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Original Research Article

Isolation and Characterization of a Novel Tertiary Substituted Amine from the Leaves of *Sarcophrynium brachystachys* (Benth.) K Schum

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ABSTRACT

Sarcophrynium brachyatachys, commonly known as Yoruba soft cane or the praying plant, is traditionally used for packaging food items. Only a few natives recognize the usefulness of the leaves for the treatment of cough and bronchitis in herbal medicine. This study was designed to isolate and characterize a bioactive component. Successful percolation was carried out with 96 percent ethanol to get the crude extract. Afterwards, fractionation was carried out using chloroform and distilled water in a ratio of 1:1. The chloroform fraction was further fractionated with methanol and n-hexane. Column chromatography was carried out on the methanol portion of the extract with organic solvents of different polarities to isolate a pure compound, which was ascertained by thin layer chromatography. The isolated component was characterized based on FT-IR, ^{1}H and ^{13}C NMR spectra, DEPT-135, HSQC, COSY and mass spectrometry. FTIR spectrum revealed a weakly intense peak at 3100 cm^{-1}, which is typical of aromatic C-H. The ^{1}HNMR spectrum of showed the chemical shift of six member ring aromatic protons as doublets at 7.23 ppm (1H, d), 7.27 ppm (1H, d), and 7.32 ppm (1H, d). The methine tertiary amine proton signal was observed at 3.26 ppm. In the ^{13}CNMR spectrum, the tertiary methine amine carbon was observed at 62.62 ppm, while the tertiary methyl amine carbon resonated at 56.87 ppm. The isolated compound was identified as a tertiary substituted amine with the name 5-(2-(sec-butyl)phenoxy)-N-(1-ethoxypropyl)-6-(2-isobuthyl-2,3-dimetylcyclopropoxy)-N-methyldec-1-en-2-amine.

Keywords: Extract, isolation, spectroscopy, *S. brachystachys*, tertiary amine

Introduction

Truly, plants, through perception, speculation, and analysis, have procured information that advances wellness and prosperity. Scientific research and development are integral parts of isolating and characterizing bioactive components from natural products. Since ancient times, medicinal plants have been used as a source of healing by local populations all over the world[1]. Plants supply 80% of all synthetic drugs,[2] implying a link between their modern therapeutic and traditional applications. Plants also serve as a resource for drug discovery, providing primary healthcare for about 85% of the world's population and serving as a source for new drug discovery.[3] Since the dawn of time, people have used plants with therapeutic properties in traditional medicine. Medicinal plant products now command a market worth more than $100 billion annually on a global scale.[4]

Some of the most important human medications contain tertiary amines, including antibiotics, drugs for leukemia and breast cancer, drugs for oploidy pain, blood thinners, HIV treatment, and drugs for migraines and other conditions. They can activate the drug's essential biological functions and increase its solubility. The functional potential of tertiary amines likely remains largely unrealized despite this unique class of molecules being widely used in medicine today.

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The reason for this is that the conventional method of producing them necessitates carefully controlled conditions, which limits the discovery of novel tertiary amines, which have the potential to treat a variety of diseases that are currently incurable.[5]

S. brachystachys (Benth.) K Schum is a rhizomatous, perennial herbaceous plant species known as Yoruba soft cane. This plant is utilized for well-being or to manage some health challenges. *S. brachystachys* is an erect stem with simple basal leaves on a 1.5-meter-long elliptic to oblong-lanceolate petiole with a pointed apex and entire margin (20–50 cm long and 10–25 cm broad). In Ivory Coast, the seeds are ground and mixed with water before being taken as a remedy for pulmonary complaints such as cough and bronchitis. When used for these therapeutic purposes, it has been discovered to have restorative and recuperative properties. In the southern part of Nigeria, leaves are used for the roofing of houses and for wrapping or packaging food items. Examples of such food items are sliced oil beans (ugba), bean balls (moi moi), ogiri, dawadawa, and agidi, to mention but a few, whether at home or for commercial purposes.

Phytochemical analysis of the crude extract showed the presence of alkaloid, flavonoid, saponin, terpenes, cardiac glycosides, steroid and phenolic glycosides. Qualitatively, alkaloid and flavonoid were found to be present in high amount while saponin appeared to be present in moderate quantity. Terpenes, steroid, cardiac and phenolic glycosides were present in minute quantities.[6]

Spectroscopic methods have been used to determine the structures of plant extracts. [6-22] More research is needed to elucidate the structures of this plant. It's often not clear how its bioactivity and its phytochemical components are related. To the best of our knowledge, there are no reports on the isolation of **tertiary substituted amine** from *S. brachystachys*. Hence, we hereby report the isolation and characterization of a **tertiary substituted amine** from the leaves of *S. brachystachys*.

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Materials and Methods

Reagents

The reagents used in this research work were analytical grade reagents from BDH Chemicals London. These reagents include; Analytical grade chloroform (99.5 %), ethyl acetate (99.5 %), ethanol (96 %) n-hexane (99.5 %), methanol (99.5 %) and distilled water. The silica gel (100-120 mesh), was a product of Merck.

Sample collection

The fresh plant material (leaves) of *S. brachysplant was* collected in bulk from a forest area in Umuokpo, Emeabiam, in Owerri West local government area of Imo state, Nigeria on 23rd March 2020. The plant was authenticated and identified by a botanist at the Department of Forestry, Micheal Okpara University of Agriculture, Umudike, Abia State, Nigeria. The voucher number was ICZ 23454.

Extraction and Partitioning

The leaves of the plant were washed and air dried in the shade for four weeks. These were further ground and sieved into fine particles using a 2 mm mesh sieve. Extraction was performed at room temperature, where 1000 g of the plant material was percolated with 3 liters of 96% ethanol for 72 hours. The filtrate was decanted and filtered using Whatman's filter paper. The filtrate was concentrated at reduced pressure using a rotatory evaporator (Buchi, R 200) at 40°C. The concentrated dark-green sample was preserved in the refrigerator at 40 °C until further use. The ethanol extract weighed 163.32 g. Exactly 120 g of the ethanol extract were weighed and used for fractionation and partitioning between chloroform and distillation water in the ratio of 1:1 using a measuring cylinder. 200 ml of chloroform were measured and poured into a beaker containing 120 g of the crude extract. It was transferred into a 500-ml separating funnel that already had 200 ml of distilled water in it. This was corked and agitated for 45 minutes until a homogeneous mixture was obtained. This was allowed to stand for 48 hours to ensure a clear-cut separation. The chloroform fraction was allowed to completely evaporate using a rotatory evaporator. The dried chloroform extract obtained above was further fractionated using 60 g of the extract. The solvents, 10% aqueous methanol and n-hexane in an equal ratio of 200 ml each, were used. The sample was agitated for about 45 minutes and allowed to stand for 48 hours. Methanol and n-hexane extracts were separately collected and allowed to evaporate by using a rotatory evaporator; they were thereafter kept in the refrigerator at 40 °C until analysis.

Isolation and purification

Twenty (20) g of methanol extract was put on 400 g of 60–120 mesh silica gel and eluted with n-hexane/chloroform, chloroform/ethyl acetate, and then an ethyl acetate/methanol gradient. This produced 61 fractions. Fraction 27 produced a single spot with an Rf value of 0.56 using thin layer chromatography on a precoated aluminum plate. It was labeled as JSO4.

Spectroscopic characterization

The ^{1}H NMR of the pure compound was recorded on a Bruker Fourier 300 instrument using $CDCl_3$ as solvent. The solvent peak was seen at 7.26 ppm, operating at 300 MHz. ^{13}C NMR and DEPT 135 spectra were also obtained using the Bruker Fourier 300 and $CDCl_3$ as the solvent, both at 300 MHz. The solvent peak was seen at 77.58 ppm as a triplet. Varian Resolution Pro was used for FT-IR (Agilent Technologies), while a Bruker Fourier 300 was used for COSY at 300 MHz. $CDCl_3$ was used as a solvent. HSQC was recorded with a Bruker Fourier 300 at 300 MHZ, and $CDCl_3$ was used as a solvent. A Shimadzu QP2010 mass spectrometer was used for the mass spectrometric analysis. Column chromatography was performed using silica gel (60–120 mesh) from Sigma Aldrich in a glass column of varying size fitted with Teflon taps. Thin-layer chromatography (TLC) was performed on pre-coated surfaces, and detection was done with iodine crystals in an iodine tank.

Results and Discussion

The FT-IR, ^{1}H NMR, ^{13}CNMR, DEPT-135, HSQC, COSY and mass spectra of JSO4 are shown in Figures 1-7 respectively.

FT-IR (cm^{-1}); 3100,2924,2854,1690,1587,1487,1463,1348,1190,1170,1042.

^{1}H NMR (300 MHz, $CDCl_3$) in ppm: δ 7.27 (1H,d), 7.23 (1H,d), 7.328(1H,t), 7.353(1H,t), 4.64 (2H,s), 4.02(1H,q), 3.97(1H,q), 3.86 (1H,Cd), 3.48(2H,q)3.26(1H,t),3.10 (3H,s), 2.09 (1H,m), 2.02 (1H,m) 2.01(2H,q) 1.95 (1H,m), 1.77(2H,q), 1.74(2H,q), 1.72(2H,m), 1.70 (2H,m) 1.68 (2H,m), 1.51 (2H,d), 1.29 (3H,d), 1.26 (3H,d),1.07 (3H,t), 1.04(3H,s), 1.01(3H,d), 0.97(3H,t), 0.9(3H,t), 0.85(3H,t),

^{13}CNMR (300 MHz, $CDCl_3$): δ 149.02 (C-1), 149.07(C-2), 129.81 (C-3), 129.71 (C-4),126.74 (C-5), 126.93 (C-6), 31.54 (C-7), 14.10 (C-8), 22.46 (C-9), 10.83 (C-10), 61.06 (C-11), 31.72 (C-12), 31.84 (C-13), 14.04 (C-14), 13.93 (C-15), 30.88 (C-16), 13.99 (C-17), 28.09 (C-18), 13.98 (C-19), 61.36 (C-20), 29.05 (C-21), 22.50 (C—22), 21.87 (C-23), 10.72 (C-24), 61.56 (C-25), 26.32 (C-26), 31.64 (C-27), 149.20 (C-28), 121.91 (C-29), 56.87 (C-30), 62.62 (C-31a), 61.62 (C-31b), 23.38 (C-32), 13.70 (C-33), 50.28 (C-34), and 13.86 (C-35).

The compound JSO4 (21 mg) appeared as a golden-yellow oil with an RF value of 0.56. JSO4's

FTIR spectrum (Figure 1) revealed a weakly intense peak at 3100 cm^{-1}, which is typical of aromatic C-H and confirmed by the presence of the C=C stretch at 1487 cm^{-1} and out of plane bending at 687 cm^{-1}. Out of plane =C-H at 85.51 cm^{-1} confirmed the presence of the alkenes group C=C in a weakly intense band at 1690 cm^{-1}. The C-N stretch medium band was observed at 1190 cm^{-1} while the C-O stretch was observed at 1142 cm^{-1}. The presence of more than four adjacent methylene groups was observed at 727 cm^{-1} while at 2924 cm^{-1} the presence of symmetric and asymmetric C-H stretching vibrations of methyl groups was observed.

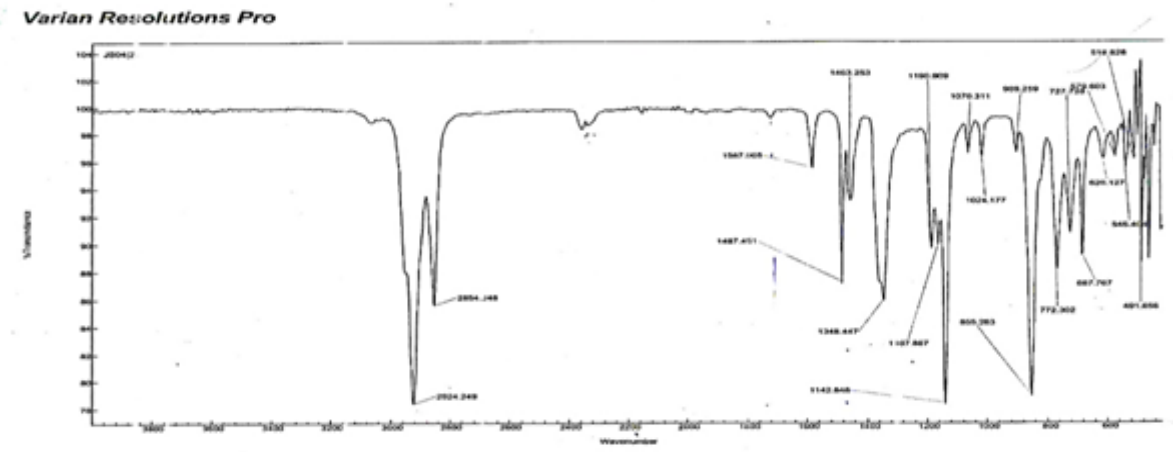

Figure 1: FT-IR spectrum of JSO4

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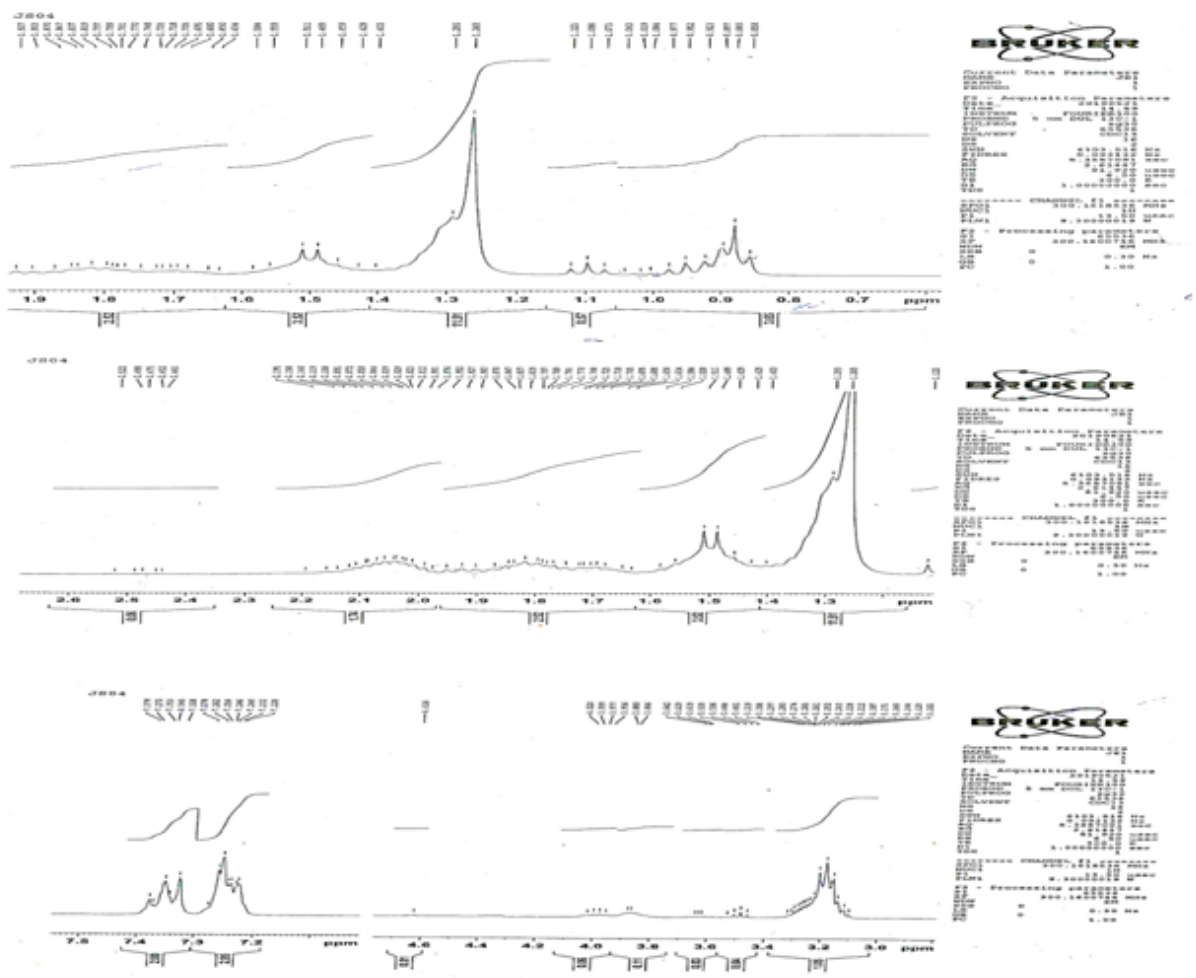


Figure 2: ^{1}H NMR spectra of JSO4 (Expanded)

The ^{1}HNMR (300 MHz, $CDCl_3$) spectrum of JSO4 (Figure 2) showed the chemical shift of six member ring aromatic protons as doublets at 7.23 ppm (1H, d), 7.27 ppm (1H, d), and 7.32 ppm (1H, d). At 7.35 ppm, the chemical shift of a six-membered aromatic proton was observed and assigned to (C-6) (1H, t). Methine alkoxyprotons signals were observed at 3.62 ppm (1H, t), 3.86 ppm (1H, d), and 4.02 ppm (1H, q). Methylene alkoxy proton resonated at 3.48 ppm (2H, q).

Other signals were assigned to nine different methyl protons ranging from 0.85 ppm (t), 0.95 ppm (t), 1.00 ppm (d), 0.01 ppm (d), 0.97 ppm (t), 1.07 ppm (t), 1,26 ppm (d), and 1.29 ppm (d). The aliphatic methylproton on a six-membered aromatic ring was observed at 0.923 and 1.29 ppm. The methine tertiary amine proton signal was assigned to 3.26 ppm (1H, t)).

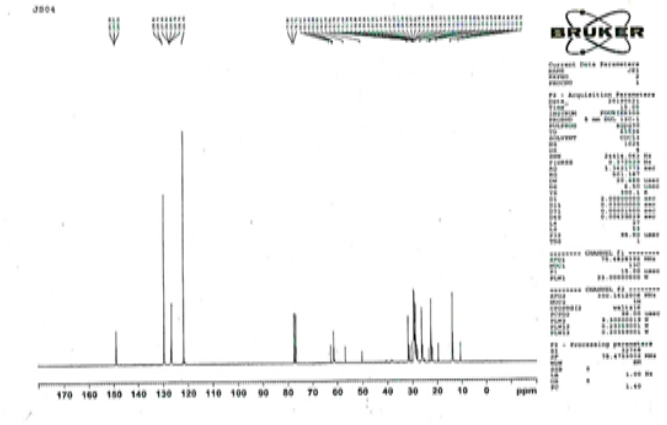


Figure 3: ^{13}C NMR spectrum of JSO4

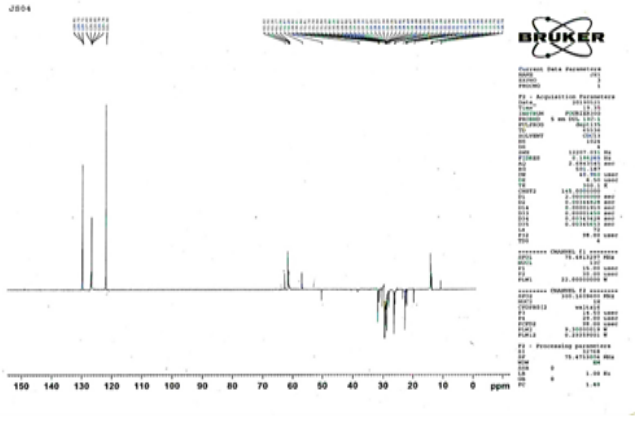


Figure 4: DEPT-135 spectrum of JSO4

The structural identity of JSO4 was further confirmed by its carbon NMR signals, which showed signals for thirty-five identifiable carbon atoms (Figure 3). The presence of an alkenic carbon signal (C-29) resonated at 121.91 ppm, and quaternary olefinic carbon was observed at 149.20 ppm (C-28). The chemical shifts at 126.74, 126.93, and 129.71 ppm were attributed to the carbon of a six-membered aromatic ring. The presences of four methine alkoxy carbons were observed at 61.62 ppm (C-31), 61.62 ppm (C-31b), 61.56 ppm (C-25), and 61.36 ppm (C-20). The methylene alkoxy carbon resonated at 50.28 ppm (C-34).

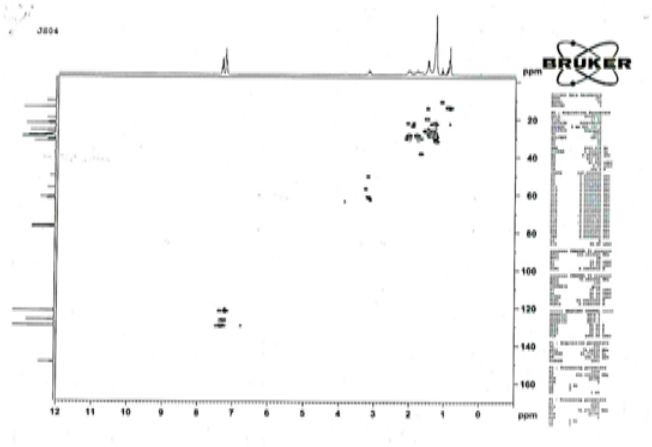


Figure 5: HSQC spectrum of JSO4

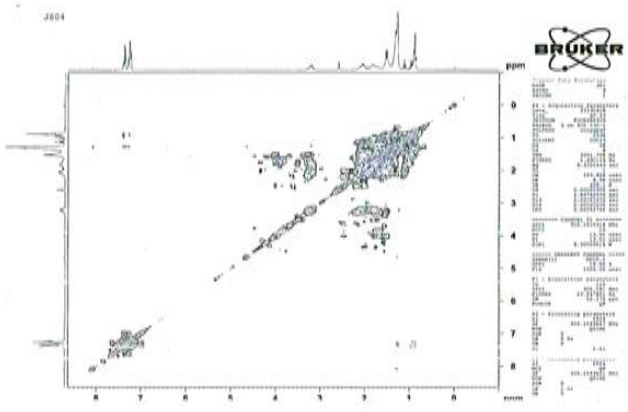


Figure 6: COSY spectrum of JSO4

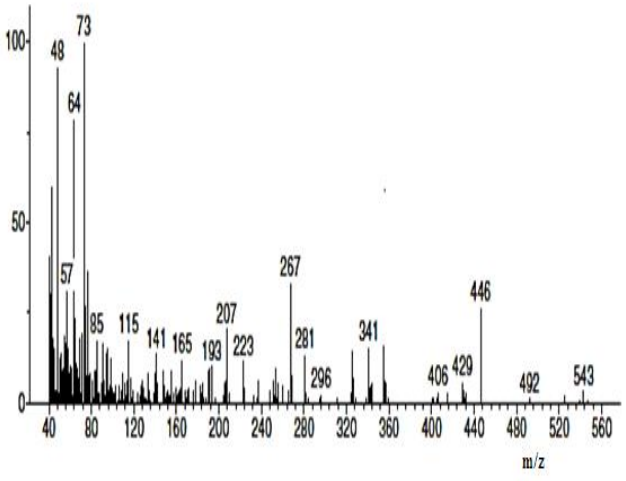
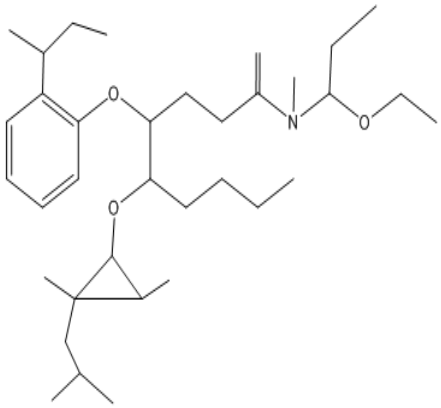
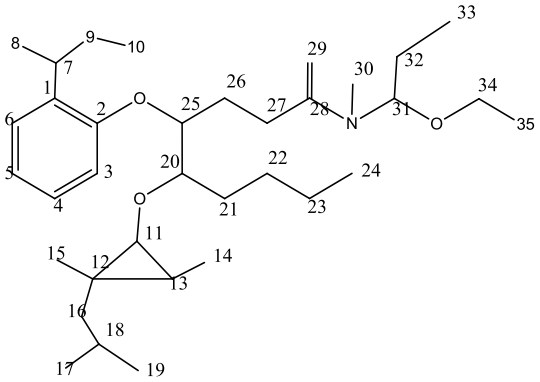


Figure 7: Mass spectrum of JSO4



5-(2-(*sec*-butyl)phenoxy)-*N*-(1-ethoxypropyl)-6-(2-isobutyl-2,3-dimethylcyclopropoxy)-*N*-methyldec-1-en-2-amine

Figure 8: Proposed structure for JSO4



$C_{35}H_{61}NO_3$

M/Z=543

Figure 9: Numbering the carbon atoms of JSO4

The distortionless enhancement by polarization transfer (DEPT) spectrum (Figure 4) showed methlylene (CH_2) carbon as negative peaks at values of 19.69, 21.87, 22.46, 22,50, and 22.56 ppm. Other methylene carbons were observed at 22.61, 29.38, 31.72, 29.47, and 30.88 ppm. Three methine alkoxy (-OCH_3) peaks were observed at 61.62, 61.06, 61.36, and 61.56 ppm. Quaternary carbons were absent while the aromatic carbons resonated at 126.74, 126.9, 129.71, and 129.81 ppm. The tertiary methine amine carbon was observed at 62.62 ppm, while the tertiary methyl amine carbon resonated at 56.87 ppm. The methylene alkoxy carbon signal was observed at 50.28 ppm.

The heteronuclear single quantum coherence (HSQC) spectroscopy of JSO4 (Figure 5) indicated that the aromatic protons with chemical shifts of 7.328, 7.353, 7.232, and 7.278 ppm were coherent with carbons at 126.74, 126.93, 129.71, and 129.81 ppm, respectively. Also, the methine alkoxy protons at 3.866 ppm were coherent with the carbon at 61.06 ppm. The tertiary methyl amine at 3.102 ppm was coherent with the carbon at 56.87 ppm, while the tertiary methine proton at 3.261 ppm was coherent with the carbon at 62.62 ppm. Also, the proton at 3.629 ppm was attached to the carbon at 61.62 ppm. The methylene protons at 1.680, 1.695, 1.705, 1.725, and 1.741 ppm were attached to their corresponding carbons at 21.87, 22.46, 22.50, and 26.32 ppm, respectively. Also, methyl protons at 0.858, 0.952, 1.004, and 1.019 ppm were coherent with their various carbons at 10.72, 10.83, 13.94, and 13.99 ppm, respectively.

Correlation spectroscopy (COSY) (Figure 6) is a two dimensional NMR that is used to identify the protons that are coupled or split with each other through a cross peak. The COSY of compound JSO4 depicts that the aliphatic CH_3 protons at 0.923 ppm coupled with CH_3 protons at 1.203 ppm are all located on the aromatic group. The methine alkoxy

proton at 3.977 ppm was coupled with the methine alkoxy proton at 4.020 ppm. Furthermore, the methylene protons at 1.695 ppm are coupled with the protons at 1.043 ppm and 1.741 ppm. Moreover, the protons at 1.043 ppm are coupled with the methylene protons at 1.695 ppm and the methyl protons at 0.858 ppm. The protons of the aromatic region are also coupled with each other at 7.232 and 7.328 ppm. The aromatic protons at 7.278 ppm and 7.353 ppm are coupled together. The aromatic protons at 7.328 ppm are coupled with the aromatic protons at 7.232 and 7.353 ppm. This showed that there was a correlation between the protons of the compound.

The GC-MS spectrum (Figure 7) showed a molecular ion peak [M^+] at m/z 543, corresponding to the formula $C_{35}H_{61}NO_3$ with six degrees of unsaturation. The six degrees of unsaturation are four C=C bonds, one cyclopropane ring, and one six membered ring. Other prominent peaks were m/z 492 (491), 446 (445), 223 (222), 207 (206), 193 (192), 165 (164), 141 (140), 115 (114), 73 (72), and 56 (57). The fragmentation patterns of 5-(2-(sec-butyl) phenoxyl)-N-(1-ethoxypropyl)-6-(2-isobutyl-2-3-dimethylcyclopropoxy)-N-methyldec-1-en-2-amine agreed with these peaks

The Hinsberg test**:** JSO4 was shaken well with the Hinsberg reagent. No reaction occurred. It remained insoluble. Dilute HCl was then added. The insoluble JSO4 became soluble. This test confirmed the presence of tertiary amine.

Based on spectra analysis, the structure in Figure 8 has been proposed for JSO4. The numbering of carbon atoms for NMR identification is shown in Figure 9. The mass spectrum fragmentation pattern is shown in Figure 10.

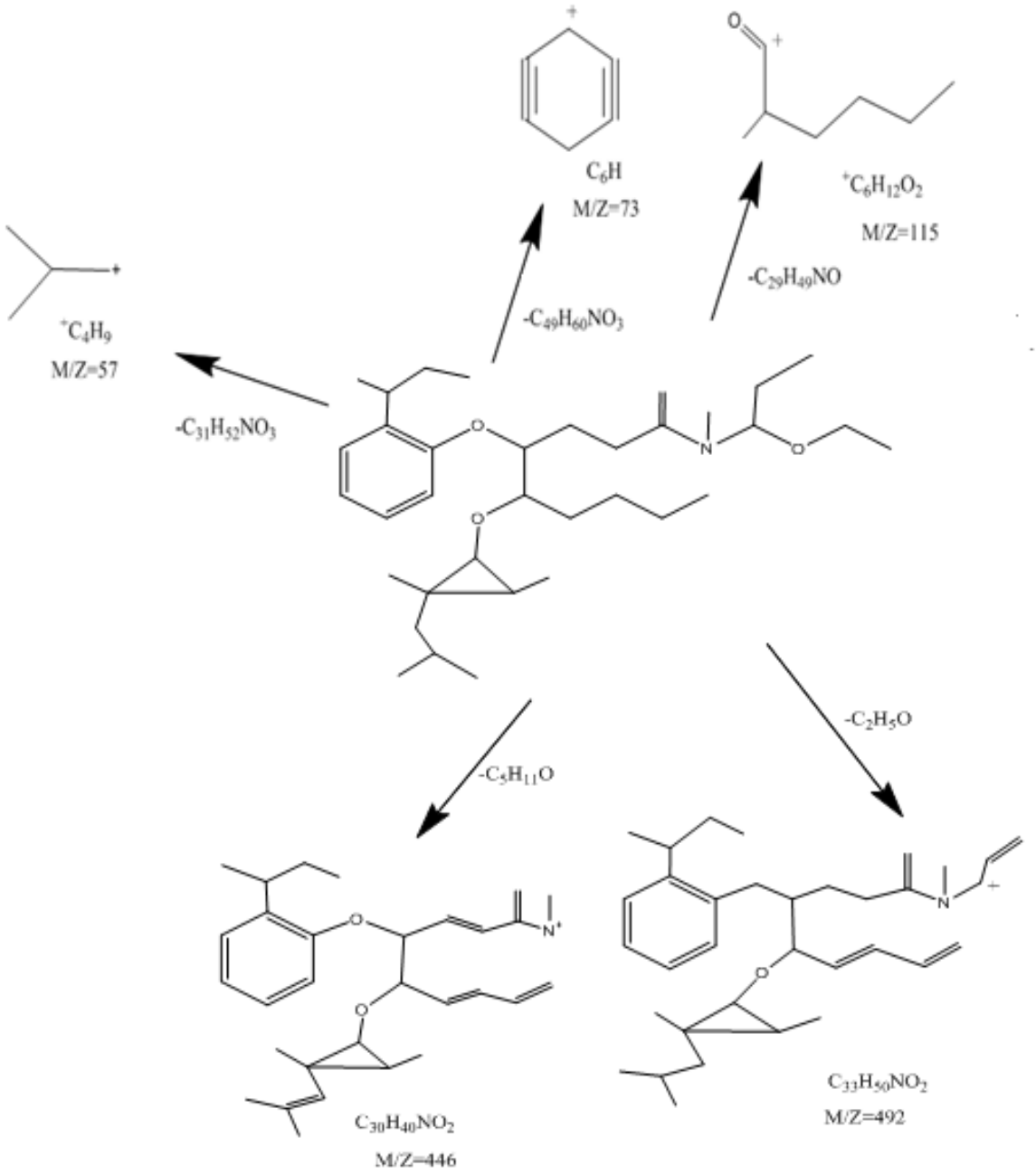


Figure 10: Mass spectrum fragmentation pattern for JSO4

Conclusion

The chloroform fraction of the leaves of *S. brachystachys* chromatographed on silica gel and then ethyl acetate and methanol solvents on a precoated aluminum plate gave rise to the compound labeled JSO4. No report from the literature with regards to any compound isolated from the leaves of this plant has yet to be documented in this work, and so it is termed a novel one. The isolated component was characterized based on FT-IR, ^{1}H and ^{13}C NMR spectra, DEPT-135, HSQC, COSY and mass spectrometry. The compound isolated was identified as 5-(2-(sec-butyl)phenoxy)-N-(1-ethoxypropyl)-6-(2-isobuthyl-2,3-dimetylcyclopropoxy)-N-methyldec-1-en-2-amine. We recommend the bioactivity and toxicity studies of this novel compound.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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