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# Antimicrobial effect of isolated compound of *Anadelphia afzeliana* (Rendle) Stapf on selected wood fungi and bacteria in Makurdi, Nigeria

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## Abstract

**_Anadelphia afzeliana_ was assessed for antimicrobial activity as an alternative to synthetic chemicals. _A. afzeliana_ was collected from Orkar village, Gboko, Benue State. The specimen was pulverized. Methanol, ethyl acetate, and n-hexane solvents were used for extraction. Each solvent measured 1800 ml was added to 300 g of _A. afzeliana_. The mixture was left to soak for 48 hours. Extract was filtered and allowed to dry by evaporation. Dried extract was mixed with silica gel to run column chromatography. Compounds were analysed by Nuclear magnetic resonance (NMR). Concentrations of 200, 100, 50 and 12.5 µg/m were constituted for antimicrobial sensitive test on ten wood bacteria and nine fungi. NMR showed the presence of stigmasterol ($C_{29}H_{48}$ O). Antifungal test revealed _A. fumigatus, C. puteana, G. sepiarium, P. schweinitzii, Rhizopus_ spp. and _S. rolfsii_ as sensitive to stigmasterol at zones of inhibition (ZOI) of 19, 21, 22, 23, 24 and 20 mm, respectively. Antibacterial showed _A. proteobacteria, B. subtilis, B. proteobacteria, E. faecium, E. coli, P. aeruginosa_ and _P. mirabilis_ sensitive at ZOI of 30, 28, 29, 26, 27, 23, and 23 mm, respectively. Minimum inhibition concentration (MIC) of 25 µg/ml completely inhibited _Alpha proteobacteria, Bacillus subtilis_ and _Beta proteobacteria_ while Minimum Bactericidal Concentration (MBC) of 50 µg/ml completely killed _A. proteobacteria, B. subtilis_, and _B. proteobacteria_. MIC of stigmasterol at 50 µg/ml completely inhibited _Coniophora puteana, Gloeophyllum sepiarium, Phaeolus schweinitzii, Rhizopus_ spp. and _Sclerotium rolfsii_ while at 100 µg/ml of Minimum fungicidal concentration (MFC), _Phaeolus schweinitzii_ and _Rhizopus_ spp. were completely killed. _A. afzeliana_ proved potent in the control of wood fungi and bacteria. MFC and MBC for tested pathogens were observed to be most effective at 50 µg/ml and is therefore recommended as concentration for _A. afzeliana_ compound in the control of fungi and bacteria infections.**

**Keyword:** *Anadelphia afzeliana*, bacteria, fungi, stigmasterol, sensitive, resistant.

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## Introduction

Wood is described a permeable and tough basic tissue located in the roots and stem of trees as well as woody plants. Wood is organic in nature which is composed of complex cellulose and hemicellulose entrenched in an atmosphere of lignin that prevents its compression. According to Jerrold (2005), wood is recognized as a multipurpose material with varied of mechanical and physical properties. It is a natural resource that is renewable with an excellent strength that can be used for a very long time. Although wood is proven to be very usefulness, its service life

can be degraded by various bio-deteriorating agents such as bacterial and fungal infection, termites, insects, marine borer, fire attack and mechanical failure that contribute to its limited selectivity (Areo, 2002).

Fungi are eukaryote, they digest food outwardly and injects the nutrient straight across its cell walls. Majority of fungi replicate by spores and have a body (thallus) that if comprised of microscopic tubular cells referred to as hyphae. Fungi are heterotrophic in nature and they derived the energy and carbon from other organisms like animals (Carris et al., 2012). Fungi has hundreds of species of which some are pathogens or parasites of other organisms and decomposers. Fungi are a vital collection of plant pathogens which cause many plant diseases (Knogge, 1996). Some types of fungi degrade moist wood thereby causing wood rot. Brown rot fungi degrade dead while Armillaria commonly known as honey fungus is a parasite of living trees. Excess moisture in wood that is above the fibre saturation point increases the activities of wood fungal (Harris, 2001). The fungi which grow and penetrate wood fibrous structure causing decay are termed lignicolous fungi.

Bacteria are prokaryotic and known as the most abundant organisms present everywhere. They can attack wood either in anaerobic or aerobic conditions. Although fungi are the major causes of wood decomposition, however, it was also discovered that bacteria attack dead wood. It is now known that there is boundless diversity of bacteria within wood. Nevertheless, in the same environment, fungi are more understood compared to bacteria (Johnston et al., 2016).

Fungi and bacteria are largely controlled by synthetic chemical. It is reported that there are currently over 113 active ingredients listed as fungicides globally (Knight et al., 1997). However, synthetic chemical lead to serious and continuing environmental pollution. They are extremely and intensely toxic (Daoubi et al., 2005). These chemicals are also known to be carcinogenic to wild animals and humans. Besides, pathogens developed resistance to several of these chemicals. Thus, there is an apparent demand to explore alternate compounds that are harmless to animals and environmentally friendly, biodegradable to control bacterial and fungal diseases (Makovitzki et al., 2007).

Researchers are now seeking for alternative non-hazardous and biological methods of control of fungi and bacteria related diseases (Arldo et al.,2009; Femi-Ola et al., 2008). Over a thousand plants species have been stated to have chemicals in leaves, stems, flowers, seeds, and roots which have insecticidal properties. However, only a few of them have been explored for practical disease control on a commercial scale. Biological control agents are potent, biodegradable, environment friendly, economically worthwhile, and socially tolerable method of pest management and disease control. Biological control method is encouraged by humid and warm micro condition favoured by bacteria and fungi (Verma et al., 2009).

*Anadelphia afzeliana* commonly called thatch grass belongs to the family Poaceae. It occurs mainly in the Sudanian zone and from Senegal eastward, southward to DR Congo (Bague, 2011). It is locally dominant near waterholes and in low-lying regularly flooded and poorly drained wetland savanna. In Senegal it is dominant in savannas receiving 900- 1100 mm annual rainfall in a wet season of 5-7 months; in southern Cote d'Ivoire it occurs in the coastal zone in drier savannas (Bague, 2011). *A. afzeliana* is a poor grazing grass but grazed when it is young. It is used as a thatching grass used for roofing and has a history of pesticidal activities. Therefore, this study was aimed at assessing the fungicidal and bactericidal properties of *A. afzeliana* solvent extracts.

## Materials and Methods

### Study area

The research was carried out at the Federal University of Agriculture Makurdi, Benue State, Nigeria. It is located in the Guinea Savannah zone between latitudes $8^0$ 35" E and $8^0$ 41" E longitudes $7^0$ 45" N and $7^0$ 52" N (Jimoh et al., 2009). Makurdi is located in the middle belt of Nigeria and situated sideways of River Benue. As of 2007, Makurdi human population was estimated to be 500,797. The main ethnic groups of Makurdi are in decreasing order of Tiv, Idoma, Igede and Etulo. Land area of Makurdi is 34,059 $km^2$ (13,150 sq. mi). Makurdi vegetation is characteristically of guinea savannah and this in addition to its climate of

favours the production and harvest of agricultural crops (cassava, yam, banana, sweet potatoes, rice, maize) and forest products which mainly timber produce (Mage and Agber 2017).

*Plant collection and preparation*

Fresh *A. afzeliana* was collected from Orkar village near Gando, Mbayion, Gboko LGA, Benue State. *A. afzeliana* was air dried for three weeks and pounded using mortar and pestle as shown in plate 1. The pounded material was ground using a grinding machine to a fine powder.


Plate 1:

A: Dried *A. afzeliana*
B: Pounded *A. afzeliana*

*Method of extraction*

Three solvents were used in the extraction. They include methanol, n-hexane and ethyl acetate. The pounded plant (300 g) was extracted using the various solvents (1800 ml) respectively, for 48 hours with intermittent four hourly shaking. The extract was filtered into clean glass jars using a filter paper (Whatman no. 1) as shown in plate 2, 3 and 4. The extracts were evaporated to dryness in a stream of air as shown in plate 2, 3 and 4. Dried extracts were dissolved using chloroform and pre-absorbed into silica gel (3 g) prior to column chromatography.

*Column chromatography*

A ball of cotton wool was gently dropped into chromatography column and tucked into place using a metal wire. A bigger ball of wool about the diameter of the column (3 cm) was also tucked into the bottom of the column to provide an even base for the silica gel bed. A gel was prepared using (hexane: ethyl acetate 95:5) and silica gel (60 g) and introduced into the column. The column was tapped lightly on the side with a thick rubber nose to release trapped air bubbles. The solvent level was reduced to 2 cm above the column bed before introducing pre-absorbed crude solvent extract. The column was run with solvent mixtures of increasing polarity of hexane and ethyl acetate in ratio of 95%:5% - 0%:100%. The fractions were collected in labeled vials and allowed to dry as shown in Table 2. Dried fractions were on the basis of Tin Layer Chromatography (TLC) similarities. Fraction (Aa26) with clean coolening crystals was sent to Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, Scotland for NMR analysis and Aa25 was sent for anti-microbial screening.

*Nuclear Magnetic Resonance (NMR) spectroscopic analysis*

Nuclear Magnetic Resonance (NMR) analysis was carried out on Aa26. NMR spectrometer (400m$H_z$) spectra acquired were further processed using mestrenora 12 software. It was composed of white needle-shaped crystals. The characterization was done using $^{1}H$ NMR. NMR was carried out in Glasgow, Scotland.

*Antimicrobial Screening*

The anti microbial screening was carried out at Nigerian Institute for Leather Science and Technology (NILEST), Zaria. The antimicrobial activities of Aa25 compound was determined using selected wood pathogens.

*Screening of bacteria*

Screening of compound was done by diffusion method. Antibacterial activities were evaluated by using initial concentration of compounds determined by dissolving 0.002 mg of each compound in 10 ml of DMSO to obtain a concentration of 200 µg/ml. Mueller Hinton agar and Sabouraud Dextrose agar were the media used as the growth media bacteria. Media were prepared in accordance to manufacturer's guidelines. It was purified at 121°C for 15 mins and introduced into germ-free Petri dishes and was left to cool and congeal.

Muller Hinton agar was seeded with 0.1 ml standard inoculums of the test bacteria. The inoculum was expanse equally on the surface of the medium with aid of a disinfected swab. A well at the middle of each injected medium was cut with a typical cork borer of 6 mm in diameter. Solution of 0.1ml of compound of 200 µg/ml concentration was then introduced into the well on the inoculated medium. At a temperature of 37°C and within 24hrs, incubation was made for bacteria after which the plate of the media was viewed for the zone of inhibition of growth. The zone was measured with a translucent meter rule and results documented in millimeters.

*Screening of fungi*

Screening of compound was done by diffusion method. Initial concentration of compounds was determined by dissolving 0.002 mg of the compound in 10 mls of DMSO to obtain a concentration of 200 µg/ml. This was used to assessed its antifungal activities. Mueller Hinton agar and sabouraud dextrose agar were the media used as the growth media for fungi. Media were prepared in accordance to manufacturer's manual and purified at temperature of 121°C for a period of 15mins. Thereafter, it was emptied into germ-free Petri dishes and was left to cool and harden.

Sabouraud Dextrose agar was seeded with a 0.1 ml standard inoculum of the test fungi. The inoculum was uniformly spread on the surface of the medium with aid of a disinfected swab. A typical cork borer measuring 6 mm in diameter was utilized to make cut a well at the middle of each injected medium. Solution of 0.1 ml of compound of 200 µg/ml of concentration was introduced into the well on the inoculated medium. Incubation was made at 30°C for 1-7 days for the fungi after which the plate of the media was observed for the zone of inhibition of growth. The zone was measured with a transparent meter rule and results recorded in millimeters.

*Procedure for Minimum Inhibition Concentration (MIC)*

MIC of compound was determined using the broth dilution method. Muller Hinton broth and Sabouraud dextrose broth were prepared. Ten millilitre was dispensed into test tubes and the broth was sterilized at 121°C for 15mins.The broth was allowed to cool. Mc-farland turbidity scale number 0.5 was made ready to produce turbid solution. Normal saline was prepared while 10 ml was poured into sterilized test tube and the test microbe was inoculated and incubated at the temperature of 37°C for a period of 6 hours for the bacteria and at a temperature of 30°C for a period of 6 hours for the fungi.

Dilution of the test microbes carried out in the standard saline until the turbidity synchronised with the scale of Mc-farland by visual assessment. At this time, the test microbe had a concentration of about $1.5x10^8$ cfu/ml. Two-fold serial dilution of the compound was carried out in the sterilized broth to attain a concentration levels of 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml and 12.5 µg/ml.

Initial concentration was obtained by dissolving 0.00 2mg of the compound in 10 ml of the sterile broth and 0.1 ml of the Microbe in the normal saline was then introduced into the different concentrations. The bacteria were introduced into the Mueller Hinton broth while the fungi were introduced into the Sabouraud Dextrose broth. Incubation was made at 37°C for 24hrs for the bacteria and at 30°C for 1-7 days for the fungi after which the test tubes of the broth were observed for turbidity (growth). The least concentration of the compound in the sterilized broth that indicate no turbidity was documented as the MIC.

*Procedure for Minimum Bacterial Concentration (MBC) and Minimum Fungicidal Concentration (MFC)*

MBC and MFC was done to assess if the test microbes were killed or only their growth was

subdued. Mueller Hinton agar and Sabouraud Dextrose agar were prepared, sterilized at a temperature of 121°C for a period of 15mins, emptied into sterilized Petri dishes and left to cool and congeal. The contents of the MIC in the serial dilution were then sub cultured on to the prepared medium. Bacteria were sub-cultured onto the Mueller Hinton agar and the fungi on Sabouraud Dextrose agar. The bacteria were incubated at 39°Cfor 24hrs while the fungi at 30°C for 1-7 days, after which the plates of the media were observed for colony growth. MBC/MFC were the plates with lowest concentration of the compound without colony growth.

**Result**

Figure1 shows Aa26 spectral. The spectral indicate the presence of stigmasterol ($C_{29}H_{48}O$). $^{1}$H NMR (500 MHz, Chloroform-d) δ 5.35 (d, J = 5.1 Hz, $^{1}$H), 5.15 (dd, J = 15.2, 8.6 Hz, 0H), 5.02 (dd, J = 15.2, 8.5 Hz, 0H), 3.52 (tt, J = 10.8, 4.7 Hz, 1H), 2.34 – 2.18 (m, 2H), 2.10 – 1.95 (m, 3H), 1.92 – 1.78 (m, 5H), 1.50 (ddd, J = 25.4, 17.0, 12.5 Hz, 16H), 1.25 (s, 3H), 1.21 – 1.06 (m, 7H), 1.01 (s, 5H), 0.92 (d, J = 6.1 Hz, 4H), 0.83 (d, J = 4.4 Hz, 3H), 0.81 (s, 3H), 0.79 (s, 2H), 0.78 (d, J = 2.0 Hz, 0H), 0.70 (s, 2H), 0.68 (s, 2H).

Figure 2 shows the structure of stigmasterol ($C_{29}H_{48}O$). Figure 2 indicate stigmasterol numbering, while Figure 3 showcase prominent signals of Aa26 consonant with the literature for stigmasterol.

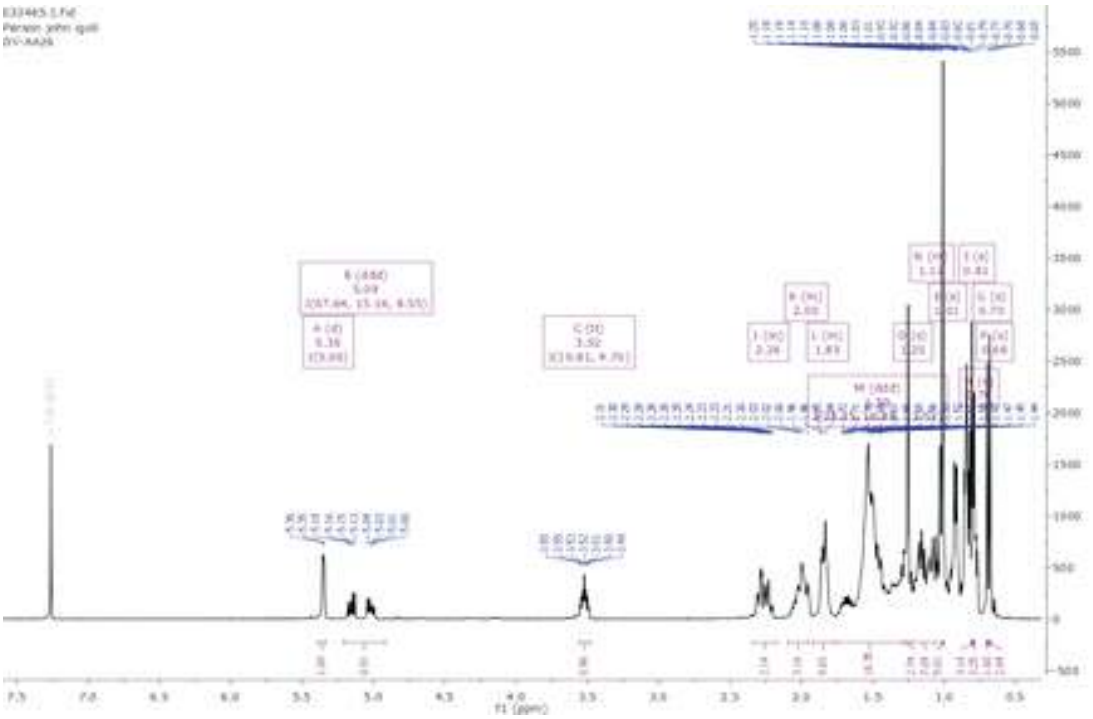

Figure 1: *A. afzeliana* compound (Aa26) spectral

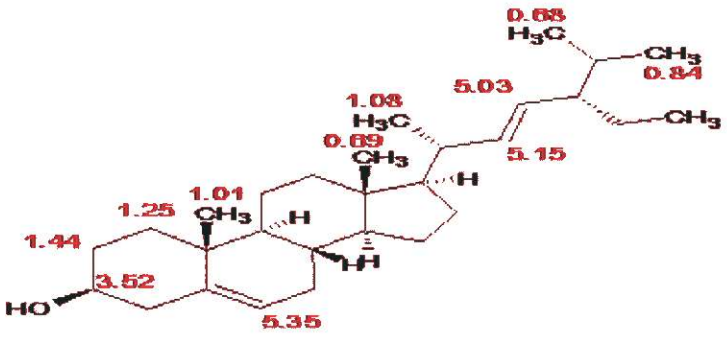


**Figure 2:** Prominent signals of Aa26 consonant with the literature for Stigmasterol

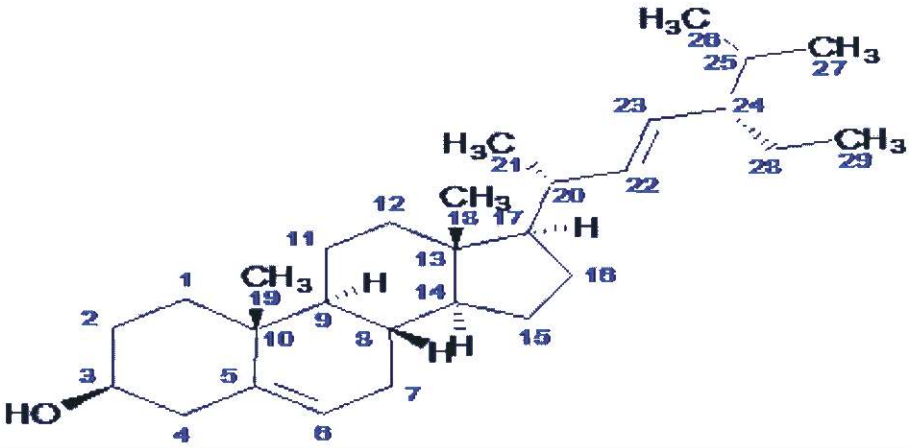

**Figure 3:** Stigma sterol numbering

Table 1 reveals antifungal activities and Zone of Inhibition (ZOI) of standard antibiotics and *A. afzeliana* compound (Aa25). The results indicated that fulcin had the highest (29 mm) ZOI against *Aspergillus fumigatus*, followed by ketoconazole (25 mm), fluconazole (20 mm) and then *A. afzeliana* compound (Aa25) with 19 mm. *Coniophora puteana* was sensitive to fulcin and Aa25 at zones of inhibition of 31 mm and 21 mm, respectively. It is however resistant to keteconazole and fluonazole. *Fibroporia vaillantii* was observed to be resistant to fulcin, keteconazole and Aa25, but sensitive to fluonazole at ZOI of 18 mm. *Fomitopsis pinicoca* was sensitive to fulcin at ZOI of 28 mm but was resistant to keteconazole, fluonazole and Aa25. Keteconazole sensitive to *Gloeophyllum sepiarium* at ZOI of 28 mm followed by Aa25 (25 mm) and fluonazole (19 mm) but showed resistance to fulcin. Fulcin tested against *Phaeolus schweinitzii* was sensitive at ZOI (25 mm) followed by Aa25 (23 mm) but keteconazole and fluonazole showed resistance. *Rhizopus* sp. was sensitive to Fulcin at highest ZOI of 29 mm followed by keteconazole (27 mm), Aa25 (24 mm) and fluonazole (20 mm) respectively. *Serpula lacrymans* was sensitive to Fulcin at highest ZOI of 31 mm, followed by keteconazole (30 mm). Keteconazole tested against *Sclerotium rolfsii* was observed to have the highest zone of inhibition (25 mm) followed by Aa25 (20 mm) while the fungus was resistant to fulcin and fluonazole.

**Table 1:** Antifungal Activities, and Zone of Inhibition of Antibiotics standard and *A. afzeliana* Compound (Aa25)

| S/No. | Pathogens | Fulcin | | Keteconazole | | Fluonazole | | Aa25 | |
|---|---|---|---|---|---|---|---|---|---|
| | | AFA | ZOI (mm) | AFA | ZOI (mm) | AFA | ZOI (mm) | AFA | ZOI (mm) |
| 1 | Aspergillus fumigatus | S | 29 | S | 25 | S | 20 | S | 19 |
| 2 | Coniophora puteana | S | 31 | R | 0 | R | 0 | S | 21 |
| 3 | Fibroporia vaillantii | R | 0 | R | 0 | S | 18 | R | 0 |
| 4 | Fomitopsis pinicoca | S | 28 | R | 0 | R | 0 | R | 0 |
| 5 | Gloeophyllum sepiarium | R | 0 | S | 28 | S | 19 | S | 22 |
| 6 | Phaeolus schweinitzii | S | 25 | R | 0 | R | 0 | S | 23 |
| 7 | Rhizopus sp. | S | 29 | S | 27 | S | 20 | S | 24 |
| 8 | Serpula lacrymans | S | 31 | S | 30 | R | 0 | R | 0 |
| 9 | Sclerotium rolfsii | R | 0 | S | 25 | R | 0 | S | 20 |

**Key:** S: Sensitive, R: Resistant, Aa: *A. afzeliana*, ZOI: Zone of inhibition, AFA: Anti fungi activities

Table 2 shows Minimum inhibition concentration (MIC) of *A. afzeliana* compound against test fungi. The MIC was found to be 50 µg/ml for *Coniophora puteana, Gloeophyllum sepiarium, Phaeolus schweinitzii*, and *Sclerotium rolfsii*. While MIC for *Aspergillus fumigatus* was 100 µg/ml. However, *Fibroporia vaillantii, Fomitopsis pinicoca*, and *Serpula lacrymans* were resistant to all concentration levels of *A. afzeliana* compound.

**Table 2:** Minimum inhibition concentration (MIC) of *A. afzeliana* compound against test

| S/No. | Pathogens | Aa 25 Concentrations (µg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 200 | 100 | 50 | 25 | 12.5 |
| 1 | Aspergillus fumigatus | - | o+ | + | ++ | +++ |
| 2 | Coniophora puteana | - | - | o+ | + | ++ |
| 3 | Fibroporia vaillantii | R | R | R | R | R |
| 4 | Fomitopsis pinicoca | R | R | R | R | R |
| 5 | Gloeophyllum sepiarium | - | - | o+ | + | ++ |
| 6 | Phaeolus schweinitzii | - | - | o+ | + | ++ |
| 7 | Rhizopus sp. | - | - | o+ | + | ++ |
| 8 | Serpula lacrymans | R | R | R | R | R |
| 9 | Sclerotium rolfsii | - | - | o+ | + | ++ |

**Key:** Aa: *A. afzeliana,* **R:** Resistant, -: No turbidity (no growth), **o+:** MIC, **+:** Turbid (Light growth), **++:** Moderate turbidity, +++**:** High turbidity

Table 3 shows Minimum Fungicidal Concentration (MFC) of (Aa25) against test Fungi. The MFC of was observed to be 100 µg/ml for *Phaeolus schweinitzii* and *Rhizopus* sp. whereas the value was 200 µg/ml *Aspergillus fumigatus, Coniophora puteana* and *Sclerotium rolfsii*. Nevertheless, *Fibroporia vaillantii, Fomitopsis pinicoca* and *Serpula lacrymans* pathogens were resistant.

**Table 3:** Minimum fungicidal concentration (MFC) of *A. afzeliana* (Aa25) compound against test Fungi

| S/No. | Test Fungi | Aa 25 Concentrations (µg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 200 | 100 | 50 | 25 | 12.5 |
| 1 | Aspergillus fumigatus | o+ | + | ++ | +++ | ++++ |
| 2 | Coniophora puteana | o+ | + | ++ | +++ | ++++ |
| 3 | Fibroporia vaillantii | R | R | R | R | R |
| 4 | Fomitopsis pinicoca | R | R | R | R | R |
| 5 | Gloeophyllum sepiarium | o+ | + | ++ | +++ | ++++ |
| 6 | Phaeolus schweinitzii | - | o+ | + | ++ | +++ |
| 7 | Rhizopus sp. | - | o+ | + | ++ | +++ |
| 8 | Serpula lacrymans | R | R | R | R | R |
| 9 | Sclerotium rolfsii | o+ | + | ++ | +++ | ++++ |

**Key:**

Aa: *A. afzeliana*; –: No colony growth; **o+:** MFC; **+:** Scanty colonies growth; **++:** Moderate colonies growth); **+++:** Heavy colonies growth; **++++:** Very heavy colonies growth

Table 2 shows Minimum inhibition concentration (MIC) of *A. afzeliana* compound against test fungi. The MIC was found to be 50 µg/ml for *Coniophora puteana, Gloeophyllum sepiarium, Phaeolus schweinitzii*, and *Sclerotium rolfsii*. While MIC for *Aspergillus fumigatus* was 100 µg/ml. However, *Fibroporia vaillantii, Fomitopsis pinicoca*, and *Serpula lacrymans* were resistant to all concentration levels of *A. afzeliana* compound.

**Table 2:** Minimum inhibition concentration (MIC) of *A. afzeliana* compound

| S/No. | Pathogens | Aa 25 Concentrations (µg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 200 | 100 | 50 | 25 | 12.5 |
| 1 | Aspergillus fumigatus | - | o+ | + | ++ | +++ |
| 2 | Coniophora puteana | - | - | o+ | + | ++ |
| 3 | Fibroporia vaillantii | R | R | R | R | R |
| 4 | Fomitopsis pinicoca | R | R | R | R | R |
| 5 | Gloeophyllum sepiarium | - | - | o+ | + | ++ |
| 6 | Phaeolus schweinitzii | - | - | o+ | + | ++ |
| 7 | Rhizopus sp. | - | - | o+ | + | ++ |
| 8 | Serpula lacrymans | R | R | R | R | R |
| 9 | Sclerotium rolfsii | - | - | o+ | + | ++ |

**Key:** Aa: *A. afzeliana*, **R:** Resistant, -: No turbidity (no growth), **o+:** MIC, **+:** Turbid (Light growth), **++:** Moderate turbidity, +++**:** High turbidity

Table 3 shows Minimum Fungicidal Concentration (MFC) of (Aa25) against test Fungi. The MFC of was observed to be 100 µg/ml for *Phaeolus schweinitzii* and *Rhizopus* sp. whereas the value was 200 µg/ml *Aspergillus fumigatus, Coniophora puteana* and *Sclerotium rolfsii*. Nevertheless, *Fibroporia vaillantii, Fomitopsis pinicoca* and *Serpula lacrymans* pathogens were resistant.

**Table 3:** Minimum fungicidal concentration (MFC) of *A. afzeliana* (Aa25) compound against test Fungi

| S/No. | Test Fungi | Aa 25 Concentrations (µg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 200 | 100 | 50 | 25 | 12.5 |
| 1 | Aspergillus fumigatus | o+ | + | ++ | +++ | ++++ |
| 2 | Coniophora puteana | o+ | + | ++ | +++ | ++++ |
| 3 | Fibroporia vaillantii | R | R | R | R | R |
| 4 | Fomitopsis pinicoca | R | R | R | R | R |
| 5 | Gloeophyllum sepiarium | o+ | + | ++ | +++ | ++++ |
| 6 | Phaeolus schweinitzii | - | o+ | + | ++ | +++ |
| 7 | Rhizopus sp. | - | o+ | + | ++ | +++ |
| 8 | Serpula lacrymans | R | R | R | R | R |
| 9 | Sclerotium rolfsii | o+ | + | ++ | +++ | ++++ |

**Key:**

Aa: *A. afzeliana;* –: No colony growth; **o+:** MFC; **+:** Scanty colonies growth; **++:** Moderate colonies growth); **+++:** Heavy colonies growth; **++++:** Very heavy colonies growth

Table shows antibacterial activities, ZOI of antibiotics and *A. afzeliana* compound (Aa25)**.** The results indicate that *Acido bacteria* was resistant to ciproflaxin, cefuroxime, and Aa25, sensitive to sparfloxacin at ZOI of 32 mm. *Actino bacteria* was observed to be resistant to ciproflaxin, cefuroxime, and Aa25 but, sensitive to sparfloxacin at ZOI of 30 mm. *Alpha proteobacteria* was sensitive (32 mm and 30mm)

respectively, but resistant to sparfloxacin. *Bacillus subtilis* was sensitive (28 mm, 35 mm and 28 mm to ciprofloxacin, sparfloxacin and Aa25 respectively, but resistant to cefuroxime. *Beta proteobacteria* was sensitive (10 mm, 30 mm, 31 mm and (29 mm) to ciprofloxacin, sparfloxacin, cefuroxime and Aa25, respectively. *Enterococcus faecium* was sensitive to ciprofloxacin cefuroxime and Aa25 at ZOI of 26 mm, 30 mm, and 26 mm respectively, but resistant to sparfloxacin. *Escherichia coli* was observed to be sensitive to ciprofloxacin, sparfloxacin, cefuroxime and Aa25 at ZOI of 32 mm, 29 mm, 39 mm and 27 mm respectively. Gama proteobacteria was sensitive (31 mm) to sparfloxacin but resistant to ciproflaxacin, cefuroxime, and Aa25. *Pseudomonas aeruginosa* was sensitive (23 mm) to Aa25 but resistant to ciproflaxacin, sparfloxacin, and cefuroxime. *Proteus mirabilis* was sensitive (32 mm, 30 mm, 31 mm and 23 mm) to ciprofloxacin sparfoxacin cefuroxime and Aa25 respectively.

**Table 4:** Antibacterial Activities and Zone of Inhibition of Antibiotics, *A. afzeliana*, (Aa25) Compound

| S/No. | Pathogens | Ciproflaxacin | | Sparfloxacin | | Cefuroxime | | Aa25 | |
|---|---|---|---|---|---|---|---|---|---|
| | | ABA | ZOI(mm) | ABA | ZOI (mm) | ABA | ZOI (mm) | ABA | ZOI (mm) |
| 1 | Acido bacteria | R | 0 | S | 32 | R | 0 | R | 0 |
| 2 | Actino bacteria | R | 0 | S | 30 | R | 0 | R | 0 |
| 3 | Alpha proteobacteria | S | 32 | R | 0 | S | 34 | S | 30 |
| 4 | Bacillus subtilis | S | 28 | S | 35 | R | 0 | S | 28 |
| 5 | Beta proteobacteria | S | 10 | S | 30 | S | 31 | S | 29 |
| 6 | Enterococcus faecium | S | 26 | R | 0 | S | 30 | S | 26 |
| 7 | Escherichia coli | S | 37 | S | 29 | S | 39 | S | 27 |
| 8 | Gama proteobacteria | R | 0 | S | 31 | R | 0 | R | 0 |
| 9 | Pseudomonas aeruginosa | R | 0 | R | 0 | R | 0 | S | 23 |
| 10 | Proteus mirabilis | S | 32 | S | 30 | S | 31 | S | 23 |

**Key:**
S: Sensitive, R: Resistant, Aa25: *A. afzeliana* isolated compound, ZOI: Zone of inhibition, ABA: Anti Bacteria activities

Table 5 shows MIC of Aa25 compound against test pathogens. The results showed that the MIC of *A. afzeliana* compound was 25 µg/ml for *Alpha proteobacteria, Bacillus subtilis, Beta proteobacteria* and *Escherichia coli* while is it 50 µg/ml for *Enterococcus faecium, Pseudomonas aeruginosa*, and *Proteus mirabilis. Acido bacteria, Actino bacteria* and *Gama proteobacteria* pathogens were all resistant.

**Table 5:** Minimum inhibition concentration of *A. afzeliana* (Aa25) compound against Pathogens

| S/No. | Pathogens | Aa25 Concentrations (µg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 200 | 100 | 50 | 25 | 12.5 |
| 1 | Acido bacteria | R | R | R | R | R |
| 2 | Actino bacteria | R | R | R | R | R |
| 3 | Alpha proteobacteria | - | - | - | o+ | + |
| 4 | Bacillus subtilis | - | - | - | o+ | + |
| 5 | Beta proteobacteria | - | - | - | o+ | + |
| 6 | Enterococcus faecium | - | - | o+ | + | ++ |
| 7 | Escherichia coli | - | - | - | o+ | + |
| 8 | Gama proteobacteria | R | R | R | R | R |
| 9 | Pseudomonas aeruginosa | - | - | o+ | + | ++ |
| 10 | Proteus mirabilis | - | - | o+ | + | ++ |

**Key:** Aa: *A. afzeliana*, **R:** Resistant, -: No turbidity (no growth), **o+:** MIC, **+:** Turbid (Light growth), **++:** Moderate turbidity, +++**:** High turbidity

Table 6 shows Minimum Bactericidal Concentration (MBC) of *A. afzeliana* (Aa25) compound against test pathogens. From the result, MBC was 50 µg/ml for *Alpha proteobacteria, Bacillus subtilis*, and *Beta proteobacteria*. It was 100 µg/ml for *Enterococcus faecium* and *Escherichia coli* whereas the value was 200 µg/ml for *Proteus mirabilis*. However, *Acido bacteria, Actino bacteria* and *Gama proteobacteria* pathogens at all levels of concentration

**Table 6:** Minimum Bactericidal concentration of *A. afzeliana* (Aa25) compound against Pathogens

| S/No. | Pathogens | Aa25 Concentrations (µg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 200 | 100 | 50 | 25 | 12.5 |
| 1 | Acido bacteria | R | R | R | R | R |
| 2 | Actino bacteria | R | R | R | R | R |
| 3 | Alpha proteobacteria | - | - | o+ | + | ++ |
| 4 | Bacillus subtilis | - | - | o+ | + | ++ |
| 5 | Beta proteobacteria | - | - | o+ | + | ++ |
| 6 | Enterococcus faecium | - | o+ | + | ++ | +++ |
| 7 | Escherichia coli | - | o+ | + | ++ | +++ |
| 8 | Gama proteobacteria | R | R | R | R | R |
| 9 | Pseudomonas aeruginosa | - | o+ | + | ++ | +++ |
| 10 | Proteus mirabilis | o+ | + | ++ | +++ | ++++ |

**Key:** Aa: *A. afzeliana;* –: No colony growth; **o+:** MFC; **+:** Scanty colonies growth; **++:** Moderate colonies growth); **+++:** Heavy colonies growth; **++++:** Very heavy colonies growth.

## Discussion

*Aa25 signal*

Aa 25 signal areas are consistent with olefinic protons (δH 5.35 ppm, δH 5.15 ppm and at δH 5.03 ppm), an oxymethine proton (δH 3.52 ppm) and methyl protons at δH 0.68, 0.69, 1.01, 0.81, 0.84, and 0.83. The $^{1}$H NMR spectrum of 25 showed that it was similar to that of stigmasterol in the literature (Khatun et al., 2012). A downfield proton signals at δH 5.35 ppm, indicative of olefinic proton (H-6) and olefinic protons at δH 5.15 ppm and at δH 5.03 ppm (H-22 and H-23); a multiplet at δH 3.52 ppm [oxymethine proton (H-3)]. Also, like that for stigmasterol the spectrum of AA25 indicated six methyl protons at δH 0.69 (H-18), 1.01 (H19), 0.84 (H-27), 0.68 (H-26), 1.08 (H-21) and 0.82 (H-29) respectively.

*Antibacterial characteristics of Stigmasterol (Aa26)*

The expanding bacteria resistance to antibiotics has become a growing concern worldwide (Gardam, 2000). Standard antibiotics (ciproflaxacin, sparfloxacin, and cefuroxine) and *A. afzeliana* extract had different anti bacteria activities and zone of inhibition against the test pathogens. Cefuroxime had the highest zone of inhibition followed by ciproflaxin which also showed to be sensitive to *Escherichia coli* whileA. afzeliana extract had a ZOI (23 mm – 30 mm) and was sensitive to *Escherichia coli*. Guevara et al (2005) stated that ZOI > 19 mm is very active. *A. afzeliana* extract had the highest (30 mm) ZOI against *Alpha proteobacteria*, followed by 29 mm on *Beta proteobacteria* which had a higher ZOI compared to ciprofloxacin. *A. afzeliana* extract was more sensitive (23 mm) against *Pseudomonas aeruginosa* compared to standard antibiotics which were all resistant. Yinusa et al., (2014) reported a ZOI of 21 mm –24 mm for stigmasterol (at 50 µg/ml) against *B. subtilis* (24 mm), value above the finding in this study and *E. coli* (21 mm), *Proteus vulgaris* (21 mm), *S. typhimurium* (21 mm), *S. dysenteriae* (21 mm), and *C. albicans* (21 mm), values which are slightly lower than what was obtained in this study. The high ZOI of *A. afzeliana* could be due to the presence of antibacterial activity of stigmasterol content in the plant.

Goins (2017) reported that MIC refers

agent stops noticeable growth of bacteria. In this study, MIC of *A. afzeliana* compound completely inhibited *Alpha proteobacteria, Bacillus subtilis, Beta proteobacteria* and *Escherichia coli* at 25 µg/ml. *Enterococcus faecium, Pseudomonas aeruginosa*, and *Proteus mirabilis* were completely inhibited at 50 µg/ml.

Goins (2017) also described Minimum Bactericidal Concentration (MBC) as the lowest concentration of an antibacterial agent desirable to kill a bacterium over a fixed, slightly extended period, for example 18 hours or 24 hours, below a precise set of conditions. The MBC of A. afzeliana compound at 50 µg/ml completely killed *Alpha proteobacteria, Bacillus subtilis*, and *Beta proteobacteria* while at 100 µg/ml *Enterococcus faecium* and *Escherichia coli* were completely killed. However, *Proteus mirabilis* was completely killed at 200 µg/ml. Shan et al., (2014) noted that MBC indicates the bacterial killing capability of the test substance. The MBC result of this study indicates that *A. afzeliana* extract might represents a potential source of plant antibiotics for the treatment of wood bacterial diseases. This agrees with Tamokou et al., (2011) who stated that such finding supports the traditional use of plant in the treatment of infectious diseases.

*Anti-fungi characteristics of A. afzeliana compound*

Standard antibiotics (fulcin, ketecanazole and fluonazole) and *A. afzeliana* compound had different antifungi activities and zone of inhibition against the tested pathogens. Comparing the standard antibiotics and *A. afzeliana* compound, the highest ZOI was observed with fulcin against *Coniophora puteana* and *Serpula lacrymans*. Anti-fungi activities of *A. afzeliana* compound was observed to be sensitive against *Aspergillus fumigatus*, *Coniophora puteana, Gloeophyllum sepiarium, Phaseolus schweinitzii, Rhizopus* sp., *Serpula lacrymans* and *Sclerotium rolfsii* but was resistence against *Fibroporia vaillantii* and *Fomitopsis pinicoca. A. afzeliana* compound had the highest zone of inhibition on *Rhizopus* sp. Guevara et al., (2005) stated that ZOI >19 mm is very active. Therefore, all ZOI of *A. afzeliana* compound were very active against the test pathogens except for *Fibroporia vaillantii, Fomitopsis pinicoca* and *Serpula lacrymans.*

*Coniophora puteana, Gloeophyllum sepiarium, Phaeolus schweinitzii*, and *Sclerotium rolfsii* were found to be completely inhibited at 50 µg/ml MIC of *A. afzeliana* compound while Aspergillus fumigatus was inhibited at 100 µg/ml. Compounds with MICs less than 100 µg/ml are regarded as having strong antimicrobial property (Tang et al., 2003). This implies that *A. afzeliana* compound was very active against *Coniophora puteana, Gloeophyllum sepiarium, Phaeolus schweinitzii*, and *Sclerotium rolfsii* microbes.

*Aspergillus fumigatus, Coniophora puteana* and *Sclerotium rolfsii* microbes were killed at MFC of 100 µg/ml, whereas *Phaeolus schweinitzii* and *Rhizopus* sp. microbes were observed to be completely killed at MFC 200 µg/ml. The result indicates that *A. afzeliana* compound was more active against bacteria than fungi. This implies that wood bacteria will readily be controlled than wood fungi. Tamokou et al., (2011) reported that antimicrobial activities vary with the bacterial and fungal species and claimed the variations could be due to genetic differences between the microorganisms.

## Conclusion

This study showed that *A. afzeliana* contains stigmasterol ($C_{29}H_{48}O$) with antibiotic properties and controlled some wood fungi (*Aspergillus fumigatus, Coniophora puteana, Fomitopsis pinicoca, Gloeophyllum sepiarium, Phaeolus schweinitzii, Rhizopus* sp. *Sclerotium rolfsii*) and wood bacteria (*Alpha proteobacteria, Bacillus subtilis, Beta proteobacteria, Enterococcus faecium, Pseudomonas aeruginosa Escherichia coli* and *Proteus mirabilis*). Its ZOI on test microbes was >19 mm which makes *A. afzeliana* very active and compete favourably with standard antibiotics. Stigmasterol in *A. afzeliana* was more potent in the control of wood bacteria than fungi. MBC/MFC for all tested pathogens were observed to be most effective at 50µg/ml level of concentration.

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