



ISOLATION AND CHARACTERIZATION OF 5-HYDROXY-RUTIN-6,7-DIMETHYL ETHER AND CILICICONE β FLAVONOIDS FROM *Indigofera stenophylla* GUIL. AND PERR. (FABACEAE)

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ABSTRACT

Indigofera stenophylla, a plant belonging to the Fabaceae family, is widely used in West African countries, particularly in Nigeria, to manage various health challenges. These include bacterial infections, pain, snakebites, epilepsy, diuretic effects, wound healing, swelling, and gout. The study aimed to identify, isolate and characterize the phytochemical compounds present in the methanol aerial parts extract of *Indigofera stenophylla*. The aerial parts of *Indigofera stenophylla* was extracted using 70% v/v methanol to obtain the methanol aerial parts extract of *Indigofera stenophylla* (MAEIS) which was partitioned into different fractions using n-hexane, dichloromethane, acetone and n-butanol. The butanol fraction was further subjected to column chromatography with a view to isolate phytochemical compounds from the aerial parts of the plant and the isolated compounds were characterized using spectroscopic methods of analysis. The extensive phytochemical investigation of the butanol fraction affords the isolation of 5-hydroxyrutin-6,7-dimethyl ether and cilicicone β . The structures of these compounds were elucidated and characterized using various spectroscopic methods, which include thin layer chromatography, ultra violet spectroscopy, infra red spectroscopy, Proton Nuclear Magnetic Resonance (¹H-NMR), Carbon 13 Nuclear Magnetic Resonance (¹³C-NMR), and Two-Dimensional Nuclear Magnetic Resonance (2-D-NMR) experiments. To the best of our literature review, this is the first report on the isolation and characterization of the compounds cilicicone β and 5-hydroxyrutin-6,7-dimethyl ether from the methanol extract of the aerial parts of the plant species *Indigofera stenophylla*. The identification and structural elucidation of these two compounds contributes to the phytochemical profile of the plant.

Keywords: Characterization, Cilicicone β , *Indigofera stenophylla*, Isolation, 5-hydroxyrutin-6,7- methyl ether

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INTRODUCTION

The plant *Indigofera stenophylla* is a member of the genus *Indigofera*, which contains over 750 species of flowering plants, shrubs, and herbs that are part of the Fabaceae/Leguminosae family. The natural blue coloring substance used in indigo dye is derived from the genus *Indigofera* [1]. The four species of *Indigofera* that are most frequently encountered are *Indigofera tinctoria* (true indigo), *I. arrecta* (natal indigo), *I. Suffruticosa*, and *I. Rothii*. The common species found in the eastern part of Nigeria are *I. hirsute*, *I. paniculata*, *I. priureana*, *I. pulchra*, *I. senegalensis*, and *I. terminalis* while *Indigofera arrecta* is commonly encountered in the northern part of Nigeria [2]. The Leguminosae/Fabaceae family, which have over 19 325 species in 727 genera and are the third-largest family among the angiosperms [3].

In West Africa and Nigeria respectively, there are roughly 78 and 60 *indigofera* species that can be found, with the Northern part of Nigeria having the highest abundance of about 60%. *Indigofera* species are native to tropical and subtropical regions of the world [1]. The common names of the plant include: Narrow-leaf *Indigo* in English, *Kama-da-baba* in Hausa and *Ako roro* in Yoruba language [4]. The species of the genus *Indigofera* are herbaceous plants, small trees, and annual and perennial semi-woody shrubs with pinnate or trifoliate leaves and racemes or spikes of tiny pea-like flowers. The plant is roughly 5 to 6 feet tall [1]. The stem's hue might be bright green or have a red tint, and it can be solitary, semi-woody, thin, upright, or noticeably branched. The stem's 10 cm long leaves are dark green, pinnate, typically evenly spaced, and may be round or velvety smooth to the touch [5]. The fruits are legume pods of various

sizes and shapes with elongated, four-angled, or flattened, smooth, reddish-brown, butterfly-like blooms [1]. In most species, the fruits have two to fifteen seeds and are frequently curled. The blooms are axillary, and racemes frequently feature open or condensed pedicels with a crimson or pink corolla. Most species' fruits are typically a smooth, reddish-brown pod with 2–15 seeds [5].

Indigofera stenophylla is an annual shrub with a stem that is rigidly upright and branching. It has more or less woody stems that range in height from 40 to 90 cm. As a medicinal plant, it is gathered from the wild for local consumption. It is widespread from Tropical Africa through Senegal and Liberia to Niger and Nigerien [6]. The folkloric information on the use of the Plant *Indigofera stenophylla* decoctions, infusions, and poultices for the management of diseases include bacterial infection, pain, inflammatory, snakebite, epileptic, breast cancer, swelling, and gout [4, 7]. The species of the genus *Indigofera* have been reported to be effective in treating bronchitis, epilepsy, nervous weakness, boils, wounds, burns, dysuria, tympanites, lowering body fat, kidney stones, piles, swellings, snakebites, and spitting snakes [8].

The genus *Indigofera* has been reportedly used ethnomedicinally as anti-arthritic, anthelmintic, anti-cancer, anti-convulsant, anti-diabetic, antilipidemic, antidiarrhea, anti-inflammatory, analgesic, anti-microbial, anti-necrotic, anti-oxidant, anti-plasmodial, anti-Trypanosoma, anti-ulcer, gastroprotective, anti-viral, chemo preventive, cytotoxicity, genotoxicity, mutagenicity, hepatoprotective, immunoreactive, insecticidal, neuroprotective, Reno-protective, repro-protective, wound healing [7]. The infusion of the roots in sesame oil is used as an anti-asthmatic in traditional Sudanese medicine [7]. The roots of *Indigofera arrecta* are traditionally used to treat diarrhea and gonorrhea [9]. A literature search has shown that there is a limited pharmacological study on the root, shoot, stem, leaves, flower, and fruit of *Indigofera stenophylla* reported. The pharmacology of several species of *Indigofera* have been reported to have an anti-arthritic, anthelmintic, anti-cancer, anti-convulsant, anti-diabetic, antilipidemic, antidiarrhea, anti-inflammatory, analgesic, anti-microbial, anti-necrotic, anti-oxidant, anti-plasmodial, anti-Trypanosoma, anti-ulcer, gastroprotective, anti-viral, chemopreventive, cytotoxicity, genotoxicity, mutagenicity, hepatoprotective, immunoreactive, insecticidal, neuroprotective, reno-protective, repro-protective and wound healing [7].

The phytochemical composition of *Indigofera stenophylla*'s root, shoot, stem, leaves, flower, and fruit has not been the subject of any prior research. However, several isolation and characterization of some compounds of the genus *Indigofera* have been reported. *Indigofera colutea* (Burm.), *Indigofera macrocalyx* Guill. Perr.,

Indigofera nigrifolia Hook f., *Indigofera pulchra* Willd., and *Indigofera tinctoria* L. have all tested positive for flavonoids, saponins, quinones, sterols/triterpenes, and tannins, except for *I. pulchra*, which showed negative test for quinones. It has also been reported that *I. hirsute* contains flavonoids, tannins, saponins, steroids/triterpenes, and alkaloids using preliminary phytochemical analysis.

Some pharmacological activities such as anti-neoplastic, anticonvulsant, antiviral, anti-inflammatory, antipyretic, analgesic, and anti-malarial drugs have been linked to the presence of certain bioactive constituents such as tannins, alkaloids, flavonoids, carbohydrates, terpenoids and steroids in the plants [10]. Herbal remedies used in traditional folk medicine provide an interesting and still largely unexplored source for developing potentially new drugs for treating diseases [11].

The aim of the study as 'in this paper, we report the isolation and characterization of some flavonoids from the aerial parts of *Indigofera stenophylla*

MATERIALS AND METHODS

Materials

The TLC tank, column (3.5×75 cm), burette, and pre-coated aluminum thin-layer chromatographic plate 60 GF257 20×20 cm in dimension (Sigma Aldrich, Germany) are some of the equipment utilized. The reagents include; Borntrager's reagent, Wagner's reagent, Mayer's reagent, Dragendoff's reagent, Shinoda's reagent, Salkowski's reagent, and Molisch's reagent. The reagents were freshly prepared before use. The solvents include methanol, acetone, ethyl acetate, dichloromethane, hexane, acetic acid (Qualikems), and Silica gel of mesh size 60-120 μ m (Qualikems). The chemicals and reagents were of analytical grade.

Equipment

The equipment includes an analytical balance, oven, rotary evaporator, and Electro-thermal melting point apparatus (Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria), ABB-MB 3000 UV spectrophotometer, Bruker AVANCE III (400 MHz) NMR spectrometer (Multi-user Laboratory, Department of Chemistry, Faculty of Physical Sciences, Ahmadu Bello University, Zaria) and IR spectrophotometer (NARICT, Zaria).

Collection, identification, and preparation of plant material

The aerial parts of the plant *Indigofera stenophylla* were collected in September 2023 at Gadau village, Itas Gadau Local Government Area of Bauchi State. The aerial parts of the plant *Indigofera stenophylla* were identified and authenticated by Namadi Sanusi at the Habierium Unit, Department of Botany, Faculty

of Life Sciences, Ahmadu Bello University, Zaria by comparing with herbarium reference voucher specimen (No. 0763). The plant was dried under shade, pulverized to powdered, labeled, and stored for further use at 25°C.

Extraction

The powdered aerial parts of *Indigofera stenophylla* (1500 g) were extracted with 70% v/v methanol (10 L) using the maceration method for 3 days, the process was repeated on the marc to obtain more yield. The extract was evaporated *in-vacuo* using a rotary evaporator at 45°C to afford a crude dark-brown solid extract weighing 191 g subsequently referred to as *Indigofera stenophylla* methanol aerial part extract (MAEIS). About 150 g of MAEIS was partitioned successively with hexane (5 L), and butanol (2.5 L) to obtain hexane fraction (HF), and butanol fraction (BF) respectively.

Isolation and purification of compounds AB2 and AB5

The column (3.5x75 cm) was packed with silica gel (60-120 μ m) by wet slurry method. Butanol sample BF weighing 12.7 g of the methanol aerial part extract was subjected to column chromatography because of its promising TLC profile by adsorbing the sample on silica gel using methanol. The elution was carried out using gradient elution starting with hexane (100%), then hexane: ethyl acetate (95:5) and the polarity was gradually increased by 10 unit, until ethyl acetate (100%), the column was washed with methanol (100%). Column Fractions K and Q were purified using gel filtration which was carried out using isocratic elution with methanol and monitor using colour band and small volume of 1 ml collected while sephadex LH-20 and preparative thin layer chromatography respectively.

Elucidation and characterization of compounds AB2 and AB5

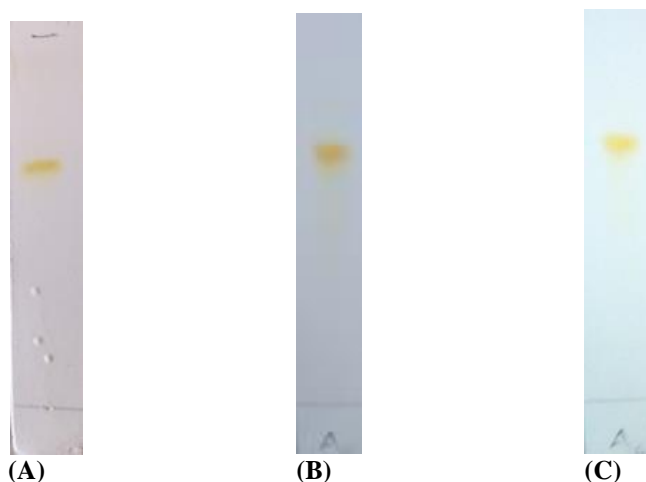
The compounds AB2 and AB5 were elucidated and characterized using UV, IR, and NMR experiments, solubility test, chemical test, melting point and TLC profile.

RESULTS

A total of 70 column collections were pooled into 17 major column fractions based on their TLC profiles. Column fraction K (3.67 g) eluate 44 – 46 obtained from butanol fraction was purified using gel filtration with Sephadex LH-20 to afford a compound coded AB2. Thin-layer chromatographic analysis of compound AB2, a yellowish substance which weighed 7 mg revealed a single homogenous spot with R_f values of 0.72, 0.68 and 0.63 using ethyl acetate chloroform: methanol: water (15:4:4:1), ethyl acetate chloroform: methanol: water (15:8:4:1) and ethyl acetate chloroform: methanol: water (15:10:4:1) solvents system respectively as shown in Table 1. The chromatograms are shown in Plate I. Color after spraying with 10% sulphuric acid spray reagent. The melting point was observed at a range of 190-191°C. It is positive to Shinoda and ferric chloride test. Column fraction Q (Eluate 69 – 70) obtained from butanol fraction column chromatography was purified using PTLC which to afford a compound coded AB5. Compound AB5 is a yellowish substance which weighed 5 mg and was coded AB5. The chromatograms are shown in Plate II. Color after spraying with 10% sulphuric acid spray reagent and the R_f values of the spots are shown in Table 2. Thin-layer chromatographic analysis of compound AB5 revealed a single homogenous spot with R_f values of 0.82, 0.78 and 0.66 using ethyl acetate: chloroform: methanol: water (15:4:4:1), ethyl acetate: chloroform: methanol: water (15:8:4:1) and ethyl acetate: chloroform: methanol: water (15:10:4:1) solvents system respectively and the physicochemical profiles in Table 4.

Table 1: TLC profiles of isolated compound AB2

Solvent System	Number of spots	Colour of spot after heating	R_f Value
(EA:C:Me:W) 15:4:4:1	1	Yellow	0.72
(EA:C:Me:W) 15:8:4:1	1	Yellow	0.68
(EA:C:Me:W) 15:10:4:1	1	Yellow	0.63

**Plate 1:** TLC Profile of Compound AB2 with Three Different Solvent Systems:

- (a) Ethyl acetate: Chloroform: Methanol: Water (15: 10: 4: 1)
 (b) Ethyl acetate: Chloroform: Methanol: Water (15: 8: 4: 1)
 (c) Ethyl acetate: Chloroform: Methanol: Water (15: 4: 4: 1)

Table 2: Physicochemical properties of compound AB2

Physicochemical properties	Compound AB2
Melting point	190-191°C
Solubility profile	
(a). Ethyl acetate	Sparingly soluble
(b). Methanol	Completely soluble
Chemical test	
(a). TLC Ferric chloride test	Green colour on TLC
(b). Shinoda Test	Red colour

Table 3: ^1H and ^{13}C -NMR (400 MHz) spectroscopic data for compound AB2 in MeOD with literature data

Table S1. ^1H and ^{13}C -NMR (400 MHz) spectroscopic data for compound AB2 in H_2O with literature data					
Position	δ ^1H -NMR (J in Hz)	δ ^{13}C -NM	δ ^1H -NMR (J in Hz)	δ ^{13}C -NMR	
Compound AB2			[12]		
2	-----		165.99	-----	157.08
3	-----		136.96	-----	134.46
4	-----		177.99	-----	178.08
5	-----		152.55	-----	164.81
6	-----		131.72	6.23 (d, 2.0, 1H)	98.59
7	-----		159.21	-----	161.55
8	6.71 (1H, s)		90.68	6.43 (d, 2.0, 1H)	93.44
9	-----		164.37	-----	157.57
10	-----		102.19	-----	104.14
1'	-----		121.30	-----	121.41
2'	7.34 (m, 1H, 8.12, 2.64)	115.76	7.90 (d, 8.4, 1H)	116.55	
3'	-----		144.95	-----	144.35
4'	-----		148.39	-----	148.60
5'	6.91 (d, 8.12)		114.65	6.89 (d, 8.4)	114.72
6'	7.34 (m, 1H, 8.12, 2.64)	121.62	7.62 (dd, 8.4, 2.0, 1H)	121.63	
1''	5.36 ((1H, s)		104.94	5.10 (d, 1H)	104.56
2''	3.64		73.30	3.60	73.69
3''	3.75		72.29	3.58	72.47
4''	3.65		71.88	3.61	71.73
5''	3.66		73.70	3.67	73.92
6''	3.87		61.86	3.85	62.91
1'''	4.56 (d, 1H)		101.48	4.55 (d, 1H)	100.53

2'''	4.21	70.56	3.77	70.89
3'''	3.69	69.86	3.55	68.79
4'''	3.50	70.17	3.53	70.65
5'''	3.47	69.50	3.51	68.31
6'''	0.93 (d, 3H)	16.98	1.21 (d, 3H)	16.56
6-OCH ₃ 3.82 (s, 3H)	59.74	-----	-----	
7-OCH ₃ 3.99 (s, 3H)	55.60	-----	-----	



(a)



(b)



(c)

Plate II: TLC profile of compound AB5 with three different solvent systems:

(a) Ethyl acetate: Chloroform: Methanol: Water (15: 4: 4: 1)

(b) Ethyl acetate: Chloroform: Methanol: Water (15: 8: 4: 1)

(c) Ethyl acetate: Chloroform: Methanol: Water (15: 10: 4: 1)

Table 4: TLC profiles of isolated compound AB5

Solvent System	Number of spots	Colour of spot after heating	R _f Value
(EA:C:Me:W) 15:4:4:1	1	Yellow	0.82
(EA:C:Me:W) 15:8:4:1	1	Yellow	0.78
(EA:C:Me:W) 15:10:4:1	1	Yellow	0.66

Table 5: Physicochemical properties of compound AB5

Physicochemical properties	Compound AB5
Solubility profile	
(a). Ethyl acetate	Sparingly soluble
(b). Methanol	Completely soluble
Chemical test	
(a). Ferric chloride test TLC	Green color
(b). Shinoda Test	Red colour

Table 6: ^1H and ^{13}C -NMR spectral data of compound AB5 compared with reported literature data

Position	$\delta^1\text{H-NMR}$ (J in Hz)	$\delta^{13}\text{C-NMR}$	$\delta^1\text{H-NMR}$ (J in Hz)	$\delta^{13}\text{C-NMR}$
	Compound AB5		[13]	
1	-----	128.3	-----	129.8
2	7.04 (s)	115.3	6.86 (s)	115.9
3	-----	145.7	-----	146.3
4	-----	146.4	-----	147.2
5	6.97 (7.6)	109.1	6.70 (8.0)	116.1
6	6.49 (6.04)	106.6	6.75 (8.0)	120.9
7	4.45 (d, 11.5)	72.3	4.41 (d, 11.5)	73.6
8	4.82 (d, 11.5)	83.9	4.82 (d, 11.5)	85.1
9	-----	197.4	-----	198.4
1'	-----	100.0	-----	101.8
2'	-----	164.1	-----	165.3
3'	5.88 (d, 2.5)	94.8	5.77 (d, 2.5)	96.3
4'	-----	163.0	-----	164.5
5'	5.86 (d, 2.5)	95.9	5.82 (d, 2.5)	97.3
6'	-----	167.9	-----	168.7

DISCUSSION

The column chromatographic separation of the butanol fraction led to the isolation two compounds coded AB2 and AB5. Compound AB2 was isolated as a yellow solid substance with R_f of 0.72, 0.68, and 0.63 which gave a single homogenous spot with three different TLC solvent systems indicating the purity of the compound. AB2 was completely soluble in methanol and had a melting point of 190 – 191°C. The compound tested positive Shinoda test and ferric chloride test indicating the presence of phenolic OH group suggesting that compound AB2 might be a flavonoid [14]. The UV spectrum of compound AB2 showed wavelength maximum absorption (λ_{max}) at 256 and 344 nm which is due to the presence of chromophores (carbonyl and olefinic functional groups). The UV spectrum is in agreement with those reported by Abdullahi *et al.* [12]. The IR spectrum of compound AB2 showed stretching and bending vibrational frequencies at 3362, 2929, 2959, 1733, 1664, 1517, 1133, 1360, 1599, 1461, 1274, 1058, 1092, 998, 961, 805, 879 and 1215 cm^{-1} . The broad signal at 3362 cm^{-1} is due to O-H stretching, 2929 cm^{-1} and 2959 cm^{-1} indicate the presence symmetrical and asymmetric C-H stretching, 1360 cm^{-1} is due to symmetrical CH_3 bending vibrations while 1461 cm^{-1} is due to CH_2 and 1733 cm^{-1} due to carbonyl compound vibrations [12].

The proton NMR spectrum of compound AB2 displayed four aromatic proton signals. The presence of one aromatic singlet at δ_{H} 6.71 (1H, s) which was assigned to H-8 (of ring A). Aromatic ring B protons were clearly showed by the proton resonances at δ_{H} 7.34 (1H, dd, $J = 8.0$ and 2.56 Hz, H-6'), 7.34 (1H, dd, $J = 8.0$ and 2.56 Hz, H-2'), and 6.91 (1H, d, $J = 8.0$ Hz H-5'). The chemical shift values of two methoxy groups at δ_{H} 3.94 (3H, s, 6-OCH₃) and 3.81 (3H, s, 7-OCH₃). These values were in close agreement to the values reported for flavones and derivatives [12]. The compound AB2 ^1H NMR

spectrum was quite similar to that reported by Abdullahi *et al.* [12] except that there were down field proton signals at 3.94 and 3.82 instead of the usual shield aromatic meta coupled signal for H-6 and H-8 in flavones skeleton, where only a ^1H signal at 6.71 suggesting that of substitution and for the presence of two signals at 4.59 and 5.36 suggestive of typical α and β anomeric protons for rhamnose and glucose respectively. The proton signal observed at 0.93 was due to a rhamnose methyl proton. Nine overlapped sugar signals at 4.21, 3.73, 3.43, 72.29, 71.89, 73.69, 61.16, 70.86 and 69.46 reflect the presence of two sugar moiety. The ^{13}C -NMR (400 MHz, CD_3OD) spectrum revealed the presence of 18 carbon atoms which include signals at δ_{C} 165.47 (C-2), 136.96 (C-3), 177.99 (C-4), 152.55 (C-5), 131.72 (C-6), 159.21 (C-7), 92.68 (C-8), 164.37 (C-9), 102.19 (C-10), 121.30 (C-1'), 115.76 (C-2'), 144.95 (C-3'), 148.80 (C-4'), 114.34 (C-5'), 121.62 (C-6'), 104.94 (C-1''), 73.30 (C-2''), 72.60 (C-3''), 71.88 (C-4''), 73.70 (C-5''), 61.86 (C-6''), 101.48 (C-1'''), 70.64 (C-2'''), 69.85 (C-3'''), 70.55 (C-4'''), 69.45 (C-5'''), 16.98 (C-6''') ppm and two methoxy carbons at 59.74 (6-OCH₃) and 55.60 (7-OCH₃) ppm. The compound AB2 ^{13}C NMR spectrum was quite similar to that reported by [12] except that there were additional oxygenated aromatic quaternary signals at 131.72 assigned to C-6.

HSQC experiment of compound AB2 established the attachment of various protons to their respective carbon atoms. The major correlations observed include δ_{H} 6.71 (H-8) to 90.68 (C-8), 7.34 (H-2') to 115.76 (C-2'), 7.34 (H-6') to 121.62 (C-6'), 6.91 (H-5') to 114.34 (C-5'), 5.36 (H-1'') to 104.94 (C-1''), 3.64 (H-2'') to 73.30 (C-2''), 3.74 (H-3'') to 72.60 (C-3''), 3.65 (H-4'') to 71.88 (C-4''), 3.66 (H-5'') to 73.70 (C-5''), 3.87 (H-6'') to 61.86 (C-6''), 4.56 (H-1''') to 101.48 (C-1'''), 4.21 (H-2''') to 70.64 (C-2'''), 3.69 (H-3''') to 69.85 (C-3'''), 3.50 (H-4''') to 70.55 (C-4'''), 3.47 (H-5''') to 69.45 (C-5'''), 0.93 (H-6''') to 16.98 (C-6'''), 3.95 (OCH₃ Proton) to 55.60 (OCH₃).

Carbon); 3.82 (OCH₃-Proton) to 59.74 (OCH₃-Carbon). The assignment of protons on ring B was confirmed via ¹H-¹H-COSY experiment which indicated cross peak correlations between the protons at δ_H 7.35 (H-6') and δ_H 6.91 (H-5') at an ortho-coupled position. HMBC spectrum of compound AB2 established the long-range connectivity between the various units of the molecule; the attachment of ring B to C-2 of ring C was confirmed via long range correlations observed between δ_H 7.34 (H-6') and δ_C 165.99 (C-2), 115.76 (C-2') and 148.39 (C-4'); the correct assignment of ring B and A were confirmed via long range correlations observed between δ_H 6.71 (H-8) and δ_C 105.77 (C-10'), 131.81 (C-6), 159.62 (C-7) and 152.5 (C-5). The long-range correlation between δ_H 3.94 and δ_C 159.3 (C-7) for 6-methoxy, δ_H 3.81 and δ_C 131.8 (C-6') for 7-methoxy, and δ_H 5.36, δ_C 135.6 (C-6) and δ_H 4.56 and δ_C 61.86 (C-6''), for anomeric protons at positions 6, 7 and 3 respectively of the Aglycone and the rhamnose moiety at C-6'' of the glucose sugar. The assignment of is in consistent agreement with structure arrangement reported by Abdullahi *et al.* [12] except for the attachment of methoxy groups, glucose and rhamnose sugar at positions 6, 7 and 3 of the Aglycone and C-6'' of glucose moiety respectively. Quercetin-3-O-rutinoside called rutin is a flavonoid that usually found in nature with the potential ability to reduce the fragility and permeability of capillaries and currently used in the treatment of capillary fragility, retinal hemorrhoids, venous insufficiency and for improving microvascular blood flow. The usefulness of rutin has been reported in the prevention and treatment of colorectal cancer and also relieves painful peripheral neuropathy induced by antitumor drugs due to its antioxidant activities [15].

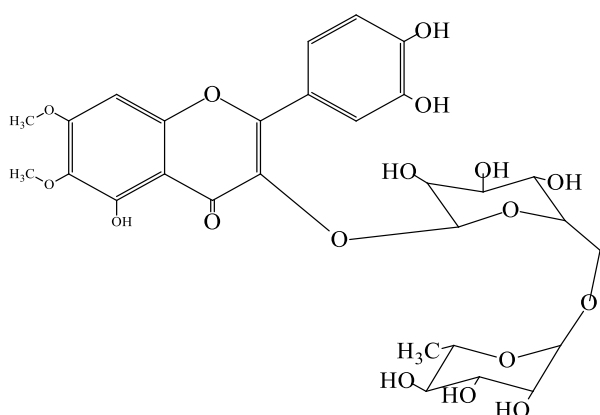


Figure 1: 5-hydroxyrutin-6,7-dimethyl ether

Column chromatographic separation of the butanol fractions led to the isolation of a dark brown solid compound coded AB5 which gave a single homogenous spot with different TLC solvent systems indicating the purity of the compound. Compound AB5 was soluble in methanol. The appearance of red color to Shinoda test and purple color to ferric chloride

test is indicative of the presence of phenolic OH group suggesting that the compound AB5 might be a flavonoid [14]. The UV spectrum of compound AB5 showed wavelength maximum absorption (λ_{max}) at 291 which is due to the presence of chromophores. The UV spectrum is agreement with those reported by [13]. The IR spectrum of compound AB5 showed stretching and bending vibrational frequencies at 3220, 2928, 1628, 1449, 1363, 1282, 1248, 1162, 1129, 1088, 1032, 771, 730, and 738 cm⁻¹. The signal at 3220 cm⁻¹ is due to O-H stretching, 2928 cm⁻¹ and 2959 cm⁻¹ indicate the presence asymmetric C-H stretching, 1363 cm⁻¹ is due to symmetrical C-H stretching. Vibrations at 1449 cm⁻¹ is due to C-O and 1628 cm⁻¹ is due to carbonyl compound vibration [13].

The ¹H-NMR spectrum of compound AB5 displayed two doublets at 5.88 ($J = 2.5$ Hz) and 5.86 (J value = 2.5) suggesting correlation between position 3' and 5' of ring B and further established coupling of 6.97 (d, $J = 8.0$, H-5) with 6.49 (d, J delete = 6.0) indicating their vicinal position in ring A. The presence of carbinolic protons at 4.45 (d, J value = 12 Hz) and 4.82 (d, $J = 12$ Hz) correlate with each other indicating vicinal correlation at position C-7 and C-8 respectively with the help of ¹H-NMR [3]. The ¹³C-NMR spectrum revealed the presence of 15 carbon signals at 128.3 (C-1), 115.3 (C-2), 109.1 (C-5), 106.6 (C-6), 72.3 (C-7), 83.9 (C-8), 197.4 (C-9), 100.0 (C-1'), 164.1 (C-2'), 94.8 (C-3'), 95.9 (C-5'), 167.9 (C-6') ppm with five signals at downfield; 164.1 (C-2'), C-4 (146.4), 146.4 (C-4), 163.0 (C-4'), and 145.7 (C-3) indicate their linkage with oxygen atoms of two hydroxyl group in ring A and three hydroxyl group in ring B respectively [13].

The ¹H-¹H COSY spectrum confirmed the correlation between H-7 and H-8 with the substantial attachment of ring A to H-7 and attachment of ring B to H-8. The HSQC spectrum revealed the correlation between the following signals: 7.04 (H-2) to 115.3 (C-2), 6.97 (H-5) to 109.1 (C-5), 6.49 (H-6) to 106.6 (C-6), 4.45 (H-7) to 72.3 (C-7), 4.82 (H-8) to 83.9 (C-8), 5.88 (H-3') to 94.8 (C-3') and 5.86 (H-5') to 95.9 (C-5'). The HMBC spectrum of compound AB5 revealed long range correlation of H-7 with C-9 (197.4), C-2 (115.3) and C-6 (106.6) indicating the presence of carbonyl carbon at position 9 and attachment of ring A with carbon C-7. The H-8 displayed long range correlation with C-9 (197.4) and C-6 (106.6) the presence of carbonyl group at position 9 and attachment of ring A to C-8 through C-7. H-3' exhibited a long-range correlation the downfield carbon signal at 164.4 (C-2') and 167.9 (C-6'). H-5' displayed a long-range correlation with C-4' (163). H-6 also displayed correlation with C-1 (128.3), C-5 (109.1), C-6 (106.6) and C-8 (83.9) thereby substantiating the structure of ring A and B. The assignment of 3,4,2',4',6',7,8-heptahydroxy-7,8-dihydro-chalcone called cilicone β collaborate with that reported in the literature [13].

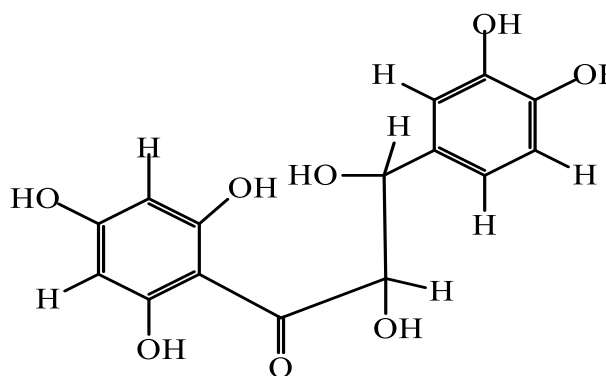


Figure 2: Cilicicone β

CONCLUSION

Based on a thorough review of the available literature, this is the first report on the isolation and characterization of 5-hydroxyrutin-6,7-dimethyl ether and cilicicone β from the plant species *Indigofera stenophylla*.

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REFERENCES

- SOLADOYE, M.O., SONIBARE, M.A. & CHUKWUMA, E.C. (2010). Morphometric study of the Genus *Indigofera* Linn (Leguminosae Papilionoideae) in South-Western Nigeria. *International Journal of Botany*, **6**(3): 343-350. <https://doi.org/10.3923/ijb.2010.343-350>.
- NWACHUKWU, C.U. & MBAQWU, F.N. (2007). Leaf anatomy of eight species of *Indigofera* L. *Agricultural Journal*, **2**: 149-159.
- VEITCH, N.C. (2007). Isoflavonoids of the Leguminosae. *Natural Product Reports*, **24**(2): 417-464. <https://doi.org/10.1039/b511238a>.
- BURKILL, H.M. (1995). The useful plants of West tropical Africa. Royal Botanic Gardens, Kew, UK. **3**: 422.
- DORFLING, C. (2001). Growing Native Plants. Hepper and Row Publisher, Pp, 797.
- AFRICAN PLANT DATABASE (2022). Conseratoire et Jardin Botaniques De La Ville De, Geneva and South African. Natural Biodiversity Institute, Pretoria. Retrieved June, 2023, from <http://africanplantdatabase-ch>.
- GEROMETTA, E., GRONDIN, I., SMADJA, J., FREDERICH, M., GAUVIN-BIALECKI, A., PHARMACOGNOSIE, L. DE, SARTILMAN, C. & HIPPOCRATE, A. (2020). A review of traditional uses, phytochemistry and pharmacology of the genus *Indigofera*. *Journal of Ethnopharmacology*, **1**-82. <https://www.sciencedirect.com/science/article/pii/S0378874119326674>.
- PADAL, S.B., CHANDRASEKHAR, P. & VIJAYAKUMAR, Y. (2013). Ethnomedicinal Uses of Some Fabaceae Family Plants of Narsipatnam Division, Visakhapatnam District, Andhra Pradesh, India. *International Journal of Innovative Research and Development*, **2**(6): 808-822.
- 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): 16-34.
- SANUSI, A., HASSAN, H.S., SANI, Y.M., MUSA, A.M. & UMAR, U. (2018). Preliminary phytochemical and anticonvulsant studies on the root extracts of *Ficus capensis* Thunb. (Moraceae). *Journal of Pharmacy and Bioresources*, **15**(1): 1-9. <https://doi.org/10.4314/Jpb.v15i1.1>.
- HASSAN, H.S., SULE, M.I., USMAN, M.A., USMAN, M. & IBRAHIM, A. (2009). Preliminary Phytochemical and antimicrobial screening of the stem bark extracts of *Bauhinia rufescens* Lam. *Bayero Journal of Pure and Applied Sciences*, **2**(2): 53-55.
- ABDULLAHI, S.M., MUSA, A.M., SANI, Y.M., ABDULLAHI, M.I. & MAGAJI, M.G. (2019). Isolation of rutin from the leaf of *Ziziphus mucronata* Willd (Rhamnaceae). *Bayero Journal of Pure and Applied Sciences*, **12**(1): 573-576. <http://dx.doi.org/10.4314/baJopas.vi2i.865>.
- AHMED, B. & AL-HOWIRINY, T. (2007). Two New Hydroxy Chalcone Derivatives from *Thymus cilicicus*. *Asian Journal of Chemistry*, **62**(2): 121-124.
- SAPTARINI, N., HERAWATI, I.E. & PERMATASARI, U.Y. (2016). Total flavonoids content in acidified extract of flowers and leaves of *Gardenia jasminoides* Ellis). *Asian Journal of Pharmaceutical and Clinical Research*, **9**(1): 213-215.
- GEORGETA, S., ANA, I.P., TUNDE, H. & SANDA, B. (2016). The isolation and identification of Rutin from Pharmaceutical Products. *Analele*, **15**(1): 109-114.