

## Phytochemical Composition and Biological Evaluation of *Daniellia oliveri* Root Bark Extracts

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**ABSTRACT:** The research focused on examining the phytochemical components and biological properties of *Daniellia oliveri* (DO) root bark extracts. The study utilized quantitative phytochemical analysis and Gas Chromatography-Mass Spectrometry (GC-MS) to identify the chemical constituents. Furthermore, the investigation included assessments of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and the antimicrobial effects against reference strains of bacteria. This research contributes to the understanding of the potential medicinal properties of *Daniellia oliveri* and its role in combating free radical species and microbial infectious diseases. The quantitative phytochemical analysis of the sample revealed the presence of various components including flavonoids, phenolics, alkaloids, saponins, and tannins. Furthermore, the GC-MS profiling identified thirty-two compounds, with notable proportions of 1-Isopropyl-4,7-dimethyl, 1-1,2,3,5-hexahydronaphthalene (21.73%), Hexadecanoic acid, methyl ester (6.8%), 1-Heptadecanamine (5.36%),  $\alpha$ -Calacorene (5.52%), and Tridecane (4.45%). The sample also exhibited a DPPH radical inhibition effect with a comparative IC<sub>50</sub> value of 0.58  $\mu$ g/mL, when compared to the synthetic reference compound with an IC<sub>50</sub> value of 0.1  $\mu$ g/mL. The antibacterial evaluation of DA root extract on some Gram positive strains; *B.stearothermophilus*, *C. sporogenes*, *B. cereus*, *B. subtilis*, *Staphylococcus aureus*, *M.luteus*, displayed moderate growth inhibition and bactericidal action toward *B.stearothermophilus*. *B. cereus*, *M.luteus* strains only. However, displayed a non-inhibitory effect toward Gram negative strains (*P.aureginosa* and *K.pneumoniae*) investigated. Hence, *Daniellia oliveri* displayed inhibitory effect on radical solution and reduced growth rate of bacterial colony, complementing the existing findings on the pharmacological effects of local herbs.

**Keywords:** *D. oliveri*, antibacterial, phytochemicals, inhibition, quantification

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### INTRODUCTION

Medicinal plants play significant roles in promoting the overall well-being of individuals by offering therapeutic antioxidant effects against a wide range of ailments, including infections caused by microbial agents (Chang et al., 2020; Njoya, 2021). These natural remedies have been recognized for their potential in combating various health issues and have been utilized for their medicinal properties for centuries. The antioxidant properties of medicinal plants contribute to their effectiveness in addressing health concerns, making them valuable resources in the field of healthcare and wellness.

Plants play a crucial role as the primary source of essential human needs such as food, shelter, and clothing, while also providing raw materials for herbal medications (Jain et al., 2019). These natural organisms contain biologically active substances known as secondary metabolites, which are therapeutically valuable and serve as the basis for the development of new drugs (Roy et al., 2019). Extensive research conducted in both in-vitro and in-vivo models has demonstrated the promising pharmacological effects of many medicinal plants, often with minimal side effects

(Beiranvand and Beiranvand, 2022). Investigations into the medicinal properties of plants typically focus on various parts such as the root, leaf, and stem bark, utilizing different organic solvents for extraction (Akpan et al., 2021).

The literature reports chemical elucidation assays on antioxidant and biological evaluation of selected medicinal plants' parts (Ghalloo et al., 2022; Kumar et al., 2021). These studies have shown that plant extracts and their isolated compounds exhibit promising free radical scavenging effects, as well as potential in various pharmacological activities such as anti-malaria (Pan et al., 2018), anti-microbial (Vaou et al., 2021), anti-diabetes (Msomi et al., 2019), wound healing effects (Oguntibeju, 2019), and anti-diarrhea (Rawat et al., 2017). These findings contribute to the growing body of evidence supporting the potential therapeutic value of medicinal plants in various health conditions.

The root of *Daniellia oliveri*, a medicinal plant in the *Caesalpinaceae* family, is widely distributed in the Savannah and central region of Nigeria as well as other West African sub-regions. Previous studies have reported the efficacy of leaf, stem bark, and root extracts in various toxicological and pharmacological assays, including anti-diabetes, anti-malaria, liver regenerative ability, anti-cancer, and anti-inflammatory response. Despite these findings, there is a lack of adequate reports on the biological assessment using *in vitro* dependent assay model for the root extract of *D. oliveri*. Therefore, the current study was designed to investigate the phytochemical constituents and biological effects of *D. oliveri* root using selected *in vitro* assays.

## MATERIALS AND METHODS

### Chemicals

Reagents and chemicals used in this study were all of high quality purchased from Merck (Nigeria outlets).

### Collection of plant material

Fresh roots of *D. oliveri* were collected from a local farm at Agbeji in Dekina local government area, Kogi State, Nigeria. The plant was authenticated by Mr Theophilus Adejoh and a voucher specimen (OG-001) was kept for reference at the herbarium unit, Department of Plant Science and Biotechnology, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria.

### Extraction of *D. oliveri* extracts

The root was cleaned, shade-dried in the laboratory. The dried root was pulverized into samples. The portion of the powdered aerial parts of 200 g (1:5 w/v) was exhaustively

extracted using methanol solvent. The extract was filtered through Whatman no. 1 filter paper and concentrated at a reduced pressure with rotary evaporator to obtain a dried extract. The filtrates (methanol extract) obtained was kept to dry in fumed cupboard. The extracts were stored in 4 °C refrigerator until required for usage.

### Phytochemical screening

#### Quantitative phytochemical analysis of methanol root extract of *D. oliveri*

Quantitative phytochemical analysis was conducted using previously outlined work of Larayetan *et al.* (Larayetan et al., 2021).

#### Gas chromatography / mass spectrometry analysis

An Agilent gas chromatograph (GC) (6890N) attached with a 5973 mass selective detector (MSD) and HP-5MS column (30 m x 250 µm x 0.25 µm) was employed for compound identifications and separations. Helium was employed as a carrier gas and was set at 1 mL/min flow rate at an average of velocity and nominal initial pressure at 26 cm/sec and 13 psi correspondingly. The ion source and quadrupole temperatures were 230°C and 150°C respectively, while the acquisition scan mass ranged from 50 to 500 amu. Starting temperature was set at 70 °C (2 min hold) and conditioned at 30°C/min to 300°C, thus giving a total runtime of 49.67 min. The plant crude extracts (1 µL) was injected in a splitless mode at 250°C with a 50 mL/min purge flow. Analysis of the components of the extracts was done by comparing their retention times (RT) with homologous series of *n*-alkanes domicile in the NIST library 2014. Identity of the mass fragmentation patterns and calculated retention times of each compound was recorded and matched with those available in the databases.

#### Free radical scavenging assay

##### 2,2-diphenyl-1-picryl hydrazyl Assay (DPPH)

The scavenging effects of *D. oliveri* extract were evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical assay, following the procedure described by Simelane et al. (2010). Different concentrations ranging from 100 to 3200 µg/mL of the extract in 2 mL of methanol and n-hexane were incubated with an equal volume of DPPH radical solution. After thorough mixing, the solution was allowed to stand for 30 to 60 minutes for color development. The absorbance values of the combined solution were measured at a wavelength of 517 nm, with ascorbic acid (AA) used as a reference synthetic antioxidant.

## The Bacteria strains

The study employed bacteria strains; *Bacillus stearothermophilus* (NCIB 8222), *Clostridium sporogenes* (NCIB 532), *Bacillus cereus* (NCIB 6349), *Bacillus subtilis* (ATCC 3610), *Staphylococcus aureus* (NCIB 8588), *Micrococcus luteus* (NCTC 196), being gram positive bacteria strains. While the selected gram negative strains includes *Pseudomonas aureginosa* (NCIB 950) and *Klebsiella pneumonia* (NCIB 418).

## Antibacterial activity

The antibacterial activity of the methanol extract was determined using agar-well diffusion method of Akinpelu *et al.* [32]. The bacterial organisms were cultured into nutrient broth and malt extract agar medium for a period of one week for proper sporulation before spores harvesting. Bacterial culture of about 18-hour-old was standardized using McFarland standard (106 cfu/mL of 0.5 McFarland standard). Following the preparation of the agar and its introduction into the plate, a sterile cork borer was used to pierce the agar medium to a diameter of 5 mm. Extract solution was introduced into the plates containing bacteria cultures and maintained at a final concentration (25 mg/mL). Bacteria plates containing extract solution were allowed to stand for one hour for an extract solution to diffuse into the medium. The bacteria strains were incubated at 37 °C for 24 hrs. A zones of inhibition of the extract against bacteria strain were determined comparatively with the standard antibiotic (streptomycin) at an estimated concentration (30 mg/mL), in this study.

## Minimum inhibitory concentration (MICs) and bacterial concentration (MBCs) of *D. oliveri* extracts extract

The MIC (minimum inhibitory concentration) of the extracts was determined using a previously reported assay (Akinpelu *et al.*, 2015; Balouiri *et al.*, 2016). The plates were inoculated by streaking across the extracts-seeded plates once with test organisms that have been adjusted to 0.5 McFarland standard and incubated at 37 °C for 24 h. Streptomycin was used as the reference control (antibiotic), while 5% DMSO was used as the negative control.

## Data analysis

Data were represented in mean  $\pm$  standard error of mean (SEM). A linear graph summarizing the percentage inhibitions were plotted to obtain the IC<sub>50</sub> values.

## RESULTS AND DISCUSSION

Based on the phytochemical screening results presented in (Table 1), the presence of flavonoids, phenolics, alkaloids, saponins, and tannins was quantitatively detected. Furthermore, the GC-MS analysis (Table 2) revealed the presence of various compounds in the extract, with 1-Isopropyl-4,7-dimethyl-1,2,3,5-hexahydronaphthalene being the most abundant at 21.73%. Other significant compounds include Hexadecanoic acid, methyl ester (6.8%), 1-Heptadecanamine (5.36%),  $\alpha$ -Calacorene (5.52%), and Tridecane (4.45%), along with several other compounds detected in different proportions. The crude extract shows a good scavenging activity on DPPH radical solution, with an IC<sub>50</sub> value of 0.58  $\mu$ g/mL compared to the synthetic reference substance (0.1  $\mu$ g/mL) (Table 3).

The antibacterial activity of *D. oliveri* root extract against various Gram-positive pathogens, including *Bacillus stearothermophilus*, *Clostridium sporogenes*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Micrococcus luteus*, was modest. The extract had no inhibitory impact on the selected Gramme negative bacteria, *Pseudomonas aureginosa* and *Klebsiella pneumoniae*, under the conditions of this study (Table 4).

The crude extract had minimal inhibitory and bactericidal activity against *Bacillus stearothermophilus*, *Bacillus cereus* and *Micrococcus luteus* strains (Table 5). The in vitro study demonstrated that the root extract of *D. oliveri* exhibits a strong ability to inhibit DPPH radicals in a co-incubated solution. This inhibitory effect is likely attributed to the presence of phytochemical constituents, particularly phenolics and flavonoids, which are known for their redox action. Previous research by Ayeni *et al.* (2019) and Sanni *et al.* (2018) also supports the idea that these effects are driven by the inherent properties of the plant extract.

Antibacterial drug resistance is one of the most serious risks to human health in the modern era, as a result of antibiotic addiction and overuse (Shaaban *et al.*, 2021), necessitating the search for new antibacterial agents among locally available plants. The study examines the biological effects of hexadecanoic acid, methyl ester on multidrug-resistant bacteria. 1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene has been found to have antibacterial properties against *Escherichia coli* and *Salmonella typhimurium* (M'hamdi *et al.*, 2024).

Tridecane, a phytochemical, has been shown to have antibacterial properties against certain bacterial organisms (Rani *et al.*, 2019). In silico studies combined with nanoparticles reveal alpha calacorene as a potent inhibitor of gram-negative bacteria growth (Saravanan *et al.*, 2023). The crude extract's ineffectiveness against gram-negative bacteria strains under the conditions investigated is most likely related to the composition of

**Table 1:** Phytochemical analysis of *D. oliveri* methanol root extract.

Phytochemicals	<i>D. oliveri</i> Extract
Flavonoids (mg GAE/g)	12.97 ± 0.01
Phenolics(mg GAE /g)	77.15 ± 0.05
Alkaloids (%)	1.75 ± 0.02
Saponins(mg/100 g)	5.39 ± 0.1
Tannins (mg TAE/g)	3.08 ± 0.04

GAE = Gallic acid equivalent; TAE= tannic acid equivalents.

**Table 2:** Gas Chromatography Mass Spectroscopy (GC-MS) of methanol extract of *D. oliveri* root

R.T	Bioactive constituents	%Composition	M.W	M.F
2.479	3-Pentanol 2,2-dimethyl	2.89	116	C <sub>7</sub> H <sub>6</sub> O
2.599	1,3-Pentandiol, 2-methyl	2.72	118	C <sub>6</sub> H <sub>14</sub> O <sub>2</sub>
2.668	2-Propanol, 1-methoxy-2-methyl	1.84	104	C <sub>5</sub> H <sub>12</sub> O <sub>2</sub>
2.799	Tetraglyme	2.10	222	C <sub>10</sub> H <sub>22</sub> O <sub>5</sub>
2.936	Methane, diethoxy	1.39	104	C <sub>5</sub> H <sub>12</sub> O <sub>2</sub>
2.993	3-Hexanol, 5-methyl	1.02	116	C <sub>7</sub> H <sub>16</sub> O
6.891	1-(3,3-Dimethyl-but-1-ynyl) -1, 2-dimethyl-3-methylene-cyclopropane	2.86	162	C <sub>12</sub> H <sub>18</sub>
7.703	1H-Cyclopenta(1,3) cyclopropane (1, 2)benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)	4.02	204	C <sub>15</sub> H <sub>24</sub>
9.446	1H-Cycloprop(e) azulene, decahydro-1,1,7-trimethyl-4-methylene	1.55	204	C <sub>15</sub> H <sub>24</sub>
9.863	1-Heptadecanamine	5.36	255	C <sub>17</sub> H <sub>37</sub> N
10.458	1-Isopropyl-4,7-dimethyl-1,2,3,5-hexahydronaphthalene	21.73	204	C <sub>15</sub> H <sub>24</sub>
10.926	α-Calacorene	5.52	200	C <sub>15</sub> H <sub>20</sub>
11.389	Quinoline, 4-ethyl	1.11	157	C <sub>11</sub> H <sub>11</sub> N
12.109	Tridecane	4.45	184	C <sub>13</sub> H <sub>28</sub>
13.321	Carbonic acid, prop-1-en-2-yl tetradecyl ester	2.26	298	C <sub>18</sub> H <sub>34</sub> O <sub>3</sub>
13.344	Tetratetracontane	1.09	618	C <sub>44</sub> H <sub>90</sub>
14.115	1,3,5-Triazin-(1H)-one, 4,6-bis(ethylamino)	0.93	183	C <sub>7</sub> H <sub>13</sub> N <sub>5</sub> O
14.527	Octadecane, 6-methyl	4.40	268	C <sub>19</sub> H <sub>40</sub>
14.647	Heptadecane, 2, 6-dimethyl	4.00	268	C <sub>19</sub> H <sub>40</sub>
15.087	Benzofuran-2-one, 3-methyl-3-aza-2,3-dihydro	1.40	149	C <sub>8</sub> H <sub>7</sub> NO <sub>2</sub>
15.584	Cyclopentanol, 1-(1-methylene-2-propyl)	1.00	138	C <sub>9</sub> H <sub>14</sub> O
16.676	4,8-Dioxatricyclo(5.1.0.0)(3,5) octane, 1-methyl-5-(1-methylethyl)	0.91	168	C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>
16.790	Nonadecane	3.13	268	C <sub>19</sub> H <sub>40</sub>
18.922	Carbonic acid, prop-1-en-2-yl tetradecyl ester	2.83	298	C <sub>18</sub> H <sub>34</sub> O <sub>3</sub>
19.516	Hexadecanoic acid, methyl ester	6.80	270	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
20.299	Phthalic acid, butyl cycloheptyl ester	3.22	318	C <sub>19</sub> H <sub>26</sub> O <sub>4</sub>
20.928	1-Octanol, 2-butyl	1.83	186	C <sub>12</sub> H <sub>26</sub> O
22.757	1-Tridecyne	1.13	180	C <sub>13</sub> H <sub>24</sub>
22.871	2-Piperidinone, N-(4-bromo-n-butyl)	2.29	233	C <sub>9</sub> H <sub>16</sub> BrNO
24.637	Dotriacontane	2.27	450	C <sub>32</sub> H <sub>66</sub>
36.257	n-Propyl 11-octadecanoate	0.99	324	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>
36.474	Dodecyl isobutyl carbonate	0.84	286	C <sub>17</sub> H <sub>34</sub> O <sub>3</sub>
<b>Total</b>		96.59%		

Footnote: RT= Retention time; MW= Molecular weight; MF= Molecular formula

**Table 3:** DPPH scavenging effect of methanol root extract of *D. oliveri*.

Scavenging effects	<i>D. oliveri</i> methanol crude	A. acid
DPPH radical	0.58	0.1

Values are presented as IC<sub>50</sub> (µg/mL) values of the scavenging activities of *D. oliveri* crude. DPPH radical = 2,2-diphenyl-1-picrylhydrazyl

the crude plant sample or the absence of the effector (copper oxide), which boosts its bactericidal impact.

The sensitivity of most bacterial organisms to plant preparations is utilized in clinical medicine to understand the cause and cure of several infectious diseases (Akinpelu et al., 2015). Gram-negative bacteria such as

*Pseudomonas aeruginosa*, *Escherichia coli*, and *K. Pneumonia* have been extensively studied and are known to exhibit high levels of insensitivity and resistance to antibiotics, including those derived from plants, as evidenced in this study. Research has delved into the antibiotic resistance mechanisms, including the structure,

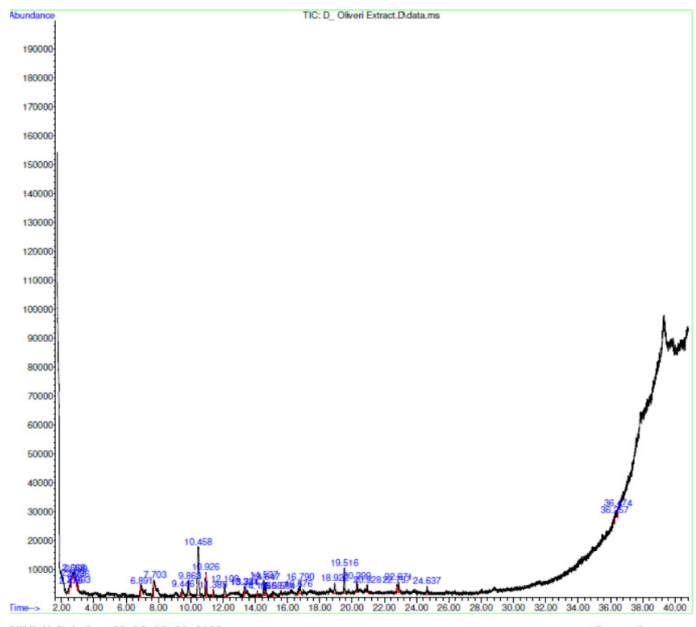


Figure 1: Total ion chromatogram (TIC) of methanol root bark extract of *D. oliveri*.

Table 4: Antibacterial sensitivity test methanol root extract of *Daniella oliveri* against the test organisms.

Test Organisms	Positive Control Streptomycin (10 µg/mL) mm	Methanol Extract (30 mg/mL) mm
<b>Gram-Positive</b>		
<i>Bacillus stearothermophilus</i> (NCIB 8222)	18.7 ± 0.4	9.0 ± 0.01
<i>Clostridium sporogenes</i> (NCIB 532)	19.10 ± 0.1	5.13 ± 0.03
<i>Bacillus subtilis</i> (NCIB 3610)	20.0 ± 0.0	8.0 ± 0.0
<i>Bacillus cereus</i> (NCIB 6349)	18.2 ± 0.0	4.06 ± 0.02
<i>Staphylococcus aureus</i> (NCIB 8588)	18.02 ± 0.0	8.02± 0.0
<i>Micrococcus luteus</i> (NCTC196)	19.0 ± 0.1	10.0 ± 0.3
<b>Gram-Negative</b>		
<i>Klebsiella pneumonia</i> (NCIB 418)	0.00 ± 0.0	NA
<i>Pseudomonas aeruginosa</i> (NCIB 950)	00.0 ± 0.0	NA

NCIB= National Collection of Industrial Bacteria, R: Resistance, I: Intermediate

Table 5: Minimum inhibitory concentrations (MICs) and minimum bactericidal *D. oliveri*

Bacteria Strains	Methanol Extract		Streptomycin		Control (DMSO)
	MIC (mg mL <sup>-1</sup> )	MBC (mg mL <sup>-1</sup> )	MIC (µg mL <sup>-1</sup> )	MBC (µg mL <sup>-1</sup> )	
<i>Bacillus stearothermophilus</i> (NCIB 8222)	0.46	>20	6.00	12.00	-
<i>Clostridium sporogenes</i> (NCIB 532)	0.00	0.00	19.00	>30	-
<i>Bacillus subtilis</i> (NCIB 3610)	0.00	0.00	ND	ND	-
<i>Bacillus cereus</i> (NCIB 6349)	0.27	2.00	7.00	14.00	-
<i>Staphylococcus aureus</i> (NCIB 8588)	0.00	0.00	6.98	14.00	-
<i>Micrococcus luteus</i> (NCTC196)	0.25	2.6	8.00	16.00	-
<i>Pseudomonas aeruginosa</i> (NCIB 950)	0.00 ± 0.00	NA	NA	NA	-

Key: ND = Not determined, - implies no effect

regulation, and inhibition of efflux pumps in both *Escherichia coli* and *Pseudomonas aeruginosa* (Puzari and Chetia, 2017), further validating the observed insensitivity of certain gram-negative bacteria in this research.

### Conclusion

*Daniellia oliveri* displayed high free radical inhibitory effect on radical incubated-extract solution and reduced growth rate of bacterial cultured colony.

The phytochemical constituents detected are known to aid the biological activities exhibited by the plant in the present study. This finding on account of the data generated, complements the existing studies on the pharmacological effects of locally utilized herbs in the diseased state. Nonetheless, elucidation of the lead compound through isolation procedure is recommended for future studies.

### Declaration of competing interests

Authors declare that the manuscript is devoid of any conflict of interest

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