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Isolation of Catechin Glucoside from the Root Bark of *Bombax costatu*m (Bombacaceae)

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ABSTRACT

Phytochemical investigation into the root bark of *Bombax costatum* led to the isolation of catechin-7-O-glucoside. The root bark of the plant was macerated using methanol. Preliminary phytochemical screening of the crude methanol extract revealed the presence of saponins, flavonoid, alkaloid, carbohydrate, triterpenes and steroid. The methanol extract was partitioned using n-hexane, chloroform, ethyl acetate and n-butanol. Column chromatography of the n-butanol fraction over silica gel LH 60-120 yielded four pooled fractions coded G1- G4. Repeated gel filtration of column fraction of G4 led to the isolation of compound HB4. The structure of the isolated compound was established on the basis of Nuclear Magnetic Resonance (NMR), Infrared spectroscopy (IR) and Ultra violet spectrophotometry (UV). Based on the spectral data and data obtained from literature, the structure of the compound was proposed to be catechin-7-O-glucoside

Keywords: NMR, IR, UV, *Bombax costatum*, catechin-7-O-glucoside, phytochemical

INTRODUCTION

The root bark of *Bombax costatum* (bombacaceae) was investigated phytochemically. The plant belongs to a small family of flowering plants which contains about 28 genera and 200 species [1]. Plants of this family are perennial, deciduous and woody trees. They occur naturally throughout the tropical and sub-tropical regions of the world especially in tropical America [2]. Preliminary phytochemical screening of the stem bark of the plant revealed the presence of saponins, flavonoid, alkaloid, carbohydrate, cardiac glycoside triterpenes and steroid.

These phytochemicals may be responsible for the observed anti-inflammatory activity as reported by Meshack [3]. In spite of the use of the plant in ethnomedicine in Nigeria and other parts of the world, there are scanty reports on the isolation of any compound from the root bark of the plant.

The present paper describes the isolation and identification of catechin-7-O- glycoside through spectral analysis.

MATERIALS AND METHOD

Thermoelectron UV machine, Ohaus digital weighing balance (Champ 11 CH15R, Ohaus Corporation, Pinebrook NJ, USA), Metler balance (Model P162 supplied by Gallenhamp), Syringes and needles, Mortar and pestle, Sample bottles, Beakers, separating funnel and conical flask. Bruka AVANCE III NMR spectrometer (500 MHz). Solvents used were of analytical grade and they included: methanol, ethanol, hexane, chloroform, ethyl acetate and n-butanol. Reagents used were freshly prepared and include those for phytochemical screening such as Molisch's reagent, Meyer's reagent, and Borntrager's reagent. Chromatographic materials used are pre-coated TLC plates (Aluminium), Silica gel (60-120 mesh) and Sephadex LH 20.

Collection of plant material

The root bark of *Bombax costatum* was collected from Basawa in Zaria. It was identified and authenticated at the Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria. A sample of the plant was deposited with a voucher number 1749 assigned.

Extraction and Partitioning

The root bark was air dried under shade and pounded to coarse powder using mortar and pestle. The powdered root bark was extracted with methanol (80%) using maceration method for 4 days. The solvent was allowed to air dry. The extract (200 g) was partitioned with n-hexane, chloroform, ethyl acetate and n-butanol to give the n-hexane, chloroform, ethyl acetate and n-butanol fractions respectively.

Preliminary phytochemical screening

Phytochemical constituents of the crude methanol extract of root bark of *Bombax costatum* were tested using simple qualitative methods as outlined below:

Test for Saponins (Frothing Test)

The sample (0.4 g) was added to 10 ml of water in a test tube; the mixture was shaken vigorously for 5 min and observed for the presence of froth which persisted for more than 10 min without ceasing [4].

Test for Tannins (Lead acetate test)

A solution of 1% lead acetate solution was added to 5 ml solution of the sample in a test tube, a cream colored precipitate indicated the presence of tannins [4].

Test for Flavonoids (Shinoda Test)

A small quantity of the sample was dissolved in methanol. Few pieces of magnesium chips was added followed by few drops of concentrated hydrochloric acid, a pink, orange, or red to purple coloration indicates the presence of flavonoid [4].

Test for Carbohydrates (Molisch Test)

To a small aqueous portion of the sample in a test tube, few drops of freshly prepared Molisch reagent followed by concentrated sulphuric acid were added down the test tube in a slanting position, formation of redish colored ring at the interface indicated the presence of carbohydrate [5].

Test for Terpenoids/steroid (Salkowski test)

The sample (0.4 g) was extracted with 70% ethanol and mixed with 2 ml of chloroform in a test tube. This was then warmed for 30 min. 1 ml conc. H_2SO_4 was added to the solution and the appearance of a reddish-brown colouration suggested the presence of terpenoids [5].

Isolation

Purification of the n-butanol fraction (15 g) was carried out on column chromatography over a silica gel-packed column of dimension 75 cm x 3.5 cm; in a polarity gradient manner. Ethyl acetate and methanol were used as the eluents at gradient mixtures from 100% ethyl acetate: 0% methanol to 0% ethyl acetate: 100% methanol [6]. Eluents of 100 ml aliquots were collected, a total of twenty-one (21) fractions were collected, and based on their TLC profiles, were pooled together into four pooled fractions coded G1-G4 [7]. Repeated gel filtration of column fraction G-4 on sephadex LH20 led to the isolation of HB4. HB4 gave single spot on TLC with two

different solvent system (ethylacetate:chloroform:methanol:water and ethylacetate:chloroform: methanol (Figure 1), indicating the purity of the isolated compound.

Solubility of Compound HB4

Compound HB4 was completely soluble in methanol and partially soluble in ethyl acetate

Chemical Tests on Compound HB4

Compound HB4 gave a blue colour when subjected to ferric chloride test indicating the presence of a phenolic nucleus. It also produced red colour when subjected to Shinoda test. This is indicative of a flavonoid nucleus [8].

Figure 1: Thin layer chromatographed of the isolated compound

(EA:CH:M:W (15:4:4:1)

EA:CH:M (7:3:1)

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of saponins, carbohydrates, steroids/terpenes, flavonoids, tannins and flavonoids. Column chromatography of n-butanol fraction yielded four sub-fraction coded G1-G4. Repeated gel filtration of column sub-fraction G4 on sephadex LH-20 column using methanol as the eluting solvent followed by preparative thin-layer chromatography led to the isolation of compound coded HB4. It was obtained as a dark brown solid weighed (7.1 mg) and revealed single homogenous spot on TLC using two (2) different solvent systems.

The UV spectra revealed peak at λmax of 301 nm and 321 nm characteristic of a flavonoid nucleus [11]. The IR spectrum of compound HB4 displayed vibration stretches around

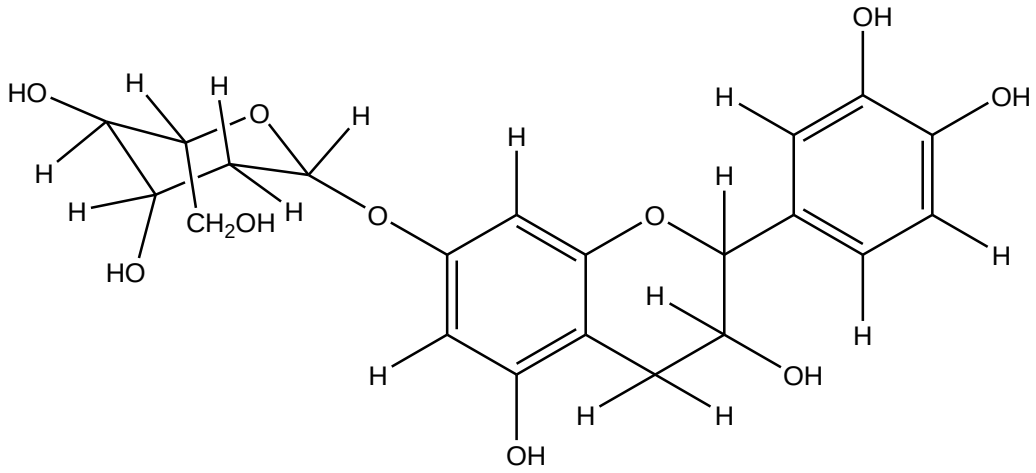
3160 cm^{-1} for OH, 2855.1 cm^{-1} and 2926.16 cm^{-1} due to saturated symmetric and asymmetric C-H stretching, and a weak band at 1584 cm^{-1} due to the aromatic C=C stretching.

The 1H NMR spectrum of HB4 exhibited five aromatic proton signals and were assigned to an AX type ring A [δH6.05 (1H, d, *J* = 2.3Hz, H-6), 6.20 (1H, d, *J* = 2.3 Hz, H-8)] and an ABX type ring B δH 6.99 (1H, d, *J* = 1.8 Hz, H-2'), δH6.80 (1H, d, *J* = 8.0 Hz, 5'), δH6.79 (1H, dd, *J* = 8.1Hz and 1.7 Hz, H-6')] [9]. The four (4) aliphatic peak of an ABMX spin system of the heterocyclic C ring supported the presence of a typical flavanol of the catechin type [10]. Proton chemical shift signal at δH 2.88 (1H, dd J= 2.1Hz, 4.8Hz, H-4ax), δH 2.93 (1H, dd, J= 3.5Hz, 5.8Hz, H-4eq), δH4.84 (1H, d, *J* = 7.3 Hz, H-2) and δH 4.20 (1H,m, H-3) were attributed to the C ring.The geometry of the C-2 and C-3 of the C ring was confirmed as 2-3-trans base on the large coupling constant (*J* =7.3 Hz, H-2) of H-2 and H-3 as opposed to epicatechin (j = 2.0Hz) which has a cis-2,3 configuration [9]. The 1H-NMR further revealed the presence of one sugar unit –with the anomeric proton at δ 4.8 (1H, d, J=6.5 Hz, H-1″); and the coupling constant on the anomeric proton is an indication of a β-glucosyl moiety [10].

The 13C-NMR spectra indicated the presence of 21 carbon atoms. The DEPT analysis revealed the presence of twelve (12) methine carbon atom at C-2, C-3, C-6, C-7, C-2', C-5' C-6', C-I'', C-2'',C-3'', C-4'' and C-5''. Two (2) methylene carbon at C-4 and C-6'', seven (7) quarternary carbons were also recorded at C-5, C-7, C-9, C-10, C-1', C-3' and C-4'. (HSQC) spectrum of HB4 was used in the assignment of protons to their respective carbon atoms. The COSY, HSQC and HMBC, were used to establish the structure of the isolated compound.

The connectivity between the sugar unit and the catechin moiety was established through the correlation observed between the anomeric Proton H-1″ and C-7 of the catechin aglycon. Further correlations were observed between the anomeric proton and sugar carbons at C-1″. The proposed structure of compound HB-4 was found to be catechin-7-O- glucoside.

Proposed structure of compound HB4



Catechin-7-O- glucoside

Table 1: ^{1}H and ^{13}C Chemical shift of the isolated compound and catechin glycoside from literature [12]

POSITION	δ ^{1}H (HB4)	δ ^{13}c (HB4)	δ ^{1}H (ref.)	δ ^{13}c (ref.)
1				
2	4.84(1H,d)	80.11	4.57 (1H, d)	83.60
3	4.20 (1H, d)	67.56	3.99 (1H, d)	69.60
4 ax	2.88 (1H, dd)	29.41	2.49 (2H, m)	29.80
4eq	2.93 (1H, dd)	29.41	2.83 (2H, m)	29.80
5		158.03		158.4
6	6.05 (1H,d)	98.67	5.84 (1H, d)	97.20
7		157.32		157.7
8	6.20 (1H, d)	97.05	5.93 (1H, d)	96.30
9		158.45		158.6
10		102.86		102.3
1’		132.29		132.5
2’	6.99 (1H, d)	115.44	7.29 (1H, d)	118.0
3’		145.9		147.3
4’		146.12		150.1
5’	6.79 (1H,d)	116.05	6.84 (1H, d)	119.2
6’	6.80 (1H,d)	119.49	6.97 (1H, d)	124.8

glu1''	4.80 (1H, d, 3.7)	103.2		5.34 (1H, d, 3.7)	101.7
2''	3.43(1H, d, 3.7,9.8)	74.89	s	3.57 (1H, d, 3.7, 9.8)	74.30
3''	3.65 (1H, t)	73.99		3.45 (1H, t)	75.20
4''	3.60 (1H, t)	71.17		3.88 (1H, t)	72.0
5''	3.41 (1H, m)	78.06		3.31 (1H, dd)	75.7
6''	3.90 (2h, m)	67.08		3.76 (2h, dd)	63.0

CONCLUSION

Catechin-7-O- glucoside was isolated for the first time from *Bombax costatum*. The purification of the isolated compound was carried out by gel filtration on Sephadex LH-20. The identity of the compound was confirmed by spectroscopic techniques

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