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Isolation and Characterization of Two Coumarin Compounds from the Chloroform Fraction of *Scadoxus multiflorus* (Martyn) Raf. (Amaryllidaceae) †

Olaiya Akeem Ayodele *, Tijani Tawakaltu Omolara, Abdullahi Sakynah Musa and Sule Mohammed Ibrahim

Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria 810107, Nigeria; omolaratijani77@gmail.com (T.T.O.); sakynahabdullahi@gmail.com (A.S.M.); mohammedsule@abu.edu.ng (S.M.I.)

* Correspondence: p19phpu8009@abu.edu.ng or dmfact86@gmail.com; Tel.: +234-08068937896

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Abstract: In this study, the aerial parts of *Scadoxus multiflorus* were extracted using methanol through maceration process. The resulting methanol crude extract was subsequently partitioned with solvents including n-hexane, chloroform, ethyl acetate, and n-butanol. Extensive column chromatography separation of the chloroform fraction, followed by isocratic elution of two pooled fractions, led to the isolation of two coumarin derivatives: 2-methyl-2H-chromen-7-ol and 7-methoxy-2H-chromen-2-one. These compounds underwent various physicochemical analyses, such as chemical tests, melting point determination, and solubility assessments. Structural elucidation of the isolated compounds were conducted using UV spectroscopy, FT-IR, and 1D/2D NMR techniques. The final molecular structures were confirmed and named using ChemDraw.

Keywords; *Scadoxus multiflorus*; coumarin; chromen

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1. Introduction

Plants have thrived on Earth for hundreds of millions of years, evolving sophisticated biochemical systems to withstand both external and internal stresses (Zaynab et al., 2018). These chemicals, referred to as secondary metabolites or natural products, are produced by plants to adapt to environmental fluctuations while minimally affecting their cellular and developmental physiological processes (Adedeji and Babalola, 2020). Some key secondary metabolites include; alkaloids, coumarins, flavonoids, steroids, tannins, anthraquinones, and saponins, among others.

Coumarins represent a large and diverse class of compounds widely distributed in the plant kingdom. They are predominantly found in higher plants, and the compound umbelliferone being particularly rich sources. Although coumarins can be found throughout various plant tissues, they are most concentrated in the fruits, followed by the roots, stems, and leaves (Jain and Joshi, 2012). In recent years, coumarin compounds have gained increasing attention for their medicinal properties, particularly for their significant roles in disease prevention and treatment (Matos et al., 2015), including bacteriostatic effects and anti-tumor activities (Jain and Joshi, 2012).

Scadoxus multiflorus, a member of the Amaryllidaceae family, is a flowering plant first described by Thomas Martyn in 1795 as *Haemanthus multiflorus*. In 1838, Constantine Samuel Rafinesque reclassified it into the new genus *Scadoxus*. Commonly known as fire-ball lily, African blood lily, powderpuff lily, and pincushion flower, *S. multiflorus* is primarily native to the tropical regions of Africa (Tsukamoto, 1989) and is cultivated globally for both ornamental and medicinal purposes (Yokosuka et al., 2017). The species is widely

distributed across northern and southern Nigeria, where it is traditionally used by the Fulani to demarcate farm plot boundaries. Additionally, in various parts of Nigeria, it serves as an ointment for treating ulcers, as an antimalarial agent, a cardiotonic, and as a stimulant for alleviating debility (Burkill, 1985).

The objective of this study is to isolate and characterize coumarin compounds from the chloroform fraction of the methanolic extract of the aerial parts of *Scadoxus multiflorus*.

2. Material and Methods

2.1. Plant Material

S. multiflorus aerial parts which comprises the flower, leaves and stem was collected from Kundugi Villiage, Sabon Gari Local Government, Kaduna State Nigeria, on September 2022. The plant material was identified by Mr. Namadi Sanusi of Herbarium unit, Department of Botany, Ahmadu Bello University, Zaria Kaduna State Nigeria, by comparing with existing Voucher specimen number (ABU019006**)**. The aerial parts was shade dried at room temperature to a constant weight and was pounded. A 800 kg of the powdered plant sample was stored at room temperature for use.

2.2. Extraction and Partitioning

The pulverized plant material (800 kg) of *S. multiflorus* was extracted with 15 L of methanol using maceration method for four days with occasional shaking and swirling. The extract was concentrated *in-vacuo* using rotary evaporator at 40 °C to afford a greenish-brown crude methanol extract. The concentrated methanol extract (97 g) was then subjected to successive fractionation with different solvents in sequence and the yield were; n-hexane-18.28 g, chloroform- 10.19 g ethylacetate-5.88 g, and n-butanol- 25.57 g (Alhakmani et al., 2013).

2.3. Isolation and Characterization of Compound **B12** *and* **C11** *from Chloroform Fraction*

The chloroform fraction (10.19 g) was chromatographed over silica gel of 60–120 μm mesh size in a glass column of 75 cm by 3.5 cm and eluted sequentially with n-hexane/ethyl acetate solvent system in respect to other of polarity from nonpolar to polar region. The fractions were monitored by thin layer chromatography (TLC). The TLC plates were sprayed with dilute sulfuric acid followed by heating at 100 °C and viewed under UV light. Similar fractions were grouped according to their TLC profile. The two fractions B and C that were purified using small column of 25 mL with n-hexane: ethyl acetate (50:50) as solvent system using isocratic elusion techniques yielded compound B12 (10 mg) and C11 (13.5 mg). The two compounds were subjected to physicochemical studies (solubility, melting point and chemical test) and the spectral analysis were carried out using UV, IR and NMR analysis (Cheriyan et al., 2017).

3. Results and Discussion

Column chromatographic separation of the chloroform fraction followed by purification of the pooled fraction **B** led to the isolation of a white solid material coded **B12** which gave a single homogenous spot on TLC using two different solvent systems with R_f value of 0.78 using HEX: EA (1:1) and R_f value of 0.63 using DCM: EA (9.5:0.5) and the compound was only visible under UV showed a violet colour. **B12** was completely soluble in chloroform and melted at 122–124 °C. The appearance of a greenish color with $FeCl_3$ solution suggests that compound **B12** might be a phenolic compound (Silva et al., 1998). The UV spectrum of compound B12 recorded in methanol showed absorption maxima at 200.11 nm and absorbance of 3.939 indicating the presence of absorbing chromophore. The IR spectrum of **B12** showed characteristics absorption frequencies at 3428.2cm^{-1} typical of the O-H stretching; stretching vibrations due to asymmetric and symmetric C-H were represented by the bands at 2926.0cm^{-1} and 2855.1cm^{-1} respectively. 1602.8 cm^{-1} and 1509.6 cm^{-1} were due to aromatic ring C=C in plane stretching vibrations, 1461.1cm^{-1} band was

due to aliphatic C-H bending, 1312.0 cm^{-1} was due to C-O-C stretching band of ether, and 820.0 cm^{-1} was due to olefinic C-H bending.

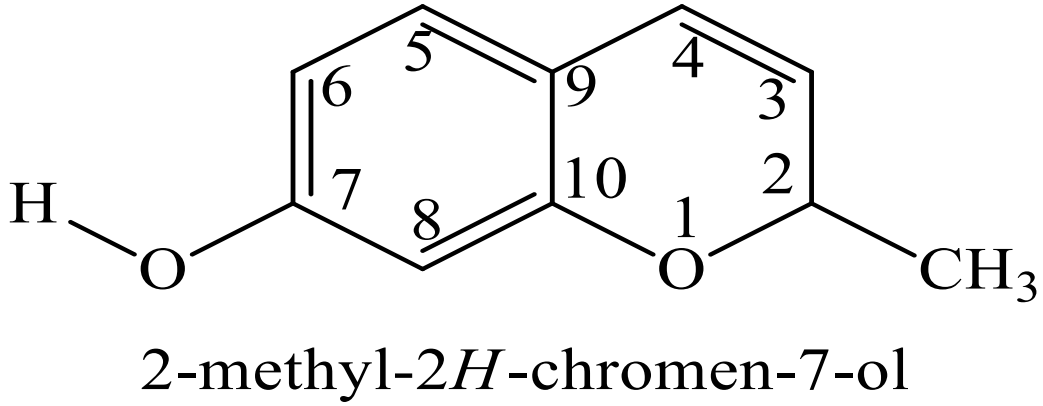
The ^{1}H- NMR revealed a singlet at 0.86 assigned to the methyl group at position two. The singlet, doublet and triplet peaks at 6.89, 8.05, 7.95, 7.46 and 7.75 are assigned protons at 3rd, 4th, 5th, 6th and 8th position protons which showed meta and ortho coupling to each other. C-NMR Spectrum showed the presence of 10 carbon atoms. The peak at 18.08 is assigned to methyl group. The peak at 56.79, 108.15, 124.68, 118.73, 110.45, 129.32, 111.42, 116.42, 151.16 were assigned to C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9, and C-10 respectively. The COSY spectral shows the correlations of protons H-3 with H-4, H-4 with H-3 and H-5, H-5 with H-4 and H-6 and H-6 with H-5. The complete assignment of the signals of compound **B12** was based on proton, ^{13}C-NMR H-H COSY and HSQC. All the data of ^{1}H, ^{13}C, COSY NMR of compound **B12** are shown in Table 1**.** Therefore the proposed structure of **B12** was suggested to be 2-methyl-2*H*-chromen-7-ol as shown in Figure 1.

Table 1: ^{1}H and ^{13}C NMR ($CDCl_3$, 400MH_Z) Chemical Shifts and Coupling Constants for Compound B12 & C11.

Compound B12			Compound C11		
C	δ_H (ppm)	δ_C (ppm)	C	δ_H (ppm)	δ_C (ppm)
1	-----	-----	1	-----	-----
2	4.09 (s)	56.79	2	-----	167.04
3	6.89 (d, J=4.0Hz)	108.15	3	6.90 (d, J=4.0Hz)	118.73
4	8.05 (t, J= 4.0, 8.0Hz)	124.68	4	8.02 (t, J= 4.0, 8.0Hz)	124.01
5	7.95 (t, J= 4.0, 8.0Hz)	118.73	5	7.93 (t, J= 4.0, 8.0Hz)	130.28
6	7.46 (d, J=4.0Hz)	110.45	6	7.49 (d, J=4.0Hz)	110.45
7	OH	129.32	7	-----	160.11
8	7.75(s)	111.42	8	7.72 (s)	111.43
9	-----	116.72	9	-----	116.43
10	-----	151.16	10	-----	152.16
CH_3	0.86 (s)	18.08	OCH_3	3.69 (s)	55.14

Column chromatographic separation of the chloroform fraction followed by column chromatography of column fraction **C** led to the isolation of a white solid material coded **C11** which gave a single homogenous spot on TLC using two different solvent systems with R_f value of 0.78 using HEX: EA (1:1) and R_f value of 0.63 using DCM: EA (9.5:0.5) and the compound was only visible under UV and appeared violet. **C11** was completely soluble in chloroform and melted at 120–123 °C. The appearance of a greenish color with $FeCl_3$ solution suggests that compound **C11** might be a phenolic compound (Silva et al., 1998). The UV spectra of compound C11 recorded in methanol showed absorption maxima at 205.13 nm and absorbance of 3.939 indicating the presence of absorbing chromophore. The IR spectrum of **C11** showed characteristics absorption frequencies at 2922.2cm^{-1} and 2851.4cm^{-1} stretching vibrations due to asymmetric and symmetric C-H. 1673.6cm^{-1} was

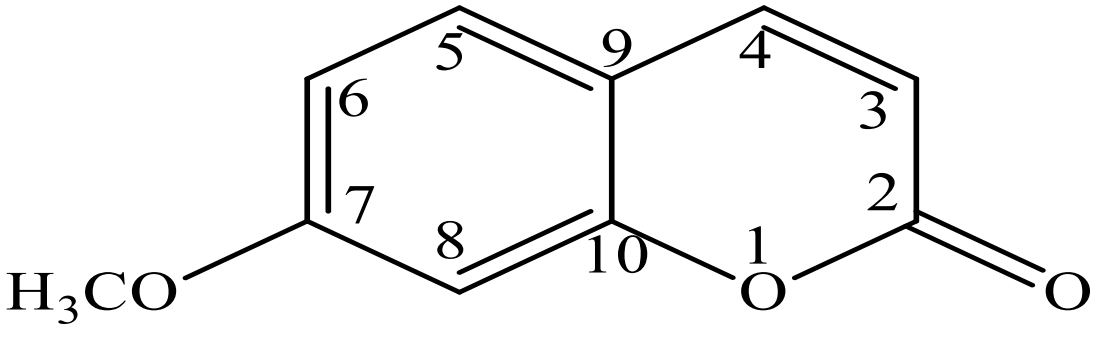
due to stretching vibration of carbonyl group. 1509.6 cm^{-1} was due to aromatic ring C=C in plane stretching vibrations, 1457.4 was due to aliphatic C-H bending vibration, 1315.8 cm^{-1} was due to C-O-C stretching band of ether, 805.1cm^{-1} was due to olefinic C-H bending vibration.



2-methyl-2*H*-chromen-7-ol

Figure 1. Proposed Structure of Compound B12 (2-methyl-2*H*-chromen-7-ol).

The ^{1}H- NMR revealed a singlet at 3.69 assigned to the methoxy group at 7th position. The singlet, doublet and triplet peaks at 6.90, 8.02, 7.93, 7.49 and 7.72 are assigned protons at 3rd, 4th, 5th, 6th and 8th position protons which showed meta and ortho coupling to each other. C-NMR Spectrum showed the presence of 10 carbon atoms. The peak at 55.14 is assigned to methoxy group. The peak at 167.04, 118.73, 124.01, 130.28, 110.45, 160.11, 111.43, 116.43, 152.16 were assigned to C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9, and C-10 respectively. The COSY spectral shows the correlations of protons H-3 with H-4, H-4 with H-3 and H-5, H-5 with H-4 and H-6 and H-6 with H-5. The complete assignment of the signals of compound **C11** was based on proton, ^{13}C-NMR, H-H COSY and HSQC. All the data of ^{1}H, ^{13}C, COSY NMR of compound **C11** are shown in Table 1. These data were compared with that of literature reported by (Bose and Roy, 1936; Natarajan, 1976; Cheriyan *et al.*; 2017) which shows a uniformity with the isolated herniarin from *Eupatorium* plants. Therefore the proposed structure of **C11** was suggested to be 7-methoxy-2*H*-chromen-2-one as shown in Figure 2.



7-methoxy-2*H*-chromen-2-one

Figure 2. Structure of Compound C11 (7-methoxy-2*H*-chromen-2-one).

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Benzopyran, commonly referred to as chromene, is a crucial compound in organic chemistry. Both natural and synthetic benzopyran derivatives exhibit a wide range of biological activities, including anti-inflammatory, anticancer, anti-arthritic, antibacterial,

anti-Alzheimer's, antiviral, and anti-skin disease properties, as well as potential therapeutic effects for autosomal dominant polycystic kidney disease (ADPKD) (Xiu et al., 2017). The two compounds, B12 and C11, feature a benzopyran ring structure similar to coumarins. Coumarins are known to be slightly soluble in water and ethanol, but highly soluble in solvents such as chloroform, diethyl ether, and pyridine (Lide and Milne, 1996; Netea et al., 1996; Budavari, 2005). Coumarins possess an extended π-π conjugated system with electron-rich and charge-transfer properties, making them useful as fluorescent sensors in biological applications. Notably, substitution at the 7-position with electron-donating groups significantly enhances their fluorescence (Raunio et al., 2020). The fact that compounds B12 and C11 were isolated from the chloroform fraction and exhibited fluorescence, as observed under UV light during thin-layer chromatography experiments, strongly suggests they belong to the coumarin class. Furthermore, the biosynthetic intermediate of coumarins derived from polycerasoidol and tocopherol in plants (Bermejo et al., 2019) bears structural similarity to compound B12, further supporting the hypothesis that B12 is a coumarin derivative.

4. Conclusions

This work revealed the Isolation and characterization of 2-methyl-2*H*-chromen-7-ol **(B12)** and 7-methoxy-2*H*-chromen-2-one **(C11)** from the chloroform fraction. With extensive literature findings, it was been concluded that this is the first report on the isolation of these two compounds from chloroform fraction of the methanol aerial parts extract of *Scadoxus multiflorus*.

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Funding:

Institutional Review Board Statement:

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Data Availability Statement:

Conflicts of Interest:

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