# ISOLATION WITH CHARACTERIZATION OF COLUMBIN AND NOVEL CLERODANE FURANO-DITERPENE WITH GC-MS AND ANTIMICROBIAL ANALYSES OF ESSENTIAL OILS FROM *Sphenocentrum jollyanum* Pierre

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## ABSTRACT

The methanol extract of the seed of *Sphenocentrum jollyanum* Pierre, was subjected to partition chromatography in gradient elutions, using vacuum liquid chromatography (VLC) and HPLC techniques. It led to the isolation of two compounds labelled as DO5d-2 and DO6, whose structures were determined by complete analyses of their spectroscopic data, utilizing modern techniques (LC-MS, $^{1}$H- and $^{13}$C-NMR, DEPT-NMR, HSQC, HMBC, COSY and NOESY). The Essential oils of the leaf and stem were extracted by hydro-distillation using Clevenger-typed apparatus and analysed by GC-MS technique. It was subsequently subjected to antimicrobial analysis using the agar diffusion method. The major isolated compound – DO6, was further characterized as 'columbin', while DO5d-2 was characterized as a new clerodane type furano-diterpene, which has not been previously reported. Essential oils obtained by hydro distillation from leaf and stem of *Sphenocentrum jollyanum* Pierre, were dominated by aldehydes in 10.93% and 15.14% respectively. Fifty (50) compounds were identified in leaf oil comprising 68.73% of it, with phytol (6.62%) and n-hexanol (6.28%) as most abundant compounds. Twenty-eight (28) compounds identified in the stem oil comprised 44.90% containing pentadecanal (14.72%) and phytol (6.38%) as its prominent compounds. The antimicrobial result showed the oil from the leaf was active against all the bacterial and fungi tested, while that from the stem revealed moderate activity against the tested bacterial and fungi.

**KEYWORDS:** *Sphenocentrum jollyanum*, Menispermaceae, furano-diterpene, columbin, Essential oils, Antimicrobial.

## INTRODUCTION

In our quest for drug discovery from natural products, 40 plants, which are utilized in ethno-medicinal treatment of malaria, were investigated, out of which six, including *Sphenocentrum jollyanum* were found to be promising [1,2]. *Sphenocentrum jollyanum* Pierre (Menispermaceae) is an evergreen indigenous medicinal plant commonly referred to as 'akerejupon in Yorubaland of Nigeria [3,4]. It is utilized in the treatment of abdominal


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disorders, coughs, high blood pressure, tumors; relieve stomach-ache and constipation for better appetite; an antidote for poisoning (including snake bite) and as food sweetener. Different parts of the plant are utilized in ethno-medicine as anti-diabetic, hepato-protective, antioxidant, molluscicidal, antimalarial, antimicrobial, anti-inflammatory, anthelmintic, antiviral, anti-cholinesterase, anti-hyperglycaemic, anti-arthritic, antitumor and hypotensive activities [5,6]. There are experimental justifications for these bioactivities [7-16]. Compounds isolated and reported from different parts of the plant include isoquinoline alkaloids: palmatine, jatrorrhizine, tetrahydrojatrorrhizine and columbamine; the inositol-derivative: (–)-viburnitol; the sterols: sitosterol, campestrol and stigmasterol; and furano-diterpenes: columbin, isocolumbin and fibleucin [17-20]. A total of 19 compounds have been reported present in the root essential oil of *Sphenocentrum jollyanum*, consisting of monoterpenoids (33.5%) and sesquiterpenoids (56.3%) [21]. However, essential oils from the leaf and stem have not been reported, which this study presents.

So, this study is reporting the chromatographic purifications on the methanol extracts of seed, and the gas chromatographic-mass spectrometric identification of the chemical constituents with the antimicrobial activities of essential oils from the leaf and stem of *Sphenocentrum jollyanum.*

## MATERIALS AND METHODS

### Plant Collection and Identification:

*Sphenocentrum jollyanum* Pierre (from Nigeria) were identified and authenticated at the Herbarium, Department of Botany, University of Ibadan where voucher specimens were deposited, with identity number UIH-22643. The plant was sorted into seeds, leaf and stem parts.

### Plant Extractions:

Seed part (100 g) of *Sphenocentrum jollyanum* were air-dried and ground to fine powder. This was extracted with methanol and concentrated under reduced pressure, at 40 $^{o}$C to give 4 g of the crude extract. Leaf and stem essential oils were extracted by hydro-distillation method using all glass Clevenger apparatus designed to British pharmacopoeia specifications [22], with a small quantity of distilled n-hexane (1mL), for 3 hours.

### Chromatographic Purification of isolates from the Plant Extract:

Methanol extract (3.83 g) of *Sphenocentrum jollyanum* seed was subjected to partition chromatography, using VLC in gradient elutions utilizing hexane, ethyl acetate and methanol to give 18 sub-fractions (DO1 to DO18). The fractions were dried and dereplicated with the use of LC-MS spectroscopy and reaxys database search to identify any unique masses. As a result of the search, DO5 was purified by the reversed-phase HPLC to obtain DO5d-2, which is compound-**1** (8 mg); DO6, DO7 and DO8 were combined and purified by same method to obtain 52.1 mg of compound-2 (DO6) a whitish elongated threadlike crystal.

### Instrumentation:

NMR spectra were recorded on Brucker BioSpin GmbH 400 and 600 MHz and Bruker AVANCE III spectrometer at 600 MHz. HRESI-MS spectra were obtained using a Thermo Instruments MS system (LTQ XL/LTQ Orbitrap Discovery) coupled to a Thermo instruments HPLC system (Accela PDA detector, Accela PDA autosampler and Accela pump). Fractionation was carried out with VLC column packed with silica gel and connected to a vacuum pump. Purification was done with semi-preparative gradient Agilent HPLC apparatus (1100series) equipped with Binary pump, Diode array detector (G1315B), Sunfire reversed phase column C18 (5µm 10×250 mm) and Agilent C18 (5 µm 9.4×250 mm), and a mobile phase solvent gradient 95:5% $H_2O$


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(Milli-Q filter water 18 MΩcm, Millipore, Germany) and 100% MeOH (Sigma Aldrich, UK).

**GC-MS Analyses:**

GC-MS analyses were carried out by using an Agilent 7890B-5977B GC-MS (Santa Clara, CA, USA) system operating in the EI mode at 70 eV, using an HP-5MS capillary column (5% phenylmethyl polysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness) (J & W Scientific, Folsom).

GC-MS was programmed with the following conditions: 60 °C for 4 min, then 4 °C/min to 160 °C, followed by 11 °C/min up to 280 °C, held for 15 min, and finally 15 °C/min up to 300 °C. The carrier gas was helium at a flow rate of 1.2 ml/min; the injector temperature was 280 °C, while the transfer line temperature was 300 °C; injection volume: 1 μl; split ratio: 1:100; run time: 57 min; acquisition mass range: 29–400 amu. Identification of the essential oil components were based on their retention indices (experimentally determined using homologous series of C8–C30 alkanes), and by comparison of their mass spectral fragmentation patterns in computer matching against library Linear retention index and mass spectra taken from Adams and NIST 17[25] FFNSC2 and MAGGI libraries (Adams 2007; NIST 17 2017; FFNSC2 2012). Relative peak area percentages were obtained by peak area normalization without using correction factors and were the mean of three determinations with a RSD% in all cases below 10%.

**Antimicrobial Assay:**

Agar well diffusion method according to Valgas *et al*., 2007 was used to screen the antimicrobial activity of the essential oils [23].

The antimicrobial activity was performed using Gram-positive and Gram-negative bacterial and fungi respectively, which are pathogenic and associated with various diseases such as urinary tract infections and alimentary tract infection. The microbes used are *Staphylococcus aureus*, *Escheria coli*, *Bacillus Substilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia*, and the fungi are *Candida albicans*, *Rhizophus stolonifer*, *Aspergillus niger*, and *Penicillin notatum*.

**RESULTS AND DISCUSSION**

Compound-**1** DO5d-2 (8 mg) was obtained as a yellowish crystal. Its LC-MS orbit trap experiment gave its HREIMS [M+H] as 375.14413 [Exact mass = 374.1366], with molecular formula of $C_{20}H_{22}O_7$, corresponding to 10 double bond equivalence, which can be accounted for as four ring systems, three carbonyl groups and three carbon-to-carbon double bonds (Fig. 1).

Infra-red (IR) spectrum confirms the functional group for compound **1** by showing absorptions at 3149 $cm^{-1}$ (C=C-H); 1505 $cm^{-1}$ (C=C); very strong and sharp absorptions at 1746 and 1716 $cm^{-1}$ corresponding to C=O stretches. The O-$H_{stretch}$ absorption is observed as the medium signal at 3464 $cm^{-1}$, which also indicate it is a weakly H bonded O-H. While 1343, 1301 and 1279 $cm^{-1}$ accounts for the O-H bending vibrations. The alkoxy C-$O_{stretch}$ is a very strong and sharp absorption at 1150 $cm^{-1}$ (Fig.2a).

$^{1}$H-NMR spectrum (Table 1) showed two peaks at 1.19 and 1.35 ppm representing two methyl protons (H-19 and H-20). The three methylene peaks are at 1.34 and 1.63 (H-6), 1.89 and 2.06 (H-7) and 2.89 and 3.49 ppm (H-11) (Fig.1) (diastereotopic protons). The spectrum indicates presence of two cis coupled olefinic protons (δ 6.47, 1H (dd, *J* = 5.2, 7.9 Hz, H-2) and (δ 6.29, 1H (dd, *J* =7.6,1.4 Hz, H-3); the three furan methine protons have their absorptions at (δ 6.72, 1H (d, *J* = 1.8), 8.04 and 7.43, for H-14, H-15, H-16, respectively.

Its $^{13}$C-NMR spectrum shows absorptions at δ 129.4 (C-2) and 134.3 (C-3) ppm, with three furan methine carbons at 107.4 (C-14), 146.9 (C-15) and 143.4 ppm (C-16) respectively and two methyl peaks at 25.6 (C-19) and 23.6 ppm (C-20). The three methylenes are observed at 24.8 (C-6), 20.8 (C-7) and 45.1 ppm (C-11) respectively. Absorptions of the three carbonyl



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carbons are at δ 195.1 (C-12), 173.6 (C-17) and 176.7 (C-18). The two-alkoxy carbons are at δ 74.5 (C-1) and 81.4 (C-4). The information from the HMBC and COSY (table 2) spectra indicated a strong correlation of furan protons signals δ 6.72 (H-14) and δ 7.43 (H-16) to the quaternary carbon δ 195.1 (C-12) and a weak correlation from a methylene proton signal δ 3.49 (H-11), methine proton δ 2.61 (H-10) and methyl proton δ 1.35 (H-20) to the latter (C-12) were observed. Also, the diastereotopic methylene protons signal δ 2.06 (H-7) show connectivity to the carbon signal δ 173.7 (C-17), of the carboxylic acid.

Compound-1 have similar spectroscopic data to that of columbin, with exceptions and major differences at carbon 12, which support compound 1 as the open analogue of the δ-lactone (6-membered cyclic ester) of columbin. Such skeleton is similar to that in spiciflorin, a previously isolated compound from *Cleidion spiciflorum* [24]. Based on these spectra data, the structure of compound 1 is characterized as a new clerodane furano-diterpene (Fig. 1).

52.1 mg of compound **2** was obtained as a whitish threadlike crystal. Its LC-MS orbit trap experiment gave the HREIMS as 358.12344 [Exact mass= 358.1416], which led to the determination of its molecular formula as $C_{20}H_{22}O_6$. $^{1}$H- and $^{13}$C-NMR data for compound-**2** are identical with the structure of columbin, which was previously reported [19,25]. Its IR spectrum shows major characteristic absorptions that confirm the functional groups in columbin (Fig.2b). Hence its overall characterization correlated with columbin previously reported in literature [19,25].

Columbin has been previously reported as constituent of many ethno-medicinally utilized plants from different parts of the world, with its structural elucidation being constantly reviewed [24-35]. Columbin and its derivatives are also known to have several bioactivities such as reduction of toxicity and remedies, including venom of snakebite from cobra, puff adder and shortening of sleeping time of anaesthetized patients [30], [36-38].

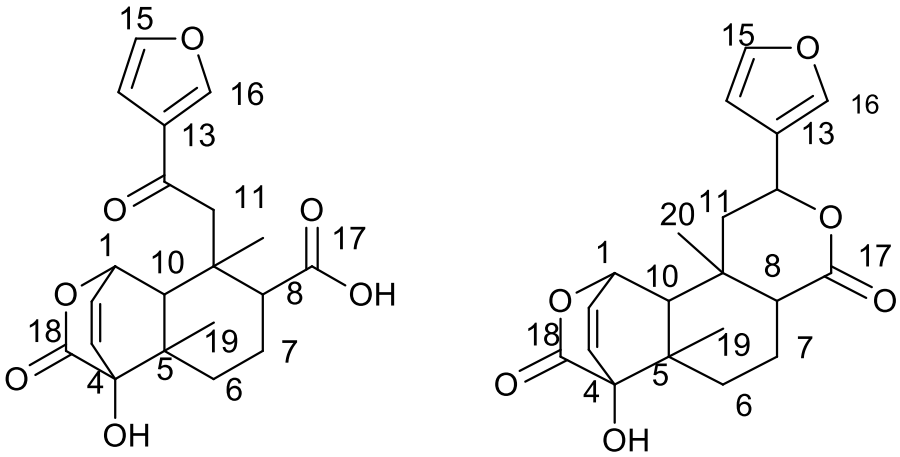


Fig. 1: Structures of **1** and **2** isolated from *Sphenocentrum jollyanum* Pierre



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**Table 1: $^{1}$H-NMR and $^{13}$C-NMR data for compound 1 and 2**

| Position | **Compound 1** | | **Compound 2** | |
|---|---|---|---|---|
| | $^{13}$C | $^{1}$H | $^{13}$C | $^{1}$H |
| 1 | 74.5 | 5.31, (1H, m) | 74.4 | 5.15(1H, d, *J*=5.4 ) |
| 2 | 129.4 | 6.47,(1H, dd, *J* = 5.2, 7.9) | 129.1 | 6.46 (1H,dd, *J*=13.4, 7.7) |
| 3 | 134.3 | 6.29, (1H, dd, *J* = 7.6, 1.4 ) | 137.4 | 6.35 (1H,dd, *J*=8.0, 6.3, 1.7) |
| 4 | 81.4 | - | 80.5 | - |
| 5 | 37.5 | - | 37.0 | - |
| 6 | 24.8 | 1.34,1.63 | 25.6 | 1.75 (1H, m), 1.40 ( 1H,m) |
| 7 | 20.8 | 1.89,2.06 | 17.3 | 2.63 (1H, m, J=27.2, 15.7, 8.0) |
| 8 | 49.5 | 2.5 | 44.5 | 2.42 (dd, J=11.0, 1.8) |
| 9 | 35.1 | | 35.2 | |
| 10 | 47.6 | 2.61 (d, J=7.7 Hz) | 47.9 | 1.73 (1H,d, J=6.2) |
| 11 | 45.1 | 2.89,3.49 | 42.0 | 2.26(1H,dd,J=14.9, 4.4) 1.94 (1H, dd , J=15.5, 12.0) |
| 12 | 195.1 | | 70.9 | 5.40 (1H, dd) [J=12.0, 4.2] |
| 13 | 128.8 | | 125.0 | - |
| 14 | 107.4 | 6.72, 1H (d, J=1.8) | 108.7 | 6.44(1H,d) [J=7.5] |
| 15 | 146.9 | 8.04 (s) | 144.3 | 7.43(1H,d/ 1H,s) |
| 16 | 143.4 | 7.43 (s) | 140.2 | 7.47(1H,s) |
| 17 | 173.6 | | 173.5 | - |
| 18 | 176.7 | | 175.6 | - |
| 19 | 25.6 | 1.19 (s) | 24.1 | 1.07 (3H,s) |
| 20 | 23.6 | 1.35 (s) | 28.3 | 1.24 (3H,s) |

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

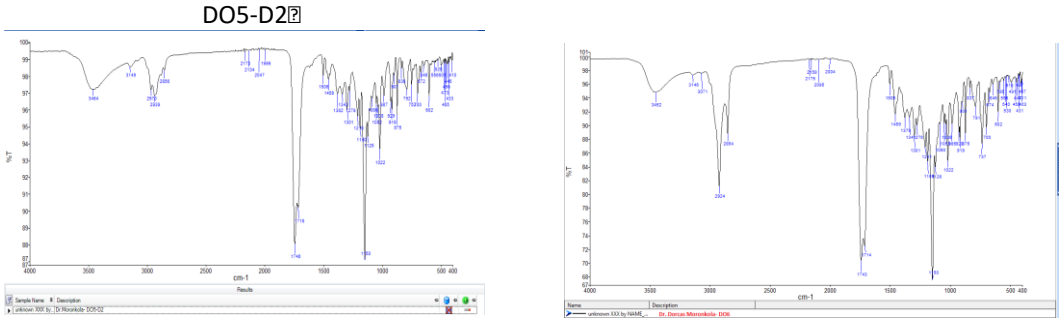


Fig. 2**:** Infra red (IR) spectrum of compounds **1(a)** and **2(b)**



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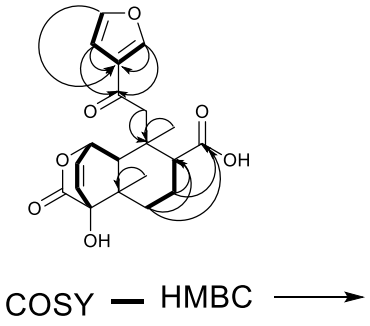


Fig. 3: COSY and HMBC correlation of compound **1**

**Table 2: COSY and HMBC correlation of compound 1**

| Carbon # | $^{13}C$ | $^{1}H$ | COSY | HMBC |
|---|---|---|---|---|
| 1 | 74.5 | 5.31, (1H, m) | H2 | |
| 2 | 129.4 | 6.47,(1H, dd, *J* = 5.2, 7.9) | H1, H3 | |
| 3 | 134.3 | 6.29, (1H, dd, *J* = 7.6, 1.4 ) | H2 | H6a, H6b |
| 4 | 81.4 | - | | H19 |
| 5 | 37.5 | - | | H11, H20 |
| 6 | 24.8 | 1.34,1.63 | H7, H8 | H8 |
| 7 | 20.8 | 1.89,2.06 | H6, H10 | |
| 8 | 49.5 | 2.5 | H6 | H11 |
| 9 | 35.1 | | | H20 |
| 10 | 47.6 | 2.61 (d, J=7.7 Hz) | H7 | H20 |
| 11 | 45.1 | 2.89,3.49 | | |
| 12 | 195.1 | | | H11a, H11b |
| 13 | 128.8 | | | H6a |
| 14 | 107.4 | 6.72, 1H (d, J=1.8) | | |
| 15 | 146.9 | 8.04 (s) | | |
| 16 | 143.4 | 7.43 (s) | | |
| 17 | 173.6 | | | H7 |
| 18 | 176.7 | | | H10 |
| 19 | 25.6 | 1.19 (s) | | |
| 20 | 23.6 | 1.35 (s) | | |

**Chemical Composition of Leaf and Stem Essential Oils of *Sphenocentrum jollyanum* Pierre**

Leaf and stem essential oils were obtained from *Sphenocentrum jollyanum* in 0.266% and 0.128% yields, respectively. (Table 3).

Fifty compounds (Table 4) identified in the leaf oil are responsible for 68.73% of it and was dominated by phytol (6.62%), n-hexanol (6.28%), dihydroedulan (6.19%), methyl salicylate (4.48%) and 4-(dimethoxymethyl)-methylbenzoate (4.48%). Aldehydes (10.93%), hydrocarbons (12.25%) and norisoprenoids (09.57%) were the most abundant classes of compounds present in the leaf oil. Twenty-eight



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compounds were identified in the stem oil responsible for 44.90% of it, with pentadecanal (14.72%), phytol (6.38%) and pentacosane (3.45%) as its major compounds.
Both leaf and stem oils are rich in aldehydes (10.93%, 15.14%), hydrocarbons (12.25%, 10.49%) (and oxygenated diterpenes (6.62%, 6.38%) respectively. The phenylpropanoid E-anethole (0.32%) is exclusively identified in the stem oil. Alcohols (7.62%) and the amide (Z)-9-octadecenamide (0.33%) is peculiar to the leaf oil. Norisoprenoids are more abundant in leaf oils (9.57%), while sequiterpenes are present in greater amount in stem oil (1.35%) (Tables 4 and 5).

The root oil of *S. jollyanum* reported by Aboaba and Ekundayo 2010, showed that α-pinene is in appreciable quantity (11.2%); it is in trace amount in the stem oil studied.
Ionones (3.38% and 2.71% in leaf and stem oils respectively) were also reported in comparable amount in the root oil (2.5%) [21].
Other major reported constituents in the root are 1,8-Cineole (6.3%), Isocaryophyllene 7.7%, α-eudesmol (26.1%) and γ-terpinene (4.1%) [21], most of which are in minimal amount in the leaf and stem oils.
Phytol is a prominent compound in the two oils (leaf and stem). It is an acyclic diterpenoid, a vital precursor for vitamins E and K1, an antioxidant and a preventive agent against epoxide-induced breast cancer carcinogenesis [39,40]. Methyl salicylate a known constituents and fragrance and perfumery industry has been reported to have significant antioxidant and antimicrobial activities [41]. Pentacosane, nonacosane, tricosane are known to exhibit antibacterial activity [39]. Presence of norisoprenoids such as dihydroedulan and ionones could also be responsible for the anticancer activity of the plant [42]. Linalool in leaf and anethole in stem oil are both bioactive agents in experimental models and has shown antibacterial, sedative and anti-inflammatory activities [43]. The vast importance of the components identified in the leaf and stem essential oils of *Sphenocentrum jollyanum* justifies its use in the treatment of many diseases.

**Table 3: Yields of essential oils procured from leaf and stem parts of *Sphenocentrum jollyanum Pierre***

| Plant | Parts | Weight of sample(g) | Weight of volatile oil procured (g) | **%** Yield of essential oil procured |
|---|---|---|---|---|
| *Sphenocentrum jollyanum* | Leaf | 1000 | 1.33 | 0.266 |
| | Stem | 800 | 1.02 | 0.128 |


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**Table 4: Essential oil composition of Leaf and Stem of *Sphenocentrum jollyanum* Pierre**

| S/N | RT | RI (Calc) | % Composition (Leaf) | % Composition (Stem) | Compound identified | Class of Compounds |
|---|---|---|---|---|---|---|
| 1 | 2.72 | 783 | 0.15 | - | 3-Hexanone | ketone |
| 2 | 2.78 | 788 | 0.44 | - | 2-Hexanone | ketone |
| 3 | 2.83 | 794 | 0.1 | - | 3-Hexanol | alcohol |
| 4 | 2.91 | 800 | 3.81 | - | Hexanal | aldehyde |
| 5 | 3.84 | 844 | 3.86 | - | (E)-2-Hexenal | aldehyde |
| 6 | 4.13 | 857 | 0.65 | - | Hex-2-en-1-ol | alcohol |
| 7 | 4.18 | 859 | 6.28 | - | n-Hexanol | alcohol |
| 8 | 4.26 | 863 | 1.06 | - | P-xylene | hydrocarbon |
| 9 | 5.87 | 923 | 0.32 | - | Acetonyl acetone | ketone |
| 10 | 6.17 | 932 | 0.25 | - | α-pinene | monoterpene |
| 11 | 7.11 | 958 | 0.2 | - | Benzaldehyde | aldehyde |
| 12 | 7.83 | 978 | 0.39 | - | 1-Octen-3-ol | alcohol |
| 13 | 8.12 | 987 | 0.81 | - | 6-methyl-5-Hepten-2-one | ketone |
| 14 | 8.31 | 992 | 1.55 | - | Myrcene | monoterpene |
| 15 | 8.66 | 1002 | 0.11 | - | Trans-2-(2-pentenyl)-Furan | furan |
| 16 | 8.99 | 1010 | 0.09 | - | 3-Carene | monoterpene |
| 17 | 9.2 | 1016 | - | 0.04 | α-Terpinene | monoterpene |
| 18 | 9.5 | 1024 | - | 0.13 | p-Cymene | monoterpene |
| 19 | 9.7 | 1028 | - | 0.28 | D-Limonene | monoterpene |
| 20 | 9.77 | 1030 | 0.81 | 0.1 | 1,8-Cineole | monoterpenoid |
| 21 | 9.9 | 1034 | 0.17 | - | 2,2,6-trimethyl cyclohexanone | ketone |
| 22 | 10.3 | 1043 | 0.21 | - | Benzene acetaldehyde | aldehyde |
| 23 | 10.9 | 1058 | 0.24 | 0.3 | γ-terpinene | monoterpene |
| 24 | 12.5 | 1100 | 0.55 | - | Linalool | monoterpenoid |
| 25 | 12.7 | 1105 | 0.18 | - | Nonanal | aldehyde |
| 26 | 15 | 1166 | 1.27 | 0.16 | 1,3-dimethoxy-Benzene | aromatic ether |
| 27 | 15.5 | 1179 | 2.69 | - | Naphthalene | hydrocarbon |
| 28 | 16 | 1193 | 4.48 | - | Methyl salicylate | ester |
| 29 | 16.9 | 1220 | 0.66 | - | β-cyclocitral | monoterpenoid |
| 30 | 19.2 | 1285 | - | 0.32 | E-Anethole | phenylpropanoid |
| 31 | 19.3 | 1287 | 6.19 | - | Dihydroedulan | norisoprenoid |
| 32 | 21.5 | 1352 | 0.19 | - | 1,2-dihydro-1,1,6-trimethyl-Naphthalene | hydrocarbon |
| 33 | 23.1 | 1400 | 0.44 | 0.17 | Tetradecane | hydrocarbon |
| 34 | 23.2 | 1405 | - | 0.11 | Methyl eugenol | monoterpenoid |



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| | | | | | | |
|---|---|---|---|---|---|---|
| 35 | 23.9 | 1428 | - | 1.14 | α-Ionone | norisoprenoid |
| 36 | 24 | 1428 | 4.48 | - | 4-(dimethoxy methyl)-methyl benzoate | ester |
| 37 | 24.8 | 1454 | 2.02 | 1.65 | 6,10-dimethyl-5,9-undecadien-2-one | ketone |
| 38 | 25.5 | 1477 | 0.07 | - | γ-Muurolene | sesquiterpene |
| 39 | 25.8 | 1486 | 3.38 | 1.57 | Trans-(E)-β-Ionone | norisoprenoid |
| 40 | 26.1 | 1496 | - | 0.42 | β- alaskene | sesquiterpene |
| 41 | 26.5 | 1509 | 0.19 | 0.93 | β-bisabolene | sesquiterpene |
| 42 | 28.5 | 1580 | - | 0.76 | (3E,7E)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene | hydrocarbon |
| 43 | 31.2 | 1682 | 1.27 | 0.86 | (Z)-3-Heptadecene, | hydrocarbon |
| 44 | 31.9 | 1716 | 2.43 | 14.72 | Pentadecanal | aldehyde |
| 45 | 33.3 | 1800 | 0.19 | 0.43 | Octadecane | hydrocarbon |
| 46 | 33.5 | 1818 | 0.24 | 0.42 | Hexadecanal | aldehyde |
| 47 | 33.9 | 1848 | 0.68 | 1.53 | Hexahydrofarnesyl acetone | sesquiterpenoid |
| 48 | 34.5 | 1890 | - | 8.57 | N.I | |
| 49 | 34.6 | 1896 | - | 8.94 | N.I | |
| 50 | 34.9 | 1922 | 0.82 | 2.31 | Farnesyl acetone | sesquiterpenoid |
| 51 | 34.9 | 1928 | 0.32 | 0.96 | Methyl-Palmitate | ester |
| 52 | 36 | 2086 | 0.2 | - | Z-2-Octadecen-1-ol | alcohol |
| 53 | 36.9 | 2154 | 6.62 | 6.38 | Phytol | diterpenoids |
| 54 | 37.6 | 2200 | 1.81 | 2.12 | Docosane | hydrocarbon |
| 55 | 38.5 | 2324 | 1.08 | 1.23 | Tricosane | hydrocarbon |
| 56 | 39 | 2374 | 0.33 | - | (Z)-9-octadecenamide | amide |
| 57 | 40 | 2496 | 1.64 | 3.45 | Pentacosane | hydrocarbon |
| 58 | 40.4 | 2556 | - | 5.52 | N.I | |
| 59 | 40.7 | 2599 | 1.31 | - | Hexacosane | hydrocarbon |
| 60 | 42.5 | 2830 | 0.93 | 0.94 | Squalene | triterpene |
| 61 | 43.1 | 2900 | 0.57 | 1.47 | Nonacosane | hydrocarbon |
| | | Total % Identified | **68.73** | **44.90** | | |
| | | Total No of Compounds Identified | **50** | **28** | | |
| | | Total % EO | **68.73** | **67.93** | | |



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**Table 5: Comparisons between the class of organic compounds of the leaf and stem essential oils of *Sphenocentrum jollyanum* Pierre**

| S/N | Class of Organic compounds | % Identified in Leaf Essential oil | % Identified in Stem Essential oil |
|---|---|---|---|
| 1. | Alcohols | 7.62 | - |
| 2. | Aldehyde | 10.93 | 15.14 |
| 3. | amide | 0.33 | - |
| 4. | ester | 9.28 | 0.96 |
| 5. | Hydrocarbon | 12.25 | 10.49 |
| 6. | Ketone | 3.91 | 1.65 |
| 7. | Monoterpenes | 2.13 | 0.75 |
| 8. | norisoprenoid | 9.57 | 2.71 |
| 9. | Monoterpenoids | 2.02 | 0.21 |
| 10. | Diterpenoids | 6.62 | 6.38 |
| 11. | Sesquiterpenes | 0.26 | 1.35 |
| 12. | Sesquiterpenoid | 1.5 | 3.84 |
| 13. | Triterpenes | 0.93 | 0.94 |
| 14. | phenylpropanoid | - | 0.32 |
| 15. | furan | 0.11 | - |
| 16. | Aromatic ether | 1.27 | 0.16 |

**Antimicrobial activities**

The essential oil of the leaf and stem were tested against six (6) bacteria (*Staphylococcus aureus, Escheria coli, Bacillus Substilis, Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumonia)* and four (4) fungi (*Candida albicans, Rhizophus stolonifer, Aspergillus niger,* and *Penicillin notatum*).

The leaf oil was effective against all the six (6) bacteria and four (4) fungi tested at concentrations between 31.25 µg/mL and 1000 µg/mL. Antibacterial and antifungal activities of the oils are comparable to the activities of the standards (gentamycin and tioconazole) used in this study. Leaf essential oil exhibited good antibacterial and antifungal activities.

The stem essential oil was effective against all the six (6) bacteria at concentrations between 500µg/mL and 1000µg/mL. While at concentration of 250µg/mL, it was effective on *Staphylococcus aureus, Bacillus substilis, Pseudomonas aeruginosa and Salmonella typhi*. (Table 7).

Based on the results obtained, it can be inferred that the essential oil of the stem of *Sphenocentrum jollyanum* has moderate antibacterial and antifungal properties.



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**Table 6: Antibacterial activities of leaf and stem essential oils *Sphenocentrum jollyanum* (Clear zones of inhibition (mm)**

| S/N | Conc. (µg/ml) of oil | *Leaf EO* | *Stem EO* | *Leaf EO* | *Stem EO* | *Leaf EO* | *Stem EO* | *Leaf EO* | *Stem EO* | *Leaf EO* | *Stem EO* | *Leaf EO* | *Stem EO* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *S. aureus (mm)* | | *E. coli (mm)* | | *B. subtilis (mm)* | | *P. aureginosa (mm)* | | *S. typhi (mm)* | | *K. pneumonia (mm)* | |
| 1 | 1000 | 28 | 18 | 26 | 14 | 28 | 20 | 26 | 16 | 24 | 14 | 27 | 12 |
| 2 | 500 | 24 | 16 | 22 | 12 | 24 | 18 | 22 | 14 | 21 | 12 | 25 | 10 |
| 3 | 250 | 22 | 14 | 18 | - | 21 | 16 | 18 | 10 | 17 | 10 | 23 | - |
| 4 | 125 | 18 | 12 | 14 | - | 18 | 14 | 15 | - | 14 | - | 20 | - |
| 5 | 62.5 | 14 | 10 | 12 | - | 16 | 10 | 13 | - | 12 | - | 18 | - |
| 6 | 31.25 | 12 | - | 10 | - | 14 | - | 11 | - | 10 | - | 14 | - |
| 7 | Methanol(-ve) | - | - | - | - | - | - | - | - | - | - | - | - |
| 8 | Gentamycin (+ve) | 38 | 38 | 38 | 38 | 38 | 38 | 36 | 36 | 36 | 36 | 38 | 38 |

**Key:** S.a = *Staphylococcus aureus*, E.c = *Escherichia coli*, B. sub = *Bacillus Substilis*, Ps.a= *Pseudomonas aeruginosa*, Sal = *Salmonella typhi*, Kleb = *Klebsiella pneumonia*, - = Not detected

It is noteworthy that essential oils are more active against gram-positive than gram-negative bacteria [44, 45], this was also established in our results. Gram-negative bacteria, which are *Esherichia coli*, *Pseudomonas aeruginosa*, *Salmonellae typhimurium* and *Klebsiellae pneumonae* are less susceptible to the effect of the stem essential oil (Table 6). This can be due to the presence of a double layer of phospholipids which is linked to the inner membrane by lipoprotein and lipopolysaccharide in the cell wall of gram-negative bacteria that serve as a barrier from hydrophobic substances [44].

**Table 7: Antifungal activities of essential oil of leaf and stem of *Sphenocentrum jollyanum* (zone of inhibition (mm)**

| S/N | *Conc(µg/ml)* | Leaf EO | Stem EO | Leaf EO | Stem EO | Leaf EO | Stem EO | Leaf EO | Stem EO |
|---|---|---|---|---|---|---|---|---|---|
| | | *C.a* | | *A.n* | | *Pen* | | *Rhiz* | |
| **1** | 1000 | 20 | 16 | 20 | 18 | 18 | 14 | 18 | - |
| **2** | 500 | 18 | 14 | 18 | 16 | 16 | 12 | 16 | - |
| **3** | 250 | 16 | 12 | 16 | 14 | 14 | 10 | 14 | - |
| **4** | 125 | 14 | 10 | 14 | 10 | 13 | - | 12 | - |
| **5** | 62.5 | 12 | - | 12 | - | 11 | - | 10 | - |
| **6** | 31.25 | 10 | - | 10 | - | 10 | - | 10 | - |
| **7** | Methanol(-ve) | - | - | - | - | - | - | - | - |
| **8** | Tioconazole(+ve) | 28 | 28 | 26 | 26 | 28 | 28 | 28 | 28 |

**Key:** *C.a = Candida albicans, Rhiz = Rhizophus stolonifer, A.n = Aspergillus niger Pen = Penicillin notatum,* - = Not detected



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## CONCLUSIONS

Two compounds (a novel clerodane furano-diterpene and columbin) were isolated and characterized from *Sphenocentrum jollyanum* Pierre (Menispermaceae). The structural elucidation of this new clerodane compound-**1** is established in this study, and it has structural similarity to spiciflorin in literature [24].

Columbin has been earlier reported in fruit extract of *Sphenocentrum jollyanum* [19] and other plants [24, 25, 27-37], but this is the first time it is reported as a major compound in the seed extract of *Sphenocentrum jollyanum*. We suggest biogenetic relationship between the new clerodane diterpenoid (compound-**1**) and columbin (compound-**2**) in the methanol extract of seed of *Sphenocentrum jollyanum* Pierre (Menispermaceae).

The essential oils from leaf and stem parts are fairly terpenoidal, also dominated by aldehydes, norisoprenoids, alcohols and hydrocarbons. The antimicrobial result showed that the oil from the leaf exhibited good antimicrobial activity while the stem oil gave moderate activity. These compositions and observed inhibition of selected bacteria and fungi by the oils of *Sphenocentrum jollyanum* makes it a promising antimicrobial agent.

Our studies on chemical constituents of this plant reveal and assist in understanding the vast ethno-medicinal and folkloric utilization of it. This study is important in natural product drug discovery in a bid to discover active biological compounds.

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