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# A Novel Flavonoid-Ester Derivative from the Ethyl acetate Fraction of Nelsonia canescens: Isolation and Structural Elucidation Techniques †

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**Abstract**

The increasing resistance of pathogens to conventional antibiotics has necessitated the search for novel antimicrobial agents from medicinal plants. *Nelsonia canescens*, a plant traditionally used in African and Asian in the management of diseases such as viral infections, cardiovascular, and inflammation, have been reported with antimicrobial activity, was investigated for its bioactive constituents. The whole plant was collected, air-dried, and extracted using 70% methanol. The crude methanol extract was partitioned into hexane, chloroform, ethyl acetate, and butanol fractions respectively. The ethyl acetate fraction was subjected to column chromatography and gel filtration, leading to the isolation of a compound coded $A_1$. The structure of compound $A_1$ was established through UV, FTIR, NMR ($^1H$, $^{13}C$, DEPT, COSY, HMQC, HMBC), and chemical tests. Compound $A_1$ was identified as 2*-hydroxy-4*-phenyl-(2**-hydroxy-ethyl)-3′-(4‴→1″) glucose-rhamnose-3-hydroxy phenyl ester, a flavonoid derivative. Spectral analysis confirmed its structure, with key signals including olefinic protons (δ 6.30 and 7.62) in the trans configuration, aromatic protons, and sugar moieties. The compound exhibited a melting point of 105–107 °C and was sparingly soluble in chloroform but fully soluble in methanol suggesting that the compound is highly polar in nature. This is the first report of isolation of 2*-hydroxy-4*-phenyl-(2**-hydroxy-ethyl)-3′-(4‴→1″) glucose-rhamnose-3-hydroxy phenyl ester from *Nelsonia canescens*, and new to the genus contributing to the taxonomy of the plant. The compound's structural features suggest potential bioactive properties, warranting further investigation into its pharmacological applications through in vitro and molecular docking studies.

**Keywords:** antibiotics; antimicrobial; chalcone; flavonoid; isolation; *Nelsonia canescens*

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## 1. Introduction

Flavonoids constitute one of the most important classes of naturally occurring phenols, serving as protective agents in plants and offering significant benefits for human

health [1]. Over 4000 flavonoids have been identified from various plant sources. Their potential therapeutic applications have been of considerable interest in recent years, as the diverse structures derived from medicinal plants are often perceived to possess immense drug-likeness and biological friendliness, making them excellent candidates for drug development [2–5].

The analysis of flavonoids in complex mixtures relies on various analytical procedures, with chromatographic techniques such as high-performance liquid chromatography (HPLC), column chromatography (CC), and gas chromatography (GC) being among the most successful [6–8]. In contrast, nuclear magnetic resonance (NMR) spectroscopy is a powerful technique that provides insight into mixtures of natural products without requiring prior separation of individual components. Its advantages include simpler, non-destructive sample preparation. NMR has been successfully used for the structural identification of diverse natural products in mixtures, including essential oils, terpenoids, sterols, saponins, anthocyanins, and phenolic acids [8].

Nigeria's rich biodiversity, particularly its tropical rainforests, harbors a vast array of medicinal plants with documented traditional uses. Nelsonia canescens (family Acanthaceae) is one such plant, used in traditional medicine to manage diarrhea, fever, smallpox, hypertension, inflammation, and viral infections [9,10]. Previous phytochemical studies have revealed the presence of saponins, tannins, alkaloids, polyphenols, coumarins, and flavonoids in this plant [11], though only two iridoids: shanzhiside methyl ester and galiridoside [11] have been fully identified to date. There remains a scarcity of studies focused on the systematic isolation and full structural characterization of active secondary metabolites from its ethyl acetate fraction.

This paper reports the structural elucidation of a novel 2*-hydroxy-4*-phenyl-(2**-hydroxy-ethyl)-3′-(4‴→1″) glucose-rhamnose-3-hydroxy phenyl ester, isolated from Nelsonia canescens. The determination of its structure was based on comprehensive spectral data from UV, IR, 1D (COSY, DEPT), and 2D NMR techniques—including HSQC (Heteronuclear Single Quantum Correlation) and HMBC (Heteronuclear Multiple Bond Correlation)—supplemented by chemical tests and comparative analysis of literature data.

## 2. Material and Method

### *2.1. General Experimental Procedures*

The compound was subjected to color and physical appearance were observed and recorded, subjected to chemical test, (Shinoda, ferric chloride test), and melting point determination (Gallenkhamp electro-thermal melting point apparatus). The isolated compound was dissolved in absolute methanol for the UV analysis. The absorbance at a specific wavelength were obtained using Ultraviolet-Visible (UV-Vis) spectroscopy, infrared (Agilent Technologies Cary 6030 FTIR spectrometer, Santa Clara, CA, USA), and for the isolated compounds, the dried sample were dissolved in deuterated methanol (MeOD) for the NRM analysis. Nuclear magnetic resonance (NMR) spectra was recorded at 400 MHz for 1D and 2D (Bruker AVANCE III Instruments Incorporation, Billerica, MA, USA 400 MHz), using TMS as internal standard or by reference to solvent signals.

### *2.2. Plant Material*

The whole plant of Nelsonia canescens was collected in March 2016, from Zaria local government area of Kaduna State. It was confirmed and authenticated by Namadi Sanusi of the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria where a voucher specimen No:1181 was deposited.

*2.3. Extraction and Purification*

The collected plant was air dried under shade for 7 days then it was powdered and weighed. The powdered plant material (3000 g) was extracted with 70% Methanol (10 L) using maceration method for 72 h and the extract was filtered using Whatman No.1 filter paper. The extract was concentrated under reduced pressure to yield 180 g subsequently referred to as crude methanol extract and then partitioned with n-hexane, Chloroform, Ethyl acetate and Butanol to obtain the following yield of fractions: n-hexane fraction (51.65 g), chloroform fraction (1.20 g), ethyl acetate fraction (7.2 g), and butanol fraction (29.23 g) [12,13]. The ethyl acetate fraction was subjected to column chromatographic analysis based on the fact that is demonstrated highest antimicrobial activity among the fractions tested.

*2.4. Isolation of Compound*

2.4.1. Column Chromatography of Ethyl Acetate Fraction

The column was packed using wet slurry method, 7 g of the ethyl acetate fraction was dissolved with ethyl acetate and absorbed over silica gel (60–120 mesh size) was added to form slurry which was allowed to dry. The column was prepared by placing cotton wool at the bottom end of the column; estimated quantity of silica gel was poured into a small amount of n-hexane, stirred vigorously and then poured into the column while stirring and allowed to settle. The absorbed fraction was introduced on to the packed column and was gradiently eluted at a reduction ration of 5% starting from n-hexane (100%) to ethyl acetate: n-hexane (90%:10%) and methanol (100%). A total number of 87 collections were collected and pooled up together based on their TLC profiles to give 14 major pooled fractions A-N.

2.4.2. Gel Filtration Chromatography of $A_1$

Collections from beakers 65–76 of the column chromatography of the Ethyl acetate fraction were pulled together and tagged as M. M was then divided into two M1 and M2, the first portion of M (M1) was then subjected to gel filtration based on TLC profile. The elution was done using methanol 100%, 20 (2 mL) collection were obtained and pooled into 7 fraction coded MA-MG. MC which had a better profile was subject to gel filtration using Sephadex to yield Mc1, MC2 and MC3 which were further purified using Sephadex and 12 collections were made and pooled into 4 fraction based on similarities coded I. I was later subjected to repeated gel filtration which yielded a dark red amorphous compound coded A1 and then $A_1$ was subjected to chemical test and spectroscopic analysis.

## 3. Results and Discussions

Silica gel column chromatographic separation of the ethyl acetate fraction followed by gel filtration over Sephadex LH-20 led to the isolation of a deep red amorphous compound coded $A_1$. $A_1$ gave a single homogenous spot was obtained using Ethyl Acetate: Chloroform: Methanol: Water in a ratio of 15:4:4:1 which was then characterized by physical, chemical and spectroscopic techniques.

Compound A1 tested positive to Ferric chloride test indicating a phenolic nucleus. Also, the melting point range of 105–107 °C further suggested that the compound was isolated pure.

Characterization of isolated compound-one compound was isolated and identified as a novel chalcone and characterized by spectral data (UV, IR, $^{1}$H NMR, $^{13}$C, DEPT, COSY, HMBC, and HSQC) as 2*-hydroxy-4*-phenyl-(2**-hydroxy-ethyl)-3′-(4‴→1″) glucose-rhamnose-3-hydroxy phenyl ester (Figure 1).

The UV absorption maxima at 206 and 219 nm suggested a substituted benzoyl moiety and 332 nm of a carbonyl moiety of an ester [14]. The IR spectrum of $A_1$ revealed major bands at; 3295.0 $cm^{-1}$, which is a characteristic of OH stretch, 2944.6 $cm^{-1}$ which is due to C-H aliphatic stretch, 2150.7 $cm^{-1}$ which is due to aromatic overtone, 1692 $cm^{-1}$, which is due to C=C olefinic stretch in aliphatic chain, 1602 $cm^{-1}$ which is due to C=O stretch and 1446.2 $cm^{-1}$ which is characteristic of C-H scissoring and bending and a sharp peak at 1010.1 due to C=O stretch of an ester [15].

The $^1$H-NMR spectrum showed two doublets at $\delta_H$ 6.30(1H, d, J = 16Hz, H-2) and $\delta_H$ 7.62(1H, d, J = 16Hz, H-1) signifying trans olefinic protons [13,16]. Signals at $\delta_H$ 6.70 (2H, dd, J = 1.6, 8 Hz, H-3*) and δH 6.59 (1H, dd, J = 1.6 Hz, H-5*) ABX trisubstituted benzene ring corresponding to aromatic protons of ring A. Also signals at $\delta_H$ 7.09 (1H, d, J = 1.6 Hz, H-2′), $\delta_H$ 6.98 (1H, dd, J = 1.6,8.0 Hz, H-6′) meta-coupled to each other and $\delta_H$ 6.81 (1H, dd, J = 1.6, 8 Hz, H-5′) ortho coupled to $\delta_H$ 6.98 (1H, dd, J = 1.6, 8.0 Hz H-6′) suggest another tri substitution in ring B [14,17]. The signals for the anomeric proton of the glucosyl and rhamnosyl units appear at δH 5.21 (1H, s) and $\delta_H$ 4.39 (1H, t, J = 8, 17.6 Hz) [13,14]. The high coupling constant observed at (8.0 Hz and 17.6 Hz) are due to the axial coupling with H-4″ proton of the glucosyl moiety and H-1‴ of rhamnosyl which confirms the configuration of both sugars as β-D glucosyl (1–4) rhamnose [14]. Other up field signals around at $\delta_H$ 2.81 (2H, t, J = 6.4, 12 Hz) and 1.12 (3H, d, J = 6.4 Hz) are due to methylene and methyl groups indicating the presence of an aliphatic moiety as a side chain of the molecule [18].

The $^{13}$CNMR spectral analysis revealed the presence of 29 carbon signals, the multiplicities of the carbon atoms were confirmed by the DEPT experiments, which revealed 7 quaternary carbon(C), 18 methine groups (CH), 3 methylene groups ($CH_2$) and 1 methyl group ($CH_3$). The downfield resonances at $\delta_C$ 166.99 (C-3) resembling carbonyl group C=O of a phenyl ester [19] and resonances at $\delta_C$ 113.65 (C-2) and $\delta_C$ 146.67 (C-3) resembling olefinic carbon between the carbonyl moiety (C-3) and benzene. Also the presence of a methylene carbon at resonance at $\delta_C$ 60.99 and methyl carbon at $\delta_C$ 17.08 indicates the presence of sugar (β-glucose –rhamnose) [14].

In the COSY Spectrum of $A_1$, the cross peaks between $\delta_H$ [6.30 and 7.62] of the olefinic moiety, $\delta_H$ [6.59 and 6.70], $\delta_H$ [6.81 and 6.98], $\delta_H$ [6.98 and 7.09] of the aromatic protons in ring A and B $\delta_H$ [5.21 and 3.95], $\delta_H$ [4.39 and 3.36] of the anomeric sugar residue of glucosyl and rhamnosyl, $\delta_H$ [3.84 and 2.81] of the aliphatic side chain in ring A.

The Heteronuclear Multiple Bond Correlation (HMBC) spectral data allowed the establishment of long range connectivity between various units of the molecule. Some of which include $\delta_H$ 7.62 correlated with $\delta_C$ 113.36, 121.88, 166.99 which confirms the attachment of the carbonyl moiety to the olefinic carbon. A signal at $\delta_H$ 7.09 correlated with $\delta_C$ 121.88, 145.41, 146.67 which confirms the attachment of the proton at position 2′ of ring B with the quaternary carbon carrying the sugar residue and that of the hydroxyl group. Signal at $\delta_H$ 6.98 correlated with $\delta_C$ 113.95, 146.67, this confirms that position 1′ of ring B to the olefinic bond and a proton at $\delta_H$ 6.71 correlated with $\delta_C$ 145.41,121.88,130.17 which confirms the attachment of the aliphatic chain at 4‴ and that of the hydroxyl group at 2‴. Other correlations confirmed by the HMBC are those of the sugar residue.

**Table 1.** Summary of 1D and 2D Spectral Data for Compound $A_1$ (MeOD, 400 MHz).

| Position | $^1$H, J(Hz) | $^{13}$C | DEPT | COSY | HMBC |
|---|---|---|---|---|---|
| 1 | 7.62(1H, d, J = 16 Hz) | 146.67 | CH | H-2 | C-2,3,6′ |
| 2 | 6.30(1H, d, J = 16 HZ) | 113.36 | CH | H-1 | C-1,1′ |
| 3 | | 166.99 | | | |
| 1* | | 143 | | | |
| 2* | | 144.71 | | | |
| 3* | 6.70(2H, dd, J = 1.6, 8 Hz) | 115.29 | CH | H-5* | C-1*,4*6′ |

| | | | | | |
|---|---|---|---|---|---|
| 4* | | 130.17 | | | |
| 5* | 6.59(1H, dd, J = 1.6, 8 Hz) | 119.95 | CH | H-6* | C-1*,2*3*6* |
| 6* | 6.70(2H, dd, J = 1.6, 8 Hz) | 115.29 | CH | H-5* | C-1*,4*6′ |
| 1** | 2.81(2H, t, J = 6.4, 12 Hz) | 35.16 | $CH_2$ | H-2** | |
| 2** | 3.84(1H, t, J = 9.2, 18.4 Hz) | 70.96 | $CH_2$ | H-1** | |
| 1′ | | 126.31 | | | |
| 2′ | 7.09(1H, d, J = 1.6 Hz) | 113.95 | CH | H-6′ | C-1′,3′,4′ |
| 3′ | | 145.41 | | | |
| 4′ | | 148.37 | | | |
| 5′ | 6.81(1H, d, J = 8 Hz) | 115.01 | CH | H-6′ | C-1′,3,′4′ |
| 6′ | 6.98(1H, dd, J = 1.6, 8 Hz) | 121.88 | CH | H-2′ | C-2′,3′ |
| 1″ | 4.39(1H, t, J = 8, 17.6 Hz) | 102.79 | CH | H-6″ | |
| 2″ | 4.07(1H, m, J = 8.4, 16 Hz) | 74.60 | CH | | |
| 3″ | 4.07(1H, m, J = 8.4, 16 Hz) | 80.32 | CH | H-4″ | |
| 4″ | 3.36(2H, t, J = 6.4, 12 Hz) | 69.04 | CH | H-3″ | |
| 5″ | 3.36(2H, t, J = 6.4, 12 Hz) | 74.80 | CH | | |
| 6″ | 3.84(1H, t, J = 9.2, 18.4 Hz) | 60.99 | $CH_2$ | H-1″ | |
| 1‴ | 5.21(1H, s) | 101.63 | CH | | |
| 2‴ | 3.66(1H, m, J = 2.4, 8 Hz) | 70.69 | CH | | |
| 3‴ | 4.07(1H, m, J = 8.4, 16 Hz) | 70.87 | CH | | |
| 4‴ | 3.36(2H, t, J = 6.4, 12 Hz) | 72.43 | CH | | |
| 5‴ | 3.66(1H, m, J = 2.4, 8 Hz) | 69.24 | CH | | |
| 6‴ | 1.12(3H, d, J = 6.4 Hz) | 17.08 | $CH_3$ | H-3″ | |

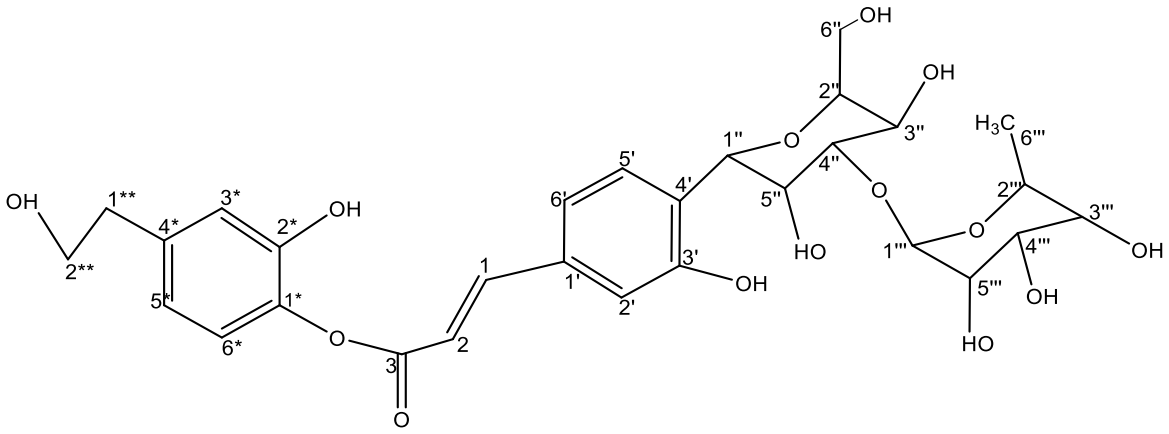


**Figure 1.** 2*-hydroxy-4*-phenyl-(2**-hydroxy-ethyl)-3′-(4‴→1″) glucose-rhamnose-3-hydroxy phenyl ester.

## 4. Conclusions

To the best of our search, this is the first report on the isolation of 2*-hydroxy-4*-phenyl-(2**-hydroxy-ethyl)-3′-(4‴→1″) glucose-rhamnose-3-hydroxy phenyl ester from *Nelsonia canescens*.

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