Bima Journal of Science and Technology, Vol. 9(2B) Jul, 2025 ISSN: 2536-6041

E-ISSN: 3115-4662 DOI: 10.64290/bima.v9i2B.1329

Isolation of Catechin and Catechin Glycoside from *Indigofera welwitschii* (Fabaceae)

A. Muntaka[1,2]*, Amina.J. Yusuf[1] and A. Sanusi[2,3]

[1] Department of Pharmaceutical Chemistry, Usman Danfodiyo University, Sokoto, Nigeria
[2] Department of Pharmaceutical Chemistry, Ahmadu Bello University, Zaria, Nigeria
[3]Department of Pharmaceutical and Medicinal Chemistry, University of Ilorin, Kwara-Nigeria

Corresponding Author: armuntaqa2f@yahoo.com

ABSTRACT

Proper phytochemical investigations of medicinal plants derived constituent has become of paramount importance owing to their versatile application as lead molecules in conventional medicine and as herbal medicine traditional practices. *Indigofera welwitschii* is not an exception. It belongs to genus Indigofera belongs and family *fabaceae (Leguminaceae)* that are well known for their ethnomedicinal claims and establish pharmacological activities. The aim of the study is to isolate and characterize phenolic compounds from the ethylacetate fraction of the methanol extract of *Indigofera welwitschii*. The preliminary phytochemical screenings of aerial parts (whole plant, leaves, flowers, seed and seedling) alone revealed the presence of different classes of compounds of which phenolic compounds are the most abundant. The method employed the use of silica gel chromatographic separation of the ethylacetate fraction using gradient elusion followed by repeated gel filtration using sephadex LH-20 to purify and obtain pure isolate; while the structure of the compound as was confirmed on the basis of chemical test, 1D- & 2D-NMR spectroscopy and comparison with an existing reported literature. catechin (a flavan-3-ol) and catechin glycoside were isolated. On the bases of literature search, this experimentation appears to be first report of isolation of epicatechin from the ethylacetate fraction of aqueous methanolic extract of the aerial parts of *Indigofera welwitschii*.

Keywords: Phytochemical, *Indigofera welwitschii*, Epicatechin, NMR spectroscopy

INTRODUCTION

The proper understanding of phytochemicals is essential for drug discovery and for the development of novel therapeutic agents against major diseases (Awuchi *et al*., 2020). The main objective of standardize extraction procedure for crude drugs is to obtain medicinal and therapeutically desired constituents using appropriate analytical grade solvent. *Indigofera welwitschii* also known and cited as *Microcharis welwitschia* (Ateba *et al*., 2021). belongs to the family *fabaceae* (*Leguminaceae*) which is ranked the third largest family of the blossoming plants after *Orchidnaceae* and *Asteraceae* with approximately 650 genera and 18000 different species and the second-largest family of medicinal plants, with more than 490 species used in traditional medicine (Dzoyem *et al*., 2014).

The plant exists as a shrub and grows very well during raining season. It's mainly distributed in Africa around Ghana, Nigeria, Cameroun, Congo, Mozambique, Malawi and Angola (Darbyshire *et al*., 2015; Schrice, 2013). Traditional medicine revealed an existing ethnobotanical contention of using *Indigofera welwitschii* leaf powder in petroleum jelly which is claimed to be effective treating dermatological problems. This contention was however not proven scientifically and over 60 *Indigofera* species which are reported in traditional medicine are not proven scientifically; thus, their uses depend on the country and the species, but similarities have been noticed (Gerometta *et al*., 2020).

Indigofera species are widely employed in traditional medicine all around the world, against many ailments; the family is well distributed across all tropical and subtropical regions of the world (Su *et al*., 2008). Some 75% of these species are restricted to Africa and Madagascar (Ponmari *et al*., 2014). Phytochemical studies have led to the identification of more than 200 compounds, notably flavonoids and terpenoids. A number of phytochemicals such as lignins, triterpenes, steroids, alkaloids, flavonoids, acylphloroglucinols, saponins, tannins, quinines, rutin, cafffeic acid, gallic acid, myricetin, galangin and quercetin have been reported from the genus (Rahman *et al*., 2018).

MATERIALS AND METHODS

Preparation of Plant Materials

The aerial part of the plant material (*Indigofera welwitschii*) was collected in July, 2019 from Batagarawa Local Government area, Katsina State, Nigeria. It was identified via taxonomic means at the Herbarium of the Department of Botany, Ahmadu Bello University Zaria, Nigeria by a Taxonomist, Mallam Sanusi Namadi (voucher number ABU01702). The aerial parts of the plant were then air dried under shade, grounded to powder, labeled and ready for use.

Extraction Procedures

Extraction and Partitioning

The aerial parts of *Indigofera welwitschii* (2.2 kg) was then extracted exhaustively with methanol. The resulting methanol extract (dried) was suspended in distilled water filtered and successively partitioned into hexane (HEF), chloroform (CHF), ethylacetate (EAF), n-Butanol (NBF) fractions and residual aqueous (AQF) fraction; respectively.

Preliminary Phytochemical Screening

The crude extract and the fractions were subjected to preliminary phytochemical screening using standard procedures to identify the presence of various chemical constituents like alkaloids, flavonoids, and tannins etc (Silva *et al*., 1998).

Chromatographic Procedures

Column chromatographic separations of Ethylacetate fraction (EAF)

Ethylacetate fraction EAF (10 g) was dissolved in small amount of methanol and it was then adsorbed in small quantity of Silica gel powder (60 – 120 mesh size), dried, triturated and then loaded on top of the column, previously packed using wet slurry method (Cannell, 1998). Silica gel gravity column chromatography was carried out by gradient elution method in the silica gel packed column (5×100 cm) using different solvent ratios starting with n-hexane (100%), hexane: ethylacetate (9:1) to hexane: ethylacetate (1:1) followed by ethylacetate (100 %), ethylacetate: methanol (9:1) up to 100 % methanol. The progress of elution was monitored using TLC chromatogram viewed under ultra violet light (254 and 366 nm) and heated upon spray with sulphuric acid reagent. The gradient elution method using the various solvent systems as mobile phase yielded one hundred and ten collections with collection volume of 30 mL each. These fractions were pooled together based on their TLC profile to afford six (6) bulk-fractions, labeled B_1-B_6. Fractions B_1, B_2 and B_3 were then subjected to further stages of purification to obtain pure compounds by the fallowing procedures.

Fraction B_2 (300 mg) obtained from the column chromatography of EAF was subjected to purification by gel filtration using serphadex LH-20 which afforded compounds AM2 and compound AM6. 2 mL each of a total of 24 collections was made and combined based on their TLC profile to afford 3 sub fractions coded (B_2A_1, B_2A_2, B_2A_3). B_2A_2 was further purified using gel filtration repeatedly to afford 3 major fractions coded C1, C2 (Compound AM2) and C3. Fraction C3 was purified further using repeated gel filtration to afford 3 major fractions coded D1, D2 (Compound AM6) and D3. Thus, Fractions C2 and D2 afforded two compounds coded AM2 and AM6, respectively and their TLC profile indicated homogenous spots each after heating the TLC plates in an oven at 105 °C.

Characterization of Compounds

The isolated compounds obtained from the chromatographic separations of EAF was characterized based on physical appearance, solubility, chemical tests and spectral analysis (using 1D- and 2D-NMR spectroscopy).

RESULTS AND DISCUSSION

Purification of Fraction B_2

Repeated gel filtration chromatography of fraction B_2 obtained from the chromatographic separation of EAF afforded 3 major fractions coded B_2A_1 – B_2A_3 (Table 1).

Table 1: Gel filtration Chromatography of fraction B_2.

S/N	No of Eluates (B2)	Code
1	1 – 6	B_2A_1
2	7 – 19	**B_2A_2**
3	20 – 24	B_2A_3

Isolation and purification of Fraction B_2A_2 (Compound AM2)

Compound AM2 was obtained after purification of fraction B_2A_2 obtained from the sub-fraction B_2 from column chromatography of EAF (Table 2)

Table 2: Column collections of fractions B_2A_2 using gel filtration.

S/N	B_2A_2	
1	1 – 2	C1
2	3 – 7	**C2(AM2)**
3	8 – 18	C3

Isolation and Characterization of fraction C3 (Compound AM6)

Compound AM6 was isolated after purification of sub-fraction C3 obtained from the purification of B_2A_2 from column chromatographic analysis of EAF; twelve collections were made and merged based on their TLC profile to afford three major fractions coded D1 – D3 (Plate I, Table 3).

Table 3: Column collection of fractions C3.

S/N	No. of eluates (C3)	Code
1	1-2	D1
2	3-9	**D2(AM6)**
3	10-12	D3

Plate I: TLC Chromatogram of C3 (Gel filtration)

Physicochemical Properties and Spectral Analysis of Compound AM2

TLC analysis of AM2 revealed single homogenous spot using chloroform: methanol

(3: 1) as solvent system (Table 4 and Plate II). The compound appears as a yellowish amorphous powder soluble in acetone and methanol and it also it tested positive with ferric chloride solution.

Table 4: Physicochemical and spectral feature of compound AM2.

Solvent System	No of Spots	R_f Value	Solubility
CHL: MeOH 3:1	1	0.49	Acetone, Methanol

Key: CHL = Chloroform, MeOH = Methanol

Plate II: TLC chromatogram of compound AM2 using chloroform: methanol 3:1.

The compound appears as a yellowish-brown amorphous powder. On the TLC, the spot of compound AM2 was active under UV lamp at 365 nm and appeared yellow after spraying with 10% H_2SO4 (Plate II) which was characteristic of flavanol compound and test positive to ferric chloride reagent and Shinoda test suggesting the presence of phenolic nucleus (Silva *et al*., 1998). It also tested positive to Fehling's reagent indicating the presence of sugar moiety (Silva *et al*., 1998).

The ^{1}H NMR data was used to confirm the basic skeletal structure of compound AM2, the presence of two meta-coupled protons (ring A) at δ_H 5.83 (1H, *br s*) and 5.94 (1H, *br s*) corresponding to H-6 and H-8, respectively, was clearly discerned from the spectrum. An ABX ring system was observed from the proton signals at δ_H 6.81 (1H, *br s* H-2'), δ_H 6.77 (1H, *d*, *J*= 4.0 Hz, H-5') and δ_H 6.73 (1H, *dd*, *J*= 4.0 Hz, 8.8 Hz, H-6'); the presence of an oxygenated aliphatic moiety was confirmed via the proton resonances at δ_H 4.55 (1H, *d*,*J*=8.0 H-2) and δ_H 3.96 (m, H-3) and the 2, 3-*trans* stereochemistry was depicted by the high coupling constant value ($J_{H\text{-}2\text{-}H\text{-}3}$ = 8.0 Hz) of the heterocyclic ring C which confirmed compound AM2 to be a catechin rather than an epicatechin (Jung *et al.,* 2012; Yusuf *et al*., 2019). Two methylene proton resonances at δ_H 2.85 (IH, *dd, J*= 4.0, 16.0 Hz) and δ_H 2.53 (1H, *dd, J*= 8.0, 16.0 Hz) representing H-4b and H-4a, respectively, further confirmed the presence of an AX-type ring C typical of 3-flavan-type flavonoids (Kombal, 1993; Surabhi and Bhadoria, 2005; Jung *et al.,* 2012).

The sugar moiety was identified by the resonances at δ_H 3.63 – 4.62 including an anomeric proton of 4.62 (*br s*) alongside others and a broad singlet at δ_H1.27 typical of the – CH_3 group which suggests the sugar to be a rhamnoside (Manfred *et al*., 1986; Jung *et al*., 2012; Yusuf *et al*., 2019) (Table 9). The ^{13}C-NMR and DEPT experiments of compound AM2 indicated the presence of twenty-one carbon atoms (Table 8); the spectrum indicated a resonance at δ_C 82.82 which appeared in a lower field suggesting the compound to be a catechin (Surabhi and Bhadoria, 2005; Jung *et al.,* 2012).

The chemical shift values at δ_C 1042.0, 73.36, 77.14, 80.90, 70.22 and were characteristic of sugar absorptions and the – CH_3 absorption at δ_C 18.53 suggests the sugar to be a rhamnose which is in consistent with the ^{1}H-NMR data. The anomeric proton signal of δ_H 4.62 (1H, *d*, *J*=1.3 Hz, H-1″); and the coupling constant on the anomeric proton is an indication of a α-rhamnosyl moiety All protons were assigned to their carbons via the HSQC spectra (Table 6). The ^{1}H-^{1}H-COSY of AM2 indicated the protons that are adjacent to one another. Major correlations observed between H-2′, H-6′ confirmed the assignment on ring B; and the presence of ring A was further confirmed via the cross peaks between H-6 and H-8; Cross peaks depicted between H-4b, H-4a and H-3, confirmed the presence of ring C, typical of flavan3-ols (Jung *et al*., 2012). Based on the 1D- and 2D-NMR spectral data (Table 8) and comparison with existing data in the literature (Yusuf *et al*. 2019 and Jung *et al*., 2012) (Table 9), the structure of compound AM2 (Figure 1) was confirmed as (+)-Catechin-3 ' -O-rhamnopyranoside,

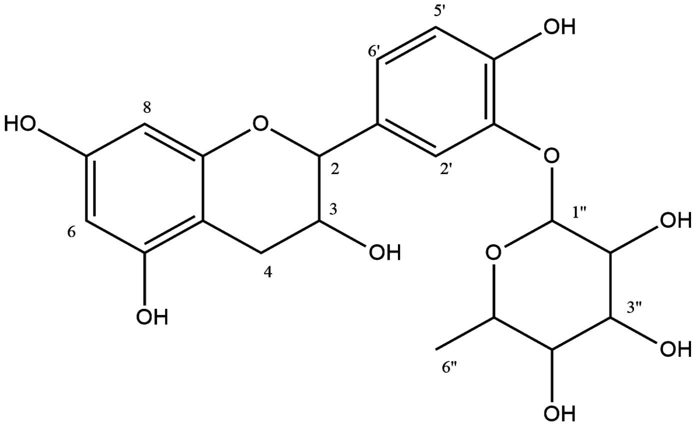

Figure 1: Catechin- 3-O-α-L-rhamnopyranoside ((+)-Catechin-3 ' -O-rhamnopyranoside).

Physicochemical Properties and Spectral Analysis of Compound AM6

Compound AM6 gave a single homogenous spot on the TLC using two solvent systems viz; Chloroform: Methanol 3:1 and Chloroform: Ethylacetate 2:8 (Plate III) The compound appears as a yellowish-brown amorphous solid which is partially soluble in ethylacetate and completely soluble in acetone and methanol and test positive to ferric chloride and shinoda test respectively.

Table 5: Physicochemical and spectral feature of compound AM6

Solvent System	No of Spots	R_f Value	Solubility
CHL: MeOH 3:1	1	0.74	Acetone, Methanol
CHL: EA 2:8	1	0.48	Acetone, Methanol

Key: CHL = Chloroform, MeOH = Methanol
CHL = Chloroform, EA = Ethylacetate

Compound AM6 was obtained as a yellowish brown amorphous solid substance which test positive to ferric chloride reagent suggesting the presence of phenolic nucleus (Silva *et al*., 1998). The ^{1}H-NMR spectrum indicated chemical shift values at δ_H 5.84 (1H, *s*) and 5.92 (1H, *s*) which is characteristic of an AX-system (1, 2, 3, 5-tetrasubstituted benzene ring A) and were assigned to H-6 and H-8, respectively. The presence of an ABX-system due to 1.3,4-trisubstituted benzene ring B was clearly observed via the proton signals at δ_H 6.96 (1H, *s*, 2'), δ_H 6.83 (1H, *d*, *J*=4.0 Hz, 5') and δ_H 6.71 (1H, *m*, 6'), typical of flavonoids (Yusuf *et al*., 2020). An AX-type ring C typical of 3-flavan type flavonoid was confirmed by the presence of two methylene proton signals at δ_H 2.86 (1H, *d*, *J*=4.0, 16.0 Hz) and δ_H 2.54 (1H, *d*, *J*=4.0, 16.0 Hz) representing H-4b and H-4a, respectively (Hye *et al*., 2009; Yusuf *et al*., 2020). The presence of an aliphatic moiety was also detected via the proton signals at δ_H 4.56 (1H, *d*, 8.0 Hz) and δ_H 4.17 (1H, *brs*) which corresponds to H-2 and H-3, respectively, characteristic of saturated ring C (Hye *et al*., 2009; Jung *et al*., 2012, Yusuf *et al*., 2020). The $J_{2\text{-}3}$ coupling constant of 8.0 Hz is an indication of 2,3-*trans* configuration observed between H-2 and H-3 suggesting compound AM6 to be a (+)-catechin rather than an (-)-epicatechin (Hye *et al*., 2009; Jung *et al*., 2012, Yusuf *et al*., 2020).

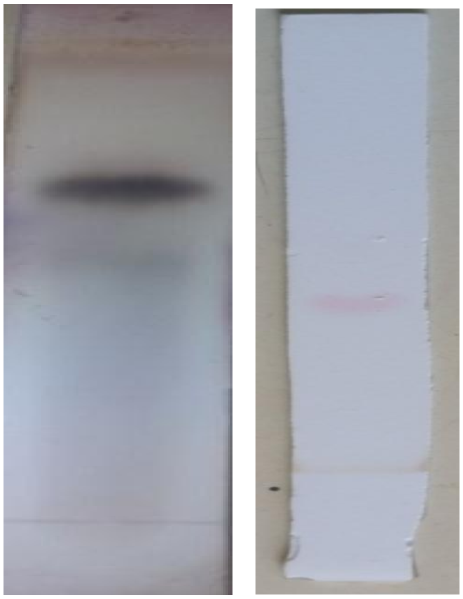

Plate III: TLC chromatogram of Compound AM6 using Chloroform: Methanol 3:1.

The ^{13}C-NMR (125 MHz, CD_3OD) and DEPT experiments of compound AM6 revealed the presence of 15 carbon atoms and their multiplicities as seven aromatic methine, five quaternary oxygenated, three aliphatic and one methylene carbon atoms (Table 6). The signal δ_C 81.43 further suggests the compound to be (+)-catechin (Hye *et al*., 2009; Jung *et al*., 2012, Yusuf *et al*., 2020).

The HSQC spectrum of compound AM6 was used to attach each proton to their respective carbon atoms (Table 6). ^{1}H-^{1}H-COSY experiment of AM6 revealed the protons that are situated in the same environment; Correct assignment of protons on the 1,2,3,5-tetrasubstituted benzene ring A was confirmed via cross peaks observed between H-6 and H-8; and the presence of 1,3,4-trisubstituted benzene ring B was confirmed via the cross peaks observed between the protons at H-2', and H-6' and H-5' and H-6' (Table 6). The HMBC spectrum of AM6 established the connectivity between the protons, carbons and the various sub-structures in the molecule. Long range correlation observed between H-2' and C-4', H-5', and H-5' and C-3', C-6' and C-2 and H-6' and C-1', C-2', C-3', and C-2 confirmed the correct assignment of ring B while the correlation of H-6 to C-10 and C-6 and H-8 and C-8 and C-10 confirmed the assignment of ring A. Attachment of protons on ring C was confirmed via the correlations observed between H-2 and C-3, C-1', C-2', and C-6', and H-4 and C-2, C-3 and C-10. Based on the 1D- and 2D-NMR spectral data (Table 7) and comparison with existing data in the literature as indicated Table 4.24 (Hye *et al*., 2009; Jung *et al*., 2012, Yusuf *et al*., 2020), the structure of compound AM6 (Figure 2) was confirmed as (+)-Catechin.

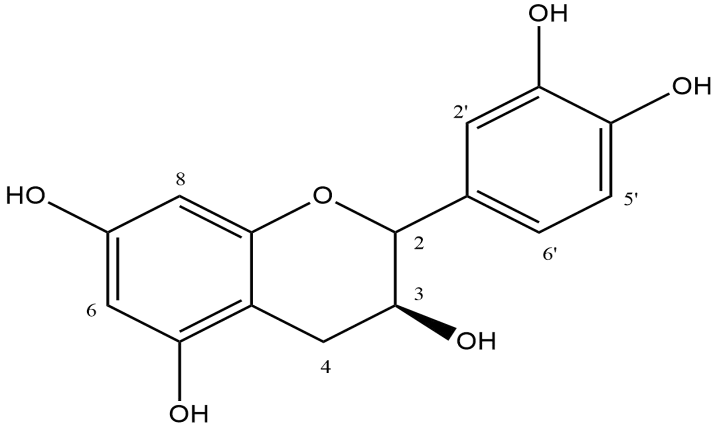

Figure 2: (+)-Catechin.

Table 6: 1D and 2D NMR spectral data summary for compound AM2.

Position	δ^1H-NMR (*J* in Hz)	δ ^{13}C-NMR	DEPT	COSY	HMBC
2	4.55 (*d*, 8.0)	82.82	CH	-	C-9, C-1', C-6', C-2', C-4
3	3.96 (*m*)	67.38	CH	H-4b	
4	2.88 (*dd*, 4.0, 16.0)	29.35	CH_2	H-3, H-4a	C-7, C-10, C-2, C-4
	2.53 (*dd*, 4.0, 16.0)			H-4b	C-7, C-10, C-2, C-4
5	-	156.98	C	-	
6	5.94 (*br s*)	98.18	CH	H-8	C-5,C-7,C-8,C-10
7	-	156.31	C	-	
8	5.83 (*br s*)	94.87	CH	H-6	C-9, C-10
9	-	157.63	C	-	
10	-	98.88	C	-	
1′	-	133.04	C	-	
2′	6.81 ((*br s*)	114.63	CH	H-6′	C-2, C-6’, C-3’, C-4
3′	-	144.79	C	-	C-2”, C-6”
4′	-	148.89	C	-	
5′	6.77 (*d*, 4.0)	116.54	CH		C-4’, C-3’, C-1
6′	6.73 (*dd*, 4.0, 8.0)	118.60	CH	H-2′	C-2, C-2’, C-3’
Rhamnose					
1″	4.62 (*d* 1.3)	104.20	CH	-	
2″	3.69 (*m*)	73.36	CH	-	

3″	3.82 (*m*)	77.14	CH	-	
4″	3.63 (*m*)	80.90	CH	-	C-4”
5″	3.87 (*m*)	70.22	CH	-	C-2”, C-6”
6″	1.27 (*br s*)	18.53	CH_3	-	

Table 7: ^{1}H and ^{13}C-NMR data of compound AM2 compared with reported literature

Position	$\delta^{1}H$-NMR AM2 (*J* in Hz)	^{1}H-NMR (*J* in Hz) (Jung *et al.*, 2012	$\delta\ ^{13}C$-NMR AM2	^{13}C-NMR (Jung *et al.*, 2012
1	-	-	-	-
2	4.55 (*d*, 8.0)	4.62(*d*, 8.0)	82.82	83.1
3	3.96 (*m*)	3.93(*m*)	67.38	76.1
4	2.88 (*dd*, 4.0, 16.0)	2.88(*dd*, 5.5, 16.0)	29.35	28.1
	2.53 (*dd*, 4.0, 16.0)	2.64(*dd*, 8.5, 16.0)	-	-
5	-	-	156.98	157.1
6	5.94 (*br s*)	5.94(*d*, 2.3)	98.18	96.5
7	-	-	156.31	157.0
8	5.83 (*br s*)	5.86(*d*, 2.3)	94.87	95.6
9	-	-	157.63	158.1
10	-	-	98.88	100.8
1′	-	-	133.04	132.1
2′	6.81 ((*br s*)	6.84(*d*, 1.8)	114.63	112.8
3′	-	-	144.79	146.4
4′	-	-	148.89	146.5
5′	6.77 (*d*, 4.0)	6.77(*d*, 8.0)	116.54	116.2
6′	6.73 (*dd*, 4.0, 8.0)	6.72(*dd*, 1.8, 8.0)	118.60	120.0
Rham				
1″	4.62 (*d* 1.3)	4.29(*d*, 1.4)	104.20	102.3
2″	3.69 (*m*)	3.51(*dd,* 1.8, 3.2)	73.36	72.1
3″	3.82 (*m*)	3.57(*dd*, 3.2, 9.6)	77.14	72.5
4″	3.63 (*m*)	3.31(*m*)	80.90	74.5
5″	3.87 (*m*)	3.68(*m*)	70.22	70.5
6″	1.27 (*br s*)	1.25(*d*, 6.2)	18.53	18.9

AM2 = CD_3OD; 400 MHz; Jung *et al.,* 2012= CD_3OD; 400 MHz

Table 8: 1D and 2D NMR spectral data summary for compound AM 6.

S/N	^{1}H-NMR	^{13}C-NMR	DEPT	^{1}H-^{1}H-COSY	HMBC
2	4.56 (1H, *d*, 8.0)	81.43	CH	H-3	C-3, C-1', C-2', C-6'
3	4.17 (1H, *brs*)	67.38	CH	H-2, H-4b, H-4a	-
4	2.86 (1H, *d*, 4.0, 16.0)	27.12	CH_2	H-4b, H-3	C-2, C-3, C-10
	2.54 (1H, *d*, 4.0, 16.0)			H-4a	C-2, C-3, C-10
5	-	155.49	C	-	-
6	5.84 (1H, *s*)	94.81	CH	H-8	C-6, C-10
7	-	156.42	C	-	-
8	5.92 (1H, *s*)	94.03	CH	H-6	C-8, C-10
9	-	156.17	C	-	-
10	-	99.36	C	-	-
1'	-	130.90	C	-	-
2'	6.96 (1H, *s*)	113.81	CH	H-6'	C-4'
3'	-	145.97	C	-	-
4'	-	144.83	C	-	-
5'	6.83 (1H, *d*, 4.0)	114.63	CH	H-6'	C-2, C-3', C-6'
6'	6.71 (1H, *m*)	118.61	CH	H-2', H-5'	C-2, C-1', C-2', C-3', C-4'

Table 9: ^{1}H and ^{13}C-NMR data of compound AM6 compared with reported literature.

S/N	^{1}H-NMR AM6	^{13}C-NMR AM6	^{1}H-NMR A*	^{13}C-NMR A*	^{1}H-NMR B*	^{13}C-NMR B*
2	4.56 (1H, *d*, 8.0)	81.43	4.60	82.0	4.56	80.9
3	4.17 (1H, *brs*)	67.38	4.21	66.1	4.00	66.3
4	2.86 (1H, *d*, 4.0, 16.0) 2.54 (1H, *d*, 4.0, 16.0)	27.12	2.91 2.78	27.9	2.90 2.54	27.7
5	-	155.49	-	155.9	-	156.4
6	5.84 (1H, *s*)	94.81	5.95	95.1	5.87	93.9
7	-	156.42	-	156.6	-	155.3
8	5.92 (1H, *s*)	94.03	5.98	94.6	6.01	95.1
9	-	156.17	-	156.3	-	156.1
10	-	99.36	-	98.8	-	99.1
1'	-	130.90	-	130.9	-	130.6
2'	6.96 (1H, *s*)	113.81	7.00	113.9	6.89	114.5
3'	-	145.97	-	144.6	-	144.8
4'	-	144.83	-	144.4	-	144.6
5'	6.83 (1H, *d*, 4.0)	114.63	6.82	114.5	6.79	115.1
6'	6.71 (1H, *m*)	118.61	6.84	118.1	6.73	119.9

A*= Yusuf *et al.*, 2020 = CD_3OD; 600 MHz
B*= Hye *et al.*, 2009 = Acetone; 400 MHz
Compound AM6= CD_3OD; 400 MHz

CONCLUSION

The two phenolic compounds isolated and characterized from the ethylacetate fraction of aerial part of *Indigofera welwitschii* were proposed to be catechin and catechin-3 ' -O-rhamnopyranoside. It's to the best of our knowledge and literature search the first report of isolation of this compound from the plant.

REFRENCES

Abdullahi, S. M., Musa, A. M., Abdullahi, M. I., Sani, Y. M., & Atiku, I. (2017). Catechin from the leaf extract of *Ziziphus mucronate* willd. (Rhamnaceae). *Nigerian Journal of Pharmaceutical Sciences*, *16*(2), 01-05.

Antonelli, U. M., Yamaguti, E., Uemura, L. M., Nakamura, C. V., Dias Filho, B. P., Palazzo De Mello, J. C. (2007). Chemical and Microbiological Study of Extract from Seeds of Guarana (*Paulliniacupana*var. *sorbilis*). *Latin American Journal of Pharmacy*, 26(1): 5-9.

Ateba, S. B., Njamen, D., & Krenn, L. (2021). The genus Eriosema (Fabaceae): From the ethnopharmacology to an evidence-based phytotherapeutic perspective?. *Frontiers in Pharmacology*, *12*, 641225.

Awuchi, C. G., Chinelo, K. E., Obinna, C. A., Nwabgaoso, O. & Ikechukwu, O. A. (2020). Medicinal plant phytochemicals: the biochemistry and uses of the pharmacologically active alkaloids, terpenes, polyphenols, and glycosides. *Proceedings of the Nigerian Institute of Food Science and Technology*.

Cannell R.J.P. (2000) How to approach the Isolation of Natural Product.In Cannell R.J.P. (ed.) *Natural Product Isolation*. Humana press Totowa, New Jersey (USA). Pp. 1-52.

Darbyshire, I., Kordofani, M., Farag, I., Candiga, R. & Pickering, H. (2015). The plants of Sudan and South Sudan: an annotated checklist.

De Mello, J. P., Petereit, F., & Nahrstedt, A. (1996). Flavan-3-ols and prodelphinidins from Stryphnodendron adstringens. *Journal of phytochemistry*, *41*(3), 807-813.

Dzoyem, J. P., McGaw, L. J., & Eloff, J. N. (2014). In vitro antibacterial, antioxidant and cytotoxic activity of acetone leaf extracts of nine under-investigated Fabaceae tree species leads to potentially useful extracts in animal health and productivity. *BMC Complementary and Alternative Medicine*, *14*(1), 1-7.

Gerometta, E., Grondin, I., Smadja, J., Frederich, M., &Gauvin-Bialecki, A. (2020).A review of traditional uses, phytochemistry and pharmacology of the genus Indigofera.*Journal of ethnopharmacology*, *253*, 112608.

Hye, M.A., Taher, M.A., Ali, M.Y., Ali, M.U. and Shahed, Z. (2009).Isolation of (+) – Catechin from *Acacia catechu* (Cutch Tree) by a Convenient Method.*Journal of Scientific Research*. 1(2): 300 – 305.

Jung, E.K., Sang, S.K., Chang-Gu, H. and Nam, H.L. (2012). Antioxidant Chemical Constituents from the Stems of *Cleyera japonica* Thunberg. *International Journal of Pharmacology*. 8(5): 410-415.

Nasir Tajuddeen, Muhammad S. Sallau, Aliyu M. Musa, Sani M. Yahaya, James D. Habila and Abdullahi Musa Ismail (2015): A novel antimicrobial flavonoid from the stem bark of *Commiphorapedunculata* (Kotschy&Peyr.) Engl.,*Natural Product Research*: Formerly *Natural Product Letters*, DOI: 10.1080/14786419.2015.1041138.

Orisakeye, O. T., & Olugbade, T. A. (2014). Epicatechin and procyanidin B2 in the stem and root bark of SterculiatragacanthaLindl (Sterculiaceae). *Med Chem*, *4*(02), 334-7.

Petereit, F. (1996).“Polyphenolische Inhaltssto ffeund Untersuchungenzurentsündungshe mmendenAktivität der traditionellen Arzneipflanze *Cistusincanus*L. (Cistaceae)” PhD thesis, University of Münster, Germany.

Ponmari, G., Annamalai, A., Gopalakrishnan, V. K., Lakshmi, P. T. V., & Guruvayoorappan, C. (2014). NF-κB activation and proinflammatory cytokines mediated protective effect of Indigofera caerulea Roxb. on CCl4 induced liver damage in rats. *International Immunopharmacology*, *23*(2), 672-680.

Rahman, T. U., Zeb, M. A., Liaqat, W., Sajid, M., Hussain, S., & Choudhary, M. I. (2018). Phytochemistry and pharmacology of genus Indigofera: a review. *Records of Natural Products*, *12*(1), 1-13.

Schrire, B. (2013). A review of tribe Indigofereae (Leguminosae–Papilionoideae) in Southern Africa (including South Africa, Lesotho, Swaziland & Namibia; excluding Botswana). *South African Journal of Botany*, *89*, 281-283.

Silva G.L., Lee I. (1998). Special problems in phytochemistry and extraction from plants sources. In: Cannell J.P.R (eds). Natural Products Isolation, Human Publishers, New Jersey USA, pp 251-293.

Silva, A. R., Fagundes, C. M. S., Andrade-da-Costa, B. L. S., & Rodrigues, M. C. A. (2014). 071—(SIL0192) Anticonvulsant properties of *Indigofera suffruticosa* in the intrahippocampal pilocarpine model in rats. *Epilepsy & Behavior*, *38*, 212-213.

Su, Y., Lü, M., Yang, F., Li, C., Di, L., Wu, D. & Guo, D. (2008). Six new glucose esters of 3-nitropropanoic acid from Indigofera kirilowii.*Fitoterapia*, *79*(6), 451-455.

Yusuf, A. J., Abdullahi, M. I., Musa, A. M., Haruna, A. K., Mzozoyana, V., & Sanusi, A. (2019). Isolation of epicatechin from the stem bark of *Neocarya macrophylla* (Sabine) Prance (Chrysobalanaceae). *Nigerian Journal of Basic and Applied Sciences*, *27*(2), 101-107.