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Antibacterial Activity of Triterpenes from the Stem Bark and Heartwood of *Erythrophleum suaveolens* (Guill. & Perr.) Brenan

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ABSTRACT: The antibacterial activity of compounds isolated from the stem bark and sawdust of *Erythrophleum suaveolens* were evaluated. Extraction of the pulverized plants materials was carried out using hexane, ethyl acetate and methanol. Extracts were fractionated using column chromatography and fractions were examined using Nuclear Magnetic Resonance spectroscopy. Antibacterial activities against *Acidobacterium capsulatum, Actinobacterium sp., Agrobacterium tumefaciens, Bacillus subtili, Ralstonia solanacearum, Enterococcus faecium, Escherichia coli, Pseudomonas syringae, Pseudomonas aeruginosa* and *Proteus mirabilis* were also carried out. A novel triterpene, 21-acetoxylupenone and betulin were identified in the fractions from the stem bark of *E. suaveolens* while cycloeucalenol and stigmasterol were obtained from the sawdust extract fractions. *Pseudomonas aeruginosa* was resistant to all control antibiotics but was inhibited by 21-acetoxylupenone and cycloeucalenol with 23 mm and 24 mm diameter of Zones of Inhibition. *Acidobacterium capsulatum, Actinobacterium sp., Agrobacterium tumefaciens, Ralstonia solanacearum,* and *Proteus mirabilis* were completely inhibited at Minimum Bactericidal Concentration (MBC) of 50 µg/mL, while *Bacillus subtilis, Enterococcus faecium, Escherichia coli, Pseudomonas syringae, and Pseudomonas aeruginosa* were completely inhibited at MBC values of 100 µg/mL. Thus *E. suaveolens* stem bark and saw dust isolates can be used in the control of plants diseases where these pathogens are the causative agents.

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The usefulness of wood cannot be overemphasized as wood materials serve a lot of purpose in construction and furniture. They could last for years without deterioration when they are properly treated (Government of Canada, 2016). However, they can be degraded by insects, pests, bacteria and fungi. Several methods have been used to control pest invasion on wood using organic and inorganic chemicals but the application of these synthetic chemicals in wood preservation has lots of challenges such as persistence of toxicants in the host, poison to organisms, woodworkers and users, cost and undesirable effects on non-target species (Obomanu *et al.,* 2017). Due to the problems and risks involved in the application of synthetic chemicals in wood preservation (Bozkurt, *et al.,* 1993; Ongley, 1996), interest in botanicals as alternative sources of wood preservatives has been rekindled. Bio-pesticides are possible alternatives as they are biodegradable, less toxic to the environment and effective especially against bacteria (Arango *et al.,* 2005). Bacteria that inhabit wood are capable of affecting wood permeability, destroying wood structure, or together with other bacteria and soft-rot fungi predispose wood to fungal attack (Clausen, 1996). Lignivorous bacteria cause irreversible damage, as they crack and soften the wood until it is completely decayed or decomposed. *Erythrophleum suaveolens* (Guill. & Perr.) Brenan belongs to the family Leguminosae-Caesalpinioideae. It is commonly known as ordeal tree; red water tree; sassy bark or sasswood tree. In French it is known as *bois rouge*; *poison d'épreuve*. An alcoholic extract of the bark is used as a drink or stimulant while the leaf and bark are used as medicines for arthritis, rheumatism, and eye treatment, oral treatment for naso-pharyngeal infections and as pain-killer (Aiyegoro *et al.* 2007). Erythrophleguine has been reported from the plant and it showed anti-fungal activities (Onuorah, 2000). This report is on the effect of stem bark and sawdust isolates on selected bacteria that cause damage in wood and wood products

MATERIALS AND METHODS

Collection and preparation of plant materials: Sawdust of *E. suaveolens* was collected from a timber shed in Makurdi Benue State Nigeria. The stem bark was collected from within the Federal University of Agriculture Makurdi. The sawdust and stem bark were air dried and the stem bark was ground to powder.

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Extraction of the stem bark and sawdust: Extraction of the powdered sawdust and stem bark was carried out by maceration using 600 g of sawdust and 1 kg of stem bark in 1 litre of hexane, ethyl acetate and then methanol for 24 hours each. The extracts were filtered, allowed to dry and then weighed.

Column chromatography: A slurry of silica gel (50 g) in hexane: ethyl acetate 95:5 was introduced into a glass column and the hexane extract (pre-adsorbed on silica gel) was loaded onto column bed. The pre-adsorbed hexane extract on silica gel was loaded onto the column and eluted with gradient mixtures of ethyl acetate in hexane. Fractions were collected and examined by TLC based on which similar fractions were combined. The combined fractions were allowed to stand for any precipitation or crystal formation.

Nuclear Magnetic Resonance (NMR) spectroscopic analysis: Fractions ES24, ESS25, ESS31 and ESS37 were analyzed using a Bruker-Avance 400 MHz) spectrophotometer with deuterated chloroform as solvent. The NMR data were analyzed using Mestrenova 12 software. Characterization and structure elucidation of the compounds was based on their ^{1}H, ^{13}C and 2D NMR experiments.

Antibacterial Screening: Antibacterial screening was carried out at Nigerian Institute for Leather Science and Technology (NILEST), Zaria. The antibacterial activities of ES24, ESS25 and ESS31 isolates were examined on *Acidobacterium capsulatum, Actinobacterium sp., Agrobacterium tumefaciens, Bacillus subtili, Ralstonia solanacearum, Enterococcus faecium, Escherichia coli, Pseudomonas syringae, Pseudomonas aeruginosa* and *Proteus mirabilis.* Isolates were screened using a disk diffusion method. Initial concentrations of compounds were prepared by dissolving 0.002 g of the compounds in 10 mL of DMSO to obtain a concentration of 200 µg/mL and used to determine antibacterial activities. Media were prepared according to manufacturer's instructions and purified at 121^{o}C for 15 minutes. The media was emptied into germ-free Petri dishes and was left to set. Mueller Hinton agar was seeded with a 0.1 mL standard inoculum of the test bacteria and spread uniformly on the surface of the medium with the aid of a disinfected swab. A 6 mm cork borer was used to make a well at the middle of each injected medium. In the well on the inoculated medium, a solution of 0.1 mL of compound of 200 µg/ml of concentration was introduced at 30^{o}C for 1-7 days and the media plates were observed for zones of inhibition (ZOI) of bacteria growth. The zone was measured in millimeters with a transparent ruler.

Minimum Inhibition Concentration (MIC): A broth dilution method was used to determine MIC of compounds. Mueller Hinton agar broth was prepared while 10 mL was dispensed into test tubes; the broth was sterilized at 121 ^{o}C for 15 minutes and allowed to cool. To produce turbid solution, Mc-Farland turbidity scale number 0.5 was made ready. Normal saline was prepared as 10 mL and emptied into sterilized test tube. The test microbe was inoculated and nurtured at 30^{o}C for 6 hours for the test bacteria. Dilution of the test bacteria was carried out in the standard saline until the turbidity synchronised with the scale of Mc-Farland by visual assessment. At this point, the test bacteria had a concentration of about $1.5x10^{8}$ cfu/mL. To obtain concentration levels of 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/mL and 12.5 µg/mL, two-fold serial dilution of compounds was carried out in the sterilized broth. Initial concentration was obtained by dissolving 0.002g of the compound in 10 ml of the sterile broth and 0.1 mL of the bacteria in the normal saline was then introduced into the different concentrations. The test bacteria were introduced into the Mueller Hinton broth. Incubation was made at at 37 ^{o}C for 24 hours for the bacteria to observe turbidity (growth). The least concentration of the compound in the sterilized broth that indicates no turbidity was recorded as the MIC.

Minimum Bactericidal Concentration (MBC): Mueller Hinton agar was prepared and sterilized at 121^{o}C for a period of 15 minutes, emptied into sterilized Petri dishes and left to cool and set. The contents of the MIC tubes were then sub-cultured onto the prepared medium. The bacteria were incubated at 37 ^{o}C for 24 hours, after which the plates of the media were observed for colony growth. MBC were determined as plates with lowest concentration of the compound without colony growth.

Data Analysis: Analysis of Variance (ANOVA) was used to determine significant effects of treatments on ZOI. Follow up test was carried out using Duncan Multiple Range Test (DMRT) where significant differences existed.

RESULTS AND DISCUSSION

The yields of E. suaveolens stem bark and sawdust extracts are given in Table 1. Extract yields were highest with methanol followed by ethyl acetate and then hexane. The stem bark gave higher percentage yield of extracts.

Characterization of ES24 as 21-acetoxylupenone: ES24 was obtained as white needles. Its proton nuclear magnetic resonance (^{1}H-NMR) gave the following data (Figure 1): ^{1}H NMR (500 MHz, chloroform-*d*) δ 4.85 (dd, *J* = 8.5, 5.3 Hz, 1H), 2.47 (ddd, *J* = 19.7, 10.8, 5.9 Hz, 2H), 2.38 (ddd, *J* = 15.5, 7.7, 4.4 Hz, 1H), 1.99 (d, *J* = 1.7 Hz, 3H), 1.85 (dtd, *J* = 28.2, 12.6, 4.4 Hz, 2H), 1.68 (s, 5H), 1.63 – 1.59 (m, 1H), 1.47 (d, *J* = 14.4 Hz, 3H), 1.42 (d, *J* = 6.8 Hz, 2H), 1.39 – 1.22 (m, 4H), 1.06 (d, *J* = 6.3 Hz, 6H), 1.01 (s, 4H), 0.94 (s, 6H), 0.92 (s, 3H). ES24 was characterized as 21-acetoxylupenone (Figure 2).

*Characterization of ES28 as Betulin***:** ES28 was obtained as white needles. Its proton nuclear magnetic resonance (^{1}H-NMR) gave the following data (Figure 3): ^{1}H NMR (500 MHz, Chloroform-*d*) δ 4.73 (s, 1H), 4.60 (s, 1H), 3.19 (dd, *J* = 11.4, 4.9 Hz, 1H), 2.34 (t, *J* = 7.6 Hz, 4H), 1.07 (s, 4H), 1.02 (d, *J* = 4.7 Hz, 5H), 0.97 (s, 4H), 0.96 (s, 3H), 0.93 (d, *J* = 3.4 Hz, 6H), 0.88 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H). ES28 was characterized as betulin (Figure 4).

Table 1: Percentage Yield of *E. suaveolens* stem bark and sawdust extracts under various solvents

S/No.	Solvent	Extract	Weight of plant material (g)	Weight of dry extract (g)	Percentage yield of extract (%)	Appearance
1	Ethyl acetate	*E. suaveolens* stem bark extract	1000	3.60	0.36	Dark Brown
		E. suaveolens sawdust	600	6.40	1.06	Dark Brown
2	Methanol	*E. suaveolens* stem bark extract	1000	51.90	5.19	Dark Brown
		E. suaveolens sawdust	600	20.50	3.42	Purple
3	N-Hexane	*E. suaveolens* stem bark extract	1000	1.60	0.16	Dark Yellow
		E. suaveolens sawdust	600	0.70	0.12	Golden Yellow

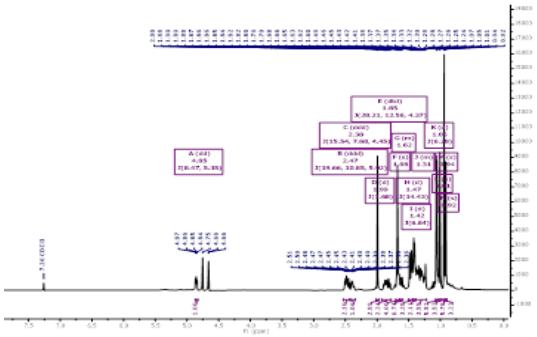

Fig 1: Proton NMR spectrum of ES24

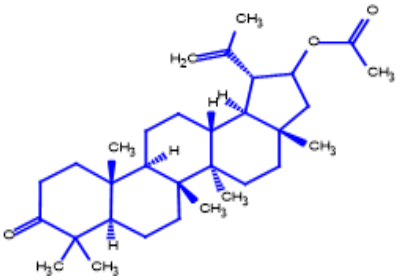

Fig 2: Structure of ES24 (21-acetoxylupenone)

Characterization of ESS25 as Cycloeucalenol: ESS25was obtained as white needles. Its proton nuclear magnetic resonance (^{1}H-NMR) gave the following data (Figure 5): ^{1}H NMR (500 MHz, Chloroform-*d*) δ 4.52 (d, *J* = 26.0 Hz, 1H), 3.09 – 2.99 (m, 0H), 2.07 (h, *J* = 6.5 Hz, 0H), 1.95 (ddd, *J* = 15.5, 11.4, 4.7 Hz, 1H), 1.84 – 1.77 (m, 1H), 1.72 (ddd, *J* = 15.2, 8.2, 4.4 Hz, 1H), 1.51 (td, *J* = 9.0, 8.6, 3.9 Hz, 1H), 1.48 – 1.34 (m, 4H), 1.29 – 1.22 (m,

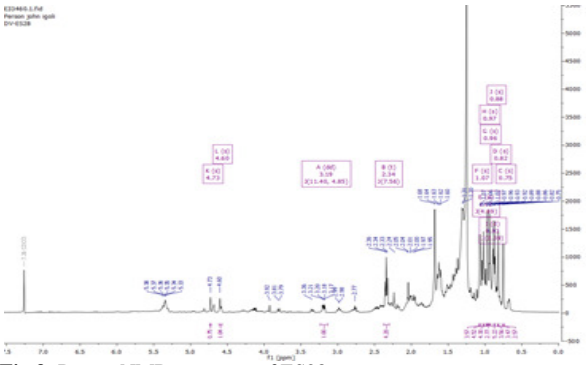

Fig 3: Proton NMR spectrum of ES28

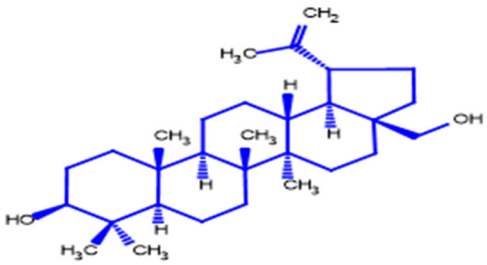

Fig 4: Structure of ES28 (Betulin)

1H), 1.16 – 1.07 (m, 4H), 1.01 (dddd, *J* = 19.0, 15.5, 7.4, 4.1 Hz, 1H), 0.86 (dd, *J* = 6.9, 2.8 Hz, 3H), 0.81 (d, *J* = 7.3 Hz, 3H), 0.73 (d, *J* = 4.8 Hz, 3H), 0.41 (dd, *J* = 12.1, 2.8 Hz, 1H), 0.22 (d, *J* = 4.1 Hz, 1H). ESS25 was characterized as Cycloeucalenol (Figure 6).

Characterization of ESS37 as a mixture of sitosterol, stigmasterol and cycloeucalenol: Fraction ESS37 was observed from its proton NMR spectrum (Fig. 7) to be a mixture of sitosterol, stigmasterol and cycloeucalenol.

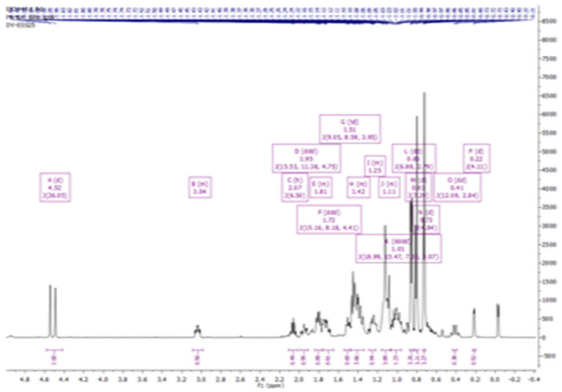

Fig 5: Proton NMR spectrum of ESS25

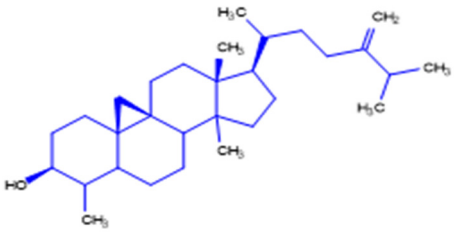

Fig 6: Structure of ESS25 (Cycloeucalenol)

The characteristic signals for these compounds were all present in the spectrum. Table 2 summarizes the compounds obtained from the plant extracts.

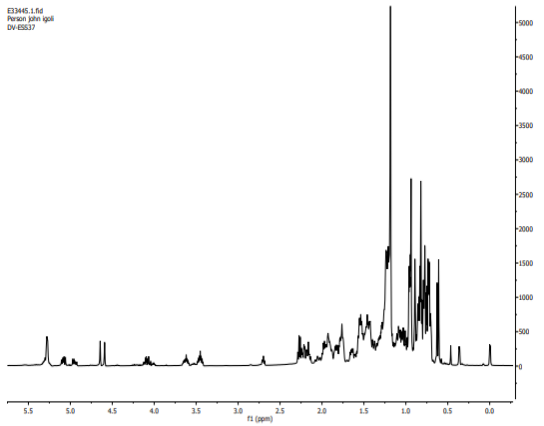

Fig 7: Proton NMR spectrum of ESS37 (A mixture of sitosterol, stigmasterol and cycloeucalenol).

Betulin and 21-acetoxylupenone were identified and characterized from *Erythrophleum suaveolens* stem bark isolates (ES28 and ES24), respectively, while Cycloeucalenol and unidentified lupane type triterpene and stigmasterol were from the sawdust (Table 2).

Table 2. Summary of Identified and Characterized Compounds from *Erythrophleum suaveolens*

Plant Species	Plant part	Isolate	Identified Compound	Class of compound
Erythrophleum suaveolens	Stem bark	ES24	21-acetoxylupenone	Triterpene
	Stem bark	ES28	Betulin ($C_{30}H_{50}O_2$)	Triterpene
	Sawdust	ESS25	Cycloeucalenol ($C_{30}H_{50}O$)	Triterpene
	Sawdust	ESS37	Sitosterol, stigmasterol and cycloeucalenol	Triterpenes

*Sensitivity and Mean ZOI of standard antibiotics against test Bacteria***:** *Acidobacterium capsulatum, Actinobacterium sp.* and *Pseudomonas syringae* were sensitive to Sparfloxacin at ZOI of 32 mm, 30 mm and 31 mm, respectively but were resistant to Ciproflaxacin, and Cefuroxime (Table 3). *Escherichia coli* and *Proteus mirabilis* were sensitive to the three antibiotics at range of ZOI of 29 – 39 mm. However, *Pseudomonas aeruginosa* was resistant to the three antibiotics. ZOI of the three antibiotics ranged between 26 mm and 39 mm which proved that they were very active against the test bacteria.

Antibacterial activities and mean ZOI of E. suaveolens isolates (ES24, ESS25, and ESS31) against test bacteria: In Table 3, ES24 isolate and ESS31 fraction were very active against *Acidobacterium capsulatum, Bacillus subtilis* and *Ralstonia solanacearum* at ZOI of 28 mm and 23 mm, 25 mm and 27 mm, as well as 27 mm and 26 mm respectively. Similarly, ES24 isolate and ESS31 fraction were very active against *Ralstonia solanacearum, Enterococcus faecium* and *Proteus mirabilis* at ZOIs of from 28 mm to 24 mm. However, *Pseudomonas syringae* was sensitive to the isolates of ES24 and ESS25, and fraction of ESS31 at ZOI of between 24 and 25mm.

*Minimum Inhibition Concentration (MIC) of E. suaveolens isolates (ES24 and ESS25) and fraction (ESS31) against test bacteria***:** At MIC of 25 µg/mL (Table 4) ES24 isolate completely inhibited the growth of *Actinobacterium sp.* and *Proteus mirabilis;* ES28 isolate prevented the growth *Acidobacterium capsulatum, Agrobacterium tumefaciens* and *Ralstonia solanacearum,* respectively; while ESS 31 hindered the growth of *Pseudomonas aeruginosa*. At MIC of 50 µg/mL ES24 inhibited the growth the growth of *Agrobacterium tumefaciens, Enterococcus faecium, Pseudomonas syringae* and *Pseudomonas aeruginosa.*

*Minimum Bactericidal Concentration (MBC) of E. suaveolens isolates (ES24 and ESS25) and and fraction (ESS31) against test bacteria***:** The most potent MBC was 50 µg/mL at which ES24 and ESS25 isolates and ESS31 fraction completely inhibited *Actinobacterium sp.* and *Proteus mirabilis; Acidobacterium capsulatum, Agrobacterium tumefaciens* and *Ralstonia solanacearum;* and *Bacillus subtilis,* respectively (Table 5). At MBC 100 µg/mL *Agrobacterium tumefaciens, Pseudomonas syringae* and *Pseudomonas aeruginosa* pathogens were inhibited by ES24 while, *Bacillus subtilis, Pseudomonas syringae* and *Pseudomonas aeruginosa* were inhibited by ESS25. Similarly, *Ralstonia solanacearum Enterococcus faecium, Enterococcus faecium* and *Pseudomonas syringae* growths were completely prevented by ESS31 fraction.

Table 3: Mean Zone of Inhibition of E. *suaveolens isolates* (ES24, ESS25, and ESS31) and standard antibiotics against test bacteria

S/No.	Test Bacteria	ES 24 (200 µg/mL)	ESS25 (200 µg/mL)	ESS31 (200 µg/mL)	Ciproflaxacin (100 µg/mL)	Sparfloxacin (100 µg/mL)	Cefuroxime (100 µg/mL)
		ABA(ZOI) mm Mean ± SD	ABA(ZOI) mm Mean ± SD	ABA(ZOI) mm Mean ± SD	ABA(ZOI) mm Mean ± SD	ABA(ZOI) mm Mean ± SD	ABA(ZOI) mm Mean ± SD
1	*Acid bacterium capsulatum*	R(0.00±0.00^{a})	S(28.00±1.00^{bc})	S(23.00±1.00^{b})	R0.00±0.00^{a})	S(32.00±1.00^{bc})	R(0.00±0.00^{a})
2	*Ralstonia solanacearum*	S(28.00±2.00^{d})	R(0.00±0.00^{a})	S(24.00±2.00^{b})	R(0.00±0.00^{a})	S(30.00±5.00^{ab})	R(0.00±0.00^{a})
3	*Agrobacterium tumefaciens*	S(24.00±2.00^{bc})	S(30.00±2.00^{c})	R(0.00±0.00^{a})	S(32.00±5.00^{de})	R(0.00±0.00^{a})	S(34.00±1.00^{bc})
4	*Bacillus subtilis*	R(0.00±0.00^{a})	S(25.00±3.00^{b})	S(27.00±3.00^{b})	S(28.00±2.00^{cd})	S(35.00±5.00^{c})	R(0.00±0.00^{a})
5	*Ralstonia solanacearum*	R(0.00±0.00^{a})	S(27.00±2.00^{bc})	S(26.00±4.00^{b})	R(0.00±0.00a)	S(30.00±3.00^{a})	S(31.00±2.00^{b})
6	*Enterococcus faecium*	S(26.00±1.00^{bcd})	R(0.00±0.00^{a})	S(25.00±1.00^{b})	S(26.00±2.00^{b})	R(0.00±0.00^{a})	S(30.00±9.00^{b})
7	*Escherichia coli*	R(0.00±0.00^{a})	R(0.00±0.00^{a})	S(27.00±3.00^{b})	S(34.67±6.81^{e})	S29.00±1.00^{b})	S(39.00±2.00^{c})
8	*Pseudomonas syringae*	S(25.00±1.00^{bc})	S(24.00±6.00^{b})	S(24.00±4.00^{b})	R(0.00±0.00^{a})	S(31.00±4.00^{bc})	R(0.00±0.00^{a})
9	*Pseudomonas aeruginosa*	S(23.00±1.00^{b})	S(24.00±1.00^{b})	R(0.00±0.00^{a})	R(0.00±0.00^{a})	R(0.00±0.00^{a})	R(0.00±0.00^{a})
10	*Proteus mirabilis*	S(27.00±4.00^{bcd})	R(0.00±0.00^{a})	S(25.00±4.00^{b})	S(32.00±1.00^{cd})	S(33.33±3.51^{bc})	S(31.00±3.00^{b})

Key: *S = Sensitive, R = Resistance; ES = E.suaveolens stem back; ESS = E. suaveolens, sawdust; ABA = Antibacterial Activities, ZOI = Zone of Inhibition ZOI < 10 mm is inactive; 10 -13 mm is partially active; 14 -19 mm is active, and >19 mm is very active. ± - Standard error; Values without common letters are significantly different at P = 0.05*

Table 4: Minimum Inhibition Concentration (MIC) of *E. suavelens* isolates (ES24, ESS25, and ESS31) against test bacteria

S/No.	Test Bacteria	ES24 Concentration (µg/mL)					ESS25 Concentration (µg/mL)					ESS31 Concentration (µg/mL)				
		200	100	50	25	12.5	200	100	50	25	12.5	200	100	50	25	12.5
1	*Acidobacterium capsulatum*	R	R	R	R	R	-	-	-	δ	+	-	-	δ	+	#
2	*Ralstonia solanacearum*	-	-	-	δ	+	R	R	R	R	R	-	-	δ	+	#
3	*Agrobacterium tumefaciens*	-	-	δ	+	#	-	-	-	δ	+	R	R	R	R	R
4	*Bacillus subtilis*	R	R	R	R	R	-	-	δ	+	#	-	-	δ	+	#
5	*Ralstonia solanacearum*	R	R	R	R	R	-	-	-	δ	+	-	-	δ	+	#
6	*Enterococcus faecium*	-	-	δ	+	#	R	R	R	R	R	R	R	R	R	R
7	*Escherichia coli*	R	R	R	R	R	R	R	R	R	R	-	-	δ	+	#
8	*Pseudomonas syringae*	-	-	δ	+	#	-	-	δ	+	#	R	R	R	R	R
9	*Pseudomonas aeruginosa*	-	-	δ	+	#	-	-	δ	+	#	-	-	-	δ	+
10	*Proteus mirabilis*	-	-	-	δ	+	R	R	R	R	R	-	-	δ	+	#

Key: ES = Erythrophleum suaveolens stem back; ESS = Erythrophleum suaveolens sawdust; R = Resistance; - = No turbidity (no growth) δ = Minimum inhibitory Concentration (MIC); + = Turbid (Light growth); # = Moderate turbidity; ### = High turbidity; ## = Very High turbidity

Table 5: Minimum Bactericidal Concentration (MBC) of *E. suaveolens isolates* (ES24, ESS25 and ESS31) against test bacteria

S/No.	Test Bacteria	ES24 Concentration (µg/mL)					ESS25 Concentration (µg/mL)					ESS31 Concentration (µg/mL)				
		200	100	50	25	12.5	200	100	50	25	12.5	200	100	50	25	12.5
1	*Acidobacterium capsulatum*	R	R	R	R	R	-	-	δ	+	#	-	δ	+	#	###
2	*Ralstonia solanacearum*	-	-	δ	+	#	R	R	R	R	R	δ	+	#	###	###
3	*Agrobacterium tumefaciens*	-	δ	+	#	###	-	-	δ	+	#	R	R	R	R	R
4	*Bacillus subtilis*	R	R	R	R	R	-	δ	+	#	###	-	-	δ	+	#
5	*Ralstonia solanacearum*	R	R	R	R	R	-	-	δ	+	#	-	δ	+	#	###
6	*Enterococcus faecium*	-	δ	+	#	###	R	R	R	R	R	-	δ	+	#	###
7	*Escherichia coli*	R	R	R	R	R	R	R	R	R	R	-	δ	+	#	###
8	*Pseudomonas syringae*	-	δ	+	#	###	-	δ	+	#	###	-	δ	+	#	###
9	*Pseudomonas aeruginosa*	-	δ	+	#	###	-	δ	+	#	###	R	R	R	R	R
10	*Proteus mirabilis*	-	-	δ	+	#	R	R	R	R	R	-	δ	+	#	###

Key: ES = Erythrophleum suaveolens stem back; ESS = Erythrophleum suaveolens sawdust; R = Resistance; - = No turbidity (no growth) δ = Minimum inhibition concentration (MIC); + = Turbid (Light growth); # = Moderate turbidity; ### = High turbidity; ## = Very High turbidity

The triterpene 21-acetoxylupenone is isolated and characterized for the first time, while cycloeucalenol is isolated from *E. suaveolens* for the first time. Cox *et al.,* (1956) first reported cycloeucalenol in *Eucalyptus microcorys* and thereafter in *Erythrophloeum guineense.* ES24 and ESS25 isolates of *E. suaveolens* were very active against *Pseudomonas aeruginosa* which showed resistance to controls (of three antibiotics) at ZOI of 23 mm and 24 mm, respectively. Atibioke (2016) reported ZOI of 18 - 28 mm from *Uapaca pilosa* isolates against bacteria. In this study, the antibacterial activity of *E. suaveolens* stem bark isolate against gram positive and gram negative bacteria was evidenced. Ngoupayo *et al.* (2015) reported that Minimum Bactericidal Concentration is the minimum concentration corresponding to the lowest concentration of a substance capable of killing more than 99.9% of bacterial inoculum or, initial (less than 0.1% of survivors) after 18 to 24 hours of incubation at a temperature of 37 °C. At MBC of 50 µg/mL *Acidobacterium capsulatum, Actinobacterium sp., Agrobacterium tumefaciens, Ralstonia solanacearum,* and *Proteus mirabilis* were killed. Similarly, *Bacillus subtilis, Enterococcus faecium, Escherichia coli, Pseudomonas syringae, and Pseudomonas aeruginosa* were killed at MBC of 100 µg/mL. The finding implies that *E. suaveolens* stem bark and sawdust isolates (ES24 and ESS25) and sawdust fraction (ESS31) can be used in the treatment of lignin decay caused by Actinobacterium (Brown and Chang 2014); wood decay colonization caused by *Agrobacterium tumefaciens;* crown gall disease caused by *Acidobacterium capsulatum (*Kersters *et al.,* 2006). The isolated compounds might also be applied in the control of bacterial wilt disease caused by *Ralstonia solanacearum* (Kirby, 2006); bark canker, fire blight and soft rot diseases caused by *Pseudomonas syringae (*Kersters *et al.,* 2006); and fire blight and apple ring rot caused by *Bacilli subtilis* (Liu *et al.*, 2009; Broggini *et al*., 2005). Also, *E. suaveolens* isolates can control bacteria soft disease (a destructive disease of fruits, vegetables, and ornamentals) and root infections of plant causing plant mortality caused by *Pseudomonas aeruginosa* as reported by Walker, (2004).

*Conclusion***:** The results obtained from this work have revealed that *E. suaveolens* stem bark and sawdust isolates and fraction possess potential antibacterial properties and can be used in the control and treatment of plant and animal diseases caused by bacteria.

REFERENCES

Aiyegoro, OA; Akinpelu, DA; Okoh, AI (2007). In vitro Antibacterial Potentials of the Stem Bark of Red Water Tree (Erythrophleum suaveolens). *J. of Biol. Sci.,* 7: 1233-1238.

Anokwuru, CP; Anyasor, GN; Ajibaye, O; Fakoya, O; Okebugwu, P (2011). Effect of Extraction solvents on phenolic, flavonoid and antioxidant activities of three Nigerian medicinal plants. *Nat. and Sci,* 9(7): 53-61.

Arango, AR; Green, FI; Hintz, K; Lebow, PK; Miller, BR (2005). Natural Durability of Tropical and Native Woods against Termite damage by Reticulutermers Flavipes. USDA. Forest service. *Int. Biodet. and Biodeg.* 57: 146-150.

Atibioke, AJ (2016). Isolation and Characterization of Bioactive compounds from Stembark Extract of *Uapaca pilosa* (Hutch.), M.Sc Dissertation, Ahmadu Bello University, Zaria Nigeria. p 1-92.

Bozkurt, Y; Göker, Y and Erdin, N (1993). Impregnation Technique University of Istanbul, Faculty of Forestry, Publication No: 425, Istanbul, Turkey.

Broggini, GAL; Duffy B; Holliger, E; Scha¨rer, HJ; Gessler, C Patocch, A (2005). Detection of the fire blight biocontrol agent Bacillus subtilis BD170 (Biopro) in a Swiss apple orchard. *Euro. J. of Plan. Path.* 111: 93–100

Brown, ME; Chang, MCY (2014). Exploring bacterial lignin degradation. *Curr Opin Chem Biol.* 19:1–7.

Charles, L; Kayanja, IBF (2012). In Vitro Antimicrobial Activity of Crude Extracts of Erythrina abyssinica and Capsicum annum in Poultry Diseases Control in the South Western Agro-Ecological Zone of Uganda, A Bird's-Eye View of Veterinary Medicine, Dr. Carlos C. Perez-Marin (Ed.), ISBN: 978-953-51-0031-7, InTech.

Clausen, CA (1996). Bacterial associations with decaying wood: a review. *Inl. Biod. and Biodeg.* 37(1-2), 101–107.

Cox, JSG; King, FE; King, T J (1959). The structure of cycloeucalenol. *J. of the Chem. Socie. (Resumed), 514.*

Government of Canada (2016). The benefits of wood in building construction. *www.nrcan.gc.ca/forest/16213.*

Gregory, AP; Clarke, RN (2005). Traceable measurements of the static permittivity of dielectric reference liquids over the temperature range 5–50°C. *Meas. Sci. and Techn.* 16, 1506–1516.

Jha, AK; Bais, HP; Vivanco, JM (2004). Enterococcus faecalis Mammalian Virulence-Related Factors Exhibit Potent Pathogenicity in the Arabidopsis thaliana Plant Model. *Inf. and Imm.* 73(1), 464–475.

Johnston, SR; Boddy, L; Weightman, AJ (2016). Bacteria in decomposing wood and their interactions with wood-decay fungi. *FEMS Micro. Ecol.* 92(11): fiw179. doi:10.1093/femsec/fiw179.

Kersters, K; De Vos, P; Gillis, M; Swings, J (2006). Proteobacteria. Encyclopedia of Life Sciences.doi:10.1038/npg.els.0004312.

Kirby, R. (2006). Actinomycetes and lignin degradation. *Adv. Appl. Microbiol.* 58: 125–168.

Liu, YZ; Chang,YH; Wei, BQ; Liu, YF; Chen, ZY; (2009). Screening and evaluation of antagonistic bacteria against Botryosphaeria berengeriana f. sp. piricola. *Chin. J. Fruit Sci.* 3:344–348.

Ngoupayo, J; Chelea, M; Djiele, NP; Félicien, MK; Ndjonkep, JY; Fotsing, KPR; Ndelo, J (2015). Characterization and evaluation in vitro of the antibacterial activity of tannins from Garcinia brevipedicellata (Bark.G) Hutch. & Dalz. *J. of Phar. and Phyto.* 5; 4(2): 81-85.

Obomanu, FG; Edori, OS; Edori, OS (2017). Protective property of Icacina trichantha against temites (Macrotermes spp) attack on the wood Bombax buonopozense. *Curr. Stud. in Comp. Edu. Sci. and Tech.* 4(1): 174-186.

Ongley, ED (1996). Control of water pollution from agriculture - FAO irrigation and drainage paper 55, ISBN 92-5-103875-9. Available at http://www.fao.org/ docrep/w2598e/w2598e00.htm#Contents.

Onuorah, EO (2000). The wood preservative potentials of heartwood extract of Milicia excelsa and *Erythrophleum suaveolens. Bior. Tech.,* 75(2), 171–173. doi:10.1016/s0960-8524(99)00165-0.

Qingfeng, M; Xuan, Z; Le, L; Shao, BFU. (2018). Research Progress in the Promissing Natural Product-Betulin. *Biomed. Sci. and Tech. Res.* 8(2). DOI: 10.26717/ BJSTR.2018.08.001619.

Sharma, A; Cannoo, DS (2016). A comparative study of effects of extraction solvents/techniques on percentage yield, polyhenolic composition, and antioxidant potential of various extracts obtained from stems of Nepeta leucophylla: RP-HPLC-DAD assessment of its polyhenolic constituents. *J. of Food Bioch.,* 41(2), e12337.

Walker, TS (2004). Pseudomonas aeruginosa-Plant Root Interactions. Pathogenicity, Biofilm Formation, and Root Exudation. *Plant Phys.* 134(1): 320–331.