Isolation of Novel 6-methylideneoxecane-3,4,5,7,8,9-hexol from the Leaves of Rauwolfia vomitoria, Apocynaceae

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Abstract

Rauwolfia vomitoria (Wennberg) belongs to the family Apocynaceae, the dried leaves were extracted successively using n-hexane, dichloromethane, 70% methanol, and concentrated in vacuo. Extracts were subjected to antibacterial assay and the butanol fraction was subjected to chromatographic purification to obtain NBF12 which was subjected to spectral analysis. The antibacterial of n-Hexane, dichloromethane, and 70% methanol extracts was inactive against the screened organisms assessed (Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa). NBF12 is yellowish crystals (10.5 mg), Rf (0.69), UV, max Abs (248) The carbon 13C-NMR spectrum displayed ten carbon atoms; one methylene, two methylene oxide carbon, 6-carbinolic carbon (Sp3), and a quaternary carbon (Sp2). The spectrum showed a tertiary carbon at δ131.09 ppm, due to C-6 and at δ 114.74 ppm due to exocyclic methylidene carbon (Sp2) (C=CH2) linked to the C-6 position. Based on the spectral data NBF12 is 6-methylideneoxecane-3,4,5,7,8,9-hexol with a molecular formula C10H18O7, molecular weight 250 g/mol.

Keywords: Isolation; Rauwolfia vomitoria; antibacterial; 6-methylideneoxecane-3,4,5,7,8,9-hexol.

INTRODUCTION

Rauwolfia vomitoria (Wennberg) belongs to the family Apocynaceae and is a medicinal plant used for the management of unknown pyrexia by the Ijaws of Southern Nigeria. This is due to its versatile utility in traditional medicine. It has been reported as an aphrodisiac, antimicrobial, antipsychosis, antihypertensive, antianxiety, and antioxidant effect (Etim et al., 2018; Emencheta et al., 2020; Oyeniran et al., 2020; Balogun and Akintunde, 2022). This medicinal plant has a battery of chemical constituents isolated and reported in the literature which includes; ursolic acid, sistrosterol, stigmasterol, reserpine, reserpine, deserpidine, ajmalicine, and ajmaline, 2,6-dimethoxybenzoquinone (Ajayi et al., 2021). The research aimed to determine the antibacterial effect and to characterize the chemical constituents of R. vomitoria leaves.

METHOD AND MATERIALS

Chemicals and Reagents
Methanol (Sigma U.K), Dichloromethane (Sigma U.K), n-hexane (Sigma U.K), Ethyl acetate (Sigma U.K), Dimethylsulphoxide (JHD), Sephadex LH-20 (Sigma), Silica gel 200-400 (Sigma U.K).

Equipment
UV spectrophotometry, NMR spectrophotometer 400 MHz (Agilent)

Microorganism
Staphylococcus aureus NCTC6571, Bacillus subtilis NCTC8236, Escherichia coli ATCC25922, Psuedomonas aeruginosa ATCC 10145

Sample Collection
The leaves of R vomitoria were collected from the wild at Otabi Community in Oloibiri District, Ogbia Local Government Area, Bayelsa State, Nigeria.

Identification of Plant
The plant was identified and authenticated by Prof. A.T Oladele of the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, and Herbarium specimen was deposited in the Herbarium of Pharmacognosy and Herbal Medicine and herbarium number (NDUP/24/01) was assigned to it.
Plant Preparation
The fresh leaves of the plant were washed and cut into small portions before air drying at room temperature for 14 days. The dried leaves were pulverized using the electrical blender to coarse powder, weighed, and stored in an airtight glass bottle.

Antibacterial Evaluation of the Extracts
The antibacterial evaluation of the extract was carried out according to (Balogun and Akintunde, 2022). Staphylococcus aureus NCTC6571, Bacillus subtilis NCTC8236, Escherichia coli ATCC25922, Psuedomonas aeruginosa ATCC 10145. A loop full of bacteria strains was inoculated in Nutrient agar media and was incubated for 48 hours. 80 mL of sterile molten Muller Hinton Agar was transferred from the Mckonkey bottle to the Petri dishes and allowed to solidify. The bacteria strain was diluted equivalent to 0.5 Mcfarland standard in 0.9% normal saline and 0.03 mL of the active test strain was transferred to the solid media, swirled allowed to dry. Wells were bored on the agar using a sterile cork borer (9 mm), and the 100 mg/mL (0.1, 0.2, 0.3, and 0.4 mL) stock concentration of each of the extracts were prepared in dimethylsulphoxide and water in a ratio (3:7). 10, 20, 30, and 40 mg of each extract was used against the bacteria strain using Ciprofloxacin 5µg/disc as the standard drug and was incubated at 37°C for 24 hours (Emencheta et al., 2020; Balogun and Akintunde, 2022; Karim et al., 2023; Dhital et al., 2024).

Isolation 6-methylideneoxecane-3,4,5,7,8,9-hexol
The methanol extract weighing 25 g was suspended in 200 mL of distilled water and it was partitioned sequentially using n-hexane, ethyl acetate, and n-butanol and the extracts were concentrated in vacuo using a rotary evaporator. The n-butanol fraction weighed 2 g was subjected to gel filtration using Sephadex LH-20 in a column (1.5 cm x 86 cm) and eluted with methanol (100%), 10 ml of the eluate collected to a total of 21 fractions coded (NBF 1-21). Based on the TLC profile NBF8,12 weighed 0.146 g was subjected to column chromatography using Silica gel as stationary phase with a dimension (1.5 cm x 86 cm) and gradually eluted with ethyl acetate, (100%); 95:5; 90:10; 80:20; 70:30; 65:35; 60:40; 50:50; 30:70; 10-90 (Ethyl acetate: Methanol) to 100% methanol and the progress of elution monitored using TLC in a solvent system ethyl acetate: methanol: water (100:16.5:13.5), fraction 12 gave a single spot on TLC, and concentrated in vacuo to give yellowish crystals weighed 10.5 mg which was coded NBF12, subjected to spectroscopic analysis.

RESULT AND DISCUSSION

Antibacterial
Antibacterial evaluation of the n-hexane, dichloromethane, and 70% methanol extracts were screened at 10, 20, 30, and 40 mg and showed that the extracts were devoid of antibacterial activity because there was no zone of inhibition compared to the standard drug ciprofloxacin 5µg/disc which showed zone of inhibition; 24 mm, 21 mm, 22 mm and 21.5 mm against S aureus, B subtilis, E coli and P aeruginosa respectively, as illustrated in Table 1, 2 and 3. The results are in line with the report by Balogun and Akintunde, 2022.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Agent/conc.</th>
<th>Zone of Inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
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<td>S. aureus</td>
</tr>
<tr>
<td>1</td>
<td>10 mg</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>20 mg</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>30 mg</td>
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<tr>
<td>4</td>
<td>40 mg</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Ciprofloxacin 5µg</td>
<td>24</td>
</tr>
</tbody>
</table>

Keys - = No zones of inhibition
Table 3. Antibacterial activity of 70 % methanol extract.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Agent/conc.</th>
<th>S. aureus</th>
<th>B. subtilis</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
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<tbody>
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</tr>
<tr>
<td>5</td>
<td>Ciprofloxacin 5µg</td>
<td>24</td>
<td>21</td>
<td>22</td>
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</table>

Chemistry of 6-methylideneoxecane-3,4,5,7,8,9-hexol (NMR Analysis)

Figure 1. $^1$H-NMR of Sample NBF$_{12}$. 
Yellowish crystals (10.5 mg), Rf (0.69), UV, max Abs (248), 1H-NMR (CD3OD, 400 MHZ, δ, ppm): 6.1, (s, 2H), 4.87 (s, 6H, OH proton), 4.48 (s, 4H), 3.69-3.27 (m, 6H).

13C-NMR (CD3OD, 400 MHZ, δ ppm); 130.04 (C-6); 114.74 (=CH2), 73.62 (C-4; C-8), 72.41 (C-2; C-10), 70.82 (C-5; C-7), 63.23 and 62.94 (C-3; C-9) respectively.

The proton NMR spectrum of sample NBF12 exhibited four prominent peaks. A downfield signal resonates at δ 6.17 ppm integrated for 2H and assigned to methylene protons linked to C-6 due to the exocyclic, unsaturated (C=CH2) group. An intense peak at δ 4.87 due to carbinol hydroxyl protons at (C-3; C-5and C-7; C-9) integrated for 6H protons and intense multiplet peak at δ 3.69-3.27 ppm due to methine (CH) of carbinolic proton at C-3-5; 7-9) positions integrated for six protons (6H) as shown in Figure 1.

The carbon 13C-NMR spectrum displayed ten carbon atoms; one methylene, two methylene oxide carbon, 6-carbinolic carbon (Sp3), and a quaternary carbon (Sp2).

CONCLUSION

The extract was not active against the microorganism assessed and isolation from the n-butanol gave a novel 6-
methyldieneoxecane-3,4,5,7,8,9-hexaol and the first time of reporting this compound from *Rauwolfia vomitoria*.

**Acknowledgment:** The authors are grateful to Prof. Kola’ K. Ajibesin and Prof. Augustine A. Ahmade for their technical assistance in carrying out this study.

**Author’s Contribution:** ADCO and ROR designed the work, ADCO and AF collected the sample, ADCO, ROR, and AF carried out the isolation while DDE carried out the antibacterial assessment of the extracts. SJB performed a critical review and manuscript proofreading. The manuscript was written by ADCO and all authors read and approved the final copy.

**Competing Interest:** The Authors have no competing interests.

**REFERENCES**


