

**Extraction, Isolation and Characterization of sesquiterpene lactone  
(1 $\alpha$ ,4 $\alpha$ -dihydroxyguaia-2,10(14),11(13)-trien-12,6 $\alpha$ -olide) From *Artemisia  
afra* Jacq. ex Willd.**

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**ABSTRACT:** This communication presents the isolation and detailed structural elucidation of a novel sesquiterpene lactone derived from the leaves of *Artemisia afra*, a medicinal plant extensively used in traditional African medicine. The structure of the compound was determined through comprehensive spectroscopic analyses, including one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) spectroscopy, alongside high-resolution mass spectrometry (HRMS). These complementary techniques enabled the clear identification of the compound's molecular architecture and stereochemistry, thereby enriching the phytochemical profile of *A. afra* and highlighting its potential pharmacological relevance. The HRMS data further supported the proposed molecular formula, with an observed mass of 285.1098 [M+Na<sup>+</sup>], closely matching the calculated value for C<sub>15</sub>H<sub>18</sub>O<sub>4</sub>Na (285.1103), thereby confirming the compound's structural integrity

**Keywords:** *Artemisia afra*, Antimicrobial, Spectroscopy, sesquiterpene, lactone

## Introduction

Asteraceae, whose members are known to contain a rich diversity of bioactive secondary metabolites. Several of these species are commercially available as herbal formulations and are especially noted for their high levels of sesquiterpene lactones, a class of naturally occurring terpenoids with significant pharmacological relevance. These compounds have been extensively studied for their broad range of biological activities, including anti-inflammatory, antimalarial, anticancer, and antimicrobial properties. *Artemisia afra*, an important medicinal plant in African traditional healing practices, is particularly recognized for its therapeutic versatility, largely attributed to its abundant content of sesquiterpene lactones and other bioactive constituents. The continued investigation of such plants holds promise for the discovery of novel therapeutic agents.

Artemisinin is a particularly unique compound due to the presence of an endoperoxide bridge within its molecular structure. This sesquiterpene lactone (STL) was originally isolated from *Artemisia annua* (Asteraceae), a plant whose aerial parts have long been used in traditional Chinese medicine as a febrifuge. Today, artemisinin and its semi-synthetic derivatives are widely employed as frontline antimalarial agents, particularly effective against *Plasmodium falciparum* strains resistant to chloroquine. Beyond its antimalarial efficacy, artemisinin has demonstrated additional pharmacological properties, including leishmanicidal activity and anticancer effects. The latter has been attributed to its ability to inhibit cell proliferation and induce apoptosis in human hepatocellular carcinoma cells (SMMC-7721) as well as other cancer cell lines. Furthermore, artemisinin exhibits antischistosomal activity and has shown effectiveness against *Helicobacter pylori*. Currently, artemisinin derivatives are undergoing phase I and II clinical trials for the treatment of lupus nephritis and several cancers, including breast, colorectal, and lung malignancies.

Against this backdrop, the present communication aims to report the isolation and structural elucidation of a previously unreported sesquiterpene lactone (STL) from *Artemisia afra*, a well-known medicinal plant extensively used throughout Africa in traditional healing practices. Despite the rich ethnopharmacological significance of

*A. afra*, its chemical diversity particularly with regard to bioactive sesquiterpene lactones remains only partially explored. The discovery of this novel compound not only enriches the phytochemical profile of the species but also lays a foundation for future investigations into its potential biological and pharmacological activities. By contributing new molecular insights, this work supports ongoing efforts to explore plant-derived natural products for therapeutic development

## Materials and Method

### Materials

#### *Instrumentation:*

NMR experiments were carried out using Bruker Avance III spectrometers. Spectra were acquired on a 500 MHz instrument operating at 500 MHz for <sup>1</sup>H and 124 MHz for <sup>13</sup>C. Both one-dimensional (1D) and two-dimensional (2D) NMR techniques were employed for structural elucidation. The 1D experiments included <sup>1</sup>H, <sup>13</sup>C, and DEPT, while the 2D experiments consisted of COSY, NOESY, HSQC, and HMBC. Peak multiplicities such as doublets (d), multiplets (m), double doublets (dd), triplets (t), and doublet of triplets (dt), along with coupling constants (J), were assigned based on the observed splitting patterns. All NMR spectra were recorded in acetone-*d*<sub>6</sub>. Melting points were determined using an Ernst Leitz Wetzlar micro-hot stage apparatus. High-resolution mass spectrometry (HRMS) was performed using a Micromass LCT (Waters) spectrometer equipped with electrospray ionization (ESI) in both positive and negative ion modes, employing time-of-flight (TOF) detection.

**Materials:** All separations were monitored using thin-layer chromatography (TLC) on silica gel plates (Kieselgel 60 F254, Merck, aluminum-backed), which were cut into strips of appropriate sizes. After chromatographic development, the plates were visualized under ultraviolet light at 254 nm or 366 nm. Visualization was further enhanced by staining with reagents such as iodine or *p*-anisaldehyde (prepared by mixing 0.5 mL of *p*-anisaldehyde with 85 mL of methanol, 4 mL of sulfuric acid, and 10 mL of glacial acetic acid). Following staining, the plates were gently heated using a heat gun to induce colour development. Merck silica gel 60 (0.040–0.063 mm) was used for column chromatography and crude samples were separated on 3 cm diameter columns, while purifications were carried out on 2 cm diameter columns.

All solvents were used without further purification and were purchased from merck South Africa.

**Collection of Plant Materials:** The plant material used in this study was collected from the University of KwaZulu-Natal (UKZN) Botanical Garden under the guidance of Ms. Alison Young, the garden's curator. Ms. Young also performed the taxonomic identification of the specimens. Voucher samples have been deposited in the Bews Herbarium, located within the School of Life Sciences at the UKZN Pietermaritzburg Campus.

## Method

### *General Experimental Procedure:*

**Extraction Procedure:** Extraction procedure was by maceration. 300 g of the powdered plant material was extracted using (1:1) DC-Methanol. The content was allowed to stand for 24hours at 23°C in a shaker. The process was repeated, and the content was put together and concentrated under pressure to yield a crude extract of 40 mg.

### *Isolation of 1 $\alpha$ ,4 $\alpha$ -dihydroxyguaia-2,10(14),11(13)-trien-12,6 $\alpha$ -olide.*

A pre-purification was conducted on the extract using column chromatography eluting with non-polar to a moderately polar solvent system to generate a fraction of mass 358mg. The fraction was subjected to column chromatography using hexane-ethyl acetate (2:8) as the eluent, resulting in the collection of 107 subfractions (5 mL each). Thin-layer chromatography (TLC) was used to analysed the elution profile, and fractions exhibiting similar TLC patterns were pooled. Fractions 24 to 58 were combined to yield 15 mg of a partially purified fraction, designated as F4C5. Further purification was achieved using preparative TLC (hexane-ethyl acetate, 2:8), which led to the isolation of compound **1 $\alpha$ ,4 $\alpha$ -dihydroxyguaia-2,10(14),11(13)-trien-12,6 $\alpha$ -olide**. A dark spot was observed under UV illumination on the TLC plate with an R<sub>f</sub> of 0.58. From this, 5 mg of material was recovered, displaying a golden coloration after evaporation under a nitrogen atmosphere.

## Discussion

### *Empirical Analysis of the NMR Spectra*

The ESI-(+)-HRMS spectrum revealed that the molecular formula of the isolated compound is  $C_{15}H_{18}O_4$ , based on an observed  $[M+Na^+]$  ion at  $m/z$  285.1098, which closely aligns with the calculated value of 285.1103 for  $C_{15}H_{18}O_4Na$ . This molecular formula was further supported by the  $^{13}C$  NMR spectrum, which displayed fifteen distinct carbon signals, consistent with a sesquiterpenoid structure. Notably, the  $^{13}C$  NMR data indicated the presence of a carbonyl carbon characteristic of an ester group at  $\delta_C$  170.2, along with six  $sp^2$ -hybridized carbon signals:  $\delta_C$  148.9 (C), 140.6 (CH), 138.9 (C), 134.8 (CH), 121.1 ( $CH_2$ ), and 114.4 ( $CH_2$ ). The calculated double bond equivalent (DBE) value of seven, derived from the molecular formula, suggests the presence of three rings in the molecule, in addition to the carbonyl and three double bonds.

Additional features observed in the  $^{13}C$  NMR spectrum include three oxygenated carbons at  $\delta_C$  86.6 (C), 85.5 (C), and 83.3 (CH); two methine carbons at  $\delta_C$  67.2 and 43.2; two methylene carbons at  $\delta_C$  32.1 and 30.2; and one methyl carbon resonating at  $\delta_C$  24.7. These data collectively support a complex, polycyclic sesquiterpene lactone framework

Many sesquiterpene lactones are distinguished by the presence of a five-membered methylene lactone ring. Analysis of DEPT-135 and HSQC spectra confirmed the presence of two terminal alkene groups. One methylene carbon resonated at  $\delta_C$  121.1 ( $CH_2$ ) and was associated with proton signals at  $\delta_H$  6.27 and 5.54, while the second terminal methylene appeared at  $\delta_C$  114.4, correlating with protons at  $\delta_H$  4.98 and 4.78. The  $^1H$  NMR spectrum displayed two characteristic proton signals at  $\delta_H$  3.30 (1H, multiplet;  $\delta_C$  43.2, C-7) and  $\delta_H$  4.13 (1H, doublet of doublets,  $J = 11.1, 9.0$  Hz;  $\delta_C$  83.3, C-6), indicative of a lactone moiety. In the COSY spectrum, the protons at  $\delta_H$  6.27 and 5.54 showed correlations with the proton at  $\delta_H$  3.30, suggesting spin coupling or spatial proximity. Additionally, the  $\delta_H$  3.30 signal exhibited COSY correlations with the  $\delta_H$  4.13 proton and two methylene protons at  $\delta_H$  2.78 and 1.44 ( $\delta_C$  32.1, C-8).

Additionally, another aliphatic methylene group was identified, with proton signals at  $\delta_{\text{H}}$  2.90 and 2.32 corresponding to a carbon resonance at  $\delta_{\text{C}}$  30.2 (C-9). COSY data confirmed that this methylene group is adjacent to the previously mentioned methylene at C-8. The proton at  $\delta_{\text{H}}$  4.13 exhibited coupling constants of 11.1 and 9.0 Hz, consistent with axial relationships to neighbouring protons. A COSY correlation was also observed between this proton and another at  $\delta_{\text{H}}$  2.48 (doublet,  $J = 11.2$  Hz;  $\delta_{\text{C}}$  67.2, C-5). Taken together, these spectroscopic data allowed for the construction of a partial structural fragment, as illustrated in Figure 1. For clarity and consistency, atom numbering corresponds to that of the final compound.

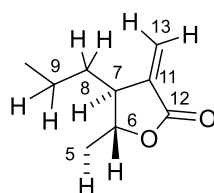


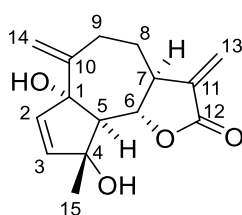
Figure 1. Fragment of structure

The HMBC spectrum revealed correlations between the protons at  $\delta_{\text{H}}$  6.27 and  $\delta_{\text{H}}$  5.57 and the carbon signals at  $\delta_{\text{C}}$  170.2 (C-12),  $\delta_{\text{C}}$  138.9 (C-11), and  $\delta_{\text{C}}$  43.2 (C-7). These correlations confirmed that the methylene group ( $\delta_{\text{H}}$  6.27 and  $\delta_{\text{H}}$  5.57) is positioned adjacent to the carbonyl carbon of the lactone ring. Additionally, a notable HMBC correlation was observed between the protons at  $\delta_{\text{H}}$  4.99 and  $\delta_{\text{H}}$  4.78 attributed to a second exocyclic methylene group and the carbons at  $\delta_{\text{C}}$  148.9,  $\delta_{\text{C}}$  86.4, and  $\delta_{\text{C}}$  30.2 (C-9). HSQC data confirmed that both protons are attached to the same carbon. The HMBC spectrum also indicated that the nearby alkene carbon resonates at  $\delta_{\text{C}}$  114.4 and is flanked by an oxygen-bearing quaternary carbon ( $\delta_{\text{C}}$  86.4, C-1) and a methylene carbon ( $\delta_{\text{C}}$  30.2, C-9). The remaining portion of the molecule included a *cis*-alkene moiety, as indicated by two vinylic proton resonances in the  $^1\text{H}$  NMR spectrum:  $\delta_{\text{H}}$  5.99 ( $J = 6.0$  Hz), which showed a direct one-bond HSQC correlation with the carbon at  $\delta_{\text{C}}$  140.6, and  $\delta_{\text{H}}$  5.62 ( $J = 6.0$  Hz), which correlated with  $\delta_{\text{C}}$  134.8. The coupling constant ( $J = 6.0$  Hz) supports a *cis* configuration for the double bond.

Additionally, a methyl singlet observed at  $\delta_{\text{H}}$  1.34 showed an HSQC correlation with a carbon resonance at  $\delta_{\text{C}}$  24.7, assigned to C-15. The only unassigned signal in the

$^{13}\text{C}$  NMR spectrum appeared at  $\delta_{\text{C}}$  82.2, corresponding to a quaternary carbon (C-4) likely bonded to an oxygen atom. In the HMBC spectrum, the methyl protons at  $\delta_{\text{H}}$  1.34 exhibited long-range correlations with carbons at  $\delta_{\text{C}}$  67.2 (C-5),  $\delta_{\text{C}}$  82.2 (C-4), and  $\delta_{\text{C}}$  140.6 (C-3), one of the alkene carbons. Furthermore, both alkene protons ( $\delta_{\text{H}}$  5.99 and 5.62) displayed HMBC correlations to two oxygenated carbons at  $\delta_{\text{C}}$  86.6 (C-1) and  $\delta_{\text{C}}$  82.2 (C-4), supporting their spatial proximity to an oxygenated ring system or framework

Based on the spectroscopic data, the structure of the compound was determined to be **1 $\alpha$ ,4 $\alpha$ -dihydroxyguaia-2,10(14),11(13)-trien-12,6 $\alpha$ -olide**. The relative configuration was established through NOESY correlations, notably between the methyl protons (H-15,  $\delta_{\text{H}}$  1.34) and H-6 ( $\delta_{\text{H}}$  4.13), as well as between H-5 ( $\delta_{\text{H}}$  2.48) and H-7 ( $\delta_{\text{H}}$  3.30). The compound features a fused five- and seven-membered ring system, where the ring junctions can adopt either *cis* or *trans* configurations. The junction involving the lactone ring was assigned a *trans* configuration, as evidenced by the large coupling constant ( $J = 9.0$  Hz) observed between H-6 and H-7. Molecular modelling indicated that, if the junction between the cyclopentene and the seven-membered ring were also *trans*, a NOESY correlation would be expected between H-2 and one of the H-14 protons ( $\delta_{\text{H}}$  4.78). The absence of this correlation led to the conclusion that this ring junction is *cis*



The NMR data for **1 $\alpha$ ,4 $\alpha$ -dihydroxyguaia-2,10(14),11(13)-trien-12,6 $\alpha$ -olide** are presented in Table 1. Previous accounts of this molecule's isolation include that of Jakupovic et al. (1991), who obtained it from *Artemisia rutifolia*, and Todorovan and Tsankova, who sourced it from *Achillea chrysocoma*. However, neither publication included  $^{13}\text{C}$  NMR information. As such, this study provides the first complete  $^{13}\text{C}$  spectral assignment for the compound.

Table 1. <sup>1</sup>H and <sup>13</sup>C NMR data of  $\alpha,4\alpha$ -dihydroxyguaia-2,10(14),11(13)-trien-12,6 $\alpha$ -olide in CDCl<sub>3</sub> (500 MHz).

Experimental						Literature <sup>16</sup>	
Position	$\delta_C$	DEPT	$\delta_H$	COSY	HMBC correlation <sup>a</sup>	$\delta_C$	$\delta_H$
1	86.4	C			H-2, 3, 5, 14a, 14b		
2	134.8	CH	5.99, d, <i>J</i> 6.0 Hz	3	H-3		5.79 doublet
3	140.6	CH	5.62, d, <i>J</i> 6.0 Hz	2	H-2, 15		5.93 doublet
4	82.2	C			H-2, 3, 6, 5, 15		
5	67.2	CH	2.48, d, <i>J</i> 11.2 Hz	6	H-2, 3, 6, 15		2.17 doublet
6	83.5	CH	4.13, dd, <i>J</i> 11.1, 9.01 Hz	5, 7	H-3, 5, 7		4.67 triplet
7	43.2	CH	3.9, m	8, 6	H-2, 5, 6, 13a, 13b		3.17 multiplet
8	32.1	CH <sub>2</sub>	2.78 m 1.44 m	7, 9	H-6, 9a, 9b		
9	30.2	CH <sub>2</sub>	2.90, td, <i>J</i> 13.0, 2.32, m	8	H-8a, 8b, 14a, 14b		
10	148.9	C			H-5, 8a, 14a, 14b		
11	138.9	C			H-7, 13a, 13b		
12	170.2	C		13, 9	H-13a, 13b		
13	121.2	CH <sub>2</sub>	6.27, <i>d</i> , <i>J</i> 3.5 Hz 5.57, <i>d</i> , <i>J</i> 3.1 Hz		H-7		6.25 doublet 5.47 doublet
14	114.4	CH <sub>2</sub>	14a: 4.99, br, <i>s</i> 14b: 4.78, <i>d</i> , <i>J</i> 1.2 Hz		H-8a, 8b, 9a, 9b		1.45 singlet
15	24.7	CH <sub>3</sub>	1.36, <i>s</i>		H-5		1.24 singlet

<sup>a</sup>HMBC correlations are from the protons stated to the indicated carbon.

## Conclusion

Although the quantity of the isolated sesquiterpene lactone (STL) was insufficient to permit biological evaluation in the present study, its structural features, along with

extensive literature evidence, suggest promising bioactivity. Sesquiterpene lactones are well-documented for their broad-spectrum biological activities, including antimicrobial, anti-inflammatory, anticancer, antimalarial, and antiparasitic properties. Numerous studies have demonstrated that STLs frequently exhibit potent activity against a range of pathogenic microorganisms, including bacteria, fungi, protozoa, and parasites. Therefore, despite the current limitation in quantity, the discovery of this compound provides a strong rationale for future studies aimed at its synthesis or re-isolation in larger amounts, to fully assess its pharmacological potential.

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