J. Chem. Soc. Nigeria, Vol. 50, No. 1, pp 201 – 207 [2025]

# ANTIMICROBIAL ACTIVITY OF TRITERPENOIDS FROM *Brachystegia eurycoma*

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## ABSTRACT

Antibiotic resistance poses a significant global health threat, necessitating the search for novel antimicrobial agents. Natural products offer promising alternatives due to their structural diversity and evolutionary refinement. The antibacterial activity of crude extracts of the stem bark of *Brachystegia eurycoma* against Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Escherichia coli* (VRE) was investigated. The stem bark was extracted using hexane, ethyl acetate, and methanol, then column chromatography led to the isolation of friedelan-3-one, 2-hydroxy lupeol, and prunetin. The ethyl acetate extract exhibited the highest activity with a minimum inhibitory concentration of 45.0 µg/mL for MRSA and 10.0 µg/mL for VRE. The study highlights the potential of *B. eurycoma* as a source of bioactive compounds for antibiotic drug development.

## INTRODUCTION

Antibiotics have undoubtedly revolutionized modern medicine, saving countless lives by treating bacterial and fungal infections that were once fatal. However, the success of antibiotics has inadvertently given rise to a new challenge: antibiotic resistance. Microbes are evolving and developing adaptations to withstand antibiotics, rendering these once-potent drugs less effective or inactive. This natural selection process is exacerbated in part by the overuse and misuse of antibiotics in healthcare and agriculture, including improper prescriptions, patient non-compliance, and the widespread use of antibiotics in livestock [1]. This constitutes a problem for human healthcare and threatens the efficacy of medical procedures such as organ transplants and chemotherapy that rely on effective antibiotics to prevent and treat infections. Some microbial pathogens, especially Gram-negative bacteria such as *Acinetobacter baumannii, Pseudomonas aeruginosa Escherichia coli* and *Klebsiella* spp have limited therapeutic alternatives to antibiotics [2]. The World Health Organization in an attempt to combat antimicrobial resistance in humans, initiated global measures including robust infection prevention and control measures, and substantial investment in research and development for the discovery of new medicines, diagnostics, and prevention tools.[3] To integrate and unify approaches toward optimal and sustainable health outcomes for people, animals, and ecosystems, it has become imperative to



ISSN 0795 - 22066

consider the interrelated nature of the health of humans, domestic and wild animals, plants, and the wider environment. The One Health approach is a convergence of stakeholders from all these sectors to communicate and work together in designing, implementing, and monitoring programs, policies, legislation, and research to curtail antimicrobial resistance [3]. Regardless, the need for innovation in research and development approaches to combat antimicrobial resistance subsists. The discovery of novel chemical moieties that target drug-resistant bacteria without promoting further resistance is complex, especially from chemical libraries. Natural products are an invaluable source of compounds with diverse moieties and chemical scaffolds, offering great potential to combat antimicrobial resistance. Their structural diversity, biological activity, and evolutionary refinement are particularly well-suited for combating drug-resistant pathogens. Several of the most successful antibiotics were derived from natural products [4], [5], [6], [7]. This study examined the bioactivity of natural products from the stem bark of *Brachystegia eurycoma* against two pathogenic bacteria: Methicilin-resistant *Staphylococcus aureus* and Vancomycin-resistant *Escherichia coli*.

## MATERIALS AND METHODS

### *Plant Collection*

The stem bark of *Brachystegia eurycoma* was collected in February 2017 in Ipav, Gboko Local Government Area of Benue State, Nigeria. The plant was identified at the Department of Forest Products and Production, Federal University of Agriculture, Makurdi. The specimen was assigned the voucher number FHI 0238 and deposited in the herbarium.

### *Extraction and Isolation*

The stem bark was air-dried under a shade and pulverized into a coarse powder. The pulverized stem bark (500 g) was extracted by maceration in hexane for 72 h at room temperature with intermittent stirring. The extract was filtered and concentrated using a rotary evaporator at 40 ℃ under reduced pressure. The concentrated extract was allowed to evaporate to dryness. The process was repeated using ethyl acetate and methanol.

The dry extract was pre-adsorbed on silica gel by dissolving the extract (6.0 g) in ethyl acetate (10 mL) and adding silica gel (6 g) to it in a beaker. The mixture was stirred vigorously, resulting in a homogeneous slurry. The slurry was then allowed to stand; the solvent evaporated to dryness, leaving behind a fine powder of the extract adsorbed on silica gel. The powder was loaded onto a glass column packed wet with 100 % hexane and eluted gradient-wise with mixtures of hexane and ethyl acetate beginning with 95 % hexane and gradually increasing the volume of ethyl acetate by 5 % after every 200 mL. The eluate was collected as 10 mL fractions in vials and allowed to dry at room temperature. Similar fractions were combined after TLC and resulted



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in three pure fractions which were analyzed by NMR and mass spectrometry.

***Antimicrobial Assay:***

An Agar-well diffusion method according to Valgas et al. [8] was used to assay the antimicrobial activity of the hexane, ethyl acetate and methanol extracts.

## RESULTS AND DISCUSSION

*Characterization of Compound 1*

$^{1}$H NMR spectrum ($CDCl_3$, 400 MHz) showed the presence of eight methyl protons at $\delta_H$ 0.72, 0.86, 0.88, 0.96, 1.00, 1.04, 1.08, and 1.18 integrated for 3H-each . The large number of methyl groups suggests that it is a cyclic triterpene. Other signals were due to methylene and methine protons. The $^{13}$C NMR spectrum of the compound showed 30 signals comprised of eight methyl groups at δc 6.96 (C-23), 23.0 (C-24), 18.08 (C-25), 20.39 (C-26), 18.80 (C-27), 31.92 (C-29) and 35.16 (C-30); the signal due to a carbonyl carbon was observed at δc 213.4 (C-3) which is typical of Friedelan-3-one **(1)**. The compound was confirmed by comparison of its spectral data with literature reports [9].

*Characterization of Compound 2*

The $^{1}$H NMR spectrum revealed the presence of seven tertiary methyl protons at δ 0.78, 0.79, 0.86, 0.94, 0.96, 1.07and 1.68 (all singlets) Two carbinylic proton resonances were centered at δ 3.66, 2.99 (1H, m, $J_{ax,\ ax=}$ 8.0 Hz, $J_{ax,\ eq}$= 4.0 Hz). The chemical shift and coupling constants of the two carbinylic protons revealed β and equatorial configuration of hydroxyl groups at C-2 and C-3. A sextet of one proton at $\delta_H$ 2.37 ascribed to 19β-H is characteristic of lupeol [10], [11]. The resonance at $\delta_H$ 4.57 and $\delta_H$ 4.69 indicates two olefinic protons of an exocyclic double bond at C-29 (a, e). These assignments are in good agreement with literature reports [10], [11] for the structure of lupeol. The DEPT experiment further confirmed the multiplicities of carbons, revealing six quaternary carbons, two oxymethine, five methine, ten methylene, and seven methyl carbons. The DEPT further supported the total number of carbons in the $^{13}$C NMR of the isolated compound. The COSY correlation spectra established the correlations between the protons such as H-3 (2.99) δ H-2 (3.66), H-2 (3.66) δ H-1 (2.02), H-21 (1.91) δ H-19 (2.37), H-13 (1.46) δ H-19 (2.37). Protons were assigned to their respective carbons using HSQC. In the HMBC spectrum, the signal at $\delta_H$ 2.99 (H-3) showed cross peaks with a methyl carbon signal at δc 28.6 (C-23) by $J_3$ correlation and a quaternary carbon at δc 38.7 (C-4) $J_2$ correlation. The signal at $\delta_H$ 2.37 (H-19) showed cross peaks with two methylene carbon signals at $\delta_C$ 29.7 (C-21), 109.9 (C-29) and a quaternary carbon signal 151.0 (C-20). The pair of olefinic protons at $\delta_H$ 4.57 and 4.69 showed cross peaks with methylene carbons at $\delta_C$ 47.5 (C-19) and $\delta_C$ 19.5 (C-30) by $J_3$ correlation. Utilization of the 1D and 2D NMR



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data compared to literature reports [10], [11] confirmed compound **2** to as 2-hydroxy lupeol.

*Characterization of Compound 3*

The $^{1}$H NMR spectrum of compound **3** showed two broad singlets at $\delta_H$ 6.40 (1H, brs, H-8) and $\delta_H$ 6.38 (1H, brs, H-6) and a singlet methoxy proton at $\delta_H$ 3.87 (3H, s), suggesting a 5- hydroxy substitution in ring A of a flavone moiety. The 4′-hydroxylation of ring B was inferred from the two ortho-coupled doublet protons at $\delta_H$ 7.39 (2H, d, $J$ = 8.4Hz, H-2'/6') and $\delta_H$ 6.91 (2H, d, $J$ = 8.0, 4.0 Hz, H-3'/5'). This pattern of coupling system indicates a 4′, 5-dihydroxyl-7-methoxyflavone typical of a prunetin nucleus [12]. The multiplicities of the carbon atoms were confirmed by the DEPT experiment, which revealed eight quaternary carbons, and seven methine carbons including the symmetric signals for C-2′/C-6′ and C-3′/C-5′ and a methoxy carbon. The oxygenated quaternary carbon signals at $\delta_C$ 165.6 and 162.7 are due to phenolic carbons. The signal at $\delta_C$ 55.4 is due to a methoxy carbon [13] and the carbonyl carbon signal at $\delta_C$ 180.0 is typical of an iso-flavone nucleus.. The chemical shifts at $\delta_C$ 130.27 and 115.66 are due to the methine carbons of the B-ring of the isoflavone.[12] The COSY correlation spectrum of prunetin established the correlations between protons such as 7.39 (H-2'/6') and 6.91 (H-3'/5') 6.39 (H-6) and 6.38 (H-8). Protons were assigned to their respective carbons using HSQC. The HMBC spectrum was used to establish linkages between the fragments. The HMBC spectrum showed a long-range ($^{3}J$) correlation between the methoxy proton at $\delta_H$ 3.84 and $\delta_C$ 165.55 (C-7), confirming the connectivity of the methoxy on ring A at C-7. The proton at $\delta_H$ 7.85 showed a $^{2}J$ and $^{3}J$ with C-2, C-4, and C-1′, while the proton signal at 7.39 showed a long-range correlation to C-2, C-1′, C-2′and C-4′ which made it possible to assign all the substituents on the compound and comparison with literature reports confirmed compound **3** as prunetin.

*The result of the antimicrobial assay*

Table 1: Minimum Inhibitory Concentration (MIC)

| Antibiotic resistant baceteria | Hexane extract (µg/mL ) | Ethyl acetate extract (µg/mL ) | Methanol extract (µg/mL ) |
|---|---|---|---|
| Methicilin-resistant *Staphylococcus aureus* | 100 | 45 | 15 |
| Vancomycin-resistant *Escherichia coli* | 25 | 10 | 25 |
| Ciprofloxacin (vended drug) | 28 | 31 | 28 |



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Table 2: Minimum Bactericidal Concentration (MBC)

| Antibiotic resistant baceteria | Hexane extract (µg/mL ) | Ethyl acetate extract (µg/mL ) | Methanol extract (µg/mL ) |
|---|---|---|---|
| Methicilin-resistant *Staphylococcus aureus* | 32 | 15 | 18 |
| Vancomycin-resistant *Escherichia coli* | 38 | 36 | 22 |
| Ciprofloxacin (vended drug) | 29 | 10 | 19 |

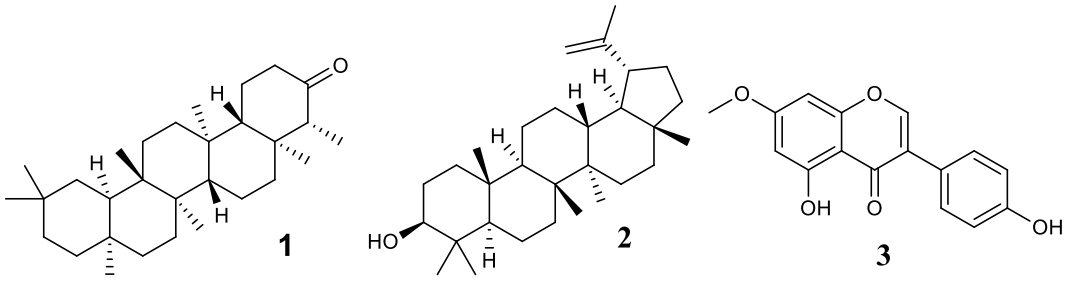
Table 3: Summary of NMR (in $CDCl_3$) of Compounds 1, 2 and 3

| | **Compound 1** | | **Compound 2** | | **Compound 3** | | |
|---|---|---|---|---|---|---|---|
| **Position** | **δH** | **$^{13}$C** | **δH** | **$^{13}$C** | **Position** | **δH** | **$^{13}$C** |
| 1 | 1.98a,1.68e(2H, m) | 23.0 | 2.05 (2H, m) | 46.21 | 2 | 7.85 (1H, s) | 152.65 |
| 2 | 2.24a,2.27e(2H,m) | 41.40 | 3.66 (1H,m) | 69.22 | 3 | - | 122.62 |
| 3 | | 213.40 | 2.99 (1H,m) | 84.0 | 4 | - | 180.0 |
| 4 | 2.27 (1H, m) | 58.34 | | 38.67 | 5 | - | 155.76 |
| 5 | | 42.29 | | 55.48 | 6 | 6.38 (1H, brs) | 98.22 |
| 6 | | 41.65 | 1.37(2H, m) | 18.49 | 7 | - | 165.55 |
| 7 | 1.54a, 1.22e(1H,m) | 18.36 | | 34.60 | 8 | 6.40 (1H, brs) | 92.44 |
| 8 | 1.33 (1H, m) | 53.22 | | 41.0 | 9 | - | 156.73 |
| 9 | | 37.56 | 1.28 | 50.48 | 10 | - | 109.42 |
| 10 | 1.38 (1H, s) | 9.58 | | 37.27 | 1’ | - | 123.75 |
| 11 | 1.21 (2H, m) | 35.74 | 1.50 (2H, m) | 21.18 | 2’/6’ | 7.39 (2H, d) | 130.27 |
| 12 | | 30.63 | 1.31 | 27.55 | 3’/5’ | 6.91 (2H, d) | 115.66 |
| 13 | | 39.81 | 1.46 (1H, m) | 39.34 | 4’ | - | 162.73 |
| 14 | | 38.41 | | 42.03 | $OCH_3$ | 3.87 (3H, s) | 55.36 |
| 15 | | 32.89 | 1.01 | 27.05 | $OH^5$ | 12.84 (1H, s) | _ |
| 16 | 1.24 (2H, m) | 36.13 | | 35.71 | | | |
| 17 | | 29.80 | | 43.14 | | | |
| 18 | | 42.90 | | 48.13 | | | |
| 19 | | 35.46 | 2.37 (1H, ddd) | 47.53 | | | |
| 20 | | 28.30 | | 150.96 | | | |
| 21 | | 32.54 | 1.91 (1H, m) | 29.71 | | | |
| 22 | | 39.38 | | 40.06 | | | |
| 23 | 0.88 (3H, s) | 6.96 | 0.96 (3H, s) | 28.62 | | | |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 24 | 0.72 (3H, s) | 23.0 | 1.07 | 16.17 | | | |
| 25 | 0.86 (3H, s) | 18.08 | 0.78 (3H, s ) | 16.96 | | | |
| 26 | 1.00 (3H, s) | 20.39 | 0.86 (3H, s ) | 16.70 | | | |
| 27 | 1.04 (3H, s) | 18.80 | 0.94 (3H, s ) | 14.67 | | | |
| 28 | 1.08 (3H, s) | 32.22 | 0.79 (3H, s ) | 18.17 | | | |
| 29 | 1.18 (3H, s) | 31.92 | 4.57a, 4.69b (2H, d) | 109.55 | | | |
| 30 | 0.97 (3H, s) | 35.16 | 1.68 (3H, s ) | 19.53 | | | |




**Structures of isolated compounds**

## CONCLUSION

Extracts of the stem bark of *Brachystegia eurycoma* showed good antimicrobial activity against Methicilin-resistant *Staphylococcus aureus* and Vancomycin-resistant *Escherichia coli*. Friedelan-3-one, 2-hydroxy lupeol, and prunetin were isolated from the extracts through column chromatography.

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ISSN 0795 - 22066