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Full Length Research Article

Isolation of Epicatechin from the Stem Bark of *Neocarya macrophylla* (Sabine) Prance (Chrysobalanaceae)

***[1]A. J. Yusuf, [1]M. I. Abdullahi, [2]A. M. Musa, [2]A. K. Haruna, [3]V. Mzozoyana and [2]A. Sanusi**
[1]Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria
[2]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria
[3]School of Chemistry and Physics, University of KwaZulu-Natal, Westville, Durban, South Africa
[*Corresponding Author: E-mail: amynajega@gmail.com; ☎:+234(0)8036386793]

ABSTRACT
Neocarya macrophylla has a wide range of medicinal uses in traditional medicine. The aim of the study was to isolate and characterize compound from the stem bark of the plant. (-)-Epicatechin (a flavan-3-ol) was isolated from the ethylacetate soluble fraction of the methanol stem bark extract of the plant using a combination of silica gel and sephadex LH-20 column chromatography. The structure of the compound as (-)-epicatechin was confirmed on the basis of chemical test, 1D- & 2D-NMR spectroscopy and comparison with existing data in literature. This is the first report of isolation of epicatechin from the stem bark of the plant.

Keywords: *Neocarya macrophylla*, stem bark, (-)-Epicatechin, NMR analysis

INTRODUCTION
The chrysobalanaceae family (first described by a botanist Robert Brown in 1816) is composed of 17 genera and about 525 species. They are mostly woody plants, shrubs or trees found in tropical and subtropical regions, mainly in the southern America (Yakandawala *et al*., 2010) with most of the species having edible fruits (Prance, 1988). There are few researches that revealed the chemical composition of the species of Chrysobalanaceae beyond genres *Licania* and *Parinari*. Flavonoidic compounds are known to play a very important role in the chemotaxonomy of the Chrysobalanaceae family (Coradin *et al*., 1985). A total of 167 secondary metabolites were reported so far for the plant family Chrysobalanaceae including triterpenes (lupane, oleanane, ursane and curcubitanetriterpenoids), diterpenes (kaurane and clerodanediterpenoids), steroids and polyphenols such as flavonoids (myricetin, quercetin, kaemferol, flavanones, flavanonols, flavanols, flavones) and chromones (Carnevale *et al*., 2013).

Neocarya macrophylla (Sabine) Prance formerly *Parinari macrophylla* Sabine, is a West African species used extensively in the Northern part of Nigeria in ethno-medicine to treat numerous diseases which include asthma, skin infections, treatment of wounds, dysentery, inflammations, pulmonary troubles, ear and eye infections (Warra *et al*., 2013). In Nigerian traditional medicine, the fruit is used to treat diarrhoea, and could be eaten fresh or boiled with cereal (Arbonnier, 2004; Tidjani *et al*., 2010). The seed kernels are also eaten while the nuts are usually roasted and enjoyed like cashews or almonds (NRC, 2008). The stem bark is used to treat dysentery, cancer, tooth decay, breathing disorders and snake bites [Personal Communication], conjunctivitis and pain (Arbonnier, 2004). In Senegal, a cigarette prepared from the stem bark of *N. macrophylla* is used as remedy for snake bite (Mohagheghzadeh *et al*., 2006). The leaves may also be chewed or applied topically for the relief of pain (Tidjani *et al*., 2010) and it is also used in the treatment of snakebite [Personal Communication]. Decoction of the bark and leaves is used as mouth wash, internal troubles and for inflamed eye (Arbonnier, 2004; Tidjani *et al*., 2010). The roots are used in treatment of circumcision wounds, as antivenom and haemostatic agent (Arbonnier, 2004). Stigmasterol and Bis-(5, 7-diacetyl-catechin-4'-α-

rhamnopyranoside) were isolated from the stem bark of the plant (Yusuf *et al*., 2015b).The leaves were reported to have anthelminthic (Barnabas *et al*., 2010) and antimicrobial (Audu *et al*., 2005; Halilu *et. al*., 2010) activities while the stem bark has antimicrobial (Yusuf *et al*., 2015c), analgesic (Yusuf *et al*., 2015d) and antivenin activities (Yusuf *et al*., 2015a). Recently, antimicrobial activity of stigmasterol from the stem bark of the plant was reported (Yusuf *et al*., 2018) and Olowo-Okere *et al.* (2018) also reported the antibacterial and anti-biofilm activities of the methanol leaf extract and its fractions against *S. aureus*and *P. aureginosa*. We now report the isolation and characterization of (-)-epicatechin from the ethylacetate fraction of the methanol stem bark extract of *N. macrophylla*.

MATERIALS AND METHODS

General Experimental Procedures

NMR data were recorded on a Bruker AVANCE spectrometer (600 MHz) with residual solvent as internal standard. Melting point was determined on an Electro thermal melting point apparatus. TLC was carried out using silica gel 60 GF_{254} pre-coated aluminum sheets (Sigma Aldrich, Germany). Column chromatography was conducted using LOBA Cheme silica gel (60 – 200) mesh while gel filtration chromatography was performed using sephadex LH-20 (Sima, Spruce street, St. Louis, MO, USA). Spots on TLC plates were visualized by spraying with 10 % H_2SO_4 followed by heating at 105^oC for 10 min.

Plant Sample

The stem bark of *N. macrophylla* was collected in October, 2015 at Jega Local Government Area of Kebbi State. It was identified at the Herbarium section, Department of Biological Sciences, Ahmadu Bello University by comparing with herbarium reference voucher specimen (No. 3197). The stem bark was shade dried, pulverized to powder, labelled and stored at room temperature for use.

Extraction and Isolation

The powdered stem bark (3000 g) was extracted with 90% methanol using maceration method for 6 days. The extract was evaporated *in-vacuo* using rotary evaporator at 40°C to yield a reddish-brown residue (396g) subsequently referred to as the crude methanol extract (MES). Some part of MES (200g) was dissolved in distilled water, filtered and partitioned successively with n-hexane (1 L), dichloromethane (1 L), ethylacetate (2.5 L) and n-butanol (2.5 L) to obtain hexane fraction (HFS, 13.5 g), dichloromethane fraction (DFS, 2.8 g), ethylacetate fraction (EFS, 13.0 g), n-butanol fraction (BFS, 32.0 g) and the residual aqueous fraction (AFS), respectively. The ethylacetate fraction (13.0g) was gradiently eluted in a silica gel packed column using different solvent combinations starting with hexane: ethylacetate (1:1), ethylacetate (100%) to ethylacetate: methanol (8:2). Thirty (30) mL each of a total of 426 fractions were collected and combined based on their TLC profile to give (12) major fractions coded EA- EL. Repeated gel filtration of fraction EE with sephadex LH-20 using methanol led to the isolation of compound **1** (11 mg).

Characterization of Compound 1

Compound **1** was subjected to characterization using chemical tests, melting point and spectroscopic analysis.

RESULTS AND DISCUSSION

Spectral data

(-)-Epicatechin (**1**). Brown amorphous solid substance, m.p. 176 – 178 ^oC; ^{1}H-NMR spectrum (in CD_3OD); δ 5.97 (1H, d, J=2.2 Hz, H-8), δ 5.95 (1H, d, J=2.2 Hz. H-6), δ 2.91 (lH, dd, J=4.6, 16.7 Hz, H-4b), δ2.78 (1H, dd, J=3.3, 16.7 Hz, H-4a), δ 4.85 (1H, brs, H-2), δ 4.21 (1H, m, H-3), δ 7.00 (1H, d, J=1.7 Hz, H-2'), δ 6.84 (1H, dd, J=1.8, 8.5 Hz, H-6') and δ6.82 (1H, d, 8.5 Hz, H-5').^{13}C-NMR (600 MHz, CD_3OD); δ_C 155.9 (C-5), 156.6 (C-7), 156.3 (C-9), 98.7 (C-10), 130.9 (C-1'), 144.4 (C-4'), 144.6 (C-3'), 78.5 (C-2), 66.1 (C-3), 27.9 (C-4), 95.1 (C-6), 94.6 (C-8), 113.9 (C-2'), 118.0 (C-6') and 114.6 (C-5').

Compound **1** was obtained as a brown amorphous solid substance; the melting point range of the compound observed indicated its purity (John, 1964) and it tested positive to ferric chloride reagent suggesting the presence of phenolic nucleus (Silva *et al*., 1998). The ^{1}H-NMR and ^{13}C-NMR spectra of compound **1**showed chemical shift values typical of flavonoids (Hye *et al*., 2009; Jung *et al*., 2012; Nasir *et al*., 2015). The presence of an AX system (1, 2, 3, 5-tetrasubstituted benzene ring A) was assigned from the protons at *δ*5.97 (1H, d, J=2.2 Hz, H-8) and *δ* 5.95 (1H, d, J=2.2 Hz, H-6) while an ABX system (1, 3, 4-trisubstituted benzene ring B) was depicted via the protons at *δ* 7.00 (1H, d, J=1.7 Hz, H-2'), *δ* 6.84 (1H, dd, J=1.8, 8.5 Hz, H-6') and *δ*6.82 (1H, d, J=1.8, 8.5, H-5'). The presence of an aliphatic ring was clearly discerned from the proton chemical shift values observed at *δ* 4.85 (1H, s, H-2) and *δ* 4.21 (1H, m, H-3) representing an oxymethine and a carbinol proton respectively, consistent with a saturated ring C (Jung *et al*., 2012). Two hydrogen atoms with chemical shift values at *δ*2.78 (1H, dd, 2.9, 16.7 Hz, H-4a) and *δ* 2.91 (1H, dd, 4.6, 16.7 Hz, H-4b) assignable to C-4 is characteristic of 3-flavan-type flavonoid (Hye *et al*., 2009; Jung *et al*., 2012; Nasir *et al*., 2015).The chemical shift value for H-2 (*δ* 4.85) which appeared as a broad singlet is an indication that compound **1** is an (-)-epicatechin rather than (+)-catechin (Petereit *et al*., 1991; De Mello *et al*., 1996). The ^{13}C-NMR (600 MHz, CD_3OD) and DEPT experiments indicated the presence of 15 carbon atoms. Compound **1** exhibited seven aromatic methine carbon peaks at *δ*94.5 (C-6), 95.1 (C-8), 98.7 (C-10), 130.9 (C-1'), 113.9 (C-2'), 114.6 (C-5') and 118.0 (C-6'), five oxygenated carbon atoms at *δ* 156.3 (C-5), 155.9 (C-7), 156.6 (C-9), 144.4 (C-4') and 144.6 (C-3') and the three aliphatic carbons at *δ* 78.5 (C-2), 66.1 (C-3) and the methylene carbon at *δ* 27.9 (C-4) further suggested the compound **1** to be an epicatechin (Antonelli *etal*., 2007; Abdullahi *etal*., 2017). The absence of a downfield signal at around *δ* 82 (C-2) confirms compound **1** to be an (-)-epicatechin rather than (+)-catechin(Petereit*et al*., 1991; De Mello *et al*., 1996).

The results of the 2D-NMR spectroscopy of compound **1** were used to assign the carbon atoms with their respective protons and established the connectivity between the various protons and carbons within the molecule. HSQC experiment established the attachment of various protons to their respective carbons, the proton at δ_H4.85 correlated with δ_C 78.5 and δ_H2.91, 2.78 correlated with δ_C 27.9 (Table 1). The ^{1}H-^{1}H COSY established the correlations between the protons at *δ* 4.21 (H-3) # *δ* 2.78 (H-4a), 2.91 (H-4b) and *δ* 2.78 (H-4a) and 2.91 (H-4b) which confirmed the assignment of ring C while the cross peaks correlations observed between *δ* 7.00 (H-2') and *δ* 6.84 (H-6') further substantiate the assignment of ring B. The assignment of the protons, carbons and their linkages in the molecule was confirmed through the cross peaks detected on the HMBC spectrum (Table 1, Figure 2) which further confirmed the structure of compound **1** as an (-)-epicatechin (Figure 1). Comparison with a reference NMR data (Orisakeye and Olugbade, 2014) in Table 2 showed a good match and based on the above, the structure of compound **1** was confirmed to be (2R,3R)-3,4-dihydro-2-(3,4-dihydroxyphenyl)-2H-chromene-3,5,7-triol or (-)-epicatechin (Figure 1). Epicatechin has been reported to possess different pharmacological actions such as cardiovascular and neuropsychological effects (Bernatova, 2018), antioxidant, antimicrobial (Maria John *et al*., 2011) activities among others.

Table 1: 1D and 2D NMR spectral data summary for compound 1

Position	δ ^{1}H	δ ^{13}C	DEPT	COSY	HMBC
1	-	-	-	-	-
2	4.85	78.5	CH	-	C1', C5', C6'
3	4.21	66.1	CH	H-4a, H-4b	C10
4	2.91	27.9	CH_2	H-4a	C9, C10, C3, C2
	2.78		CH_2	H-4b	C9, C10, C3, C2
5	-	155.9	C	-	-
6	5.95	95.1	CH	-	C5, C6, C10
7	-	156.6	C	-	-
8	5.97	94.5	CH	-	C7, C8, C9
9	-	156.3	C	-	
10	-	98.7	C	-	
1'	-	130.9	C	-	
2'	7.00	113.9	CH	H-6'	C4', C6', C2
3'	-	144.6	C	-	
4'	-	144.4	C	-	
5'	6.82	114.6	CH	-	C3', C5', C2
6'	6.84	118.0	CH	H-2'	C1', C2', C3'

Compound 1 = CD_3OD; 600 MHz

Table 2: Comparison of ^{1}H and ^{13}C-NMR data of compound 1 with reported literature

Position	δ ^{1}H(*J* in Hz)	δ ^{13}C	δ ^{1}H	δ ^{13}C
	Compound 1		Literature	
1	-	-	-	-
2	4.85(1H, brs)	78.5	4.87(brs)	78.8
3	4.21(1H, m)	66.1	4.20(m)	66.3
4	2.91(1H, dd, 2.9, 16.7)	27.9	2.72(dd, 3.3, 16.7)	28.3
	2.78(1H, dd, 4.6 16.7)		2.85(dd, 4.5, 16.7)	
5	-	155.9	-	156.9
6	5.95(1H, d, 2.2)	95.1	6.02(d, 2.2)	95.1
7	-	156.6	-	156.5
8	5.97(1H, d, 2.2)	94.5	5.91(d, 2.2)	95.0
9	-	156.3	-	156.9
10	-	98.7	-	99.1
1'	-	130.9	-	131.6
2'	7.00(1H, d, 1.7)	113.9	7.05(d, 1.7)	114.6
3'	-	144.6	-	144.8
4'	-	144.4	-	144.6
5'	6.82(1H, d, 8.5)	114.6	6.78(d, 8.1)	114.8
6'	6.84(1H, dd, 1.8, 8.5)	118.0	6.84(dd, 1.7, 8.1)	118.7

Compound 1 = CD_3OD; 600 MHz Orisakeye and Olugbade = Acetone; 300 MHz

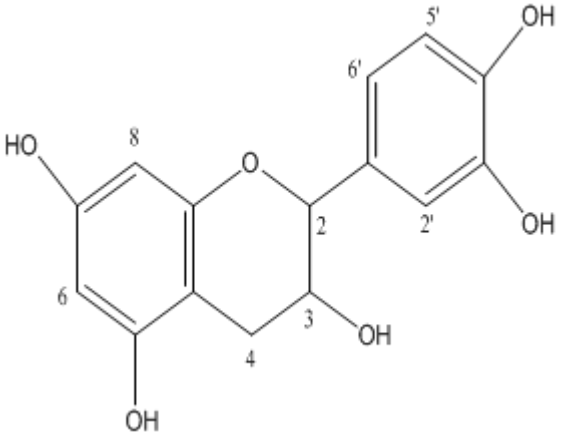

Figure 1: Structure of epicatechin

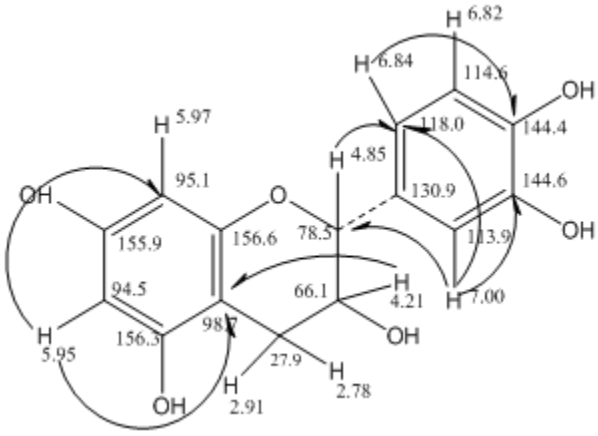

Figure 2: Some major HMBC correlations of epicatechin

CONCLUSION

The isolated and characterized compound epicatechin from the stem bark of *N. macrophylla* to the best of our knowledge is the first report of isolation of this compound from the plant.

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CONFLICT OF INTERESTS

None declared.

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