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Research Article

Anti-wood-degrading fungi activity of triterpenes and sterols from *Alstonia boonei* stem bark

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Abstract

This study investigates the bioactive compounds from *Alstonia boonei* stem bark and their antifungal activity against wood-degrading fungi. The air-dried, pulverised, stem bark was sequentially extracted with n-hexane, ethyl acetate and methanol. The ethyl acetate extract was subjected to silica gel column chromatography using hexane-ethyl acetate solvent gradients and characterized using Nuclear Magnetic Resonance (NMR) spectroscopy. Antifungal activity was evaluated against *Aspergillus flavus*, *Coniophora puteana*, *Fibroporia vaillantii*, *Sclerotium rolfsii*, and other wood-degrading fungi using the broth dilution method to determine Minimum Inhibitory Concentrations (MICs) and Minimum Fungicidal Concentrations (MFCs). The study led to the isolation and characterisation of 24-methylenecycloartenol (**1**), β-amyrin (**2**), cycloeucalenol (**3**), stigmasterol (**4**), β-sitosterol (**5**), betulinic acid (**6**), α-amyrin acetate (**7**) and lupeol acetate (**8**). All fractions showed significant antifungal activity, with zones of inhibition (ZOI) ranging from 24–30 mm. Fraction ABO69 exhibited the highest activity (ZOI = 30 mm) and the lowest MIC (50 µg/mL) and MFC (100 µg/mL), demonstrating effectiveness against six fungal species. The methanol crude extract displayed moderate activity, with ZOIs of 18–21 mm and MIC and MFC values of 2.5 mg/mL and 10 mg/mL, respectively. *A. boonei* stem bark extracts and fractions exhibit potent antifungal properties, comparable to standard antibiotics. Fraction ABO69 shows the most promise for development as a bio-pesticide. Further research is recommended to explore its practical applications and safety profile.

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Alstonia boonei, triterpenes, sterols, anti-wood-fungi, bio-pesticides.

1. Introduction

Alstonia boonei De Wild, commonly known as "God's tree" or "*Onyame dua*," holds a significant place in the cultural and medicinal traditions of West Africa [1]. Revered in many forest communities, the plant is considered sacred, with its parts often reserved for therapeutic purposes rather than culinary use [1]. This cultural reverence underscores its importance as a cornerstone of traditional medicine, where it has been employed for a wide range of treatments, including antimalarial, antidiabetic, antimicrobial, aphrodisiac,

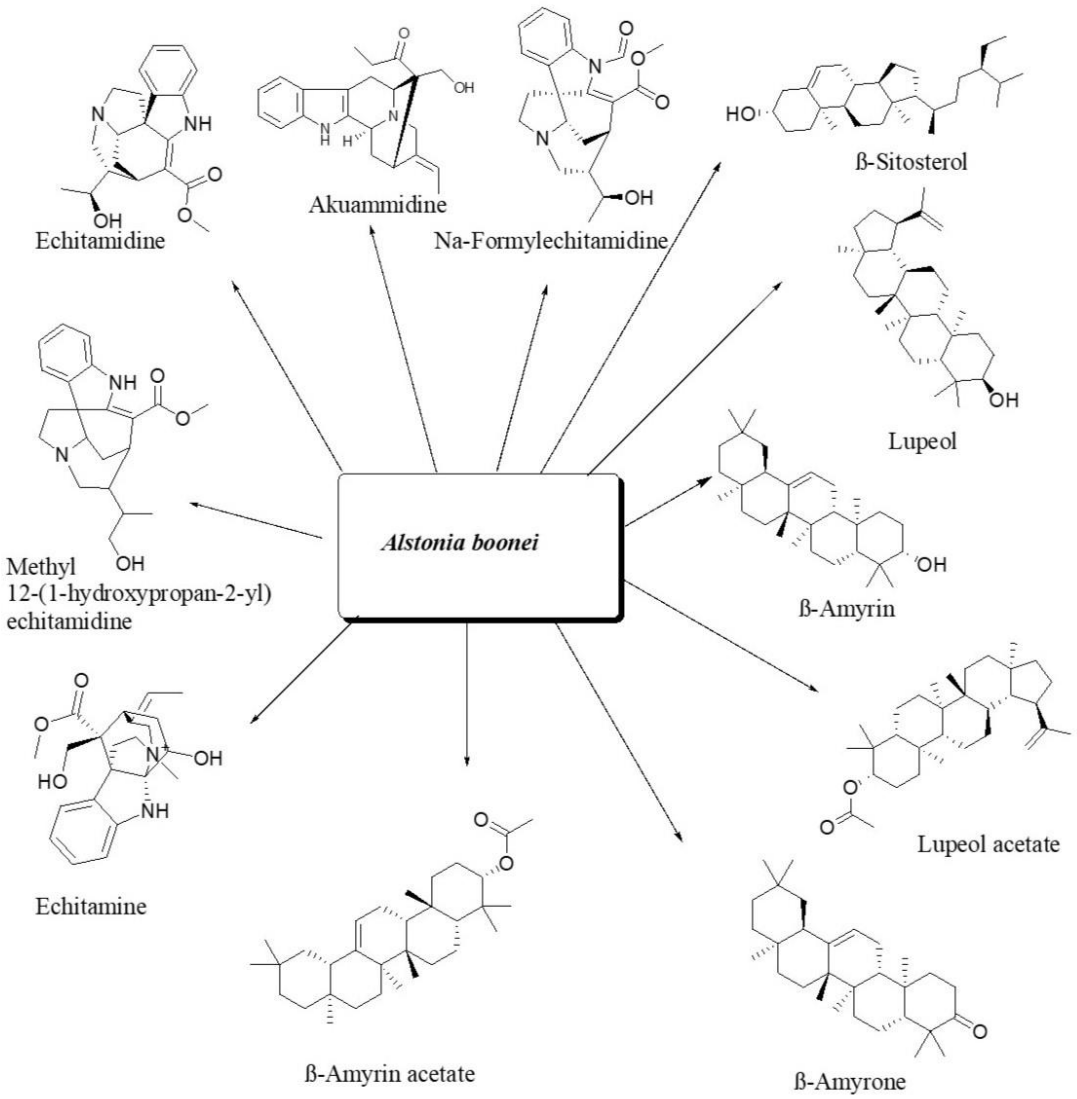

Figure 1. Previous compound Isolations from *Alstonia boonei*

and antipyretic applications [1]. Despite its extensive medicinal applications, the bioactivity of *A. boonei* exhibits specificity, as it lacks fungicidal effects against certain fungal isolates responsible for crown rot disease in bananas [2]. This selective efficacy suggests that its bioactive compounds may target specific pathogens or physiological conditions more effectively. Such specificity warrants a closer examination of its phytochemical constituents, which are responsible for its diverse pharmacological properties [3, 4]. The medicinal value of *A. boonei* is attributed to its rich phytochemical profile, encompassing alkaloids, tannins, saponins, flavonoids, cardiac glycosides, steroids, phenols, and terpenoids [3, 4]. Many bioactive compounds, including echitamine, echitamidine, akuammidine, Nα-formylechitamidine, β-amyrin, lupeol, β-amyrone, lupeol acetate, β-sitosterol and β-amyrin acetate (Fig 1) have been previously isolated from *Alstonia boonei*., have been isolated from various parts of the plant [1].

These compounds play crucial roles in its therapeutic effects, offering potential pathways for targeted drug development. The extraction method and choice of solvent significantly influence the phytochemical composition and, consequently, the biological activity of *A. boonei* extracts. Methanol extracts, for example, have been shown to yield higher levels of phenolic compounds compared to ethyl acetate extracts. Advanced analytical techniques, such as Ultra-High-Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS), have identified key

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compounds in methanol extracts, including phenolic acids (caffeic, chlorogenic, and ferulic acids), flavonoids (rutin and isoquercetin), and flavonolignans (Cinchonain isomers). Notably, a novel compound, 2-methyl-3-propylbutane-1,4-diol (MPBD), was recently isolated from the methanol fraction of the stem bark extract, underscoring the plant's potential for yielding unique bioactive agents [5, 6]. While the therapeutic applications of *A. boonei* are well-documented, its fungicidal properties, particularly against wood-degrading fungi, remain underexplored. The lack of comprehensive studies in this area represents a critical research gap. Investigating the bioactive compounds of *A. boonei* and their potential antifungal activity could not only expand its pharmacological utility but also provide novel solutions for managing wood-degrading fungi.

2. Materials and methods

2.1 Plant collection and preparation

The stem bark of *Alstonia boonei* was harvested from a standing tree in Edo State, southern Nigeria, where this species is prevalent and traditionally used in medicine for treating various ailments. The collected stem bark was air-dried under shade for three weeks at an ambient temperature of 27-34 °C, with controlled humidity conditions to preserve its phytochemical integrity. Once dried, the material was pulverized using a wooden mortar and pestle to a fine powder, ensuring uniform particle size for optimal extraction efficiency. Fig. 2 depicts the leaves and stem of *A. boonei* as photographed during the study and Scheme 1 denotes the total work procedures.

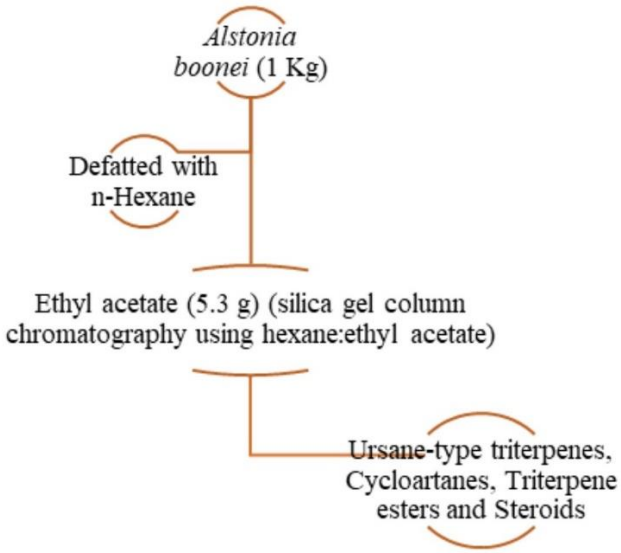
2.2 Extraction of A. boonei stem bark

Methanol, ethyl acetate, and n-hexane solvents were purified by distillation to remove impurities. Pulverized *A. boonei* stem bark (1 Kg) was macerated in n-hexane (2 L) for 24 hours (w/v ratio). The resulting extract was filtered using Whatman No. 1 filter paper and transferred into labeled glass bottles. The filtrate was concentrated using a rotary evaporator to obtain the dried crude extract. This was repeated for the ethyl acetate and methanol extracts.

2.3 Column chromatography procedure

The ethyl acetate crude extract (5.3 g) of *A. boonei* stem bark was subjected to column chromatography. The

Figure 2. Leaves and stem of *A. boonei*



Scheme 1. Isolation protocol for triterpenes and steroids isolated from *A. boonei*

column was prepared by packing a glass column with silica gel 60-120 mesh (Merck) to a height of 60 cm, with a cotton wool layer at the neck. Ethyl acetate (5%) in n-hexane was used to prepare the slurry. The introduced slurry was left for eight hours to ensure proper packing and preparation for effective separation, Scheme 2 illustrates the basic isolation protocol. The column was eluted with solvent mixtures of n-hexane and ethyl acetate, varying the ratios from 95%:5% to 0%:100%; a stepwise increase in ethyl acetate to achieve increasing polarity. The collected fractions were air-dried in a fumehood and weighed with a precision balance. Fractions ABO 39, ABO 41, ABO 43, ABO 45, ABP 68, ABP 70, ABP 74, ABP 76, ABP 87, ABP 89, ABP 91, ABP 93, ABP 105, ABP 107 and ABP 109 were further analyzed using Nuclear Magnetic Resonance (NMR) spectroscopic analysis, while fractions ABO-42, ABO-44, ABO-69, and ABO-71 were reserved for antifungal testing.

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Table 1. Compounds isolated from *A. boonei*

Fraction Codes	Compounds	Description	Compound number
ABO 39	α-Amyrin acetate	Creamy White	7
ABO 41	α-Amyrin acetate	Creamy White	7
ABO 43	α-Amyrin acetate and Lupeol acetate (minor)	Creamy White	7 & 8
ABO 45	Lupeol acetate	Creamy White	8
ABP 68	24-Methylenecycloartanol (major) and β-Amyrin (minor)	White powdery	1 & 2
ABP 70	24-Methylenecycloartanol (minor) and β-Amyrin (major)	White powdery	1 & 2
ABP 74	Cycloeucalenol (major) and β-Amyrin (minor)	White powdery	3 & 2
ABP 76	Cycloeucalenol and β-Amyrin (1:1)	White powdery	3 & 2
ABP 87	β-Sitosterol	White powdery	5
ABP 89	β-Sitosterol (major) and Stigmasterol (minor)	White powdery	5 & 4
ABP 91	β-Sitosterol (major) and Stigmasterol (minor)	White powdery	5 & 4
ABP 93	β-Sitosterol : Stigmasterol (1:1)	White powdery	5 & 4
ABP 105	Betulinic acid	White powdery	6
ABP 107	Betulinic acid	White powdery	6
ABP 109	Betulinic acid	White powdery	6

2.4 Antifungal screening of test fungi

The antifungal activity of *A. boonei* stem bark fractions and crude extract were evaluated against selected wood-degrading fungi, including *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Coniophora puteana*, *Fibroporia vaillantii*, *Fomitopsis pinicola*, *Fusarium* sp., *Rhizopus* spp., *Sclerotium rolfsii*, *Trichoderma* sp., and *Serpula lacrymans*. ketoconazole and sparfloxacin served as standard antifungal controls. The disk diffusion method by Chand *et al.* [7] was adopted to screen the extracts and fractions for antifungal activity over seven days by observing zones of fungal growth inhibition.

2.5 Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The broth dilution method, based on the protocol described by Chand *et al.* [7], was also employed to determine the MIC and MFC of the extract and fractions. Petri dishes with the lowest concentration of extracts or fractions that showed no colony growth were recorded as the MIC, while those without colony regrowth after subculturing were noted as the MFC.

3. Results

3.1 Compounds isolated from A. boonei stem bark

Table 1 presents an array of compounds isolated from *A. boonei* stem bark. The compounds: 24-Methylenecycloartenol (**1**), β-amyrin (**2**), cycloeucalenol (**3**), stigmasterol (**4**), β-sitosterol (**5**), betulinic acid (**6**), α-amyrin acetate (**7**), and lupeol acetate (**8**) range from cycloartane triterpenes, lupane triterpenes, ursane, oleanane and sterols (Scheme 2).

*24-Methylenecycloartenol (**1**)* (Fig. 3*)* (ABP 68; ABP 70): White powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 4.74 (1H, m, H-31a), 4.63 (1H, m, H-31b))[olefinic cycloartanes signals], 3.20 (1H, ddd, *J* = 14.1, 11.0, 4.8 Hz, H-3) [oxymethine proton], 2.37 (1H, td, *J* = 11.0, 5.7 Hz, H-23b), 2.26 (1H, m, H-23a), 1.76 (1H, td, *J* = 13.7, 4.5 Hz, H-17), 1.68 (1H, s, H-20), 1.13 (2H, s, H-18, H-19), 0.99 (2H, s, H-25, H-26), 0.96 (3H, s, H-28), 0.93 (2H, s, H-27, H-30), 0.87 (3H, s, H-29), 0.83 (2H, s, H-22, H-21), 0.79 (3H, s, H-16), 0.76 (1H, s, H-15), 0.55 (1H, d, *J* = 4.3 Hz, H-19a), 0.33 (1H, d, *J* = 4.2 Hz, H-19b) [non-equivalent high-field, cycloartane cyclopropyl protons] [37, 38].

*β-Amyrin (**2**)* (Fig. 4*)* (ABP 68; ABP 70; ABP 74): White powder. ^{1}H NMR (400 MHz, $CDCl_3$): δH 5.18 (t, 1H, *J* = 3.7 Hz, H-12) [olefinic], 3.21 (m, 1H, H-3)) [oxymethine proton], 0.96 (s, 3H, H-29), 0.87 (s, 3H, H-30), 0.79 (s, 3H, H-24). Comparison of It's characteristic signals with the literature [30] allowed for unambiguous characterisation of the compound as *β*-amyrin (Fig. 4).

Cycloeucalenol **(3)** **(**Fig. 5a**)** *(ABP76):* While crystal. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.72 (s, 0H), 4.66 (s, 0.4H) [olefinic cycloartanes signals], 3.21 (td, *J* = 9.3, 7.7, 4.5 Hz, 1H) [oxymethine proton], 1.25 (s, 2H), 1.14 (s, 1H), 1.04 – 1.01 (m, 2H), 1.00 (s, 1H), 0.97 (s, 3H), 0.94 (s,

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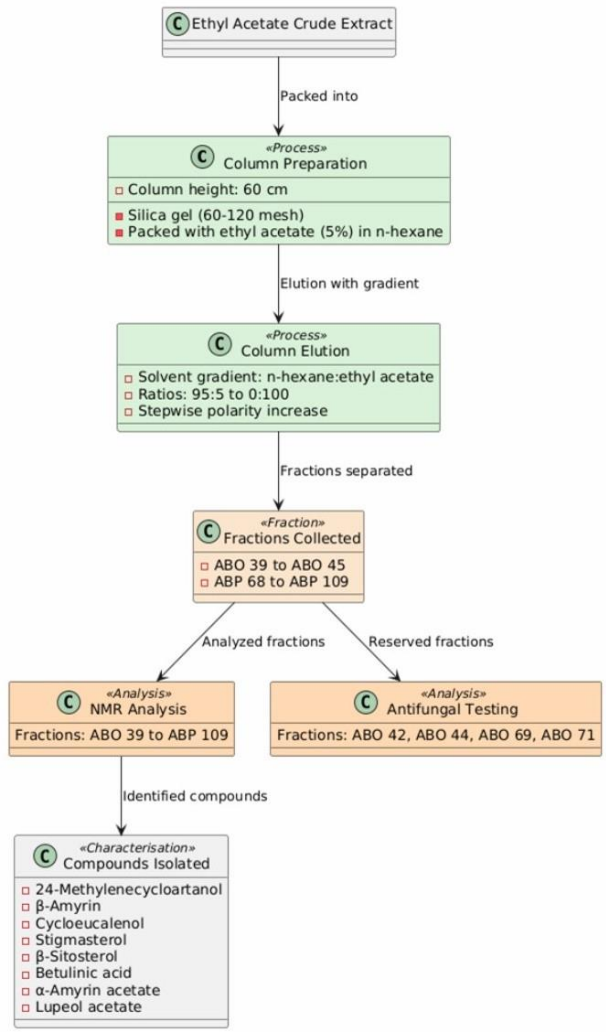
2H), 0.89 (d, *J* = 2.2 Hz, 1H), 0.87 (s, 3H), 0.83 (s, 1H), 0.79 (s, 2H), 0.39 (d, *J* = 4.1 Hz, 0.4H), 0.14 (d, *J* = 4.1 Hz, 0.4H) [non-equivalent high-field, cycloartane cyclopropyl protons]. Characteristic signals and literature comparisons confirmed the compound as cycloeucalenol [31,38] .



Scheme 2. Work summary

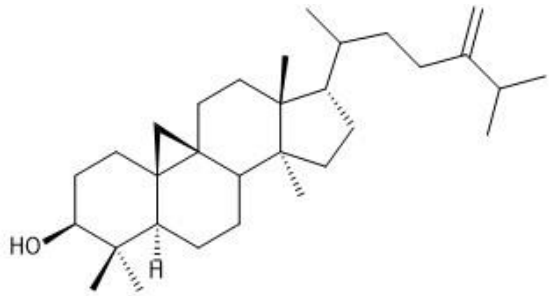


Figure 3. 24-Methylenecycloartenol (ABP 68; ABP 70)

Stigmasterol ***(4)*** **(**Fig. 5b**)** *(ABP 89, 91, 93):* White powder. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.35 (1H, t, *J* = 5.2 Hz, H-6), 5.15 (1H, dd, *J* = 15.2, 8.5 Hz, H-22), 5.02 (1H, dd, *J* = 15.2, 8.5 Hz, H-23), 3.53 (1H, tt, *J* = 10.8, 4.8 Hz, H-3), 2.26 (1H, dd, *J* = 18.2, 4.5 Hz, H-4), 1.64 (1H, q, *J* = 7.7 Hz, H-7), 1.25 (18H, s, H-28), 1.01 (3H, s, H-19), 0.92 (2H, d, *J* = 6.2 Hz, H-26), 0.83 (2H, d, *J* = 7.9 Hz, H-27), 0.81 (1H, s, H-18), 0.69 (3H, d, *J* = 7.4 Hz, H-29). Comparison of its pertinent signals with those of stigmasterol in the literature confirmed the compound to be stigmasterol [32].

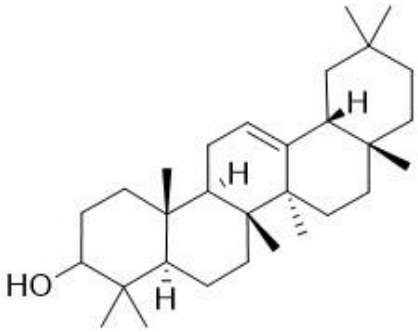


Figure 4. *β*-Amyrin (ABP 68; BP70; ABP 74)

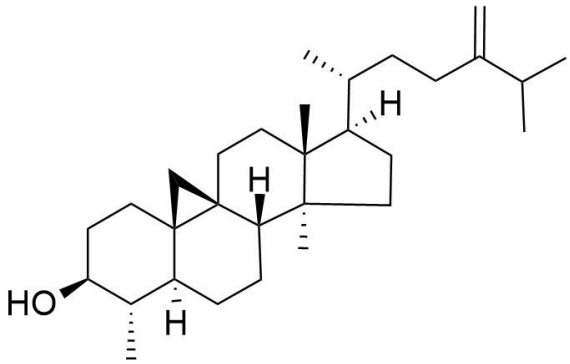


Figure 5a. Cycloeucalenol (ABP76)

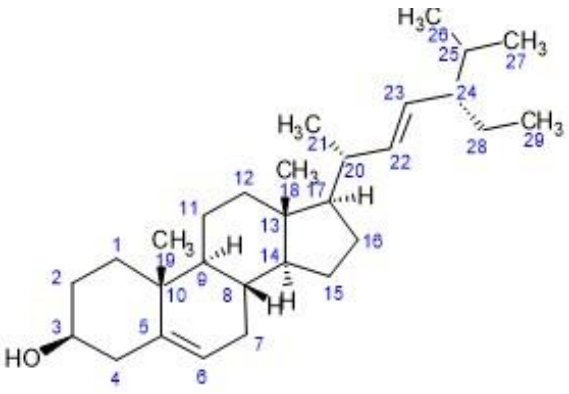


Figure 5b. Stigmasterol (ABP 89, 91, 93)

β-Sitosterol ***(5)*** *(*Fig. 6*)* *(*ABP87, 89, 91, 93*):* White powder. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.35 (1H, d, J = 5.2 Hz, H-6), 3.53 (1H, tt, J = 10.8, 4.8 Hz, H-3), 2.26 (1H, dd, J = 18.2, 4.5 Hz, H-4β), 1.64 (1H, q, J = 7.7 Hz, H-11), 1.25 (18H, s, overlapping CH_2 signals, likely

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from the steroid side chain), 1.01 (3H, s, H-19), 0.92 (2H, d, J = 6.2 Hz, H-26), 0.83 (2H, d, J = 7.9 Hz, H-27), 0.81 (1H, s, H-18), 0.69 (3H, d, J = 7.4 Hz, H-21)[the coupling constant (J = 7.4 Hz) is somewhat large for a typical methyl doublet, due to overlap of stigmasterol signals and baseline distortions]. A comparison of its pertinent signals with those of β-sitosterol in the literature confirmed the compound to be stigmasterol [32].

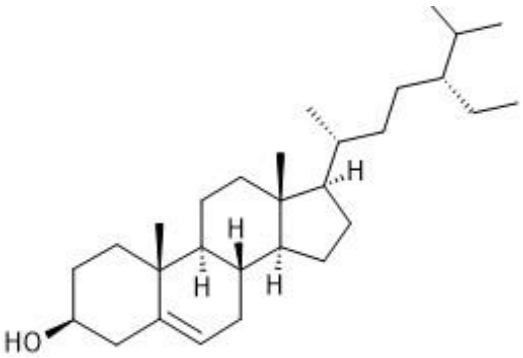

Figure 6. *β*-Sitosterol (5) (ABP87, 89, 91, 93)

*Betulinic acid (**6**) (*Fig. 7*) (*ABP 105, 107, 109*):* White amorphous powder. ^{1}H NMR ($CDCl_3$, 400 MHz): **δ** 4.79 (1H, m, H-29b), 4.73 (1H, m, H-29a), 3.19 (1H, br s, H-3), 2.98 (1H, br s, H-19), 2.35–2.27 (2H, m, H-13, H-15), 1.76 (3H, s, H-30), 1.75–1.67 (6H, m, H-2, H-18, H-21, H-22), 1.63–1.59 (5H, m, H-6, H-7, H-16), 1.52–1.43 (4H, m, H-9, H-11, H-12), 1.34 (3H, s, H-23), 1.29 (3H, s, H-26), 1.27 (3H, s, H-27), 1.25 (3H, s, H-24), 1.03 (3H, s, H-25), 0.97 (3H, s, H-28), 0.72–0.69 (3H, m, H-1, H-5). ^{13}C NMR ($CDCl_3$, 101 MHz): δ 180.8 (C-28), 150.2 (C-20), 109.8 (C-29), 78.5 (C-3), 55.6 (C-5), 50.1 (C-9), 49.5 (C-19), 48.1 (C-18), 47.4 (C-17), 42.2 (C-14), 40.4 (C-8), 39.1 (C-4), 39.0 (C-1), 38.8 (C-13), 38.4 (C-10), 38.3 (C-22), 33.8 (C-7), 32.3 (C-16), 30.3 (C-21), 29.9 (C-15), 27.9 (C-23), 27.4 (C-2), 24.7 (C-12), 21.1 (C-11), 19.1 (C-30), 16.8 (C-26), 16.3 (C-24), 16.5 (C-25), 15.8 (C-27). The characteristic signals and literature comparison allowed for unambiguous characterisation of the compound as betulinic acid [33-35].

*α-Amyrin acetate (**7**) (*Fig. 8*) (*ABO 39, 41, 43*):* Creamy white gummy powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.12 (1H, t, *J* = 3.7 Hz, H-12), 4.51 (1H, dd, *J* = 10.1, 6.3 Hz, H-3), 2.28 (2H, dt, *J* = 11.3, 7.6 Hz, H-2), 2.04 (2H, s, H-24), 1.13 (3H, s, H-26), 1.11 (3H, s, H-27), 1.07 (2H, s, H-25), 1.01 (2H, s, H-23), 0.98 (2H, s, H-28), 0.92 (3H, s, H-30), 0.91 (1H, s, H-29), 0.89 (3H, s, H-18), 0.80 (3H, s, H-28), 0.79 (4H, d, *J* = 4.2 Hz, H-29), 0.69 (0.25H, d, *J* = 7.6 Hz, probably form minor isomer). The characteristic signals and literature comparison allowed for unambiguous characterisation of the compound as *α*-amyrin acetate [36].

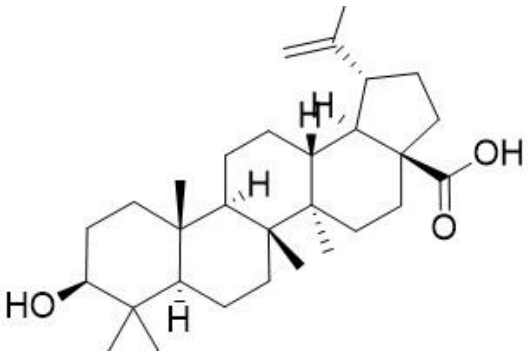

Figure 7. Betulinic acid (ABP 105, 107, 109)

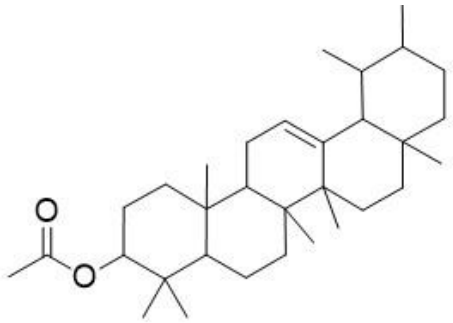

Figure 8. *α*-Amyrin acetate (7). (ABO 39, 41, 43)

Lupeol acetate (**8**) (Fig. 9). (ABO 43, 45): Creamy white gummy powder**.** ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.67 (1H, d, J = 2.5 Hz, H-29a), 4.55 (1H, t, J = 2.0 Hz, H-29b), 4.53–4.41 (1H, m, H-3), 2.35 (1H, td, J = 11.0, 5.9 Hz), 2.0 (3H, s, CH_3COO), 1.90 (1H, td, J = 8.0, 3.7 Hz), 1.66 (3H, s, Me), 1.01 (2H, s, Me), 0.92 (2H, s, Me), 0.86 (1H, s, Me), 0.85 (1H, s, Me), 0.84 (3H, s, Me), 0.83 (3H, s, Me), 0.82 (1H, s, Me), 0.78 (1H, s, Me), 0.77 (3H, s, Me). The characteristic signals and literature comparison allowed for unambiguous characterisation of the compound as lupeol acetate [37].

3.2 Antifungal activities and zone of inhibition of A. boonei stem bark fractions on treated wood fungi

Results of antifungal activities of *A. boonei* stem bark fractions (ABO-42, ABO-44, ABO-69, and ABO-71) were evaluated against wood fungal pathogens, comparing their efficacy to standard antifungal agents sparfloxacin and keteconazole are presented in Table 2.

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Table 2. Antifungal activities and Zone of inhibition of *A. boonei* fractions on treated fungi

S/No.	Test Fungi	*A. boonei* fractions				Standard Antifungal (Control)	
		ABO-42	ABO-44	ABO-69	ABO-71	Sparfloxacin (10 µg/mL)	Keteconazole (10 µg/mL)
		AFA(ZoI)	AFA(ZoI)	AFA(ZoI)	AFA(ZoI)	AFA(ZoI)	AFA(ZoI)
1.	*Aspergillus flavus*	S(27)	S(29)	S(26)	S(25)	R(0)	S(30)
2.	*Aspergillus fumigatus*	R(0)	R(0)	S(24)	S(22)	R(0)	S(25)
3.	*Aspergillus nigre*	R(0)	R(0)	R(0)	R(0)	R(0)	S(27)
4.	*Coniophora puteana*	S(28)	S(26)	S(29)	S(24)	S(26)	R(0)
5.	*Fibroporia vaillantii*	S(26)	S(28)	S(30)	S(27)	R(0)	S(27)
6.	*Fomitopsis pinicola*	S(24)	S(24)	S(28)	S(28)	S(31)	R(0)
7.	*Fusarium* sp	R(0)	R(0)	R(0)	R(0)	R(0)	S(32)
8.	*Rhizopus* spp	R(0)	R(0)	R(0)	R(0)	S(28)	S(30)
9.	*Sclerotium rolfsii*	S(28)	S(26)	S(26)	S(25)	S(25)	S(29)
10.	*Trichoderma sp*	R(0)	R(0)	R(0)	R(0)	R(0)	S(27)
11.	*Serpula lacrymans*	R(0)	R(0)	R(0)	R(0)	R(0)	R(0)

Key: S - Sensitive, R – Resistance; AFA = Antifungal 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 [18].

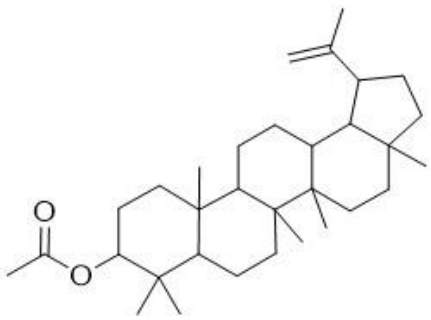

Figure 9. Lupeol acetate (8). (ABO 43, 45)

The results demonstrated that *Aspergillus flavus*, *Coniophora puteana*, *Fibroporia vaillantii*, and *Sclerotium rolfsii* were sensitive to the fractions, with ZoI values ranging from 24 mm to 30 mm. *Aspergillus fumigatus* exhibited sensitivity only to ABO-69 and ABO-71, while *Aspergillus niger*, *Fusarium* sp., *Rhizopus* spp., *Trichoderma* sp., and *Serpula lacrymans* showed resistance to all fractions. The standard antifungal Keteconazole demonstrated effectiveness against *Aspergillus flavus and Aspergillus fumigatus*, with ZoI values of 30 mm and 25 mm, respectively.

3.3 MIC and MFC of A. boonei fractions on treated fungi

Table 3 presents results on the antifungal efficacy (MIC and MFC) of *A. boonei* stem bark fractions (ABO-42, ABO-44, ABO-69, ABO-71) against wood fungi. Results indicated that *Aspergillus flavus, Coniophora puteana, Fibroporia vaillantii, Fomitopsis pinicola,* and *Sclerotium rolfsii* were susceptible to all fractions with MICs ranging from 50 to 100 µg/mL and MFCs ranging from 100 to 200 µg/mL. In contrast, *Aspergillus niger, Fusarium* sp, *Rhizopus* spp, *Trichoderma sp,* and *Serpula lacrymans* were resistant to all fractions tested, showing no inhibition or fungicidal activity. The fraction ABO-69 exhibited the lowest MIC values (50 µg/mL) against *Coniophora puteana* and *Fibroporia vaillantii,* indicating its active potential as a potent antifungal agent.

3.4 Fungicidal activities and zone of inhibition of stem bark A. boonei methanol crude extracts against test fungi

The results of the fungicidal activity of stem bark methanol crude extract *of A. boonei* against eleven fungal strains, comparing the results with standard antibiotics sparfloxacin and keteconazole are presented in Table 4. *A. boonei* methanol stem bark crude extract demonstrated sensitivity (S) with a zone of inhibition (ZoI) ranging from 18 to 21 mm against seven fungal strains, including *Aspergillus flavus* (20 mm), *Aspergillus niger* (19 mm), and *Rhizopus spp* (21 mm). However, it showed resistance (R) against *Aspergillus fumigatus, Coniophora puteana, Fomitopsis pinicola, Tricoderma sp,* and *Serpula lacrymans*. In comparison, Keteconazole displayed higher efficacy with ZoI values up to 32 mm, while Sparfloxacin was effective only against *Coniophora puteana* and *Rhizopus spp*.

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Table 3. MIC and Minimum MFC of *A. boonei* stem bark fractions on treated fungi

S/No.	Test Fungi	ABO-42	ABO-44	ABO-69	ABO-71	ABO-42	ABO-44	ABO-69	ABO-71
		MIC (µg/mL)				MFC (µg/mL)			
1.	*Aspergillus flavus*	100	50	100	100	200	100	200	200
2.	*Aspergillus fumigatus*	R	R	100	100	R	R	200	200
3.	*Aspergillus nigre*	R	R	R	R	R	R	R	R
4.	*Coniophora puteana*	100	100	50	100	200	200	100	200
5.	*Fibroporia vaillantii*	100	100	50	100	200	200	100	200
6.	*Fomitopsis pinicola*	100	100	100	100	200	200	200	200
7.	*Fusarium sp*	R	R	R	R	R	R	R	R
8.	*Rhizopus spp*	R	R	R	R	R	R	R	R
9.	*Sclerotium rolfsii*	100	100	100	100	200	200	200	200
10.	*Trichoderma sp*	R	R	R	R	R	R	R	R
11.	*Serpula lacrymans*	R	R	R	R	R	R	R	R

Table 4. Fungicidal activities and zone of inhibition of stem bark *A. boonei* methanol crude extracts against the test fungi

S/No.	Test Fungi	Crude extract	Standard antibiotics (Control)	
		A. boonei AFA(ZoI)	Sparfloxacin (10 µg/mL) AFA(ZoI)	Keteconazole (10 µg/mL) AFA(ZoI)
1.	*Aspergillus flavus*	S(20)	R(0)	S(30)
2.	*Aspergillus fumigatus*	R(0)	R(0)	S(25)
3.	*Aspergillus nigre*	S(19)	R(0)	S(27)
4.	*Coniophora puteana*	R(0)	S(26)	R(0)
5.	*Fibroporia vaillantii*	S(20)	R(0)	S(27)
6.	*Fomitopsis pinicola*	R(0)	S(31)	R(0)
7.	*Fusarium* sp	S(18)	R(0)	S(32)
8.	*Rhizopus* spp	S(21)	S(28)	S(30)
9.	*Sclerotium rolfsii*	S(19)	S(25)	S(29)
10.	*Tricoderma* sp	R(0)	R(0)	S(27)
11.	*Serpula lacrymans*	R(0)	R(0)	R(0)

Key: S = Sensitive R = Resistance; AFA = Antifungal 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 [18].

3.5 Fungicidal activities and zone of inhibition of stem bark A. boonei methanol stem bark crude extracts against test fungi

Results of antifungal activity (MIC and MFC) of *A. boonei* methanol stem bark crude extracts against eleven wood fungal strains are presented in Table 5. The results revealed that the extracts were effective against *Aspergillus flavus*, *Aspergillus niger*, *Fibroporia vaillantii*, *Fusarium sp*, *Rhizopus spp*, and *Sclerotium rolfsii* with MIC value of 2.5 mg/mL and consistent MFC values of 10 mg/mL. However, the extracts showed resistance against *Aspergillus fumigatus*, *Coniophora puteana*, *Fomitopsis pinicola*, *Trichoderma sp*, and *Serpula lacrymans*, indicating no inhibitory or fungicidal activity.

3.6 Pictorial presentation of cultured wood fungi and the effect of A. boonei stem bark fractions and extract by zone of inhibition

Figs. 10 - 13 show Zones of inhibition of *A. boonei* stem bark fractions on test fungi. Whereas Fig. 14 shows the Zones of inhibition of *A. boonei* stem bark methanol extract on test fungi.

4. Discussion

This study characterized 24-methylcycloartenol, α-amyrin acetate, β-amyrin, stigmasterol, β-sitosterol and phytosterol–fractions from the stem bark of *A. boonei.* Bouvier-Navé *et al.* [8] and Nair [9] reported 24-methylcycloartenol from *Ficus krishnae* which exerts antidiabetic activity and was also found

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Table 5. Minimum inhibitory concentration and minimum fungicidal concentration of *A. boonei* methanol extracts against the test fungi

S/No	Test organism	*A. boonei* methanol crude extracts	
		MIC (mg/mL)	MFC (mg/mL)
1.	*Aspergillus flavus*	2.5	10
2.	*Aspergillus fumigatus*	Resistant	Resistant
3.	*Aspergillus nigre*	5	10
4.	*Coniophora puteana*	Resistant	Resistant
5.	*Fibroporia vaillantii*	2.5	10
6.	*Fomitopsis pinicola*	Resistant	Resistant
7.	*Fusarium sp*	5	10
8.	*Rhizopus spp*	2.5	10
9.	*Sclerotium rolfsii*	5	10
10.	*Trichoderma sp*	Resistant	Resistant
11.	*Serpula lacrymans*	R	R

together in many vegetable oils [10, 11]. Stigmasterol, β-sitosterol, and betulinic acid are naturally occurring compounds found in various plants, each exhibiting distinct biological activities. Stigmasterol and β-sitosterol are phytosterols with structural similarities to cholesterol, while betulinic acid is a pentacyclic triterpenoid. Stigmasterol has been identified by Griebel and Zeier [12] as a significant metabolic

Figure 10. Zone of inhibition of *A. boonei* stem bark fraction (ABO71) of test fungi

Key: 1. *Aspergillus flavus;* 2. *Aspergillus fumigatus;* 3. *Aspergillus nigre;* 4. *Coniophora puteana;* 5. *Fibroporia vaillantii;* 6. *Fomitopsis pinicola;* 7. *Fusarium sp;* 7. *Rhizopus spp;* 8. *Sclerotium rolfsii;* 9. *Tricoderma* sp; 10. *Serpula lacrymans*

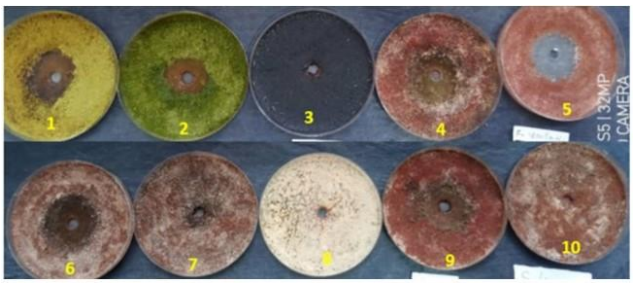

Figure 11. Zone of inhibition of *A. boonei* stem bark fraction (ABO69) of test fungi

Key: 1. *Aspergillus* *flavus;* 2. *Aspergillus fumigatus;* 3. *Aspergillus nigre;* 4. *Coniophora puteana;* 5. *Fibroporia vaillantii;* 6. *Fomitopsis pinicola;* 7. *Fusarium sp;* 7. *Rhizopus spp;* 8. *Sclerotium rolfsii;* 9. *Tricoderma* sp; 10. *Serpula lacrymans*

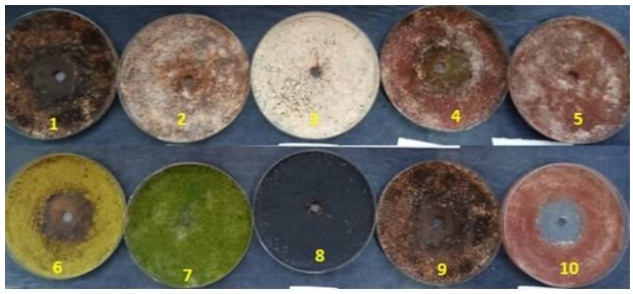

Figure 12. Zone of inhibition of *A. boonei* stem bark fraction (ABO44) of test fungi

Key: 1. *Aspergillus flavus;* 2. *Aspergillus fumigatus;* 3. *Aspergillus nigre;* 4. *Coniophora puteana;* 5. *Fibroporia vaillantii;* 6. *Fomitopsis pinicola;* 7. *Fusarium sp;* 7. *Rhizopus spp;* 8. *Sclerotium rolfsii;* 9. *Tricoderma* sp; 10. *Serpula lacrymans*

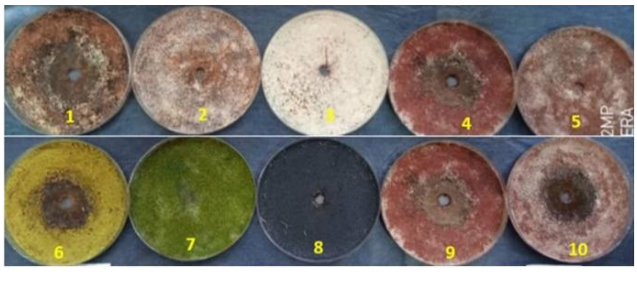

Figure 13. Zone of inhibition of *A. boonei* stem bark fraction (ABO42) of test fungi

Key: 1. *Aspergillus flavus;* 2. *Aspergillus fumigatus;* 3. *Aspergillus nigre;* 4. *Coniophora puteana;* 5. *Fibroporia vaillantii;* 6. *Fomitopsis pinicola;* 7. *Fusarium sp;* 7. *Rhizopus spp;* 8. *Sclerotium rolfsii;* 9. *Tricoderma* sp; 10. *Serpula lacrymans*

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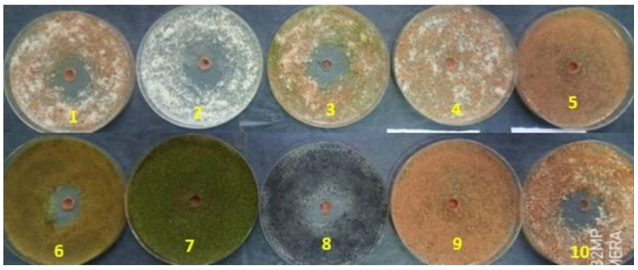

Figure 14. Zone of inhibition of *A. boonei* stem bark methanol crude extract of test fungi
Key: 1. *Aspergillus flavus;* 2. *Aspergillus fumigatus;* 3. *Aspergillus nigre;* 4. *Coniophora puteana;* 5. *Fibroporia vaillantii;* 6. *Fomitopsis pinicola;* 7. *Fusarium* sp; 7. *Rhizopus spp;* 8. *Sclerotium rolfsii;* 9. *Tricoderma* sp; 10. *Serpula lacrymans*

product in plants during pathogen attacks, such as in the *Arabidopsis thaliana -Pseudomonas syringae* interaction, where its accumulation is linked to increased plant susceptibility to pathogens. Conversely, *β*-sitosterol is the most prevalent phytosterol in plants and has been synthesized in pure form to study its oxides' biological impacts [13]. Betulinic acid, isolated from various plant extracts, has been reported for its therapeutic potential, including anti-inflammatory and antitumor activities [14, 15].

While both stigmasterol and *β*-sitosterol are implicated in plant defense mechanisms, their effects on lipid membranes and metabolic processes differ. Griebel and Zeier [12] noted that stigmasterol may promote disease susceptibility in plants by favoring pathogen multiplication, whereas Yoshida and Niki; Hąc-Wydro *et al.* [16, 17] reported that *β*-sitosterol has been shown to stabilize lipid membranes and act as an antioxidant. Chaturvedula *et al.* [12] identified betulinic acid as an inhibitor of DNA polymerase *β*-lyase activity, highlighting its potential in therapeutic applications.

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 24 mm to 30 mm. The values of ZOI obtained in this study were very active test fungi, which agrees with the findings of Guevara [18] who reported ZoI values above 19 mm as being very active. *A. 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 [19] 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 [20] 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 *et al.* [21] 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.

In terms of MIC and MBC, *A. boonei* was effective against a subset of fungi with an MIC of 2.5 mg/mL and MBC of 5 mg/mL, reinforcing its potential but limited antifungal application. This selective efficacy aligns with other findings where plant extracts exhibit significant activity against certain pathogens but lack broad-spectrum effectiveness [22, 23]. The study on *A. boonei* methanol stem bark crude extracts demonstrates notable fungicidal activity against several fungal strains, including *A. flavus* and *Rhizopus spp.*, with a Zone of Inhibition (ZoI) ranging from 18 to 21 mm. Despite this, the extract was less effective than Keteconazole, a standard antifungal, which showed ZoI values up to 32 mm. This suggests that while *A. boonei* exhibits some antifungal properties, it is not as potent as conventional antifungal treatments. Fagbohun [24] reported that methanolic crude extracts of the stem bark of *A. boonei* were tested for antifungal activity at concentrations of 50, 100, 150 and 200 mg/mL against *Aspergillus flavus*, *Absidia corymbifera*, and *Aspergillus niger*. According to Osagie

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[25], the methanol leaf extract of *A. boonei* demonstrated antifungal properties. The ZoIs recorded by Aruhanga [26] for three compounds isolated from the stem bark of *Alstonia boonei* were 12.6 mm, 12.3 mm, and 9.0 mm, while the crude extract showed a ZoI of 13.1 mm against *Candida albicans* and *Aspergillus fumigatus*.

The MIC and MFC results further reinforce this finding. *A. boonei* was effective at MIC values between 2.5 to 25 mg/mL and MFC of 10 mg/mL against specific strains like *Aspergillus niger* and *Fusarium sp.*, but it was ineffective against others, such as *A. fumigatus* and *Trichoderma sp.*, which displayed resistance. These findings are consistent with other studies on plant-based antifungals, which often show selective efficacy but are generally less broad-spectrum compared to synthetic antifungal agents like Keteconazole [27-29]. Osagie [25] reported a concentration (IC_{50}) value of 64.47 µg/mL for radical scavenging activity from methanol leaf extract of *A. boonei.*

5. Conclusions

Motivated by the ethnobotany of *Alstonia boonie*, this investigation has successfully led to the isolation and characterisation of eight compounds: 24-Methylenecycloartenol, β-Amyrin, Cycloeucalenol, Stigmasterol, β-Sitosterol, Betulinic acid, α-Amyrin acetate, and Lupeol acetate. from the stem bark of the plant. *A. boonei* stem bark fractions on test fungi demonstrated that *Aspergillus flavus, Coniophora puteana, Fibroporia vaillantii,* and *Sclerotium rolfsii* were sensitive to the fractions, with ZOIs ranging from 25 - 29 mm. Evidence from the current research shows that solvent extracts of *A. boonei* stem bark have notable antifungal activity. These fractions and extracts hold promise as a novel tool in the fight against drug resistance, a significant worldwide concern. The results of this research provide scientific backing for the traditional application of *A. boonei* by the indigenous community of Okhuesan, Edo State, Nigeria, to combat microbial infections.

Authors' contributions

Designed, coordinated the study and proof read the work, D.O.E; C.E.; Proof read the work, carried out the extraction and antifungal activities and wrote the report, 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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