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Antimicrobial Activities of *Azadirachta indica* Juss Leaves Extracts and Fractions on Selected Wood Fungi and Bacteria

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ABSTRACT
Antimicrobial activities of *A. indica* leaves extracts and fractions on selected wood fungi and bacteria was investigated. Fresh neem leaves were collected washed, air-dried at 37°C for 2 weeks, and finely powdered. Extracts were prepared using2g of leave powder to 1800 ml of solvent for 24-72 hours. Column chromatography was performed on the extracts. Fractions were collected, dried, and analyzed by TLC and NMR. Antimicrobial activity of the methanol extracts and fractions was tested against wood using media.Nonacosane was characterized from AIE12; palmitic acid from AIE26 and Tridecanoic acid from AIE60 fractions. The antibacterial activity of the fractions showed inhibition zones (ZoI) of 22-26 mm against *Bacillus Subtilis*, 20-26 mm against *Pseudomonas aeruginosa,* 25 -30 mm against *Serratia marcescens,* 23-27 mm against *Enterobacter sp*, 24-29 mm against *Rhanella sp*, and 23-30 mm against *Pectobacterium carotovorum,* Sparfloxacin showed ZoI values of 27-31 mm. Also, fractions exhibited antifungal activity with ZoI values from 26 mm (AIE-54 against *Fibroporia vaillantii* and *Rhizopus sp.)* to 29 mm (AIE-52 against *Fomitopsis pinicola* and AIE-54 against *Fusarium sp.),* and MIC values of 50-100 mg/mL and MFC values from 100-200 mg/mL. *A. indica* methanol extract showed fungicidal activity with ZoI values from 19 - 22 mm against *Serpula lacryman, Coniophora puteana, Aspergillus flavu, Fibroporia vaillantii, Rhizopus sp., Tricoderma sp* and *Fusarium* sp. and MIC of 2.5-5 mg/mL and MFC of 10 mg/mL against fungi. These results suggest that *A. indica* leaves have potential as natural antimicrobial agents against a range of bacteria and fungi.

Keywords:
Antimicrobia,
Bacteria,
Compound,
Extract,
Fraction,
Fungi,
Neem leaves.

INTRODUCTION

Azadirachta indica, commonly known as neem, has been extensively studied for its antimicrobial properties. Locally in Nigeria, it is referred to as "Dongoyaro" (Odugbemi *et al*., 2008). The plant is recognized for its therapeutic applications, which are attributed to the presence of phytochemicals such as saponins, steroids, and terpenes (Ujah *et al*., 2021). Neem is also used in the treatment of malaria, as indicated by its inclusion in a list of medicinal plants used for malaria therapy in Okeigbo, Southwest Nigeria (Odugbemi *et al*., 2008). Additionally, a fractionated neem-leaf extract known as IRAB, with reported activities against malaria, HIV/AIDS, and cancer, has been developed into a drug marketed in Nigeria as IRACAP (Anyaehie, 2010).

Research has focused on both seed and leaf extracts, revealing a broad spectrum of antimicrobial activity. Neem seed extracts have demonstrated effectiveness against a range of bacteria and fungi. A study using neem seed extract and a commercially available product showed significant inhibition of bacterial growth, including *Bacillus mycoides*, and fungal pathogens causing 'take-all' and 'snow mould' diseases. Notably, the unformulated seed extract reduced conidial germination of *Sphaerotheca fuliginea* to 11%, indicating strong antifungal activity (Coventry and Allan, 2001). However, major neem metabolites such as azadirachtin, nimbin, and salannin were not identified as the antibacterial agents in this context.

In contrast, neem leaf extracts have also been investigated, with varying ethanol concentrations used to prepare hydroalcoholic extracts. Despite the absence of azadirachtin, these extracts showed antibacterial activity against *Staphylococcus aureus,* although not in a dose-dependent manner (Alves *et al*., 2009). Further studies on leaf extracts have confirmed their

antimicrobial efficacy, with significant activity against various bacterial strains, including multi-drug resistant *Klebsiella pneumoniae*. The activity was found to be concentration and organism-dependent, with Gram-positive bacteria being more susceptible (Abdullah-Al-Emran *et al*., 2011).

Methanol extracts from neem leaves have been reported to exhibit potent antibacterial activity against bacterial strains such as *E. coli* and Salmonella, with effects comparable to the antibiotic gentamicin (Akhter and Sarker, 2019). Additionally, various extracts of neem leaves, including ethanol, methanol, and ethyl acetate, have shown inhibitory effects on pathogenic bacteria, with methanol extract displaying the strongest activity (Maleki *et al*., 2017). Interestingly, silver nanoparticles synthesized from neem leaf extracts have demonstrated antibacterial and anti-quorum sensing activities, particularly against Gram-negative pathogens (Mishra *et al*., 2022).

Comparative studies have also been conducted, with neem leaf extracts showing higher antimicrobial activity against *E. coli* compared to tulsi (*Ocimum sanctum*) extracts, and enhanced effects when both extracts were combined (Kumar *et al*., 2018). Furthermore, aqueous neem leaf extracts have been found to inhibit fungal growth and mycotoxin production *in Penicillium expansum* (Mossini *et al*., 2004).

Despite these studies on neem leaves, not much has been done on the control of wood fungi and bacteria using neem leaves extracts and fractions. This study examined the efficacy of neem leaves extracts and fractions on the control of selected wood fungi and bacteria.

MATERIALS AND METHODS

Study Area

This study was carried out at the termite infested area, located in North core, Joseph Sawuan Tarka University Makurdi. Joseph Sawuan Tarka University Makurdi lies between longitude 8° 21' and 9° E and latitude 7° 21' and 8° N in Benue State, within the southern guinea savanna ecological zone.The climate is characterized by distinct rainy and dry seasons. The mean annual rainfall is between 1200 mm to 1500 mm. The vegetation of the area has been described as Southern guinea savanna (Gyang, 1997). The major occupations of the people are: farming, civil service, trading and hunting; and the major tribes are the *Tiv*, *Idoma* and *Igede.*

Collection of Materials

Fresh leaves of neem tree were collected randomly in the premises of the College of Forestry and Fisheries of Joseph Sawuan Tarka University, Makurdi.

Extraction of neem leaves powder

The leaves were washed under running water, to remove dirt and soil debris and air dried at room temperature 37c to get rid of moisture samples.Two (2) weeks period of ensuring sufficient airflow to avoid damping. The dry leaves were then finely powdered by mortaring. According to (Murktar and Turkur, 2000). Extracts was prepared using ethyl acetate, N-Hexane, and methanol.Two gramms leaf powder was be added to 1800ml of solvent and allowed for the extraction with shaking after 24 hours for 72 hours, followed by filtration and evaporation of solvent and stored at 4ºC.

Plate 1: Fresh leaves of *A. indica*

Distillation of Solvents

Ethyl acetate, N-hexane, and methanol solvents used for extraction were bought from Showcrown Laboratory. Ltd., Ibadan. The solvents were distilled in the laboratory to remove impurity. Distilled solvents were collected and stored in bottles before extraction.

Column Chromatography of*A. indica* extracts

The column was prepared, a ball of cotton wool was gently dropped into the 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 silica

gel bed.Dried extract was mixed with powered silica gel and introduced into the column. A solvent mixture was prepared using (hexane: ethyl acetate 95:5) and silica gel (60 g) and introduced into the column. The column was diluted with solvent mixtures increasing polarity; hexane: ethyl acetate (95%:5% - 0%:100%). The fractions were collected in labeled vials and allowed to dry.Dried fractions were on the basis of TLC similarities. Fractions with clean cooling crystals were sent for Nuclear Magnetic Resonance NMR analysis.

Antimicrobial screening of seeds and leaves extracts and fractions *A. indica*

The antimicrobial activity of *A. indica* methanol extracts and fractions was determined using some plant and animal pathogenic microbes, the microbes were obtained from *Institute for Agricultural Research (IAR)* and Department of Medical Microbiology *Ahmadu Bello University (ABU)* Teaching Hospital Zaria. Mueller Hinton agar and Sabouraud dextrose agar were the media used as the growth media for the microbes.The media were prepared according to the manufacturer instructions sterilized at 121ºCfor 15mins, poured into the sterile petri dishes and was allowed to cool and solidify.The sterilized media were seeded with 0.1ml of the standard inoculum of the test microbe, the inoculum was spread evenly over the surface of the media by the used of sterile swab.

The volume of 0.1 ml of solution of the crude extract of the concentration of 10mg/ml was then introduced into the well on the inoculated media.Incubation was made at 37ºCfor 24hrs for the bacteria and at 30ºC for 1 – 7days for the fungi.Plates of the media were observed for the zone of inhibition of growth, the zone of inhibition was measured with a transparent ruler and the result recorded in millimeter.Two antibacterial: Sparfloxacin (10 µg/ml) and Sparfloxacin (10 µg/ml) were used as control for the bacteria.while antifungal: Keteconazole (10 µg/ml) and Fulcin (10 µg/ml) used as control for fungi.

Determination of Minimum Inhibition Concentration (MIC)

The minimum inhibition concentration of the crude extract was determined using the broth dilution method. Mueller Hinton broth and Sabouraud dextrose broth were prepared 10mls was dispensed into test tubes and was sterilized at 121ºC for 15mins, the broth was allowed to cool.

Mc-Farland's turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline was prepared, 10mls was dispensed into sterile test tube and the test microbe was inoculated and incubated at 37 ºC for 6hours.

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC/MFC was carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar and Sabouraud dextrose agar were prepared sterilized at 121ºC for 15mins, poured into sterile petri dishes and was allowed to cool and solidly. The contents of the MIC in the serial dilutions were then sub cultured onto the prepared media, incubation was made at 37^{0C} for 24hrs for the bacteria and at 30ºC for 1-7days for the fungi. After which the plates of the media were observed for colony growth, MBC/MFC were the plates with lowest concentration of the crude extract without colony growth.

RESULTS AND DISCUSSION

RESULTS

^{1}H-NMRCharacterisation of AIE12 as Nonacosane

AIE12 had the following NMR data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (s, 54H), 0.88 (t, J = 6.6 Hz, 6H). The spectrum was composed of an alkyl region reminiscent of an alkane skeleton. There was a methyl region made of 6 protons [0.88 (t, J = 6.6 Hz, 6H)] and a methylenic one of 54 protons [δ 1.26 (s, 54H)]. The 54 protons correspond to 27 methylenic carbons while the six methyl protons correspond to two methyls making a 29-carbon chain. That long chain alkane is nonacosane (Figure 1).

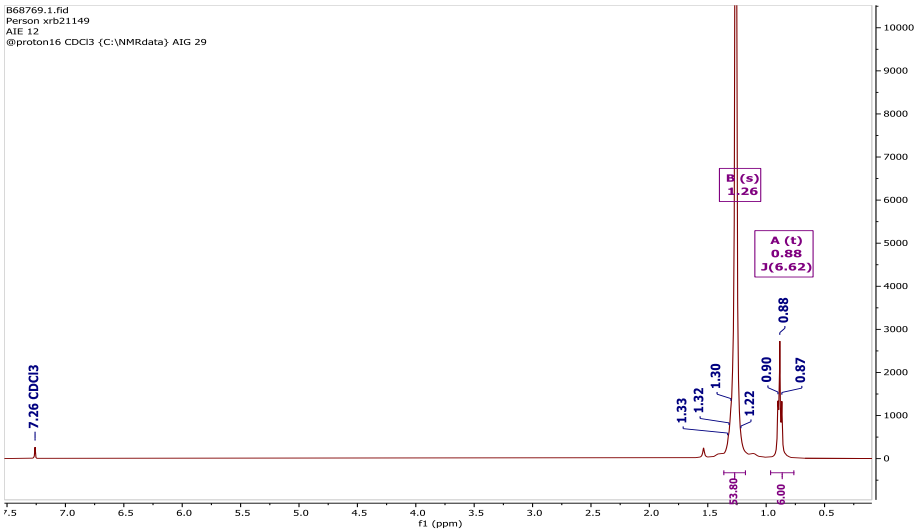

Figure 1 (a): HNMR Spectrum of AIE12

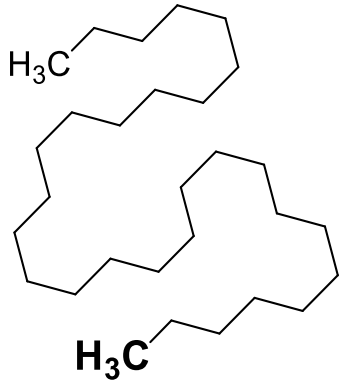

Figure 1 (b): Structure of Nonacosane

^{1}H-NMRCharacterisation of AIE26 as Palmitic acid

AIE26 had the following NMR data:^{1}H NMR (400 MHz, $CDCl_3$) δ 2.07 – 1.99 (m, 1H), 1.69 – 1.53 (m, 3H), 1.37 – 1.21 (m, 24H), 0.90 – 0.88 (t, 3H). The spectrum was composed of an alkyl region split into methylic protons, undisturbed methylenes and disturbed methylenes shifted in a manner reminiscent of fatty acid proton spectra. The methyl region was made of 3 protons [0.88 (t, 3H)] and an undisturbed methylenic one of 24 protons [δ 1.21-1.26 (m, 24H)]; the two disturbed signal areas were consistent with H-2 and H-3 fatty acid proton signals. The 22 protons correspond to 12 methylenic carbons while the 3 methyl protons correspond to a methylgroup with the H-2, H-3 and carbonyl making a 16-carbon chain. Put together, the spectrum is consistent with that for palmitic acid (Figure 2).

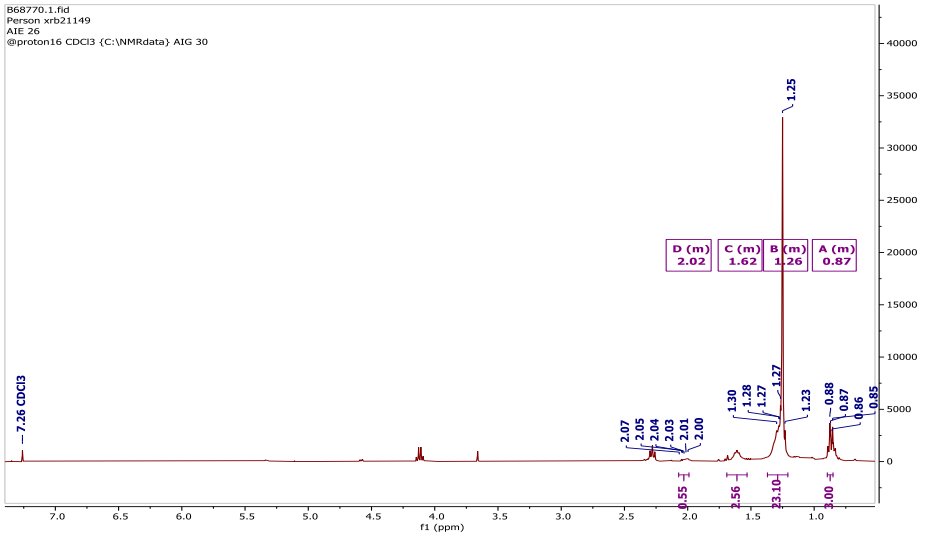

Figure 2 (a): HNMR Spectrum of AIE26

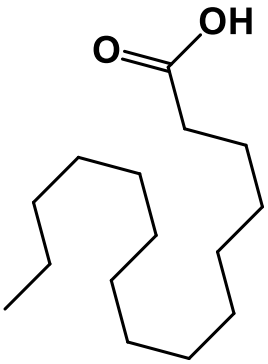

Figure 2(b): Structure of Palmitic acid

^{1}H-NMRCharacterisation of AIE60 as Tridecanoic acid

AIE60 had the following NMR data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33 (dt, J = 15.3, 7.8 Hz, 2H), 1.63 (p, J = 7.3 Hz, 2H), 1.25 (s, 18H), 0.87 (q, J = 5.8 Hz, 3H). The spectrum was composed of an alkyl region split into methylic protons, undisturbed methylenes and disturbed methylenes shifted in a manner reminiscent of fatty acid proton spectra. The methyl region was made of 3 protons [0.88 (t, 3H)] and an undisturbed methylenic one of 18 protons [δ 1.21-1.26 (m, 18H)]; the two disturbed signal areas were consistent with H-2 and H-3 fatty acid proton signals. The 18 protons correspond to 9 methylenic carbons while the 3 methyl protons correspond to a methyl group with the H-2, H-3 and carbonyl making a 13-carbon chain. Put together, the spectrum is consistent with that for Tridecanoic acid (Figure 3).

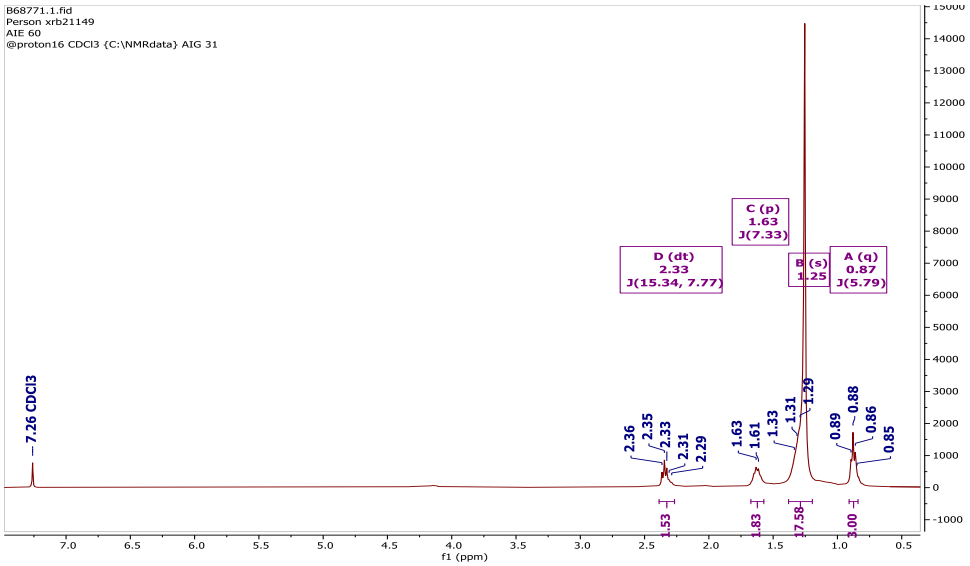

Figure 3(a): HNMR Spectrum of AIE60

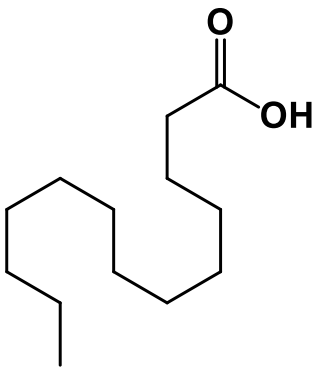

Figure 3(b): Structure of Tridecanoic acid

Bacterial activities and zone of inhibition *A. indica*leaves Fractions (AIE-51, AIE-52, AIE-53 and AIE-54) against test bacteria

Table 1 presents antibacterial activity of different fractions of *A. indica* leaves against a range of bacteria. The antibacterial activity was compared with the standard antibiotics Sparfloxacin and Ketoconazole. The results, as presented in the table, show that the fractions of *A. indica* leaves exhibited varying levels of antibacterial activity against the test bacteria. The data indicate that the fractions were sensitive against several bacteria, including *Bacillus Subtilis* (22-32 mm), *Pseudomonas aeruginosa* (21-30 mm), *Serratia marcescens* (25-30 mm), *Enterobacter sp* (25-30 mm), *Rhanella sp* (24-28 mm), and *Pectobacterium carotovorum* (23-31 mm). In contrast, they were resistant against *Streptococcus salvarius* (0 mm), *Erwinia carotovora* (0 mm), and *Pseudomonas contexa* (0 mm). The antibacterial activity of Sparfloxacin and Ketoconazole was also evaluated. Sparfloxacin showed significant antibacterial activity against most of the test bacteria, with inhibition zones ranging from 27 to 32 mm. Ketoconazole exhibited moderate activity, with inhibition zones ranging from 27 to 30 mm.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the fractions against the test bacteria

Table 2 shows the antibacterial activity of different fractions against various bacteria, determining the MIC and MBC for each fraction against the test bacteria. The results indicate that the fractions exhibited varying levels of antibacterial activity, with MIC values ranging from 50 mg/mL (*Pectobacterium carotovorum*) to 100 mg/mL (*Bacillus Subtilis, Pseudomonas aeruginosa, Enterobacter sp*, and *Rhanella sp*), and MBC values ranging from 100 mg/mL (*Serratia marcescens* and *Pectobacterium carotovorum*) to 200 mg/mL (*Bacillus Subtilis, Pseudomonas aeruginosa,* and *Enterobacter sp*). The fractions were resistant against *Streptococcus Salvarius, Erwinia carratovora, Pseudomonas contexa*, and *Klebsiella sp*.

Table 1: Bacterial activities and zone of inhibition *A. indica*leaves Fractions (AIE-51, AIE-52, AIE-53 and AIE-54) against test bacteria

S/No.	Test bacteria	*A. indica*leaves Fractions								Antibacterial (Control)			
		AIE-51		AIE-52		AIE-53		AIE-54		Sparfloxacin (10 µg/ml)		Ketoconazole (10 µg/ml)	
		ABA	ZoI	ABA	ZoI	ABA	ZoI	ABA	ZoI	ABA	ZoI	ABA	ZoI
1.	*Bacillus Subtilis*	S	22	S	25	S	23	S	26	R	0	S	32
2.	*Pseudomonas aeruginosa*	S	21	S	20	S	26	S	24	R	0	S	30
3.	*Streptococcus salvarius*	R	0	R	0	R	0	R	0	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	27	S	25	S	30	S	26	R	0
6.	*Pseudomonas contexa*	R	0	R	0	R	0	R	0	R	0	S	27
7.	*Enterobacter sp*	S	25	S	23	S	27	S	26	S	30	R	0
8.	*Rhanella sp*	S	24	S	28	S	25	S	29	S	28	R	0
9.	*Klebsiella sp*	R	0	R	0	R	0	R	0	R	0	S	27
10.	*Pectobacterium carotovorum*	S	23	S	26	S	29	S	30	S	31	R	0

Key:S = Sensitive R = Resistance; 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. (Guevara, 2005)

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the fractions against the test bacteria

S/No.	Test bacteria	Minimum Inhibitory Concentration (MIC)				Minimum Bactericidal Concentration (MBC)			
		AIE-51 mg/mL	AIE-52 mg/mL	AIE-53 mg/mL	AIE-54 mg/mL	AIE-51 mg/mL	AIE-52 mg/mL	AIE-53 mg/mL	AIE-54 mg/mL
1.	*Bacillus Subtilis*	100	100	100	100	400	200	200	200
2.	*Pseudomonas aeruginosa*	100	100	100	100	400	400	200	200
3.	*Streptococcus Salvarius*	R	R	R	R	R	R	R	R
4.	*Erwinia carratovora*	R	R	R	R	R	R	R	R
5.	*Serratia marcescens*	100	100	100	50	200	200	200	100
6.	*Pseudomonas contexa*	R	R	R	R	R	R	R	R
7.	*Enterobacter sp*	100	100	100	100	200	200	200	200
8.	*Rhanella sp*	100	100	100	50	200	200	200	100
9.	*Klebsiella sp*	R	R	R	R	R	R	R	R
10.	*Pectobacterium carotovorum*	100	100	50	50	200	200	100	100

Key: R = *Resistance*

Antibacterial activities and Zone of Inhibition of *A. indica*leaves methanol crude extracts against test bacteria

In Table 3, results of antibacterial activity of *A. indica* leaves methanol crude extract against selected wood bacteria are presented. The results indicate that the extracts exhibited varying levels of antibacterial activity, with the highest Zone of Inhibition (ZoI) values of 32 mm *against Bacillus subtilis,* 31 mm against *Pectobacterium carotovorum,* and 30 mm against *Pseudomonas aeruginosa* and *Enterobacter sp.A. indica* methanol leave extract was sensitive against *Bacillus subtilis* (18 mm), *Pseudomonas aeruginosa* (19 mm), *Serratia marcescens* (20 mm), *Enterobacter sp* (21 mm), and *Pectobacterium carotovorum* (20 mm), while they were resistant against *Streptococcus salvarius, Erwinia carotovora, Pseudomonas contexa,* and *Klebsiella sp.* These resistant bacteria were controlled by standard antibacterial.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *A. indica*leavesmethanol crude extracts against the test bacteria

Results of antibacterial activity of *A. indica* leaves methanol crude extracts against various bacteria, determining the MIC and MBC for each fraction against the test bacteria are shown in Table 4. The results indicate that the extract exhibited different levels of antibacterial activity, with MIC values ranging from 2.5 mg/mL (*Serratia marcescens* and *Pectobacterium carotovorum*) to 5 mg/mL (*Bacillus subtilis, Pseudomonas aeruginosa,* and *Rhanella sp*), and MBC values ranging from 10 mg/mL (*Bacillus subtilis, Pseudomonas aeruginosa, and Rhanella sp*) to 10 mg/mL (*Serratia marcescens and Pectobacterium carotovorum*). The extract was resistant against *Streptococcus salvarius, Erwinia carotovora, Pseudomonas contexa,* and *Klebsiella sp*.

Table 3: Antibacterial activities and Zone of Inhibition of *A. indica*leaves methanol crude extracts against test bacteria

S/No.	Test bacteria	*A. indica*leaves methanol crude extract		Standard Antibacterial			
				Sparfloxacin (10 µg/ml)		Keteconazole (10 µg/ml)	
		ABA	ZoI	ABA	ZoI	ABA	ZoI
1.	*Bacillus subtilis*	S	18	R	0	S	32
2.	*Pseudomonas aeruginosa*	S	19	R	0	S	30
3.	*Streptococcus salvarius*	R	0	S	27	S	29
4.	*Erwinia carotovora*	R	0	S	30	R	0
5.	*Serratia marcescens*	S	20	S	26	R	0
6.	*Pseudomonas contexa*	R	0	R	0	S	27
7.	*Enterobacter sp*	S	21	S	30	R	0
8.	*Rhanella sp*	S	18	S	28	R	0
9.	*Klebsiella sp*	R	0	R	0	S	27
10.	*Pectobacterium carotovorum*	S	20	S	31	R	0

Key: S = Sensitive R = Resistance; 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. (Guevara, 2005)

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *A. indica*leavesmethanol crude extracts against the test bacteria

S/No	Test bacteria	Minimum Inhibitory Concentration (MIC)	Minimum Bactericidal Concentration (MBC)
		mg/mL	mg/mL
1.	*Bacillus subtilis*	5	10
2.	*Pseudomonas aeruginosa*	5	10
3.	*Streptococcus salvarius*	R	R
4.	*Erwinia carotovora*	R	R
5.	*Serratia marcescens*	2.5	10
6.	*Pseudomonas contexa*	R	R
7.	*Enterobacter sp*	2.5	10
8.	*Rhanella sp*	5	10
9.	*Klebsiella sp*	R	R
10.	*Pectobacterium carotovorum*	2.5	10

Key: R = Resistance

Antifungal activities and Zone of inhibition of *A. indica*leaves Fractions (AIE-51, AIE-52, AIE-53 and AIE-54) on treated fungi

Table 5 presents antifungal activity of *A. indica* leaves fractions (AIE-51, AIE-52, AIE-53, and AIE-54) against selected wood fungi. The results indicate that the fractions exhibited antifungal activity, with the highest Zone of Inhibition (ZoI) values ranging from 30 mm (AIE-54 against *Fibroporia vaillantii* and *Rhizopus sp*.) to 29 mm (AIE-52 against *Fomitopsis pinicola* and AIE-54 against *Fusarium sp.*). The AIE-52 fraction exhibited the highest ZoI values overall, with 29 mm against *Fomitopsis pinicola* and 28 mm against *Fibroporia vaillantii* and *Rhizopus sp.* The AIE-54 fraction also showed strong antifungal activity, with ZoI values of 29 mm against *Fusarium sp.* and 28 mm against Aspergillus flavus. In contrast, the AIE-51 and AIE-53 fractions generally had lower ZoI values compared to AIE-52 and AIE-54. *Aspergillus fumigatus, Aspergillus nigre, Coniophora puteana, Sclerotium rolfsii,* and *Trichoderma sp.* were resistant to *A. indica* fractions.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *A, indica*leaves Fractions against the test fungi

Table 6 present the antifungal activity of *A. indica* leaves fractions (AIE-51, AIE-52, AIE-53, and AIE-54) against selected wood fungi. The results indicate that the fractions exhibited antifungal activity, with the lowest MIC values ranging from 50 mg/mL to 100 mg/mL. The most sensitive fungi to the fractions were *Fomitopsis pinicola, Fusarium sp* and *Fibroporia vaillantii,* with MIC values as low as 50 mg/mL for the AIE-52, AIE-53 and AIE-54 fractions. In comparison, the MIC values for *Aspergillus flavus, Fibroporia vaillantii, Rhizopus sp.,* and *Serpula lacrymans* were consistently 100 mg/mL across the different fractions. The MFC values ranged from 100 mg/mL to 200 mg/mL. The AIE-52, AIE-53 and AIE-54 fractions exhibited the lowest MFC values of 100 mg/mL against *Fibroporia vaillantii, Fusarium sp*and *Fomitopsis pinicola*, respectively. In contrast, the other fractions and test fungi generally had MFC values of 200 mg/mL. The results suggest that the *A. indica* leaves fractions, particularly AIE-52, AIE-52 and AIE-54, have potential as natural antifungal agents against certain fungal species.

Table 5: Antifungal activities and Zone of inhibition of *A. indica*leaves Fractions (AIE-51, AIE-52, AIE-53 and AIE-54) on treated fungi

S/No.	Test fungi	*A. induce*leaves Fractions								Standard Antifungal (Control)			
		AIE-51		AIE-52		AIE-53		AIE-54		Keteconazole (10µg/mL)		Fulcin (10µg/mL)	
		ABA	ZoI	ABA	ZoI	ABA	ZoI	ABA	ZoI	ABA	ZoI	ABA	ZoI
1.	*Aspergillus flavus*	S	25	S	27	S	26	S	28	R	0	S	30
2.	*Fibroporia vaillantii*	S	25	S	28	S	30	S	26	R	0	S	27
3.	*Rhizopus sp*	S	25	S	28	S	24	S	26	S	28	S	30
4.	*Fomitopsis pinicola*	S	24	S	29	S	26	S	24	S	31	R	0
5.	*Fusarium sp*	S	24	S	26	S	28	S	29	R	0	S	32
6.	*Serpula lacrymans*	S	23	S	25	S	23	S	27	R	0	R	0
7.	*Aspergillus fumigatus*	R	0	R	0	R	0	R	0	R	0	S	25
8.	*Aspergillus nigre*	R	0	R	0	R	0	R	0	R	0	S	27
9.	*Coniophora puteana*	R	0	R	0	R	0	R	0	S	26	R	0
10.	*Sclerotium rolfsii*	R	0	R	0	R	0	R	0	S	25	S	29
11.	*Trichoderma sp*	R	0	R	0	R	0	R	0	R	0	S	27

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. (Guevara, 2005)

Table 6: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *A. indica*leaves Fractions against the test fungi

S/No.	Test fungi	Minimum Inhibitory Concentration (MIC)				Minimum Fungicidal Concentration (MFC)			
		AIE-51 mg/mL	AIE-52 mg/mL	AIE-53 mg/mL	AIE-54 mg/mL	AIE-51 mg/mL	AIE-52 mg/mL	AIE-53 mg/mL	AIE-54 mg/mL
1.	*Aspergillus flavus*	100	100	100	100	200	200	200	200
2.	*Fibroporia vaillantii*	100	100	50	100	200	200	100	200
3.	*Fomitopsis pinicola*	100	50	100	100	200	100	200	200
4.	*Fusarium sp*	100	100	100	50	200	200	200	100
5.	*Rhizopus sp*	100	100	100	100	200	200	200	200
6.	*Serpula lacrymans*	100	100	100	100	200	200	200	200
7.	*Aspergillus fumigatus*	R	R	R	R	R	R	R	R
8.	*Aspergillus nigre*	R	R	R	R	R	R	R	R
9.	*Coniophora puteana*	R	R	R	R	R	R	R	R
10.	*Sclerotium rolfsii*	R	R	R	R	R	R	R	R
11.	*Trichoderma sp*	R	R	R	R	R	R	R	R

Key: *R:Resistant*

Fungicidal activities and zone of inhibition of *A. indica*leaves methanol crude extract against test fungi

Table 7 presents the fungicidal activity of *A. indica* leaves methanol crude extract and compared it with the standard antifungal agents Ketoconazole and Fulcin. The results indicate that the extract displayed variable levels of fungicidal activity, with the highest Zone of Inhibition (ZoI) values ranging from 32 mm against *Fusarium sp.* for Fulcin to 19 mm against *Serpula lacrymans* for the extract. The extract was sensitive against *Fusarium sp.* (22 mm), *Coniophora puteana* (21 mm), *Aspergillus flavus*, *Fibroporia vaillantii*, *Rhizopus sp*. and *Tricoderma sp.* at ZoI of 20 mm, respectively. In comparison, Ketoconazole showed sensitivity against *Coniophora puteana* (26 mm), *Rhizopus sp.* (28 mm), *Fomitopsis pinicola* (31 mm), and *Sclerotium rolfsii* (25 mm), while Fulcin was sensitive against *Fusarium sp.* (32 mm), *Aspergillus flavus* (30 mm), *Rhizopus sp.* (30 mm), and *Sclerotium rolfsii* (29 mm).The extract was resistant against *Aspergillus fumigatus, Aspergillus nigre, Fomitopsis pinicola,* and *Sclerotium rolfsii,* while Ketoconazole and Fulcin showed resistance against some of the test fungi as well. These results suggest that the *A. indica* leaves methanol crude extract has potential as a natural fungicidal agent, particularly against *Fusarium sp., Coniophora puteana, and Rhizopus sp.*

Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of the *A. indica*leavesmethanol extract against test fungi

In Table 8, the MIC and MFC results *A. indica* leaves methanol extract against various fungi are presented. The results indicate antifungal activity of extract, with the lowest MIC values of 2.5 mg/mL against *Aspergillus flavus, Aspergillus fumigatus, Aspergillus nigre, Coniophora puteana, Fibroporia vaillantii, Fusarium sp, Rhizopus sp, Trichoderma sp,* and *Serpula lacrymans.* The highest MIC value was 5 mg/mL against *Serpula lacrymans.* The MFC value was 10 mg/mL against *Aspergillus flavus, Aspergillus fumigatus, Aspergillus nigre, Coniophora puteana, Fibroporia vaillantii, Fusarium sp, Rhizopus sp, Trichoderma sp,* and *Serpula lacrymans*. However, the extract was resistant against *Fomitopsis pinicola* and *Sclerotium rolfsii*.

Table 7: Fungicidal activities and zone of inhibition of *A. indica*leaves methanol crude extract against test fungi

S/No.	Test fungi	*A. indica*leaves methanol crude extract		Standard Antifungal (Control)			
				Keteconazole (10µg/ml)		Fulcin (10µg/ml)	
		ABA	ZoI	ABA	ZoI	ABA	ZoI
1.	*Fusarium sp*	S	22	R	0	S	32
2.	*Coniophora puteana*	S	21	S	26	R	0
3.	*Aspergillus flavus*	S	20	R	0	S	30
4.	*Fibroporia vaillantii*	S	20	R	0	S	27
5.	*Rhizopus sp*	S	20	S	28	S	30
6.	*Tricoderma sp*	S	20	R	0	S	27
7.	*Serpula lacrymans*	S	19	R	0	R	0
8.	*Aspergillus fumigatus*	R	0	R	0	S	25
9.	*Aspergillus nigre*	R	0	R	0	S	27
10.	*Fomitopsis pinicola*	R	0	S	31	R	0
11.	*Sclerotium rolfsii*	R	0	S	25	S	29

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. (Guevara, 2005)*

Table 8: Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of the *A. indica* leaves methanol extract against test fungi

S/No	Test fungi	Minimum Inhibitory Concentration (MIC)	Minimum Fungicidal Concentration (MFC)
		mg/mL	mg/mL
1.	*Aspergillus flavus*	2.5	10
2.	*Aspergillus fumigatus*	Resistant	Resistant
3.	*Aspergillus nigre*	Resistant	Resistant
4.	*Coniophora puteana*	2.5	10
5.	*Fibroporia vaillantii*	2.5	10
6.	*Fomitopsis pinicola*	Resistant	Resistant
7.	*Fusarium sp*	2.5	10
8.	*Rhizopus sp*	2.5	10
9.	*Sclerotium rolfsii*	Resistant	Resistant
10.	*Trichoderma sp*	2.5	10
11.	*Serpula lacrymans*	5	10

Plates 2-11 show the cultured wood fungi and bacteria on Petri dish and the zone of inhibition of*A. indica* leaves fractions extracts

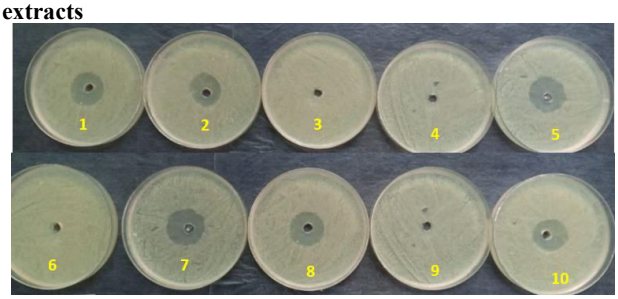

Plate 2: Zone of inhibition of *A. indica* leaves(AIE-51 Fraction)on plates of test bacteria

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*

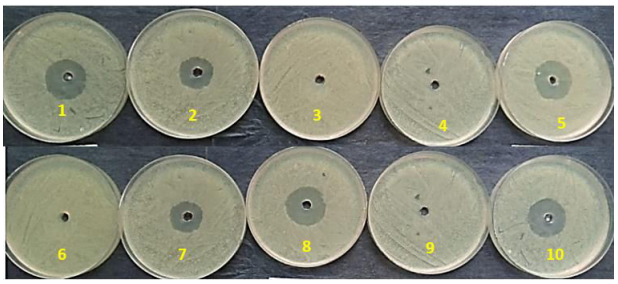

Plate 3: Zone of inhibition of *A. indica* leaves(AIE-52 Fraction)on plates of test bacteria

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*

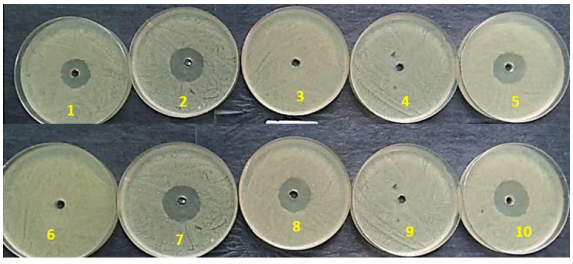

Plate 4: Zone of inhibition of *A. indica* leaves(AIE-53 Fraction)on plates of test bacteria

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*

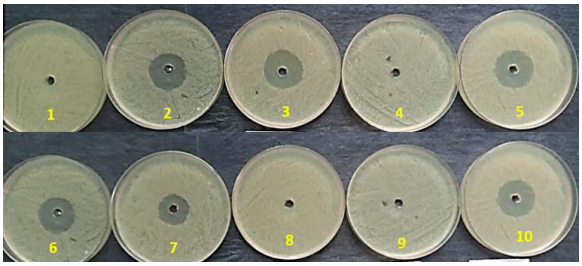

Plate 5: Zone of inhibition of *A. indica* leaves(AIE-54 Fraction)on plates of test bacteria

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*

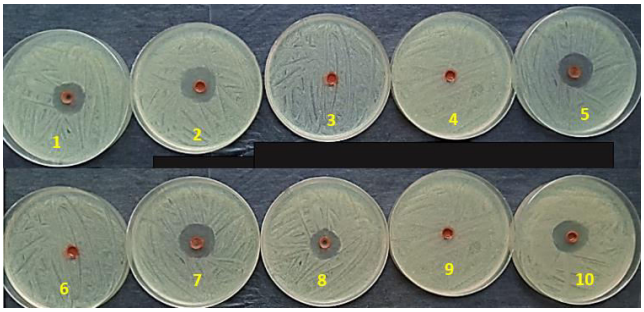

Plate 6: Zone of inhibition of *A. indica* leavesmethanol extract on plates test bacteria

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*

Plate 7: Zone of inhibition of *A. indica* leaves(AIE-54 Fraction)on plates 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*

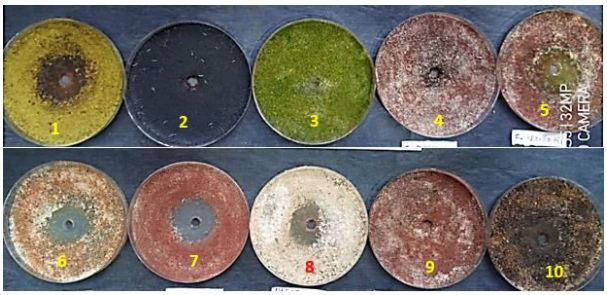

Plate 8: Zone of inhibition of *A. indica* leaves(AIE-53 Fraction)on plates of test fungi

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*

Plate 9: Zone of inhibition of *A. indica* leaves(AIE-52 Fraction)on plates 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*

Plate 10: Zone of inhibition of *A. indica* leaves(AIE-51 Fraction)on plates 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*

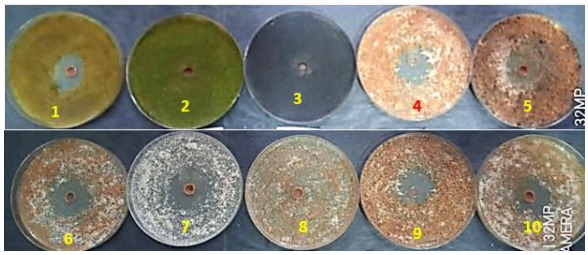

Plate 11: Zone of inhibition of *A. indica* leavesmethanol extract on plates test fungi

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*

Characterisation of *A. indica* leaves fractions (AIE12, AIE26 and AIE60)

From this study a long chain alkane is nonacosane was characterized from AIE12 fraction; palmitic acid from AIE26 and Tridecanoic acid from AIE60 fraction. Nonacosane, palmitic acid, and tridecanoic acid are significant compounds found in plants. Zhukov (2015) reported that Nonacosane, a long-chain hydrocarbon, is commonly present in plant cuticles and serves as a protective barrier. Adolf (1986) noted that nonacosane, a compound found in plants, can play a role in protecting cultivated plants against harmful effects of

aggressive agricultural chemicals. According to Ranson (1955) palmitic acid is a saturated fatty acid that is abundant in various plant lipids, especially in high-polar fractions like mitochondrial membranes, with levels increasing under cold stress. Deineka and Deineka (2004) noted that tridecanoic acid, also known as tridecyl alcohol was the distribution of fatty acids in plant triglycerides and predominant esterification of specific fatty acids like palmitic acid and stearic acid at primary positions. Plants utilize nonacosane for protection through various mechanisms. These compounds play essential roles in plant physiology and biochemistry, contributing to structural integrity, energy storage, and stress responses.

Effect of *A. indica* leaves extract and fractions on test wood bacteria

Nonacosane, a nonacosane derivative, has been reported to exhibit antimicrobial properties against various microorganisms. For example, a study published by Itelima *et al.,* (2016) found that nonacosane exhibited significant antimicrobial activity against several bacteria, including *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* which agrees with the finding from this study. Palmitic acid and are fatty acid found in *A. indica* leaves, has been reported to exhibit antimicrobial properties. For instance, a study published Itelima *et al.,* (2016) found that both acids exhibited significant antimicrobial activity against several bacteria, including *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.* The antimicrobial properties of Nonacosane, Palmitic acid, and Tridecanoic acid obtained from *A. indica* leaves have been reported in various published articles. These studies have consistently shown that these compounds exhibit significant antimicrobial activity against various microorganisms, including bacteria, fungi, and viruses. The antimicrobial properties of these compounds are attributed to their ability to disrupt the cell membranes of microorganisms, thereby inhibiting their growth and replication. This study agrees with the report of Megha and Venkatesha (2023) that ethanol extract showed antimicrobial of on *A. indica* activities effectiveness against *K. lebsiella* and *S. aureus.* They also reported that Ethyl acetate extract was more active against *E. coli* and MRSA and that both ethanolic and petroleum ether extracts exhibited activity against *C. albicans.*

Effect of *A. indica* leaves extract and fractions on test wood Fungi

The fungicidal activity of *A. indica* leaves methanol crude extract against wood fungi has been extensively studied. Research by Salazar *et al.* (2015) and Abd *et al.* (2022) have shown that *A. indica* leaves contain bioactive compounds that exhibit potent antifungal properties against various pathogenic fungi, including *Alternaria alternate*, *Neoscytalidium dimidiatum, Sordaria fimicola, and Candida albicans.* Owoyale *et al.,* (2020) reported that methanol crude extract of *A, indica* leaves demonstrated significant inhibitory effects on fungal growth, with MIC and MFC against different strains of wood fungi, such as P37005 and RM1000. A study by Ali *et al.* (2024) found that *A. indica* extracts, particularly from leaves and seeds, exhibited antifungal activity against plant pathogenic fungi such as *Cladosporium fulvum, Colletotrichum coccodes, Fusarium oxysporum,* and *Rhizoctonia solani*. The study reported variable MIC and MFC values for the different extracts. Another study by Wylie and Merrell (2022) reviewed the antimicrobial potential of *A. indica,* including its antifungal activity against *Candida albicans* and other oral microbes. A study published by Salazar *et al.* (2015) found that methanolic extracts of *A. indica* leaves had higher antifungal activity against dermatophyte fungi compared to seed oil extracts, which is consistent with the findings of the current research. Wylie and Merrell (2022) also reported antimicrobial potential of *A. indica,* including its antifungal properties, and highlighted the need for further research to explore its therapeutic applications. Mostafa *et al.* (2023) reported that neem leaf extracts and neem Ag-NPs demonstrated antimicrobial activity against food-borne pathogenic bacteria and mycotoxigenic fungi, with neem Ag-NPs showing the lowest minimum inhibitory concentration (MIC) values. These findings highlight the potential of *A. indica* as a natural source of antifungal agents that can be effective against wood fungi, showcasing its promising role in environmentally friendly and sustainable fungicidal applications.

Effect of *A. indica* leaves extract termites infestation on test wood samples

The termiticidal activity of *A. indica* (neem) extracts has been extensively studied, with various extracts exhibiting significant inhibitory effects against various termite species. However, termicidal properties of Nonacosane, Palmitic acid, and Tridecanoic acid obtained from *A. indica* leaves have not been extensively studied. Nevertheless, some studies have reported that these compounds exhibit some degree of termicidal activity. For example, a study published by Kumar *et al*. (2018) found that nonacosane exhibited some degree of termicidal activity. A study by Okweche *et al.* (2021) found that the oil extracts from the kernels of *A. indica* and *Jatropha curcas* exhibited a dose-dependent effect on adult *Coptotermes sjostedti,* with the highest mortality rate observed at a concentration of 15 mL. Another study by Ahmed *et al.* (2016) found that the extracts of *A. indica* and *J. curcas* were effective against *Heterotermis indicola,* with the highest mortality rate observed at a concentration of 20%. The termiticidal activity of *A.*

indica extracts has also been compared to that of other plant extracts. A study by Karyah, (2021) by establish that the extracts of *A. indica* and Jatropha curcas were more effective against termites than the extracts of other plants, such as garlic and tobacco. The toxicological effects of the extracts have also been studied. A study by Ahmed *et al.* (2016) found that the extracts of *A indica* and *J. curcas* were toxic to termites, with the highest mortality rate observed at a concentration of 100%.

CONCLUSION

The study characterized a long chain alkane, nonacosane from AIE12; palmitic acid from AIE26 and Tridecanoic acid from AIE60 *A. indica* fractions. *Azadirachta indica* leaves methanol crude extract and fractions were very active five wood bacteria: *Bacillus Subtili, Pseudomonas aeruginosa, Serratia marcescens, nterobacter sp, Rhanella sp, and Pectobacterium carotovorum* at zone of inhibition between 19 – 25 mm and MIC and MBC of between 100 – 400 mg/mL of fractions and 5 – 10 mg/mL of crude extract out of 10 test samples.Similarly, *A. indica* leaves methanol crude extract and fractions were very active six wood fungi namely: *Aspergillus flavus, Fibroporia vaillantii, Rhizopus sp, Fomitopsis pinicola, Fusarium sp and Serpula lacrymans* at zone of inhibition between 19 – 25 mm and MIC & MBC of between 50 – 200 mg/mL of fractions and 2.5 – 10 mg/mL of crude extract out of ten tested.

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