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Research Article

Isolation, characterization and antibacterial evaluation of stem bark of *Alstonia boonei* (De Wild) (Apocynaceae) against selected wood associated bacteria

David Oriabure Ekhuemelo*, **Terzungwe Atondo** and **Emmanuel Terzungwe Tembe**

Department of Forest Production and Products, Federal University of Agriculture Makurdi (Now Joseph Sarwuan Tarka University, Makurdi) Nigeria.

Abstract

Isolation, characterization, and antibacterial evaluation of the stem bark of *A. boonei* against selected wood-associated bacteria was studied. Stem bark of *A. boonei* was collected, dried under shade and pulverised into powder for extraction. Extraction was carried out by macerating 1000 g in 1000 mL (w/v) of n-hexane for 24 hours and filtering off the hexane extract with No. 1 Whatman and subjected to column chromatography to isolate pure fractions. Fractions were sent for Nuclear Magnetic Resonance (NMR) analysis. Fractions and extracts were tested on Mueller Hinton agar inoculated with microbes at 37 °C for 24 hours. The zone of inhibition was measured to determine the antibacterial properties with sparfloxacin and sparfloxacin serving as control. Minimum inhibitory concentration and minimum bactericidal concentration were determined. Results revealed ABO39 and ABO41 fractions to yield alpha amyrin acetate, while ABO43 contained both lupeol acetate and beta amyrin acetate. Similarly, ABO45 showed lupeol acetate along with alpha amyrin acetate, contributing to a rich profile of triterpenoids. *A. boonei* fractions were very active against *Pseudomonas aeruginosa, Strepto -coccus salvarius, Serratia marcescens, Rhanella* sp, and *Klebsiella* sp, with zones of inhibition ranging from 24 mm to 30 mm. *P. aeruginosa and S. salvarius* showed variable susceptibility, with MIC values ranging from 100 to 200 µg/mL and MBC values corresponding closely to their MICs. *Klebsiella* sp. demonstrated a MIC of 100 µg/mL for both ABO-42 and ABO-44, indicating a more pronounced sensitivity. The antibacterial activity of the methanol extract followed the same trend as the fraction.

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Corresponding Author
Prof. Dr. David Oriabure Ekhuemelo
E-mail: ekhuemelo.david@uam.edu.ng
Tel: +234-703 133 2803

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1. Introduction

Alstonia boonei De Wild (Apocynaceae) is a medicinal plant with significant antibacterial properties, particularly in its stem bark. *A. boonei*, commonly known as "God's tree" or "Onyame dua" in West Africa, is an herbal medicinal plant of West African origin [1]. It is also referred to as "stool wood" in Ghana, where its bark is sold in large quantities in markets [2]. *A. boonei* is found primarily in West African forest communities, where it is considered sacred in some areas. Due to its sacred status, the plant parts are not typically eaten in these communities [1]. The plant is widely distributed across West Africa, with its various parts being used for traditional medicinal purposes.

Interestingly, while *A. boonei* is revered for its medicinal properties, it is also utilized for other purposes. For instance, in Southern Nigeria, it is used as a botanical preservative for palm wine, alongside *Sacoglottis gabonensis* [3]. This demonstrates the versatility of the plant in different cultural contexts

within West Africa. *A. boonei* a medicinal plant of West African origin, has numerous traditional uses supported by scientific research: The plant is widely used in traditional medicine for treating various ailments. It is employed for its antimalarial, aphrodisiac, antidiabetic, antimicrobial, and antipyretic properties [1]. Specifically, the stem bark is confirmed to be effective against malaria and fever [4]. In some West African forest communities, *A. boonei* is considered sacred and its parts are not consumed [1]. Although *A. boonei* is primarily known for its medicinal properties, it is also recognized as an underutilized indigenous timber tree with high medicinal value [5]. Different parts of the tree, including barks, roots, leaves, flowers, stems, and gums, are used to treat various conditions such as painful urination, epilepsy, and convulsions [5].

Studies have shown that extracts from *A. boonei* exhibit varying degrees of effectiveness against different bacterial strains [6]. The stem bark of *A. boonei* has been found to contain bioactive compounds with potential therapeutic applications, including β-amyrin and α-amyrin acetate, which demonstrate anti-inflammatory activities [7]. While some studies reported the potent antibacterial activity of *A. boonei* stem bark extracts [8] and others have found limited effectiveness against certain bacterial species [6]. This discrepancy highlights the importance of extraction methods and the specific bacterial strains tested. Additionally, endophytic fungi isolated from *A. boonei* have shown promising antibacterial potential against pneumonia-causing bacteria [9] suggesting that the plant's antimicrobial properties may extend beyond its direct chemical constituents.

A. boonei has demonstrated antimicrobial activity against various bacteria, including some that may affect wood. Several studies have investigated its efficacy: The stem bark extracts of *A. boonei* showed antibacterial activity against *Staphylococcus aureus, Salmonella typhi, and Klebsiella pneumoniae* [10]. Compounds isolated from *A. boonei* stem bark exhibited significant antimicrobial effects against *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus,* and *Klebsiella pneumoniae* [11]. These findings suggest potential applications in protecting wood from bacterial degradation. Interestingly, there are some contradictions in the literature regarding A. boonei's antimicrobial properties. While Adomi [8] reported that ethanol extracts of *A. boonei* were not active against any tested bacteria, Adomi and Umukoro [6] found that crude extracts from *A. boonei* were not potent against the bacteria tested. However, Adomi [12] demonstrated that leaf extracts of *A. boonei* showed antibacterial activity, although its latex was not effective. The objectives of this study was to isolate and characterize compounds from the stem bark and investigate the antibacterial potential of the methanol extract and fractions on selected wood bacteria.

2. Materials and methods

2.1 Plant materials collection and preparation

The stem bark of *A. boonei* was collected from standing tree in Edo State, the southern part of Nigeria where the species is dominant and parts are used for the treatment of different ailments. The stem bark was dried under shade and the dried samples were pulverised into powder for extraction according to the method described by Siddig and Ahmed [13,14]. Fig 1 shows the photograph of *A. boonei`* leaves and stem.

Figure 1. Leaves and stem of *A. boonei`*

2.2 Distillation of Solvents

Methanol solvent was used for the extraction and ethyl acetate with n-hexane was bought from Showcrown Laboratory. Ltd., Ibadan and distilled in multi-purpose chemistry laboratory in JOSTUM to remove impurities. Distilled solvents were collected and stored in bottles before extraction. The methanol extraction was sequentially macerated for 24 hours.

2.3 Crude extraction of powdered A. boonei stem bark

Extraction of pulverized stem bark was done sequentially by macerating 1000 g into 1000 mL (w/v) of n-hexane for 24 hours and filtering off the hexane

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extract. Crude extract was filtered with No. 1 Whiteman filter papers into well labelled glass bottle. The filtrate was evaporated using rotary evaporator to obtain dried extracts and the bottle was re-weighed (w_2).

2.4 Column chromatography used for separation of methanol stem bark extract

The methanol crude extract was subjected to column chromatography to isolate pure fractions. A clean column was prepared with cotton wool at the neck and packed with powdered Silica Gel 60 (Academic Grade) to a height of 20 cm. N-hexane was added to saturate the cotton wool and silica gel, ensuring proper column preparation. The column was diluted with solvent mixtures increasing polarity in the ratios as hexane: ethyl acetate (95%:5% to 0%:100%). Fractions assigned ABOs codes were collected, dried using a rotary evaporator, and weighed with a sensitive balance. Vials containing crystals were set aside for NMR analysis and antimicrobial testing.

2.5 Antimicrobial screening of stem bark of A. boonei

The antibacterial activity of *A. boonei (*ABO) extracts and fractions were evaluated against wooden bacteria. The antimicrobial activity was evaluated following the method described earlier by Panda [15]. The extract and fractions were prepared in DMSO, added to Mueller Hinton agar media inoculated with test microbes, and incubated at 37°C for 24 hours. The zone of inhibition was measured to determine the antibacterial properties of ABO extract and fractions, with Sparfloxacin (10 µg/mL) and Sparfloxacin (10 µg/mL) antibacterial serving as controls. The methodology involved preparing the extract and fractions, seeding the media with test microbes, creating a well to add the extract/fractions, and incubating and measuring the zone of inhibition. This study aimed to assess the potential antimicrobial effects of ABO extract and fractions against various pathogenic microbes.

2.6 Determination of minimum inhibition concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the crude extract and fractions was determined according to Vipra and Parvekar [16,17] by using the broth dilution method, involving preparation and sterilization of Mueller Hinton broth, creation of a turbid test microbe solution, dilution to 1.5 x 10^8 cfu/mL, and two-fold serial dilutions of the crude extract and fractions in sterile broth, resulting in concentrations ranging from 0.63 mg/mL to 10 mg/mL and 25 µg/mL to 400 µg/mL, respectively. The test microbe was inoculated into each concentration, incubated at 37°C for 24 hours, and the lowest concentration showing no turbidity (growth) was recorded as the MIC.

2.7 Determination of minimum bactericidal concentration (MBC)

The MBC test distinguished between microbial growth inhibition and actual microbial death caused by the crude extract and fractions. The method described by Parvekar [17] was adopted. Mueller Hinton agar was prepared, sterilized, and poured into petri dishes. The contents of the MIC serial dilutions were sub-cultured onto the media and incubated at 37°C for 24 hours. After incubation, the plates were observed for colony growth, and the MBC/MFC was determined as the lowest concentration of the crude extract and fractions that showed no colony growth, indicating microbial killing.

3. Results

3.1 Compounds isolated from A. boonei stem bark

Table 1 presents an array of compounds isolated from various isolates of *A. boonei* stem bark. The isolates ABO39 and ABO41 yield alpha amyrin acetate. ABO43 contains both lupeol acetate and beta amyrin acetate. Similarly, ABO45 revealed lupeol acetate along with alpha amyrin acetate, contributing to a rich profile of triterpenoids.

Table 1. Compounds isolated from *A. boonei*

S/No.	Isolate	Compounds
1.	ABO39 and ABO41	Alpha amyrin acetate
2.	ABO43	Lupeol acetate % beta amyrin acetate
3.	ABO45	Lupeol acetate and alpha amyrin acetate

3.2 ^{1}H-NMR Characterisation of ABO39 as alpha amyrin acetate

The fraction was obtained as white crystals. It had the following NMR spectrum: ^{1}H NMR (400 MHz, $CDCl_3$)

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δ 5.12 (t, *J* = 3.7 Hz, 1H), 4.51 (dd, *J* = 10.1, 6.3 Hz, 1H), 2.28 (dt, *J* = 11.3, 7.6 Hz, 2H), 2.04 (s, 2H), 1.07 (s, 2H), 1.01 (s, 2H), 0.98 (s, 2H), 0.91 (s, 1H), 0.79 (d, *J* = 4.2 Hz, 4H), 0.69 (d, *J* = 7.6 Hz, 1H). It's characteristic signals and comparison with the literature allowed for unambiguous characterisation of the fraction as α-amyrin acetate (2) (Fig. 2a and 2b).

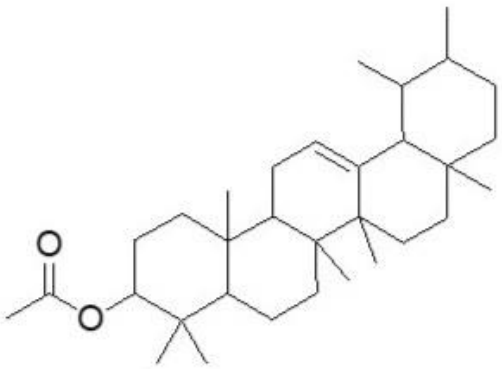

Figure 2a: Structure of alpha amyrin acetate

3.3 ^{1}H-NMR characterisation of ABO41 as alpha amyrin acetate

Fraction was obtained as while crystals. It had the following NMR spectrum: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.12 (t, *J* = 3.6 Hz, 1H), 4.57 – 4.43 (m, 1H), 2.04 (s, 3H), 1.98 (dd, *J* = 13.4, 4.4 Hz, 0H), 1.90 (d, *J* = 3.7 Hz, 0H), 1.81 (dd, *J* = 13.2, 4.8 Hz, 0H), 1.13 (s, 1H), 1.06 (s, 2H), 1.01 (s, 2H), 0.98 (s, 2H), 0.91 (s, 1H), 0.87 (d, *J* = 3.4 Hz, 7H), 0.79 (d, *J* = 4.0 Hz, 4H). It's characteristic signals and comparison with the literature allowed for unambiguous characterisation of the fraction as α-amyrin acetate (2) (Fig. 2a and 2b).

3.4 ^{1}H-NMR characterisation of ABO43 as lupeol acetate and beta amyrin acetate

The fraction was obtained as white crystals. It had the following NMR spectrum: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.17 (t, *J* = 3.7 Hz, 0H), [5.11 (t, *J* = 3.6 Hz, 1H) beta amyrin endocyclic protons], [4.67 (d, *J* = 2.5 Hz, 0H), 4.55 (t, *J* = 2.1 Hz, 0H) lupane-type exo-cyclic terminal olefinic protons], 4.54 – 4.43 (m, 2H), 2.36 (td, *J* = 11.0, 5.8 Hz, 0H), 2.03 (d, *J* = 3.4 Hz, 6H), 2.00 – 1.95 (m, 1H), 1.89 (d, *J* = 3.7 Hz, 1H), 1.80 (dd, *J* = 13.2, 4.8 Hz, 1H), 1.76 – 1.71 (m, 0H), 1.67 (s, 2H), 1.12 (s, 1H), 1.06 (s, 3H), 1.02 (s, 1H), 1.00 (s, 3H), 0.97 (s, 3H), 0.90 (s, 2H), 0.86 (s, 8H), 0.86 (s, 11H), 0.82 (d, *J* = 2.2 Hz, 2H), 0.79 (d, *J* = 2.2 Hz, 5H), 0.78 (s, 4H) methyl singlets for both triterpenes].

^{1}H NMR revealed specific chemical shifts and coupling patterns. Notably, the spectrum displayed characteristic endocyclic and exocyclic proton signals indicative of triterpene structures, including signals at δ 5.17 and δ 5.11, attributed to beta-amyrin, and olefinic proton signals at δ 4.67 and δ 4.55, suggestive of lupane-type structures. The complex multiplicity of signals ranging from δ 4.54 to 0.78, including various singlet, doublet, triplet, and multiplet patterns, further corroborated the presence of triterpenes. Comparative analysis with literature data facilitated the definitive identification of the fraction as co-occurrence of lupeol acetate (4) and β-amyrin acetate (3), with the latter being the predominant compound. This conclusion was substantiated by matching the observed NMR spectral data with reported values (Fig 3).

3.5 ^{1}H-NMR characterisation of ABO45 as lupeol acetate

The fraction, appearing as white crystals, exhibited a characteristic NMR spectrum, indicative of lupeol acetate (**5**). The ^{1}H NMR (400 MHz, $CDCl_3$) data showed distinct peaks: exocyclic olefinic protons at δ 4.67 (d, J = 2.5 Hz, 1H) and δ 4.55 (t, J = 2.0 Hz, 1H), a deshielded oxy-methine proton at δ 4.53 – 4.41 (m, 1H), alongside other key signals including acetate methyl and multiple singlets and doublets. These spectral features, coupled with literature comparisons, confirmed the identity of the fraction as lupeol acetate (Fig 4).

3.6 ^{1}H-NMR characterisation of ABP68 as 24-methyl cycloartenol (minor) and alpha amyrin (major)

The fraction (ABP68) was isolated in the form of white crystals. Its NMR spectrum, recorded at 400 MHz in $CDCl_3$, displayed characteristic signals: a triplet at δ 5.18 (J = 3.7 Hz, 1H) indicative of α-amyrin, along with multiple signals consistent with cycloalkanes. Detailed spectral analysis, including a series of multiplets and singlets across the δ 0.33-5.18 range, and comparisons with existing literature, led to the definitive identification of the fraction as a mixture of 24-methyl cycloartenol (**7**) and α-amyrin (**6**). For further details, refer to Fig 2.

3.7 Bacterial activities and zone of inhibition A. boonei stem bark fractions

Antibacterial activities of various *A. boonei* stem bark fractions (ABO-42, ABO-44, ABO-69, and ABO-71) against wood test bacteria, comparing the results

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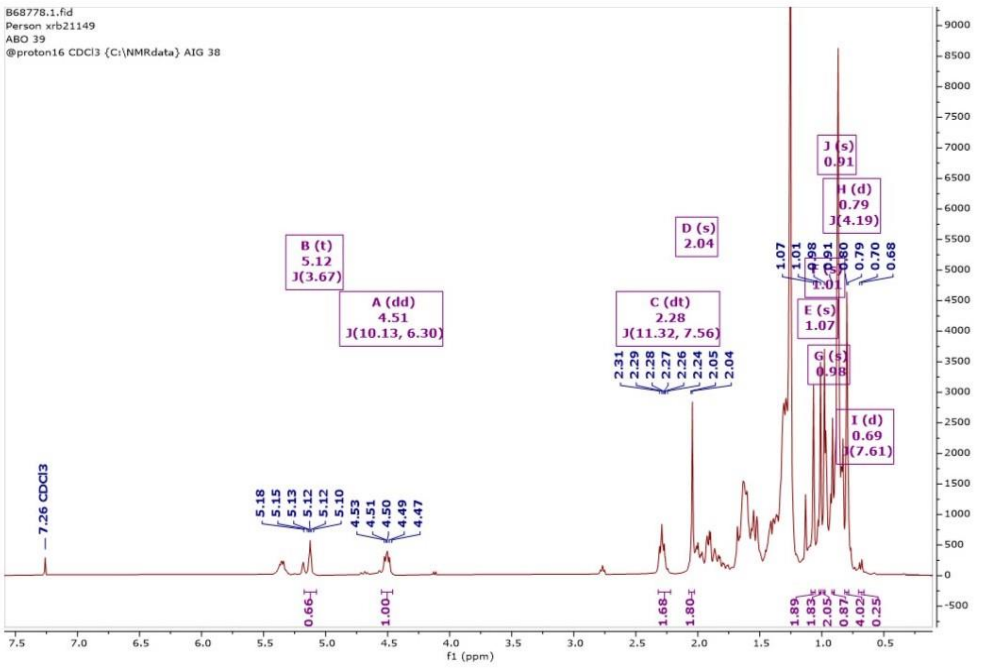

Figure 2b. 1H NMR spectrum of ABO39 and ABO41 characterised as alpha amyrin acetate

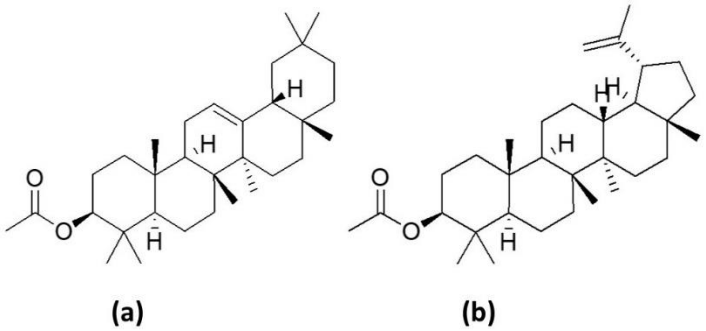

Figure 3a. Lupeol acetate, (**b**) Beta amyrin acetate

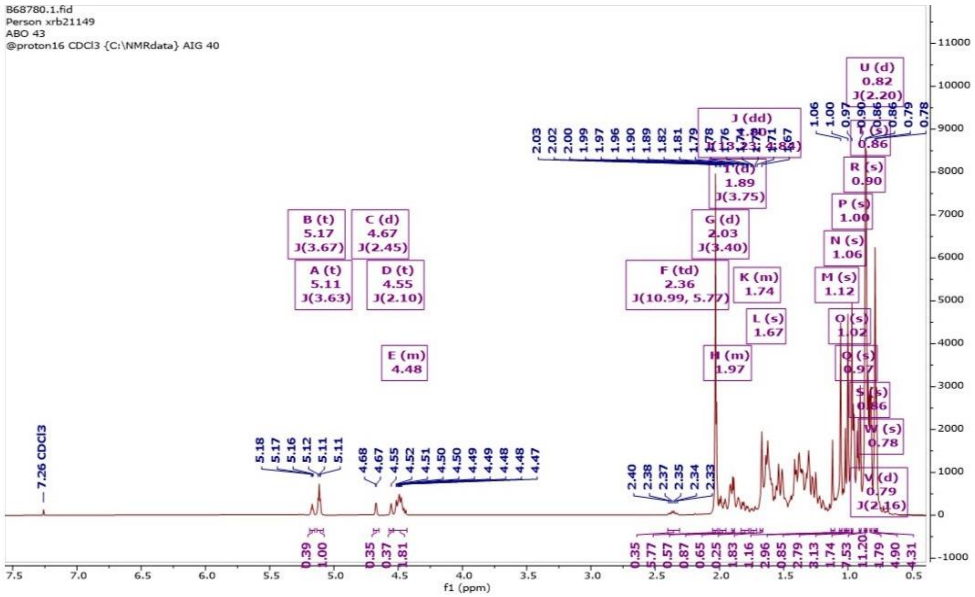

Figure 3c. 1H NMR spectrum of ABO43 characterised as lupeol acetate and beta amyrin acetate

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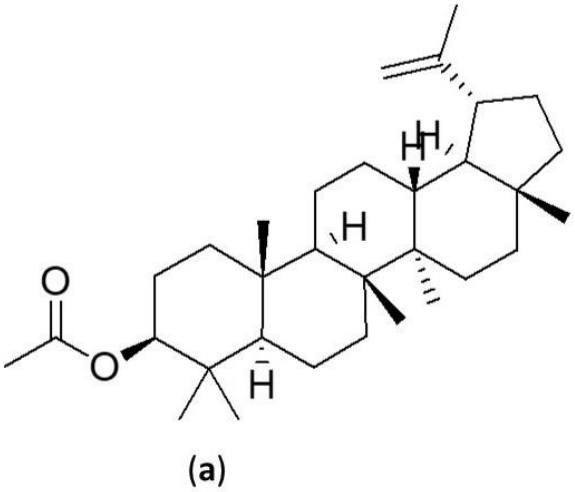

Figure 4a. Lupeol acetate

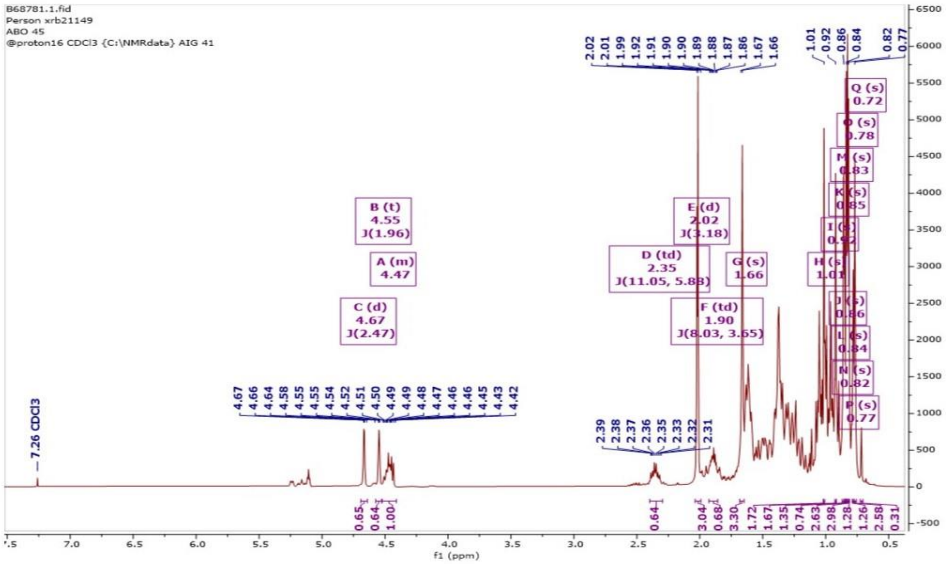

Figure 4b. 1H NMR spectrum of ABO45 characterised as lupeol acetate

with standard antibacterial agents sparfloxacin and keteconazole are shown in Table 2. The results indicated that *A. boonei* fractions exhibited varying degrees of antibacterial activity, particularly against *Pseudomonas aeruginosa, Streptococcus salvarius, Serratia marcescens, Rhanella* sp, and *Klebsiella* sp, with zones of inhibition ranging from 24 mm to 30 mm. In contrast, fractions ABO-42, ABO-44, ABO-69, and ABO-71 showed no activity against *Bacillus subtilis, Erwinia carotovora, Pseudomonas contexa*, and *Enterobacter* sp, which were resistant to these fractions. The standard controls demonstrated antibacterial activity, with Sparfloxacin achieving the highest inhibition zone of 32 mm against *Bacillus subtilis.*

3.8 MIC and MBC of the A. boonei stem bark fractions against the test bacteria

In Table 3, results of antibacterial efficacy through MIC and MBC of *A. boonei* stem bark fractions (ABO-42, ABO-44, ABO-69, and ABO-71) against wood test bacteria are presented. The results indicated that *Bacillus subtilis, Erwinia carotovora, Pseudomonas contexa, Enterobacter* sp., and *Pectobacterium carotovorum* exhibited resistance to all tested fractions. In contrast, *Pseudomonas aeruginosa and Streptococcus salvarius* showed variable susceptibility, with MIC

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Table 2. Bacterial activities and zone of inhibition *A. boonei* (ABO-42, ABO-44, ABO-69, and ABO-71 fractions) against test bacteria

S/No.	Test bacteria	*A. boonei* Fractions				Standard Antibacterial (Control)	
		ABO-42	ABO-44	ABO-69	ABO-71	Sparfloxacin (10 µg/mL)	Keteconazole (10 µg/mL)
		ABA(ZoI)					
1.	*Bacillus Subtilis*	R(0)	R(0)	R(0)	R(0)	R(0)	S(32)
2.	*Pseudomonas aeruginosa*	S(27)	S(25)	S(29)	S(26)	R(0)	S(30)
3.	*Streptococcus salvarius*	S(28)	S(30)	S(27)	S(25)	S(27)	S(29)
4.	*Erwinia carotovora*	R(0)	R(0)	R(0)	R(0)	S(30)	R(0)
5.	*Serratia marcescens*	S(25)	S(28)	S(23)	S(30)	S(26)	R(0)
6.	*Pseudomonas contexa*	R(0)	R(0)	R(0)	R(0)	R(0)	S(27)
7.	*Enterobacter* sp	R(0)	R(0)	R(0)	R(0)	S(30)	R(0)
8.	*Rhanella* sp	S(24)	S(25)	S(24)	S(29)	S(28)	R(0)
9.	*Klebsiella* sp	S(29)	S(30)	S(26)	S(25)	R(0)	S(27)
10.	*Pectobacterium carotovorum*	R(0)	R(0)	R(0)	R(0)	S(31)	R(0)

Key: S = Sensitive R = Resistance; ABA = Antibacterial activities; ZoI = Zone of Inhibition; When zone of inhibition (ZOI) values are < 10 mm the antibiotics are said to be inactive, at 10-13 mm they are partially active, 14-19 mm they are active, and >19 the antibiotics are very active [34].

Table 3. MIC and MBC of the *A. boonei* fractions against the test bacteria

S/No.	Test Bacteria	ABO 42	ABO 44	ABO 69	ABO 71	ABO 42	ABO 44	ABO 69	ABO 71
		MIC (µg/mL)				MBC (µg/mL)			
1.	*Bacillus Subtilis*	R	R	R	R	R	R	R	R
2.	*Pseudomonas aeruginosa*	200	200	100	200	200	200	200	200
3.	*Streptococcus Salvarius*	200	100	200	200	200	100	100	200
4.	*Erwinia carratovora*	R	R	R	R	R	R	R	R
5.	*Serratia marcescens*	200	200	200	100	200	200	200	100
6.	*Pseudomonas contexa*	R	R	R	R	R	R	R	R
7.	*Enterobacter sp*	R	R	R	R	R	R	R	R
8.	*Rhanella sp*	200	200	200	100	200	200	200	100
9.	*Klebsiella sp*	100	100	200	200	100	100	200	200
10.	*Pectobacterium carotovorum*	R	R	R	R	R	R	R	R

values ranging from 100 to 200 µg/mL and MBC values corresponding closely to their MICs. *Klebsiella* sp. demonstrated a MIC of 100 µg/mL for both ABO-42 and ABO-44, indicating a more pronounced sensitivity.

3.9 Antibacterial activities and zone of inhibition of plant methanol crude extracts against test bacteria

In Table 4, the results of the antibacterial activity of *A. boonei* methanol stem bark crude extracts against ten wood bacterial strains, with sparfloxacin and keteconazole serving as standard antibacterial controls are presented. *A. boonei* exhibited sensitivity (S) with a zone of inhibition (ZoI) ranging from 21 to 26 mm against five bacterial strains: *Pseudomonas aeruginosa* (23 mm), *Streptococcus salivarius* (25 mm), *Serratia marcescens* (21 mm), *Rhanella sp* (21 mm), and *Pectobacterium carotovorum* (26 mm). In contrast, it showed resistance (R) with no inhibition (0 mm) against the remaining five strains, including *Bacillus subtilis* and *Klebsiella sp*. Sparfloxacin was effective against five bacterial strains with ZoI ranging from 26 to 31 mm, while keteconazole exhibited high activity against four strains with ZoI up to 32 mm. The comparison shows that while *A. boonei* demonstrates moderate antibacterial activity, it is less effective than the standard antibiotics, particularly against *Bacillus subtilis*, *Erwinia carotovora*, *Pseudomonas contexa*, *Enterobacter sp*, and *Klebsiella sp*.

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Table 4. Antibacterial activities and Zone of Inhibition of plant methanol crude extracts against test bacteria

S/No.	Test bacteria	Crude extract	Standard Antibacterial (Control)	
		A. boonei	Sparfloxacin (10 µg/mL)	Keteconazole (10 µg/mL)
		ABA(ZoI)		
1.	*Bacillus subtilis*	R(0)	R(0)	S(32)
2.	*Pseudomonas aeruginosa*	S(23)	R(0)	S(30)
3.	*Streptococcus salvarius*	S(25)	S(27)	S(29)
4.	*Erwinia carotovora*	R(0)	S(30)	R(0)
5.	*Serratia marcescens*	S(21)	S(26)	R(0)
6.	*Pseudomonas contexa*	R(0)	R(0)	S(27)
7.	*Enterobacter sp*	R(0)	S(30)	R(0)
8.	*Rhanella sp*	S(21)	S(28)	R(0)
9.	*Klebsiella sp*	R(0)	R(0)	S(27)
10.	*Pectobacterium carotovorum*	S(26)	S(31)	R(0)

Key: S = Sensitive R = Resistance; ABA = Antibacterial activities; ZoI = Zone of Inhibition; When zone of inhibition (ZOI) values are < 10 mm the antibiotics are said to be inactive, at 10-13 mm they are partially active, 14-19 mm they are active, and >19 the antibiotics are very active [34].

3.10 MIC and MB) of A. boonei methanol crude extracts against the test bacteria

Antibacterial efficacy (MIC and MBC) of *A. boonei* methanol stem bark crude extracts against various bacterial strains results are presented in Table 5. The extracts exhibited significant activity against *Pseudomonas aeruginosa*, *Streptococcus salivarius*, *Serratia marcescens*, *Rhanella sp*, and *Pectobacterium carotovorum* with a consistent MIC of 2.5 mg/mL and MBC of 5 mg/mL. In contrast, the extracts were ineffective against *Bacillus subtilis*, *Erwinia carotovora*, *Pseudomonas contexa*, *Enterobacter sp*, and *Klebsiella sp*, showing resistance with no detectable MIC or MBC. Fig 5 - 9 show the zones of inhibition of *A. boonei* stem bark fractions on test bacteria, while Fig 9 shows the zones of inhibition of *A. boonei* stem bark methanol extract on test bacteria.

Table 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *A. boonei* methanol crude extracts against the test bacteria

S/ No	Test bacteria	*A. boonei* methanol crude extract	
		MIC (mg/mL)	MBC (mg/mL)
1.	*Bacillus subtilis*	Resistant	Resistant
2.	*Pseudomonas aeruginosa*	2.5	5
3.	*Streptococcus salvarius*	2.5	5
4.	*Erwinia carotovora*	Resistant	Resistant
5.	*Serratia marcescens*	2.5	5
6.	*Pseudomonas contexa*	Resistant	Resistant
7.	*Enterobacter sp*	Resistant	Resistant
8.	*Rhanella sp*	2.5	5
9.	*Klebsiella sp*	Resistant	Resistant
10.	*Pectobacterium carotovorum*	2.5	5

4. Discussion

4.1 Characterised compounds from stem bark fractions of A. Boonei

This study characterised alpha amyrin acetate, lupeol acetate, beta amyrin acetate and beta amyrin compounds from the stem bark of *A. boonei*. Vitor [18] reported that alpha-amyrin acetate is a triterpene acetate derivative of alpha-amyrin, which is a pentacyclic triterpene found in plants. As an acetate ester, it would show characteristic 1H-NMR signals for the acetyl group, likely around 2.0-2.1 ppm for the methyl protons of the acetate. The structure of alpha-amyrin acetate contains multiple methyl groups and a complex aliphatic region typical of triterpenes. While exact chemical shift values are not provided, we can expect to see signals in the aliphatic region (0.5-2.5 ppm) corresponding to these structural features [19,20].

Lupeol acetate, a derivative of the natural triterpene lupeol, has been extensively studied using 1H NMR spectroscopy for structural elucidation and confirmation. Several papers report the use of 1H NMR in characterizing lupeol acetate and related compounds [21-24].

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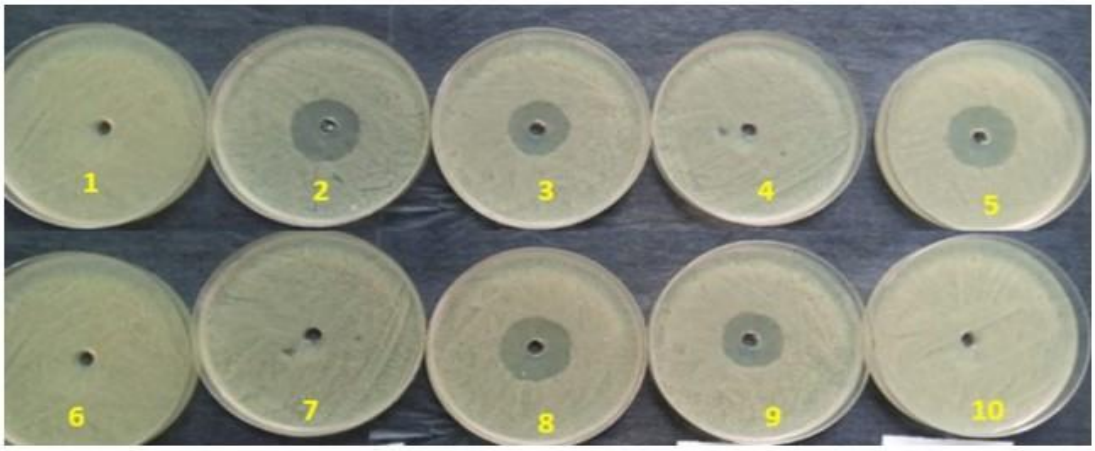

Figure 5. Zone of inhibition of *A. boonei* stem bark fraction (ABO71) of test bacteria
Key: 1. *Bacillus subtilis,* 2. *Pseudomonas aeruginosa,* 3. *Streptococcus salvarius,* 4. *Erwinia carotovora,* 5. *Serratia marcescens,* 6. *Pseudomonas convexa,* 7. *Enterobacter spp.,* 8. *Rahnella spp.,* 9. *Klebsiella spp,* 10. *Pectobacterium carotovorum*

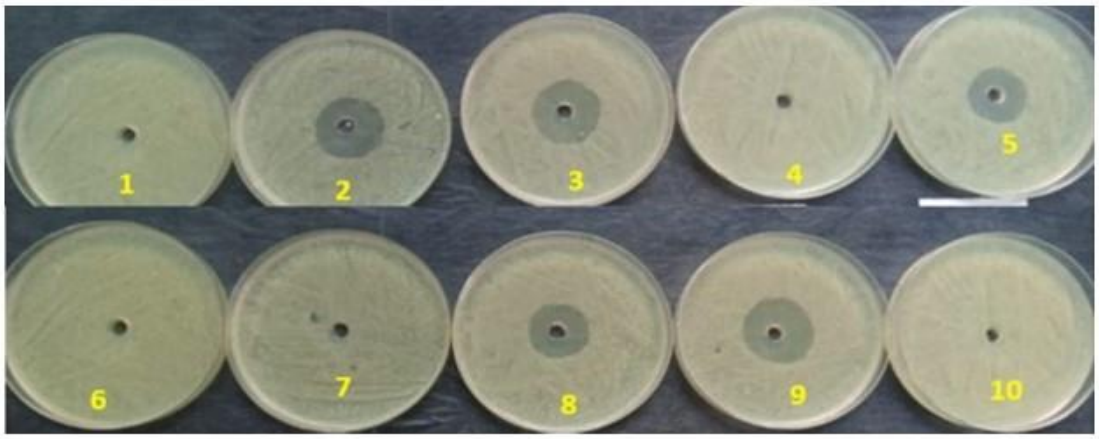

Figure 6. Zone of inhibition of *A. boonei* stem bark fraction (ABO42) of test bacteria
Key: 1. *Bacillus subtilis,* 2. *Pseudomonas aeruginosa,* 3. *Streptococcus salvarius,* 4. *Erwinia carotovora,* 5. *Serratia marcescens,* 6. *Pseudomonas convexa,* 7. *Enterobacter spp.,* 8. *Rahnella spp.,* 9. *Klebsiella spp,* 10. *Pectobacterium carotovorum*

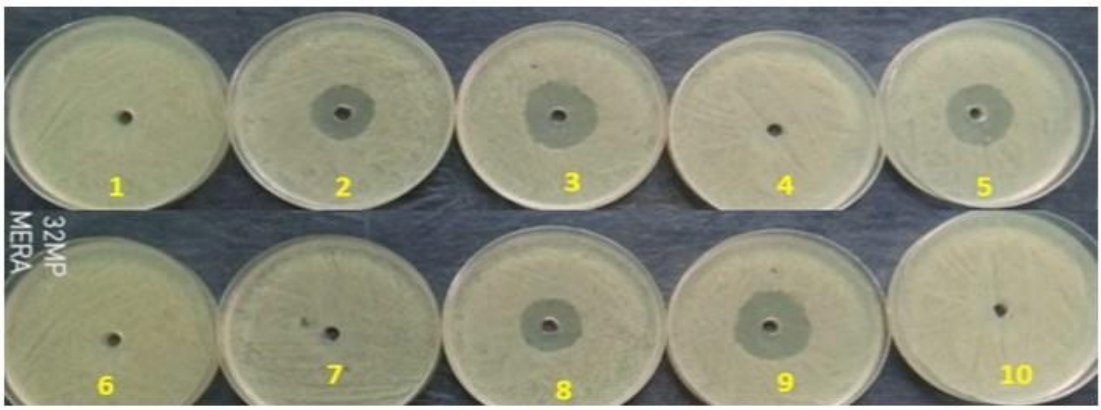

Figure 7. Zone of inhibition of *A. boonei* stem bark fraction (ABO44) of test bacteria
Key: 1. *Bacillus subtilis,* 2. *Pseudomonas aeruginosa,* 3. *Streptococcus salvarius,* 4. *Erwinia carotovora,* 5. *Serratia marcescens,* 6. *Pseudomonas convexa,* 7. *Enterobacter spp.,* 8. *Rahnella spp.,* 9. *Klebsiella spp,* 10. *Pectobacterium carotovorum*

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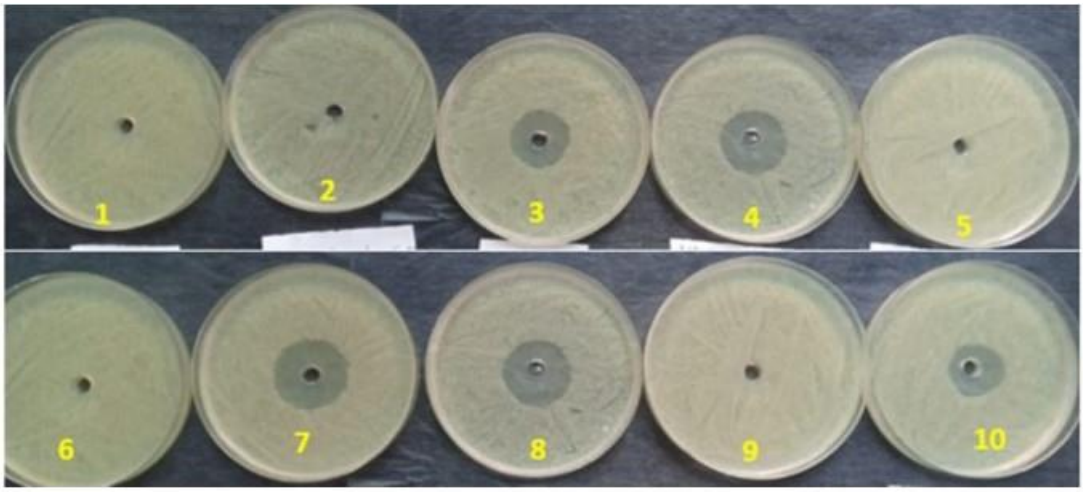

Figure 8. Zone of inhibition of *A. boonei* stem bark fraction (ABO69) of test bacteria
Key: 1. *Bacillus subtilis*, 2. *Pseudomonas aeruginosa*, 3. *Streptococcus salvarius*, 4. *Erwinia carotovora*, 5. *Serratia marcescens*, 6. *Pseudomonas convexa*, 7. *Enterobacter spp.*, 8. *Rahnella spp.*, 9. *Klebsiella spp*, 10. *Pectobacterium carotovorum*

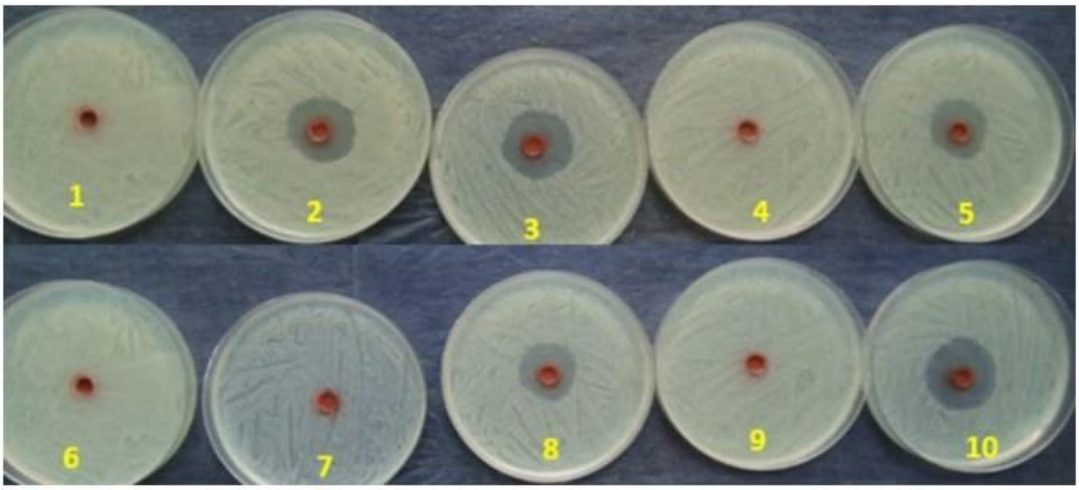

Figure 9. Zone of inhibition of *A. boonei* stem bark methanol crude extract of test bacteria
Key: 1. *Bacillus subtilis*, 2. *Pseudomonas aeruginosa*, 3. *Streptococcus salvarius*, 4. *Erwinia carotovora*, 5. *Serratia marcescens*, 6. *Pseudomonas convexa*, 7. *Enterobacter spp.*, 8. *Rahnella spp.*, 9. *Klebsiella spp*, 10. *Pectobacterium carotovorum*

The 1H NMR spectroscopy, often combined with 13C NMR and other techniques like HRMS, has proven to be a powerful tool for confirming the structure of lupeol acetate and its derivatives [21, 23].

1H NMR spectroscopy has been extensively used to characterize and identify various triterpenes, including β-amyrin acetate. The complete and definitive assignment of 1H NMR chemical shifts for β-amyrin acetate has been achieved through the application of one- and two-dimensional NMR experiments such as gCOSY, gNOESY, gHSQC, and gHMBC [19]. β-amyrin acetate has been isolated from various plant sources and has shown significant biological activities. For instance, it was found to exhibit anti-inflammatory properties in a mouse model of cystitis induced by cyclophosphamide [25]. Research on *Celastrus hindsii* by Viet [26] isolated α- and β-amyrin, confirming their presence and potential therapeutic benefits through methods like GC-MS and NMR spectroscopy, which corroborate the structural analysis of compounds identified in *A. boonei* by Okoye and Okoye [27]. Another study by Hernández-Vázquez [28] has shown that compounds like lupeol and stigmasterol exhibit anti-

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inflammatory and analgesic activities, reinforcing the therapeutic potential of the phytochemicals derived from *A. boonei* and their relevance in ethnomedicine.

Aalpha-amyrin acetate, lupeol acetate, and beta-amyrin acetate are triterpene acetates that have been isolated from various plant sources and have been the subject of numerous studies due to their biological activities. alpha-amyrin acetate and beta-amyrin acetate were identified by Akihisa [29] in the kernel fat of the Shea tree and have shown marked anti-inflammatory activity in mice and inhibitory effects on tumor promotion. Lupeol acetate, also isolated from the Shea tree by Akihisa [29] exhibited similar anti-inflammatory properties and was further evaluated for its effects on Epstein-Barr virus early antigen in Raji cells. These compounds were found in the Amazonian medicinal mistletoe and the swallow roots, indicating their widespread occurrence in nature [30, 31].

4.2 Effect of A. boonei stem bark fractions on test fungi

The results on the effect of *A. boonei* stem bark fractions on test fungi demonstrated that *A. flavus, C. puteana, F. vaillantii,* and *S. rolfsii* were sensitive to the fractions, with zones of inhibition (ZoI) ranging from 25 mm to 29 mm. *Aspergillus fumigatus* exhibited sensitivity only to ABO-69 and ABO-71. Further analysis of the MIC and MFC of the *A. boonei* fractions revealed that *A. flavus, C. puteana, F. vaillantii, F. pinicola*, and *S. rolfsii* were susceptible to all fractions, with MIC values ranging from 50 to 100 µg/mL and MFC values from 100 to 200 µg/mL. The fraction ABO-69 exhibited the lowest MIC value of 50 µg/mL *against C. puteana* and *F. vaillantii,* indicating its active potential as a potent antifungal agent.

These findings are consistent with previous studies that have reported the antimicrobial and antifungal properties of *A. boonei.* For instance, a study by Opoku and Akoto [32] found that ethanol and aqueous extracts of *A. boonei* root exhibited significant antimicrobial activity against various bacterial and fungal strains, including *Candida albicans.* Another study by Okoye and Okoye [27] isolated antioxidant and antimicrobial flavonoid glycosides from *A. boonei* leaves, further supporting the plant's potential as a source of bioactive compounds with therapeutic applications. Moreover, studies by Mollica [33] highlighted the traditional use of *A. boonei* in treating various ailments, including malaria, fever, and ulcers. The current study provides scientific validation for these traditional uses by demonstrating the antifungal efficacy of *A. boonei* stem bark fractions against wood fungal pathogens.

5. Conclusions

Alpha amyrin acetate, lupeol acetate and beta amyrin acetate of triterpenoids were characterized from *A. boonei* stem bark. *A. boonei* stem bark extract and fractions exhibited very active antibacterial activity against *P. aeruginosa, S. salvarius, S. arcescens, Rhanella* sp, and *Klebsiella* sp, with zones of inhibition ranging from 24 - 30 mm. *Therefore, A. boonei* stem bark *should be explored for the production of biopesticides for wood bacteria control.*

Authors' contributions

Designed, coordinated the study and proofread the work, D.O.E; E.T.T, Extraction and antibacterial activities experiment, M.T.A.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors confirm that there is no conflict of interest to declare.

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