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Isolation and Characterization of Buchholztrienone A, a Novel Trienone Glycosidic Polyketide from the Stem Bark of *Buchholzia coriacae* Engler (*Capparaceae*)

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Abstract : *Buccholzia coriacea has been used in traditional medicine to treat hypertension, headache, and infectious diseases among others. This study was undertaken to isolate and characterize a new secondary metabolites from the stem bark of Buchholzia coriacea. Cold maceration in ethanol was used to obtain the crude extract which was partitioned between chloroform and n-hexane (1:1). Column chromatography was employed to purify the chloroform fraction, while thin layer chromatography (TLC) was used to monitor the purification process. The structure of the isolated compound was elucidated through characterization of its infra-red (IR) spectrum, proton nuclear magnetic resonance (^{1}HNMR) spectrum, carbon-13 nuclear magnetic resonance (^{13}CNMR) spectrum, distortionless enhancement by polarization transfer (DEPT); 2-dimensional hetronuclear single quantum coherence (HSQC) as well as quadrupule time-of-flight positive electron spray mass spectrometry (Q-Tof MS ES$^+$). The isolated compound was proposed as Buchholztrienone A, a trienone glycosidic polyketide. The IUPAC name is (2Z, 4Z, 6E)-(tetrahydro - 2,3,5,6 - tetramethoxy-2H-pyran-2yl)methoxy)-17-(1,3-dioxan-5-yl)-21-hydroxy-8-(hydroxymethyl)-9-methlyhenicosa-2,4,6-trien-11-one, with molecular formular $C_{37}H_{64}O_{11}$, showing a molecular ion (M^+) peak at 684, a white crystalline solid with R_f 0.86 in n-hexane/chloroform/methanol (40:30:30). The isolated compound is a remarkable contribution to Natural Products research.*

Keywords: *Buchholzia coriacea, Trienone glycoside, Natural product, Structural elucidation, Polyketide compound*

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1.0 Introduction

Buchholzia coriacea, commonly called 'Wonderful kola' of the *Capparaceae* family (Erhirhie et al. 2015), is a perennial evergreen shrub growing up to 20 metres tall. The bark of the plant is smooth, blackish-brown, dark green or grey (Lemmens, 2013). It was named after R.W. Buchholz, who collected the plant in Cameroun in the late 19th century (Anowi et al, 2012). It is also called Elephant kola in English, Its local names include 'obo' or *'Uworo'* in Yoruba, *'Owi'* in Edo, *'Uke'* in Igbo (Quattrochi – Umbeto, 2007). Wonderful kola grows in forest regions with large leaves; conspicuous leaves at the end of its branches (Mbata, Duru and Onwumelu, 2009). Wonderful kola promotes the function of human memory and the nervous system. In traditional medicine, it has been used to treat cardiovascular conditions and hypertension, headache, nasal congestion, sinusitis, bronchitis, eye problems, pleurisy, kidney pains, ear ache, small pox, skin itches, and by local women to treat problems related to menstrual cycles in Nigeria, Gabon and Ivory Coast (Umeokoli et al., 2016).

(a) **(b)** **(c)**

Fig. 1. Morphological features of *Buchholzia coriacea*: (a) tree, (b) seeds, and (c) leaf.

Phytochemicals identifed in were Alkaloids, glycosides, saponin, steroids, tannin, flavonoids, terpenes, reducing sugars and phenol have previously been idenfied in ethanolic extract of seeds of *Buchholzia coriacea* by (Ibrahim and Fagbohun, 2013). Lupeol, β-sitosterol, α–sulphur and cyclooctasulphur were isolated from different parts of *Buchholzia coriacea* (Ojinnaka, Kenne and Abbey, 1992); (Ajaiyeoba, Onocha and Olarewanju, 2001). The presence of three aliphatic acids namely: tetradecanoic acid, hexadecanoic acid and 9,12-octadecadienoic acid were revealed in *Buchholzia coriacea* by (Familoni and Okpuzor, 2000). In a study by Ajaiyeoba et al. (2003), fractions prepared from the methanol extract of *Buchholzia coriacea* stem bark exhibited a high concentration-dependent antibacterial and antifungal activity compared to the standard antibiotics, ampicillin and tioconazole. In the brine shrimp lethality (BSL) assay, the methanol extract was found to be non-toxic with an LC_{50} of 1031 microg/ml. The two main compounds present in the most active fraction were isolated and identified as lupeol and beta-sitosterol.

The use of synthetic medicines have been associated with problems such as adverse drug reactions, deleterious side effects and multiple antibiotic resistance by pathogenic organisms. These have become a major public health concern (Adam and Alan, 2019), (Aiyegoro and Okoh, 2009). In addition, more strains of pathogens have become resistant to many antibiotics and therapeutic agents (Hiroshi, 2009). Meanwhile, literature on isolated compounds from stem bark of *Buchholzia*

coriacae is scanty. The aim of the study was to isolate a novel compound, from the stem bark of *Buchholzia coriacae*. The new compound is an addition to the number of phytochemicals previously isolated from the plant, and may form new lead for further exploration and pharmaceutical development into drugs for therapeutic purposes.

2.0 Materials and Methods

2.1 Sample Collection and Authentication

Fresh stem bark of *Buchholzia coriacea* was collected on 15th October 2024 from a cultivated tree located in a family garden at Umulolo, Ihitte-Ubi, Ahiazu Mbaise Local Government Area, Imo State, Nigeria. Collection was conducted in the early morning hours (6:00 am – 7:00 am) to ensure optimal preservation of phytochemicals. The plant sample was authenticated by a taxonomist at the Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, and a voucher specimen was deposited.

2.2 Sample Preparation

The harvested stem bark was shade-dried at ambient room temperature (27 ± 2°C) for two weeks. The dried material was then ground into a fine powder using a Thomas Wiley milling machine (Model No. 4). The powdered sample was stored in an airtight amber glass container to prevent photodegradation and moisture absorption.

2.3 Extraction Procedure

One kilogram (1.0 kg) of the powdered bark was weighed using an Ohaus Precision Balance (Model PJX203/E) and transferred into a 5 L amber glass bottle. Four litres (4.0 L) of absolute ethanol (analytical grade, Sigma-Aldrich) were added to the sample, and the mixture was left to macerate at room temperature for 72 hours with intermittent shaking.

After extraction, the mixture was filtered through Whatman No. 42 filter paper using a glass funnel. The dark brown residue (marc) was discarded, and the clear filtrate (ethanolic extract) was concentrated under reduced pressure using a rotary evaporator (Büchi Rotavapor R-300) at 45°C, 5 kPa pressure, and 40–55 rpm. The resulting semi-solid crude extract was further dried at room temperature for 48 hours.

2.4 Solvent Partitioning

The dried crude extract was partitioned between equal volumes of *n*-hexane and chloroform (1:1, v/v) following the method of Johnbull et al. (2001), with minor modifications. The chloroform fraction was collected and dried under reduced pressure for further purification.

2.5 Column Chromatography

A portion of the chloroform fraction (18.6 g) was subjected to column chromatography using silica gel (60–120 mesh, Merck) as the stationary phase. The column (60 cm × 3 cm) was packed using the slurry method with *n*-hexane. The sample was pre-adsorbed on a small amount of silica and loaded onto the top of the column. Elution was carried out with increasing polarity solvent systems starting from *n*-hexane/chloroform (90:10) to chloroform/methanol (90:10). A total of 42 fractions (10 mL each) were collected.

2.6 Thin-Layer Chromatography (TLC)

Fractions were monitored using Thin-Layer Chromatography on silica gel plates (Merck, 0.25 mm thickness) under UV light at 254 and 365 nm. The mobile phase system used was *n*-hexane:chloroform:methanol (40:30:30, v/v/v). Plates were developed, air-dried, and sprayed with vanillin-sulphuric acid reagent, then heated at 110°C to visualize the spots. Fractions with similar TLC profiles were pooled and subjected to further purification.

2.7 Spectroscopic Characterization

The pure compound obtained was subjected to various spectroscopic techniques for structural elucidation. Infrared (IR) spectra were recorded using a PerkinElmer FTIR spectrometer, Model Spectrum Two, within the spectral range of 4000 to 625 cm^{-1}. The

sample was prepared as a nujol mull placed between potassium bromide (KBr) plates. Proton (^1H) and carbon-13 (^13C) nuclear magnetic resonance (NMR) spectra were obtained at 500 MHz and 125 MHz, respectively, using a Bruker AM-500 FT-NMR spectrometer with deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent. Additional two-dimensional NMR experiments, including DEPT, HSQC, and HMQC, were conducted to assist in the assignment of proton and carbon signals. Tetramethylsilane (TMS) served as the internal reference standard. High-resolution mass spectrometry (MS) analysis was carried out using an Agilent Quadrupole Time-of-Flight Mass Spectrometer (Q-TOF MS ES^+), operated in positive electrospray ionization (ESI^+) mode, with the scan range set from m/z 50 to 1000.

3.0 Results and Discussion

White crystalline solid with mass 0.032 g was isolated, from column chromatographic elution mixture n-hexane : chloroform (40:60), gave a single spot with in a solvent ratio of *n*-hexane/chloroform/methanol 40:30:30. Rf measurement of the isolated compound was recorded as 0.86.

3.1 FTIR analysis

The infrared spectrum in Fig. reveals the presence of O-H of alcohol which is evident from the broad band extending from 3100 to 3600 cm^{-1}. The recorded absorption frequencies for OH in this study compares with the report of (Sultama and Ali, 2018) in their isolation and characterization of 6-α-D-Pentaglucose from fruits of *Phoenix dactylifera*. The C=C absorption band around 3100 cm^{-1} is subsumed within the O-H absorption in this spectrum. The absorption at 2921.7 cm^{-1} with a strong intensity is indicative of C-H asymmetric stretching vibration. The adjacent strong absorption frequency at 2852.2 cm^{-1} indicates C-H symmetric vibration. The medium intensity absorption at 1463.0 cm^{-1} is due to scissoring vibrational bending modes of the C-H bond typical of methyl. Carbonyl group of ketone was observed as a strong band at 1739.6 cm^{-1}. A strong stretching vibration at 1162.2 cm^{-1} is related to C-O bond of ether, this is supported by the strong absorption band at 1097.3 cm^{-1} which gives a strong evidence for C-O-C asymmetric stretching of a dialkyl ether.

3.2 NMR analysis

The ^1H NMR spectrum of the isolated compound with data presented in table 1, reveals the presence of two methyl protons at δ 1.0 (H-1, singlet) and δ 0.84 (H-9b, doublet); eleven aliphatic methylene protons at δ 3.35 (H-8b, doublet), δ 1.19, 2.00 (H-10, doublet), δ 1.30 (H-13, quartet), δ 1.29 (H-14, multiplet), δ 1.29 (H-15, multiplet), δ 1.20 (H-16, quartet), δ 1.20 (H-18, quartet), δ 2.79 (H-19, multiplet), δ 1.10, 1.29 (H-20, multiplet), δ 3.88 (H-21a, triplet) and δ 2.10, 1.61 (H-3', triplet). Seven aliphatic methine protons at δ 2.30 (H-8a, quartet), δ 2.33 (H-9a, multiplet), δ 1.30 (H-17, multiplet), δ 3.27 (H-2'a, triplet), δ 3.017 (H-4'a, quartet), δ 5.411 (H-5'a, doublet) and δ 2.29 (H-5'', multiplet). Six olefinic methine protons at δ 5.42 (H-2, multiplet), δ 5.413 (H-3, doublet of doublet), δ 5.401 (H-4, doublet of doublet), δ 5.407 (H-5, doublet of doublet), δ 5.406 (H-6, doublet of doublet) and δ 5.412 (H-7, doublet of doublet). One alkoxy methine proton at δ3.88 (H-12, triplet); six methylene alkoxy protons, at δ 3.64, 3.71 (H-1'c, singlet), and δ 3.20, 3.41 for both (H-4'', doublet) and (H-6'' doublet). Four methoxy protons at δ 3.19 (H-1'b, singlet), δ3.19 (H-2'b, singlet), δ 3.19 (H-4'b, singlet) and δ 3.19 (H-5'b, singlet); two methylene- dioxy protons at δ 4.50 and 4.60 (H-2''a and b respectively singlet). Finally, two alcohol hydroxyl protons were observed at δ 2.40 (H-8b' singlet) and δ 2.41 (H-21b singlet). The two olefinic methine protons at H-4 and H-5 showing resonances at δ 5.407 and δ 5.412 respectively are coupled with a coupling

constant (J) of 2.09 Hz, which is in line with data from (Cheng and Thomas, 2012) and suggests a cis configuration.

The ^{13}C NMR and DEPT spectra of the isolated compound whose results are displayed in table 1, reveals the presence of two methyl primary carbons at δ 19.0 (C-1), (C-9b); four methoxy carbons at δ 58.45 (C-1'b), δ 50.40 (C-2'b), δ 50.36 (C-4'b) and δ 58.47 (C-5'b); eleven secondary aliphatic methylene carbons at δ 68.81(C-8b), δ 40.0 (C-10), δ 28.0 (C-13), δ 22.0 (C-14), (C-15), δ 34.8 (C-16), (C-18), δ 26.0 (C-19), δ 38.0 (C-20), δ 62.30 (C-21 a) and δ 27.0 (C-3'); three secondary alkoxy carbons at δ 61.48 (C-1'c), δ 73.87 (C-4'') , δ 73.84 (C-6'') and one secondary dioxy methylene carbon at δ97.98 (C-2''). Seven tertiary aliphatic carbons at δ 38.1 (C-8a), δ 45.59 (C-9a), δ 29.0 (C-17), δ 81.14 (C-2'a), δ 87.90 (C-4'a), δ 114.02 (C-5'a) and δ 35.0 (C-5''); six tertiary olefinic carbons at δ 129.00 (C-2), δ 131.00 (C-3), δ 136.00 (C-4), δ 136.00 (C-5), δ 134.00 (C-6), δ 142 (C-7) and one tertiary alkoxy carbon at δ 88.0 (C-12); one quartenary carbon at δ 115.71 (C-2a) and one carbonyl carbon at δ 211.83 (C-11). These assignments are in agreement with (Minstry, 2009). Based on data from the DEPT spectrum (Fig. 4.6), six methylene carbons can be identified. They include the $–CH_2-$ carbons at δ 97.00 (C-2''), δ 78.00 (C-4''). δ 93.00 (C-6''), δ 82.69 (C-1'c), δ 63.08 (C-8b) and δ 68.81 (C-21a).

Table 1: ^{1}H and ^{13}C Assignment of the isolated compound

No.	C (ppm)	H (ppm)	Type	Number of Hydrogen	Multiplicity
1	19.0	1.0	$-CH_3$	3H	s
2	129	5.42	H–C=	1H	m
3	131	5.413	=C–H	1H	dd
4	136	5.401	H–C=	1H	dd
5	136	5.407	=C–H	1H	dd
6	134	5.406	H–C=	1H	dd
7	142	5.412	=C–H	1H	dd
8a	38.1	2.30	-CH	1H	q
8b	63.08	3.35, 3.91	$-CH_2-$	2H	d
8b′	–	2.40	–OH	1H	s
9a	45.59	2.33	-CH	1H	m
9b	19.0	0.84	$-CH_3$	3H	d
10	40.0	1.19, 2.00	$-CH_2-$	2H	d
11	211.83	–	C=O	–	–
12	88.0	3.88	-O–CH	1H	t
13	28.0	1.30, 1.18	$-CH_2-$	2H	q
14	22.0	1.29, 1.26	$-CH_2-$	2H	m
15	22.0	1.29, 1.16	$-CH_2-$	2H	m
16	34.8	1.20, 1.26	$-CH_2-$	2H	q
17	29.0	1.30	-CH	1H	m
18	34.8	1.20, 1.26	$-CH_2-$	2H	q
19	26.0	2.79, 1.17	$-CH_2-$	2H	m
20	38.0	1.10, 1.29	$-CH_2-$	2H	m
21a	68.81	3.88, 2.40	$-CH_2-$	2H	t

21b	–	2.41	–OH	1H	s
1′c	82.69	3.71, 3.64	-CH_2–O–	2H	s
1′a	115.71	–	-C-	–	q
1′b	58.45	3.19	CH_3O–	3H	s
2′a	81.14	3.27	-CH	1H	t
2′b	50.40	3.19	CH_3O–	3H	s
3′	27.0	2.10, 1.16	-CH_2-	2H	t
4′a	87.9	3.017	-CH-	1H	q
4′b	50.36	3.19	–OCH_3	3H	s
5′a	114.02	5.411	-CH-	1H	d
5′b	58.47	3.19	–OCH_3	3H	s
2″	97.00	4.60, 4.50	–O–CH_2–O–	2H	s
4″	78.00	3.20, 3.41	–O–CH_2–	2H	d
5″	35.00	2.29	-CH-	1H	m
6″	93.00	3.20, 3.41	-CH_2–O–	2H	d

***** m= multiplet, t = triplet, d = doublet, dd = doublet of doublet, s = singlet, q= quartet***

Heteronuclear Multiple Quantum Coherence (HMQC) is a 2-dimensional correlation spectroscopy which relates protons to their attached heteronuclei. Data from this experiment was useful in proposing the carbon framework of the molecule. Investigation of data from the 2-dimensional HMQC spectrum of the isolated compound led to the establishment of correlations between the aliphatic secondary carbon (C-19) at δ 26.0 and one of the two methylene protons multiplet (H-19) attached to it showing resonance at δ 2.79. Correlation was also revealed between the aliphatic tertiary carbon at δ 38.1(C-8a) and the 1H quartet proton at δ 2.30. Similar coupling was established between the aliphatic tertiary C-5'' carbon at δ 35.0 of the 1,3-dioxan system and its bonded aliphatic methine proton at δ 2.29. The aliphatic tertiary carbon C-17 at δ29.0 correlated to one proton multiplet at δ 1.30. Also, the secondary aliphatic carbon C-10 at δ 40.0 correlates to the two attached protons at δ 1.19 and 2.00 resonating as a doublet. The C-20 aliphatic secondary carbon at δ 38.0 was observed to correlate to the two protons methylene multiplet at δ 1.10 and 1.29. Finally, two methyl carbons C-1 and C-9b both resonating at δ 19.0 are each correlated to three equivalent protons at δ 1.0 (singlet) and δ 0.84(doublet) respectively. All correlations above substantiated the assignments made in the aglycone part of the molecule. In the pyran ring system in which methoxy groups are almost ubiquitous, key structural information was obtained by the correlation between the aliphatic secondary carbon C-3' at δ 27.0 and the two bonded protons at δ 2.10 and 1.61, these two protons also having two adjacent protons resonate as a triplet.

The mass spectrum shows a molecular ion peak M^+ at m/z 684, accounting for the molecular mass of the characterized molecule with a molecular formular $C_{37}H_{64}O_{11}$. The molecular ion was identified as a tetramethoxy glycoside derivative named as (2Z, 4Z, 6E)-12-((tetrahydro-2,3,5,6-tetramethoxy-2*H*-pyran-2-yl)methoxy)-17-(1,3-dioxan-5-yl)-21-hydroxy-8-(hydroxymethyl)-9-methylhenicosa-2,4,6-trien-11-one.

As shown by the fragmentation pattern given in Fig. 2, a C-C bond cleavage of the molecular ion between C13 and C14 gives (2Z,4Z,6E)-12(tetrahydro-2,3,5,6-tetramethoxy-2Hpyran-2yl) methoxy -8-(hydroxymethyl)-9 methyltetradeca-2,4,6-trien-11-one, with molecular formular $C_{26}H_{44}O_8$ and m/z 484; the remainder from the molecular ion is 5-(1,3-dioxan-5yl)hept-6-en-1ol), with molecular formular $C_{11}H_{22}O_3$, with m/z 200.

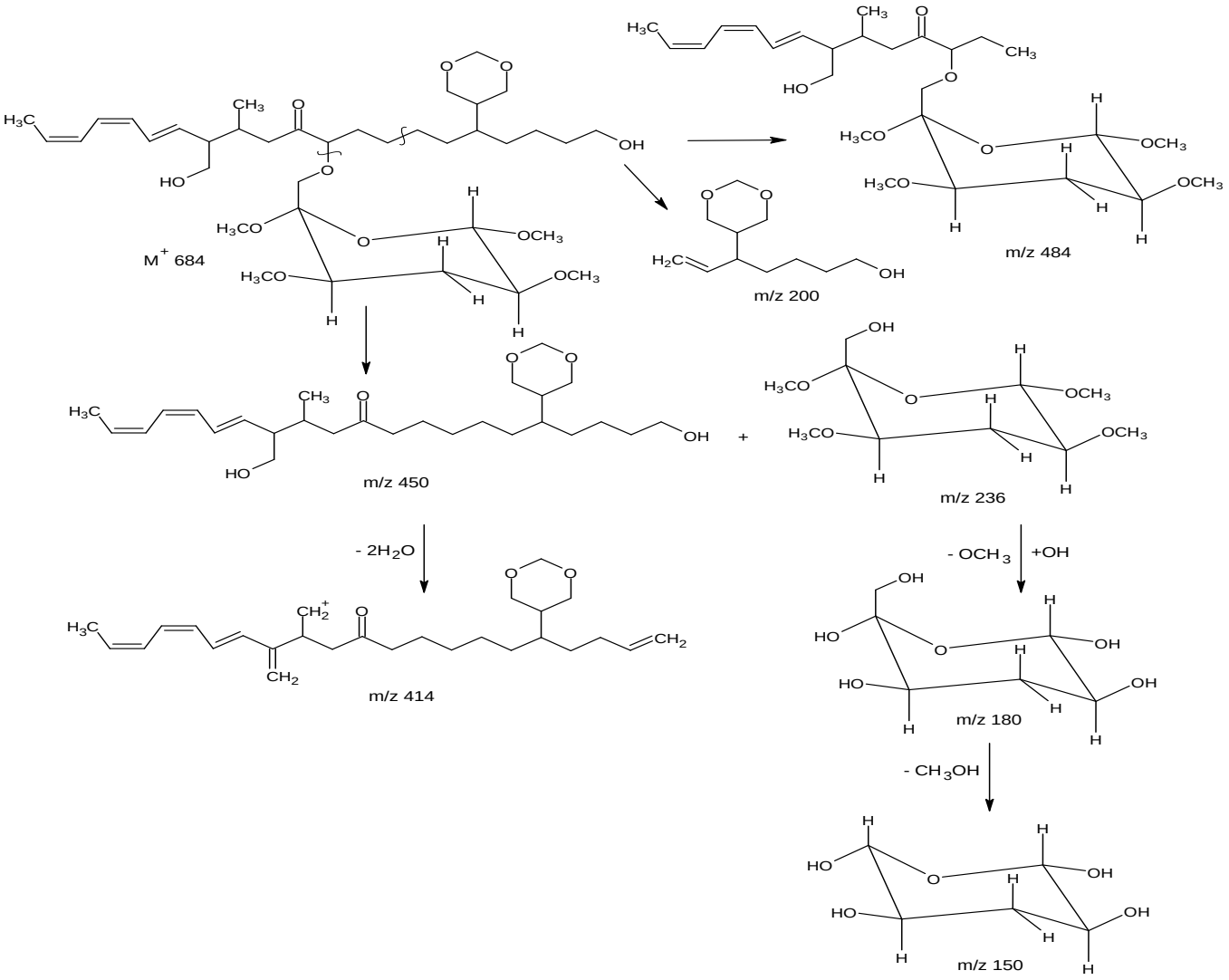

Fig. 2. Fragmentation pattern of the isolated compound

From the molecular ion also, a carbon – oxygen cleavage gave two fragments, The aglycone (2Z,4Z,6E)-17-(1,3-dioxan-5-yl)-21-hydroxy-8-(hydroxymethyl)-9-methylhenicosa-2,4,6-trien-11-one, with molecular formular $C_{27}H_{46}O_5$ and m/z 450; the second fragment is a tetramethoxylated sugar, (tetrahydro-2,3,5,6-tetramethoxy-2*H*-pyran-2-yl) methanol, with molecular formular $C_{10}H_{20}O_6$, and m/z 236. Loss of two molecules of water from the aglycone above affords a tetraenone derivative, (15E, 17Z, 19Z)-5-(1,3-dioxan-5yl)-13-methyl-14-methylenehenicosa-1,15,17,19-tetraen-11-one,

$C_{27}H_{42}O_3$, m/z 414, which accounts for the base peak and was formed by loss of a proton from the fragment with m/z 414. Exchanging all the four methoxy groups with hydroxyl groups in the sugar moiety gives the hydroxylated sugar, (tetrahydro-2,3,5,6-tetrahydro-2*H*-pyran-2-yl) methanol, with molecular formular $C_{10}H_{10}O_6$ and m/z 180. Loss of methanol from the last sugar gives tetrahydro-*2H*-pyran-2,3,5,6-tetraol with molecular formular $C_5H_{10}O_5$, m/z 150.

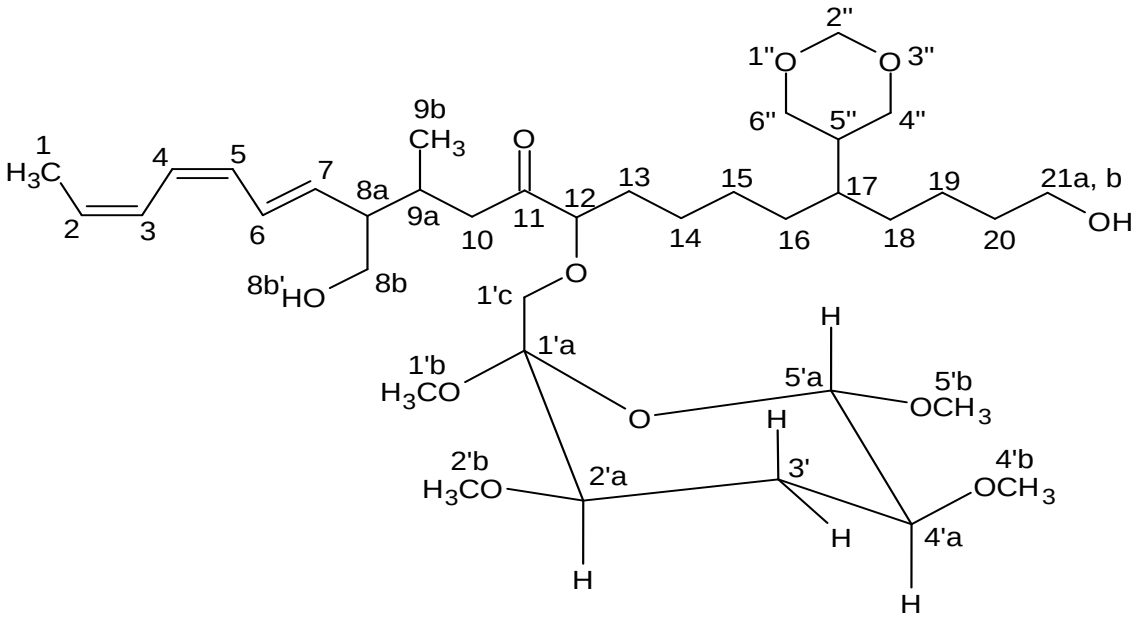

Fig. 3. Buchholztrienone A, a novel trienone glycosidic polyketide isolated from chloroform fraction of the stem bark of *Buchholzia coriacae* Engler

IUPAC Name: (2Z,4Z,6E)-12((tetrahydro-2,3,5,6-tetramethoxy-2*H*-pyran-2yl)methoxy)-17-(1,3-dioxan-5yl)-21-hydroxy-8-(hydroxymethyl)-9-methylhenicosa-2,4,6-trien-11-one, $M^+ = 684$,
Molecular Formular $C_{37}H_{64}O_{11}$

4.0`Conclusion

In this study, a novel trienone glycosidic polyketide named Buchholztrienone A was successfully isolated and structurally elucidated from the stem bark of *Buchholzia coriacea*. Spectroscopic analyses confirmed its unique structure, which includes a rare conjugated trienone backbone and a tetramethoxylated sugar moiety. This compound represents a new addition to the phytochemical profile of the plant and highlights the species' potential as a source of bioactive natural products. Given its structural complexity, Buchholztrienone A may serve as a lead compound for further pharmacological investigations. Future research should explore its biological activities and therapeutic relevance, especially in antimicrobial and anti-inflammatory applications.

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Declaration

Consent for publication

Not applicable

Availability of data

Data shall be made available on demand.

Competing interests

The authors declared no conflict of interest

Ethical Consideration

Not applicable

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Authors' Contribution

Chidi P. Njoku conceived the study, supervised the research, and led data interpretation and manuscript preparation. Bright C. Onyekwere handled spectroscopic analysis and data validation. Rosemary I. Uchegbu carried out plant collection, extraction, and compound isolation. Chintua E. Igara assisted in experimental work and literature review. All authors reviewed and approved the final manuscript.

SPECTRA DATA

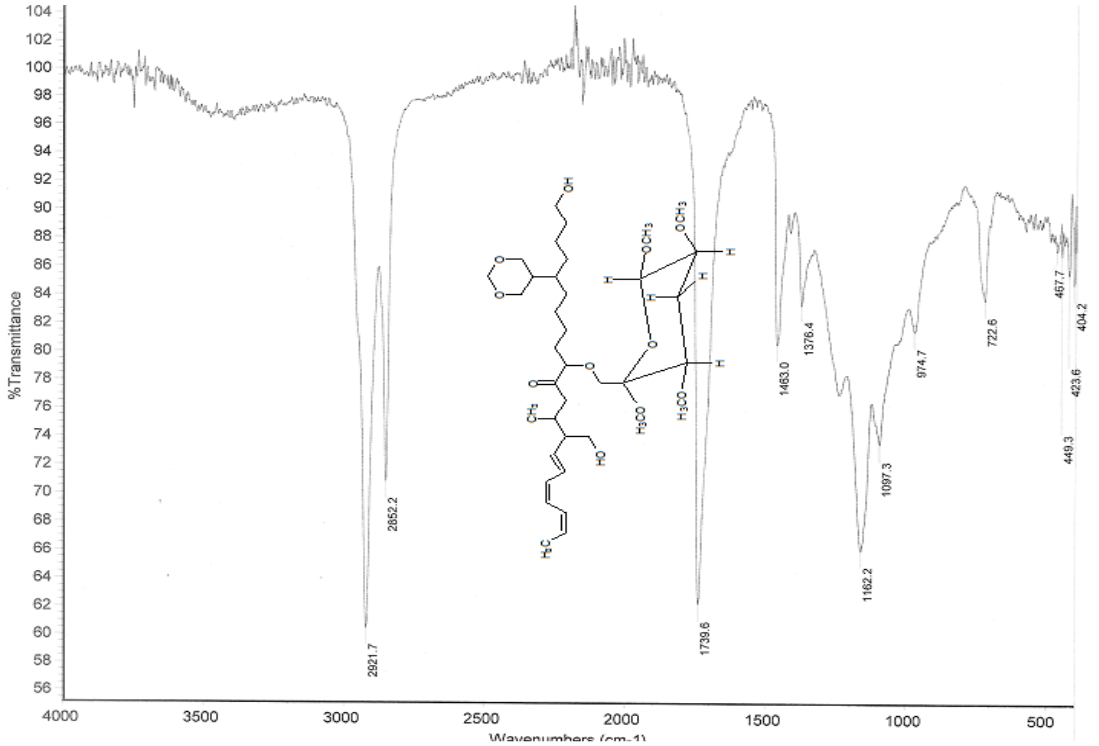

Fig. 4. IR spectrum of the isolated compound

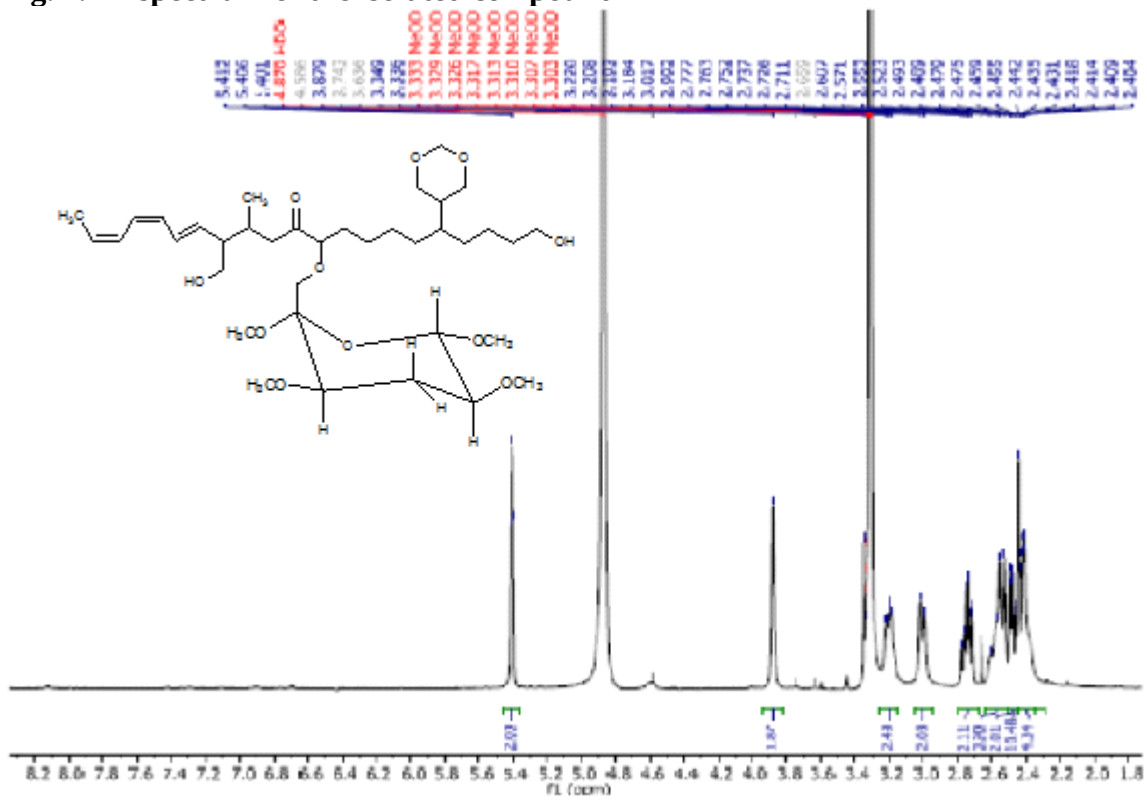

Fig. 5. [1]HNMR spectrum of the isolated compound

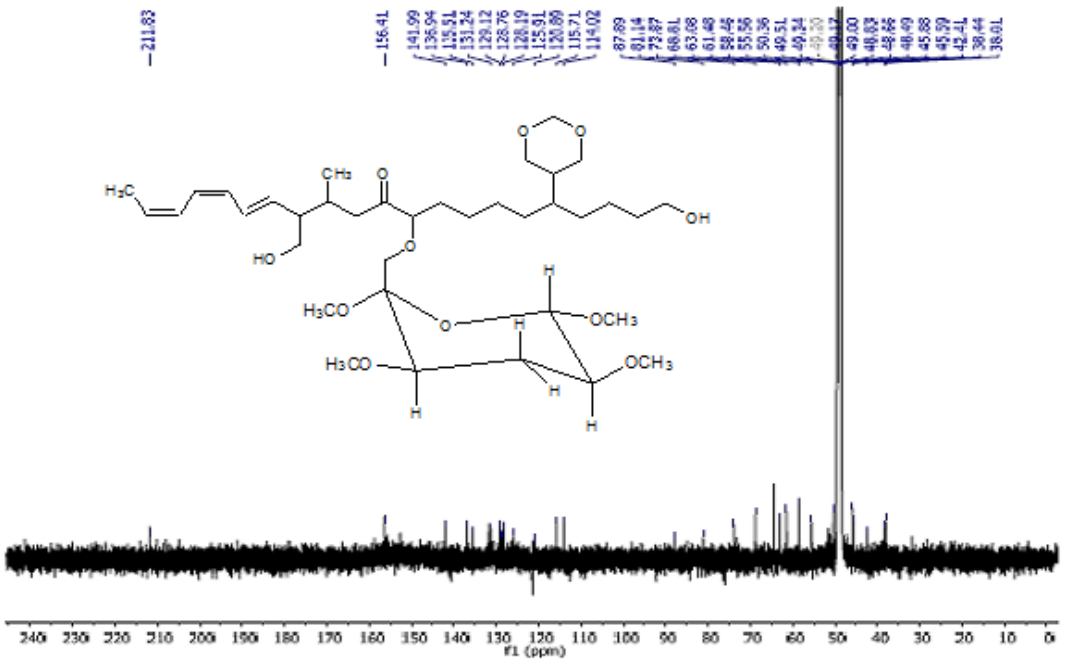

Fig. 6. DEPT spectrum of the isolated compound

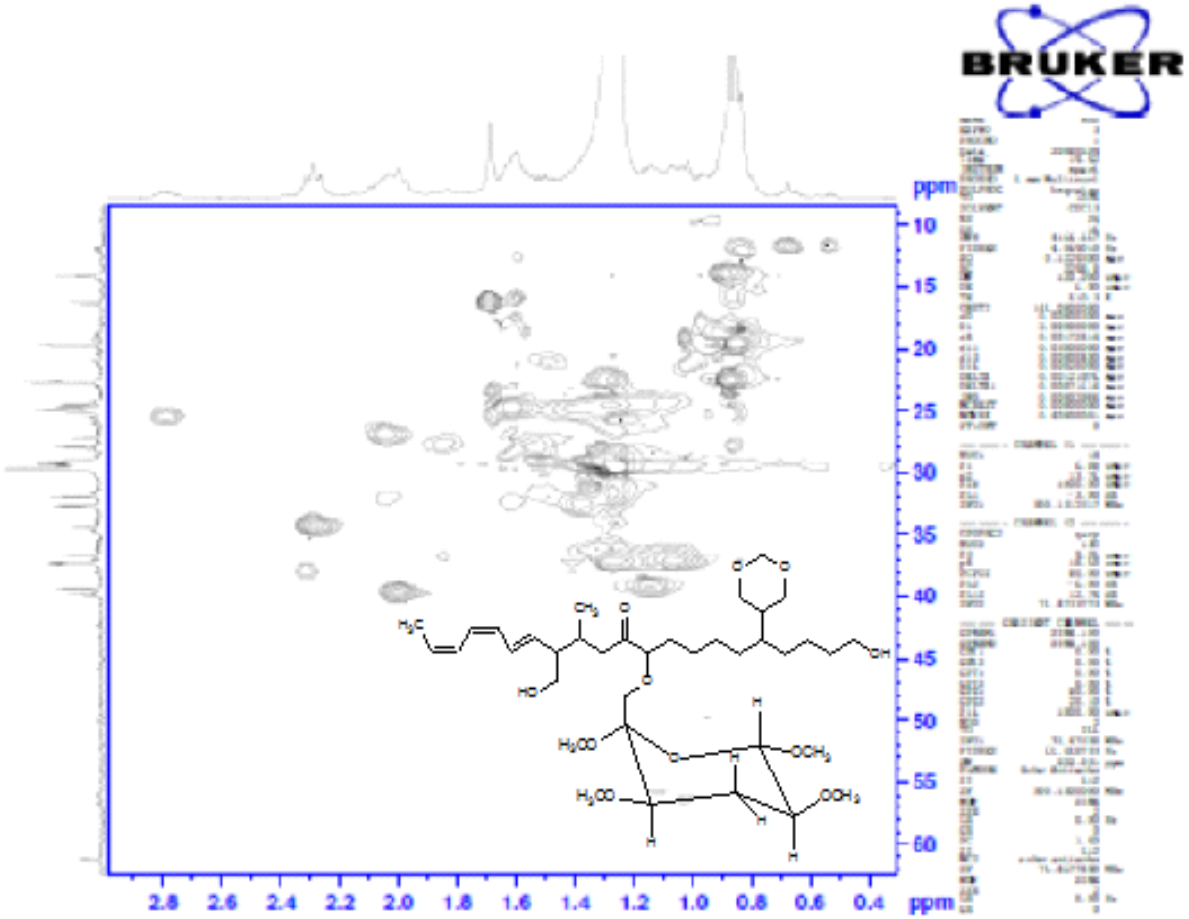

Fig. 7. HMQC Spectrum of the isolated compound

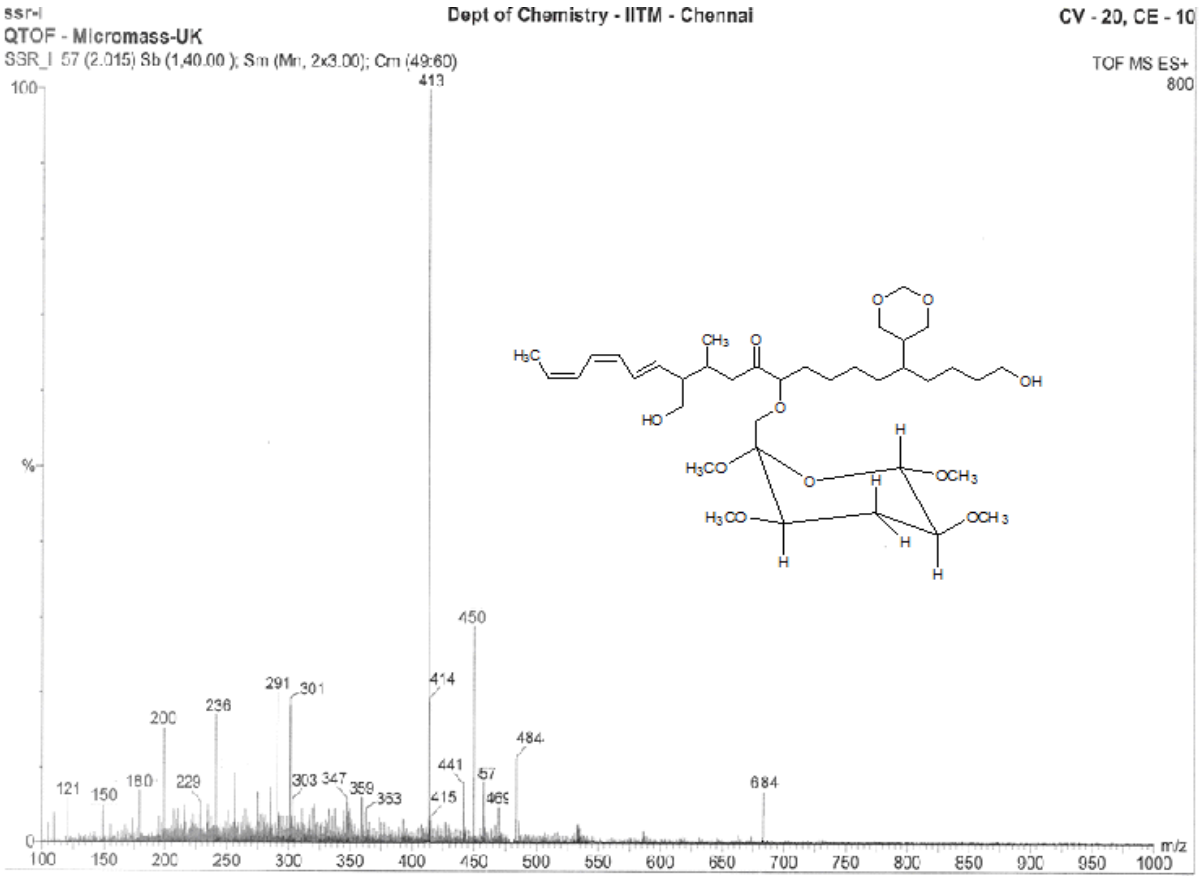

Fig. 8. Mass spectrum of the isolated compound