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A phenolic derivative from the aerial parts of *Indigoferra Arrecta* Hochst. ex A.rich (papilionoiceae)

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ABSTRACT

Indigofera arrecta (Papilionoideae) is a widely recognized medicinal plant traditionally used in various cultures for the treatment of ailments such as epilepsy, snakebites, and jaundice. The therapeutic potential of this plant is attributed to its rich phytochemical composition, which includes bioactive secondary metabolites with significant pharmacological properties. In this study, we report the isolation and structural characterization of a novel phenolic compound,4-[3'-(2'-hexyloxy-ethoxy)-1'-hydroxy-2'-methoxypropyl]-2-methoxyphenol, from the aerial parts of I. arrecta. The compound was purified using chromatographic techniques, and its chemical structure was elucidated through comprehensive spectroscopic analyses, including ultraviolet (UV), infrared (IR), and one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) spectroscopy. To the best of our knowledge, this is the first report of this compound being identified from a natural source. Its structural features indicate a high likelihood of possessing significant pharmacological activities. Given its phenolic nature, it is hypothesized that this compound exhibits antioxidant, anti-inflammatory, and antimicrobial properties, making it a promising candidate for further biological evaluation and potential drug development. These findings contribute to the growing body of research on I. arrecta and its bioactive constituents, reinforcing the plant's traditional medicinal use and expanding the scope of its pharmacological potential. Further studies, including in vitro and in vivo biological assays, are warranted to confirm its therapeutic efficacy and explore its mechanism of action. This research highlights the importance of natural product chemistry in the discovery of novel bioactive compounds with potential pharmaceutical applications.

1. Introduction

Indigofera arrecta (Fabaceae-Papilionoideae) is a perennial herb widely distributed across tropical Africa and utilized in traditional medicine for ailments such as epilepsy, snakebites, jaundice, and

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microbial infections [1]. The plant grows throughout tropical Africa, in northern and eastern South Africa, Swaziland and southern Arabia. It is commonly called natal indigo in English, Elu-eja in Yoruba, Baba in Hausa and Ewere in Igbo. Its therapeutic relevance is reflected in its local use for treating conditions ranging from ophthalmia to gonorrhea, making it a significant species in ethnomedicine [2,3]. Previous phytochemical screening of the aerial parts, seeds, and branches of I. arrecta have revealed saponins, tannins, alkaloids, flavonoids, and cardiac glycosides as key bioactive constituents [4,5]. Recent studies have also reported the isolation of stigmasterol from the aerial parts, further underscoring its chemical and pharmacological potential [6] .

Numerous species within the Indigofera genus have yielded bioactive phenolic and flavonoid derivatives with diverse therapeutic properties, such as anti-inflammatory, antimicrobial, and antioxidant activities [7,8]. For instance, phenolic glycosides and prenylated flavonoids have been isolated from Indigofera tinctoria and Indigofera linnaei, demonstrating strong biological activities [9,10]. Despite the growing interest in this genus, reports on the isolation of phenolic derivatives from I. arrecta remain limited, with most studies focusing on its extracts rather than isolated compounds. This gap limits our understanding of its phytochemical diversity and bioactive potential .

In this study, we report the isolation and characterization of a phenolic derivative, 4-[3'-(2'-hexyloxy-ethoxy)-1'-hydroxy-2'-methoxypropyl]-2-methoxyphenol ("Indigo phenol"), from the aerial parts of I. arrecta. To the best of our knowledge, this is the first report of this compound from natural sources. The isolation of Indigo phenol not only expands the chemical repertoire of the Indigofera genus but also provides insights into its potential therapeutic applications, thus addressing a critical gap in the phytochemical investigation of this ethnomedicinally significant species.

2. Materials and methods

2.1 General experimental procedures

TLC was conducted using silica gel 60 GF254 pre-coated aluminum sheets (Sigma Aldrich, Germany). Spots on TLC were visualized by spraying with 10 % H2SO4 followed by heating within an oven at 105 °C. Column chromatography was conducted using LOBA Cheme Silica gel (60 – 120 μm) mesh. UV was recorded on a UV spectrophotometer while IR was recorded on an FT-IR machine NMR data were recorded on Bruker AVANCE III spectrometer (400 MHz) with residual solvent as internal standard. The melting point was determined on an Electro thermal melting point apparatus.

2.2 Collection, Identification and Preparation of plant material

The plant sample comprising the leaves, stem bark, seeds, fruits (aerial parts) of I. arrecta were collected from Sakaru village old Jos Road, Zaria-Nigeria in March 2017. It was authenticated by Mr. Namadi Sanusi in the Herbarium section of the Department of Botany, Ahmadu Bello University, Zaria by comparison with herbarium reference, voucher specimen (Number 0663) was obtained. The aerial part was shade dried and pulverized.

2.3 Extraction and Isolation

The pulverized aerial part of the plant (1.9 kg) was macerated with 70 % methanol with occasional shaking for 5 days and the solvent was removed using rotary vacuum evaporator at 42 °C to afford the methanol aerial extract (MAE). The extract (178 g) was partitioned using n-hexane (2L), chloroform (1.5L), ethyl acetate (1L) and n-butanol (1L) to obtain n-hexane (HF, 6.04 g), chloroform (CF, 4.04 g), ethyl acetate (EF, 4.93 g) and n-butanol (BF, 8.00 g) fractions.

The chloroform fraction of I. arrecta was selected for further studies and was based on its phytochemical profile (using TLC), particularly the presence of phenolics identified through preliminary tests. The fraction (4.04 g) was subjected to low pressure column chromatography using different solvent systems starting with n-hexane (100 %), mixture of n-hexane: ethyl acetate to ethyl acetate (100 %). A total of 72 collections of 50 mL each were made. The collections were pooled together based on their TLC profile to obtain 23 major fractions coded A-W. Fraction M was subjected to further purification using silica gel column repeatedly to afford a yellow crystalline solid substance labeled IRA.

2.4 Identification and Characterization of IRA

Compound IRA was subjected to physicochemical tests, melting point determination and spectroscopic analysis (UV, IR and NMR) to elucidate its chemical structure.

3. Results and Discussion

Repeated silica gel column chromatographic separation of the chloroform fraction led to the isolation of a yellow crystalline solid substance coded IRA (3 mg). TLC analysis of the compound using n-hexane: ethyl acetate (1:5) and (5:3) as mobile phase gave single homogenous spots with Rf values of 0.84 and 0.53, respectively; Compound IRA was sparingly and completely soluble in chloroform and methanol respectively and the melting point was found to be 138 – 140 °C. The appearance of a greenish color with FeCl3 solution suggests the compound to be phenolic [11]. The UV-VIS spectrum of compound IRA recorded in methanol showed absorption maxima at 275 nm indicating the presence of a chromophore as shown in Figure 1.

The IR spectrum of IRA (Figure 2) showed characteristics absorption frequencies at 3384.4 cm^{-1} typical of the O-H stretching; stretching vibrations due to asymmetric and symmetric methylene groups (C-H) were represented by the bands at 2922.2 cm^{-1} and 2851.4 cm^{-1} respectively. The absorption at 1602.8 cm^{-1} was due to aromatic ring C=C in plane stretching vibrations, 1375.4 cm^{-1} was due to symmetric aliphatic C-H bending of methyl group, 1271.0 cm^{-1} and 1036.2 cm^{-1} was due to C-O-C stretching band of ether, 1121.9 cm^{-1} was due to C-O stretching vibration of alcohol and 820.0 cm^{-1} was due to aromatic out of plane ring [12].

The ^{1}H-NMR spectrum (MeOD, 400MHZ) of compound IRA revealed the presence of three aromatic protons at δH 7.01 (1H, d, J=1.8Hz, H-3), δH 6.87 (1H, dd, J=1.8, 8.1Hz, H-5) and δH 6.84 (1H, d J= 8.1 Hz, H-6) assignable to the tri-substituted benzene ring; the presence of two methoxy protons were clearly depicted at δH 3.94 (s, 3H) and 3.21 (s, 3H) and the proton signal at δH 4.78 (d, J=4.4Hz) was attributed to the presence of an anomeric proton at C-1' (Figures 3-5) [13].

The ^{13}C-NMR and DEPT experiments (MeOD, 400MHZ) revealed nineteen (19) signals among which seven are assigned to the aromatic moiety attributing to one methoxy, three methine and three quaternary carbons atoms; twelve carbon signals were assigned to the C-prenylated units which attributes to one methyl, one methoxy, two methine and eight methylene carbon atoms (Figure 6 – 7) [14]. The HSQC spectrum was used to assign each proton to their respective carbon atoms (Figure 8; Table 1).

HMBC spectrum of IRA was used to establish correlations within the molecule; the long-range correlation HMBC between proton at δH 3.94 and δC149.3 (C-2) and the proton at δH 3.21 showing HMBC and COSY correlations with protons attached to C-1' and C-3' confirmed the attachment of the two methoxy groups to C-2 and C-2' (Figure 9 – 11); attachment of the prenylated chain to C-4 was confirmed via HMBC correlations of δH 4.78 to 1112 (C-3), 120.2 (C-5) and 72.8 (C-3') (Figure 9).

COSY spectrum indicated the correlations between protons that are situated in the same environment; major correlations observed include δH 7.01 # δH 6.87 and δH 4.78 # δH 4.44 among others (Figure 10). Based on the 1D and 2D-NMR data of IRA (Table 1), the chemical structure of compound IRA was proposed as 4-[3'-(2'-Hexyloxy-ethoxy)-1'-hydroxy-2'-methoxypropyl]-2-methoxyphenol (Figure 12).

This study successfully isolated and characterized a phenolic compound, 4-[3'-(2'-hexyloxy-ethoxy)-1'-hydroxy-2'-methoxypropyl]-2-methoxyphenol, from the aerial parts of *Indigofera arrecta*. Its structural features align with those of known bioactive phenolic derivatives, suggesting significant pharmacological potential. The findings provide a foundation for further exploration of its therapeutic relevance, particularly in antioxidant, anti-inflammatory, and antimicrobial applications [15-17] .

By connecting the properties of the compound to the traditional uses of I. arrecta, this research underscores the importance of integrating ethnomedicinal knowledge with modern phytochemical studies. However, the absence of bioactivity data limits definitive conclusions on its pharmacological efficacy. Future studies should prioritize bioactivity assays and expand the investigation to other fractions to identify additional bioactive constituents. This work not only contributes to the growing body of knowledge on Indigofera species but also highlights the plant's potential as a source of novel therapeutic agents, paving the way for its application in drug discovery and development.

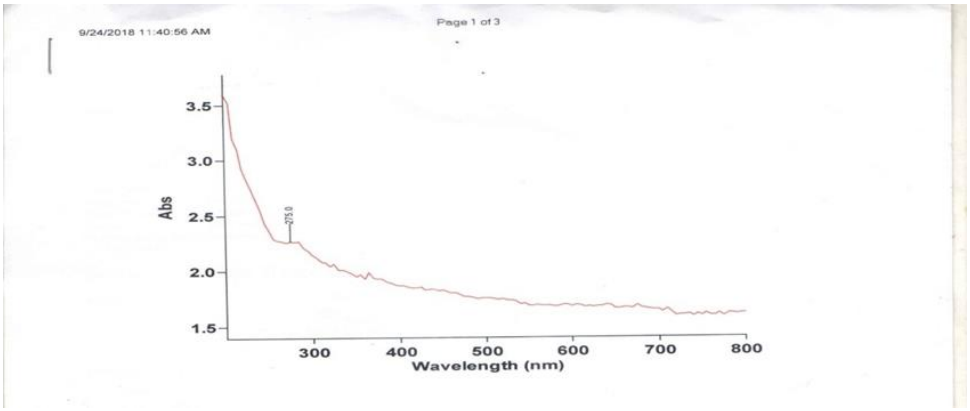

Figure 1. UV-Vis spectrum of compound IRA

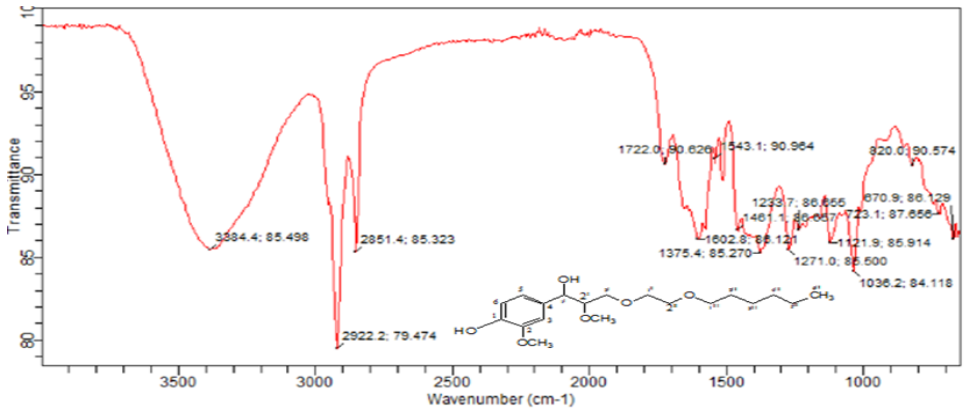

Figure 2. IR spectrum of compound IRA

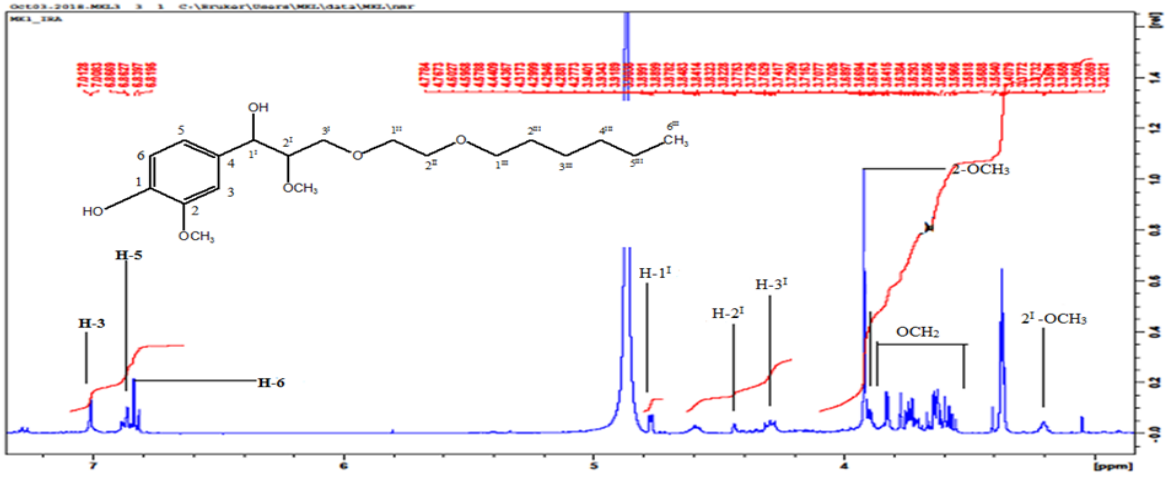

Figure 3. [1]H NMR spectrum of Compound IRA in MeOD

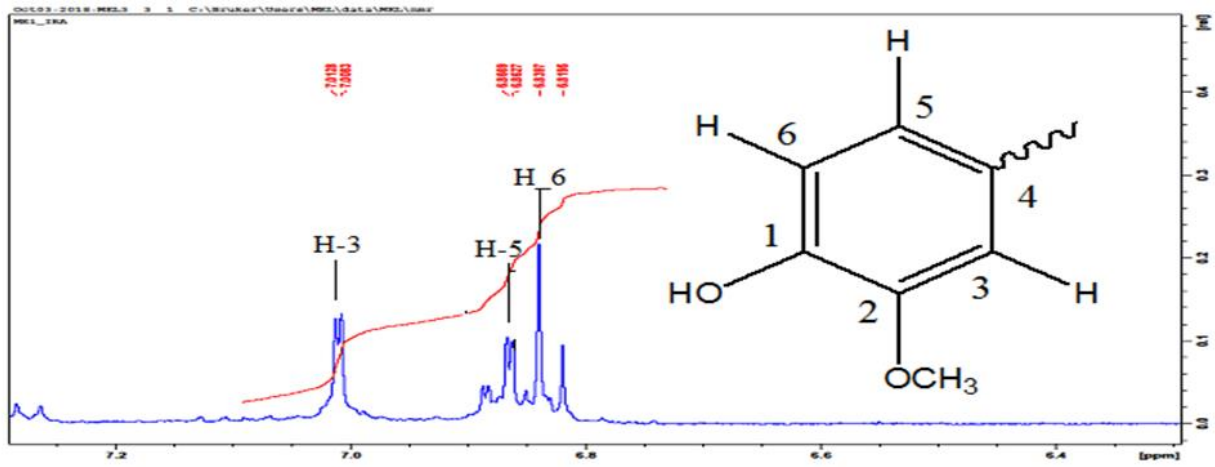

Figure 4. Expanded [1]HNMR of aromatic region of compound IRA in MeOD (400 MHz)

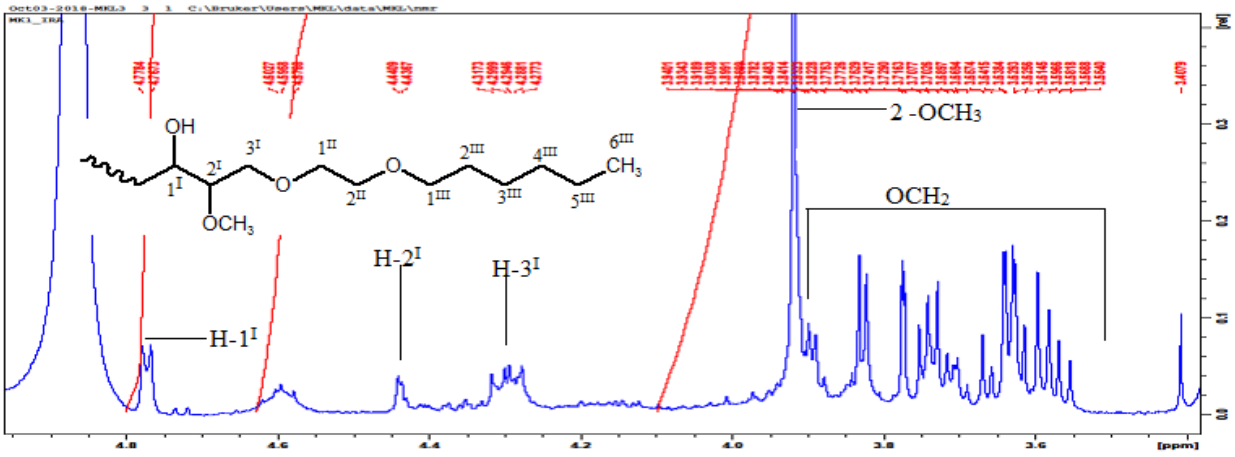

Figure 5. Expanded [1]HNMR of the side chain of compound IRA in MeOD (400MHz)

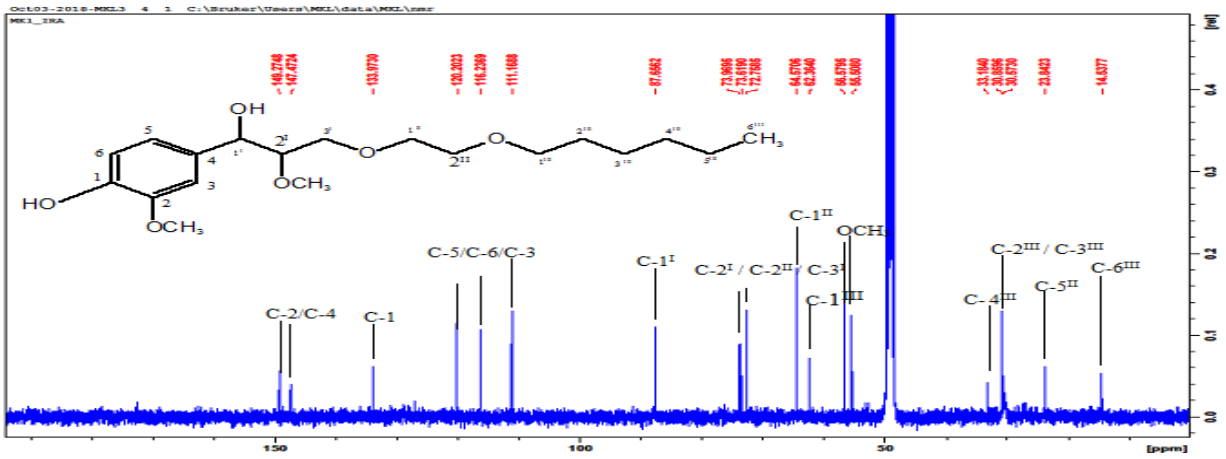

Figure 6. ^{13}C NMR spectrum of compound IRA in MeOD

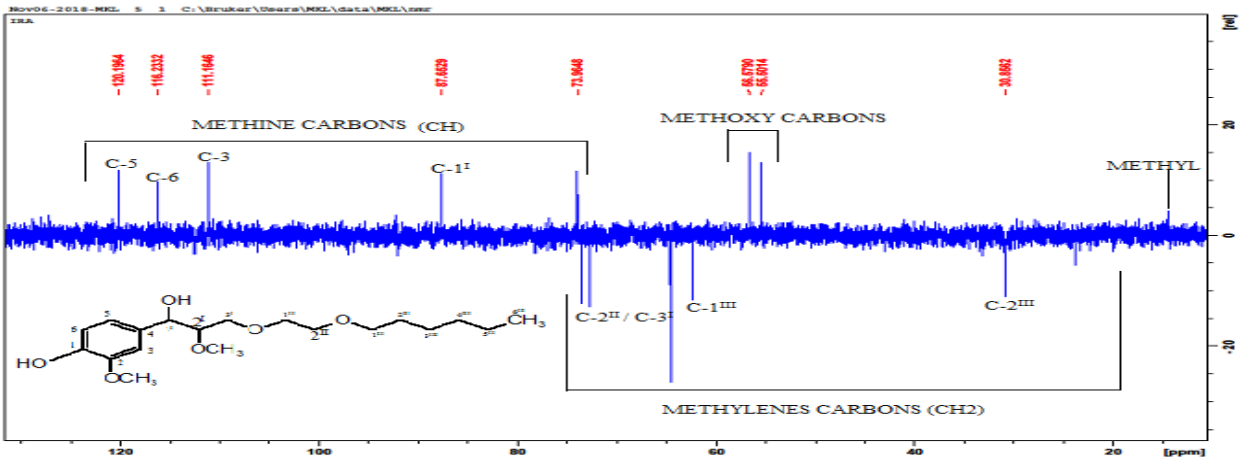

Figure 7. DEPT spectrum of compound IRA in MeOD

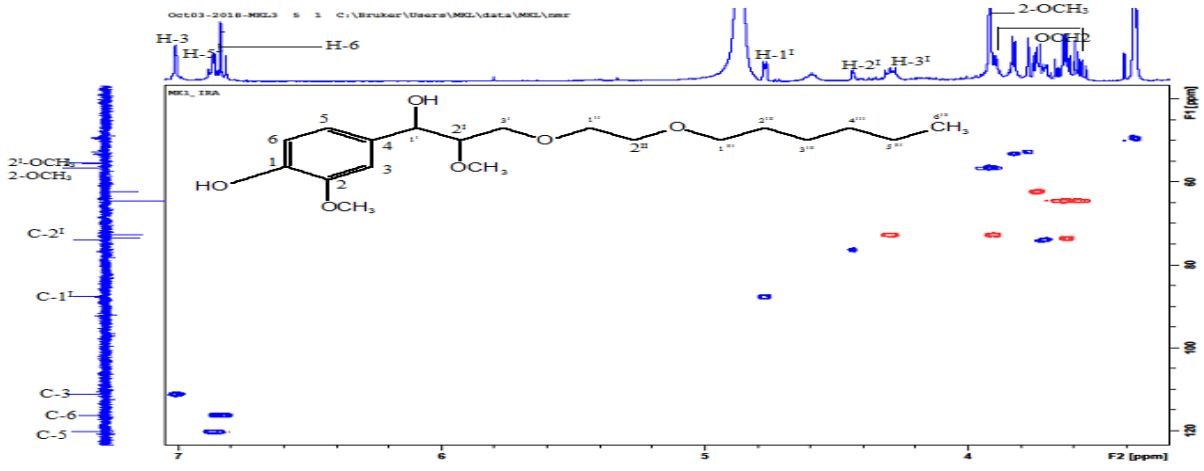

Figure 8. HSQC/DEPT spectrum of compound IRA in MeOD

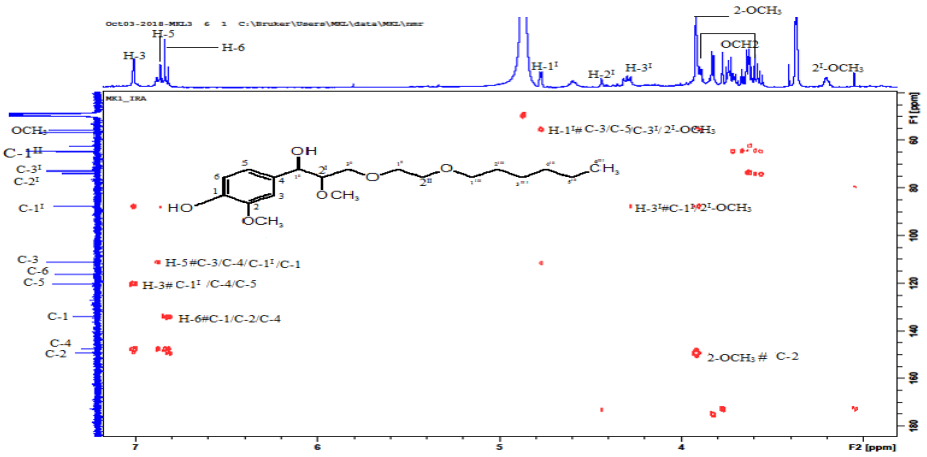

Figure 9. HMBC spectrum of compound IRA in MeOD

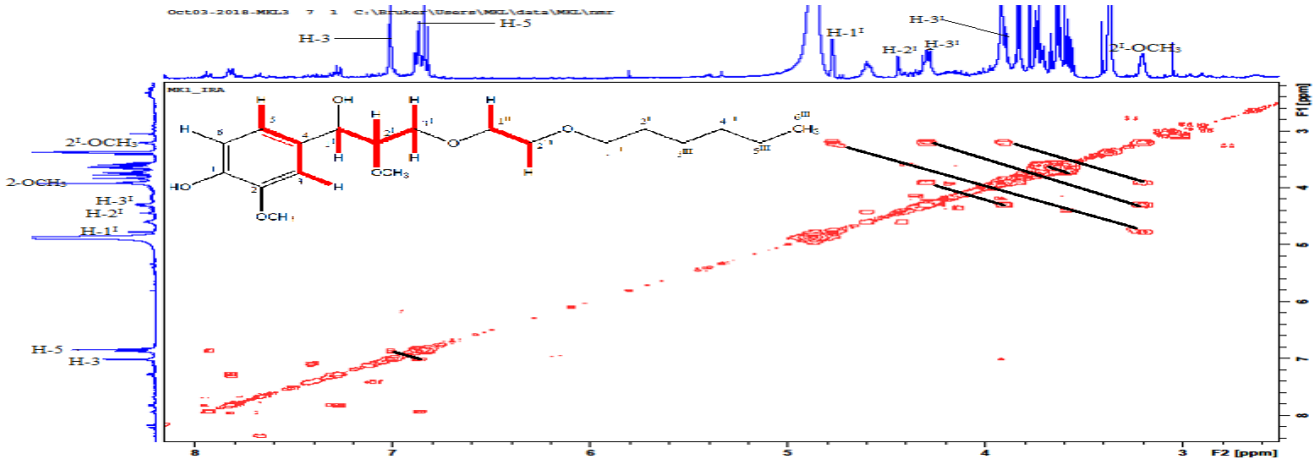

Figure 10. COSY spectrum of compound IRA in MeOD

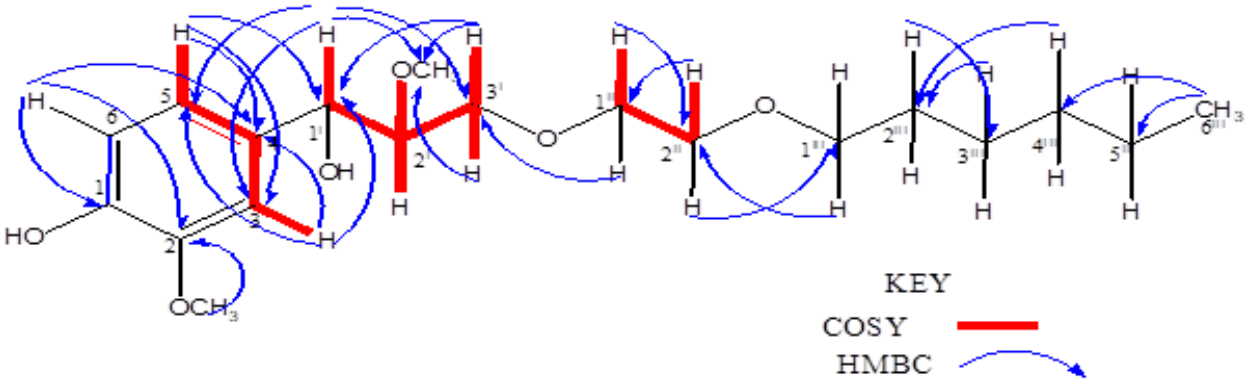

Figure 11. Major COSY & HMBC Correlations of compound IRA

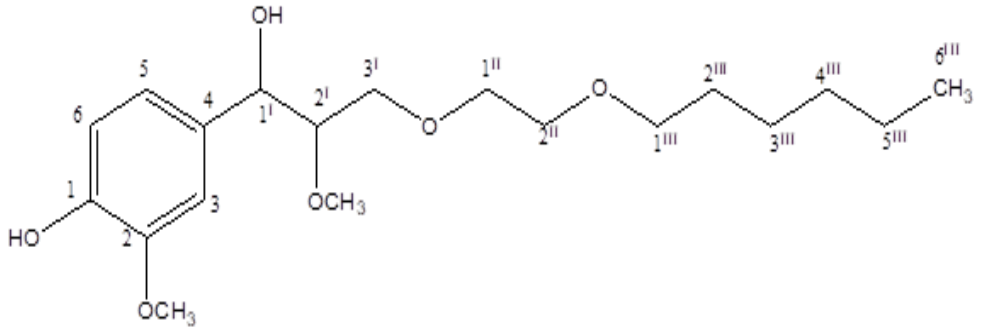

Figure 12. Chemical structure of IRA

Table 1: Summary of 1D and 2D NMR spectra analysis of compound IRA

Position	DEPT	^{13}C	^{1}H-NMR	COSY	HMBC
1	C	133.9	--------	--------	--------
2	C	149.3	--------	--------	--------
3	CH	111.2	7.01(1H, d, *J*=1.8 Hz)	5	4,5, 1'
4	C	147.5	--------	-------	--------
5	CH	120.2	6.87(1H, dd, *J*=1.8, 8.1 Hz)	3	3,4,1'
6	CH	116.2	6.84(1H, d, *J*=8.1 Hz)	--------	1,2,4
1'	CH	87.7	4.78(1H, d, *J*=4.44)	2'	3,5,3',2'-OCH_3
2'	CH	73.9	4.44(1H, d, *J*=1.68)	1'	--------
3'	CH_2	72.8	4.29,3.90	2'-OCH_3	1',2'-OCH_3
1''	CH_2	64.6	3.63,3.58	2''	3',2''
2''	CH_2	73.6	3.72,3.62	1''	1'', 1''
1'''	CH_2	62.4	3.77	-------	2''
2'''	CH_2	30.9	1.45	-------	3'''
3'''	CH_2	30.6	1.41	-------	2'''
4'''	CH_2	33.2	1.41	-------	2'''
5'''	CH_2	23.8	1.42	-------	--------
6'''	CH_3	14.5	0.99	-------	4''',5''
2-OCH_3	OCH_3	56.6	3.94	-------	2
2'-OCH_3	OCH_3	55.5	3.21	1',3'	--------

4. Conclusion

Repeated silica gel column chromatography of the chloroform fraction from the methanol aerial extract of *Indigofera arrecta* resulted in the isolation of a phenolic compound, 4-[3'-(2'-hexyloxy-ethoxy)-1'-hydroxy-2'-methoxypropyl]-2-methoxyphenol. To the best of our knowledge, this is the first report of its isolation from a natural source. Given their structural similarity to bioactive phenolic derivatives, further studies should prioritize evaluating its antioxidant, anti-inflammatory, and antimicrobial properties through in vitro and in vivo assays. Additionally, exploration of other fractions from I. arrecta may uncover complementary bioactive constituents, expanding its potential for pharmaceutical development.

Conflict of Interest

There is no conflict of interest.

Author's Declaration

The authors declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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