002) *Cassia occidentalis*, *Heliotropium indicum*, *Xylopi aethiopica* (Apinega et al., 2018). The World Health Organisation estimates that the proportion of general population with active epilepsy (i.e. continuing seizures or the need for treatment) at a given time is 4 to 10 per 1000 people, However, some studies in developing countries suggest that the proportion is 6 to 10 per 1000 (Assogba et al., 2017). In developed countries, annual new cases are 40 to 70 per 100,000

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people in the general population. In developing countries, this figure is often close to twice as high due to higher risks of conditions that can lead to permanent brain damage (Barau et al., 2016; Nazifi et al., 2017). It is estimated that approximately 20 to 30% of patients are refractory to therapies using the currently available AEDs (Bello et al., 2022). And 88% of such patients suffer severe side effects like renal failure, adverse effect on cognition, and some of the available anti-epileptic drugs are even associated with teratogenicity on long term management of the condition with such drugs (Bello et al., 2022; Stasevich et al., 2020).

Hence the call for development and search for new AEDs especially from medicinal plant sources, which may have fewer side effects and greater efficacy, it is against this backdrop that the need to evaluate scientifically the anti-convulsant profile of *B. costatum* to validate its use for treatment of convulsions by traditional medical practitioners around Abeda Shitire in Benue state. This study, therefore, aimed to isolate and characterise some of the bioactive compounds (s) present in the stem bark of *B. costatum* and to validate scientifically the ethnomedicinal claim for the use of the plant *B. costatum* in the treatment of epilepsy.

B. costatum is a common tree which is also called red-flowered silk cotton tree in English and kapokier in French (Sayyah et al., 2004). It is known locally as Kuryaa or Gurjiyaa in the Hausa language. The plant is of the family Bombacaceae, found within the Savanna zone of West and Central Africa, including Nigeria and Chad (Raza et al., 2001). The *Bombax* genus is distributed around the tropical region and comprises about eight (8) different species. Two of the species are found in Africa, while the other six species are found in Asia. In Nigeria, *B. costatum* is known by other vernacular names such as “*Gurjiya*” in Hausa, “*Joohi*” in Fulfulde (Nieoczym et al., 2019) and “*Danaa*” in Babur. Further, in Nigeria and other African countries, different parts of the plant are used in traditional medicine for the management and treatment of different ailments, to relieve fever, promote lactation, and as a stimulant against weakness (Tripathi, 2008). The root powder is eaten or applied to the whole body against epileptic seizures (Yaro et al., 2019). In some countries, the roots and stem bark are used for the treatment of fever, dysentery, and dizziness. The bark is used in the management of insanity, treatment of skin diseases, headache, yellow fever and wound healing. The stem bark is used against pain, oedema and hernia in Benin, while the leaves are taken for the treatment of convulsion (Yemitan et al., 2006).

Materials and Methods

Solvents/Reagents and Chromatographic Materials: The solvents used include methanol, n- butanol, ethyl acetate, chloroform, and n-hexane. Reagents used were freshly prepared, including those for phytochemical screening such as Molisch’s reagent, Salkowski’s reagent, Shinoda’s reagent, Dragendorff’s reagent, Mayer’s reagent, Wagner’s reagent and Borntrager’s reagent. Pentylene tetrazole (Sigma Chemical Co., St. Louis, USA), Chromatographic materials included TLC plates (aluminium), Silica gel 60- 120 mesh (Merck KGaA, Darmstadt, Germany), Glass Plates, Chromatographic tanks, and an open column (75cm by 3.5cm). Standard drugs used include Phenytoin sodium (Parker-Davis and Co Ltd., Detroit), Diazepam (Sanofi-aventis UK).

Equipment

NMR spectra were recorded on a Bruker DRX500 spectrophotometer (Bruker BioSpin, Rheinstetten, Germany) at 400 MHz (¹H) and 100 MHz (¹³C). Samples were prepared in CDCl₃ with tetramethylsilane (TMS) as an internal standard. The chemical shift (δ) values were measured relative to the internal standard in ppm. The IR spectrum was recorded on a KBr disc on a Happ-Genzel 4000-650 spectrophotometer. The UV spectrum was recorded on a THERMO ELECTRON-VISIONpro V3.00 spectrophotometer. TLCs were performed on pre-coated TLC plates Si 60 F254 (Sigma-Aldrich). The developed plates were visualised under UV light (254/366 nm). Column chromatography (CC) was performed by gravity using glass columns of appropriate sizes with Silica gel 60 Å, 230-400 mesh (Sigma-Aldrich Co., St. Louis, MO, USA).

Plant collection and identification

The plant material was collected from Federal Housing Estate, North Bank Makurdi, Benue State, Nigeria, on the 7th December 2016. It was authenticated by Mallam Namadi Sunusi of the Herbarium unit, Department of Botany, Ahmadu Bello University, Zaria. A voucher number (No:1211) was deposited at the herbarium of the University.

Phytochemical screening

The presence of saponins, phytosterols, tannins, alkaloids, terpenoids, flavonoids, and glycosides in the crude methanol stem bark extract was tested using simple qualitative methods as previously reported by Trease and Evans.

Extraction and isolation

The stem bark of the plant was cleaned and air dried under shade, and the size was reduced to semi-powdered material using a wooden mortar and pestle. The semi-powdered material (2.5 kg) was extracted with about 12 L of methanol by cold maceration to obtain the crude methanol extract. The crude methanol extract (170 g) was subjected to a liquid–liquid fractionation using various organic solvents in order of increasing polarity, beginning with hexane, then chloroform, ethyl acetate and butanol. The chloroform fraction was subjected to column chromatography using silica gel and eluted with 100% chloroform, then a step-wise gradient of increasing polarity with ethyl acetate up to chloroform: ethyl acetate (95:5). A total of 32 column fractions were collected. Fraction coded B3 was re-chromatographed on a silica gel column, eluted with a solvent mixture of chloroform: ethyl acetate (90:10). The eluate on drying gave a white amorphous solid (compound B5, 25 mg).

Results

Table 1: Determination of the median oral lethal dose (LD50) of the crude methanol stem bark extract of *B. Costatum*

First phase

Doses (mg/kg)	Number of mice	Mortality
10	3	0/3
100	3	0/3
1000	3	0/3

Second phase

Doses (mg/kg)	Number of mice	Mortality
1600	1	0/1
2900	1	0/1
5000	1	0/1

Table 2: Effect of Methanol Stem Bark Extract of *B. costatum* on Maximal Electroshock-Induced Convulsion in Chicks

Treatment (mg/kg)	Mean Recovery Period (Min.)	Quantal Protection	% Protection against seizure
NS 10 ml/kg	17.2 ± 1.68	0/10	0.00
MSE 150	9.60 ± 1.27	0/10	0.00
MSE 300	8.90 ± 1.04*	0/10	0.00
MSE 600	6.90 ± 0.38*	0/10	0.00
PH 20	-----	10/10	100

Values are presented as Mean ± SEM, no significant difference compared to normal saline control group - One way ANOVA followed by Dunnett's post hoc test, n=10, NS = Normal Saline, MSE = Methanol stem bark extract, PH = Phenytoin.

Table 3: Effect of Methanol Stem Bark Extract of *B. costatum* on PTZ-Induced Convulsion in Mice

Treatment (mg/kg)	Mean Onset of Seizures (min.)	Quantal protection	%Protection against seizure	% Mortality
NS 10 ml/kg	0.67 ± 0.14	0/6	0.00	100.00
MSE 150	1.67 ± 0.14*	0/6	0.00	100.00
MSE 300	1.00 ± 0.18	0/6	0.00	100.00
MSE 600	1.00 ± 0.18	0/6	0.00	100.00
DZ 20	-----	6/6	100.00	0.00

Protection against seizure and mortality expressed as percentages; Mean onset of seizures presented as Mean ± SEM, * = $p < 0.05$ compared to normal saline group - One way ANOVA followed by Dunnett's post hoc test of multiple comparison, n=6, NS=Normal Saline, MSE = Methanol Stem Bark extract, DZ = Diazepam

Table 4: spectra data for compound B5 (CDCl₃, 400MHz)

Position	¹³ C NMR Pierre andMoses, 2015 Experimental	¹³ C NMR	¹ H NMR	DEPT
1	37.15	36.67	1.08	CH2
2	31.56	31.41	1.45,1.83	CH2
3	71.71	71.97	3.52	CH
4	42.19	42.47	2.26,2.31	CH2
5	140.81	140.86	--	C=C
6	121.62	121.87	5.36	C=CH
7	31.56	31.41	1.45,1.99	CH2
8	31.79	34.16	1.38	CH
9	50.02	50.30	1.27	CH
10	36.16	36.30	--	C
11	21.12	21.24	1.45	CH2
12	39.57	37.33	1.19,2.03	CH2
13	42.10	46.00	--	C
14	56.76	56.93	1.03	CH
15	24.27	23.84	1.56	CH2
16	28.83	29.19	1.79	CH2
17	55.84	56.22	1.16	CH
18	12.15	12.02	0.72	CH3
19	19.88	19.55	0.93	CH3
20	40.40	39.94	2.09	CH
21	20.99	21.24	1.03	CH3
22	138.23	138.46	5.16	C=C
23	129.60	129.43	5.02	C=C
24	51.30	51.31	1.50	CH
25	31.94	34.16	1.52	CH
26	21.23	21.24	0.91	CH3
27	19.01	19.20	0.82	CH3
28	25.40	26.24	1.56	CH2

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29	12.25	12.12	0.85	CH3
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Discussion

Phytochemical screening carried out on the crude methanol extract of *B. costatum* revealed the presence of saponins, tannins, flavonoids, carbohydrates, anthraquinones, and steroids. Steroids and triterpenes, among other phytochemicals, have been reported to possess anticonvulsant activities (Chiroma et al., 2022 and Nieoczym et al., 2019). The median lethal dose (LD₅₀) value for the methanol stem bark extract of *B. costatum* was found to be greater than 5000 mg/kg body weight in Swiss albino mice, indicating the relative safety of the plant (DeLorenzo et al., 2019 and Sasa, 2006).

Standard anti-epileptic test, such as MEST, evaluates the testing material's ability to protect against hind limb tonic extension (Jain and Bari, 2010; Wanbara et al., 2021). It predicts drug activity against generalised seizures of the tonic clonic (grandmal) type and other forms of epilepsy. Thus, protection projects the anticonvulsant activity of AEDs that prevent the spread of the epileptic seizure discharge from an epileptic focus during seizure activity (Kalmaluddeen & Abdullahi, 2015), Pierre and Moses, 2015). Drugs such as phenytoin, lamotrigine and carbamazepine have been shown to abolish tonic hind limb extension in the MEST test. Primarily, they act by prolonging the inactive state of Na⁺, consequently, preventing the repetitive firing of the neurons (Wanbara et al., 2021). Anti-seizure drugs that abolish or depress MEST seizure act by preventing the spread of seizures in the brain and the spinal cord by prolonging the inactive state of Na⁺, consequently preventing the repetitive firing of the neurons (Kamboj & Saluja, 2011; Wajuba et al., 2022). Consequently, the ability of the crude methanol extract from *B. costatum* to reduce the recovery time may suggest an interaction with sodium-gated channels or glutamatergic neurotransmission mediated by N-methyl-d-aspartate receptors (Lorke, 1983; Pierre & Moses, 2015).

On the other hand, the PTZ seizure model screens agents with activity against non-generalised seizures such as petit mal epilepsy. Antiseizure drugs such as phenobarbitone, benzodiazepines, ethosuximide, and sodium valproate are active against seizures induced by PTZ. Drugs that abolish petit mal epilepsy act by enhancing GABA_A inhibitory action and block T-type Ca²⁺ current (Luhata & Munkombwe 2015; Tripathi, 2008). The methanol stem bark extract of *B. costatum* showed prolongation in the mean onset of seizures in the PTZ model, which was considered to be statistically significant and therefore suggested that the extract might be effective in absence seizures (Mann et al., 2003; Sayyah, 2004). The anticonvulsant activity observed in this study could be a result of the phytoconstituents present.

Compound B5 is a white crystalline solid, which tested positive to both Lieberman-Burchard and Salwoski and was observed to have a melting point of 136-138°C indicating the compound to be pure (Mohammed et al., 2019; World Health Organization, 2012).

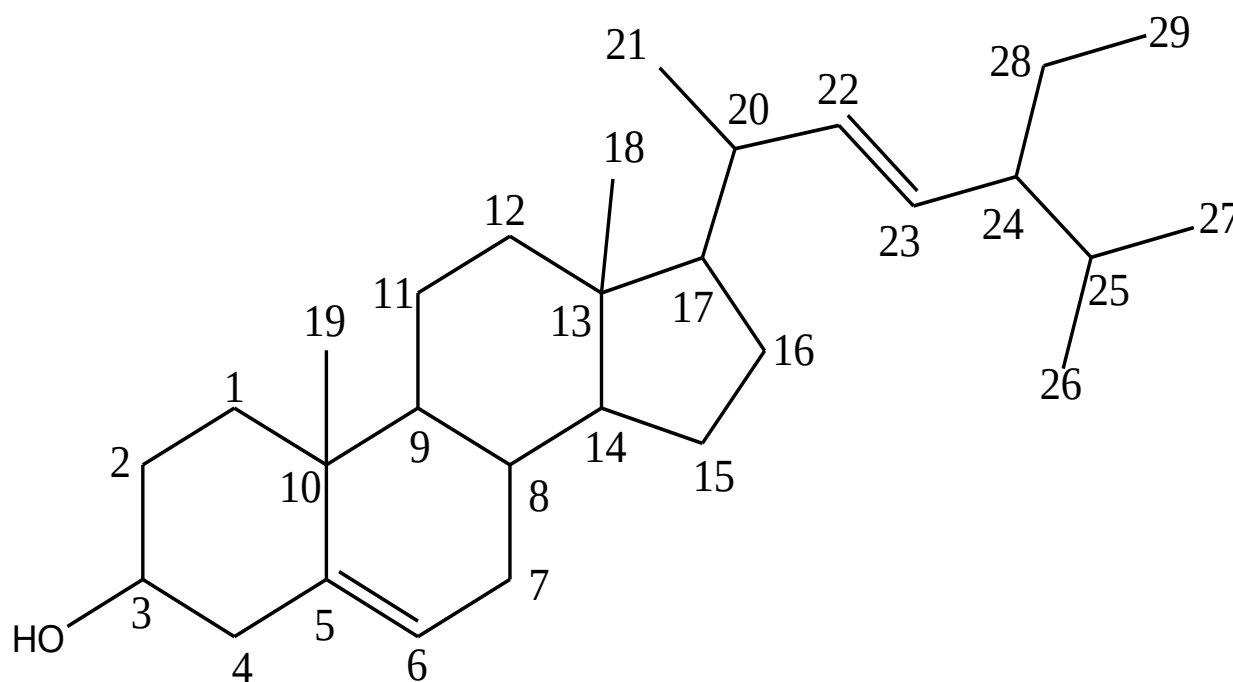
The IR spectrum of B5 showed characteristic absorption frequencies at 3354.6 and 1028 cm⁻¹, typical of OH and C-O bond vibrations, respectively; the absorption observed at 3071 is due to =CH, whereas vibrations at 2922.2 and 2855.1 were ascribed to CH str resulting from asymmetric and symmetric vibrations, respectively. The absorption observed at 1684.8 was due to the C=C bond vibration. Absorptions at 1449.9 were for CH bending due to CH₂ vibrations (Mann et al., 2003; Nieoczym et al., 2019).

The ¹H NMR data of B5 showed three olefinic protons at δ 5.16, 5.02, and 5.36 (H-22, 23, and 6); a multiplet was observed at δ 3.52 (H-3). The presence of six methyl protons with their signals at δ 0.72, 1.01, 1.03, 0.91, 0.82, and 0.85 corresponds with reported literature values (Nazifi et al., 2020). The ¹³C NMR showed three quaternary carbons at C-5, C-10, C-13, eleven methine carbons at C-3, C-6, C-8, C-9, C-14, C-17, C-20, C-22, C-23, C-24, C-25, nine methylene carbons at C-1, C-2, C-4, C-7, C-12, C-15, C-16, C-21, C-28, and six methyl carbon at C-18, C-19, C-26, C-27, C-29, distinct signals were recorded at 140.81 and 121.62 (C5, C6) Angular carbon atom signals (C-19, C-18) were also recognized at 19.41 and 12.06 respectively, A signal at 71.71 (C-3) was indicative of a hydroxyl group (Nazifi et al., 2017).

The ¹H-¹H COSY correlations were observed between H-6 (5.36) # H-7 (1.99); H-22 (5.16) # H-20 (2.09); H-20 (2.09) # H-17 (1.16); H-23 (5.02) # H-2a (1.54); H-3 (3.52) # H-4 (2.26); H-3 (3.52) # H-2 (1.84); H-7 (1.97) #

H-15 (1.56); H-24 (1.50) # H-29 (0.85); H-2b (1.84) # H-29 (0.85); H-12 (2.03) # H-14 (1.03); H-2b (1.84) # H-1 (1.08); and H-28 (1.25) # H-29 (0.85).

Other correlations between the various protons and their respective carbons were established using the 2D NMR of HSQC and HMBC. The methylene proton signal at δ 2.26 (H-4) showed cross peaks with a quaternary carbon signal at (δ c 140.86, C-5) by J2 correlation, a methine carbon signal at (δ c 71.79, C-3) and a methine carbon signal at (δ 121.87, C-6) by a J3 correlation. The methylene proton signal δ 1.08 (H-1) showed cross peaks with quaternary carbon signal at (δ c 36.67, C-1) and a methine carbon signal at (δ c 50.30, C-) by J3 correlation. The methyl proton at δ 0.82 (H-27) showed cross peaks with a methyl carbon signal at (δ c 21.24, C-26) by J3 correlation and a methyl carbon signal at (δ c 12.12, C-29) by J3 correlation, respectively. There was also a correlation between the methyl proton signal at 0.85 (H-29) and the methyl carbon signal at (δ c 17.20, C-27). Therefore, comparing the IR, ¹H, ¹³C, APT, and the 2D spectra of compound B5 with reported literature data, B5 was proposed to be a stigmasterol.



Proposed structure of compound B5 (stigmasterol).

Conclusion

Column chromatographic separation carried out on the chloroform fraction of the crude methanol stem bark extract of *B. costatum* led to the isolation of stigmasterol, which could have been responsible for the observed anticonvulsant effect of the extract. The work has, to some extent, validated the ethnomedicinal use of the plant in the management of epilepsy.

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