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Isolation and Characterization of a Novel Secondary Metabolite from the Leaves of *Sarcophrynium brachystachys* (Benth.) K Schum

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ABSTRACT

Sarcophrynium *brachystachys* (Benth.) K. Schum, commonly known as the praying plant or Yoruba soft cane, is traditionally used in the treatment of respiratory ailments such as cough and bronchitis. However, limited information exists regarding its phytochemical constituents and the compounds responsible for its medicinal properties. This study was undertaken to isolate and characterize a bioactive secondary metabolite from the leaves of *S. brachystachys*. The dried leaves were extracted with 96% ethanol and subjected to successive solvent partitioning and chromatographic separation. The methanolic fraction was purified by column chromatography, and the purity of the isolated compound was monitored using thin-layer chromatography (TLC). Structural elucidation was achieved through Fourier-transform infrared spectroscopy (FTIR), proton and carbon-13 nuclear magnetic resonance spectroscopy (^{1}H NMR and ^{13}C NMR), DEPT-135, HSQC, COSY, and mass spectrometry. Spectroscopic analyses led to the identification of an amine-containing compound, namely (E)-N-(1-(3-(8-(3,5-dimethylhexan-3-yl)-3-(3-ethyl-4-methoxypent-3-en-1-yl)-2,3-dihydrobenzo***[b][1,4]***dioxin-2-yl)propoxy)propyl)-N,O-dimethylhydroxylamine**.** The successful isolation and characterization of this metabolite contribute to the phytochemical profiling of *S. brachystachys* and provide phytochemical information relevant to the traditional medicinal use and warrant further biological investigation.

Keywords: *Sarcophrynium brachystachys,* phytochemistry, isolation, spectroscopy.

1. Introduction

In the development and discovery of novel therapeutic agents, medicinal plants have continuously played critical roles as new sources of these vital bioactive components (Najmi *et al*., 2022). Consequently, man y phytochemicals are regarded as naturally occurring bioactive compounds owing to their diverse biologic al activities and potential applications in drug discovery and development,
(Ezeobiora *et al*. (2021), Najmi *et al*. (2022).

The development and formulation of active and effective phytochemicals, which have fewer or no side effects, into therapeutic materials begins with evaluating the potential biological effects of extracts obtained from either dry or wet plant parts. This process is typically followed by isolation and purification to obtain the active phytochemical, often conducted using guided fractionation methods.

After isolation, the phytochemicals are tested for their in vitro and in vivo effects (Igwe *et al*., 2025).

Traditionally, various plant parts have been utilized in the treatment and management of numerous diseases and ailments. The use of medicinal plants is one of the oldest forms of healthcare known to humanity and underpins traditional medical systems such as Ayurveda and Traditional Chinese Medicine, both of which have been practiced for several millennia and remain relevant in contemporary healthcare (World Health Organization, 2013).

In the ancient and medieval period, the knowledge of selection of specific plants and plant parts, the appropriate time of material collection, and the method of preparation and application of phytochemicals for the treatment of specific illnesses were verbally transmitted between generations. This folklore of knowledge transfer resulted in the loss of vital information regarding the use and preparation of these naturally occurring therapies. These therapies were prepared traditionally as tinctures, teas, powders, poultices, decoctions, and several other methods (Ofori-kwakye, 2017). These modes of preparation were the most common methods for creating therapies until the 18th century. With the development of modern medicine, these traditional modes of preparation could be considered to fit the modern scientific definition of drugs, despite their lack of proper purification and dosage complications (Asogwa *et al*., 2026).

The growing interest in medicinal plants underscores the necessity for thorough phytochemical isolation and analysis of extracts from therapeutic compounds such as *S. brachystachys*. This research aims to identify significant bioactive constituents, assess their efficacy, and properly document the knowledge associated with this plant (Ikpeazu *et al*., 2017; Chali *et al*., 2021).

Sarcophrynium brachystachys is traditionally used as a medicine for treating cough and bronchitis. However, there is likely very little logical evidence to support these uses. Additionally, there is a lack of information regarding the isolation and characterization of the chemical constituents found in the leaves of this plant. A comprehensive literature search indicates that no records have been found revealing the chemical constituents of *Sarcophrynium brachystachys* (Mahomoodally, 2013).

Research has shown that several therapeutic phytochemicals are secondary metabolites. Secondary metabolites are non-essential compounds produced by plants, fungi, and bacteria for defense (Ezeobiora *et al*., 2021). It is therefore worthy of note that some of the most important human medications, such as drugs for leukemia, breast cancer, blood thinners, HIV, migraines, and even antibiotics contain secondary metabolites (Egbucha *et al.*, 2024).

Secondary metabolites are used in drug formulation because of their ability to act as a catalyst by activating the drug's essential functions and equally increase its solubility (Otuokere *et a*l. 2016). Despite the current understanding of secondary metabolites, there are still many untapped areas regarding the use of these compounds. This gap could be traced to the conventional methods of producing them, which necessitate carefully controlled conditions, which have limited the discovery of novel secondary metabolites with therapeutic potential (Igwe *et al.*, 2016).

S. brachystachys (Benth.) K Schum commonly called Yoruba soft cane is a rhizomatous, perennial herbaceous plant. Its structure consists of an erect stem and simple basal leaves of 1.5 meters elliptic to

oblong-lanceolate, with pointed ends and about 20-50 cm and 10-25 cm length and width, respectively. In the southern parts of Nigeria, the leaves of S. brachystachys are also used to make local roofs for houses and to wrap and package food items such as sliced oil beans (ugba), bean balls (moi moi), ogiri, dawadawa,

and agidi for both home and commercial purposes (Egbucha *et al*., 2024).

In several parts of Africa, such as Ivory Coast, it is used for the treatment of pulmonary diseases such as cough and bronchitis, the seeds are usually ground and mixed with water before they are taken. The continued use of *S. brachystachys* for treatments led to the discovery of its therapeutic (restorative and recuperative) properties.

Although *S. brachystachys* is used locally to treat various illnesses, there have been few studies examining its individual parts, leading to a limited body of literature regarding the bioactivity of its phytochemical constituents. This underscores the rationale for this research, which aims to isolate and characterize secondary metabolites from the leaves. This research will establish a foundation for future investigations into its bioactivity.

2. Methodology

Collection of Sample

The plant samples were collected from Umuokpo, Emebia, and Owerri West Local Government Area, Imo State, Nigeria. The plant was identified as *Sarcophrynium brachystachys* (Benth.) K. Schum. by a botanist of the Micheal Okpara University of Agriculture, Umudike, Abia State, using morphological characteristics and *Flora of West Tropical Africa* (Hepper, 1968).

Preparation of the sample

The obtained and identified leaves of *S. brachystachys* were air-dried after washing under a shade for a period of four weeks. The dry leaves were pulverized to fine particles and sieved using a 2 mm mesh sieve.

Extraction and Partitioning

A 1000 g of the prepared leaves of *S. brachyatachys* were percolated using 96 % ethanol (3 liters) for 72 hours at room temperature. The mixture was then decanted and filtered with Whitman's filter paper to remove the leaf particles. The obtained filtrate was concentrated using a rotary evaporator (Buchi, R 200) at a reduced pressure and a 40 °C temperature. A 163.32 g of concentrated dark-green ethanolic extract was obtained**.** 200 ml of chloroform was added to a beaker containing 120 g of the extract, then poured into a 500 ml separating funnel already containing 200 ml of distilled water.

This mixture was corked and agitated for 45 minutes to give a homogeneous mixture and allowed to stand undisturbed for 48 hours to ensure a proper separation into layers. The chloroform fraction of the mixture was allowed to completely evaporate the solvent with the help of a rotatory evaporator. This dried chloroform extract portion was further fractionated using 60 g of the extract. An equal volume of 10% aqueous methanol and n-hexane was added to the resulting mixture, agitated for 45 minutes, and allowed to stand for another 48 hours. The methanolic and n-hexane portions were collected separately, and the

solvents were allowed to evaporate using a rotary evaporator, and the resulting compounds were stored in a refrigerator.

Isolation and purification

The methanol extract (20 g) was used to prepare a slurry with 400 g of 60–120 mesh silica gel and eluted with various solvent mixtures, including n-hexane/chloroform, chloroform/ethyl acetate, and an ethyl acetate/methanol gradient, yielding 61 fractions. Fraction 45, obtained using a mixed solution of ethyl acetate/methanol in a 4:1 ratio, resulted in a single spot on thin layer chromatography (TLC) performed on a pre-coated aluminum plate with ethyl acetate and methanol mixed in a 7:3 ratio. This fraction had an Rf value of 0.63 and was designated as JSO5.

Spectroscopy

The 1H and 13C NMR, COSY, HSQC and DEPT 135 spectra for the obtained pure compound (JSO5) were all obtained using a Bruker Fourier 300 MHz NMR machine. $CDCl_3$ was used as a solvent, the signal was observed as a singlet peak at 7.26 ppm and a triplet peak at 77.58 ppm in the ^{1}H NMR and ^{13}C NMR, respectively**.** The FT-IR spectra were obtained using a Varian Resolution Pro. The mass spectrometric analysis was done using a Shimadzu QP2010 mass spectrometer, while silica gel (60–120 mesh) from Sigma Aldrich was placed in a glass column of different sizes and fitted with Teflon taps to form the column chromatography. The thin-layer chromatography was also carried out on a pre-coated plate, while detection and visualisation were achieved using iodine crystals in an iodine tank.

3. Results and Discussion

The FTIR, ^{1}H NMR, expanded ^{1}H NMR, ^{13}C NMR, DEPT-135, HSQC, expanded HSQC, COSY, mass spectra are shown in Figures 1, 2a, 2b, 3,4, 5, 6 and 7 respectively.

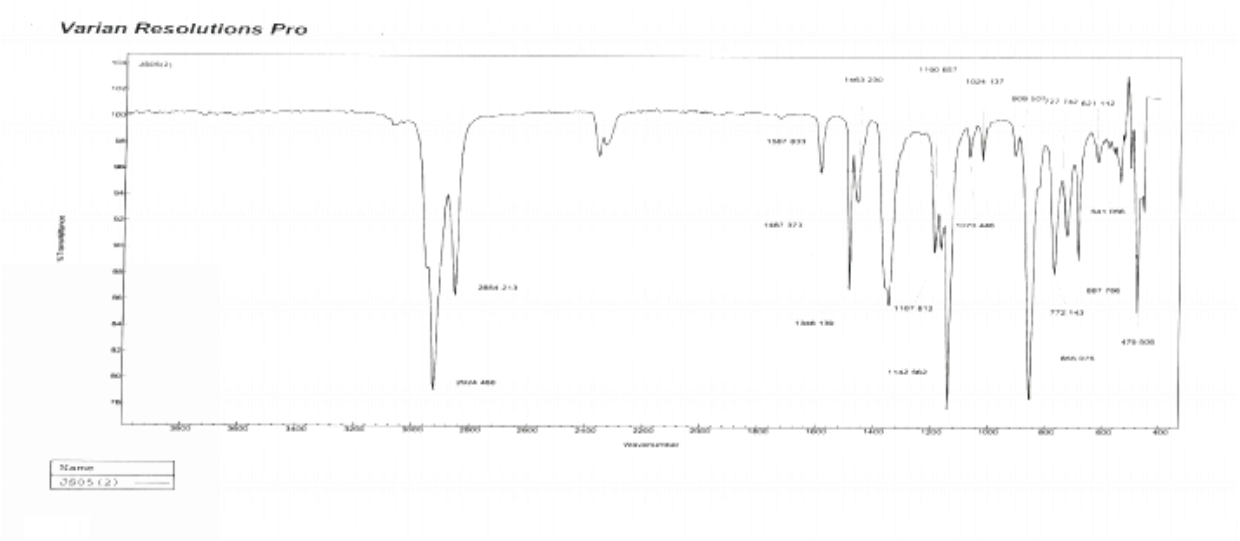


Figure 1: FTIR spectrum of JSO5

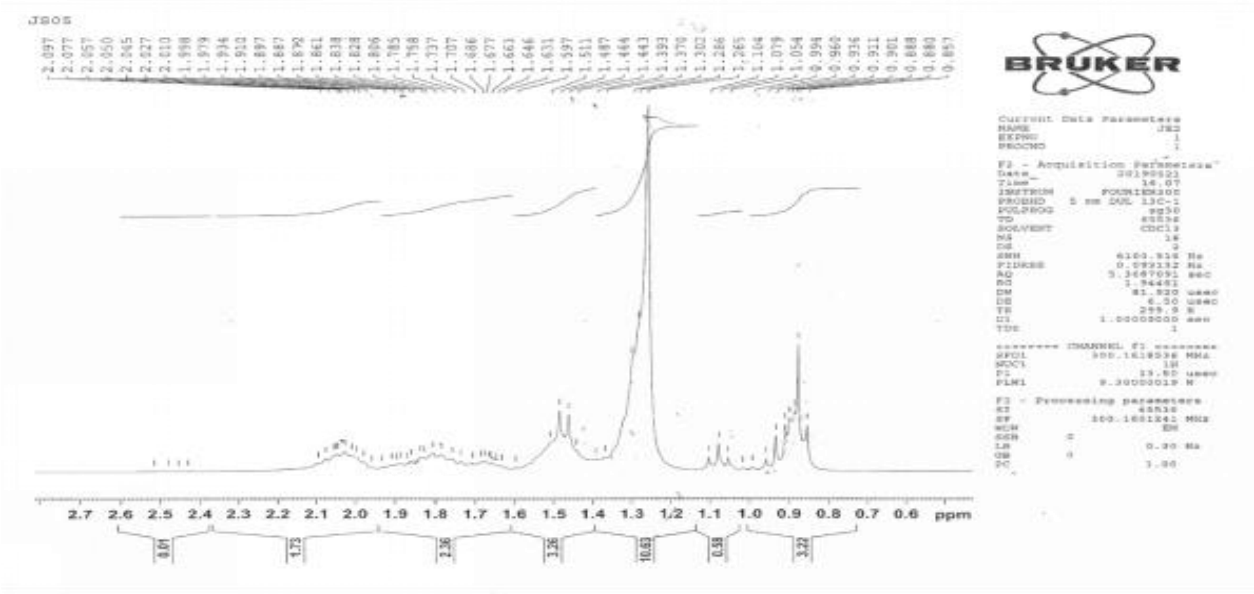


Figure 2a: expanded ^{1}H NMR spectrum of JSO5

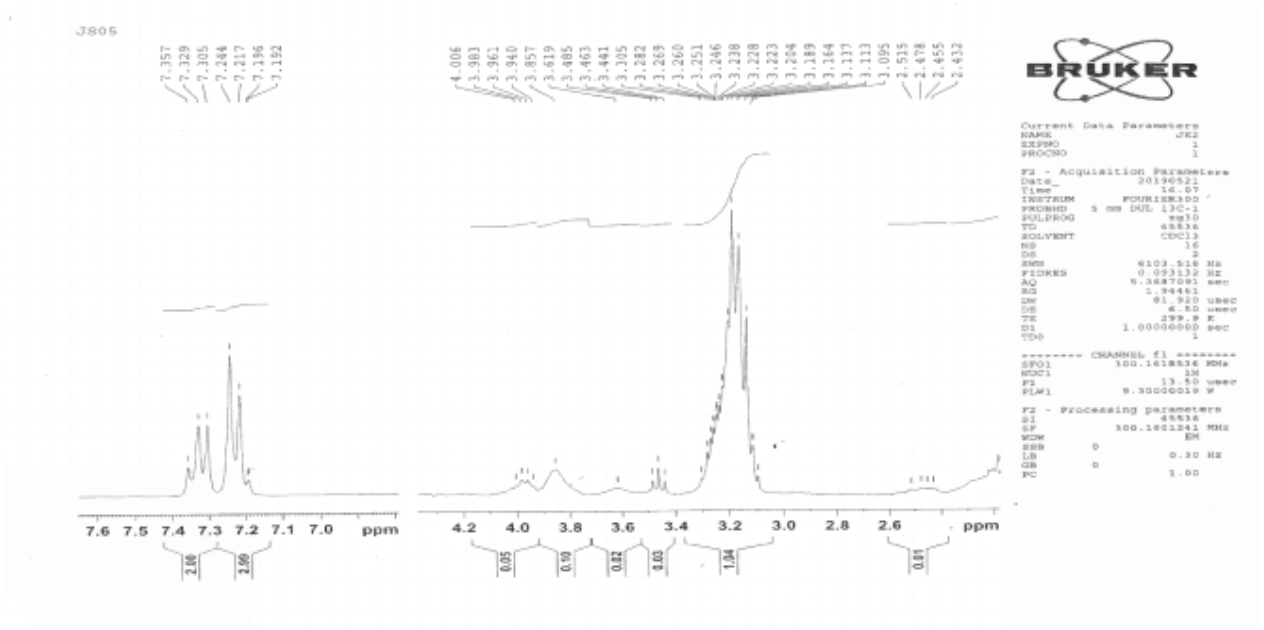


Figure 2b: expanded ^{1}H NMR spectrum of JSO5

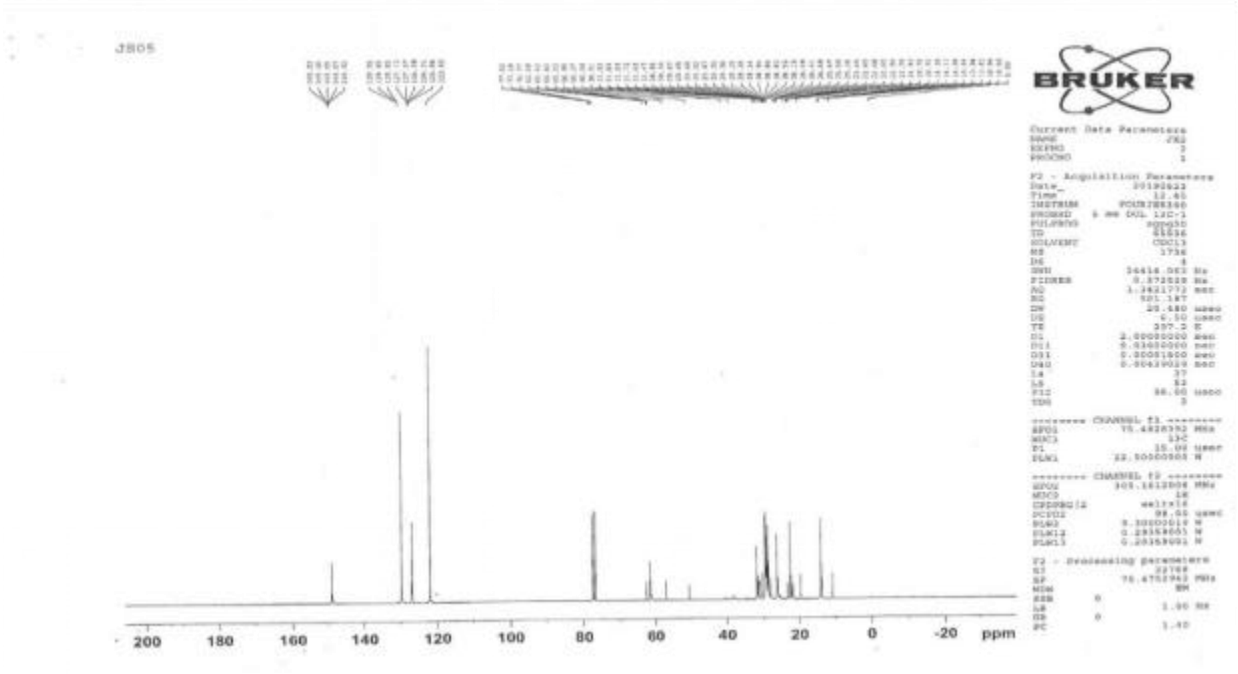


Figure 3: ^{13}C NMR spectrum of JSO5

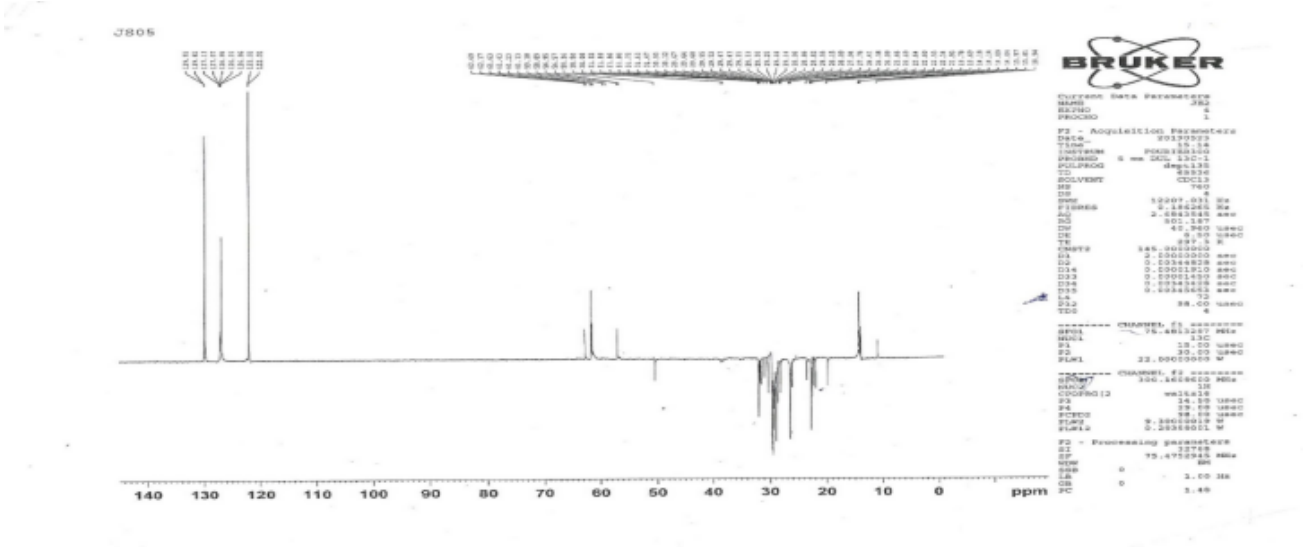


Figure 4: DEPT-135 spectrum of JSO5

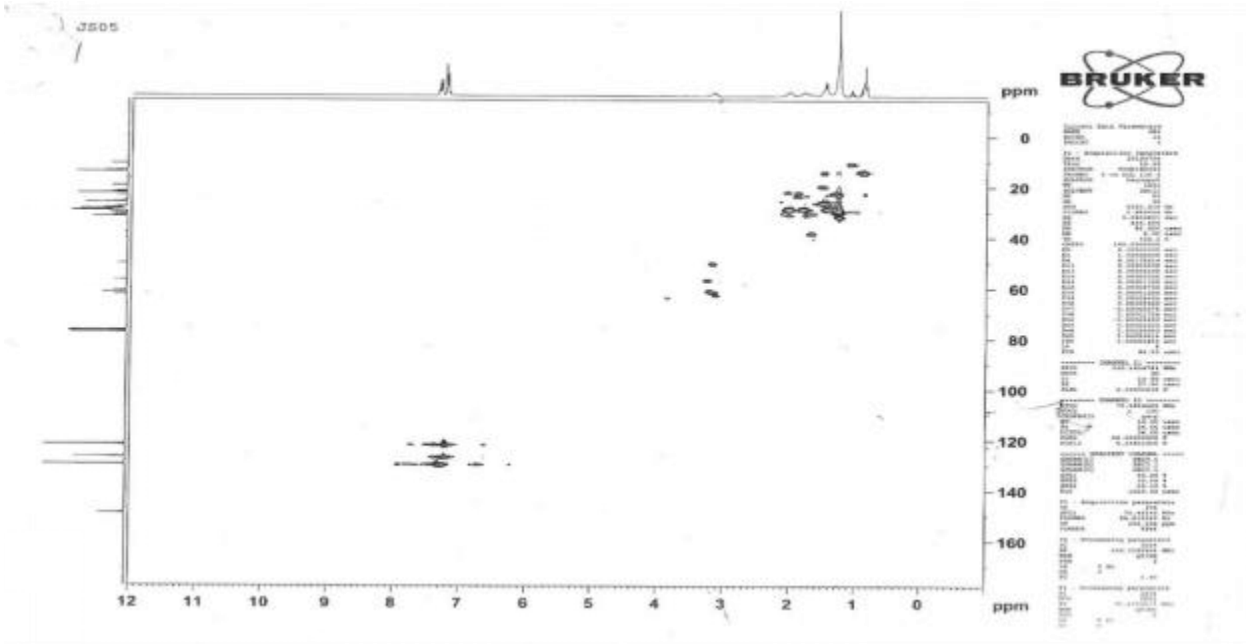


Figure 5: HSQC spectra of JSO5

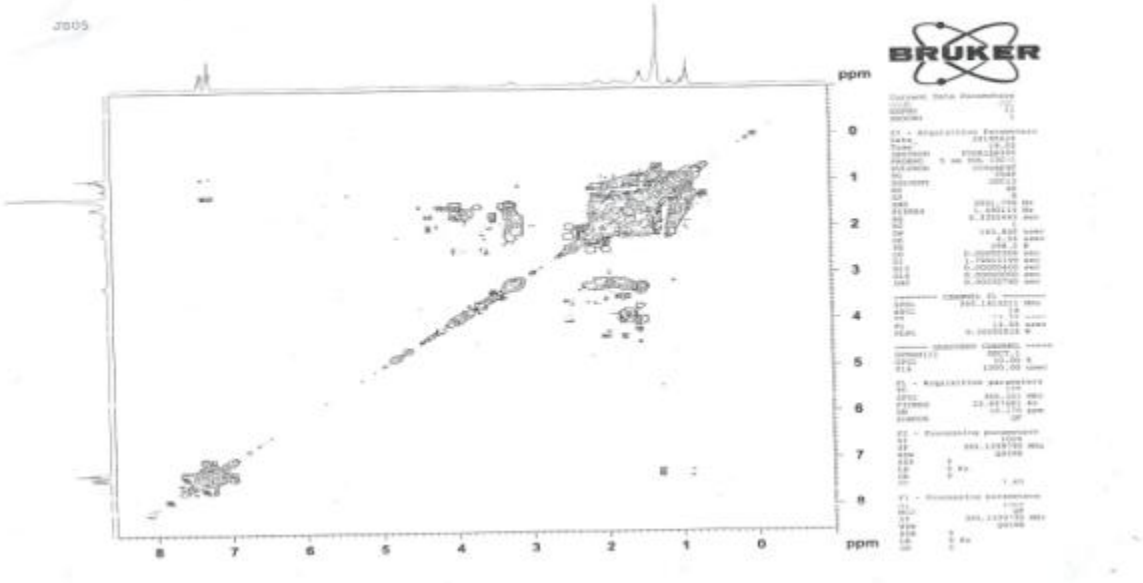


Figure 6: COSY spectrum of JSO5

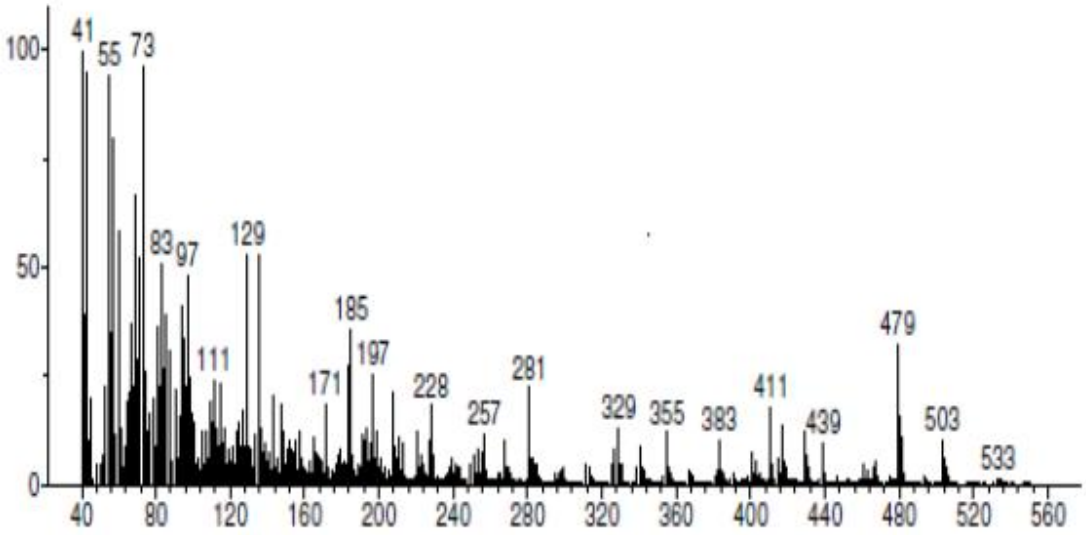


Figure 7: MS of JSO5

The compound JSO5 (27 mg) was isolated as a light green oil.
FT-IR spectra gave the following bands in cm^{-1}: 3100, 2924, 2854, 1690, 1587, 1487, 1463, 1348, 1142, 1070, 1190, 727 and 687.
^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.30 (1H, t), 7.24 (1H, d), 7.19 (1H, d), 4.00 (1H, q), 3.96 (1H, q), 3.94 (3H, s), 3.61 (3H, s), 3.46 (2H, t), 3.24 (1H, t), 3.09 (3H, s), 2.43 (3H, s), 2.09 (2H, q), 2.05 (2H, t), 1.78 (2H, m), 1.75 (2H, q), 1.70 (2H, q), 1.67 (2H, q), 1.63 (2H, m), 1.51 (2H, t), 1.43 (1H, m), 1.28 (3H, t), 1.07 (3H, t), 0.99 (3H, d), 0.96 (3H, d), 0.90 (3H, t), and 0.85 (3H, t).
^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 149.23 (C-2), 149.10 (C-27), 149.05 (C-1), 148.97 (C-3), 129.92 (C-4), 127.09 (C-6), 126.86 (C-5), 122.02 (C-30), 62.69 (C-19), 61.63 (C-15), 61.23 (C-24), 59.39 (C-23), 58.89 (C-32), 56.57 (C-22), 50.37 (C-18), 40.48 (C-7), 31.80 (C-28), 30.93 (C-31), 30.32 (C-26), 28.55 (C-20), 28.09 (C-9), 25.59 (C-8), 23.46 (C-25), 22.60 (C-16), 22.55 (C-12), 19.69 (C-17), 14.18 (C-14), 14.08 (C-29), 13.96 (C-10), 13.81 (C-11), 10.94 (C-13), and 10.82 (C-21).

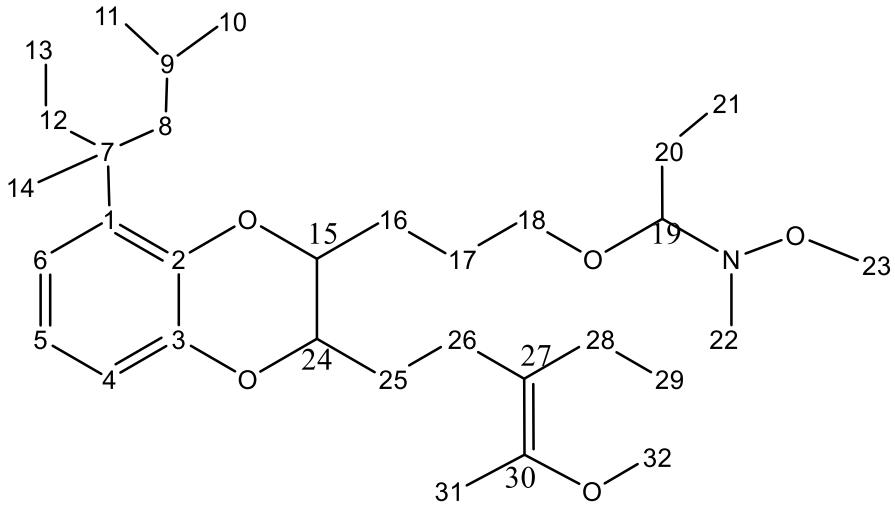
The ^{1}H NMR spectrum (300 MHz, $CDCl_3$, δ ppm) showed signals arising from the different proton environments of the proposed structure. Three aromatic proton signals were observed at δH 7.30 (1H, t), 7.24 (1H, d) and 7.19 (1H, d), consistent with a 1,2,3-trisubstituted aromatic ring. The remaining aliphatic and heteroatom-associated protons resonated between δH 0.85 and 4.00 ppm, including characteristic signals at δH 4.00, 3.96, 3.94, 3.61, 3.46, 3.24 and 3.09 ppm. The complete set of reported integrations summed to 55 H, comprising 30 methyl protons (from 10 CH_3 groups), 18 methylene protons (from 9 CH_2 groups), 4 aliphatic methine protons, and 3 aromatic protons. This proton distribution is fully consistent with the hydrogen count required by the proposed molecular formula, C_{32} H_{55} NO_5.
The ^{13}C NMR spectrum (75 MHz, $CDCl_3$) displayed 32 carbon resonances. Based on chemical shift and multiplicity data, the carbons were classified as 10 methyl (CH_3), 9 methylene (CH_2), 4 aliphatic methine (CH), 3 aromatic CH, and 6 quaternary carbons. The methyl carbons occurred over the range δC 10.82–59.39 ppm, the methylene carbons between δC 19.69 and 50.37 ppm, and the four aliphatic methine carbons

between δC 28.09 and 62.69 ppm. The three aromatic CH carbons were observed at δC 126.86–129.92 ppm. Four quaternary carbons, C-1, C-2, C-3 and C-27, occurred at δC 148.97–149.23 ppm, while the remaining two quaternary carbons, C-7 and C-30, resonated at δC 40.48 and 122.02 ppm, respectively. This classification accounts for all 32 carbons of the proposed molecular formula, in direct agreement with the proton count established above.

The DEPT-135 spectrum (Figure 4) supported these multiplicity assignments, showing the expected phase distinction between protonated carbon types, with CH and CH_3 signals appearing in the positive phase and CH_2 signals in the negative phase, while quaternary carbons were absent, as expected. Representative methyl signals were observed at δC 10.82, 13.96 and 59.39 ppm, methylene signals at δC 22.60 and 31.80 ppm, and methine signals at δC 28.09 and 62.69 ppm, consistent with the carbon classification established from the 13C data.

The HSQC spectrum (Figure 5) provided direct one-bond $^{1}H–^{13}C$ correlations that further supported these assignments. Methylene proton signals at δH 1.677, 1.707, 1.511, 1.785, 1.464 and 2.050 ppm correlated with carbon resonances at δC 22.55, 23.46, 22.60, 25.59, 28.55 and 31.80 ppm, respectively. The aromatic proton signals at δH 7.306, 7.196 and 7.244 ppm correlated with δC 126.86, 127.09 and 129.92 ppm, respectively, confirming the three protonated aromatic environments. These direct correlations were consistent with the protonated carbon assignments obtained from the ^{13}C and DEPT spectra (Pohl *et. al*.,2011)

The COSY spectrum (Figure 6) showed a correlation between δH 0.90 and 1.28 ppm, consistent with vicinal coupling within the aliphatic side chains, and mutual correlations among the three aromatic protons at δH 7.306, 7.196 and 7.244 ppm, confirming that they belong to a single contiguous spin system. Together, the COSY and HSQC data provide complementary evidence for the connectivity of the protonated structural fragments of the proposed structure.



(E)-N-(1-(3-(8-(3,5-dimethylhexan-3-yl)-3-(3-ethyl-4-methoxypent-3-en-1-yl)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)propoxy)propyl)-N, O-dimethylhydroxylamine.

Figure 8: Proposed structure of JSO5

The observed ^{13}C chemical shifts were also compared with published data for structurally related natural-product alkaloids, with the aromatic and quaternary carbon resonances falling within reported ranges, providing supportive context for the structural assignment (Carvalho Junior *et al.*, 2017).

The GC–MS spectrum (Figure 7) showed a molecular ion peak at m/z 533 $[M]^+$, corresponding to the proposed molecular formula $C_{32}H_{55}NO_5$ and a nominal molecular mass of 533. The molecular formula gives six degrees of unsaturation. Additional fragment ions were observed in the mass spectrum; however, detailed assignment of individual fragmentation pathways was not undertaken because the available data did not provide sufficient evidence to assign each fragment unambiguously to a specific structural portion of the proposed molecule. The mass spectral data were therefore used primarily to support the molecular-mass and molecular-formula assignment.

4. Conclusion

The present study resulted in the isolation and spectroscopic characterization of JS05, obtained as a light-green oil from the investigated plant material. Combined FT-IR, 1H NMR, ^{13}C NMR, DEPT-135, HSQC and COSY data were consistent with the major functional groups, proton and carbon environments, and carbon multiplicities of the isolated compound. The DEPT, HSQC and COSY experiments provided complementary information on carbon multiplicity and on proton–carbon and proton–proton correlations. The MS molecular ion at m/z 533 was consistent with the proposed molecular formula, $C_{32}\ H_{55}\ NO_5$.

On the basis of this evidence, a tentative structure is proposed for JS05, comprising a trisubstituted aromatic ring fused to a 1,4-dioxane, two branched aliphatic side chains, and an N,O-disubstituted hydroxylamine-type terminus. While the overall carbon framework is well supported, full connectivity at the nitrogen-containing terminus would benefit from additional two-dimensional correlation data, and a small number of closely spaced ^{13}C signals warrant further resolution in subsequent work. Accordingly, the structural assignment should be regarded as provisional pending: HMBC and NOESY/ROESY experiments to establish unambiguous long-range connectivity and relative configuration; supporting high-resolution mass spectrometric data to further strengthen the molecular formula assignment; and, where feasible, comparison with an authentic or independently synthesized standard. Such comparison would also help clarify whether the N,O-containing moiety is a genuine biosynthetic feature of this plant or a product of sample handling.

The findings reported here therefore represent a valuable preliminary spectroscopic characterization of JS05, providing a molecular formula and a working structural hypothesis to guide further investigation of this compound and its potential significance as a plant-derived natural product. Also, this study, lays a solid foundation for future investigations, particularly those aimed at evaluating the biological activities of JSO5, such as its antimicrobial, anti-inflammatory, or cytotoxic potential, to help in validating the traditional medicinal uses of this plant. Additionally, further studies involving molecular docking, pharmacokinetic profiling, and in vivo models will be essential to fully understand the therapeutic relevance and safety of this compound.

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