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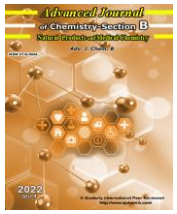

Original Research Article

Isolation and Characterization of Neoduline from the Rhizome of *Dolichos pachyrhizus* Harm

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ABSTRACT

Dolichos is a genus of twining plants found in both the Northern and Southern hemispheres, with the most frequently grown and used species being Dolichos biflorus and Dolichos lablab. Antimicrobial, antioxidant, anticancer, anti-schistosomal, and anti-inflammatory properties have been reported in various parts of the Dolichos species, including the leaves, roots, bark, and stem. The rhizome of Dolichos pachyrhizus was cut, air-dried at ambient temperature, and macerated with n-hexane, dichloromethane, ethyl acetate, and methanol for 72 hours each using a conventional maceration procedure, with regular shaking at intervals. The mixture was decanted, filtered, and concentrated at 40 °C using a rotavapor to yield crude n-hexane, dichloromethane, ethyl acetate, and methanol extracts (R110). To obtain the pure isolate, a methanol crude extract of the rhizome of Dolichos pachyrhizus (D. pachyrhizus) was chromatographed on a silica gel column using various eluents. The preliminary phytochemical investigation of the methanolic rhizome extract of Dolichos pachyrhizus revealed the presence of seven (7) different phytochemicals, including; - alkaloids, flavonoids, glycosides, phenols, tannins, saponins and steroids. The structure of the pure isolate was successfully identified, characterized and confirmed using spectroscopic techniques such as; - Fourier transform spectroscopy (FT-IR), Gas chromatography-mass spectrometry (GC-MS) and Nuclear magnetic resonance (NMR) be Neoduline.

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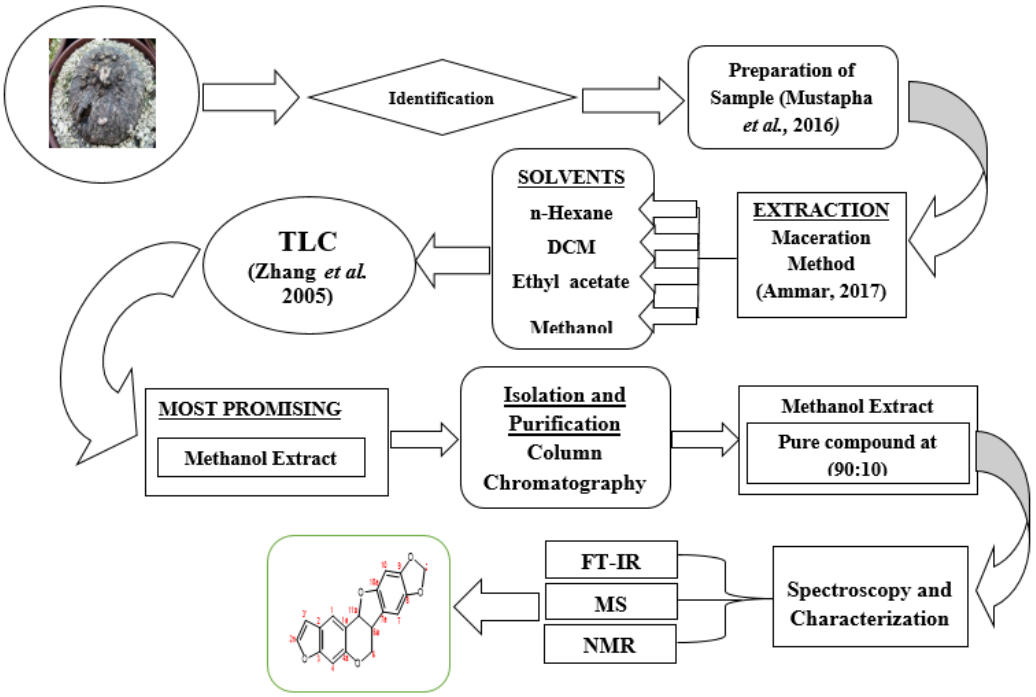
GRAPHICAL ABSTRACT



1. Introduction

Dolichos Pachyrhizus Harms (*D. pachyrhizus*) Papilionaceae (Fabaceae) is a branched, sub-erect, and downing herb distributed in the tropics of both hemispheres which belongs to fabaceae family [1]. *Dolichos pachyrhizus* Harms is a leguminous sub-shrubby plant which is found growing in the rocky soil and a common twining creeper, trailing annual with small trifoliate leaves, bearing when mature, narrow flat curved pods 1.5- 2.0 inches long tipped with a persistent style. The pods contain 5-6 flattened, ellipsoid seeds 1/8-1/4 inch long [1]. It is a tropical plant, native from Mexico and Central America. In Mexico, *D. Pachyrhizus* is known as "jicama", and profusely cultivated since pre-Columbian era due to its edible subterranean tubercles [2]. It has been introduced with the name "yam bean" as a crop in most parts of India, China, and the USA, and also it is used as a food ingredient in India than in any other country since the ancient times [3, 4]. The plant has been cultivated in Central, South and West Africa [5].

The phytochemical constituents of Dolichos species previously reported include isoflavones such as coumarin derivatives and pterocarpans from *D. mitis* [6, 7, 8, 9]. Two different ayurvedic preparations of *D. biflorus* have been used as the ingredient and also reported to possess antinephrotoxic and free radical scavenging activities [10, 11, 12].

The seeds of Dolichos species have been reported to possess antilithiatic [13], antihepatotoxic [14] and hypolipidemic properties [15] and involved in lowering the level of blood sugar and total cholesterol [16, 5, 13, 15]. The seeds of yam bean (*D. Pachyrhizus*) Harms (Fabaceae), have been often used as a source of insecticidal, because of rotenone content which was reported to be toxic to both the insects and mites [13]. In folk medicine, the pulverized seed of *D. Pachyrhizus* has been utilized in the treatment of skin eruptions, bleeding piles, vaginal bleeding, and

leucorrhoea [17, 18]. The powdered seeds of *D. Pachyrhizus* have been used as a poultice to induce sweating [13].

The petroleum ether root extracts of *D. mitis* have indicated a significant acaricidal activity against female ticks, larvacidal, and mosquitocidal activities against larvae of Anopheles gambiae and Culex quinquefaciatus mosquitoes [7, 8].

Generally, plants represent an unlimited source of natural antimicrobials that might serve as lead for the development of the novel drugs. The use of traditional medicine is widening all over the world. Recently, much attention has been focused toward extracts and biologically active compounds isolated from common plant species [19]. The use of medicinal plants plays a crucial role in covering the basic health requirements in developing countries, and these plants may offer a new source of therapeutic agents. Therefore, this research aimed to extract, isolate, and characterize the bioactive component(s) from the methanol extract of *Dolichos pachyrhizus* rhizomes.

2. Materials and Methods

2.1 Reagents and solvents

All used reagents and solvents were of the analytical grade obtained from Sigma Aldrich (Germany), and they were used as purchased without further purification which include dilute hydrochloric acid (HCl), potassium iodide (KI), concentrated sulphuric acid (H_2SO_4), sodium hydroxide (NaOH), 1% ferric chloride ($FeCl_2$), and methanol (CH_3OH).

2.2 General analysis

Thin layer chromatography (TLC) was performed using pre-coated silica gel 60 (F_{254}) from MERCK (Germany). The spots were manually applied using a capillary tube, the plates were dried with a hot air blower, and the spots were developed in a Shandon chromatography tank at room temperature. The UV light (254 nm and 366 nm) was used to visualize the spots on the TLC plates, which were also sprayed with 10 % sulphuric acid before being heated at 110 °C for 5-10 minutes. The KBr disc method was used to record the IR spectrum of the isolated product using a Perkin Elmer Spectrum 100 FT-IR spectrometer. ^1H and ^{13}C-NMR spectra were obtained on a Bruker Advance III400 MHz Spectrometer at room temperature, (400 MHz for both ^1H and ^{13}C), using the TMS as a reference. The chemical shift values (δ) were reported in parts per million (ppm) relative to the TMS standard, and coupling constant (*J*) are given in Hz. For these tests, deuterated chloroform was used as the solvent ($CDCl_3$-d6). The sample was investigated through Gas Chromatography Mass Spectrometry (GC-MS) (Scion 436- GC Bruker model), operated in a positive electron ionization (EI) mode with ionization energy of 70eV. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

2.3 Plant collection and identification

The rhizome of *Dolichos pachyrhizus* was collected in February, 2019 at Ajja bush of Batsari Local Government Area of Katsina State, Nigeria. The rhizome was identified at Herbarium Unit, Biological Sciences Department, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, and voucher number (ABU09001) was deposited.

2.4 Extraction of the plant material

The cold maceration method as reported by [20, 21] with modifications was adopted for the extraction process. The following solvents in order of increasing polarity were used for the extraction: n-Hexane – dichloromethane – ethyl acetate – methanol, to obtain the crude extracts of *Dolichos pachyrhizus* of different solvents and stored for further usage.

2.5 Phytochemical Screening

The phytochemical screening of the methanol extract of *Dolichos pachyrhizus* was carried out to determine the presence of bioactive constituents using the standard methods [22, 23].

2.6 Column chromatographic purification

The crude methanol extract (2 g) was weighed and transferred into a packed column carefully on top of silica gel, about 5 g of dried silica gel was applied to serve as a protective layer. A gradient solvent system of 100 % n-hexane (100 cm^3) was initially used to elute the column, followed by n-hexane-ethyl acetate (100 cm^3) in the following order as: 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, and 5:95. Finally, the column was cleansed with 100 % ethyl acetate (100 cm^3). Different fractions were collected in a 10 cm^3 beakers labelled (1-147) at intervals. At room temperature, each fraction was allowed to evaporate before being examined through thin layer chromatography (TLC). Similar fractions were merged together based on their TLC pattern, grouped into two (A and B) fractions and purified further using a short column [24], using n-Hexane: Ethyl acetate (90:10) as the solvent systems. This led to isolate the compound from fraction B which was thoroughly washed with pentane to obtain the optimal purity for spectroscopic analyses.

3. Results and Discussion

3.1 Results of Phytochemical screening

Table 1. Phytochemical Constituents of *D. pachyrhizus* methanol extract

Test Compounds	Methanol Extract
Saponins	+
Glycosides	+
Alkaloids	+
Anthraquinones	-
Steroids	+
Terpenes	-
Flavonoids	+
Tannins	+
Phenols	+

3.2 Results of spectroscopic analyses

The pure compound was eluted with 90 % n-hexane: ethyl acetate and isolated as white powder (15 mg), molecular mass (308 g), melting point (m.p) 216 – 217; FT-IR (cm^{-1}): 3035 (C-H), 1735 C-O, 2856 C=C, and 1725 (Aromatic ring).

^{1}H-NMR: (400 MHz, $CDCl_3$), δ (ppm): 7.49 (1H, s, H-1), 7.72 (1H, d, *J* = 2.5 Hz, H-2n), 7.56 (1H, s, H-4), 7.06 (1H, s, H-7), 6.74 (1H, s, H-10), 6.72 (1H, d, *J* = 2.5, 1 Hz H-3'), 6.44 (1H, s, *J* = 1.5 Hz, H-2m), 5.68 (1H, d, *J* = 7 Hz, H-11a), 5.66 (1H, d, *J* = 1.5 Hz H-11a), 4.29 (1H, q, *J* = 11, 5 Hz, H-6a), 3.74 (1H, d, *J* = 11, 11 Hz, H-6), and 3.68 (1H, d, *J* = 11, 7, 5 Hz, H-6a).

^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 154.20 (C-3), 153.50 (C-10a), 152.54 (C-4a), 148.12 (C-9), 145.08 (C-2n), 141.78 (C-8), 122.81 (C-1), 122.40 (C-2), 117.90 (C-7a), 116.50 (C-1a), 106.21 (C-3'), 104.70 (C-7), 101.27 (C-2m), 99.83 (C-4), 93.72 (C-10), 79.21 (C-11a), 77.17 (C-6a), and 66.96 (C-6).

4. Discussion

The rhizome of *Dolichos pachyrhizus* methanol extract indicates the presence of alkaloids, tannins, saponins, glycosides, flavonoids, phenols, and steroids (Table 1). Phytochemicals, which are chemical substances which are responsible for the biological activity, are commonly found in plants with biological activities [18]. The result indicates that the rhizome contains a considerable quantity of

secondary metabolites such as alkaloids with extreme significance in medicine which were found to aid in anti-diuretic activity of medicinal plants [26], they also make up the majority of the most valuable medications in animal physiology such as antiplasmodial analgesic, antispasmodic, antidiabetic, and anti-inflammatory properties [27]; the most diversified groups of phenolic compounds were found in plant, flavonoids with antibacterial, anti-inflammatory, anti-allergic, protect against ulcers, vineses, and anti-tumor effect [28]. Flavonoids are free radical scavengers, super anti-oxidant and potential water soluble which prevent oxidative cell, damage, and have a strong anti-cancer, anti-allergic, anti-thrombotic, vasoprotective, tumor inhibitory, and anti-viral activity [29]; glycosides have a great and direct influence on the heart, helping to sustain its strength and the rate of contraction when it is weakening [30], saponins which are steroid or triterpenoid glycosides has been reported to have anti-inflammatory, cardiac depressant, and hypo-cholesterol effect [31] as well as biological actions that are structure-dependent [32]; steroids were determined to be presented, steroidal compounds are very important and interesting in pharmacy due to their interactions with substances like sex hormones [33]. Because the steroidal structure could serve as a potent starting material in the synthesis of these hormones, decoction of the rhizome of Dolichos pachyrhizus may be useful to hoping mothers, or nursing mothers to ensure their hormonal stability [18], and tannins was reported to contain wound healing properties, and discovered that it exhibits anti-diarrhea, anti-diabetic anti-inflammatory and anti-oxidant activities [29]; [34]. When consumed, the presence of phenol in the rhizome of *Dolichos pachyrhizus* acts as an antiseptic and lowers inflammation [30]. The results of the obtained phytochemical screening implies the rhizome of plant may be used as anti-microbial agent which was in agreement with what was reported by [35] from the methanol extract of *Dolichos biflorus* Linn seeds.

The compound was isolated as a white crystalline solid with 90 % n-hexane: ethyl acetate solvent system 12.0 mg, m.p. 223—224 ℃, Lit. m.p. 225 ℃. The followings are the prominent functional groups indicated by a signal in IR spectrum 1667 cm^{-1} C-H, 1493 cm^{-1} (-C=C- aromatic) and 1084 cm^{-1} (-C-O-C). The mass fragmentation pattern of the compound is depicted in Figure 3. The high-resolution electron-ionization mass spectroscopy (HREIMS) of the compound gave a molecular ion peak at m/z (307) corresponding to the molecular formula of $C_{18}H_{12}O_5$ (308.28488). The loss of cyclopentanone from the compound was indicated by the presence of a fragment ion at m/z 262. The subsequent fragment at m/z 212 might be due to the loss of a phenyl cyclopentanone group. The signal at m/z 66 was the characteristic of furan fragmentation due to the loss of side chain followed by the loss of two hydrogen atoms.

The ^{1}H NMR spectrum (Figure 1) revealed a total of 10 proton signals resolved by DEPT as eight methines, two methylenes and no methyl protons. The spectrum indicates five singlet peaks, with two singlet protons resonating at δ 7.55 (1H, s, H-1) and 7.56 (1H, s, H-4) on the corresponding carbon C-1 and C-4, respectively and the other two singlet protons at δ 7.08 (1H, s, H-7) and 6.72 (1H, s, H10) on the same, but different ring on C-7 and C-10, respectively, whereas the last singlet proton resonate at δ 6.44 on C-2m. The spectrum shows only one quotate 4.16 (1H, q, and H-6a). The methylene proton at δ 4.29 (2H, d, and H-6) indicates the COSY cross peak with a proton at δ 3.74 (1H, d, and H-6a). The furanyl ring protons is resonated at δ 6.74 (1H, d, H-3'), δ 7.72 (1H, dd, H-2n), and 7.70 (1H, dd, H-2n).

The ^{13}C NMR of the compound revealed 18 carbon signals, characteristic of an isoflavonoid resolved by distortionless enhancement by polarization transfer (DEPT) spectrum as 8

quaternary carbons, five of which were highly desheilded oxygenated centers at C-3 (δ 145.20), C-4a (δ 152.54), C-8 (δ 141.78), C-9 (δ 148.12), and C-10a (δ 153.50), one being bridged atom in the pentacyclic ring at C-2 (δ 122.40). There were 8 methine carbons at C-1 (δ 122.81), C-3' (δ 106.21), C-2n (δ 145.08), C-4 (δ 99.83), C-6a (δ 77.17), C-7 (δ 104.70), C-10 (δ 93.72), and C-11a (δ 79.21). The two methylene carbons were assigned to C-6 (δ 66.96) and C-2m (δ 101.27).

The connectivity of proton to carbon atoms were obtained from the Heteronuclear Single Quantum Correlation (HSQC) spectrum. There were cross peaks which were observed between H-6a and H-11a in COSY spectra. In addition, there were a coupling of ortho-related protons (H1 -H2) and a coupling of meta-related protons (H-2 –H-4). The signals at δH 7.5 (d, *J* = 2.25, H-2') and 6.7 (d, *J* = 2.23, H-3') were characteristics for ortho-related protons in a furan system. The COSY spectrum of the compound indicated two isolated spin arrangements of the ^{1}H- NMR signals (dH 8.49, 8.25, 7.76, and 7.05 ppm) in aromatic rings A and B. The substitution pattern in ring B was further analyzed by its ^{1}H-NMR, HMBC, and COSY spectroscopic data. An ortho-coupled doublets observed in the ^{1}H-NMR at 7.10 (d, *J* = 8 Hz), 6.41 (1H, dd, *J* = 8, 2 Hz,), and a meta-coupled doublet at 6.38 (d, *J* = 2 Hz) were assigned to H-1 and H-10, respectively, on the basis of COSY experiment. In the HMBC spectrum, there were correlations between the proton signals at d 8.25 [1H, s, H-2] and d 8.49 [1H, s, H-5], and also the comparison with those reported for dehydroneotenone [25] in *N. mitis* are the same [7], except for the difference in the substitution pattern in the aromatic ring B.

The ^{1}H-, ^{13}C-NMR, HSQC, HMBC, and Gas Chromatography-Mass Spectroscopy (GC-MS) spectral data are similar to a pterocarpan "neoduline" compound previously reported as component of *Neorautanenia mitis* [7] obtained from [25] and is the first report of its isolation from the rhizome of *Dolichos pachyrhizus*.

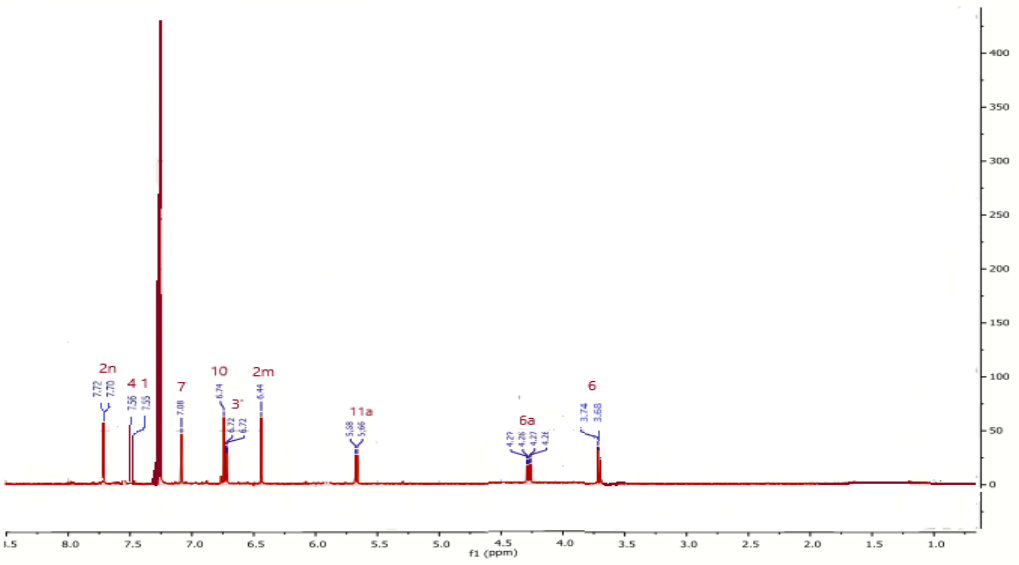


Figure 1. ^{1}H NMR Spectrum of the Compound

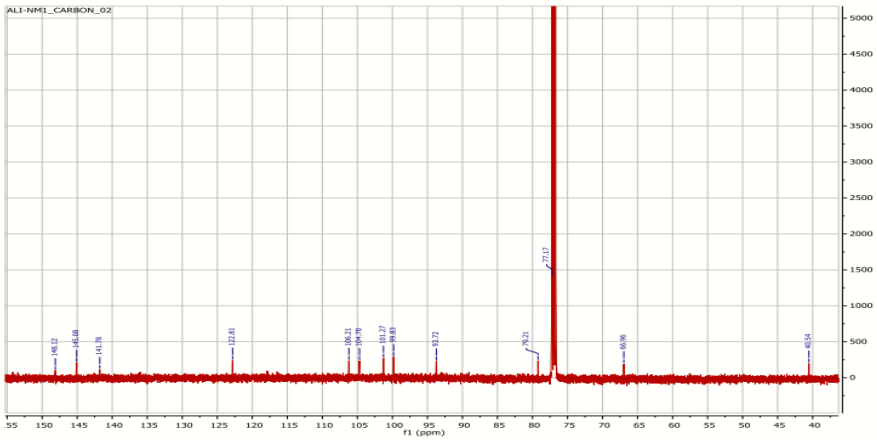


Figure 2. ^{13}C NMR Spectrum of the Compound

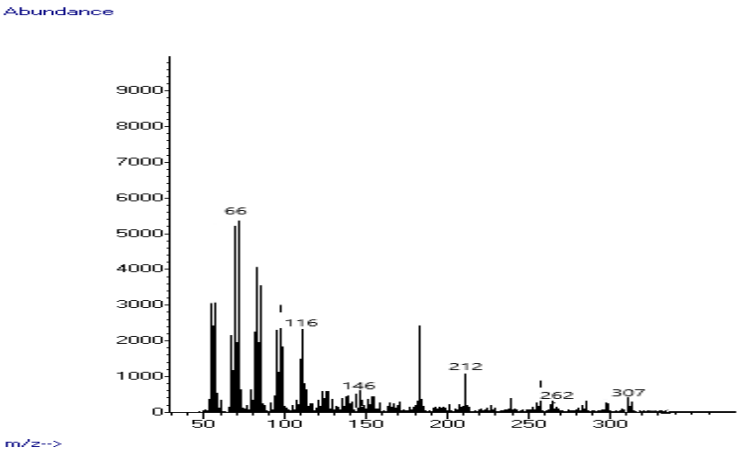


Figure 3. GC-MS Spectrum of the Compound

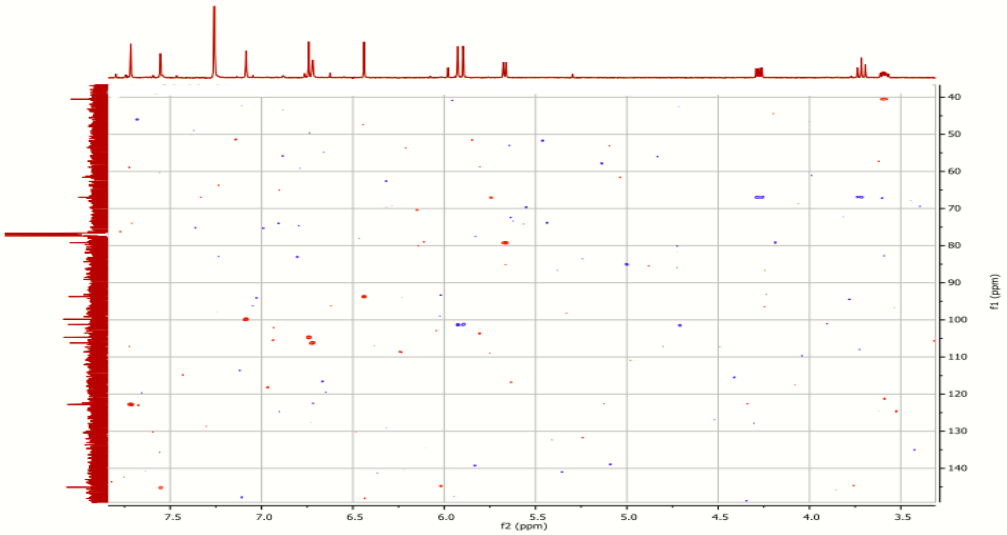


Figure 4. HSQC Spectrum of the Compound

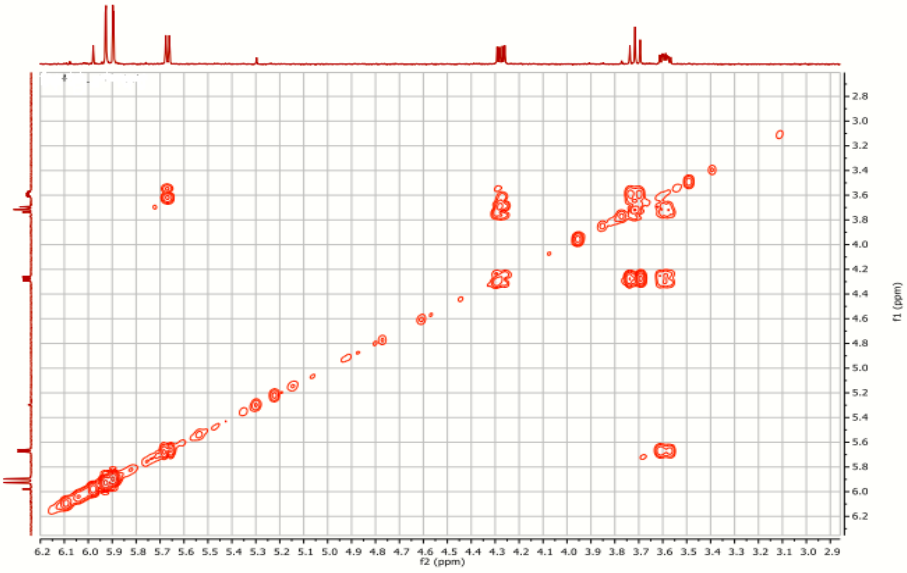


Figure 5. COSY Spectrum of the Compound

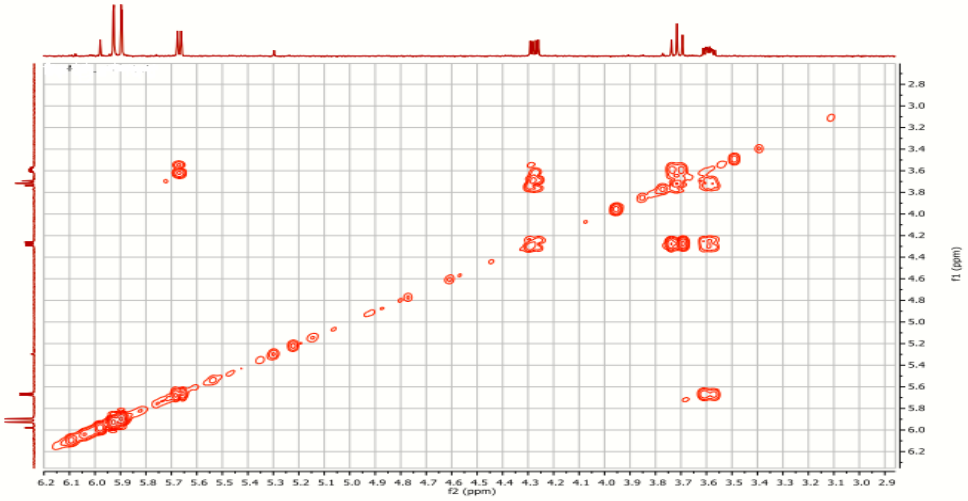


Figure 6. HMBC Spectrum of the Compound

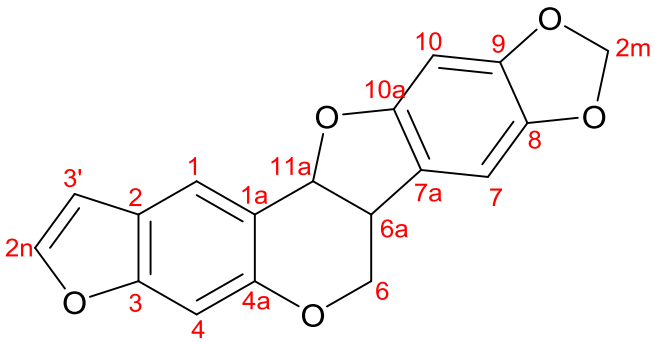


Figure 7: Structure of the Neoduline Compound

Table 2. ^{13}C NMR spectral data of the isolated compound and that of Neoduline from Literature (at 600 MHz for ^{1}H and 150 MHz for ^{13}C NMR; 298 K; δ in ppm)

S/No	Type of Carbon	^{13}C NMR experimental data	^{13}C NMR reference data [25]
1	Methine (CH)	122.8	122.9
2	Quaternary (C)	122.4	122.4
3	Quaternary (C)	154.2	155.7
4	Methine (CH)	99.8	99.9
6	Methylene (CH_2)	67.0	67 [7]
7	Methine (CH)	104.7	104.7 [7]
8	Quaternary (C)	141.8	141.8 [7]
9	Quaternary (C)	148.1	148.2 [7]
10	Methine (CH)	93.7	93.9
1a	Quaternary (C)	116.5	116.7
2n	Methine (CH)	145.1	145.1
2m	Methylene (CH_2)	101.3	101.3
3’	Methine (CH)	106.2	106.3
4a	Quaternary (C)	152.5	153.5
6a	Methine (CH)	77.2	40.6
7a	Quaternary (C)	117.9	117.9
10a	Quaternary (C)	153.5	154.3
11a	Methine (CH)	79.2	79.3

5. Conclusion

The preliminary phytochemical screening of the rhizome of *Dolichos pachyrhizus* methanol extract revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroids, and tannins. The isolated compound from the methanol extract of the sample was obtained as a white powder using both column and thin layer chromatographic methods. The ^{1}H NMR spectrum indicated five singlet protons at δH 7.56, 7.55, 7.06, 6.74, and 6.44 corresponding to their respective carbons at δC 99.83, 122.81, 104.70, 93.72, and 101.27 ppm. The structure of the compound was achieved by the interpretation of ^{1}H-^{1}H correlations in COSY and ^{1}H-^{13}C long range correlations in HMBC and HSQC spectra. Finally, the isolated compound from methanol extract of the rhizome of *Dolichos pachyrhizus* could be assigned as neoduline compound which was consistent to the reported literature values.

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